SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.22v43n5p2309

Characterization of coagulase-positive *Staphylococcus* spp., antimicrobial resistance profile, and presence of enterotoxin-producing genes in goat milk in Paraná State

Caracterização de *Staphylococcus* spp. coagulase positiva, do perfil de resistência aos antimicrobianos e da presença de genes produtores de enterotoxinas no leite de cabra no Estado do Paraná

Fernanda Yuri Rodrigues Tanaka[1]; Fernanda Montanholi de Lira[1]; Samanta Stinghen de Abreu[2]*; Stael Málaga Carrilho[2]; Edson Antonio Rios[3]; Ronaldo Tamanini[3]; Elaine Maria Seles Dorneles[4]; Natalia Gonzaga[5]; Rafael Fagnani[5]; Ulisses de Pádua Pereira[5]

Highlights

Coagulase-positive *Staphylococci* can cause food poisoning.
27% of the selected strains were resistant to at least one antimicrobial.
Staphylococcus in goat milk carries enterotoxin-producing genes *sec*, *seh*, and *see*.
Goat milk is often used in artisanal products without heat treatment.
Goat milk is a source of penicillin and tetracycline resistant *Staphylococcus*.

Abstract

Goat milk presents interesting characteristics to consumers, but the production of this food faces several challenges that influence its quality. Process failures from milking to processing and commercialization can expose milk to contamination by pathogenic microorganisms, including the coagulase-positive *Staphylococcus* group. Although *Staphylococcus aureus* is the most relevant species in mastitis and food poisoning, other species in this group are also important, especially those related to food poisoning. This

[1] Doctoral Students of the Postgraduate Program in Animal Science, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: fernanda.yurit@uel.br; fernandamon1995@hotmail.com
[2] Master Students of the Postgraduate Program in Animal Science, UEL, Londrina, PR, Brazil. E-mail: samanta.stinghen31@gmail.com; stael.te@gmail.com
[3] Drs. in Animal Science, UEL, Londrina, PR, Brasil. E-mail: edsonrios1@hotmail.com; ronaldot@uel.br
[4] Profa Dra, Universidade Federal de Lavras, UFLA, Lavras, MG, Brasil. E-mail: elaine.dorneles@dmv.ufla.br
[5] Profs. Drs., UEL, Department of Preventive Veterinary Medicine, Londrina, PR, Brazil. E-mail: natalia_gonzaga_@hotmail.com; rafaelfagnani@hotmail.com; upaduapereira@uel.br
* Author for correspondence

Received: Nov. 29, 2021 - Approved: Nov. 07, 2022

study aimed to identify the coagulase-positive *Staphylococcus* species in goat milk using biochemical tests, determine the prevalence of antimicrobial resistance using the disc diffusion test, and investigate enterotoxin-producing genes, *sea, seb, sec, sed, see, seg, seh,* and *sei* by multiplex PCR. A total of 384 coagulase-positive *Staphylococcus* strains obtained from raw goat milk collected from nine farms in Paraná during four seasons of the year were studied. Biochemical tests showed that 85.69% of the 384 strains were *S. aureus*, followed by 9.38% of *S. intermedius*, 4.17% of *S. hyicus* and 0.78% of *S. delphini*. For the antimicrobial resistance test, up to three strains of each species identified as coagulase-positive *Staphylococci* were chosen from each farm, with a total of 74 strains. Of these, 27% (20/74) showed resistance to at least one antibiotic, and among all penicillin-resistant strains, 45% (9/20) also presented resistance to tetracycline. In the search for staphylococcal enterotoxin-producing genes, 49 *S. aureus* strains were studied; among them, 40.81% (20/49) presented enterotoxin-producing genes. The highest prevalence was detected for the *sec* gene, which was present in 22.44% (11/49) of the strains, followed by the *seh* gene in 18.36% (9/49), and the *see* gene was detected in 4.08% (2/49). It was concluded that *S. aureus* was the predominant species in raw goat milk, with a high prevalence of penicillin- and tetracycline-resistant *Staphylococci* and a significant number of strains with staphylococcal enterotoxin-producing genes. The strains studied carried enterotoxin-producing genes involved in food poisoning in humans, indicating that goat milk is a possible source of contamination and, therefore, a potential danger to public health.
Key words: Antimicrobial susceptibility. Biochemical tests. Enterotoxin. Public health.

Resumo

O leite de cabra apresenta características interessantes aos consumidores, porém é um alimento cuja produção enfrenta vários desafios, influenciando na sua qualidade. Falhas durante a obtenção do leite até o processamento e comercialização, podem expor este à contaminação por microrganismos patogênicos, incluindo o grupo dos *Staphylococcus* coagulase positiva. Embora *Staphylococcus aureus* seja a espécie de maior relevância quando se trata de mastites e intoxicações alimentares, outras espécies incluídas no grupo dos *Staphylococcus* coagulase positiva também possuem importância, principalmente relacionada à intoxicação alimentar. Este trabalho visou identificar as espécies de *Staphylococcus* coagulase positiva em leite de cabra através de testes bioquímicos, e então determinar a prevalência de resistência a antimicrobianos seguindo a metodologia da disco difusão e pesquisar genes produtores de enterotoxinas, *sea, seb, sec, sed, see, seg, seh* e *sei* por PCR Multiplex. Foram estudadas 384 cepas de *Staphylococcus* coagulase positiva obtidas do leite cru de cabra, coletado de nove propriedades rurais no Paraná durante as quatro estações do ano. Nos resultados dos testes bioquímicos, do total de 384 cepas constatou-se que 85,69% eram *S. aureus*, seguido de 9,38% de *S. intermedius*, 4,17% de *S. hyicus* e 0,78% de *S. delphini.* Para o teste de resistência antimicrobiana foram escolhidas até três cepas de cada espécie identificada como *Staphylococcus* coagulase positiva de cada uma das propriedades, totalizando 74 cepas. Destas, 27% (20/74) apresentaram resistência a algum dos antibióticos testados, e dentre todas as cepas que possuíam resistência à penicilina, 45% (9/20) também apresentaram resistência a tetraciclina. Na pesquisa de genes produtores de enterotoxinas estafilocócicas, foram estudadas 49 cepas de *S. aureus*, e destas, 40,81% (20/49) apresentaram genes produtores de enterotoxinas. A maior prevalência detectada foi a do gene sec, estando presente em 22,44% (11/49) das cepas, seguido de 18,36% (9/49) de *seh* e 4,08% (2/49) de *see*. Conclui-se que *S. aureus* foi a espécie predominante em leite cru de cabra, observando-se alta

prevalência de *Staphylococcus* resistentes à penicilina e à tetraciclina e um número significativo de cepas com presença de genes produtores de enterotoxinas estafilocócicas. As cepas estudadas são portadoras de genes produtores de enterotoxinas envolvidos em intoxicação alimentares em humanos, indicando que o leite de cabra é uma possível fonte de intoxicação e, portanto, um perigo potencial para a saúde pública.
Palavras-chave: Enterotoxinas. Saúde pública. Susceptibilidade antimicrobiana. Testes bioquímicos.

Introduction

Goat milk has a growing market owing to its nutritional and technological characteristics. The technological potential of this product is mainly related to its hypoallergenic characteristics, which make it an alternative to cow milk (Claeys et al., 2014; Balthazar et al., 2017). The quality and safety of milk depends directly on adequate hygienic conditions in milking, processing, and commercialization. Failures in these steps can expose milk to contamination with pathogenic microorganisms, including *Staphylococci* (Vyletělová et al., 2016; Palii et al., 2020).

Staphylococcus aureus is the most relevant species within the group of coagulase-positive organisms, as it causes the greatest damage to dairy production (Aras et al., 2012). From a public health perspective, in addition to the production of coagulase, which is indicative of *Staphylococcus* spp. pathogenicity and resistance to antimicrobials, other species belonging to the coagulase-positive group are considered potentially pathogenic because they are capable of producing more than 22 different thermostable enterotoxins, known as staphylococcal enterotoxins (SEs) (Le Loir et al., 2003). Although *S. aureus* is the most frequent species in milk samples, the importance of other coagulase-positive species, such as *S. intermedius* and *S. hyicus,* which can also cause food poisoning (Linage et al., 2012), must be considered.

SEs are potent antigens whose activity is maintained even after heat treatments such as pasteurization (Cretenet et al., 2011). They can be separated into two groups: the five classical serological types (SEA-SEE) and newer (SEG-SElY and counting) enterotoxin groups. Only enterotoxins with demonstrated emetic potential in monkeys were designated SE, whereas enterotoxins that failed to do so or have not been evaluated in non-human primate models of emesis are designated enterotoxin-like (SEl-) antigens (Fisher et al,, 2018; Argudín et al., 2010). SEs are one of the most common causes of food poisonings worldwide, and symptoms usually appear four hours after ingestion, and include nausea, vomiting, abdominal cramps, and diarrhea (Hennekinne et al., 2012).

The classical staphylococcal enterotoxins are responsible for 95% of staphylococcal food poisoning cases (Bencardino&Vitali,2019).Twostaphylococcal enterotoxins, SEA and SEB, are among the most important gastroenteritis-causing agents. They represent more than 60% of the agents involved in food poisoning in the USA and England (Jay et al., 2005).

S. aureus carries multiple genes and produces different SEs and SE-like toxins. The SEs (SEA to SEE, SEG to SEI, SEK, SEM to SET), and SE-like toxins (SElJ, SElL, SElU to SElZ) have been reported to be associated with foodborne disease outbreaks (Kluytmans & Wertheim, 2005; Fooladi et al., 2010). In 2014,

a food poisoning outbreak was reported at a Swiss boarding school, attributed to the consumption of soft cheese made from raw cow's milk. In this study, we found high levels of staphylococcal enterotoxins in cheese (Johler et al., 2018). In China, 94.8% of *S. aureus* isolates from goat milk contain at least one of the enterotoxin-producing genes, with seb and sea being the most common (Qian et al., 2019).

Recently, several studies have reported the transmission of antimicrobial-resistant microorganisms via food, including milk and dairy products (Laganà et al., 2020). The selection of antimicrobial-resistant S. aureus strains poses a potential public health risk.

However, little is known about the frequency of coagulase-positive *Staphylococcus* species, their antimicrobial resistance profile, and the presence of enterotoxins in goat milk obtained in the state of Paraná.

Therefore, this study aimed to identify coagulase-positive *Staphylococcus* species in goat milk, determine the prevalence of antimicrobial resistance, and evaluate the presence of enterotoxin-producing genes in these isolates.

Material and Methods

Sampling and biochemical tests to identify coagulase-positive Staphylococci

A total of 384 coagulase-positive *Staphylococcus* strains isolated from raw goat milk collected from the bulk milk tanks of healthy goat herds were evaluated. The isolates were stored in BHI (Brain Heart Infusion) broth (Oxoid, Hampshire, United Kingdom) with 20% glycerol (-24 °C) and were provided by the Laboratório de Inspeção de Produtos de Origem Animal, Universidade Estadual de Londrina, Paraná. The strains were obtained from nine dairy goat farms located in the state of Paraná and collected during four periods of the year (fall, winter, spring, and summer) (Rios et al., 2018).

The isolates were subjected to the coagulase test and streaked on 5% sheep blood agar to observe purity and hemolysis. The pure colonies were then tested for species identification in phenol red broth (1% mannitol, 1% maltose, and 1% trehalose) and Voges Proskauer (VP) in Clark Lubs medium. To interpret the results, the methodology described by Costa et al. (2010) was followed.

Antimicrobial resistance test

To evaluate antimicrobial resistance, 74 strains of coagulase-positive *Staphylococci* were tested to obtain representative isolates from all the farms and the four sampling periods. Of these, 49 strains were *S. aureus,* 16 were *S. intermedius,* six *S. hyicus,* and three *S. delphini.*

The strains were seeded onto nutrient agar (Oxoid) and incubated at 37°C for 24 h. Then, antimicrobial resistance testing was performed using the disc diffusion test on Müller Hinton agar (Oxoid) according to the guidelines of the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute [CLSI], 2021). For this purpose, a colony from the nutrient agar plate was inoculated in 0.1% peptone saline solution and adjusted to 0.5 turbidity on the McFarland scale.

The isolates were tested for resistance to β-lactams [penicillin (P, 10 units)], inhibitors of the folic acid metabolic pathway [sulfamethoxazole-trimethoprim (Sxt, 25 µg)], fluoroquinolones [norfloxacin (Nor, 10 µg), enrofloxacin (Eno, 5 µg)], cephalosporins [cefotaxime (Ctx, 30 µg), ceftiofur (Ctf, 30 µg)], and tetracyclines [tetracycline (Tet, 30 µg)] (Cefar, São Paulo, Brazil). After inoculation and application of the antimicrobial discs, the Müller Hinton plates were incubated at 37 °C for 18 h.

Characterization of staphylococcal enterotoxin-producing genes

To investigate enterotoxin-producing genes, 49 strains of *Staphylococcus aureus* isolated from goat milk were tested, representing all farms across the four sampling periods.

Five pure colonies from each sample were selected for genomic DNA extraction in a nutrient agar plate, for which the boiling method was used (Ribeiro et al., 2016).

The extracts were subjected to multiplex polymerase chain reaction (PCR) assays. In the first assay, the primers described by Becker et al. (1998) were used to investigate the enterotoxin-producing *sea, seb, sec, sed,* and *see* genes. The second assay was performed to detect the presence of genes *sec, seh,* and *sei* (Jarraud et al., 1999). The primers used for the oligonucleotides and PCR conditions are listed in Table 1.

Table 1
Primers and cycle conditions used in PCR to investigate enterotoxin-producing genes.

Gene	Oligonucleotides primers sequences (5`- 3`)	Size (pb)	PCR cycle conditions	Reference
sea	CCTTTGGAAACGGTTAAAACG TCTGAACCTTCCCATCAAAAAC	127	95 °C, 2 min, 30 cycles 95 °C, 1 min; 55 °C, 1 min; 72 °C, 2 min; 72 °C, 5 min	(Becker et al., 1998)
seb	TCGCATCAAACTGACAAACG GCAGGTACTCTATAAGTGCCTGC	477		
sec	CTCAAGAACTAGACATAAAAGCTAGG TCAAAATCGGATTAACATTATCC	271		
sed	CTAGTTTGGTAATATCTCCTTTAAACG *TTAATGCTATATCTTATAGGGTAAACATC*	319		
see	CAGTACCTATAGATAAAGTTAAAACAAGC TAACTTACCGTGGACCCTTC	178		
seg	AATTATGTGAATGCTCAACCCGATC AAACTTATATGGAACAAAAGGTACTAGTTC	642	94 °C, 2 min, 30 cycles. 94 °C, 1 min; 53 °C, 1 min; 72 °C, 1 min; 72 °C, 5 min	(Jarraud et al., 1999)
seh	CAATCACATCATATGCGAAAGCAG CATCTACCCAAACATTAGCACC	375		
sei	CTCAAGGTGATATTGGTGTAGG AAAAAACTTACAGGCAGTCCATCTC	576		

Results and Discussion

Among the 384 strains evaluated, *S. aureus* was the predominant species, representing 85.68% (329) of the strains studied, followed by 9.38% (36) of *S. intermedius,* 4.17% (16) of *S. hyicus,* and 0.78% (3) *S. delphini* (Table 2). Our results agree with other studies showing that *S. aureus* is the predominant species among coagulase-positive *Staphylococcus* isolates from goat milk (Gonzales-Barron et al., 2017; Rola et al., 2015).

Table 2
Absolute and relative frequency of *Staphylococcus* strains detected in bulk raw goat milk from 9 dairy farms located in the state of Paraná, Brazil.

Dairy farm	*S. aureus*	*S. intermedius*	*S. hyicus*	*S. delphini*	Total
1	*15 (75%)*	*5 (25%)*	*0*	*0*	*20 (100%)*
2	*0*	*0*	*13 (100%)*	*0*	*13 (100%)*
3	*3 (50%)*	*3 (50%)*	*0*	*0*	*6 (100%)*
4	*119 (100%)*	*0*	*0*	*0*	*119 (100%)*
5	*11 (92%)*	*0*	*1 (8%)*	*0*	*12 (100%)*
6	*10 (71%)*	*3 (21%)*	*1 (8%)*	*0*	*14 (100%)*
7	*28 (97%)*	*1 (3%)*	*0*	*0*	*29 (100%)*
8	*119 (91%)*	*9 (7%)*	*0*	*3 (2%)*	*131 (100%)*
9	*24 (60%)*	*15 (38%)*	*1 (2%)*	*0*	*40 (100%)*
Total	*329 (86%)*	*36 (9%)*	*16 (4%)*	*3 (1%)*	*384 (100%)*

The presence of *Staphylococcus* spp. in milk, especially *S. aureus,* can be indicative of intramammary infection in the herd as well as of poor hygiene during milking (Kümmel et al., 2016). From a public health perspective, enterotoxigenic *Staphylococcus* spp. strains have the potential to cause food poisoning in humans (Le Loir et al., 2009; Schmid et al., 2009; Ostyn et al., 2010). There have been several foodborne outbreaks involving *S. aureus,* and consumption of contaminated milk has been documented to be associated with poisoning (De Buyser et al., 1985; Fetsch et al., 2014). In the United States, *S. aureus* causes approximately 241,000 cases of food poisoning each year (Scallan et al., 2011; Kadariya et al., 2014). In China, 23% of the analyzed retail food was contaminated with *S. aureus.* Among the raw milk samples selected, 14.6% contained this pathogen (Wang et al., 2014).

Antimicrobial resistance was observed for at least one of the drugs tested in 27% (20/74) of the species analyzed. The highest resistance was found against penicillin, which was characteristic of the 20 strains tested. In addition, 45% (9/20) of the penicillin-resistant microorganisms were resistant to tetracycline. Resistance to penicillin was observed in 10.20% (5/49) of *S. aureus* isolates, 31.25% (5/16) of *S. intermedius* isolates, and 16.67% (1/6) of *S.*

hyicus isolates. Penicillin and tetracycline resistance profiles were present in 14.28% (7/49) of *S. aureus* isolates, 16.67% (1/6) of *S. hyicus* isolates, and 33.33% (1/3) of *S. delphini* isolates.

Our results corroborate those of other studies in which high penicillin resistance was detected in *S. aureus* isolated from goat milk (Obaidat et al., 2018; Rola et al., 2015). *Staphylococcal* resistance to penicillin is mediated by the β-lactamase enzyme, which is synthesized when *Staphylococci* are exposed to β-lactam antibiotics (Lowy, 2003), commonly used for the control of intramammary infections in goats (Moroni et al., 2004). In recent years, the emergence of antibiotic-resistant strains in farm animals, which are easily transferable to humans, has become a growing public health concern (Sharma et al., 2018).

Regarding the investigation of genes responsible for the production of staphylococcal enterotoxins, 40.80% (20/49) of the *S. aureus* strains tested were positive for at least one of the genes studied. A higher prevalence of the sec gene was detected in 22.44% (11/49) of strains. The *sec* gene is divided into three antigenically distinct subtypes (sec_1, sec_2, and sec_3) based on antigenic differences and animal host. Some studies suggest that the heterogeneity of enterotoxin C is related to the selection of modified sequences that facilitate the survival of *S. aureus* in their respective hosts (Smyth et al., 2005). The prevalence of the *sec* gene found in this study is different from that found in other studies, in which *sei* or *seg* genes were most frequently detected in goat and bovine milk samples (De Leon et al., 2020; Arcuri et al., 2010; Silveira et al., 2014). These genes can be found together, which is explained by their location within the same gene cluster (enterotoxin gene cluster or *egc*) in genomic island type II vSaβ (Jarraud et al., 2001).

The *seh* gene was also detected in 18.36% (9/49) of the isolates and the *see* gene in 4.08% (2/49) of the isolates. Two strains showed a combination of the *sec* and *seh* genes. It is possible to find more than one combination of genes in many reports because *S. aureus* strains may produce more than one enterotoxin gene simultaneously (Bonsaglia et al., 2018; Zschöck et al., 2005; Srinivasan et al., 2006). Although potentially under-reported, the *seh* gene was described in an outbreak investigation caused by consuming mashed potatoes with raw milk, where high levels of SEH were detected (Jorgensen et al., 2005).

The high prevalence of enterotoxin genes in *S. aureus* strains may be due to their localization in mobile elements (phages, plasmids, and pathogenicity islands), which allows horizontal transfer of genes between different isolates (Malachowa & Deleo, 2010).

The *see* gene is rarely reported in food and animals, and its involvement in outbreaks has been rarely reported. However, six outbreaks of SEE poisoning occurred in France at the end of 2009, where the consumption of soft cheese made from unpasteurized milk was the common source (Ostyn et al., 2010). Furthermore, the *see* gene was widely found in bulk milk tanks during an investigation in South Korea, even though the authors evidenced that this gene was not detected or detected at a low frequency in milk in previous studies (Jung & Lee, 2022). This result can be related to a temperature above 8 °C, as refrigeration failures during

milk processing and/or transportation can occur (Homsombat et al., 2021).

The frequency of enterotoxin-producing genes is influenced by several factors including temperature, pH, water activity, and microbiota composition (Schelin et al., 2011). In addition to coagulase-positive *Staphylococci,* enterotoxin-producing genes can also be found in coagulase-negative *Staphylococci.* A recent study demonstrated a frequent combination of *the sec* and *seh* genes in isolates from ready-to-eat food samples, demonstrating the important role of coagulase-negative *Staphylococci* in food poisoning (Chajęcka-Wierzchowska et al., 2020).

Remarkably, all genes detected in *S. aureus* strains isolated from goat milk have already been shown to cause food poisoning (Kadariya et al., 2014; Omoe et al., 2002; Ostyn et al., 2010). Goat milk is often used in artisanal products without heat treatment, representing a potential source of staphylococcal food poisoning (Aragon-Alegro et al., 2007).

Conclusion

This study demonstrated that among the coagulase-positive *Staphylococcus* strains studied, *S. aureus* was the predominant species in raw goat milk. There was a high prevalence of penicillin- and tetracycline-resistant coagulase-positive *Staphylococcus* strains, which can be a health risk for consumers of raw goat milk and its derivatives. The presence of the *sec* and *seh* genes in *S. aureus* isolates makes goat milk consumption a potential risk to public health and may be associated with food poisoning.

Acknowledgments

The authors are grateful to the Instituto Nacional de Ciência e Tecnologia para a Cadeia Produtiva do Leite.

References

Aragon-Alegro L. C., Konta, E. M., Suzuki, K., Silva, M. G., Fernandes, A., Jr., Rall, R., & Rall, V. L. M. (2007). Occurrence of coagulase-positive *Staphylococcus* in various food products commercialized in Botucatu, SP, Brazil and detection of toxins from food and isolated strains. *Food Control, 18*(6), 630-634. doi: 10.1016/j.foodcont.2006.02.010

Aras, Z., Aydin, I., & Kav, K. (2012). Isolation of methicillin-resistant *Staphylococcus aureus* from caprine mastitis cases. *Small Ruminant Research, 102*(1), 68-73. doi: 10.1016/j.smallrumres.2011.08.014

Arcuri, E. F., Angelo, F. F., Guimarães, M. F. M., Talon, R., Borges, M. F., Leroy, S., Loiseau, G., Lange, C. C., Andrade, N. J., & Montet, D. (2010). Toxigenic status of *Staphylococcus aureus* isolated from bovine raw milk and Minas frescal cheese in Brazil. *Journal of Food Protection, 73*(12), 2225-2231. doi: 10. 4315/0362-028X-73.12.2225

Argudín, M. A., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins, 2*(7), 1751-1773. doi: 10.3390/toxins2071751

Balthazar, C. F., Pimentel, T., Ferrão, L. L., Almada, C. N., Santillo, A., Albenzio, M., Mollakhalili, N., Mortazavian, A. M., Nascimento, J. S.,

Silva, M. C., Freitas, M. Q., Sant'ana, A. S., Granato, D., & Cruz, A. G. (2017). Sheep milk: physicochemical characteristics and relevance for functional food development. *Comprehensive Reviews in Food Science and Food Safety, 16*(2), 247-262. doi: 10.1111/1541-4337.12250

Becker, K., Roth, R., & Peters, G. (1998). Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *Journal of Clinical Microbiology, 36*(9), 2548-2553. doi: 10.1128/JCM.36.9.2548-2553.1998

Bencardino, D., & Vitali, L. A. (2019). Staphylococcus aureus carriage among food handlers in a pasta company: pattern of virulence and resistance to linezolid. *Food Control, 96*(12), 351-356. doi: 10.1016/ j.foodcont.2018.09.031

Bonsaglia, E. C. R., Silva, N. C. C., Rossi, B. F., Camargo, C. H., Dantas, S. T. A., Langoni, H., Guimarães, F. F., Lima, F. S., Fitzgerald, J. R., Fernandes, A., Jr., & Rall, V. L. M. (2018). Molecular epidemiology of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated from milk of cows with subclinical mastitis. *Microbial Pathogenesis, 124*(2018), 130-135. doi: 10.1016/j.micpath.2018.08.031

Chajęcka-Wierzchowska, W., Gajewska, J., Wiśniewski, P., & Zadernowska, A. (2020). Enterotoxigenic potential of coagulase-negative staphylococci from ready-to-eat food. *Pathogens, 9*(734), 1-12. doi: 10. 3390/pathogens9090734

Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., Dewettinck, K., & Herman, L. (2014). Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. *Food Control, 42*(2014), 188-201. doi: 10.1016/j.foodcont.2014.01.045

Clinical and Laboratory Standards Institute (2021). *Performance standards for antimicrobial susceptibility testing* (31nd ed.). CLSI.

Costa, G. M., Paiva, L. V., Piccoli, R. H., Figueiredo, D. J., Pereira, U. P., & Silva, N. (2010). Evaluation of a simplified key for the identification of coagulase-positive Staphylococcus isolated from bovine mastitis. *Acta Scientiarum. Biological Sciences, 32*(4), 403-406. doi: 10.4025/ actascibiolsci.v32i4.6276

Cretenet, M., Even, S., & Le Loir, Y. (2011). Unveiling *Staphylococcus aureus* enterotoxin production in dairy products: a review of recent advances to face new challenges. *Dairy Science & Technology, 91*(2), 127-150. doi: 10.1007/s13594-011-0014-9

De Buyser, A. L., Janin, F., & Dilasser, F. (1985). Contamination of ewe cheese with *Staphylococcus aureus*: study of an outbreak of food poisoning. *Zentralblatt fur Bakteriologie, Mikrobiologie and Hygiene, 14*(1), 677-678.

De Leon, C. M. C. G., Sousa, F. G. C., Saraiva, M. M. S., Givisiez, P. E. N., Silva, N. M. V., Vieira, R. F. C., & Oliveira, C. J. B. (2020). Equipment contact surfaces as sources of *Staphylococcus* carrying enterotoxin encoding genes in goat milk dairy plants. *International Dairy Journal, 11*(10), 1-20. doi: 10.1016/j.idairyj. 2020.104827

Fetsch, A., Contzen, M., Hartelt, K., Kleiser, A., Maassen, S., Rau, J., Kraushaar, Layer, B. F., & Strommenger, B. (2014). *Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream. *International Journal of Food Microbiology, 187*(2014), 1-6. doi: 10.1016/j.ijfoodmicro.2014.06.017

Fisher, E. L., Otto, M., & Cheung, G. Y. C. (2018). Basis of virulence in enterotoxin-mediated staphylococcal food poisoning. *Frontiers in Microbiology, 9*(436), 1-18. doi: 10.3389/fmicb.2018.00436

Fooladi, A. A., Tavakoli, H. R., & Naderi, A. (2010). Detection of enterotoxigenic *Staphylococcus aureus* isolates in domestic dairy products. *Iranian Journal of Microbiology, 2*(3), 137-142.

Gonzales-Barron, U., Gonçalves-Tenório, A., Rodrigues, V., & Cadavez, V. (2017). Foodborne pathogens in raw milk and cheese of sheep and goat origin: a meta-analysis approach. *Current Opinion in Food Science, 18*(2017), 7-13. doi: 10.1016/j.cofs.2017.10.002

Hennekinne, J., De Buyser, M. L., & Dragacci, S. (2012). *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbioliogy Reviews, 36*(4), 815-836. doi: 10.1111/j.1574-6976.2011.00311.x

Homsombat, T., Boonyayatra, S., Awaiwanont, N., & Pichpol, D. (2021). Effect of temperature on the expression of classical enterotoxin genes among staphylococci associated with bovine mastitis. *Pathogens, 10*(8), 1-11. doi: 10.3390/pathogens10080975

Jarraud, S., Cozon, G., Vandenesch, F., Bes, M., Etienne, J., & Lina, G. (1999). Involvement of enterotoxins G and I in staphylococcal toxic shock syndrome and staphylococcal scarlet fever. *Journal of Clinical Microbiology, 37*(8), 2446-2449. doi: 10.1128/JCM.37.8.2446-2449.1999

Jarraud, S., Peyrat, M. A., Lim, A., Tristan, A., Bes, M., Mougel, C., Etienne, J., Vandenesch, F., Bonneville, M., & Lina, G. (2001). *Egc,* a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus. Journal of Immunology, 166*(1), 669-677. doi: 10.4049/jimmunol.166.1.669

Jay, M. J., Loessner, J. M., & Golden, A. D. (2005). Staphylococcal gastroenteritis. In J. M. Jay, M. J. Loessner, & D. A. Golden, *Modern food microbiology* (pp. 545-560). New York.

Johler, S., Macori, G., Bellio, A., Acutis, P. L., Gallina, S., & Decastelli, L. (2018). Characterization of *Staphylococcus aureus* isolated along the raw milk cheese production process in artisan dairies in Italy. *Journal of Dairy Science, 101*(4), 2915-2920. doi: 10.3168/jds.2017-13815

Jorgensen, H. J., Mathisen, T., Lovseth, A., Omoe, K., Qvale, K. S., & Loncarevic, S. (2005). An outbreak of staphylococcal food poisoning caused by enterotoxin H in mashed potato made with raw milk. *Microbiology Letters, 252*(2), 267-272. doi: 10.1016/j.femsle.2005.09.005

Jung, H. R., & Lee, Y. J. (2022). Characterization of virulence factors in enterotoxin-producing *Staphylococcus aureus* from bulk tank milk. *Animals, 12*(301), 1-10. doi: 10.3390/ani12030301

Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *BioMed Research International, 2014*(2014), 1-9. doi: 10.1155/2014/827965

Kluytmans, J., & Wertheim, H. F. L. (2005). Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections. *Infection, 33*(1), 3-7. doi: 10.1007/s15010-005-4012-9

Kümmel, J., Stessl, B., Gonano, M., Walcher, G., Bereuter, O., Fricker, M., Grunert, T., Wagner, M., & Ehling-Schulz, M. (2016). *Staphylococcus aureus* entrance into the dairy chain: tracking S. aureus from dairy cow to cheese. *Frontiers in Microbiology, 7*(1603), 1-11. doi: 10.3389/fmicb.2016.01603

Laganà, P., Delia, S., Di Pietro, A., Costa, A., & Coniglio, M. A. (2020). Antibiotic resistance in bacteria strains isolated from foods and correlated environments. *Progress in Nutrition, 22*(3), 1-8. doi: 10.23751/pn.v22i3.10407

Le Loir, Y., Baron, F., & Gautier, M. (2003). Staphylococcus aureus and food poisoning. *Genetics and Molecular Research, 2*(1), 63-76.

Le Loir, Y., Baron, F., & Gautier, M. (2009). Staphylococcal food poisoning. In F. Lang, *Encyclopedia of molecular mechanisms of disease* (pp. 1974-1975). Berlin, Heidelberg.

Linage, B., Rodríguez-Calleja, J. M., Otero, A., García-López, M. L., & Santos, J. A. (2012). Characterization of coagulase-positive staphylococci isolated from tank and silo ewe milk. *Journal of Dairy Science, 95*(4), 1639-1644. doi: 10.3168/jds.2011-4734

Lowy, F. D. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus. The Journal of Clinical Investigation, 111*(9), 265-1273. doi: 10.1172/JCI18535

Malachowa, N., & Deleo, F. R. (2010). Mobile genetic elements of *Staphylococcus aureus. Cellular and Molecular Life Sciences, 67*(18), 3057-3071. doi: 10.1007/s00018-010-0389-4

Moroni, P., Vellere, F., Antonini, M., Pisoni, G., Ruffo, G., & Carli, S. (2004). Antibiotic susceptibility of coagulase-negative staphylococci isolated from goats' milk. *International Journal of Antimicrobial Agents, 23*(6), 637-640. doi: 10.1016/j.ijantimicag.2003.10.007

Obaidat, M. M., Roess, A. A., Mahasneh, A. A., & Al-Hakimi, R. A. (2018). Antibiotic-resistance, enterotoxin gene profiles and farm-level prevalence of *Staphylococcus aureus* in cow, sheep and goat bulk tank milk in Jordan. *International Dairy Journal, 81*(2018), 28-34. doi: 10.1016/j.idairyj.2018.02.001

Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D., Ueda, S., & Shinagawa, K. (2002). Detection of seg, seh, and sei genes in Staphylococcus aureus isolates and determination of the enterotoxin productivities of *S. aureus* isolates harboring seg, seh, or sei genes. *Journal of Clinical Microbiology, 40*(3), 857-862. doi: 10.1128/JCM.40.3.857-862.2002

Ostyn, A., De Buyser, M. L., Guillier, F., Groult, J., Félix, B., Salah, S., Delmas, G., & Hennekinne, J. A. (2010). First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009. *Eurosurveillance, 15*(13), 10-13. doi:10.2807/ese.15.13.19528-en

Palii, A. P., Paliy, A. P., Rodionova, K. O., Zolotaryova, S. A., Kushch, L. L., Borovkova, V. M., Kazakov, M. V., Pavlenko, I. S., Kovalchuk, Y. O., Kalabska, V. S., Kovalenko, O. V., Pobirchenko, O. M., & Umrihina, O. S. (2020). Microbial contamination of cow's milk and operator hygiene. *Ukrainian Journal of Ecology, 10*(2), 392-397. doi: 10.15421/2020_113

Qian, W., Shen, L., Li, X., Wang, T., Liu, M., Wang, W., Fu, Y., & Zeng, Q. (2019). Epidemiological characteristics of *Staphylococcus aureus* in raw goat milk in Shaanxi Province, China. *Antibiotics, 8*(3), 141, 1-12. doi: 10.3390/antibiotics8030141

Ribeiro, J. C., Jr., Tamanini, R., Soares, B. F., Oliveira, A. M., Silva, F. G., Silva, F. F., Augusto, N. A., & Beloti, V. (2016). Efficiency of boiling and four other methods for genomic DNA extraction of deteriorating spore-forming bacteria from milk. *Semina: Ciências Agrárias, 37*(5), 3069-3078. doi: 10.5433/1679-0359.2016v37n5p3069

Rios, E. A., Pereira, J. R., Tamanini, R., Mareze, J., Gonzaga, N., Ossugui, E., Nero, L. A., & Beloti, V. (2018). Quality of goat's milk produced on farms in the Paraná State - Brazil. *Semina: Ciências Agrárias, 39*(6), 2425-2436. doi: 10.5433/1679-0359.2018v39n6p2425

Rola, J. G., Sosnowski, M., Ostrowska, M., & Osek, J. (2015). Prevalence and antimicrobial resistance of coagulase-positive staphylococci isolated from raw goat milk. *Small Ruminant Research, 123*(1), 124-128. doi: 10.1016/j.smallrumres.2014.11.010

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States major pathogens. *Emerging Infectious Diseases, 17*(1), 7-15. doi: 10.3201/eid1701.P11101

Schelin, J., Wallin-Carlquist, N., Cohn, M. T., Lindqvist, R., & Barker, G. C. (2011). The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence, 2*(6), 580-592. doi: 10.4161/viru.2.6.18122

Schmid, D., Fretz, R., Winter, P., Mann, M., Höger, G., Stöger, A., Ruppitsch, W., Ladstätter, J., Mayer, N., Martin, A., & Allerberger, F. (2009). Outbreak of staphylococcal food intoxication after consumption of pasteurized milk products. *Wiener Klinische Wochenschrift, 121*(3), 125-131. doi: 10.1007/s00508-008-1132-0

Sharma, C., Rokana, N., Chandra, M., Singh, B. P., Gulhane, R. D., Gill, J. P. S., Ray, P., Puniya, A. K., & Panwar, H. (2018). Antimicrobial resistance: its surveillance, impact, and alternative management strategies in dairy animals. *Frontiers in Veterinary Science, 4*(237), 1-27. doi: 10.3389/fvets.2017.00237

Silveira, V., Fº., Luz, I. S., Campos, A. P. F., Silva, W. M., Barros, M. P. S. B., Medeiros, E. S., Freitas, M. F. L., Mota, R. A., Sena, M. J., & Leal-Balbino, T. C. (2014). Antibiotic resistance and molecular analysis of *Staphylococcus aureus* isolated from cow's milk and dairy products in northeast Brazil. *Journal of Food Protection, 77*(4), 583-591. doi: 10.4315/0362-028X.JFP-13-343

Smyth, D. S., Hartigan, P. J., Meaney, W. J., Fitzgerald, J. R., Deobald, C. F., Bohach, G. A., & Smyth, C. J. (2005). Superantigen genes encoded by the egc cluster and SaPIbov are predominant among

Staphylococcus aureus isolates from cows, goats, sheep, rabbits and poultry. *Journal of Medical Microbiology, 54*(4), 401-411. doi: 10.1099/jmm.0.45863-0

Srinivasan, V., Sawant, A. A., Gillespie, B. E., Headrick, S. J., Ceasaris, L., & Oliver, S. P. (2006). Prevalence of enterotoxin and toxic shock syndrome toxin genes in *Staphylococcus aureus* isolated from milk of cows with mastitis. *Foodborne Pathogens and Disease, 3*(3), 274-284. doi: 10.1089/fpd.2006.3.274

Vyletělová, M., Hanuš, O., Karpíšková, R., & Štástková, Z. (2016). Occurrence and antimicrobial sensitivity in staphylococci isolated from goat, sheep and cow's milk. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 59*(3), 209-214. doi: 10.11118/actaun201159030209

Wang, X., Li, G., Xia, X., Yang, B., Xi, M., & Meng, J. (2014). Antimicrobial susceptibility and molecular typing of methicillin-resistant *Staphylococcus aureus* in retail foods in Shaanxi, China. *Foodborne Pathogens and Disease, 11*(4), 281-286. doi: 10.1089/fpd.2013.1643

Zschöck, M., Kloppert, B., Wolter, W., Hamann, H. P., & Lämmler, C. (2005). Pattern of enterotoxin genes seg, seh, sei and sej positive *Staphylococcus aureus* isolated from bovine mastitis. *Veterinary Microbiology, 108*(3), 243-249. doi: 10.1016/j.vetmic.2005.02.012