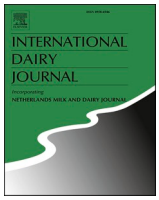
International Dairy Journal 160 (2025) 106088

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj



Research Article

Pheno- and genotypic epidemiological characterization of *Staphylococcus aureus* isolated from bulk tank milk in Colombia

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ARTICLE INFO

Handling Editor: Dr. Wei Wei

Keywords:
Bovine mastitis
Antimicrobial resistance
MRSA
Staphylococcal enterotoxins
PFGE

ABSTRACT

Staphylococcus aureus is recognized for causing contagious mastitis in cattle. Elimination of the organism in affected animals is challenging due to its potential antibiotic resistance mechanisms and virulence factors. The aim of the study was the pheno- and genotypic epidemiological characterization of *S. aureus* isolated from bulk tank milk samples obtained from three municipalities in the northern region of Antioquia. Twenty-one *S. aureus* isolates from 150 bulk tank milk samples were characterized by evaluating antimicrobial susceptibility to 17 antibiotics using the VITEK 2, and identifying several genes encoding for virulence factors, enterotoxins, and the *agr* groups by conventional PCR. The clonal relationship between the isolates was assessed using macrorestriction fragment analysis (MRFA) of chromosomal DNA by pulsed-field gel electrophoresis (PFGE). Most of the isolates showed susceptibility to the tested antibiotics; 19% exhibited resistance to tetracycline and all isolates showed beta-lactamases. Molecular typing revealed that 76% carried the *agr*I and *cap*5 genes. Seven isolates harbored genes coding for staphylococcal enterotoxins (SE), with *seh* being the most identified gene. Furthermore, MRFA demonstrated high heterogeneity among the isolates, resulting in the assignment of 18 different MRFA patterns. These results indicate phenotypic resistance to tetracycline and beta-lactams, the presence of genes encoding for staphylococcal enterotoxins, and high heterogeneity among *S. aureus* isolates from bulk tank milk.

1. Introduction

Staphylococcus (*S.*) *aureus* causes contagious mastitis in cattle (Peton & Le Loir, 2014), mainly inducing a subclinical infection with a chronic course due to the expression of virulence factors. These factors enable adherence to the mammary tissue, evasion of the immune barrier, and, in some cases, inhibition of antibiotic effects, favoring its presence in the udder for a longer period (Abril et al., 2020). This extended presence turns infected animals into infectious sources within the herd, leading to an increase in somatic cell counts (SCC) due to the subclinical infections (Keefe, 2012). This not only reduces animal welfare but also raises costs due to production losses.

The primary habitat of *S. aureus* is the mammary gland of infected animals, with some bacteria being expelled through milk. Therefore, isolation of the bacteria from tank milk samples is an evidence of infection in milking cows (Jayarao & Wolfgang, 2003). The presence of *S. aureus* in tank milk may be a risk to consumers of raw milk or their products, as the bacteria can produce enterotoxins that cause food poisoning in humans (Jørgensen et al., 2005). Enterotoxigenic *S. aureus* has been detected in udder milk samples obtained from cows with bovine mastitis (Grispoldi et al., 2019; Salasia et al., 2004; Toshkova et al., 2001) as well as from bulk tank milk samples (Pacha et al., 2020).

The mammary gland of healthy animals is highly susceptible to *S. aureus* infection and the bacterium achieves very good adaptation to host tissue (Rainard et al., 2018), which is why the specificity of some virulence factors has been related to the different lineages of the strains (Cheung et al., 2021). Although, *S. aureus* may exhibit antibiotic resistance patterns that constrain therapeutic efficacy and its elimination from the mammary gland (Vestergaard et al., 2019).

Epidemiological studies of *S. aureus* strains improve the comprehension of its infection dynamics in the herd (Rainard et al., 2018). Molecular tools for the identification of clones, based on the identification of virulence factors, antibiotic resistance, and enterotoxins, can demonstrate the variability of *S. aureus* strains in a herd or region.

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https://doi.org/10.1016/j.idairyj.2024.106088
Received 10 June 2024; Received in revised form 9 September 2024; Accepted 9 September 2024
Available online 13 September 2024

Previous research carried out on strains isolated from milk samples of cows with clinical and subclinical mastitis in Colombia has shown their pathogenic potential through biofilm-producing and low invasion abilities (Torres et al., 2019, 2020). Protein A, encoded by the *spa gene*, is a surface protein that binds to host Immunoglobulin G, preventing opsonization and phagocytosis (Frenay et al., 1996). A diversity of *spa* types was suggesting potential persistent mammary infections and a zoonotic risk to consumers (Torres et al., 2023). In addition, antimicrobial resistance studies have been carried out demonstrating an increase in the resistance profiles of *S. aureus* isolated from bovine mastitis samples (Calderón et al., 2011; Jiménez Velásquez et al., 2020; Rámirez et al., 2001).

Despite the knowledge achieved with previous research on mastitis so far in Colombia, information on virulence factors, enterotoxins and genetic variability of *S. aureus* isolated from tank milk of dairy farms is still scarce. Therefore, the objective of this study was to epidemiologically phenotypic and genotypic characterize *S. aureus* isolated from bulk tank milk samples obtained from three municipalities in the northern region of Antioquia.

2. Materials and methods

2.1. Isolation of S. aureus

Samples were collected in a previous study (Ágredo-Campos et al., 2023) from a total number of 150 bulk tank milk samples from an equal number of herds in the municipalities of Santa Rosa de Osos (n = 73), San Pedro de los Milagros (n = 44), and Entrerríos (n = 33) in the northern region of Antioquia (Colombia). For the isolation of *S. aureus* from the milk samples, 10 μL of raw milk were streaked on Columbia® agar plates supplemented with 5% sheep blood (bioMérieux SA, Marcy l'Etoile, France) and incubated at 37 ± 2 °C for 24 h. Based on culture and hemolysis properties, presumptive *S. aureus* culture was further inoculated on Columbia blood agar and incubated aerobically at 37 ± 2 °C for 24 h (Akineden et al., 2001). Subsequently, all isolates were identified by VITEK 2 GP ID card (bioMérieux). The purified culture isolates were further stored for pheno- and genotypic analyses.

2.2. Antimicrobial susceptibility testing

The Minimum Inhibitory Concentration (MIC) of the antibiotics cefoxitin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, teicoplanin, vancomycin, minocycline, tetracycline, nitrofurantoin, oxacillin, rifampicin and trimethoprim/sulfamethoxazole were measured by the VITEK 2 automated system with AST-P577 card reference 22218 (bioMérieux). All protocols were performed by following the manufacturer's instructions. A qualitative test for the detection of beta-lactamase-producing *S. aureus* was evaluated using the nitrocefin test according to CLSI guidelines (CLSI, 2020). The CLSI guidelines were used to determine antimicrobial susceptibility breakpoints (CLSI, 2020).

2.3. Identification of Methicillin-resistant Staphylococcus aureus (MRSA)

Methicillin resistance was determined in parallel by susceptibility testing using Chromogenic ChromID® MRSA agar plates (bioMérieux) and the cefoxitin disk diffusion test on Mueller-Hinton agar plates (bioMérieux) according to a previous study (Kreausukon et al., 2012). A strain was considered a MRSA when it showed a cefoxitin (30 μg) breakpoint of ≤17 mm and negative when it was ≥18 mm (CLSI, 2020). The presence of the *mecA* gene was evaluated by conventional PCR as described previously by Sanchez et al. (2013). As a positive control was used a MRSA-clinical strain kindly provided by the strain collection of the MICROBA research group of the School of Microbiology from the Universidad de Antioquia (Ocampo et al., 2014).

2.4. DNA extraction

The total genomic DNA was extracted from single colonies of each isolate by using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany) according to the manufacturers' recommendations. DNA assessment of integrity and quantity was performed with an Epoch Microplate Reader (BioTek Instruments, Germany). The extracted DNA was stored at −20 °C for further analysis.

2.5. PCR based identification

Species identification of isolates was done by amplification of the species-specific part of the gene encoding the thermonuclease (*nuc*A) with the oligonucleotide primers and conditions according to Brakstad et al. (1992). Furthermore, the 16sRNA gene partial sequence of three randomly chosen isolates was amplified using the oligonucleotide forward 16SUNI-L (5′-AGA GTT TGA TCC TGG CTC AG-3′) and reverse 16SUNI-R (5′-AAG GAG GTG ATC CAG CCG CA-3′) primers as described previously by Kuhnert et al. (1996). PCR products were purified using QIAquick gel extraction kits (Qiagen) in accordance with the manufacturer's protocol and sequenced bidirectionally by Microsynth Seqlab GmbH (Göttingen, Germany). The obtained DNA sequences were edited and combined in Molecular Evolutionary Genetics Analysis software version 10 (MEGA10). For species-level identification, the obtained sequences were compared with the GeneBank database, by using the basic local alignment research tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6. PCR analyses of virulence factors and enterotoxin genes

All isolates were subjected to molecular characterization using a set of published PCR systems for the amplification of the gene for coagulase (*coa*), clumping factor A (*clf*A) and B (*clf*B), X-region of protein A (*spa*), hemolysin A (*hla*) and B (*hlb*), fibronectin binding proteins A (*fbn*A) and B (*fbn*B), and intercellular adhesion proteins A (*ica*A) and D (*ica*D), and the genes of the capsular locus 5 and 8 (*cap*5, *cap*8), the accessory gene regulator of the *agr* group (*agr*I, *agr*II, and *agr*III). The oligonucleotide primer sequences and the corresponding sequence locations of SEs, as well as PCR conditions are listed in Table 1. Furthermore, PCR for 18 *S. aureus* enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei, selj, sek, sel, sem, sen, seo, sep, seq, ser,* and *selu*) was performed as described by Taban et al. (2017). All amplification reactions were performed as uniplex. The PCR reaction (total volume 30 μL) was performed in a 0.2 mL reaction vial and consisted of 20.1 μL sterile aqua dest., 3 μL Taq Buffer, with KCl (10x; Thermo Fisher Scientific Inc., Waltham MA, USA), 1.8 μL MgCl2 (25 mM; Thermo Fisher Scientific Inc.), 0.6 μL deoxynucleoside triphosphate (10 mM; Thermo Fisher Scientific Inc.), 1 μL primers 1 and 2 (10x μM; Integrated DNA Technologies, Inc. Iowa, USA), 0.5 μL Taq DNA polymerase (5 U/μl, Thermo Fisher Scientific Inc.) and 2 μL DNA template. The reaction was run in a MultiGene OptiMax Thermal Cycler (Labnet, Massachusetts, USA), and PCR products were determined by electrophoresis of 12 μL of the reaction product in a 1.5% agarose gel (Biozym, Hessisch-Oldendorf, Germany) at 120 V in 1x Tris-acetate-electrophoresis buffer (TAE) [(0.04 mol/l Tris, 0.001 mol/l EDTA; pH 7.8)] and a Gene-Ruler™ 50 and 100 bp DNA ladder (MBI Fermentas) as molecular markers followed by staining with 5 μL/mL GelRed Nucleic Acid Gel Stain 10,000X (Biotium, California, USA) for 5 min. Finally, the amplicons were visualized under a UV transilluminator using the Enduro® Gel Documentation Systems (Labnet).

2.7. Macrorestriction fragment pattern analysis of chromosomal DNA by pulsed-field gel electrophoresis

All isolates were characterized after digestion of their chromosomal DNAs with the restriction enzyme *Sma*I (Invitrogen) and subsequent separation of the fragments by pulsed-field gel electrophoresis (PFGE) using the Chef-Dr II pulsed-field electrophoresis system (BioRad

Table 1
Oligonucleotide primers used for PCR analysis of staphylococcal virulence factors.

Target genes	Target Protein	Primer	Amplicon size (bp)	Oligonucleotide sequence (5′-3′)	Programme	Reference
coa	Coagulase	Coa-I	Variable	ATAGAGATGCTGGTACAGGGCTTCCGATTGTTCGATGC	1	Hookey et al. (1998)
		Coa-II		ATAGAGATGCTGGTACAGGGCTTCCGATTGTTCGATGC		
clfA	Clumping factor A	ClfA-I	1000	GGCTTCAGTGCTTGTAGG	2	Stephan et al. (2001)
		ClfA-II		TTTTCAGGGTCAATATAAGC		
clfB	Clumping factor B	ClfB-I	194	TGCAAGTGCAGATTCCGAAAAAAAC	3	Klein et al., 2012.
		ClfB-II		CCGTCGGTTGAGGTGTTTCATTTG		
spa	X-region of protein A	SPA-III	variable	CAAGCACCAAAAGAGGAA	4	Frenay et al. (1996)
		SPA-IV		CACCAGGTTTAACGACAT		
hla	Hemolysin A	hla-1	535	GGTTTAGCCTGGCCTTC	5	Booth et al. (2001)
		hla-2		CGAACGAGTTCGTGATG		
hlb	Hemolysin B	hlb-1	833	GCCAAAGCCGAATCTAAG		
		hlb-2		GCCATGGGATGTATATGC		
fbnA	fibronectin binding protein A	FbnA-1	133	CGACACAACCTCAAGACAATAGCGG	6	Pereyra et al. (2016)
		FbnA-2		CGTGGCTTACTTTCTGATGCCGTTC		
fbnB	fibronectin binding protein B	FbnB-1	197	ACGCTCAAGGCGACGGCAAAG		
		FbnB-2		ACCTTCTGCATGACCTTCTGCACCT		
icaA	intercellular adhesion protein A	icaA-1	178	CTTGCTGGCGCAGTCAATAC	7	Salimena et al. (2016)
		icaA-2		CAACATCCAACACATGGCA		
icaD	intercellular adhesion protein D	icaD-1	164	CGCTATATCGTGTGTCTTTTGGA		
		icaD-2		TCGCGAAAATGCCCATAGTT		
cap5	capsular locus 5	cap5-1	300	GTCAAAGATTATGTGATGCTACTGAG	7	Verdier et al. (2007)
		cap5-2		ACTTCGAATATAAACTTGAATCAATGTTATACAG		
cap8	capsular locus 8	cap8-1	800	TGTGGGATTTTTGTAGCTTT		Ambroggio et al. (2018)
		cap8-2		CGGGTGACTAAAAATACTCG		
agr-pan	accessory gene regulator of the		–	ATGCACATGGTGCACATGC	7	Gilot et al. (2002)
*agr*I	*agr* group	agr-B	441	GTCACAAGTACTATAAGCTGCGAT		
*agr*II		agr-D	575	TATTACTAATTGAAAAGTGGCCATAGC		
*agr*III		agr-C	323	GTAATGTAATAGCTTGTATAATAATACCCAG		

Thermal cycler program: 30 cycles, initial denaturation 94 °C for 30 s, annealing (1: 57 °C, 2: 58 °C, 3: 55 °C, 4:50 °C, 5: 50 °C, 6: 46 °C, 72 °C, 7: 55 °C) for 30 s, elongation 72 °C for 60 s.

Laboratories, CA, USA). The preparation of the whole bacterial DNA in agarose gel plugs, subsequent digestion of the bacterial DNA with the *Sma*I restriction enzyme and PFGE conditions was done as described previously (Quelle et al., 2003). A dendrogram of the macrorestriction fragment restriction patterns was created using the Bionumerics software package version 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium).

3. Results

3.1. Isolation and identification of S. aureus

A total of twenty-one individual bulk tank milk samples were positive for *S. aureus*. The putative *S. aureus* culture isolates were gram-positive cocci in aggregates and formed hemolysin zones on Columbia blood agar. Of the 21 positive bulk tank milk samples, one typical isolate per sample was selected for further identification and characterization by phenotypical and genotypic investigations. All selected twenty-one isolates were further confirmed by VITEK 2 GP ID card (bioMérieux) and were positively amplified for the thermonuclease gene *nucA*. Three randomly selected isolates were further identified by 16S rDNA sequencing. The BLAST search results of 16S rRNA gene analysis revealed that three isolates showed 100% similarity to *S. aureus* (Table 2).

3.2. Antimicrobial susceptibility

Four isolates out of 21 (19%) were resistant to tetracycline (MIC ≥16 μg/mL). However, all the isolates were susceptible to oxacillin (MIC ≤0.25 μg/mL), cefoxitin (MIC 4 μg/mL), gentamicin (MIC ≤0.5 μg/mL), ciprofloxacin (MIC ≤0.5 μg/mL), levofloxacin (MIC ≤0.12 μg/mL), moxifloxacin (MIC ≤0.25 μg/mL), erythromycin (MIC ≤0.25 μg/mL), clindamycin (MIC ≤0.25 μg/mL), quinupristin/dalfopristin (MIC ≤0.25 μg/mL), linezolid (MIC 1 μg/mL), teicoplanin (MIC≤ 0.5 μg/mL), vancomycin (MIC ≤0.5 μg/mL), minocycline (MIC ≤0.5 μg/mL), nitrofurantoin (MIC 32 μg/mL), rifampicin (MIC ≤0.5 μg/mL), and trimethoprim/sulfamethoxazole (MIC ≤10 μg/mL). All isolates (100%) showed beta-lactamases production.

3.3. Identification of MRSA

No MRSA isolates were detected by the phenotypic tests using Chromogenic ChromID® MRSA agar and the cefoxitin disk agar diffusion test. None of the isolates harbored the *mec*A gene by conventional PCR.

3.4. Virulence factors

All *S. aureus* isolates tested uniformly positive for the genes *coa, clfB, spa* (X-region), *hla, fbnA, icaA*, and *icaD*. The genes *hlb, fbnB,* and *clfA* were successfully amplified in 15, 16, and 20 isolates, respectively. Polymorphisms were observed in the *coa* and *spa* (x-region) genes. The most frequent *coa* gene sizes were 790 bp, 600 bp, and 750 bp found in 16, three, and two isolates. Similarly, the most frequent *spa* (x-region) gene sizes were 310 bp, 110 bp, and 290 bp found in 17, three, and one isolates, respectively. Most isolates (n = 17) carried the *cap*5 gene while only four isolates possessed the *cap*8 gene. Regarding *agr* groups, the *agr*I gene was present in 17 isolates, with one isolate harboring *agr*II and three isolates having *agr*III. Combinations of *cap* and *agr* genes varied, with 16, three, and one isolates exhibiting *cap*5 and *agr*I, *cap*8 and *agr*III, *cap*5 and *agr*II, and *cap*8 and *agr*I gene combinations, respectively (Fig. 1). It is noteworthy that further PCR testing for enterotoxin genes revealed seven isolates to be enterotoxigenic. The gene encoding for staphylococcal enterotoxin H (*seh*) was identified in six isolates, while

Table 2
Identification *nucA* gene amplification, 16S rDNA sequencing, and antibiotic resistance profiles (MIC, μg/mL) of 21 *S. aureus* isolates obtained from bulk tank milk samples obtained from dairy farms in the northern region of Antioquia.

Designation of isolate [a]	Origin [b]	Identification by VITEK 2 [c] (%)	*nucA*-amplification [d]	Species identification by 16S rRNA sequence analysis					Antibiotic resistance (MIC, μg/mL)	
				16S rRNA sequencing [e]	Phylogenetic affiliation	Sequence length (bp)	Identity % (Query Coverage)	Closest species (NCBI accession number)	TET	B-lac
S. aureus #1	4, SR	99.0	+	+	*S. aureus*	1323	99.77	*S. aureus* ATCC 12600 (NR_115606.1)	≤1	+
S. aureus #2	14, SR	99.0	+	n.a.*					≤1	+
S. aureus #3	16, SR	950	+	n.a.					≤1	+
S. aureus #4	22, SR	99.0	+	n.a.					≥16	+
S. aureus #5	26, SR	87.0	+	n.a.						+
S. aureus #6	28, SR	95.0	+	n.a.					≤1	+
S. aureus #7	31, SR	99.0	+	n.a.						+
S. aureus #8	35, SR	99.0	+	n.a.					≤1	+
S. aureus #9	46, SR	91.0	+	n.a.					≥16	+
S. aureus #10	60, SR	91.0	+	n.a.					≤1	+
S. aureus #11	65, SR	99.0	+	n.a.					≤1	+
S. aureus #12	74, ER	99.0	+	+	*S. aureus*	1324	99.85	*S. aureus* ATCC 12600 (NR_115606.1)	≤1	+
S. aureus #13	78, ER	91.0	+	n.a.					≤1	+
S. aureus #14	86, ER	99.0	+	n.a.					≤1	+
S. aureus #15	89, ER	91.0	+	n.a.					≤1	+
S. aureus #16	104, ER	93.0	+	n.a.					≤1	+
S. aureus #17	130, SP	99.0	+	+	*S. aureus*	1329	99.85	*S. aureus* ATCC 12600 (NR_115606.1)	≤1	+
S. aureus #18	131, SP	99.0	+	n.a.					≤1	+
S. aureus #19	139, SP	99.0	+	n.a.					≤1	+
S. aureus #20	148, SP	99.0	+	n.a.					≤1	+
S. aureus #21	150, SP	99.0	+	n.a.					≥16	+

[a] According to label on sample tube.
[b] Number and location of individual farm; Municipality SR: Santa Rosa de Osos, ER: Entrerrios, SP: San Pedro de los Milagros.
[c] By Vitek 2 using AST-P577 card.
[d] Species specific PCR detection of the thermonuclease (*nucA*) gene according to Brakstad et al. (1992).
[e] Sequencing of the 16S rRNA gene according to Kuhnert et al. (1996) *n.a.: not analyzed; -: none detected; +: positive; Abbreviations: TET, tetracycline, β-lac, β-lactamase; For all isolates, identical MICs were obtained with the following substances: oxacillin (MIC ≤0.25 μg/mL), cefoxitin (MIC 4 μg/mL), gentamicin (MIC ≤0.5 μg/mL), ciprofloxacin (MIC ≤0.5 μg/mL), levofloxacin (MIC ≤0.12 μg/mL), moxifloxacin (MIC ≤0.25 μg/mL), erythromycin (MIC ≤0.25 μg/mL), clindamycin (MIC ≤0.25 μg/mL), quinupristin/dalfopristin (MIC ≤0.25 μg/mL), linezolid (MIC 1 μg/mL), teicoplanin (MIC ≤0.5 μg/mL), vancomycin (MIC ≤0.5 μg/mL), minocycline (MIC ≤0.5 μg/mL), nitrofurantoin (MIC 32 μg/mL), rifampicin (MIC ≤0.5 μg/mL), and trimethoprim/sulfamethoxazole (MIC ≤10 μg/mL).

one isolate also carried the *egc* complex genes (*seg, sei, sem, sen, seo, selu)* (Fig. 1).

3.5. Macrorestriction fragment pattern analysis of chromosomal DNA

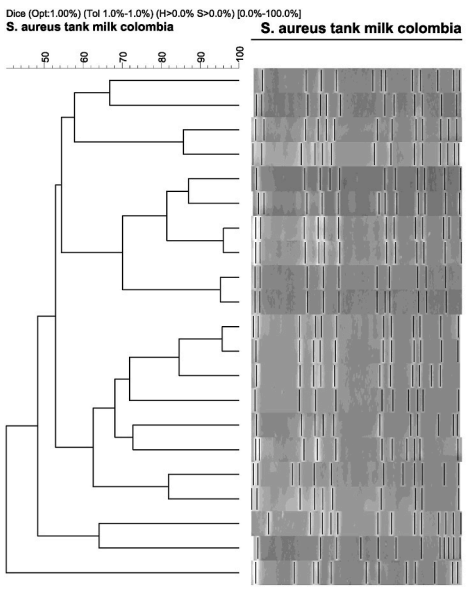
For clone-level variation, the 21 isolates were further characterized by macrorestriction fragment pattern (MRFP) analysis using PFGE of *Sma*I digested genomic DNA. Digestion with the *Sma*I restriction enzyme produced ten to 14 readable and distinctive bands for different isolates. The dendrogram analysis showed clustering of types and origin of isolates that confirmed the non-identical status of the different isolates and displayed 18 unique MRFP patterns with an overall approximately 42% similarity. However, each pair of MRFP subtypes (P7 and P7a, P8 and P8a, and P9 and P9a) shared related MRFP subtypes with an overall approximately more than 95% similarity (Fig. 1).

4. Discussion

S. aureus remains one of the main causative agents of bovine mastitis, which in addition to its negative impact on animal health and herd economics, can severely affect public health by causing food poisoning or therapeutic failure due to its antimicrobial resistance mechanisms. The study of genetic variability among *S. aureus* isolates from dairy cows from different regions or countries has been proposed to reduce the spread of infection (Monistero et al., 2018). In this sense, this study evaluated the molecular characteristics and genetic profile of *S. aureus* isolates from bulk tank milk in one of the most important dairy regions in Colombia.

The high susceptibility to most of the antibiotics found in the present study differs from previous studies conducted in Colombia, in which there was a high rate of resistance to oxacillin, cefoxitin, erythromycin, and Trimethoprim/Sulfamethoxazole (Jiménez Velásquez et al., 2020; Torres et al., 2023). Although *S. aureus* strains isolated from raw milk have been found to exhibit lower antibiotic resistance compared to isolates from humans and pets (Pahlavanzadeh et al., 2021), further exploration of the spread of antimicrobial resistance among *S. aureus* strains isolated from food-producing animals, pets and food samples are needed.

The phenotypic resistance to tetracycline reported in this investigation is distinct from that found earlier by Torres et al. (2023), who identified no resistance to this antibiotic in isolates from Colombian cow mastitis samples. However, it was lower compared to the results found in other strains isolated from cows with mastitis in China (Liao et al., 2022), milk tanks in Portugal (Oliveira et al., 2022), and strains isolated from raw milk and dairy products in a meta-analysis (Zhang et al., 2022). This finding is noteworthy given that antibiotics of the tetracycline group are not preferred or chosen for the treatment of bovine mastitis. However, it could be related to the use of oxytetracycline for



Key	Municipality	coa	spa	agrI	agrII	agrIII	cap5	cap8	hlb	fbnB	clfA	enterotoxin gene	MRFP type
S. aureus #19	SP	790	310	+	-	-	+	-	-	+	+	-	P1
S. aureus #21	SP	600	310	-	+	-	+	-	-	+	-	-	P2
S. aureus #12	ER	600	110	-	-	+	-	+	+	+	+	seh	P3
S. aureus #7	SR	600	110	-	-	+	-	+	+	+	+	seh	P4
S. aureus #16	ER	790	310	+	-	-	+	-	+	+	+	seh	P5
S. aureus #3	SR	790	310	+	-	-	+	-	+	-	+	-	P6
S. aureus #13	ER	790	310	+	-	-	+	-	+	+	+	seh	P7
S. aureus #15	ER	790	310	+	-	-	+	-	+	+	+	seh	P7a
S. aureus #17	SP	790	310	+	-	-	+	-	-	+	+	-	P8
S. aureus #18	SP	790	310	+	-	-	+	-	+	-	+	-	P8a
S. aureus #10	SR	790	310	+	-	-	+	-	+	+	+	-	P9
S. aureus #5	SR	790	310	+	-	-	+	-	+	+	+	-	P9a
S. aureus #9	SR	790	310	+	-		+	-	+	+	+	-	P10
S. aureus #2	SR	790	310	+	-	-	+	-	+	+	+	-	P11
S. aureus #14	ER	790	310	+	-	-	+	-	-	+	+	-	P12
S. aureus #8	SR	790	310	+	-	-	+	-	+	+	+	-	P13
S. aureus #1	SR	790	310	+	-	-	+	-	+	-	+	-	P14
S. aureus #4	SR	790	310	+	-	-	+	-	-	+	+	-	P15
S. aureus #11	SR	600	110	-	-	+	-	+	+	+	+	seh	P16
S. aureus #20	SP	690	290	+	-	-	-	+	-	-	+	seg, sei, sem, sen, seo, seu	P17
S. aureus #6	SR	790	310	+	-	-	+	-	+	-	+	-	P18

Fig. 1. Macrorestriction fragment patterns of 21 *S. aureus* isolates obtained from bulk tank milk samples from three municipalities in northern Antioquia digested with the *Sma*I restriction enzyme. The dendrogram was constructed with the BioNumerics software 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium) choosing the Dice coefficient setting both tolerance and optimization at 1%. The horizontal scale on the left side (100–50) indicates the level of similarity in percent among fingerprints. Details given in the 3rd through 12th column from the left are the isolate designation, municipality (SR: Santa Rosa de Osos, ER: Entrerrios, SP: San Pedro de los Milagros), presence (+) or absence (−) of several genes coding for virulence factors (*coa, spa, agr*I, *agr*II, *agr*III, *cap*5, *cap*8, *hlb, fbnB, clfA*) and enterotoxin gene and MRFP type for each isolate. For all isolates, consistent positive amplification was achieved for the following genes: *hla*, *fbn*A, *clf*B, *ica*A, *ica*D.

other types of diseases, since it has been reported as one of the most widely used antibiotics in cattle, especially in small farms (Benavides et al., 2021; Pulido-Delgado et al., 2022). The frequent use of this antibiotic may be due to its low cost and wide national distribution. However, the estimated use of tetracyclines in livestock in Colombia is unknown, and in general, there are few published studies on the use of antibiotics in animal production in Latin America (Cuong et al., 2018).

All *S. aureus* strains in this study presented beta-lactamases, which is remarkable given the evidence of an increase in the phenotypic presentation of this type of resistance in Colombia in comparison with previous studies carried out on samples from bovine mammary quarters (Jiménez Velásquez et al., 2020; Ramírez et al., 2018; Torres et al., 2023) and from bulk tank milk in other countries (Grispoldi et al., 2019; Hassani et al., 2022; Liu et al., 2017). This increase could be due to the excessive or indiscriminate use of beta-lactams for the treatment of bovine mastitis which demands the strengthening of the active surveillance measures in addition to educational campaigns on the proper use of antibiotics in dairy cattle.

The absence of MRSA or phenotypic resistance to vancomycin in this research agrees with a previous study (Monistero et al., 2020). However, since in MRSA the transmission of resistance genes is mainly mediated by plasmids transferred between *S. aureus* and other bacteria (Guo et al., 2020; Vestergaard et al., 2019) the absence of MRSA in the herds of study should not be underestimated because the *mecA* gene has already been found in strains isolated from bovine milk in the country (Jiménez Velásquez et al., 2020; Torres et al., 2023).

Some virulence factors related to the pathogenicity of *S. aureus* in the bovine mammary gland were analyzed. The *hla* and *hlb* findings are consistent with those found in strains isolated from cases of bovine mastitis in other countries (Monistero et al., 2020; Salasia et al., 2004). Virulence factors not found in some of the *S. aureus* strains analyzed were *hlb*, *fbnB*, and *clfA*, which is consistent with other studies wherein clonal distribution varied according to geographic distribution (Monistero et al., 2020; Salasia et al., 2004). The above is evidence that toxin production by *S. aureus* can vary significantly according to genotype (Oliveira et al., 2018) and the study zone.

In the present work, three *agr* types (I, II, and III) were evaluated. Of these, *agr*I was the most identified group harboring in 17 isolates. This finding agrees with that observed in other studies of strains isolated from milk mastitis samples (Ambroggio et al., 2018; Khemiri et al., 2018; Rossi et al., 2021). This gene has been isolated most frequently in strains of *S. aureus* isolated from cases of bovine subclinical mastitis (Dendani Chadi et al., 2022) that evidenced improved adaptation to persistent intracellular survival in the host (Buzzola et al., 2007). As for the gene *agr*II, was presented only in one isolate, which differs from other studies in which it has been the most frequently isolated strain causing subclinical mastitis in cows (Bonsaglia et al., 2018). Regarding the *agr*III gene, it has been associated with cases of clinical mastitis (Rossi et al., 2021). In this study, three strains were found to have it, which is consistent with findings from cow samples in Tunisia (Khemiri et al., 2018) and Brazil (Bonsaglia et al., 2018). Therefore, according to the findings at the *agr*I group level, the strains isolated in this study come mainly from cows with subclinical mastitis, this may be because milk from cows with subclinical mastitis is added to the tank but not milk from cows with clinical mastitis. It could also be hypothesized that the strains of *S. aureus* distributed in the area have similar characteristics that allowed it to adapt and specialize in the bovine mammary gland tissue so that the identification of the *agr* group is a tool that allows molecular discrimination of *S. aureus* strains and their relationship with virulence genes (Dendani Chadi et al., 2022).

Additionally, the predominance of isolates harboring the *cap*5 gene was identified which agrees with studies conducted on *S. aureus* from bovine mastitis samples from Chile (Ambroggio et al., 2018) but differs from other studies of the same type of samples from Brazil, Uruguay, and Argentina in which the predominant gene was *cap*8 (Ambroggio et al., 2018; Rossi et al., 2021). This study identified in most isolates the combination *cap*5 and *agr*I, which has been associated with a high rate of tissue invasion, showing better adaptation to the intracellular environment, and was associated with chronic mastitis (Bardiau et al., 2016). Additionally, Bardiau et al. (2016) found an association between *cap*5-*agr*I strains and the presence of beta-lactamases, which coincides with this work in which all isolates presented beta-lactamases and most were cap5 and *agr*I. Since the isolates of this study were isolated from tank milk, it could be hypothesized that the *S. aureus* strains come from

cows with subclinical mastitis of chronic course and that evidence resistance to beta-lactams.

The presence of genes encoding for staphylococcal enterotoxin is similar to the results of a previous meta-analysis (Zhang et al., 2022). The presence of *seh* gene, which codes for enterotoxin H, agrees with previous reports of *S. aureus* isolated from samples of cows with mastitis in Colombia (Monistero et al., 2018), however, it differs from other countries in which the *seh* detection rate was low (Oliveira et al., 2022; Szczuka et al., 2022; Wang et al., 2022) or undetected (Liao et al., 2022). These findings suggest a major risk of food poisoning caused by consumption of raw milk or dairy products (Schubert et al., 2016) because staphylococcal enterotoxin H (SEH) has been involved in two food poisoning cases and was directly detected in food products. The first outbreak, involving eight individuals, was caused by contaminated mashed potatoes made with raw bovine milk (Jørgensen et al., 2005). The second case, a large outbreak in Japan, was caused by reconstituted milk powder (Ikeda et al., 2005). One of the isolates presented the genes among *egc*-complex (*seg, sei, sem, sen, seo,* and *selu*) which has been related to the pathogenicity of the isolates in the bovine mammary gland since enterotoxins G to Q are most frequently identified in *S. aureus* isolated from bovine mastitis samples (Abril et al., 2020). In addition, these genes have been identified as being involved in the inflammatory response in animals (Abril et al., 2020).

In this study, further genotypic characterization was performed to genetically compare and identify strain-level clonal relatedness and genetic variability among 21 isolates using MRFPA. The dendrogram analysis of macrorestriction fragment patterns revealed five different clusters with an overall approximately 42% similarity, and high genotypic variability was found among isolates. Of the total of five clusters, 18 distinct MRFP patterns were found. However, MRFP patterns subtypes in three cases could be attributed to isolates from different dairy farms but from the same municipality was also observed. The isolates #13 and #15 from Entrerríos, #17 and #18 from San Pedro de los Milagros, #5 and #10 from Santa Rosa de Osos, shared related MRFP patterns (P7 and P7a, P8 and P8a, and P9 and P9a, respectively) with an overall approximately more than 95% similarity (Fig. 1). This finding aligns with results from previous studies on *S. aureus* isolated from cows with mastitis (Srinivasan et al., 2006; Vaughn et al., 2020). The observed related MRFP patterns in isolates from different dairy farms might be elucidated by the movement of animals among dairy herds due to the intensive trade of animals in the area. This practice likely facilitated the dissemination of clones within the same study region. This is linked to the similarities identified in related MRFP patterns, particularly in the presentation of genes such as *coa, spa, cap*5*, agr*I, and enterotoxin genes (*seh*). These similarities suggest a potential common genetic origin among the isolates. The expansion of territory might have led to the acquisition of new genetic elements, fostering genetic variability. This interpretation is in line with findings from other studies that reported diversity in a specific area, often with a few dominant MRFP patterns (Vaughn et al., 2020).

5. Conclusion

The results of this study reveal phenotypic resistance to tetracycline and evidenced beta-lactamase production in *S. aureus* isolates from bulk tank milk obtained from dairy farms in the northern region of Antioquia. Additionally, the presence of genes encoding staphylococcal enterotoxins, especially the newly identified SEH, raises significant concerns for individuals consuming raw milk and raw milk products contaminated with such enterotoxigenic *S. aureus*. The high genetic variability observed among the isolates suggests the spread of clones in the study region, posing a risk for potential therapeutic failure in cases of bovine mastitis. Therefore, it is imperative to implement containment and prevention measures through on-farm programs. These measures should focus on reducing the entry of new animals, preventing the infection of existing ones, and ultimately minimizing the use of antibiotics for the treatment of bovine mastitis.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Ángela Sofía Ágredo-Campos: Writing – original draft, Methodology, Investigation, Formal analysis. **Cecilia Camussone:** Writing – review & editing, Methodology, Formal analysis. **Ömer Akineden:** Writing – review & editing, Investigation, Formal analysis. **Jorge A. Fernández-Silva:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Nicolás F. Ramírez-Vásquez:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the Primary Founder Ministerio de Ciencia, Tecnología e Innovación of Colombia, Minciencias, for sponsoring this research Grant ID: Project 111580763373, contract 755–2018. Also, thanks to the Federation of Livestock Associations (FAGA), the Universidad de Antioquia, dairy farmers and the municipalities for allowing the sample collection and information and for their support.

References

Abril, A. G., Villa, G., Barros-vel, J., Cañas, B., Angeles, S., Calo-mata, P., & Carrera, M. (2020). Staphylococcus aureus exotoxins and their detection in the dairy industry and mastitis. *Toxins, 12*(537), 1–18.

Ambroggio, M. B., Perrig, M. S., Camussone, C., Pujato, N., Bertón, A., Gianneechini, E., Alvarez, S., Marcipar, I. S., Calvinho, L. F., & Barbagelata, M. S. (2018). Survey of potential factors involved in the low frequency of CP5 and CP8 expression in Staphylococcus aureus isolates from mastitis of dairy cattle from Argentina, Chile, and Uruguay. *Journal of Applied Genetics, 59*(3), 357–363. https://doi.org/10.1007/s13353-018-0443-8

Bardiau, M., Caplin, J., Detilleux, J., Graber, H., Moroni, P., Taminiau, B., & Mainil, J. G. (2016). Existence of two groups of Staphylococcus aureus strains isolated from bovine mastitis based on bio fi lm formation , intracellular survival , capsular pro fi le and agr -typing. *Veterinary Microbiology, 185*, 1–6. https://doi.org/10.1016/j.vetmic.2016.01.003

Benavides, J. A., Streicker, D. G., Gonzales, M. S., Rojas-Paniagua, E., & Shiva, C. (2021). Knowledge and use of antibiotics among low-income small-scale farmers of Peru. *Preventive Veterinary Medicine, 189*(September 2020), Article 105287. https://doi.org/10.1016/j.prevetmed.2021.105287

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., & Rall, V. L. M. (2018). Molecular epidemiology of methicillin-susceptible Staphylococcus aureus (MSSA) isolated from milk of cows with subclinical mastitis. *Microbial Pathogenesis, 124*(2), 130–135. https://doi.org/10.1016/j.micpath.2018.08.031

Booth, M. C., Pence, L. M., Mahasreshti, P., Callegan, M. C., & Gilmore, M. S. (2001). Clonal associations among Staphylococcus aureus isolates from various sites of infection. *Infection and Immunity, 69*(1), 345–352. https://doi.org/10.1128/IAI.69.1.345-352.2001

Buzzola, F. R., Alvarez, P., Tuchscherr, L. P. N., Barbagelata, S., Lattar, S. M., Calvinho, L., & Sordelli, D. O. (2007). Differential abilities of capsulated and noncapsulated Staphylococcus aureus isolates from diverse agr groups to invade mammary epithelial cells ☋. *Infection and Immunity, 75*(2), 886–891. https://doi.org/10.1128/IAI.01215-06

Calderón, A., Virginia, R., Germán, A., & Salim, M. (2011). Prevalence of mastitis in dual purpose cattle farms in monteria (Colombia): Etiology and antibacterial susceptibility. *Revista Colombiana de Ciencias Pecuarias, 24*, 19–28.

Cheung, G. Y. C., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of Staphylococcus aureus. *Virulence, 12*(1), 547–569. https://doi.org/10.1080/21505594.2021.1878688

CLSI. (2020). In*Clsi m100-ED29: 2021 performance standards for antimicrobial susceptibility testing* (30th ed., Vol. 40). Clsi, 1.

Cuong, N. V., Padungtod, P., Thwaites, G., & Carrique-Mas, J. J. (2018). Antimicrobial usage in animal production: A review of the literature with a focus on low-and middle-income countries. *Antibiotics, 7*(3). https://doi.org/10.3390/antibiotics7030075

Dendani Chadi, Z., Dib, L., Zeroual, F., & Benakhla, A. (2022). Usefulness of molecular typing methods for epidemiological and evolutionary studies of Staphylococcus aureus isolated from bovine intramammary infections. *Saudi Journal of Biological Sciences, 29*(8), Article 103338. https://doi.org/10.1016/j.sjbs.2022.103338

Frenay, H. M., Bunschoten, A. E., Schouls, L. M., van Leeuwen, W. J., Vandenbroucke-Grauls, C. M., Verhoef, J., & Mooi, F. R. (1996). Molecular typing of methicillin-resistant Staphylococcus aureus on the basis of protein A gene polymorphism. *European Journal of Clinical Microbiology & Infectious Diseases, 15*(1), 60–64.

Gilot, P., Cochard, T., & Poutrel, B. (2002). Analysis of the genetic variability of genes encoding the RNA III-activating components agr and TRAP in a population of Staphylococcus aureus strains isolated from cows with mastitis. *Journal of Clinical Microbiology, 40*(11), 4060–4067. https://doi.org/10.1128/JCM.40.11.4060

Grispoldi, L., Massetti, L., Sechi, P., Iulietto, M. F., Ceccarelli, M., Karama, M., Popescu, P. A., Pandolfi, F., & Cenci-goga, B. T. (2019). Short communication : Characterization of enterotoxin-producing Staphylococcus aureus isolated from mastitic cows. *Journal of Dairy Science, 102*(2), 1059–1065. https://doi.org/10.3168/jds.2018-15373

Guo, Y., Song, G., Sun, M., Wang, J., & Wang, Y. (2020). Prevalence and therapies of antibiotic-resistance in Staphylococcus aureus. *Frontiers in Cellular and Infection Microbiology, 10*(March), 1–11. https://doi.org/10.3389/fcimb.2020.00107

Hassani, S., Moosavy, M. H., Gharajalar, S. N., Khatibi, S. A., Hajibemani, A., & Barabadi, Z. (2022). High prevalence of antibiotic resistance in pathogenic foodborne bacteria isolated from bovine milk. *Scientific Reports, 12*(1), 1–10. https://doi.org/10.1038/s41598-022-07845-6

Hookey, J. V., Richardson, J. F., & Cookson, B. D. (1998). Molecular typing of Staphylococcus aureus based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. *Journal of Clinical Microbiology, 36*(4), 1083–1089.

Jørgensen, H. J., Mørk, T., & Rørvik, L. M. (2005). The occurrence of Staphylococcus aureus on a farm with small-scale production of raw milk cheese. *Journal of Dairy Science, 88*(11), 3810–3817. https://doi.org/10.3168/jds.S0022-0302(05)73066-6

Jayarao, B. M., & Wolfgang, D. R. (2003). Bulk-tank milk analysis: A useful tool for improving milk quality and herd udder health. *Veterinary Clinics of North America: Food Animal Practice, 19*(1), 75–92. https://doi.org/10.1016/S0749-0720(02)00075-0

Jiménez Velásquez, S. del C., Torres Higuera, L. D., Parra Arango, J. L., Rodríguez Bautista, J. L., García Castro, F. E., & Patiño Burbano, R. E. (2020). Profile of antimicrobial resistance in isolates of Staphylococcus spp. obtained from bovine milk in Colombia. *Revista Argentina de Microbiología, 52*(2), 121–130. https://doi.org/10.1016/j.ram.2019.05.004

Keefe, G. (2012). Update on control of staphylococcus aureus and streptococcus agalactiae for management of mastitis. *Veterinary Clinics of North America: Food Animal Practice, 28*(2), 203–216. https://doi.org/10.1016/j.cvfa.2012.03.010

Khemiri, M., Abbassi, M. S., Couto, N., Mansouri, R., Hammami, S., & Pomba, C. (2018). Genetic characterisation of Staphylococcus aureus isolated from milk and nasal samples of healthy cows in Tunisia: First report of ST97-t267-agrI-SCCmecV MRSA of bovine origin in Tunisia. *Journal of Global Antimicrobial Resistance, 14*, 161–165. https://doi.org/10.1016/j.jgar.2018.03.013

Kreausukon, K., Fetsch, A., Kraushaar, B., Alt, K., Müller, K., Krömker, V., Zessin, K. H., Käsbohrer, A., & Tenhagen, B. A. (2012). Prevalence, antimicrobial resistance, and molecular characterization of methicillin-resistant Staphylococcus aureus from bulk tank milk of dairy herds. *Journal of Dairy Science, 95*(8), 4382–4388. https://doi.org/10.3168/jds.2011-5198

Liao, G., Wu, Z., Lv, J., Ren, Q., & Chen, W. (2022). Investigation of clonal diversity , virulence genes , and antibiotic resistance of Staphylococcus aureus recovered from raw cow milk in southern Xinjiang , China. *Folia Microbiologica, 67*, 245–252. https://doi.org/10.1007/s12223-021-00924-7

Liu, H., Li, S., Meng, L., Dong, L., Zhao, S., Lan, X., & Wang, J. (2017). Prevalence , antimicrobial susceptibility , and molecular characterization of Staphylococcus aureus isolated from dairy herds in northern China. *Journal of Dairy Science, 100*(11), 8796–8803. https://doi.org/10.3168/jds.2017-13370

Monistero, V., Barberio, A., Biscarini, F., Cremonesi, P., Castiglioni, B., Graber, H. U., Bottini, E., Ceballos-Marquez, A., Kroemker, V., Petzer, I. M., 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 Staphylococcus aureus strains isolated from clinical mastitis in six countries. *Journal of Dairy Science, 103*(4), 3431–3446. https://doi.org/10.3168/jds.2019-17141

Monistero, V., Graber, H. U., Pollera, C., Cremonesi, P., Castiglioni, B., Bottini, E., Id, A. C., Lasso-rojas, L., Id, V. K., Wente, N., Petzer, I., Santisteban, C., Runyan, J., Veiga, M., Alves, B. G., Piccinini, R., & Bronzo, V. (2018). Staphylococcus aureus isolates from bovine mastitis in eight countries : Genotypes, detection of genes virulence genes. *Toxins, 10*(247). https://doi.org/10.3390/toxins10060247

Ocampo, A. M., Vélez, L. A., Robledo, J., & Jiménez, J. N. (2014). Cambios a lo largo del tiempo en la distribución de los complejos de clones dominantes de Staphylococcus aureus resistente a la meticilina en Medellín , Colombia. *Biomedica, 34*(suple 1), 34–40.

Oliveira, D., Borges, A., & Simões, M. (2018). Staphylococcus aureus toxins and their molecular activity in infectious diseases. *Toxins, 10*(6), 1–19. https://doi.org/10.3390/toxins10060252

Oliveira, Pinho, E., Almeida, G., Azevedo, N. F., & Silva, N. (2022). Prevalence and diversity of Staphylococcus aureus and staphylococcal enterotoxins in raw milk from northern Portugal. *Frontiers in Microbiology, 13*(March), 1–12. https://doi.org/10.3389/fmicb.2022.846653

Pacha, P. A., Munoz, M. A., Paredes-Osses, E., & Latorre, A. A. (2020). Short communication: Virulence profiles of Staphylococcus aureus isolated from bulk tank milk and adherences on milking equipment on Chilean dairy farms. *Journal of Dairy Science, 103*(5), 4732–4737. https://doi.org/10.3168/jds.2019-17794

Pahlavanzadeh, S., Khoshbakht, R., Kaboosi, H., & Moazamian, E. (2021). Antibiotic resistance and phylogenetic comparison of human, pet animals and raw milk Staphylococcus aureus isolates. *Comparative Immunology, Microbiology and Infectious Diseases, 79*(October), Article 101717. https://doi.org/10.1016/j.cimid.2021.101717

Pereyra, E. A. L., Picech, F., Renna, M. S., Baravalle, C., Andreotti, C. S., Russi, R., Calvinho, L. F., Diez, C., & Dallard, B. E. (2016). Detection of Staphylococcus aureus adhesion and bio fi lm-producing genes and their expression during internalization in bovine mammary epithelial cells. *Veterinary Microbiology, 183*, 69–77. https://doi.org/10.1016/j.vetmic.2015.12.002

Peton, V., & Le Loir, Y. (2014). Staphylococcus aureus in veterinary medicine. *Infection, Genetics and Evolution, 21*, 602–615. https://doi.org/10.1016/j.meegid.2013.08.011

Pulido-Delgado, E., Acero-Plazas, V., Gil-Tibocha, D., & Arenas-Suárez, N. (2022). Evaluación de la administración de medicamentos veterinarios en hatos lecheros de quince municipios de Cundinamarca, Colombia. *Revista de Salud Pública, 24*(4), 1–8.

Quelle, L. S., Corso, A., Galas, M., & Sordelli, D. O. (2003). STAR gene restriction profile analysis in epidemiological typing of methicillin-resistant Staphylococcus aureus: Description of the new method and comparison with other polymerase chain reaction (PCR)-based methods. *Diagnostic Microbiology and Infectious Disease, 47*(3), 455–464. https://doi.org/10.1016/S0732-8893(03)00137-8

Rámirez, N., Gaviria, G., Arroyave, O., Sierra, B., & Benjumea, J. (2001). Prevalencia de mastitis en vacas lecheras lactantes en el municipio de San Pedro de los Milagros, Antioquia. *Revista Colombiana de Ciencias Pecuarias, 14*(1), 76–87.

Rainard, P., Foucras, G., Fitzgerald, J. R., Watts, J. L., Koop, G., & Middleton, J. R. (2018). Knowledge gaps and research priorities in Staphylococcus aureus mastitis control. *Transboundary and Emerging Diseases, 65*(March), 149–165. https://doi.org/10.1111/tbed.12698

Ramírez, N., Fernández-silva, J. A., & Palacio, L. G. (2018). Tasa de incidencia de mastitis clínica y susceptibilidad antibiótica de patógenos productores de mastitis en ganado lechero del norte de Antioquia , Colombia. *Revista de Medicina Veterinaria, 1*(36), 75–87.

Rossi, B. F., Bonsaglia, E. C. R., Pantoja, J. C. F., Santos, M. V., Gonçalves, J. L., Fernandes Júnior, A., & Rall, V. L. M. (2021). Short communication: Association between the accessory gene regulator (agr) group and the severity of bovine mastitis caused by Staphylococcus aureus. *Journal of Dairy Science, 104*(3), 3564–3568. https://doi.org/10.3168/jds.2020-19275

Salasia, S., Khusnan, Z., Lämmler, C., & Zschöck, M. (2004). Comparative studies on pheno- and genotypic properties of Staphylococcus aureus isolated from bovine subclinical mastitis in central Java in Indonesia and Hesse in Germany. *Journal of Veterinary Science, 5*(2), 103–109.

Salimena, A. P. S., Lange, C. C., Camussone, C., Signorini, M., Calvinho, L. F., Brito, M. A. V. P., Borges, C. A. V., Guimarães, A. S., Ribeiro, J. B., & Mendonça, L. C. (2016). Genotypic and phenotypic detection of capsular polysaccharide and biofilm formation in Staphylococcus aureus isolated from bovine milk collected from Brazilian dairy farms. *Veterinary Research Communications, 40*, 97–106. https://doi.org/10.1007/s11259-016-9658-5

Schubert, J., Podkowik, M., Bystroń, J., & Bania, J. (2016). Production of staphylococcal enterotoxins in microbial broth and milk by Staphylococcus aureus strains harboring seh gene. *International Journal of Food Microbiology, 235*, 36–45. https://doi.org/10.1016/j.ijfoodmicro.2016.06.043

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), 274–283. https://doi.org/10.1089/fpd.2006.3.274

Stephan, R., Annemuller, C., Hassan, A. A., & Lammler, C. (2001). Characterization of enterotoxigenic Staphylococcus aureus strains isolated from bovine mastitis in north-east Switzerland. *Veterinary Microbiology, 78*, 373–382. https://doi.org/10.1016/S0378-1135(00)00341-2

Szczuka, E., Porada, K., Wesołowska, M., & Łęska, B. (2022). Occurrence and characteristics of Staphylococcus aureus isolated from dairy products. *Molecules, 27*(14). https://doi.org/10.3390/molecules27144649

Torres, G., Vargas, K., Cuesta, Y., Reyes, J., & Olivera-angel, M. (2020). Phenotypic characterization and whole genome analysis of a strong biofilm-forming Staphylococcus aureus strain associated with subclinical bovine mastitis in Colombia. *Frontiers in Veterinary Science, 7*(530). https://doi.org/10.3389/fvets.2020.00530

Torres, G., Vargas, K., Jim, N., Blanchard, A., & Olivera-angel, M. (2023). High genetic diversity and zoonotic potential of Staphylococcus aureus strains recovered from bovine intramammary infections in Colombians dairy herds. *Comparative Immunology, Microbiology and Infectious Diseases, 93*(2), Article 101940. https://doi.org/10.1016/j.cimid.2022.101940

Torres, G., Vargas, K., Sanchez, M., Reyes-Velez, J., & Olivera-Angel, M. (2019). Genotypic and phenotypic characterization of biofilm production by Staphylococcus aureus strains isolated from bovine intramammary infections in Colombian dairy farms. *Heliyon, 5*(10), Article e02535. https://doi.org/10.1016/j.heliyon.2019.e02535

Toshkova, K., Annemüller, C., Akineden, O., & Lämmler, C. (2001). The significance of nasal carriage of Staphylococcus aureus as risk factor for human skin infections.

FEMS Microbiology Letters, 202(1), 17–24. https://doi.org/10.1016/S0378-1097(01)00287-7

Vaughn, J. M., Abdi, R. D., Gillespie, B. E., & Kerro Dego, O. (2020). Genetic diversity and virulence characteristics of Staphylococcus aureus isolates from cases of bovine mastitis. *Microbial Pathogenesis, 144*(March), Article 104171. https://doi.org/10.1016/j.micpath.2020.104171

Verdier, I., Durand, G., Bes, M., Taylor, K. L., Lina, G., Vandenesch, F., Fattom, A. I., & Etienne, J. (2007). Identification of the capsular polysaccharides in Staphylococcus aureus clinical isolates by PCR and agglutination tests. *Journal of Clinical Microbiology, 45*(3), 725–729. https://doi.org/10.1128/JCM.01572-06

Vestergaard, M., Frees, D., & Ingmer, H. (2019). Antibiotic resistance and the MRSA problem. *Microbiology Spectrum, 7*, 747–765. https://doi.org/10.1128/9781683670131.ch47

Wang, H., Shen, J., Zhu, C., Ma, K., Fang, M., Li, B., Wang, W., & Xue, T. (2022). Antibiotics resistance and virulence of Staphylococcus aureus isolates isolated from raw milk from handmade dairy retail stores in hefei city, China. *Foods, 11*(2185), 1–13.

Zhang, J., Wang, J., Jin, J., Li, X., Zhang, H., Shi, X., & Zhao, C. (2022). Prevalence, antibiotic resistance, and enterotoxin genes of Staphylococcus aureus isolated from milk and dairy products worldwide: A systematic review and meta-analysis. *Food Research International, 162*(PA), Article 111969. https://doi.org/10.1016/j.foodres.2022.111969