FULL ARTICLE

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Virulence, *agr* groups, antimicrobial resistance and epidemiology of *Staphylococcus aureus* isolated from bovine subclinical mastitis

Virulência, grupos agr, resistência a antimicrobianos e epidemiologia de isolados de Staphylococcus aureus obtidos de vacas com mastite

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ABSTRACT

Fifty-two *Staphylococcus aureus* recovered from papillary ostium and milk samples collected from cows with subclinical mastitis and milking environments in three small dairy herds located in southeastern Brazil were subjected to PCR identification based on the thermonuclease (*nuc*) gene. All the strains were submitted to *in vitro* antimicrobial susceptibility testing, and we investigated the sequence types (STs), *agr* groups (I-IV), virulence genes encoding for Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), biofilm-associated proteins, bi-component toxins, pyrogenic toxin superantigens, and enterotoxins. Screening for oxacillin resistance (2-6 µg/ml oxacillin), beta-lactamase activity assays, and PCR for the *mecA*/*mecC* genes detected 26 methicillin-susceptible *S. aureus* (MSSA) and 26 *mec*-independent oxacillin-nonsusceptible *S. aureus* (MIONSA). While MSSA isolates were found to be susceptible to all antimicrobial agents tested, or only resistant to penicillin and ampicillin, MIONSA isolates were multidrug-resistant. ST126-*agr* group II MSSA isolates were prevalent in milk (n=14) and carried a broad set of virulence genes (*clfA*, *clfB*, *eno*, *fnbA*, *fiB*, *icaA*, *icaD*, *lukED*, *hla,* and *hlb*), as well as the ST126-*agr* group II MIONSA isolated from milking liners (n=1), which also carried the *eta* gene. ST1-*agr* group III MIONSA isolates (n=4) were found in papillary ostium and milk, but most MIONSA isolates (n=21), which were identified in both papillary ostium and milking liners, were *agr*-negative and assigned to ST126. The *agr*-negative and *agr* group III lineages showed a low potential for virulence. Studies on the characterization of bovine-associated MSSA/MIONSA are essential to reduce *S. aureus* mastitis to prevent economic losses in dairy production and also to monitor the zoonotic potential of these pathogens associated with invasive infections and treatment failures in healthcare.

Keywords: MSSA. MIONSA. Accessory gene regulator (*agr*). Bovine mastitis.

RESUMO

Cinquenta e dois isolados de *Staphylococcus aureus* obtidos de amostras colhidas do óstio papilar, do leite de vacas com mastite subclínica e do ambiente de ordenha em três fazendas de rebanhos leiteiros localizadas no sudeste do Brasil foram identificados por PCR para o gene da termonuclease (*nuc*). Todos os isolados foram testados para sensibilidade a antimicrobianos e foram investigados os *sequence types* (*STs*), grupos *agr* (I-IV) e genes de virulência que codificam *Microbial Surface Components Recognizing Adhesive Matrix Molecules* (*MSCRAMMs*), proteínas associadas a biofilme, toxinas bi-componentes, toxinas pirogênicas com propriedades de superantígenos e enterotoxinas. Triagem para detecção de resistência à oxacilina (2-6 µg/ml oxacilina), ensaios de atividade de enzimas beta-lactamases e PCR para os genes *mecA*/*mecC* detectaram 26 estirpes de *S. aureus* sensíveis à meticilina (*methicillin-susceptible S. aureus*, *MSSA*) e 26 estirpes de *S. aureus mec*-negativas não sensíveis à meticilina (*mec-independent oxacillin-nonsusceptible S. aureus*, *MIONSA*). Enquanto os isolados MSSA foram sensíveis a todos os agentes antimicrobianos testados, ou apenas resistentes à penicilina e ampicilina, os isolados MIONSA foram multirresistentes. MSSA ST126-*agr* grupo II foram prevalentes no leite (n= 14) e apresentaram um amplo conjunto de genes de virulência (*clfA*, *clfB*, *eno*, *fnbA*, *fiB*, *icaA*, *icaD*, *lukED*, *hla* e *hlb*), assim como o isolado MIONSA ST126-*agr* grupo II proveniente de um insuflador (n= 1), o qual também apresentou o gene *eta*. MIONSA ST1-*agr* grupo III (n= 4) foram identificados no óstio papilar e leite, mas a maioria dos

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isolados MIONSA (n= 21), encontrados em óstios papilares e insufladores, foram *agr*-negativos e pertenceram ao ST126. As linhagens *agr*-negativas e *agr* grupo III apresentaram baixo potencial de virulência. Estudos sobre a caracterização de MSSA/MIONSA associados a bovinos são essenciais para a redução da mastite causada por *S. aureus* e de perdas econômicas na produção leiteira e, também, para o monitoramento do potencial zoonótico desses patógenos associados a infecções invasivas e falhas de tratamento em ambientes hospitalares.
Palavras-chave: *MSSA*. *MIONSA*. *Accessory gene regulator* (*agr*). Mastite bovina.

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Introduction

Staphylococcus aureus has long been known as a cause of bovine intramammary infection (IMI) in dairy cattle worldwide (Barkema et al., 2006; Fitzgerald, 2012). *S. aureus* IMI leading to mastitis is favored by a set of the prolific genetic background of *S. aureus* genes encoding for Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), extracellular enzymes, exotoxins, bi-component toxins, and pyrogenic toxin superantigens (SAgs) (Foster et al., 2014). Virulence regulation in staphylococci has been mainly associated with the accessory gene-regulator (Agr) quorum-sensing system that functions based on the activity of two divergent transcripts regulated by two different promoters (P2 and P3) (Yarwood & Schlievert, 2003). While the transcript RNAII encodes for components of the signaling system (AgrB, AgrD, AgrC, and AgrA), the 514 bp-small non-coding RNA (sRNA) (Benito et al., 2000), RNAIII, acts as an effector molecule in the staphylococcal *agr* locus, regulating the expression of virulence factors and heterogeneous resistance to antimicrobials as well (Abdelhady et al., 2015; Yarwood & Schlievert, 2003). Four *agr* groups (I-IV) based on polymorphisms in the autoinducing peptides' (AIPs') amino acid sequences and the AgrC transmembrane receptor (Ji et al., 1997) have been associated with certain staphylococcal syndromes (Jarraud et al., 2002; Seidl et al., 2011; Painter et al., 2014). Nevertheless, autoinducing peptide variants other than those initially reported have also been identified in *S. aureus* isolates from cows with mastitis (Takeuchi et al., 2001), revealing the genetic heterogeneity of the *agr* locus.

Most livestock-associated (LA) *S. aureus* isolates causing IMI have emerged in several clonal clusters (CC) well-adapted to animal hosts. Of those, CC97, CC425, and CC705 appear to be restricted to the bovine host, whereas CC1, CC5, CC8, CC133, CC385, and CC398 have a broader host tropism (Fitzgerald, 2012; Smith et al., 2005). Virulence factors, antimicrobial resistance patterns, and *agr* types of bovine *S. aureus* lineages are critical for their adaptive evolution in the host, and insights into this can contribute to therapeutic intervention in cows with mastitis. To date, few studies on MLST-based phylogeny, in particular, studies on the bovine *S. aureus agr* locus have been carried out in Brazil. Here, we examined *agr* groups, selected virulence factors, antimicrobial susceptibility pattern, and sequence types (STs) of methicillin-susceptible *S. aureus* (MSSA) and *mec*-independent oxacillin-nonsusceptible *S. aureus* (MIONSA) isolated from bovine papillary ostium, mastitic cow's milk, and milking liners in dairy herds in southeastern Brazil.

Material and Methods

A convenience collection of 52 *S. aureus* recovered from papillary ostium, milk, and milking liners in three small dairy herds located in São Paulo State, Brazil, was included in this study. Papillary ostium and milk samples were taken from cows that were previously diagnosed with subclinical mastitis by physical examination of the mammary glands, per the Ethics Committee on Animal Use guidelines (CEEA protocol number 034). Milk was sampled and stored following the National Mastitis Council procedures (National Mastitis Council, 2004) and then submitted for strip cup, California Mastitis Test (CMT), and SCC analyses (Schalm & Noorlander, 1957). Only subclinical cases were included considering 200,000 cells/mL as the cut-off for the presence

of subclinical mastitis (Pyörälä et al.,2003; Schukken et al., 2003; Oliveira et al., 2013), which has been demonstrated as a high sensitivity and specificity threshold for mastitis caused specifically by *S. aureus* (Dohoo et al., 2011). Swabs were collected from randomly selected milking liners in each farm and eight samples yielded colonies on blood agar (5% sheep blood) at 37°C. All colonies, including those from a culture of the papillary ostium and milk samples, were submitted to biochemical conventional assays for *S. aureus* screening (e.g. Gram stain, tube coagulase test, latex slide agglutination test, catalase, and DNase production) (Becker et al., 2015).

DNA of the *S. aureus* isolates was extracted using the DNeasy Blood and tissue kit (Qiagen, USA), with the addition of lysozyme (50 mg/ml) and lysostaphin (10 mg/ml). For species confirmation, PCR assays for the *S. aureus*-specific thermonuclease (*nuc*) gene were conducted. The *agr* groups were identified by an *agr* group-specific multiplex PCR as previously described (Gilot et al., 2002). Control strains were used as follows: *S. aureus* N315 (*agr* II), *S. aureus* ATCC 25923 (*agr* III), and *S. aureus* clinical strains from our laboratory collection (*agr* I, *agr* IV). Also, *agr* function was examined by determining delta-hemolysin activity (Seidl et al., 2011). The *S. aureus* strains RN6607 and RN9120 (RN6607 Δ*agr*) were used as positive and negative controls, respectively. Multiple virulence genes were assessed by PCR. All primer sets used in this study are included in Table 1 (Cucarella et al., 2001; Jarraud et al., 2002; Omoe et al., 2002; Tristan et al., 2003; Vasudevan et al., 2003).

Table 1 - *Primers used in the PCR assays* applied for the identification of *Staphylococcus aureus* isolated in dairy herds in SP state, Brazil

Primer	F: sequence (5'→3')	R: sequence (5'→3')	References
nuc	TATGGTCCTGAAGCAAGTG	GCCACGTCCATATTTATCAG	This study
mecA	TGCTATCCACCCTCAAACAGG	AACGTTGTAACCACCCCAAGA	Kondo et al. (2007)
mecC	GAAAAAAAGGCTTAGAACGCCTC	GAAGATCTTTTCCGTTTTCAGC	García-Alvarez et al. (2011)
Pan	ATGCACATGGTGCACATGC		Gilot et al. (2002)
*agr*1		GTCACAAGTACTATAAGCTGCGAT	Gilot et al. (2002)
*agr*2		TATTACTAATTGAAAAGTGGCCATAGC	Gilot et al. (2002)
*agr*3		GTAATGTAATAGCTTGTATAATAATACCCAG	Gilot et al. (2002)
*agr*4		CGATAATGCCGTAATACCCG	Gilot et al. (2002)
cflA	ATTGGCGTGGCTTCAGTGCT	CGTTTCTTCCGTAGTTGCATTTG	Tristan et al. (2003)
cflB	ACATCAGTAATAGTAGGGGGCAAC	TTCGCACTGTTTGTGTTTGCAC	Tristan et al. (2003)
fnbA	GTGAAGTTTTAGAAGGTGGAAAGATTAG	GCTCTTGTAAGACCATTTTTCTTCAC	Tristan et al. (2003)
fnbB	GTAACAGCTAATGGTCGAATTGATACT	CAAGTTCGATAGGAGTACTATGTTC	Tristan et al. (2003)
bbp	AACTACATCTAGTACTCAACAACAG	ATGTGCTTGAATAACACCATCATCT	Tristan et al. (2003)
cna	GTCAAGCAGTTATTAACACCAGAC	AATCAGTAATTGCACTTTGTCCACTG	Tristan et al. (2003)
eno	ACGTGCAGCAGCTGACT	CAACAGCATYCTTCAGTACCTTC	Tristan et al. (2003)
ebp	CATCCAGAACCAATCGAAGAC	CTTAACAGTTACATCATCATGTTTATCTTTG	Tristan et al. (2003)
fib	CTACAACTACAATTGCCGTCAACAG	GCTCTTGTAAGACCATTTTCTTCAC	Tristan et al. (2003)
icaA	CCTAACTAACGAAAGGTAG	AAGATATAGCGATAAGTGC	Vasudevan et al. (2003)
icaD	AAACGTAAGAGAGGTGG	GGCAATATGATCAAGATAC	Vasudevan et al. (2003)
bap	CCCTATATCGAAGGTGTAGAATTG	GCTGTTGAAGTTAATACTGTACCTGC	Cucarella et al. (2001)
hla	CTGATTACTATCCAAGAAATTCGATTG	CTTTCCAGCCTACTTTTTTATCAGT	Jarraud et al. (2002)
hlb	GTGCACTTACTGACAATAGTGC	GTTGATGAGTAGCTACCTTCAGT	Jarraud et al. (2002)
hlg	GTCAYAGAGTCCATAATGCATTTAA	CACCAAATGTATAGCCTAAAGTG	Jarraud et al. (2002)
tst	TTCACTATTTGTAAAAGTGTCAGACCCACT	TACTAATGAATTTTTTTATCGTAAGCCCTT	Jarraud et al. (2002)
pvl	ATCATTAGGTAAAATGTCTGGACATGATCCA	GCATCAASTGTATTGGATAGCAAAAGC	Jarraud et al. (2002)
lukDE	TGAAAAAGGTTCAAAGTTGATACGAG	TGTATTCGATAGCAAAAGCAGTGCA	Jarraud et al. (2002)
lukMF	TGGATGTTACCTATGCAACCTAC	GTTCGTTTCCATATAATGAATCACTAC	Jarraud et al. (2002)
eta	ACTGTAGGAGCTAGTGCATTTGT	TGGATACTTTTGTCTATCTTTTTCATCAAC	Jarraud et al. (2002)
etb	CAGATAAAGAGCTTTATACACACATTAC	AGTGAACTTATCTTTCTATTGAAAAACACTC	Jarraud et al. (2002)
sea	GAAAAAAGTCTGAATTGCAGGGAACA	CAAATAAATCGTAATTAACCGAAGGTTC	Jarraud et al. (2002)
seb	ATTCTATTAAGGACACTAAGTTAGGGA	ATCCCGTTTCATAAGGCGAGT	Jarraud et al. (2002)
sec	GTAAAGTTACAGGTGGCAAAACTTG	CATATCATACCAAAAAGTATTGCCGT	Jarraud et al. (2002)
sed	GAATTAAGTAGTACCGCGCTAAATAATATG	GCTGTATTTTTCCTCCGAGAGT	Jarraud et al. (2002)
see	CAAAGAAATGCTTTAAGCAATCTTAGGC	CACCTTACCGCCAAAGCTG	Jarraud et al. (2002)
seg	AAGTAGACATTTTTGGCGTTCC	AGAACCATCAAACTCGTATAGC	Omoe et al. (2002)
seh	GTCTATATGGAGGTACAACACT	GACCTTTACTTATTTCGCTGTC	Omoe et al. (2002)
sei	GGTGATATTGGTGTAGGTAAC	ATCCATATTCTTTGCCTTTACCAG	Omoe et al. (2002)

Methicillin susceptibility of the *S. aureus* isolates was screened using Mueller-Hinton agar supplemented with 4% NaCl containing 6 μg/ml of oxacillin at 37°C (24 h), according to the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute, 2018); 2-4 μg/ml of oxacillin were tested as well. *S. aureus* N315 (MRSA) and *S. aureus* ATCC 25923 (MSSA) were used as control strains. For detection of beta-lactamase production, nitrocefin-based enzyme activity assays (Cefinase; Becton-Dickinson Microbiology Systems, Cockeysville, MD, USA) were performed according to the manufacturer's instructions. Then, all *S. aureus* isolates were investigated for the presence of the *mecA*/*mecC* genes by PCR (García-Álvarez et al., 2011; Kondo et al., 2007).

Minimum inhibitory concentrations (MIC's) of penicillin, ampicillin, cephalothin, oxacillin, vancomycin, linezolid, chloramphenicol, florfenicol, tetracycline, erythromycin, azithromycin, tylosin, clindamycin, tigecycline, daptomycin, quinupristin-dalfopristin, ciprofloxacin, ofloxacin, nitrofurantoin, sulfonamide, sulfazotrin, streptomycin, amikacin, gentamicin, and kanamycin were determined using broth microdilution testing and interpreted according to the CLSI susceptibility breakpoints (Clinical and Laboratory Standards Institute, 2018). Isolates that exhibited resistance to three or more classes of antimicrobial agents in the phenotypic susceptibility testing were considered multidrug-resistant (MDR) (Magiorakos et al., 2012). The *S. aureus* ATCC strain 29213 was used as a control for antimicrobial susceptibility testing. Sequence types (STs) were determined using PubMLST (Jolley et al., 2018) and were assigned to clonal complexes (CC) using eBURST v3.

Results and Discussion

The MSSA isolates from milk (n=12) were susceptible to all antimicrobial agents tested, or exhibited resistance only to penicillin and ampicillin (n=14). On the other hand, 2 *S. aureus* isolated from milk and all *S. aureus* from papillary ostium (n=16) and milking liners (n=8) exhibited low-level oxacillin resistance and were MDR (Table 2). These MIONSA isolates were grown on oxacillin resistance screening agar using 2-6 μg/ml oxacillin, exhibited oxacillin MIC's from 2 to 16 μg/ml by broth microdilution testing or Oxoid® M.I.C. Evaluator® strips, and were negative for the *mecA*/*meC* genes by PCR. Non-penicillin-binding protein 2a (PBP2a)-mediated oxacillin resistance has been implicated in treatment failure of chronic MSSA infections with antistaphylococcal penicillins (Altman et al., 2018). It has been assigned to β-lactamase hyperproduction (borderline oxacillin-resistant *S. aureus*, BORSA) or adaptive mutations in PBPs (modified PBP *S. aureus*, MODSA) (Chambers, 1997), but recent studies have shown that other adaptive pathways under exposure to oxacillin also contribute to the MIONSA phenotype (Giulieri et al., 2020). β-lactamase production using nitrocefin disks was detected in 14 MSSA and all MIONSA isolates in this study, but none of them were considered to be hyper-producers using amoxicillin (20 μg) and clavulanic acid (10 μg) disks. The selective pressure due to the antimicrobial use in animal husbandry practices may have favored the emergence of resistances in the autochthonous microbiota of these herds or their milking environments. Moreover, these MIONSA isolates may have been selected by intramammary antimicrobial therapy (van den Borne et al., 2019), as dry cow therapy

Table 2 - MLST, *agr* group, virulence genes and antimicrobial susceptibility of MSSA and MIONSA investigation of *Staphylococcus aureus* isolated in dairy herds in SP state, Brazil

Isolate	Origin	Agr group	ST	Virulence genes	Antimicrobial resistance
MSSA (14)	Milk	II	126	*clfA, clfB, eno, fnbA, fib, icaA, icaD lukED, hla, hlb*	PEN, AMP
MSSA (5)	Milk	negative	126	*clfA, lukED*	PEN, AMP
MSSA (7)	Milk	negative	126	*clfA, eno*	
MIONSA (1)	Milk	negative	126	*clfA*	PEN, AMP, KF, OXA, AK, DA, SUL
MIONSA (2)	Milk	III	1	*clfA*	PEN, AMP, KF, OXA, AK, DA, SUL
MIONSA (12)	Papillary ostium	negative	126	*clfA*	PEN, AMP, KF, OXA, AK, DA, SUL
MIONSA (1)	Papillary ostium	negative	126	*clfA, eta*	PEN, AMP, KF, OXA, AK, DA, SUL, TET, ERY, AZI, CHL
MIONSA (2)	Papillary ostium	III	1	*clfA*	PEN, AMP, KF, OXA, AK, DA, SUL
MIONSA (1)	Milking liner	II	126	*clfA, clfB, eno, fnbA, fib, icaA, icaD, lukED, hla, hlb, eta*	PEN, AMP, KF, OXA, AK, DA, SUL
MIONSA (4)	Milking liner	negative	126	*clfA, icaA, icaD eta*	PEN, AMP, KF, OXA, AK, DA, SUL, TET, ERY, AZI, CHL
MIONSA (3)	Milking liner	negative	126	*clfA*	PEN, AMP, KF, OXA, AK, DA, SUL, TET

PEN, penicillin; AMP, ampicillin; KF, c*ephalothin;* OXA, oxacillin; AK, a*mikacin;* DA, clindamycin; SUL, sulfonamide; TET, tetracycline; ERY, erythromycin; AZI, azithromycin; CHL chloramphenicol.

using cloxacillin was a common practice for control of mastitis among these herds.

The most prevalent *agr* group found among the MSSA and MIONSA isolates of this study were *agr*-II (n=15), followed by *agr*-III (n=4). No *agr* group could be assigned to 12 MSSA or 21 MIONSA isolates (Table 2). While *agr*-II strains were carriers of a broad set of genes encoding for MSCRAMMs, extracellular proteins, and cytolytic toxins, *agr*-III and non-typeable strains had a lower potential for virulence.

All MSSA and MIONSA isolates, regardless of the *agr* group, carried the gene encoding for the fibrinogen-binding MSCRAMM protein ClfA (clumping factor A). ClfA binding occurs via the fibrinogen γ-chain, which allows the interaction of *S. aureus* to various hosts (Geoghegan et al., 2010). The cell wall-anchored (CWA) protein ClfA promotes inhibition of opsonophagocytosis, and *S. aureus* adhesion to host blood proteins and biomaterial surfaces (Foster et al., 2014), which has recently been shown to occur under physiological stress (Herman-Bausier et al., 2018). *clfA*-carrying MSSA and MIONSA isolates were recovered from papillary ostium, milk, and milking liners, suggesting a crucial role of this surface adhesin in determining *S. aureus* IMI and enhancing the persistence of the pathogen in these milking environments.

In addition to *clfA*, all *agr*-II strains carried other MSCRAMM genes, such as *clfB* (clumping factor B), *fib* (fibrinogen-binding protein), *fnbA* (fibronectin-binding protein A), and *eno* (laminin-binding protein). These CWA proteins can determine the virulence of *S. aureus* lineages, as they are involved in the adhesion and invasion of host tissues, evasion of an immune response, and biofilm formation (Foster et al., 2014). The intercellular adhesion (*ica*) locus required to mediate cell-to-cell adhesion and polysaccharide intercellular adhesin (PIA) production was also identified in all *agr*-II strains, supporting their potential for biofilm production. Moreover, *agr*-II strains harbored genes encoding for the bicomponent pore-forming toxin LukED, alpha-toxin Hla, and beta-toxin Hlb. Leukotoxin ED, which is encoded in the pathogenicity island νSaβ, can damage bovine neutrophils by targeting CXCR2 as a receptor (Reyes-Robles et al., 2013). The leukotoxic action of *S. aureus* may be further accentuated by the pore-forming toxin Hla and sphingomyelinase Hlb expression, cytolytic toxins that are also involved with *S. aureus* adhesion to epithelial cells of bovine mammary glands (Cifrian et al., 1996).

Agr-II strains isolated from milking liners (n=1), but not from milk, carried the *eta* gene encoding exfoliative toxin A (ETA). *agr*-negative *S. aureus* isolated from milking liners (n=4) and papillary ostium (n=1) were also positive for *eta*. The extracellular protein encoded by the prophage ETA is responsible for the staphylococcal scalded-skin syndrome (SSSS) in *S. aureus* human infections. However, there is a genetic heterogeneity among temperate phages (phiETA), and bovine *S. aureus* isolates have already been able to produce ETA after being lysogenized by a yet undescribed lineage of an *eta*-converting phage (Endo et al., 2003). Therefore, bovine *S. aureus* pathogenicity might be enhanced by replication of phiETA in milking environments.

No MSSA or MIONSA isolates in this study possessed the MSCRAMM genes *clfB* (receptor for fibrinogen), *bbp* (receptor for bone sialoprotein), *ebpS* (elastin-binding protein), *cna* (collagen-binding protein), or *fnbB* (fibronectin-binding protein). Neither did the isolates possess the biofilm-associated gene *bap*, genes for the bicomponent pore-forming toxins LukAB, γ-hemolysin, LukMF′, and Panton-Valentine leukocidin (PVL), the exfoliative gene *etb*, the toxic shock syndrome toxin-1 gene (*tst*) or genes for the enterotoxins SEA-E and SEG-I.

Analysis of MLST-based phylogeny showed the occurrence of ST126 and ST1. The *agr*-II MSSA isolates were assigned to ST126 (CC97). CC97-associated *S. aureus* lineages have been mainly associated with bovine infections (Fitzgerald, 2012; Smith et al., 2005), but have also emerged as a cause of healthcare-related infections. ST126 lineages appear to be well adapted and so far restricted to cows (Fitzgerald, 2012; Smith et al., 2005). Identification of ST126-*agr* group II MSSA, most common in this study, raises concern over the livestock, as they were found to contain a significant set of virulence genes. The *agr*-defective MSSA and MIONSA isolates were assigned to ST126 and also to ST1, which has been implicated in causing infections in multiple host species (Fitzgerald, 2012). Even though *agr*-defective *S. aureus* strains are known to have attenuated virulence, studies have shown that *agr* dysfunction may arise and provide advantages to the pathogen, such as enhanced biofilm formation or polystyrene adherence during nosocomial infections (Shopsin et al., 2008).

The collection of isolates examined in this study was relatively small and restricted to the same geographic area, which limited inferring whether ST126-*agr* group II MSSA or MIONSA isolates would be endemic to certain regions or disseminated in dairy farms throughout the country, which may be considered limitations of the current study.

The present study provides data on the epidemiology and pathogenesis of *S. aureus* lineages involved in bovine mastitis in Brazil, which is indeed critical for developing effective strategies to reduce *S. aureus* IMI. Moreover, we

report the *mec* gene-independent low-level oxacillin resistance phenotype in *S. aureus* isolated in papillary ostium, milk, and milking liners. Understanding how oxacillin resistance adaptation arises in bovine *S. aureus* is also crucial for the effectiveness of mastitis treatment.

Conflict of interest

The authors declare there is no conflict of interests.

Ethics Statement

The research was conducted under the approval of the Ethics Committee on Animal Use guidelines (CEEA protocol number 034).

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