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Frequency of *Staphylococcus aureus* virulence genes in milk of cows and goats with mastitis[1]

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ABSTRACT.- Acosta A.C., Oliveira P.R.F., Albuquerque L., Silva I.F., Medeiros E.S., Costa M.M., Pinheiro Junior J.W. & Mota R.A. 2018. **Frequency of *Staphylococcus aureus* virulence genes in milk of cows and goats with mastites.** *Pesquisa Veterinária Brasileira 38(11):2029-2036.* Laboratório de Bacterioses dos Animais Domésticos, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: acabad80@gmail.com

The present study determined the frequency of *Staphylococcus aureus* virulence genes in 2,253 milk samples of cows (n=1000) and goats (n=1253) raised in three different geographical regions of the state Pernambuco, Brazil. The presence of genes of virulence factors associated to adhesion to host cells (*fnbA, fnbB, clfA* and *clfB*), toxinosis (*sea, seb, sec, sed, seg, seh, sei, tsst, hla* and *hlb*), and capsular polysaccharide (*cap5* and *cap8)* was evaluated by PCR. A total of 123 and 27 *S. aureus* strains were isolated from cows' and goats' milk, respectively. The *sec* and *tsst* genes were detected exclusively in goats' isolates, while the *seh* gene was only identified in cows' isolates. The number of toxin genes per strain showed that goats' isolates are likely more toxic than bovines' isolates. The *cap5* genotype predominated in both host species, especially in strains collected from cows raised in the *Agreste* region. The *cap8* genotype is likely more virulent due to the number of virulence genes per strain. The results of the present study demonstrate that *S. aureus* may pose a potential threat to human health in Brazil, and, therefore, these results should support actions related to mastitis control programs.

INDEX TERMS: *Staphylococcus aureus*, virulence genes, milk, cows, goats, mastitis, molecular typing, genotyping, staphylococcal enterotoxin, capsular polysaccharide, cattle, bacterioses.

RESUMO.- [**Frequência de genes fatores de virulência de *Staphylococcus aureus* em leite de vacas e cabras com mastite.**] O presente estudo determinou a frequência de genes de virulência de *Staphylococcus aureus* em 2253 amostras de leite, sendo de vacas n=1000 e de cabras n=1253, procedentes das três regiões geográficas do estado de Pernambuco, Brasil. A presença de genes de fatores de virulência associados à adesão às células hospedeiras (*fnbA, fnbB, clfA* e *clfB*), toxinosis (*sea, seb, sec, sed, seg, seh, sei, tsst, hla* e *hlb*) e polissacarídeo capsular (*cap5* e *cap8)* foram avaliadas por PCR. Um total de 123 e 27 cepas de *S. aureus* foram isoladas do leite de vacas e cabras, respectivamente. Os genes *sec* e *tsst* foram detectados exclusivamente em isolados de cabras, enquanto o gene *seh* foi identificado apenas em isolados de vaca. O número de genes de toxina por cepa mostrou que os isolados de cabras são potencialmente mais tóxicos do que os isolados obtidos de bovinos. O genótipo *cap5* predominou em ambas as espécies hospedeiras, especialmente em cepas coletadas de vacas criadas na região Agreste. O genótipo *cap8* é potencialmente mais virulento devido ao número de genes de virulência por isolado. Os resultados do presente estudo demonstram que *S. aureus* pode representar uma ameaça potencial para a saúde humana no Brasil e, portanto, estes resultados devem subsidiar ações relacionadas aos programas de controle de mastite.

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TERMOS DE INDEXAÇÃO: Genes, virulência, *Staphylococcus aureus*, leite, cabras, mastite, tipagem molecular, genotipagem, enterotoxina estafilocócica, polissacarídeo capsular, bovinos, caprinos, bacterioses.

INTRODUCTION

Intramammary infections (IMI) are the main cause of economic loss in the dairy industry worldwide (Halasa et al. 2007). The average cost of clinical mastitis in high-yielding cows was estimated in $71 per cow per year (Bar et al. 2008). *Staphylococcus aureus* is an important etiological agent of ruminant IMI in Brazil (Mota et al. 2012), and its eradication from dairy cattle and dairy small ruminants has proven to be difficult (Bergonier et al. 2003).

Several characteristics contribute for the pathogenesis and spread of *S. aureus* including virulence factors, the host and the environment (Roberson et al. 1998, Barkema et al. 2006). Previous studies suggest that existing phenotypic and genotype diversity between groups of *S. aureus* would make that some of these strains are better adapted to live in hostile environment (Bardiau et al. 2014, 2016). This bacterium can cause a broad range of diseases due to an abundance of virulence factors that facilitate attachment, colonization, tissue invasion, toxinosis, and immune evasion, including adhesion proteins, enterotoxins and capsular polysaccharides (Zecconi et al. 2006, Piccinini et al. 2010).

Adhesion is hypothesized to be a prerequisite and crucial early step for IMI development (Kerro et al. 2002). Two fibronectin-binding proteins (FnBPs), FnBPA and FnBPB, are involved in not only adhesion to cells but also internalization by cells (Garzoni & Kelley 2009). Other two important adhesion factors involved in the pathogenesis of *S. aureus* are the clumping factors, ClfA and ClfB (Ní Eidhin et al. 1998, Garzoni & Kelley 2009).

Staphylococcal enterotoxins (SEs) are an important group of virulence factors. They play a significant role in modulating the host immune response and may contribute to maintaining a suitable environment for colonization. In addition, SEs and toxic shock syndrome toxin 1 (TSST1) are superantigens, which have the ability to stimulate large populations of T cells that have a particular Vβ element of the T-cell receptor (Omoe et al. 2003). Other important toxins are hemolysins, which can negatively affect a wide range of host cells including erythrocytes, epithelial cells, endothelial cells, T cells, monocytes and macrophages (Berube & Wardenburg 2013).

Capsular polysaccharide (cap) is a cell wall bacterial component that protects bacterium from phagocytic uptake and enhances microbial virulence (Sutra et al. 1990). *Cap5* and *cap8* were the predominant capsular types in *S. aureus* isolated from clinical bovine mastitis in different countries (Gogoi-Tiwari et al. 2015).

The strong pathogenicity of *S. aureus* strains is driven by multifactorial and complex virulence factors. Appropriate molecular typing methods and information about the genetic diversity of *S. aureus* strains in a particular region may contribute to the development of effective strategies for epidemiological control. The present study aimed to determine the frequency of virulence factors genes in *S. aureus* strains isolated from cow and goat herds raised in three different geographical regions of the state of Pernambuco, Brazil.

MATERIALS AND METHODS

Ethical statement. The present study was approved by the Institutional Animal Care and Use Committee of the School of Veterinary Medicine, Federal Rural University of Pernambuco (Universidade Federal Rural de Pernambuco - UFRPE), under the protocol number 079/2014-CEUA.

Sample collection and presumptive identification of *Staphylococcus aureus*. The samples were collected from 24 bovine and 13 goat herds raised in three different geographical regions of state of Pernambuco, Brazil: the Atlantic Forest (five bovine and two goat herds), the Agreste (nine bovine and three goat herds), and the Sertão regions (ten bovine and eigth goat herds). The Atlantic Forest region (Paulista, Recife, Camaragibe and Paudalho) has a hot and humid climate, while the Agreste region (Gravatá, Caruaru and Garanhuns) and Sertão region (Sertânia, Custódia, Serra Talhada, Floresta, Salgueiro, Cabrobó, Santa Maria da Boa Vista and Petrolina) both have a semi-arid climate (Fig.1) (Nóbrega et al. 2015).

A total of 1000 and 1253 cows' and goats' milk samples were collected from individual mammary glands (udder quarters for cows or udder halves for goats) after disinfection of the ostium with 70% ethanol following the recommendations of the National Mastitis Council (National-Mastitis-Council 1999), and transported to the laboratory under refrigeration (4-10°C).

In cows, subclinical mastitis was diagnosed by the California Mastitis Test (CMT, (Schalm & Noorlander 1957); while in goats, IMI was defined as the presence of three or more colonies of the same type after primary culture (Pantoja et al. 2009).

Primary culture of milk samples (10μL) was performed in 5% ovine blood agar plates incubated aerobically at 37°C for 72h. Milk samples were considered contaminated when three or more dissimilar colony types were founded (Hiitiö et al. 2015). The presumptive identification of *S.* aureus was based on the following parameters: Gram-positive cocci, hemolytic on blood agar, catalase positive, and coagulase positive in 4-18h (Zecconi et al. 2006). The *S. aureus* ATCC 29213 strain was used as positive control.

Confirmation of the presumptive identification of *Staphylococcus aureus.* Species confirmation was performed by PCR amplification of the *nuc* gene, as previously described by Brakstad et al. (1992). The isolates presumptively identified as *S. aureus* were freshly cultured in brain infusion broth (Merck) and incubated overnight at 37°C. Subsequently, DNA was extracted using a commercial kit (Promega) following the manufacturer's instructions and stored at -20°C. The nuc_F and nuc_R primers, which amplify a single amplicon with an expected size of approximately 296bp (nt 862768-863064, HF937103.1), were specifically designed for the present study (Table 1).

PCR testing for virulence factors genes. The primers used for amplification of the virulence factors genes are shown in Table 1. The PCR was performed in 0.2mL tubes with a total volume of 25μL containing: PCR buffer (10mM Tris-HCl, pH 9.0; 50mM KCl; 0.1% Triton X-100), 1.5mM $MgCl_2$, 250μM each dNTP, 0.5μM each gene-specific primers, 1.5U Taq DNA polymerase (Promega), 20ng DNA, and distilled water.

PCR conditions for the amplification of the *nuc* gene and the virulence factors genes were as follows: 94°C for 5min (initial denaturation), followed by 32 cycles of 94°C for 1min, annealing temperature specific for each fragment (Table 1) for 1min and 72°C for 1min, and a final extension of 72°C for 5min. The PCR products were visualized by electrophoresis in 2% agarose gels stained with Blue Green Loading Dye I (LGC Biotecnologia) and photographed under UV illumination (Molecular Imaging L.PIX Loccus biotecnologia).

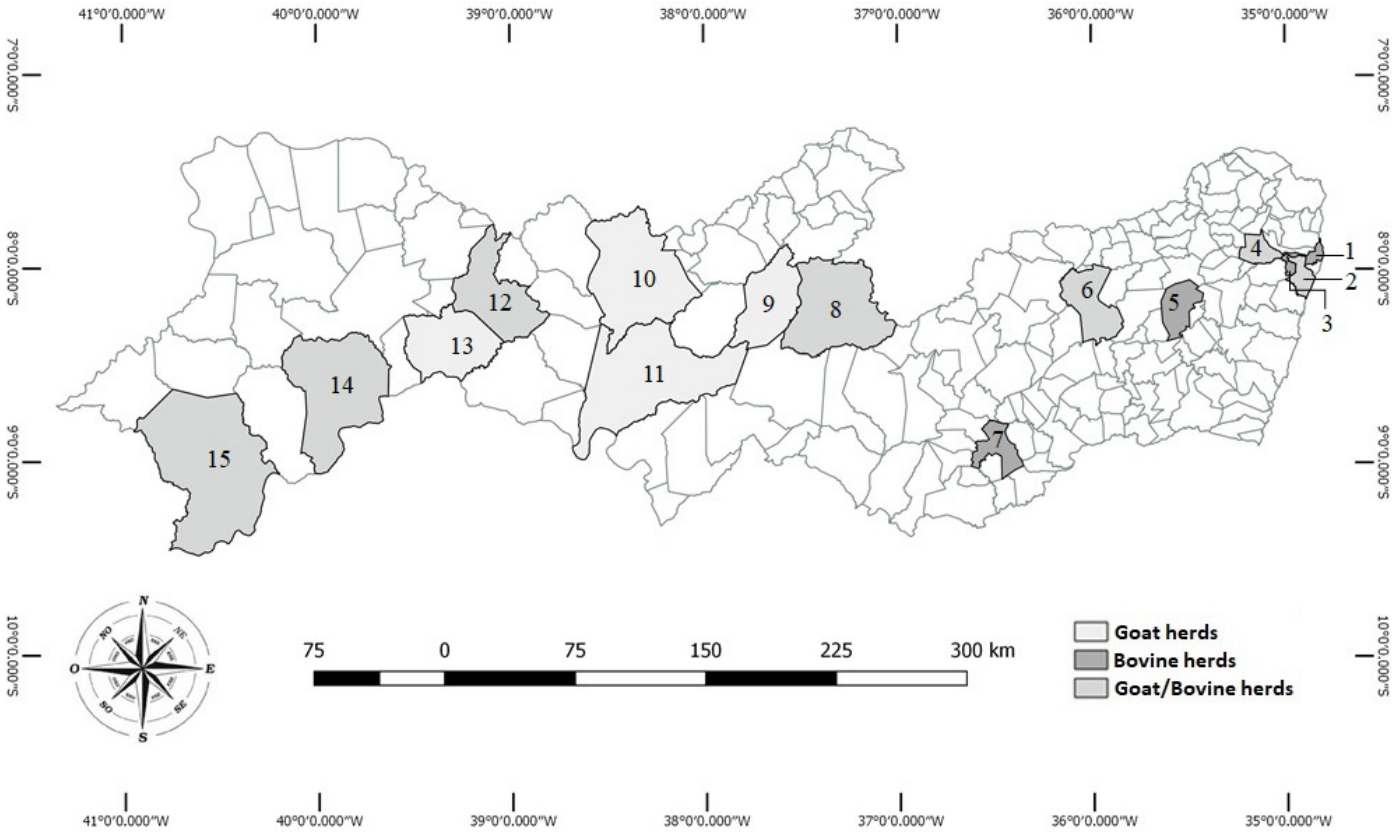

Fig.1. Map of Pernambuco state, Brazil. Distribution of the bovine and goat herds by municipal districts where the *Staphylococcus aureus* isolates were recovered. Each number represents municipal districts studied of the Atlantic Forest region: Paulista (1), Recife (2), Camaragibe (3), Paudalho (4). Agreste region: Gravatá (5), Caruaru (6), Garanhuns (7). Sertão region: Sertânia (8), Custódia (9), Serra Talhada (10), Floresta (11), Salgueiro (12), Cabrobó (13), Santa Maria da Boa Vista (14), Petrolina (15).

Three amplicons of each virulence factor were purified using the Wizard® SV gel and PCR clean-up system (Promega) and bidirectionally sequenced by standard protocols using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City/CA, USA) in an automated sequencer ABI-PRISM 3130 (Applied Biosystems, Foster City/CA, USA). Sequences were aligned using the BioEdit v.7.0.9 software (Hall 1999) and compared with those available in the GenBank database by Basic Local Alignment Search Tool (BLAST- http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis. Each *S. aureus* strain was considered a experimental unit in all analyses. Gene frequencies were calculated as categorical (binary) variables using Microsoft Excel. The frequency of each virulence gene by host species was compared using the chi-square test (Statgraphics Centurion XVI version 16.1.15, Statpoint Technologies Inc, Warrenton, Virginia). Statistical significance was set at $p<0.05$ for interpretation and discussion.

RESULTS

All the *Staphylococcus aureus* strains isolated in the present study were identified by a genotypic method in which the *nuc* gene was targeted using specific primers, a well-accepted method that has been used for confirmation of *S. aureus* infection worldwide (Rall et al. 2014). Three amplicons of the *nuc* gene and each virulence factors (*fnbA, fnbB, clfA, clfB, sea, seb, sec, sed, seg, seh, sei, tsst, hla, hlb, cap5* and *cap8*) were sequenced, and the partial sequences were confirmed by BLAST searches.

S. aureus strains were recovered from 12.3% (123/1000) and 2.15% (27/1253) of the cows' and goats' samples, respectively. Table 2 shows the number of strains recovered by geographical area, species and animals' disease status.

Table 3 shows the frequency of the virulence factors by host species and geographical area. The frequency of the virulence factors according to capsular genotyping is shown in Table 4. The fibronectin-binding proteins, *fnbA* and *fnbB*, were detected in 141 (94%) and 122 strains (81.33%), respectively. *S. aureus* strains isolated from bovines had a higher frequency of *fnbB* (91.06%, p=0.0000) than those isolated from goats (Table 3). Strains positive for *cap5* had a higher frequency of the *fnbB* gene (88.68%, p=0.0012) than *cap* (-) strains (Table 4).

The clumping factor A (*clfA*) and clumping factor B (*clfB*) were detected in 114 (76%) and 115 strains (76.67%), respectively. The lowest frequency of *clfA* (62.79%, p=0.0267) and *clfB* (65.12% p=0.0323) were observed in samples collected from animals raised in the Atlantic forest (Table 3). *Cap* (-) strains had the lowest frequency of *clfA* (47.06%, p=0.0000) (Table 4). *S. aureus* adhesion cannot be explained by the action of a single virulence determinant, and likely a number of factors act in combination during the infective process.

Table 1. Nucleotide sequences and their characteristics for the *Staphylococcus aureus* gene-specific oligonucleotide primers used in this study

Target gene	Name	Oligonucleotide sequence (5´- 3´)	Expected size	At (°C)[a]
nuc	nuc_F	GGTTCTGAAGATCCAACAGTAT	296pb	61
	nuc_R	GCTAAGCCACGTCCATATTTA		
fnbA	fnbA _F	ACTTCACCTGTCGCCATTAC	539pb	57
	fnbA _R	GCAGTACAAGCACCACAAAC		
fnbB	fnbB _F	AGGCGACGGCAAAGATAAA	317pb	60
	fnbB _R	TAGTAACCTGACCACCACCT		
clfA[b]	clfA_F	GATTCTGACCCAGGTTCAGA	945pb	55
	clfA_R	CTGTATCTGGTAATGGTTCTTT		
clfB[b]	clfB_F	ATGGTGATTCAGCAGTAAATCC	880pb	55
	clfB_R	CATTATTTGGTGGTGTAACTCTT		
sea[c]	sea_F	GGTTATCAATGTGCGGGTGG	102pb	65
	sea_R	CGGCACTTTTTTCTCTTCGG		
seb	seb_F	CCCGTTTCATAAGGCGAGTT	314pb	57
	seb_R	ACGTAGATGTGTTTGGAGCTAAT		
sec[c]	sec_F	AGATGAAGTAGTTGATGTGTATGG	451pb	57
	sec_R	CACACTTTTAGAATCAACCG		
sed	sed_F	GTCACTCCACACGAAGGTAATAA	255pb	55
	sed_R	GAGACTTTAGACCCATCAGAAGAA		
see[c]	see_F	GCTGGAGGCACACCAAATA	301pb	57
	see_R	CATAACTTACCGTGGACCCTTC		
seg	seg_F	GCCAGTGTCTTGCTTTGTAATC	491pb	56
	seg_R	GAATGCTCAACCCGATCCTAA		
seh	seh_F	CACATCATATGCGAAAGCAGAAG	365pb	60
	seh_R	CCCAAACATTAGCACCAATCAC		
sei	sei_F	AGGCAGTCCATCTCCTGTATAA	568pb	55
	sei_R	TGCTCAAGGTGATATTGGTGTAG		
tsst[c]	tsst_F	ACCCCTGTTCCCTTATCATC	326pb	57
	tsst_R	TTTTCAGTATTTGTAACGCC		
hla	hla_F	CTGTAGCGAAGTCTGGTGAAA	293pb	62
	hla_R	CGGCCTTATTGGTGCAAATG		
hlb	hlb_F	GCCAAAGCCGAATCTAAGAAAG	495pb	60
	hlb_R	ATCATGTCCAGCACCACAA		
cap5	cap5_F	CGAACCGATGATTGATGCTATTG	555pb	61
	cap5_R	TGCTATGACTGCACCAGTATTT		
cap8	cap8_F	GGAGGAAATGACGATGAGGATAG	608pb	61
	cap8_R	TAGCTTCTGTAGCGGTGAATG		

[a] Annealing temperature °C, [b] PCR primers designed by Sabat et al. (2003), [c] PCR primers designed by Mehrotra et al. (2000).

Table 2. Numbers of strains recovered by regions of the herd, species and disease status of the animal

		Animal species		Disease status of the animal	
		Bovine	Goat	Subclinical	Clinical
Regions of the herd	Atlantic Forest	36	7	34	9
	Agreste	52	5	57	0
	Sertão	35	15	49	1
Animal species	Bovine			120	3
	Goat			20	7

Of the 150 strains isolated, 78 (52%) were positive for one or more SEs genes, which were found in 46.34% (57/123) and 77.78% (21/23) of the bovines' and goats' isolates, respectively (p=0.0000). There was no pattern observed in the frequency of the virulence factors by geographical area. The SEs genes most frequently detected were *seg* (25.33%), *seh* (18%), *sei* (13.33%), *sed* (10.67%), *sec* (7.33%), *sea* (6.67%) and *seb*, which was present in only one strain (0.67%).

S. aureus strains isolated from goats had a statistically significant higher frequency of *sea, seg* and *sei* than bovines' isolates (Table 3). The *sec* and the *seh* genes were detected exclusively in goats' and bovines' isolates, respectively. The *sec* gene was most frequently found in isolates from goats raised in the *Sertão* region (p=0.0061); while the smallest frequency of the *seh* gene was detected in bovines isolates from the Atlantic forest (p=0.0206) (Table 3). Goats' isolates had a higher frequency of the SEs genes than bovines (p=0.0005). The *sea* (p=0.0011), *seb* (p=0.0007), *sec* (p=0.0040) and *seh* (p=0.0000) genes were more frequently found in *cap8* (+) strains (Table 4). The *tsst* gene was exclusively detected in goats' isolates (5.33%) (Table 3), and *cap5* (+) strains had the lowest frequency of this gene (1.89%, p=0.0115) (Table 4).

The *hla* and *hlb* genes were detected in 132 (88%) and 128 strains (85.33%), respectively. Both genes were more frequent in bovine isolates than in goats isolates (95.93%, p=0.0000 and 93.5%, p=0.0000 respectively), and less frequent in strains isolated from animals raised in the *Agreste* region (78%, p=0. 0233 and 72%, p=0.0042) (Table 3). Strains that did not carry neither *cap5* nor *cap8* genes had the lowest frequency of *hla* and *hlb* genes (58.82%, p=0.0000 and 55.88%, p=0.0000) (Table 4).

The capsular gene most frequently detected among the isolated strains was *cap5* (70.67%), while *cap8* was detected in only ten strains (6.67%). The *cap5* gene was more frequently found in bovines isolates (81.3%, p=0.0000) and in samples from animals raised in the *Agreste* region (87.72%, p=0.0002) (Table 3).

DISCUSSION

Staphylococcus aureus has been detected in 10.2% of cows diagnosed with mastitis and 17.5% of cows with subclinical mastitis in the state of São Paulo, Brazil (Silva et al. 2013, Rall et al. 2014). *S. aureus* has also been identified in 16.2% of sheep and goats from the states of Pernambuco and Bahia, Brazil, (Peixoto et al. 2010) and in 2.72% of samples from lactating goats from the state of Paraíba, Brazil (Ferreira et al. 2014). The results of the present study corroborates with previous claims that *S. aureus* is one of the main pathogens that causes mastitis in small and large ruminants (Mota et al. 2012).

The *see* gene was not present in any of the strains evaluated in the study. This gene is carried by a defective phage, and *sea* and *see* have 84% nucleotide homology (Couch et al. 1988). In Italy, studies have shown a low frequency of the *see* gene in mastitis-related strains (Zecconi et al. 2006) and have not identified the *see* gene in *S. aureus* isolated from milk or dairy products (Carfora et al. 2015) similarly to what have been reported in Brazil (Ferreira et al. 2014).

Intramammary infections caused by *S. aureus* may represent a potential risk for human health not only by the risk of pathogen transmission but also by the presence of enterotoxins in milk or dairy products, especially in goats'

Table 3. Absolute and relative frequency of virulence factors in relation to species and regions of origin

	Species					Regions					
	Bovine (n=123)		Goat (n=27)		p	Atlantic Forest (n=43)		Agreste (n=57)		Sertão (n=50)		p
	AF[a]	RF[b]	AF	RF		AF	RF	AF	RF	AF	RF	
Virulence factors involved in adhesion to host cells												
fnbA	115	93.50	26	96.30	0.8400	38	88.37	54	94.74	49	98.00	0.1451
fnbB	112	91.06	10	37.04	**0.0000**	33	76.74	51	89.47	38	76.00	0.1357
clfA	96	78.05	18	66.67	0.3454	27	62.79 [A*]	49	85.96 [B]	38	76.00 [AB]	**0.0267**
clfB	93	75.61	22	81.48	0.4316	28	65.12 [A]	43	75.44 [AB]	44	88.00 [B]	**0.0323**
Virulence factors: toxins												
sea	5	4.07	5	18.52	**0.0062**	6	13.95	3	5.26	1	2.00	0.0610
seb	1	0.81	0	0.00	0.6624	0	0.00	1	1.75	0	0.00	0.4452
sec	0	0.00	11	40.74	**0.0000**	3	6.98 [AB]	0	0.00 [A]	8	16.00 [B]	**0.0061**
sed	14	11.38	2	7.41	0.2574	4	9.30	7	12.28	5	10.00	0.8789
see	0	0.00	0	0.00		0	0.00	0	0.00	0	0.00	
seg	23	18.70	15	55.56	**0.0034**	9	20.93	14	24.56	15	30.00	0.6013
seh	27	21.95	0	0.00	**0.0240**	2	4.65 [A]	12	21.05 [B]	13	26.00 [B]	**0.0206**
sei	10	8.13	10	37.04	**0.0076**	5	11.63	6	10.53	9	18.00	0.4923
tsst	0	0.00	8	29.63	**0.0000**	3	6.98	0	0.00	5	10.00	0.0611
hla	118	95.93	14	51.85	**0.0000**	39	90.70 [AB]	54	94.74 [A]	39	78.00 [B]	**0.0233**
hlb	115	93.50	13	48.15	**0.0000**	39	90.70 [A]	53	92.98 [A]	36	72.00 [B]	**0.0042**
Virulence factors to evade innate immune defenses												
cap5	100	81.30	6	22.22	**0.0000**	30	69.77 [A]	50	87.72 [B]	26	52.00 [C]	**0.0002**
cap8	8	6.50	2	7.41	0.7048	1	2.33	5	8.77	4	8.00	0.4014

[a] Absolute frequency, [b] relative frequency, [A, B, C] relative frequency followed by the same capital letters on the lines do not differ significantly; statistical significance p<0.05.

Table 4. Absolute and relative frequency of virulence factors in relation to capsular genotyping

	Capsular genotyping						
	cap - (n=34)		*cap5* (n=106)		*cap8* (n=10)		p
	AF[a]	RF[b]	AF	RF	AF	RF	
Virulence factors involved in adhesion to host cells							
fnbA	31	91.18	100	94.34	10	100.00	0.5706
fnbB	21	61.76 [A*]	94	88.68 [B]	7	70.00 [AB]	**0.0012**
clfA	16	47.06 [A]	89	83.96 [B]	9	90.00 [B]	**0.0000**
clfB	24	70.59	81	76.42	10	100.00	0.1556
Virulence factors: toxins							
SEs	19	55.88 [A]	49	46.23 [A]	10	100.00 [B]	**0.0005**
sea	6	17.65 [A]	2	1.89 [B]	2	20.00 [A]	**0.0011**
seb	0	0.00 [A]	0	0.00 [A]	1	10.00 [B]	**0.0007**
sec	6	17.65 [AB]	3	2.83 [A]	2	20.00 [B]	**0.0040**
sed	3	8.82	12	11.32	1	10.00	0.9185
see	0	0.00	0	0.00	0	0.00	
seg	10	29.41	24	22.64	4	40.00	0.4032
seh	0	0.00 [A]	21	19.81 [A]	6	60.00 [B]	**0.0000**
sei	7	20.59	11	10.38	2	20.00	0.2586
tsst	5	14.71 [A]	2	1.89 [B]	1	10.00 [AB]	**0.0115**
hla	20	58.82 [A]	102	96.23 [B]	10	100.00 [B]	**0.0000**
Hlb	19	55.88 [A]	100	94.34 [B]	9	90.00 [B]	**0.0000**

[a] Absolute frequency, [b] relative frequency, [A, B] relative frequency followed by the same capital letters on the lines do not differ significantly; statistical significance p<0.05.

milk. Considering the high frequency of enterotoxin genes found in the present study and the fact that in Brazil the production of artisanal cheese with unpasteurized milk is a common practice (Shinohara et al. 2015), *S. aureus* infection can pose a threat to public health in the country.

SEs genes have different genetic structures, most of which are mobile genetic elements. These genes can be located in chromosomes, plasmids, pathogenicity islands and phages (Guimarães et al. 2013). The enterotoxins encoded by pathogenicity islands support the hypothesis that they

could play an important role in the evolution of *S. aureus* as a pathogen (Omoe et al. 2003), but their role in mastitis pathogenesis is still to be elucidated (Piccinini et al. 2010).

Staphylococcal enterotoxins and TSST-1 may act as super antigens for cells of the bovine immune system (Farahmand-Azar et al. 2016). These super antigenic toxins play an important role in modulating the host immune response and may therefore contribute to maintain a suitable environment for colonization (Omoe et al. 2003).

Yadav et al. (2015) reported high frequencies of *hla* (93.75%) and *hlb* genes (81.25%) in bovines isolates. The pathogenicity of *S. aureus* is related to the production of a wide variety of exoproteins including alpha and beta hemolysins that contribute to its ability to cause diseases in different mammalian species (Silva et al. 2005).

It is known that IMI caused by *S. aureus* have different patterns in different herds, which vary according to the bacterial strain, geographic location, as well as host- and tissue-related characteristics (Gilot & Van Leeuwen 2004, Van Leeuwen et al. 2005, Zecconi et al. 2005).

Capsular polysaccharides are important virulence factors of *S. aureus* because they confer resistance to phagocytosis (Sutra et al. 1990) and prolong the pathogen persistence in the host blood stream (O'Riordan & Lee 2004). However, Tuchscherr et al. (2005) hypothesized that the lack of capsule expression might permit intracellular persistence of *S. aureus* and promote subclinical mammary gland infection.

The distribution of the different *cap* genotypes of *S. aureus* around the world may differ (Ote et al. 2011, Gogoi-Tiwari et al. 2015). The CP serotype 5 (CP5) or 8 (CP8) are the target of conjugate vaccines. Therefore, it is important to know their prevalence in our geographic regions.

A characterization of 87 *S. aureus* strains from individual animals of 23 farms located in six different municipal districts of two geographical regions of the state of São Paulo found *cap5* and *cap8* in 11 (12.64%) and 76 (87.36%) strains, respectively (Cabral et al. 2004). Similar results were found in a molecular characterization of *S. aureus* strains isolated from small and large ruminants in 12 regions of France, Brazil, USA, and Belgium, which showed that the *cap8* gene was predominant and accounted for 65.4% of *S. aureus* strains, while *cap5* and the non-typeable CP types accounted for 30.7% and 3.9%, respectively. When considering the host species, *cap8* was predominant in small ruminants, with an overall prevalence of 83.1% in ovine-caprine isolates. In contrast, *cap5* was slightly predominant in bovine isolates (56.3%) (Alves et al. 2009).

In our study, 34 (22.67%) strains did not carry neither *cap5* nor *cap8* genes. *S. aureus* strains that do not express capsule induce chronic mastitis in mice, suggesting that the absence of capsule synthesis may help the bacteria to persist in the mammary glands (Tuchscherr et al. 2005).

The existence of two groups of *S. aureus* strains isolated from bovine mastitis based on capsular typing, intracellular survival and *agr*-typing was confirmed by Bardiau et al. (2016). They hypothesized that the first group "*cap5-agrI*" may correspond to strains adapted to the intracellular niche leading to chronic infection and that the second group "*cap8-agrII*" may correspond to strains better adapted to the extracellular niche leading to acute infection. The existence of these two groups is highly important as they may represent two clusters that are adapted differently to the host and/or the surrounding environment (Bardiau et al. 2016).

CONCLUSIONS

According to the results obtained in the present study, *Staphylococcus aureus* strains isolated from goats are more toxic than those isolated from bovines.

Our data also show that the *cap5* genotype is predominant in both bovines' and goats' isolates.

The *cap8* genotype is likely more virulent due to the number of virulence genes per strain.

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