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Antibiotic resistance and molecular characterization of *Staphylococcus* species from mastitic milk

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Species within the *Staphylococcus* genus are important mastitis pathogens. Studies to describe virulence and antibiotic resistance as well as rapid techniques that permit analyses strains are needed. The aims were to identify and characterize *Staphylococcus* spp. isolated from mastitic milk, and to optimize multiplex polymerase chain reactions (PCR). Staphylococci previously isolated from milk of dairy cows with subclinical mastitis were analyzed. PCR was completed to amplify *nuc, sodA, spa, agr* locus, virulence factors, and antibiotic resistance genes. DNA sequencing of *sod*A and *spa* genes was performed and antibiograms were carried out on all isolates. In a group of 49 staphylococci, *S. aureus* was the most prevalent, followed by *S. hyicus*, *S. xylosus*, *S. chromogenes*. Following optimization of multiplex PCR, virulence factor genes were identified in the majority of isolates. The enterotoxin genes, *seh* and *sel*x were highlighted. All hemolysin genes were detected in 28.6% of isolates. Antibiotic resistance was evaluated and the majority of isolates (69.4%) were resistant to penicillin. Among the genes encoding antibiotic resistance, *mec*A was identified, while two methicillin-resistant *S. aureus* were typed as *spa* type 605, *agr* type II, and one identified as SCC*mec* type IVa. The types t605 and *agr* II were detected in the majority of *S. aureus* assessed. The findings emphasized the importance of preventing *Staphylococcus* infection in dairy cows. Effective dairy herd management and information on milk quality are essential to prevent mastitis pathogens.

Key-words: Antibiotic, staphylococci, toxins, virulence, genes.

INTRODUCTION

Bovine mastitis affects the dairy industry worldwide, and is associated with reduced milk quality and production (Silva et al., 2013). Coagulase-positive staphylococci (CPS) are widely studied as a common cause of clinical

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and subclinical mastitis (Ote et al., 2011; Rajic-Savic et al., 2015), and the most important causative agent in this bacterial group is *Staphylococcus aureus* (Ote et al., 2011). In addition, the relevance of coagulase-negative staphylococci (CNS) as a cause of mastitis in dairy cows has also been shown (Silva et al., 2014). CNS research has predominantly focused on humans, and the enterotoxigenic potential of CNS has not been extensively explored, although it has been suggested that CNS from bovine intrammamary infection (IMI) could be a potential source of staphylococcal superantigens (SAgs) (Park et al., 2011).

SAgs, e.g. staphylococcal enterotoxin (SE) and toxic shock syndrome toxin-1 (TSST-1), were first identified in *S. aureus* (Park et al., 2011), and have been well characterized. The emetic activity of SEs has been demonstrated (Hu and Nakane, 2014); thus, there is a potential to cause foodborne disease (Jorgensen et al., 2005). *Staphylococcus aureus* may carry genes for production of other toxins such as Panton-Valentine leukocidin, toxic shock syndrome toxin and exfoliative toxins (Jarraud et al., 2002).

The importance of virulence factors in *Staphylococcus* genus and the highly clonal structure within the *S. aureus* population have been highlighted in medicine, and could potentially help in treatments (Ote et al., 2011). However, antibiotic resistance is a concern since studies have demonstrated the emergence of resistant isolates from bovine mastitis (Moon et al., 2007; Silva et al., 2014). Thus, the aims of the present study were to identify, and characterize *Staphylococcus* spp. isolated from mastitic milk, and to optimize several multiplex polymerase chain reaction (PCR) in order to simultaneously identify the presence of different virulence factor genes.

MATERIALS AND METHODS

Origin and collection of isolates

The collection of bacterial isolates belonging to Hygiene and Dairy Laboratory, University of São Paulo, was used. From this collection, isolates from mastitic milk previously identified as *Staphylococcus* spp. were selected. Forty-nine isolates were selected from three different dairy farms located in São Paulo State, region of Piracicaba city, Brazil.

The isolates were obtained in a previous study performed by Hygiene and Dairy Laboratory's group, in which dairy cows were diagnosed with subclinical mastitis after screening using California Mastitis Test. Mastitic milk samples collected from September to October of 2013 were used. For the bacterial culturing, standard microbiological methods included colony morphology on Baird Parker Agar (BPA, Difco BD®, Nova Jersey, EUA) with egg yolk tellurite supplement (Laborclin®, Pinhais, Brazil), Gram staining, catalase, and coagulase test were completed to identify staphylococci, and all isolates were stored at -20°C.

DNA extraction, polymerase chain reaction and molecular typing

Each isolate was inoculated into Brain Heart Infusion (BHI, Oxoid™, Hampshire, UK) broth and incubated at 37°C for 24 h. Aliquots of each culture were centrifuged and the supernatant was discarded. The pellet was used to extract DNA using "AxyPrep™ Blood Genomic DNA Miniprep kit" (Axygen Scientific Inc., Union City, USA), according to manufacturer's instructions. Agarose gel electrophoresis was completed to verify the extraction, and the genomic DNA was stored at -20°C.

Coagulase-positive and coagulase-negative staphylococci previously identified by coagulase test were confirmed by detecting the *coa* gene through PCR. The PCR amplification of *coa* gene described by Aarestrup et al. (1995) was modified by using 0.75 mM of $MgCl_2$ in each reaction, and the PCR cycles used were as follows: 95°C for 5 min; 30 cycles at 95°C for 30 s, 55°C for 2 min, and 72°C for 4 min; and finally at 72°C for 10 min. When confirmed as coagulase-positive, multiplex PCR was performed to identify *S. aureus*, *S. intermedius* and *S. hyicus* according to Sasaki et al. (2010). Other strains were identified by amplifying the *sod*A gene, and through DNA sequencing using Sanger method (Silva et al., 2014).

The SEs (SEA-SEE, SEG-SEIJ, SEIK-SEIQ, SER-SET, SEIU, SEIV and SEIX), hemolysins (alpha, beta, delta, gamma component A, B and C and gamma-variant hemolysin), Panton-Valentine leukocidin (PVL), exfoliative toxins (ETA, ETB and ETD) and toxic shock syndrome toxin (TSST-1) genes were assessed by PCR. Primers used in this study are shown in Table 1.

Single PCR was initially performed for genes and positive (extracted DNA from strains belonging to Hygiene and Dairy Laboratory collection, University of São Paulo) and negative controls were incorporate into each run. Next, primers were combined in the same reaction when possible depending on amplification characteristics e.g. annealing temperature, number of PCR cycles and concentration of $MgCl_2$. The multiplex reactions were as follows: 1X PCR Buffer, 1U GoTaq® Hot Start Polymerase (Promega Corporation, Madison, USA), $MgCl_2$ (Promega Corporation, Madison, USA) concentration was variable, 10 pmol of each primer (synthesis by Sigma-Aldrich®, São Paulo, Brazil), 200 µM deoxynucleotides (Promega Corporation, Madison, USA), template DNA (approximately 40 ng) and ultrapure water to bring the final reaction volume to 25 µL. Genes that were not incorporate into multiplex PCR, were amplified by uniplex PCR using 2.5 mM of $MgCl_2$ and thermally cycled at 94°C for 5 min, 30 cycles at 94°C for 2 min, 48°C for 1 min, and 72°C for 1 min, and then once at 72 °C for 10 min.

agr type was developed according to Shopsin et al. (2003) and the amplification of *spa* region was carried out following the website http://www.ridom.com/, the repeats were identified for *spa* types detection after sequencing by Sanger method.

Antibiotic resistance detection

Antibiotic resistance of each isolate was tested using the agar diffusion method following the Clinical and Laboratory Standards Institute guidelines (Clinical Laboratory Standards Institute, 2015). The antibiotics tested included penicillin, cefoxitin, oxacilin, erythromycin, clindamycin, chloramphenicol, ciprofloxacin, vancomycin, tobramycin, tetracycline and gentamicin. The *tet*K, *tet*L, *tet*M (Gómez-Sanz et al., 2010), *ant(4')*-Ia (van de Klundert et al., 1993), *erm*A, *erm*B, *erm*C (Gómez-Sanz et al., 2010), *mec*A (Moon et al., 2007) and *mec*C (Cuny et al., 2011) genes were detected by PCR, and Staphylococcal Cassette Chromosome *mec* (SCC*mec*) types I to V in methicillin-resistant *S. aureus* were identified as described by Kondo et al. (2007).

RESULTS

Of the total isolates, 46 (93.9%) were confirmed as

Table 1. Primers sequences used to amplify virulence factors.

Gene	Oligonucleotide sequence (5´→3´)	bp	Reference
sea	TTGGAAACGGTTAAAACGAA GAACCTTCCCATCAAAAACA	120	Johnson et al. (1991)
seb	TCGCATCAAACTGACAAACG GCAGGTACTCTATAAGTGCC	478	Johnson et al. (1991)
sec	GACATAAAAGCTAGGAATTT AAATCGGATTAACATTATCC	257	Johnson et al. (1991)
sed	CTAGTTTGGTAATATCTCCT TAATGCTATATCTTATAGGG	317	Johnson et al. (1991)
see	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209	Mehrotra et al. (2000)
seg	AAGTAGACATTTTTGGCGTTCC AGAACCATCAAACTCGTATAGC	287	Omoe et al. (2002)
seh	GTCTATATGGAGGTACAACACT GACCTTTACTTATTTCGCTGTC	213	Omoe et al. (2002)
sei	GGTGATATTGGTGTAGGTAAC ATCCATATTCTTTGCCTTTACCAG	454	Omoe et al. (2002)
selj	CATCAGAACTGTTGTTCCGCTAG CTGAATTTTACCATCAAAGGTAC	142	Nashev et al. (2004)
selk	TAGGTGTCTCTAATAATGCCA TAGATATTCGTTAGTAGCTG	293	Omoe et al. (2005)
sell	CACCAGAATCACACCGCTTA CTGTTTGATGCTTGCCATTG	240	Cremonesi et al. (2005)
selm	ATCATATCGCAACCGCTGAT TTCAGTTTCGACAGTTTTGTTGTC	626	Ote et al. (2011)
seln	ATGAGATTGTTCTACATAGCTGCAAT AACTCTGCTCCCACTGAAC	680	Ote et al. (2011)
selo	AAATGATTCTTTATGCTCCG AAAGCACATTGTCATGGTGA	300	Ote et al. (2011)
selp	TGATTTATTAGTAGACCTTGG ATAACCAACCGAATCACCAG	396	Omoe et al. (2005)
selq	AATCTCTGGGTCAATGGTAAGC TTGTATTCGTTTTGTAGGTATTTTCG	122	Omoe et al. (2005)
ser	GGATAAAGCGGTAATAGCAG GTATTCCAAACACATCTAAC	166	Omoe et al. (2005)
ses	CCCCGGATCCGATGAATCTAGACCTAAAATAG CCCCGTCGACTTATTGGGAATAAAC	794	Ono et al. (2008)
set	CCCCGGATCCGATTCTCGTGAAGGTTTAAAAG CCCCGTCGACCTATTTTTCCATATATATATC	671	Ono et al. (2008)
selu	ATGGAGTTGTTGGAATGAAGT TTTTTGGTTAAATGAACTTCTACA	796	Fischer et al. (2009)
selv	GCAGGATCCGATGTCGGAGTTTTGAATCTTAGG TAACTGCAGTTAGTTACTATCTACATATGATATTTCGACATC	720	Thomas et al. (2009)
selx	AGCAGACGCGTCAACACAAA ACTTGTTCAATGTCATTAACACTTTTCAC	612	Wilson et al. (2011)
hla	CTGATTACTATCCAAGAAATTCGATTG CTTTCCAGCCTACTTTTTTATCAGT	209	Jarraud et al. (2002)
hlb	GTGCACTTACTGACAATAGTGC GTTGATGAGTAGCTACCTTCAGT	309	Jarraud et al. (2002)

Table 1. Contd.

hld	AAGAATTTTTATCTTAATTAAGGAAGGAGTG TTAGTGAATTTGTTCACTGTGTCGA	111	Jarraud et al. (2002)
hlg	GTCAYAGAGTCCATAATGCATTTAA CACCAAATGTATAGCCTAAAGTG	535	Jarraud et al. (2002)
hlg-v	GACATAGAGTCCATAATGCATTYGT ATAGTCATTAGGATTAGGTTTCACAAAG	390	Jarraud et al. (2002)
eta	ACTGTAGGAGCTAGTGCATTTGT TGGATACTTTTGTCTATCTTTTTCATCAAC	190	Jarraud et al. (2002)
etb	CAGATAAAGAGCTTTATACACACATTAC AGTGAACTTATCTTTCTATTGAAAAACACTC	612	Jarraud et al. (2002)
etd	AACTATCATGTATCAAGG CAGAATTTCCCGACTCAG	376	Yamaguchi et al. (2002)
tst	TTCACTATTTGTAAAAGTGTCAGACCCACT TACTAATGAATTTTTTTATCGTAAGCCCTT	180	Jarraud et al. (2002)
pvl	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAAGTGTATTGGATAGCAAAAGC	443	Lina et al. (1999)

coagulase-positive through amplification of *coa* gene. The species observed were *S. aureus* (42 strains, 85.7% of isolates), *S. hyicus* (4, 8.2%), *S. xylosus* (2, 4.1%) and *S. chromogenes* (1, 2.0%) (Table 2).

In multiplex PCR optimization, a total of 11 multiplex PCR (Table 3) to detect virulence genes (*sea, seb, sec, sed, see, seg, seh, sei, selj, selk, sell, selm, seln, selo, selp, selq, ser, selu, pvl, tst, hla, hlb, hld, hlg* and *hlg-v*) were performed. Multiplex PCR for 25 genes were evaluated across 11 reactions, which permitted optimization of the analyses and reducing costs.

Forty-two isolates (85.7% of isolates) were positive for one or more enterotoxin gene. The enterotoxin genes observed were *seh* (59.2%) and *selx* (57.1%) followed by *seg* (51.0%), *ser* (46.9%), *selu* (38.8%), *sell* (24.5%), *selo* (18.4%), *seln* and *selp* (6.1% each one), *seb*, *selj*, *selk* and *selm*, (4.1% each one) and *selq* (2,0%). *sea*, *sec*, *sed*, *see*, *sei*, *ses*, *set* and *selv* genes were not detected. In this study, 30 profiles were observed across 49 isolates. Among the profiles identified in this study, *seg+seh+ser+seu+selx,* was the most abundant (10.2% of strains), followed by *seg+seh*, *seg+seh+sem+seo+ser+seu+selx*,*seg+seh+seo+ser+seu +selx*, *seh*, *seh+seo+ser+selx*, *seh+ser+selx* and *sel* (4.1% each profile). In seven isolates, enterotoxin genes were not identified. All hemolysin genes were detected, that is *hla* (38.8%), *hlb* (55.1%), *hld* (32.7%), *hlg* (42.9%) and *hlg-v* (53.1%). The presence of all hemolysins was the most frequent profile (28.6%), and 19 of the strains (38.7%) did not carry hemolysin genes. Genes encoding exfoliative toxins, *pvl* and *tst* were not identified.

Regarding antibiotic resistance, isolates were resistant to penicillin (69.4% of isolates), cefoxitin (8.2%), erythromycin, chloramphenicol, tetracycline (4.1% to each antibiotic), tobramycin, clindamycin, oxacilin (2.0% to each antibiotic). One isolate demonstrated intermediate resistance to gentamicin, erythromycin, clindamycin, while all strains were sensitive to vancomycin and ciprofloxacin. Across all strains, three isolates were multi-drug resistant. Herein, *erm*A, *erm*C, *tet*K and *tet*M genes were detected (Table 2). Of the isolates resistant to cefoxitin (4 isolates, *spa* type t605 isolated from farm A) only 2 isolates were positive for *mec*A, one was identified as SCC*mec* type IVa and another was non-typeable. In addition, the methicillin-resistant *S. aureus* (MRSA) strains belong to *spa* typing t605 and *agr* type II, and the absence of a novel *mec*A homologue was observed. In *spa* typing detected across 42 *S. aureus* (Table 2), the type most frequent was t605 (83.3%), also it was present on all farms, followed by t267 (9.5%), t521 (4.8%) and t9129 (2.4%). The *agr* types detected were I (11.9%) and II (88.1%).

DISCUSSION

In this study, in a limited group of staphylococci were identified and *S. aureus* was the dominant species; however, CNS was also present. In addition, several virulence factor genes were identified in the majority of isolates by multiplex PCR as well as antibiotic resistance to one or more antibiotics tested by diffusion method. Regarding SEs, the importance of *seh,* and *selx* genes corresponding to SEH and SEIX is emphasized due their high incidence, while low frequency or absence of classical SEs were observed. The *mec*A positive isolates detected were *spa* type 605, and *agr* type II, which were also identified in the majority isolates.

Herein, high frequency of *S. aureus* was detected, this species has been identified as the primary pathogen associated with mastitis (Ote et al., 2011; Silva et al., 2014), and previous studies have identified a high

Table 2. Species, *spa* typing, virulence factor genes and antibiotic resistance genes detected by farm.

Farm	Species (n)	*spa* type (n)	*agr* type (n)	Enterotoxin gene (n)	Hemolysin gene (n)	Antibiotic resistance gene (n)
A	*S. aureus* (28)	t605 (28)	II (28)	*seb* (2) *seg* (18) *seh* (18) *selk* (2) *sell* (5) *selm* (2) *seln* (6) *selq* (1) *ser* (13) *selu* (13) *selx* (17)	*hla* (10) *hlb* (17) *hld* (10) *hlg* (15) *hlg-v* (15)	*mec*A (2)
	S. hyicus (2)					*erm*A (1) *erm*C (1)
	S. xylosus (2)			*sell* (1) *selp* (1)		*tet*K (2) *tet*M (1)
B	*S. aureus* (10)	t605 (5) t267(4) t9129 (1)	I (5) II (5)	*seg* (7) *seh* (7) *selj* (1) *sell* (2) *seln* (2) *selo* (1) *ser* (8) *selu* (6) *selx* (9)	*hla* (7) *hlb* (8) *hld* (4) *hlg* (4) *hlg-v* (9)	*erm*C (1) *tet*M (1)
	S. hyicus (2)			*seh* (1) *selj* (1) *sell* (2) *selp*(1)		
C	*S. aureus* (4)	t605 (2) t521 (2)	II (4)	*seh* (3) *sell* (2) *seln* (1) *selo* (2) *ser* (2) *selx* (2)	*hla* (2) *hlb* (2) *hld* (2) *hlg* (2) *hlg-v* (2)	
	S. chromogenes (1)			*selp* (1)		

frequency of this pathogen in Brazil (Silva et al., 2013; Lange et al., 2015). Giannechini et al. (2002) also detected high frequency of *S. aureus*, and low frequency of *S. hyicus* coagulase-positive among isolates from sub-clinical mastitis cases. The *coa* gene amplification also showed that the minority of the isolates belonged to CNS; which are capable of causing opportunistic mastitis (Moon et al., 2007). Lange et al. (2015) reported *S. chromogenes* at a frequency of 38.5%, which highlights the importance of coagulase-negative strains; however, in this study, the detection of CNS was low. *S. xylosus*, coagulase-negative, were also detected, within this species there are strains that can potentially be hazardous, and they are related to animal opportunistic infections (Dordet-Frisoni et al., 2007).

The low frequency of classical SEs is in agreement with a previous study in which *S. aureus* associated with bovine mastitis were analyzed (Ote et al., 2011). In this study, classical SEs were not identified in *S. chromogenes*, *S. xylosus* and *S. hyicus*; however, classical SEs have been reported in these species (Park et al., 2011). Among the other SEs, the frequency of *seh* was highly detected. SEH has emetic activity and staphylococcal food poisoning associated with *S. aureus*

Table 3. Conditions of the multiplex PCR optimized in this study.

Set	Genes	Concentrated $MgCl_2$ (mM)	Condition of PCR[a]
A	*sea* + *sec*	2.0	94°C - 2 min 54°C - 1 min 30 cycles 72°C - 1 min
B	*seb* + *selk*	2.0	94°C - 2 min 55°C - 1 min 30 cycles 72°C - 1 min
C	*sed* + *seh*	2.0	94°C - 2 min 55°C - 1 min 30 cycles 72°C - 1 min
D	*see* + *selq*	2.0	94°C - 2 min 54°C - 1 min 30 cycles 72°C - 1 min
E	*seg* + *selu*	2.0	94°C - 2 min 54°C - 1 min 30 cycles 72°C - 1 min
F	*sei* + *selm* + *selo*	1.5	94°C - 2 min 54°C - 1 min 30 cycles 72°C - 1 min
G	*selj* + *sell*	3.0	94°C - 2 min 64°C - 2 min 35 cycles 72°C - 1 min
H	*seln* + *selp* + *ser*	3.0	94 °C - 30 s 58 °C - 30 s 35 cycles 72 °C - 1 min
I	*pvl* + *tst*	3.0	94°C - 30 s 55°C - 30 s 30 cycles 72°C - 1 min
J	*hla* + *hlb* + *hld*	2.0	94°C - 30 s 63°C - 30 s 30 cycles 72°C - 1 min
K	*hlg* + *hlg-v*	2.0	94°C - 30 min 48°C - 30 s 30 cycles 72°C - 1 min

[a]94°C/5 min for initial denaturation and 72°C/7 min for extension final.

carrying the *seh* gene has been reported (Jorgensen et al., 2005; Argudín et al., 2010). Considering the potential of SEH to cause foodborne disease, strains from our collection that carry the *seh* gene should be tested forenterotoxin protein expression in further investigations.

The staphylococcal enterotoxin-like toxin X (SEIX) also demonstrated a high frequency. The *selx* gene is encoded in the core genome of *S. aureus*, which explains the frequency of *selx*. However, its emetic activity has not yet been tested (Hu and Nakane, 2014). In addition, it is

suggested in this case to further study allelic diversification. Other genes (*seb*, *seg*, *sej* and *ser*) that encode for SEs with emetic activity were detected; it shows that milk quality control needs to be strict in order to avoid the pathogen or significant count of it, and consequently the possibility of milk contamination with SEs. *sek*, *sel*, *sem*, *sen*, *seo*, *sep, seq* and *seu* were detected; however, these have not exhibited emetic activity in primate models or emetic activity has not been tested for some genes. Several SEs profiles were identified, this finding demonstrates the high distribution of SEs genes in the species studied; for example, 32 superantigenic toxin genotypes were observed across 166 isolates (69 food poisoning isolates, and 97 healthy human nasal swab isolates) in the study performed by Omoe et al. (2005). All hemolysin genes were identified, *hla*, *hlb*, *hld* and *hlg*AC were also detected by Ote et al. (2011), and they identified frequencies between 78.6 and 100% in strains. In this study, hemolysin gene frequencies were between 32.7 and 55.1%. The most prevalent was *hlb*, which is in agreement with other study that assessed isolates from raw milk products (Morandi et al., 2009). Genes encoding exfoliative toxins, *pvl* and *tst* were not identified; previously Ote et al. (2011) identified *eta* and *tst* genes in isolates associated with bovine mastitis.

Regarding antibiotic resistance, penicillin resistance is commonly detected in *Staphylococcus* spp. (Moon et al., 2007; Gómez-Sanz et al., 2010), and this was demonstrated in the present study. Silva et al. (2013) did not detect resistance to erythromycin in their isolates, although they detected one strain of *S. aureus* with resistance to chloramphenicol. Erythromycin and tetracycline resistance genes were observed (Table 2); these genes have been detected in *Staphylococcus* sp. (Silva et al., 2014; Gómez-Sanz et al., 2010). It is important to highlight that all isolates were tested for the presence of *mec*A and *mec*C genes as well as other resistance genes. These results on *mec*A, and SCC*mec* type are in line with Silva et al. (2014), where they assessed methicillin-resistant coagulase-negative staphylococci in milk from cows with mastitis in Brazil. Herein, SCCmec type I to V was investigated due to the availability of positive controls, further studies to assess types I to XI are necessary due to their importance in methicillin resistance. Meanwhile, the absence of a novel *mec*A homologue could be expected because it is of rare occurrence (Cuny et al., 2011). The absence of *mec* genes in cefoxitin and oxacillin resistant strains can indicate the potential presence of modified *S. aureus* (MODSA); MODSA possesses a modification of its penicillin-binding proteins (PBPs), which is different of classical mechanism of MRSA (Bhutia et al., 2012).

Four *spa* types were detected, and on the farm A only one *spa* type (t605) was observed. This suggests that the *spa* type t605 is common and it can be endemic in the region causing subclinical bovine mastitis. The t605 type was initially detected in Austria, France, Germany, Netherlands, Norway, Spain, Sweden and United Kingdom, and represents 0.1% of relative global frequency of *spa* type occurrences in accordance with the website, http://www.ridom.com (http://spaserver.ridom.de - data collected on June 2015). Other studies in Brazil also detected this *spa* type in strains isolated from milk from bovine and others animals (Aires-de-Sousa et al., 2007; Silva et al., 2013). On the other hand, the *spa* type t127 were the most detected by Silva et al. (2013). The *agr* types detected were I and II, which were also detected in a previous study with isolates from bovine mastitis (Silva et al., 2013).

Conclusion

The majority of isolates were identified as *S. aureus*. Other isolates also identified were *S. hyicus*, *S. xylosus* and *S. chromogenes*. The majority virulence factor genes identified using multiplex PCR, in total eleven different multiplex reactions were successfully optimized and applied in this study. The most isolates carried virulence factor genes, *seh*, and *sel*x were the most detected among SEs. Hemolysins genes were widely identified, presenting several profiles as well as SEs. Antibiotic resistance was widely detected for penicillin; in addition, MRSA strains were observed which presents a concern to public health. The most prevalent *spa* type was t605, which suggests that this could be an endemic *spa* type in the herds sampled. In summary, data regarding molecular variability, and antibiotic resistance for a small group of staphylococci isolated from mastitic milk was shown, which confirms that more studies should be completed to identify and understand strains/clones in specifics regions, and thus to help prevent *Staphylococcus* infection in dairy cows.

Conflict of interest

The authors declare that there is no conflict of interest.

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