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Molecular characterization and antibiotic resistance of *Staphylococcus* spp. isolated from cheese processing plants

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ABSTRACT

The aim of this research paper was to characterize coagulase-positive and coagulase-negative staphylococci from raw milk, Minas cheese, and production lines of Minas cheese processing. One hundred isolates from 3 different cheese producers were characterized using molecular approaches, such as PCR, molecular typing, and DNA sequencing. *Staphylococcus aureus* (88% of the isolates) was the most abundant followed by *Staphylococcus epidermidis*, *Staphylococcus hyicus*, and *Staphylococcus warneri*. Among the 22 enterotoxin genes tested, the most frequent was *seh* (62% of the isolates), followed by *selx* and *ser*. Hemolysin genes were widely distributed across isolates, and Panton-Valentine leukocidin and toxic shock syndrome toxin genes were also identified. Methicillin-resistant *S. aureus* were staphylococcal cassette chromosome *mec* III, IVa, IVd, and others nontypeable. In the phenotypic antibiotic resistance, multiresistant isolates were detected and resistance to penicillin was the most observed. Using *spa* typing, we identified several types and described a new one, t14969, isolated from cheese. These findings suggest that antibiotic resistance and potentially virulent strains from different sources can be found in the Brazilian dairy processing environment. Further research should be conducted with collaboration from regulatory agencies to develop programs of prevention of virulent and resistant strain dissemination in dairy products and the processing environment.

Key words: cheese, staphylococci, antibiotic resistance, virulence factor

INTRODUCTION

Staphylococcus spp. are known worldwide as a cause of human and animal infections, such as bacteremia, wound infections (Podkowik et al., 2013), and mastitis (Podkowik et al., 2013; Luini et al., 2015). The species *Staphylococcus aureus* is the main etiological agent of mastitis in dairy cows (Luini et al., 2015), a disease economically important to the dairy industry (Hayakawa et al., 2001; Shaheen et al., 2016). In addition, staphylococci from bovine milk, excluding *S. aureus*, also represent a heterogeneous group of microorganisms, namely CNS, that are commonly associated with bovine mastitis (Lange et al., 2015). In different studies, CNS has been highlighted in human (Jean-Baptiste et al., 2011) and animal infections (Lange et al., 2015), and also in food poisoning cases (Podkowik et al., 2013).

Food poisoning is widely related to *S. aureus* and is frequently detected in milk and dairy products (Carfora et al., 2015; Jamali et al., 2015; Xing et al., 2016). In Brazil, milk and dairy products are among the foods most involved in foodborne disease outbreaks and in these cases *S. aureus* is the third most identified pathogen according to the Ministry of Health (2016); these data corroborate the importance of studies with isolates in Brazil. Specifically in cheese production, André et al. (2008) and Arcuri et al. (2010) showed that the source of *S. aureus* contamination could be multifactorial, such as raw milk, processing environment, and handlers. In general, *S. aureus* is a major concern for the food processing industry due to its virulence factors (Xing et al., 2016) and ability to form biofilm (Langsrud, 2009). Strains can produce coagulase, hemolysins, exfoliative toxins, toxic shock syndrome toxin-1, protein A, staphylococcal enterotoxins, in addition to others (Hayakawa et al., 2001).

Moreover, the presence of strains resistant to antibiotics has been reported and its transmission via food is

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a concern for public health (Jamali et al., 2015). Resistance of *Staphylococcus aureus* isolated from raw milk and dairy products to tetracycline (**TET**), kanamycin, gentamicin (**GEN**), streptomycin, methicillin, and other antibiotics was reported by Jamali et al. (2015). Other studies have described the antibiotic resistance of *Staphylococcus* spp. isolated from milk (André et al., 2008; Rola et al., 2015). Additionally, *S. aureus* is a pathogen identified by the World Health Organization as an international concern due to its resistance to antibacterial drugs (WHO, 2014). Considering this, the aim of this study was to characterize coagulase-positive and negative staphylococci isolated from raw milk, Minas cheese (fresh cheese), and production lines of Minas cheese processing. Virulence factor genes, antibiotic resistance genes, and antibiogram testing 11 different antibiotics were all assessed for all isolates; molecular typing of *S. aureus* [*spa* and staphylococcal cassette chromosome *mec* (**SCC*mec***) types] was also performed.

MATERIALS AND METHODS

Origin of Isolates and Dairy Descriptions

One hundred isolates from the bacterial collection of Hygiene and Dairy Laboratory, University of São Paulo, Brazil, were used in this study. In a previous study performed by the Hygiene and Dairy Laboratory's research group (Silva, 2013), staphylococci were isolated using standard methods (Downes and Ito, 2001) from samples collected from 3 different processing plants of traditional Brazilian fresh cheese called Minas Frescal cheese or Minas cheese. All processing plants were located in São Paulo State, Brazil. The isolates used were storage at −20°C and their origins are shown in Table 1.

Dairy A produced approximately 600 kg (kg) of cheese per week and the pasteurization used was HTST. Dairy B processed 150 kg per week and slow pasteurization was used. Dairy C produced its own milk, the pasteurization was rapid (HTST), and 400 kg of cheese was produced per week.

Molecular Characterization

The strains were reactivated in Brain Heart Infusion broth (BHI, Oxoid, Hampshire, UK). The cell pellet was harvested by centrifugation at 12.000 rpm/2 min. The cell pellet was used and DNA was extracted from samples using a AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Scientific Inc., Union City, CA) according to the manufacturer's instructions. The genomic DNA was stored at −20°C until further analysis.

Classification of isolates as negative or positive coagulase was conducted using PCR of the *coa* gene according to Aarestrup et al. (1995) with modifications. When confirmed as coagulase positive, a multiplex PCR was performed to identify *Staphylococcus intermedius*, *S. aureus*, and *Staphylococcus hyicus* (Sasaki et al., 2010). For identification of other species, amplification of the *sod*A gene as described by Silva et al. (2014) was completed. The DNA was purified using Illustra, GFX PCR DNA, and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) and Sanger sequencing was performed. Posterior comparison of the FASTA sequence against sequences stored in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi; Benson et al., 2009; Sayers et al., 2009) was completed to identify the species.

Genes encoding enterotoxin, hemolysin, exfoliative toxin, Panton-Valentine leukocidin, and toxic shock syndrome toxin were identified using oligonucleotide sequences previously described, the sequences corresponding to the genes *sea*, *seb*, *sec*, *sed* (Johnson et al., 1991), *see* (Mehrotra et al., 2000), *seg*, *seh*, *sei* (Omoe et al., 2002), *selj* (Nashev et al., 2004), *selk* (Omoe et al., 2005), *sell* (Cremonesi et al., 2005), *selm*, *seln*, *selo*

Table 1. Origin and number of *Staphylococcus* isolated per cheese processing plant enrolled in this study

	No. of isolates			
Origin	Dairy A	Dairy B	Dairy C	Total
Raw milk	23	2	4	29
Pasteurized milk	0	0	1	1
Food handler	20	7	0	27
Table	0	1	2	3
Floor	0	1	0	1
Cheese before packing	6	2	0	8
Cheese after packing	15	8	1	24
Brine	2	0	0	2
Milk tank	1	0	2	3
Packer	1	0	0	1
Cheese mold	1	0	0	1