TITLE: MOLECULAR IDENTIFICATION OF *Staphylococcus* spp. ISOLATED IN DAIRY FARMS FROM STATE OF SÃO PAULO, BRAZIL

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ABSTRACT:
Milk is an excellent substrate for microorganisms growing and microbiological characteristics play a role on milk quality. Therefore, microbiology is an indicative of handling and hygiene adopted by dairy producers. The aim of this research was to identify, using molecular methods, *Staphylococcus* spp. isolated from bulk tank milk, milking cups, water and hands of workers in four farms located in northeast region of State of Sao Paulo. The isolated colonies were submitted to DNA extraction, using thermal shock. It were used specific primers that detect the genes Tuf and 16S of *Staphylococcus* spp. The PCR products were analyzed by agarose electrophoresis for confirming the amplification and size of expected fragments. Following, the positive samples were purified and sequenced. The sequences were analyzed by using the programs CodonCode Aligner, BioEdit and aligned at ClustalW. An initial search for similarities among sequences was performed by BLAST of NCBI. From these information, the program Mega was used for building phylogenetic trees for expressing the relationship among species. From 50 samples analyzed, 21 were positive for gene Tuf, and eight submitted to sequencing. Other 21 samples were positive for 16S, and nine were submitted to sequencing. From 12 samples sequenced for the two genes, seven were confirmed as *Staphylococcus*, two were identified as *Enterococcus* and three as *Macrococcus*. It were found coagulase negative *Staphylococcus*: *S. chromogenes, S. schleiferi, S. capitis* and *S. sciuri.* Coagulase negative *Staphylococcus* are part of microbiota of cows' skin and teats and can indicate infection of mammary gland. They can be cause of intramammary infections in 10 to 20% of infected quarters. *Enterococcus* can indicate lack of hygiene and fecal contamination. *Macrococcus*, that cannot cause disease in animals and humans, present 93.4 % to 95.3% of DNA similarity with *Staphylococcus.* The presence of coagulase negative *Staphylococcus* species, that is considered as an emergent microorganism and possibly causing intramammary infections, confirmed the necessity of training workers involved in the milk chain, adopting good practices of cleaning and hygiene in milking.

Keywords: Coagulase negative, milk, PCR

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