Title: PROTEOMIC ANALYSIS IN THE IDENTIFICATION OF *Staphylococcus* spp. ISOLATED FROM MILK LINE PRODUCTION

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Abstract:

Although *Staphylococcus aureus* is the major causative agent of bovine mastitis, other species of *Staphylococcus* spp. can also be associated with this disease. Correct diagnosis of the species is often hampered by variability of biochemical characteristics and time required to obtain results. Many genotypic methods have been developed to facilitate the identification of *S. aureus* and other species of this genus. Moreover, identification by proteomic technique Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) provides faster and more reliable results. In this study MALDI-TOF MS was used to characterize 181 isolates of *Staphylococcus* spp. obtained from 201 cow milk and 38 milk production chain samples from a property in the South Fluminense of Rio de Janeiro. Samples were collected three times in consecutive weeks during Winter (2014), Spring (2014) and Summer (2015) seasons, as part of their annual monitoring of bovine mastitis. Samples were phenotypically identified as *Staphylococcus* spp. and previously differentiated by coagulase production. Pure colonies were taken to Research Laboratory of Medical Microbiology, Institute of Microbiology Paulo Goes (UFRJ) for identification by MALDI-TOF Bruker Microflex LT, Bruker. A total of 60.2% (109/181) were identified as *S. aureus* and 36.5% (66/181) as different species of CNS: 25.7% (17/66) *S. haemolyticus*, 16.7% (11/66) *S. chromogenes*, 15.2% (10/66) *S. saprophyticus*, 10.6% (7/66) *S. sciuri*, 9.1% (6/66) *S. equorum*, 6.1% (4/66) *S. xylosus*, 6.1% (4/66) *S. epidermidis*, 4.5% (3/66) *S. hyicus* (coagulase-negative variant), 1.5% (1/66) *S. capitis*, 1.5% (1/66) *S. kloosii*, 1.5% (1/66) *S. nepalensis* and 1.5% (1/66) *S. warneri*. For 3.3% (6/181) of the phenotypically identified isolated as ECN, the identification was not possible. *S. aureus*, the prevalent species in this study, is the etiologic agent most commonly associated with clinical and subclinical infections in dairy cows. Its main reservoir appears to be infected mammary quarter and transmission often occurs during milking, by contamination of machinery, equipment and hands of milkers. For a long time species of CNS were considered saprophytes and rarely pathogenic. In recent years, however, the importance of the CNS species implicated in animal infections has increased.

Keywords: *Staphylococcus* spp., bovine mastitis, MALDI-TOF MS

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