

TITLE: VIRULENCE GENES AND AGR QUORUM SENSING SYSTEM IN *Staphylococcus aureus* ISOLATES FROM BOVINE MASTITIS

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ABSTRACT:

Clinical and subclinical mastitis are one of the main factors affecting dairy farms in Brazil. It causes inflammation in the mammary glands, which can be affected by etiological agents such as bacteria, fungi and viruses that are present mainly in the environment. *Staphylococcus aureus* is an important bacterial pathogen that causes significant losses in the dairy industry. It is one of the most frequent etiologic agents isolated from raw milk. In staphylococci, the expression of most virulence factors is controlled by the Agr (accessory gene regulator) quorum sensing system. The extracellular signal of Agr is a post-translationally modified peptide involved in expression of adhesins, exoenzymes, and toxins in high cell density. In this study, 70 coagulase-positive staphylococci isolates were analyzed. Of these, 52 were isolated from milk samples from cows with subclinical mastitis in the State of São Paulo, and 18 were isolated from milk samples in the State of Pernambuco (6 from clinical mastitis and 12 from subclinical mastitis). The staphylococcal isolates were confirmed to be *S. aureus* by PCR test using primers for the *nuc* gene. Pulsed-field gel electrophoresis (PFGE) was performed in order to determine the molecular epidemiology of these *S. aureus* isolates. The group II *agr* was associated to the presence of multiple genes encoding virulence factors, whereas the group III *agr* was associated to few virulence genes. The *cflA* gene was identified in all *S. aureus* isolates, suggesting that it has a role in colonizing the mammary glands. Although the gene *eta* is uncommon in *S. aureus* of animal origin, we found it in both *agr*-negative and group II *agr* bovine strains. The association of the *hla/hlb* (alpha-hemolysins) and *lukE-D* (leukotoxin) genes was found only in the group II *agr* *S. aureus* isolates. PFGE genotyping revealed the predominance of two *S. aureus* clones that carried *agr* II and multiple virulence genes in the milk samples from SP, while different clones were identified in both clinical and subclinical mastitis milk samples. The role of the *agr* locus has been considered essential in the pathogenesis of *S. aureus* in animal infection models. Determination of *agr* group and virulence factors contributes to the understanding of the ability of *S. aureus* to establish in mammary glands of animal hosts.

Keywords: mastitis, *Staphylococcus aureus*, Agr quorum sensing system, virulence.

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