

Title: A STUDY OF BIOFILM PRODUCTION AND BETA-LACTAMIC RESISTANCE IN *Staphylococcus aureus* BOVINE MASTITIS ISOLATES

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Abstract:

Staphylococcus spp. play an important role in the etiology of bovine mastitis. *S. aureus* is considered the most relevant species due to the production of virulence factors such as slime, which is required for biofilm formation. Biofilm production in *S. aureus* isolates from mastitis can also be associated with antimicrobial resistance. The present study aimed to detect the phenotypic expression of slime and the structural and regulatory genes involved in the production of this virulence factor. Also stages of biofilm synthesis along growth curve were evaluated by Scanning Electron Microscopy. Additionally pheno-genotypic resistance to beta-lactamic and its possible relation to biofilm production were evaluated. A total of 20 *S. aureus* isolates from bovine mastitic milk were characterized by pheno-genotypic and MALDI-TOF assays and further tested for *icaA*, *icaD*, *bap*, *agr RNAIII* and *agr I*, *agr II*, *agr III* and *agr IV* genes, associated to slime production and its regulation. The genes *mecA* and *blaZ*, related to beta-lactamic resistance, were also detected. The biofilm production was measured during bacterial growth curve through qualitative and quantitative assays. All 20 isolates of *S. aureus* were subjected to disk diffusion tests using cefoxitin (30 ug), oxacillin (10 ug) and amoxicillin + clavulanic acid (30 ug) disks. Also “edge zone” test evaluated the production of beta-lactamases. MIC and MBC tests were performed for the isolates N-354, N-365 and N-341. Scanning Electron Microscopy (SEM) was performed to evaluate biofilm production along the bacterial growth curve and to determine the most suitable time intervals for its analysis. All isolates were biofilm producers. Fourteen isolates (70%) presented *agr* type II. The *icaA* and *icaD* genes were detected in 17 (85%) and 19 (95%), respectively. Otherwise, just one isolate presented *bap* gene. The absence of this gene implicates *icaA* and *icaD* genes in the observed biofilm formation. All isolates presented resistance to penicillin and the presence of the gene *blaZ* in 70% indicates that beta-lactamase production is the possible mechanism underlying this resistance. Through SEM, it was possible to observe gradual changes in the complexity of the biofilm structure during the different stages of growth. Biofilm production reached its peak at the stationary phase. The high value of cefoxitin MBC detected in the study may be associated with biofilm protection.

Keywords: Biofilm, Agr types, antimicrobial resistance, mastitis

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