

**Title:** Ability of biofilm formation *in vitro* by *Staphylococcus aureus* isolated from cows with clinical mastitis.

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**Abstract:**

Biofilm is one of the main factors that contribute to the persistence and proliferation of the bacteria in an environment biotic or abiotic surfaces. The biofilm is a sessile microbial community, adhered to a substrate and among themselves, involved by a polysaccharide matrix. Considering mastitis cases, the biofilm protects the pathogen from the action of the immune system and antimicrobials, therefore so many of the cases of this disease become chronic, causing damage to dairy farmers. *Staphylococcus aureus* are known for their high biofilm formation capacity and the *icaADBC* operon is the main genetic factor involved in biofilm production, with the support of the *bap* gene that is responsible of the initial adhesion in the surface. The aim of this study was to evaluate the capacity of biofilm formation *in vitro* and the presence of the *icaA*, *icaD* and *bap* genes in *S. aureus* strains of clinical mastitis. A total of 103 *S. aureus* strains were identified due to the presence of *nuc* gene using Polymerase Chain Reaction (PCR). To analyze the presence of the genes (*icaA*, *icaD* and *bap*), specific primers were used in PCR reactions. Regarding *in vitro* biofilm production assay, the strains were incubated at 35°C for 24h, the cultures grown in BHI were diluted in 0.5 MacFarland scale, and 200µL were distributed in quadruplicate in 96-well polystyrene microplates and incubated at 35°C for 48h. After, the plate was washed in PBS (pH 7.4), the wells were stained with 1% crystal violet for 15 min, washed 3 times with the distilled water and read at 570 nm. The results showed that all *S. aureus* were able to produce biofilm *in vitro*. The *icaD* gene appeared in all strains, the *icaA* gene in 92% and the *bap* gene in 23%. The 24 strains presenting the *bap* gene were the most strong biofilm producers. This study show the ability of *S. aureus* to produce biofilm and the importance to clean the environment and utensils used in milking, because once the biofilm formed, its removal becomes more difficult allowing dissemination of the pathogen.