TITLE: BIOFILM-ENCODING GENES IN *Staphylococcus aureus* ISOLATED FROM COWS WITH SUBCLINICAL MASTITIS TREATED WITH HOMEOPATHIC MEDICINE

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ABSTRACT

Mastitis is one of the major diseases in the bovine dairy herds, as it affects milk quality and results in economic losses due to factors such as reduction in milk production and costs associated with the treatment of animals and discarded milk. Among the bacteria that cause mastitis, Staphylococcus aureus can express several virulence factors and also produce biofilms, which influence the severity of the mammary gland inflammation and contribute to the persistence of infections. In organic herds, mastitis is controlled by alternative means such as homeopathy. The aim of this study was to investigate genes related to biofilm expression in S. aureus isolated from cow-milk samples after homeopathic treatment for subclinical mastitis. Two homogenous groups of cows with subclinical mastitis, with and without homeopathic treatment, were studied for one year from the beginning of the treatment wherein medicine was added to the feed. Sugar was added to the feed of untreated cows. The formulation, preparation, and administration of the drug was guided by a specialist in homeopathic medicine. S. aureus were identified by detection of the SA442 gene after DNA extraction. The presence of genes coding for the production of biofilm-icaA, icaD, bap, bhp, and aap-was studied. Forty seven isolates of S. aureus were identified, of which 23 (48.9%) were obtained from the group of control animals and 24 (51.1%) from the group that received homeopathic treatment. The *icaA* gene was present in 22 (95.6%) isolates from the control group and 22 (91.7%) from the treatment group, while the icaD gene was detected in all isolates from the control group and in 23 (95.8%) isolates from the treatment group. In 41 (87.2%) isolates, the *icaA* and *icaD* genes were present simultaneously. The *bap* gene was identified in 4 (17.4%) isolates from the control group and five (20.8%) from the treatment group. Only one (2.1%) isolate was positive for the *aap* gene, in the treatment group. The *bhp* gene was not detected in any of the isolates. The high occurrence of *icaA* and *icaD* genes in S. aureus suggests that they may be contribute to the persistence of infection in the mammary gland of treated and untreated animals.

Keywords: animal health, bacterial persistence, subclinical mastitis, virulence factors.

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