

TITLE: TOXIN-ENCODING GENES OF *Staphylococcus* spp. ISOLATED FROM MILK OF COWS TREATED WITH HOMEOPATHY

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ABSTRACT

Staphylococci are the main microorganisms causing bovine mastitis and are capable of expressing virulence factors such as thermostable toxins. When the disease affects herds associated with organic milk production, alternative means are employed for the treatment of diseased animals. However, homeopathic treatment does not always seek to eliminate bacteria but promotes harmonic coexistence between the host and the causal agent of the disease. The aim of this study was to investigate genes related to the production of toxins by *Staphylococcus* spp. isolated from the milk of cows treated with homeopathy for subclinical mastitis. Two homogenous groups of cows, with and without treatment, were followed for one year. The homeopathic medicine was added to the feed. The choice of the formulation, along with the method of preparation and suitable form of administration, were based on expert advice of a technician specialized in homeopathic medicine. *S. aureus* was identified by the detection of the SA442 gene after extraction of the DNA of coagulase-negative *Staphylococcus* (SCN) by the use of conserved sequence primers adjacent to the 16S and 23S genes by the Internal Transcribed Spacer PCR (ITS-PCR) technique. The toxin-encoding genes investigated were *sea*, *seb*, *sec*, *sed*, and *tst*. Fifty-one staphylococci were identified in the milk of treated cows and thirty-seven in the milk of untreated cows. The most frequent species was *S. aureus*, isolated in 56.7% and 62.2% of the milk samples from treated and untreated animals, respectively. The SCN species without toxin-encoding genes identified in the milk of treated cows were *S. epidermidis* (25.5%), *S. chromogenes* (15.7%), and *S. warneri* (2.0%), whereas those in untreated cows were *S. chromogenes* (29.7%) and *S. epidermidis* (8.1%). Also, 75.9% and 87.0% of treated and untreated cows did not present *S. aureus* with toxin-encoding genes, respectively. The genes *sea*, *seb*, and *sed* were identified in milk of 6.9%, 3.4%, and 6.9% of *S. aureus*-infected cows treated with homeopathy, while *seb* and *seb* were identified in the milk of 4.3% of cows without treatment. The *sec* and *tst* genes were not detected. Cows treated with homeopathic formulation presented *S. aureus* with genes encoding toxins after treatment for mastitis, suggesting the presence of sources of infection to other animals of the herd and potential risk to public health event after homeopathic treatment.

Keywords: Subclinical mastitis, toxins, epidemiology, animal health, zoonoses.

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