TITLE: Differentiation of *Staphylococcus aureus* identified in diagnoses of persistent mastitis in goats of other Brazilian isolates using the MLST technique.

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ABSTRACT:

Staphylococcus aureus is the most important bacteria that cause mastitis in milk producing animals and demonstrate to be a ubiquitous organism that has the capacity to be present in several samples environments and tissues, moreover behave like an indigenous microorganisms or even as an infectious pathogen in the host. Multi Locus Sequence Typing (MLST) is a method based on nucleotide changes of seven maintenance genes (house-keeping) and provides discriminant allelic profile, known as sequence type, for each bacterial isolate. The technique is a valuable tool to identify and differentiate isolates of the genus Staphylococcus from human and animal origins. The aim of this study was to analyze S. aureus isolates from persistent goats mastitis (MP), chronic disease, along with most other Brazilian isolates. We used 18 isolates of S. aureus from MP in goats together with another 25 isolates from goats, sheep and cow diagnosed with mastitis deposited in a database (https://pubmlst.org/). The alleles were aligned in the MEGAX program and the phylogenetic reconstruction was performed by Bayesian Inference (BI) and Maximum Likelihood (ML). The evolutionary model "HKY + I" was defined by the jModelTest software and the phylogenetic tree was inferred (Markov Chain Monte Carlo) using MrBayes software. The number of haplotypes and haplotype diversity were determined by the DNAsp software, and the haplotype network was obtained in Network 5.0 software. The results demonstrate a phylogenetic tree with separation of goats and cattle from a common ancestor of S. aureus and the formation of clades subdivided into five monophyletic groups, and the MP sequences were associated with a clade separated from the others. Sequences related to cows showed to have undergone more mutations and adaptive changes throughout the period studied. The alleles indicated that the genes yqiL, aroE, arcC, among the seven housekeeping genes, have a higher variability than others when analyzed for mastitis per host, and the arcC, pta, tpi and qlpF genes have moderate genetic variability. Therefore, phylogenetic analysis demonstrated high variability of S. aureus and that there is a distinction between MP sequences, and individual analysis of housekeeping by the haplotype method can better distinguish the mastitis-causing strains among hosts.

Keywords: MLST, goat, mastitis, persistent, milk.

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