

TITLE: AN IN SILICO, DIFFERENTIATION OF *Staphylococcus aureus* IDENTIFIED IN CASES OF MASTITIS IN MAMMALS.

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ABSTRACT:

Staphylococcus aureus is the most important and prevalent contagious mammary pathogen, is a ubiquitous organism that can be both a commensal bacterium and a lethal pathogen in several species of animals. Multilocus sequence typing (MLST) is a method based on nucleotide changes of seven maintenance genes (house-keeping) and provides a discriminant allelic profile, known as sequence type, for each bacterial isolate. The MLST when associated with bioinformatics approaches becomes a valuable tool to identify and differentiate strains genetically. The aim of this study was to analyze and evaluate the molecular diversity indices of wide populations by multilocus sequence typing genes of *S. aureus* deposited in a MLST public database. A total of 582 sequences and seven alleles from *S. aureus* isolates of milk and deposited from 20 different countries were analyzed in this study. Of these, 531 are sequences belonging to cows, 24 from goats, 10 from sheep and nine from humans diagnosed with different types of mastitis deposited in PubMLST database (<https://pubmlst.org/saureus/>). The alleles and concatenated sequences were aligned in the MEGAX program, and the phylogenetic reconstruction was performed by Bayesian Inference (BI) and Maximum Likelihood (ML). The evolutionary model "GTR+I+G" was defined by the jModelTest software and the phylogenetic tree was inferred (Markov Chain Monte Carlo) using MrBayes software. Polymorphism site analyzes were determined by the DNAsp software. The results demonstrate a phylogenetic tree with separation of 12 monophyletic groups, denoting the separation of some lineages. Strains of *S. aureus* belonging to cows were present in all clades, goats and humans in four different clades, while sheep occupied three different clades. No specific grouping of clades by hosts was identified, but there is greater grouping by genetic identity by country. The analysis demonstrated that the polymorphism diversity and *pta* genes and *tpi* were higher than *arc*, *aroE*, *gmk*, *glpF* and *yqiL*. The *pta* gene showed 234 mutations, while *gmk* only 23. The largest number of haplotypes was found in the *glpF* gene, 39, while *gmk* had only 24 haplotypes. Therefore, the phylogenetic analysis demonstrated a considerable number of lineages. Furthermore, the *pta* and *tpi* genes mutations may indicate adaptations associated with environmental changes, de-regulates the central metabolism and changes in the cell surface of *S. aureus*.

Keywords: Bacteria; Haplotype; MLST; Milk; Phylogenetic.

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