

TITLE: VIRULENCE GENES, CLONAL PROFILE AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CANASTRA ARTISANAL CHEESE IN BRAZIL.

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ABSTRACT:

Canastra cheese is a traditional artisanal product made in Serra da Canastra, Brazil, manufactured with fresh raw milk, rennet and a natural starter culture called "pingo". The first step in cheese production is milking the cows, where microbiological contamination can be minimized applying good milking practices. The consumption of cheeses contaminated with enterotoxigenic *Staphylococcus aureus* may pose a health risk to consumers. This study aimed to evaluate the virulence potential, the genetic variability and antimicrobial resistance profile of *S. aureus* samples isolated from Canastra cheese. From 83 cheese samples, strains were isolated for gen *nuc* amplification, specific for *S. aureus* identification using Polymerase chain reaction (PCR). The clonal profile of the isolates was characterized by Pulsed-field gel electrophoresis (PFGE), and the accessory gene regulator alleles *agr*-typing, by PCR. To detect the presence of genes coding for enterotoxins, hemolysins, biofilm formation, microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), and methicillin resistance (MRSA), the strains were analyzed by PCR. Crystal violet test was performed to classify the isolates according to biofilm formation capacity. Antibiotics susceptibility was also determined by the disc diffusion method. The results showed that there were 303 positive isolates from 445 isolates for the *nuc* gene and 54 PFGE pulsotypes distributed in five clusters. The *agr* typing showed groups distributed as follows: *agr*I (27.4%), *agr*II (31.4%), *agr*III (14.2%), *agr*IV (0%), while 27.0% could not be attributed to an *agr* group. After performing the typing techniques, 51 isolates were selected to be further tested. The positive enterotoxin genes were *seg* and *seo* (21.5%), and *sem* (15.6%). From the 51 tested isolates, the following were positive for other virulence genes: hemolysins *hla* (100%), *hly* (98.1%); MSCRAMMS genes *clfA*, *clfB*, *eno* (98.1%); *fnbB* (31.3%), *icaA* (70.5%), *icaD* (47.0%), *fib* (84.3%), *bbp* (3.9%) and *mecA* (11.7%). The crystal violet assay indicated that 86.3% of the isolates were considered biofilm producers. In the antibiogram test, 49.0% of the strains showed resistance to penicillin and 25.4% to tetracycline. These findings demonstrated a high prevalence of virulence genes and antibiotic resistance in the evaluated strains. It is important to avoid *S. aureus* contamination at every step of the cheese production chain by implementing good manufacturing practices.

Keywords: antimicrobial resistance; PFGE; virulence genes.

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