

TITLE: ADHESION AND INVASION IN HELA AND BMEC CELLS BY *STAPHYLOCOCCUS AUREUS* ISOLATED FROM MILK OF COWS WITH SUBCLINICAL MASTITIS.

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ABSTRACT:

Staphylococcus aureus is one of the main pathogens causing subclinical bovine mastitis. Its pathogenicity is due to important factors of virulence, such as the ability to adhere and invade the tissues of the mammary glands. The aim of this study was to test 20 *S. aureus* strains isolated from milk of cows with subclinical mastitis regarding their ability to adhere and invade bovine mammary epithelial cells (BMEC) and HeLa cells. The cells were cultured in 24-well microplates to form confluent layer. And the *S. aureus* cultures were diluted in Dubelcco Modified Eagle's medium (DMEM) (1.5×10^6 CFU/mL) and 1 mL of these suspensions was inoculated into each well, in triplicate, 37°C/5%CO₂. After 3 hours, the wells were washed with PBS⁺ to remove unbound bacteria. Next, the adhered cells were detached from the plate using trypsin-EDTA and the trypsinization was stopped adding DMEM and 10 µL of each well and serial dilutions were seeded on TSA by surface droplet technique. For the invasion test, the bacterial suspensions were inoculated in triplicate, and the plates were incubated for 3h/37°C/5%CO₂. After, the wells were washed with PBS⁺ buffer, and added by DMEM with 5% FBS and 100µg/ml gentamicin to remove the extracellular bacteria. After reincubation, the wells were washed with PBS⁺ and plated onto TSA to check for the absence of bacteria in the extracellular medium. Next, 0.1% Triton X-100 was added to lyse the cells to release the bacteria that invaded them, and 10 µL directly from the wells and serial dilutions were plated into TSA. The positive result was determined by the recovering and counting of the colonies, in percentage relation to the values obtained in the adhesion test. The 20 strains of *S. aureus* (100%) adhered to BMEC and 18 (90%) adhered to HeLa. In relation to the invasion, 14 strains (70%) invaded BMEC, with rates varying from 0.9 to 96% and median of 5.1%. Six strains (40%) invaded HeLa, with rates varying from 2 to 95% and an average of 11.6%. The difference between invasion rates was not statistically significant ($p = 0.0785$). The study demonstrated that *S. aureus* isolated from cases of subclinical bovine mastitis can adhere and invade bovine mammary epithelium (BMEC) but also HeLa cells. Although the biological difference, there was no statistical difference showing the same potential to cause invasion in some kind of human cells.

KEYWORDS: *S. aureus*, mastitis, adhesion, invasion, cell culture.