TITLE: EFFECT OF PRE-EXPOSURE TO ALCOHOLIC EXTRACT OF PROPOLIS ON S*taphylococcus epidermidis* BIOFILM FORMATION.

AUTHORS: OLIVEIRA, J.P.; DEONAS, A.N.; LOPES, T.P.; SILVA, D.F.; SOUZA, C.M.; FRANÇA, E.J.G.

INSTITUTION: UNIVERSIDADE ESTADUAL DO NORTE DO PARANÁ. CENTRO DE CIÊNCIAS HUMANAS E DA EDUCAÇÃO. RUA PORTUGAL, 340, CEP 86300-000, CORNÉLIO PROCÓPIO, PR.

ABSTRACT:

Staphylococcus epidermidis is naturally found in the skin of healthy individuals, however this specie have high clinical importance, been responsible for opportunistic infections in clinical environments. One of the main virulence factor associated with this species is its biofilm forming ability, which decrease its antimicrobials sensitivity and the immune system attack. The present study aimed to evaluate the effect of pre-exposure to the Propolis Alcoholic Extract on the *Staphylococcus epidermidis* biofilm forming ability. The alcoholic extract of propolis was commercially acquired, produced by Arte Nativa Ltda., presenting at the concentration of 11% of dry propolis extract. For the analysis, one *S. epidermidis* isolate stocked at -20 ° C in the Laboratory of Microbiology of UENP / CCP, was used. Cultures were performed from stock in TSB medium added by glucose (1%) by 24 hours. The cells were pelleted and used to preparation of the suspensions of $1.5x10^8$ cells / ml in TSB + glucose (1%) medium. For the assay, alcoholic extract of propolis was added to the representative concentration of 0.75% of dry propolis extract, previously established as sub inhibitory for the growth of the isolate, and the tubes were incubated at 37 ° C for 24 hours. The control consisted in the cultivation of the isolate under the same conditions, except by absence of the alcoholic extract of propolis. After, the cultures were pelleted and the cells washed in PBS buffer by centrifugation and employed for biofilm formation. Biofilms assay were made in 96-well polystyrene microplates from $1.5x10^8$ cells / mL in TSB + glucose. The microplates were incubated for 48 hours at 37 ° C. The wells were washed, the cells fixed and stained with 1% crystal violet. After removal of the dye and washing of the wells, 33% acetic acid was added and the spectrophotometric reading was performed at 490 nm. The results showed that the pre-exposure of *S. epidermidis* to the alcoholic propolis extract promoted a reduction of 63.9% in biofilm forming ability compared to untreated cells, confirming propolis interference in the biofilm forming ability of the species.

Keywords: Virulence, natural antimicrobial, formation potencial.