Title: ASSOCIATION OF Candida albicans AND Streptococcus mutans IN POLYMICROBIAL BIOFILM EXPOSED TO Rosmarinus officinalis (ROSEMARY) EXTRACT

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Abstract:

C. albicans can form a complex microbial community with S. mutans in the oral cavity. They can form a biofilm on the tooth surface and develop caries. Thus, this study evaluated the association of C. albicans and S. mutans in polymicrobial biofilm, in addition, the effect of treatment with plant extract of rosemary. For this purpose, reference strains C. albicans (ATCC 18804) and S. mutans (ATCC 35688) were grown on solid medium (37°C/24 h) and then in liquid medium under the same conditions. Were used Sabouraud-dextrose (SD) agar and Yeast Nitrogen Base (YNB) broth for C. albicans and Brain Heart Infusion (BHI) agar and broth for S. mutans. After centrifugation (2000 rpm/10 min) the supernatant was discarded and the pellet suspended in saline (0.9% NaCl) twice in succession, and the solution of each microorganism was adjusted to 1 x 10⁷ CFU/mL (forming units colony per milliliter) in a spectrophotometer. Then, in microtiter plate wells was added a part (100 µL) of C. albicans and a part (100 µL) of S. mutans. After preincubation (37°C/90 min) under stirring (75 rpm), the supernatant was discarded and added culture medium (BHI+YNB, 1:2). After 24 h incubation was replaced the medium and after 48 h the biofilm was subjected to treatment with glycolic extract of rosemary (200 mg/mL) for 5 min (n=10) and saline (n=10) was used as a control. After treatment, the biofilms were broken by sonication (25% power for 30 s), serial dilutions of suspensions were made and seeded on selective agars as SD agar with chloramphenicol (1%) for C. albicans and Mitis salivarius agar with sucrose (20%) and bacitracin (0.2 IU/mL) for S. mutans. After 48 h incubation, the CFU were counted and the data were statistically analyzed by ANOVA and Tukey Test (p<0.05). Regarding the population of C. albicans, was observed a growth of 1.26 x 10⁷ CFU/mL (± 0.08 x 10⁷) in the control group and 0.15 x 10⁷ CFU/mL (± 0.03 x 10⁷) in the treated group and a reduction of 87.72% (± 2.06) (p <0.05). Regarding the population of S. mutans there was a growth of 2.3 x 10⁸ CFU/mL (± 0.43 x 10⁸) in the control group and 0.87 x 10⁸ CFU/mL (± 0.19 x 10⁸) in the treated group, demonstrating a reduction of 61.85% (± 7.3) (p<0.05). Thus, it was demonstrated that in this polymicrobial biofilm the development of population of C. albicans was affected by the population of S. mutans, however the rosemary extract demonstrated antibiofilm effect on this polymicrobial community formed by C. albicans and S. mutans.

Keywords: Candida albicans; Polymicrobial biofilm; Rosmarinus officinalis; Streptococcus mutans;