

Title: EVALUATION OF ANTIBIOFILM ACTIVITY: DIFFERENT FORMULATIONS OF INNOVATIVE PRODUCT (WANITOX) WITH POSSIBLE APPLICABILITY IN TREATMENT OF DENTAL UNIT WATERLINES

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Abstract:

Dental unit water serves as a potential source of contamination in dentistry due to biofilm formation on waterlines. The goal of this research was to evaluate the antibiofilm activity of an innovative product (Wanitox) developed in different formulations for use with promising application in treatment of dental unit waterlines. The biofilm was formed on polystyrene microplates (96 wells) with 200µL of *Tryptic Soy Broth* (BD Difco™, USA) plus bacterial inoculum (*Escherichia coli* - ATCC 25922 or *Pseudomonas aeruginosa* - ATCC 27853) standard (10⁸CFU/mL) in each well. Incubation was performed at 37°C for 24 hours. After the incubation period, culture medium and bacterial inoculum were removed and wells rinsed with saline three times to remove bacteria planktonic cells. Wanitox products in three different formulations (Wanitox 1, Wanitox 2, Wanitox 3), 0.12% digluconate of chlorhexidine solution (CHX) and negative control (saline) were kept in contact with formed biofilm for 24 hours as well as after this period, saline rinsing with three times was performed. The biofilm was fixed with ethanol for 15min and stained with crystal violet solution (cationic dye) for 5 min. Once again, the wells were rinsed with saline for three times, and 100µL of 33% acetic acid were added to each well for indirect quantification of total biofilm biomass (absorbance read at 570nm). The results in sextuplicate were exported to BioEstat software (version 5.3) and statistical analysis (ANOVA and Tukey test) performed with significance level $\alpha = 5\%$. With regard to *E. coli*, the formulation of Wanitox 1 (0.271 ± 0.044) and CHX (0.250 ± 0.062) showed no difference in antibiofilm activity ($p > 0.05$), but were better ($p < 0.05$) than Wanitox 2 (0.487 ± 0.041), 3 (0.484 ± 0.152) and negative control (0.542 ± 0.044) - $p > 0.01$. On the other hand, Wanitox formulations and CHX did not show antibiofilm activity against *P. aeruginosa* when compared to negative control ($p > 0.05$). Therefore, future researchers are needed to improve this product with respect to its antibiofilm activity and possible applicability in treatment of dental unit waterlines.

Key words: Dental unit waterlines, biofilms, disinfectant.

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