In dentistry, biofilm formed on dental unit waterlines can allow microbial dissemination to water. The explanation for this phenomenon is assigned to the distribution system design of unit water and the microorganism ability to form structures that are highly complex and resistant to antimicrobial agents named biofilms. The objective of this study was to evaluate in vitro the antibiofilm activity of different substances with possible applicability in dental unit waterlines. On 24-well polystyrene plates, 2mL of Tryptic Soy Broth culture medium with a standardized bacterial inoculum (10^6 CFU/mL) of standard strain of *P. aeruginosa* (ATCC 27853) along with fragments (1cm) of dental unit waterlines were added. After the incubation period of plates in orbital incubator shaker at 37°C for 24h at 80rpm elapsed, the fragments of dental unit waterline were flushed with 5mL of saline solution at 0.85% by three times for removing planktonic cells. Subsequently, on another 24-well polystyrene plate, samples were kept in contact with two different substances (A and B) during 30s. As group control (without antibiofilm activity), saline solution at 0.85% for 30s was used. Each fragment of dental unit waterline was flushed with 5mL of saline solution at 0.85% and stored in microtubes containing 2mL of glutaraldehyde at 2.5% for 24h for fixing biological material. Afterward, the samples were dehydrated in alcohol series, metalized and submitted to analysis through scanning electron microscopy (SEM). SEM allowed to observe biofilm presence on all analyzed fragments of dental unit waterline, but the samples that were kept in contact with substances A and B showed a lower number of cells in biofilm when compared to the group control. In conclusion, both substances A and B in contact with *P. aeruginosa* biofilm samples presented antibiofilm activities and, consequently, possible applicability to cope with biofilm on dental unit waterlines.

Keywords: biofilms, contamination control, disinfection, dental unit waterline.

Development Agency: This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.