TITLE: EVALUATION OF BIOFILM FORMATION BY *MYCOBACTERIUM ABSCESSUS* SUBSP. *BOLLETII* IN THE PRESENCE OF SUBINHIBITORY CONCENTRATIONS OF GLUTARALDEHYDE

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ABSTRACT:

In 2008, the National Health Surveillance Agency (Anvisa) issued a technical note, that from 2003 to 2008 more than 2,000 cases of hospital-acquired Rapidly Growing Mycobacteria (RGM) infections had been notified in private hospitals in the country, mainly related to Videolaparoscopic procedures. Some of these infections had as a common factor the predominance of a particular clone of *Mycobacterium abscessus* subsp. *bolletii* (Mab) and mainly affected the skin and the subcutaneous cellular tissue of the patients. After the occurrence of these outbreaks by RGM, several measures were taken by Anvisa, among them, the suspension of chemical sterilization by immersion of surgical instruments and health products, by the use of any liquid sterilizing agent. The investigations carried out by Anvisa concluded that the outbreaks were due to failures in reprocessing of critical medical equipment, which were sterilized mainly by the use of 2% glutaraldehyde. These decisions were based on empirical observations, based exclusively on the observation of what was happening in hospital practice. To date, the relationship of Mab and its high tolerance to glutaraldehyde. Thus, this study has as main objective to investigate qualitatively and quantitatively the biofilm formation by Mab INCQS 00594 at subinhibitory concentrations of glutaraldehyde. The biofilms were developed in polycarbonate (PC) and stainless steel (AI) disks in a 24-well tissue culture plate, where suspensions of Mab were inoculated at various concentrations of glutaraldehyde (0.5, 1.0 and 1.5%) in Proskauer-Beck broth, and the plates were incubated for 7 days at 36 ºC. At the end of incubation, PC and AI discs were processed for analysis of CFUs and PC disks for microscopy. At the concentration of 0.5% glutaraldehyde, it was possible to recover about 10^7 and 10^8 CFU/mL on the surfaces of the AI and PC disks, respectively. At 1.0% and 1.5%, 10^6 and 10^4 CFU/mL were recovered in both AI and PC disks. In the microscopy analysis, it could be visualized that Mab was grew in the presence of different concentrations of glutaraldehyde after seven days of incubation. The microscopic overview revealed a large number of clusters and the presence of isolated bacteria. These preliminary results confirmed the ability of the *M. abscessus* species to survive and develop in glutaraldehyde and may be related to the occurrence of the outbreaks.

Keywords: *Mycobacterium abscessus* subsp. *bolletii*; Glutaraldehyde; Biofilm