TITLE: RAPID BACTERICIDAL EFFECT OF CANNABIDIOL (CBD) AGAINST *Staphylococcus aureus* BY *IN VITRO* TIME-KILL ASSAYS AFTER 30-DAYS OF CBD EXPOSURE

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ABSTRACT:

Introduction: Cannabidiol (CBD) is the major non-psychoactive component isolated from *Cannabis sativa*, and it is antimicrobial against Gram-positive bacteria, including multidrug-resistant (MDR) *Staphylococcus aureus.* **Objective:** The aim of this study was to evaluate the *in vitro* antibacterial activity of CBD over time against *S. aureus* using time-kill assays. **Material and methods:** *S. aureus* ATCC 29213 was submitted to 30-days passage with (i) fixed concentration of 1 µg/mL of CBD and with (ii) increasing concentrations ranging from 1 to 15.5 µg/mL of CBD, as well as (iii) in the absence of CBD (drug free passages). Minimal inhibitory concentration (MIC) was determined using both broth microdilution and agar dilution assays. Subsequently, the strains with 30 days of passaging and *S. aureus* ATCC 29213, with no passages, were evaluated in time-kill assays in four different conditions: 1 µg/mL CBD (subinhibitory concentration), 4 µg/mL CBD (MIC), 15.5 µg/mL CBD (higher concentration of passaging exposure), 40 µg/mL CBD (ten times greater than MIC), and absence of CBD, for 1, 2, 4, 6, 12, and 24 h. **Results and discussion:** Previous bacterial exposure to CBD did not affect MIC, which was the same as for *S. aureus* ATCC 29213 without exposure to CBD (MIC = 4 µg/mL), even for the strain that survived to 15.5 µg/mL of CBD in the 30-day passages. In agar dilution, no strains were able to growth in concentrations over than 2 µg/mL. Time-kill assays showed a rapid bactericidal effect within 1 hour, in a concentration dependent manner, starting in 4 µg/mL (MIC). **Conclusions:** Subinhibitory concentrations did not affect the bacterial growth of the studied strains, compared to the control strain, in the absence of CBD (drug free passages). Furthermore, the killing rate was similar among the four strains submitted to these four different conditions, suggesting that previous exposure does not affect the bactericidal effect of CBD. In addition to previous data of CBD antibacterial activity against Gram-positive cocci, these results may contribute to further studies regarding pharmacokinetic/pharmacodynamic perspectives, as well as dose-exposure response relationships.

Keywords: time-kill assay, antibacterial activity, cannabidiol, Gram-positive cocci

Development Agency: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001. CAPES Grant 8887.369851/2019-00 (NA). Pró-Reitoria de Pesquisa da USP Grant 18.1.796.60.2 grupo 057 (LNA).