TITLE: SYNERGISTIC BACTERICIDAL EFFECT OF THE COMBINATION CANNABIDIOL PLUS POLYMYXIN B (PB) AGAINST PB-RESISTANT *Klebsiella pneumoniae* BY *IN VITRO* TIME-KILL ASSAYS

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ABSTRACT:

Introduction: Polymyxin B (PB) has been used as 'last-resort' antibiotic to treat infections due to carbapenem- and third/fourth-generation cephalosporin-resistant Gram-negative bacilli (GNB). Nevertheless, *K. pneumoniae* co-resistant to carbapenems (e.g., KPC producers), aminoglycosides, polymyxins, and tigecycline ("CAPT-resistant") as well as resistant to ceftazidime-avibactam (CZA)-resistant have been increasingly detected, decreasing dramatically the therapeutic options. Cannabidiol (CBD) is the major non-psychoactive component isolated from *Cannabis sativa*, and it is antimicrobial against Gram-positive bacteria, but not against GNB. However, the combination of CBD with PB minimal antibiotic concentrations (MAC = subinhibitory concentrations of PB that promote non-lethal interference in the bacterial cell, such as destabilizing the inner membrane) leads to GNB bacterial killing. **Objective:** The present study aimed to measure the bactericidal activity of the combination CBD + PB against 12 *K. pneumoniae* isolates, corresponding to ST 258, 11, 437, and 16. **Material and methods:** Nine PB-resistant (minimal inhibitory concentration [MIC] ranging from 4 µg/mL to 128 µg/mL) and KPC-producing strains were evaluated by *in vitro* time-kill assays (1, 2, 4, 6, 12 and 24 h). We evaluated 2 µg/mL and 4 µg/mL of CBD in combination PB MAC (concentrations lower than 2 µg/mL), according to previous checkerboard assays results for each isolate evaluated. **Results and discussion:** For all PB-resistant isolates, in comparison with single drugs (CBD or PB MAC) and with control test (only bacterial inoculum), the combination CBD + PB resulted in enhanced bacterial reduction in 1h, with a maximal killing at 2h. For most isolates, synergistic effect in time-kill assays was observed within 1h. For two PB-susceptible isolates, the combination of CBD + PB showed an indifferent effect, probably due to the proximity between PB MIC and PB MAC values. **Conclusions:** These results showed the killing effect of the combination CBD + PB over time, contributing to further studies regarding dose-exposure response relationships and pharmacokinetic/pharmacodynamic parameters. Furthermore, *in vitro* time-kill results added data that suggests promising translational potential of CBD repurposing as antibacterial in the combination CBD + PB against GNB as a rescue treatment for life-threatening infections, highlighting against CZA- and CAPT-resistant (PB-resistant) *K. pneumoniae* strains.

Keywords: synergistic effect; antibacterial combination; Gram-negative bacilli; polymyxin resistance

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