TITLE: PROTEOMIC ANALYSIS OF *Clostridioides difficile* SPORES SURFACE LAYERS GENERATED UNDER SUBINHIBITORY CONCENTRATIONS OF ANTIBIOTICS

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ABSTRACT:

Clostridioides difficile is a gram-positive, anaerobic, spore-forming bacillus and the main cause of pseudomembranous colitis and antibiotic-associated diarrhea. Spores are high resistant to the adverse environment conditions and large amounts are released in the stool of patients during *C. difficile* infection (CDI), which become essential for the maintenance and transmission of the disease. As spores represent an important virulence factor, the objective of this work is to characterize proteins of the outermost spore layers (exosporium and spore coat) of the Brazilian ribotype 135 (RT135) obtained from a control condition, without antibiotics, and the exposure to sublethal concentrations of clindamycin and levofloxacin. After obtaining the spores, proteins were extracted and their profile evaluated in a polyacrylamide gel (SDS-PAGE). Protein identification was performed by mass spectrometry (MALDI TOF/TOF MS) and raw data analyzed by the MASCOT program using the NCBInr database. Through this technique, differences in the spores surface protein profile were observed, with the identification of 39 proteins. Most of the proteins identified, presented catalytic activity and antioxidant activities. Some proteins were identified only in the absence of antibiotics, such as CotH, involved in the recruitment of proteins to the surface of the spores. This study demonstrates that spore surface proteins can be modulated by environmental changes, with proteomics as a useful approach to the identification of new targets for the prevention and treatment of CDI, and for a better understanding the pathogenesis of the bacterium and its sporulation process.

Keywords: spores, proteomic, *Clostridioides difficile*, *C. difficile*, surface layers