Proteomic analysis of exosporium of the brazilian ribotypes *Peptoclostridium difficile* spores treated with hospitals antibiotics

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Peptoclostridium difficile is an anaerobic, spore-forming bacterium that is the main etiological agent of the pseudomembranous colites and diarrhea associated to antibiotics use. During the *P. difficile* infection (CDI) patients eliminates great quantities of spores, resulting in contamination of their skin, clothing, and environmental surfaces. Bacterial spores have several proteinaceous coats that surround and protect the dormant bacterial cell wall and membrane. Since the spore formation represents an important virulence factor for this pathogen, the main goal of this study was to characterize proteins of the exosporium from two Brazilian *P. difficile* ribotypes (133 and 135) and NAP1/027 strain, for comparison, after the strains were exposed to subinhibitory concentrations of clindamycin and levofloxacin antibiotics. Antibiotics were added to the 70:30 agar plates medium and all plates were incubated for 10 days at 37ºC under anaerobic conditions. Colonies formed were scraped from the plates and kept under refrigeration (4ºC) for 24h. The sediment was ressuspended in a lysis buffer at 37ºC for 2h under agitation. The content was applied to a 50% sucrose gradient and centrifuged by using a *swinging-bucket* rotor. Spores were washed and lysate with a buffer (0.1M sodium borate pH 10; 0.5% SDS and 50mM DTT). Proteins were applied to a SDS-PAGE and bands cut from the gel to process for mass spectrometry (Maldi TOF/TOF) and all spectra generated compared with a NCBInr data base (MASCOT). So far, we have identified 55 proteins with high confidence from the 135 ribotype, all related to *Peptoclostridium* spp and *Bacillus* spp spore proteins. As a conclusion, we believe that since patients eliminate spores during CDI by identifying them our study might contribute not only to elucidate *P. difficile* pathogenesis, but also to understand the sporulation process.

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