Proteomic Analysis of Surface of *Peptoclostridium difficile* Brazilian Strains Treated with Subinhibitory Antibiotic Concentrations

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Peptoclostridium difficile is the etiological agent of antibiotic-associated diarrhea disease, *P. difficile* infection (CDI). The mechanism by which the bacterium colonizes the gut during infection is poorly understood, and involves some surface-associated proteins (SP). The aim of this study was to characterize the expression of SP of Brazilian *P. difficile* exclusive ribotypes (RT133 and RT135) when grown under sub inhibitory concentrations of two antibiotics, clindamycin and levofloxacin. Moreover, the BI/NAP1/027 and 630 strains were used for comparison. The antibiotics were added to the *Brucella* broth to a final concentration of 0.5 x MIC, and the bacteria were grown overnight in an anaerobic cabinet at 37ºC for 18h. Enriched SP fractions were obtained by using a low pH glycine lysis buffer (pH 2.0) and a gel free approach combined to a nLC ESI-MS/MS mass spectrometry was used to analyze the SLPs. We were able to identify approximately 225 proteins with high confidence for each strain and condition. Lable-free quantification revealed variable amounts of a number of proteins, in special S-layer protein (SlpA), penicillin-binding protein, cold shock protein, chaperones and flagellin subunit. The analysis suggested that clindamycin had a stronger effect in protein expression, when compared to levofloxacin. Two strains, when treated with clindamycin, did not express the Hfq protein that, when depleted, has been shown to lead to growth defect, morphological changes, increase in sensitivity to stress conditions, and a better ability to sporulate and biofilm. Here we document the effect of antibiotics in the expression of *P. difficile* surface proteins. This work provides knowledge to the involvement of the SPs in the pathogenesis and maybe new protein targets for the diagnosis and/or therapeutics that may contribute to combat *P. difficile* infections.

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