Surface proteomic analysis of *Clostridium difficile* strains treated with subinhibitory concentrations of antibiotics

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Clostridium difficile is the etiological agent of antibiotic-associated diarrhea and pseudomembranous colitis. The mechanism by which this pathogen colonizes the gut is still poorly elucidated, although it seems to involve some surface-associated proteins (SP). This study aimed to characterize the SP from Brazilian ribotypes (133 and 135) comparing with two worldwide ribotypes, 027 and 014, when grown under sub lethal concentrations of antibiotics, clindamycin and levofloxacin. Enriched SP fractions were obtained by using a low pH glycine lysis buffer. In solution approaches combined with LC-ESI-MS/MS mass spectrometry coupled to LTQ Orbitrap were used to analyze the SPs. Data analysis identified 265 different proteins and the lable-free quantification revealed variable amounts of proteins, specially S-layer proteins (SlpA), cell wall family proteins (Cwps), cold shock protein, chaperones and flagellin subunit. Under the presence of antibiotics, the hypervirulent BI/NAP1/027 strain expressed the VanB protein, involved in vancomycin resistance, which raises a relevant problem since this drug is used to treat severe cases of CDI. In addition, a Western blotting (WB) was performed using a pool of antibodies from patients positive for the disease against the proteins revealing a different pattern of recognition according to the ribotype and the antibiotic used. The treatment of the different ribotypes of *C. difficile* with the antibiotics caused prominent modifications in the profile of the SPs, and others involved in the bacterial stress. Such modifications appear to be ribotype-specific, although some proteins have undergone similar changes for all ribotypes evaluated. Ribotypes 014 and 135 appear to be the most affected by antimicrobial treatment.

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