TITLE: COMPARATIVE EXOPROTEOMIC OF *Clostridioides difficile* BRAZILIAN RIBOTYPES TREATED WITH SUBINHIBITORY CONCENTRATIONS OF ANTIBIOTICS

AUTHORS: TRINDADE, C.N.R.; MOURA, H.; BARR, J.R.; FERREIRA, T.G.; FERREIRA, E.O; DOMINGUES, R.M.C.P.

INSTITUTION: INSTITUTO DE MICROBIOLOGIA PAULO DE GÓES, RIO DE JANEIRO, RJ (AVENIDA CARLOS CHAGAS FILHO, 373, SALA I2-06, CEP 21941-590, RIO DE JANEIRO – RJ, BRAZIL)

ABSTRACT*:*

Clostridioides difficile is considered one of the major etiological agent of human diarrhea associated with antibiotic use, thus being an important nosocomial pathogen and a significant cause of morbidity and mortality. Several studies have evaluated the impact of subinibitory concentrations of antibiotics on the *C. dificille* toxins expression. However, the interference on the expression of secreted proteins is poorly understood. Thus, the aim of this study was to identify and compare the exoproteome of exclusive brazilian *C. difficile* ribotypes (RT), 133 and 135, comparing them with a worldwide circulating ribotypes 014 and 027 (BI/NAP1; epidemic), when grown under subinhibitory concentrations of clindamycin and levofloxacin. After growing in different environmental conditions, secreted proteins were obtained and analysed by the technique in solution (gel free), and subsequently processed by the spectrometer Nano-LC ESI-MS/ MS coupled to LTQ Orbitrap. After analysis, approximately 290 proteins per condition and RT were obtained. All proteins were identified using the Mascot software and thereafter validated in Scaffold program. From the proteins identified, most of them were related to cellular functions, such as transport, nutrient acquisition, adherence and proteins associated with response to environmental stress, after the analisys made by the Blast2Go program. Among the proteins examined, it was found an important protein which confers resistance to vancomycin and teicoplanin only for RT135, when subjected to subinibitory concentrations of antibiotics tested. This study demonstrates the role of secreted proteins to adapt to environmental changes, and proteomics showed to be a useful approach in identification of new targets for functional analysis, and to better understand the biology of the main RTs associated with cases of infections caused by *C. difficile*.

KEYWORDS: *Clostridioides difficile*, intestinal microbiota, resistance, antibiotics, exoproteome, secreted proteins.

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