

TITLE: IDENTIFICATION AND CHARACTERIZATION OF PROTEINS SECRETED BY *Clostridioides difficile* RIBOTYPES RECOGNIZED BY PATIENTS SERA DIAGNOSED WITH CDI

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ABSTRACT:

Clostridioides difficile is the main etiological agent of bacterial diarrhea associated with the use of antimicrobials, being an important nosocomial pathogen and with high morbidity and mortality in many countries. It is well known that *C. difficile* infection (CDI) is favored by the suppression of the normal intestinal microbiome during or after antibiotic therapy. Other risk factors are advanced age, length of hospital stay and therapy for cancer treatment. In addition, infections caused by other gastrointestinal pathogens may also contribute to the development of these infections. Several virulence factors have been described for *C. difficile*, and although two potent toxins (TcdA and TcdB) are the most studied, but there are other virulence factors involved in the CDI. Thus, this work aims to identify and characterize secreted proteins recognized by sera of patients diagnosed with CDI when *C. difficile* is exposed to subinhibitory doses of two antimicrobials (levofloxacin and clindamycin). For this study, two exclusively Brazilian ribotypes (RT), RT133, RT135 were chosen and two other world circulation ribotypes, RT014 and RT027, being the latter categorized as an epidemic strain. After obtaining the proteins, western blotting and an immunoprecipitation (Dynabeads Protein G) assay, by using a pool of the patient's sera as primary antibodies, followed by mass spectrometry (Nano-LC ESI-MS/MS coupled to Orbitrap LTQ), was performed to identify proteins. Approximately 40 proteins for each ribotype and condition (control, clindamycin and levofloxacin) were identified by immunoprecipitation. Most of the proteins are associated with stress conditions and virulence. Two proteins, rubrerythrin and trehalose, common to all conditions and ribotypes were chosen for a better characterization. These proteins will be overexpressed by using the pET-26b⁺ cloning vector and mutated with the Clostron vector system (pMTL007C-E2). The overexpressed protein will be retested against the patient's sera and mutants strains tested in a biological model to evaluate the phenotype. Results obtained from this experimental strategy will allow the definition of immunogenic proteins involved in infection by *C. difficile* and will contribute to the discussion of the impact of antibiotics in the expression of potential virulence factors, providing with the basis for a long and medium-term proposal, new diagnostics, therapy and prevention of CDI.

Keywords: *Clostridioides difficile*, virulence, proteome, Orbitrap.

Financial support: CNPq, CAPES and FAPERJ.