

TITLE: IDENTIFICATION OF *Clostridioides difficile* BIOMARKERS FOR CLINICAL DIAGNOSTIC

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ABSTRACT:

*Clostridioides difficile* is anaerobic gram-positive, spore-forming bacteria associated as the main agent of nosocomial infection. *C. difficile* infection (CDI) starts with a dysbiosis in the gastrointestinal tract, mainly caused by the use of antimicrobials. CDI symptoms are generally correlated with the production of two potent toxins, TcdA and TcdB; however, other factors are associated with the induction of CDI, such as, advanced age, hospitalization and therapy for cancer. With so many cases worldwide of *C. difficile*, new diagnostic methodologies are being developed, to identify the circulating species. Thus, the main goal of this study is to use three genes, A B and C as possible biomarkers for the identification of *C. difficile* in samples from patients previously diagnosed with CDI. For this study 30 *C. difficile* ribotypes belonging to the Cell culture collection of the anaerobic bacteria laboratory were selected. All samples were reactivated in BHI-PRAS and inoculated in blood agar plates for purity test, and then all samples were identified by MALDI-TOF MS. To develop the multiplex PCR, primers aiming three genes, DNAAK, ruberitritin and enolase, were designed, based in Proteomic analysis. The BLAST of the three proteins, identified initially in the Brazilian (RT133 and RT135) and the epidemic (RT027 and RT014) ribotypes, were present in several strains. After the analysis, the study evaluated the presence of these genes in the remaining 26 ribotypes, and among those considered more virulent, the presence of the three genes chosen was observed. Until now, the preliminary results emphasize the importance and relevance of using this approach as a possible screening technique for the development of a diagnostic kit for obtaining rapid and specific results in patients with CDI.

Keywords: *Clostridioides difficile*, Proteomic, Diagnostic, Anaerobic, *C. difficile* infection

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