TITLE: PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *Clostridioides difficile* STRAINS AND ESTABLISHMENT OF MALDI-TOF MS TYPING METHOD

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ABSTRACT

Clostridioides difficile is an anaerobic spore-forming bacteria that causes an opportunistic and nosocomial infection. The development of CDI (*Clostridioides difficile* infection), a colonic inflammation called pseudomembranous colitis, is initiated by toxigenic strains, which produce toxins (A, B and binary toxin). Recently, the epidemiology of *C. difficile* has changed dramatically caused by different ribotypes, such as 014, the most prevalent and 027 known as the epidemic strain. In Brazil, there is no notification of B1/NAP1/027, although several other ribotypes have been isolated. Two of them are exclusive from our country, 133 and 135. Concerning, the main methods used to identify and characterize *C. difficile*, PCR-ribotyping and PCR-multiplex**,** are the mostly performed worldwide, but in Latin American countries, including Brazil, the PCR-Ribotyping method is not performed. However, with the development of advanced Mass Spectrometry methods, the indirectly "Ribotyping" through Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) became a possible alternative. Hence, the aim of this study is to develop and validate an in-house database for MALDI-TOF MS for *C. difficile* typing by using a fast protein extraction procedure. Besides, a complete screening, genotypic and phenotypic characterization of the *C. difficile* collection from the Biology of Anaerobes Laboratory will be performed for validation of the method. To create the database, spectra were generated initially with 20 ribotypes for further analysis on BioNumerics. Simultaneously, a genotypic characterization by PCR-Multiplex and phenotypic characterization, such as, biofilm production, antimicrobial susceptibility test, minimal inhibitory concentration, surface layer protein profile test and cytotoxicity assay is also being performed, with strains been tested through cytotoxicity assay (92% positive and 8% negative) and been characterized by the presence of toxin genes (toxins A and B) through PCR-Multiplex (52% non-toxigenic, 43% Tox A+/B+ and 5% Tox A-/B+). Also, PCR-Ribotyping method has been successfully implemented. In combination with MALDI-TOF MS typing those methods might represent a reliable and faster alternative for typing *C. difficile* strains and gathering epidemiological data.

Keywords: characterization; *Clostridioides difficile*; MALDI-TOF MS; PCR-Ribotyping; typing

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