**TITLE:** APPLICATION OF MASS SPECTROMETRY (MALDI-TOF-MS) AS CONFIRMATORY METHOD IN CLINICAL ISOLATES OF CLOS*TRIDIUM DIFFICILE*

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## ABSTRACT

**Introduction:** The Disease associated with *Clostridium difficile* (DCd) has been a major cause of concern in hospitals worldwide due to the high mortality rate associated with its virulent strains, which produce toxins (enterotoxins, cytotoxins and binary toxin). Different methods may be applied for diagnostic, but the identification of the bacterium from the coproculture in a selective medium is the gold standard in epidemiological studies. Currently, an analytical technique of biomolecules through Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) has been increasingly used, presenting results with high precision and speed for the speciation of pathogens. Thus, the main goal of this study has been to standardize the coproculture method in anaerobiosis to identify *Clostridium difficile*, using MALDI-TOF as a confirmation tool, associated with the real-time PCR technique through the GeneXpert software (Cepheid). **Materials and Methods:** We selected eight samples of diarrheal stools from patients admitted to a cancer hospital in Brazil to performed culture in CM0601 medium (Oxoid) for 48 hours in anaerobiosis atmosphere. The *Clostridium difficile* ATCC 9689 strain has been used as a positive control, besides two stool samples known to be *C. difficile* positive previously identified by immunoenzymatic assays. **Results:** Positive samples as control and an ATCC strain showed satisfactory growth without culture medium, being a species confirmed by MALDI-TOF and real-time PCR as *C. difficile*. All the samples collected from the inpatients have been negative in coproculture. **Conclusion:** Considering the results obtained, we consider that the culture method used was satisfactory, with growth of the bacterium of interest. The use of mass spectrometry was relevant to confirm the presence of the pathogen.

**Keywords:** *Clostridium difficile*; MALDI-TOF; Coproculture; Epidemiological Study; GeneXpert.

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