TITLE: IDENTIFICATION OF *C.difficile* FROM PERIANAL SWABS

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ABSTRACT:

Clostridium difficile is a significant gastrointestinal pathogen and is the primary agent of antibiotic-associated diarrhea. This bacterium can produce 3 types of toxins, which are related to the disease's pathogenesis. The isolation of *Clostridium difficile* in cultural methods is very difficult since it is an anaerobic bacterium with slow growth. So, molecular identification of toxin genes is an important option for rapid diagnosis of *C. difficile* disease. Usually, fecal samples are used to perform a variety of tests but perianal swabs have been considered a good and simple method to detect toxin genes by PCR. The objective of this study was to evaluate the use of perianal swabs for PCR detection of toxins genes, and perform cultural identification of *C.difficile* on positives samples from PCR, using perianal swabs. Multiplex Real-Time PCR was performed within 24 hours after collection of perianal swab samples, to identify binary toxin (cdtA) genes, toxin B (tcdB) and C(tcdC) genes. A toxigenic culture was performed to positive swab samples in PCR. One perianal swab were collected from each patient and stored in tube with 2 mL of Tris-EDTA, pH 8. An aliquot of each sample was used to isolate the bacterium by cultural methods. The samples were incubated for 3 days in tioglicolate broth, and then incubated for 48 hours on Brucella agar – KASVI and Clostridium agar –OXOID. MALDI- TOF MS was used to confirm identification of *Clostridium difficile* at the specie level.

From 103 samples processed, 18 were positive on PCR, and all of them showed the presence of tcdB gene, responsible for toxin B production. Eight samples showed the presence of both tcdB and tcdC genes (44.4%), one sample was positive for both tcdB and ctdA genes, and one showed the presence of the three genes (tcdB, tcdC and ctdA).

Only 3 of 18 positives samples on PCR were recovered in cultural methods. The recovery of *C. difficile* on culture was probably difficulty by the small number of cells in the tubes containing Tris-EDTA where the swabs were stored. On the other hand, the perianal swabs appear to be a good sample for PCR testing.

Keywords: *Clostridium difficile*. Molecular identification. Perianal swabs.

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