TITLE: EVALUATION OF RELATIVE GENE EXPRESSION OF *CLOSTRIDIUM DIFFICILE* STRAINS OF VIRULENCE FACTORS

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ABSTRACT:

Clostridium difficile is a Gram-positive spore-forming bacillus which is recognized to be the major cause of nosocomial diarrhea associated with antibiotic therapy. Several virulence factors of *C. difficile* contribute to the colonization and development of infection, but the most studied virulence factor are toxins (A, B and binary). This study aims to compare the genetic expression of some virulence factors among three strains of *C. difficile*: ATCC 700057 (non-toxigenic), NAP1 (hypervirulent strain involved in outbreaks in North America and Europe) and ICC45 (novel virulent ribotype/PFGE-type found in oncological Hospital in Fortaleza-CE Brazil). *C. difficile* strains were cultured anaerobically in BHIS broth for 48h, to log phase (OD_{630} 0.08-0.1) and used to prepare total RNA isolation (PROMEGA), followed by synthesis of the cDNA (Applied Biosystems). Changes in *tcdA, tcdB, tcdC, fliA* (flagella) and *spo0A* (spores) gene expression were assessed by qPCR using SYBR Green (Thermo Scientific). Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method. The 16S rRNA was used as an internal control gene. The data were analyzed in the Prism 6 software through ANOVA analysis followed by Turkey's post-test. qPCR analysis of each of the PaLoc genes *tcdA, tcdB* and *tcdC* were not expressed by ATCC 700057 because it is not toxigenic, whereas *tcdA, tcdB* and *tcdC* expression were 1.0-fold higher in ICC45 strain than in NAP-1 strain ($p<0.008$). ICC-45 exhibited an increase of 1.0-fold ($p<0.003$) *fliA* expression in comparison with NAP1 and ATCC. ICC-45 also showed greater ($p<0.01$) *spo0A* compared to NAP1 and ATCC. However, no significant differences in expression were found between NAP1 and ATCC. This high relative gene expression of these virulence factors by ICC45 may explain the tissue injury and inflammatory process similar to the NAP1, caused by this strain in animal model (data previously described in another study), although it does not possess an overproduction of toxins like the hypervirulent strains. These data emphasize the importance to a better understanding of the role of virulence factors in the establishment of a *C. difficile* infection by hypervirulent strains such as NAP1 as well as ICC45, new strain discovered by our research group.

Keywords: *Clostridium difficile*, relative gene expression, real time PCR, virulence factors.

Development Agency: PRONEX-FUNCAP/CNPq