**TITLE:** IMPACT OF THE HUMAN GUT METABOLOME ON *Clostridioides difficile* VIRULENCE GENE EXPRESSION

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**ABSTRACT:**

*Clostridioides difficile* is an important nosocomial pathogen, being considered one of the major etiological agents of antibiotic-associated diarrhea (AAD). Treatment of *C. difficile* infection consists of metronidazole for moderate diarrhea and vancomycin for severe cases, which further increases the impact on the gut microbiota. Therefore, alternative treatments to eradicate *C. difficile* while preserving the gut microbiota are necessary. In this context, secondary metabolites from the intestinal microbiota emerge as a promising source of bioactive compounds. The aim of this study was to analyze the effect of small molecules extracted from human feces on the expression of virulence genes from *C. difficile*. Fecal samples were collected from healthy donors without prior use of antibiotics for the past six months. Small molecules were extracted from fecal samples using ethyl acetate. Dried extracts were resuspended directly into TPG culture medium, filtered, and the pH was adjusted to match that of culture medium alone. Dried residues of ethyl acetate were used as controls. Initially, growth curves were performed in triplicate using the fecal extract of each donor (n=3). The fecal extract slightly inhibited the growth of *C. difficile* R20291. After approximately 6 hours of growth, the cultures had reached the mid-logarithmic growth phase and RNA was isolated. Interestingly, the yield of RNA obtained from cultures exposed to fecal extracts was higher than the RNA obtained from controls, suggesting that the fecal extract could be directly affecting the cell wall composition in a way that facilitates cell lysis. Alternatively, the fecal extract could be affecting the expression of genes associated with cell wall synthesis. The effect of the fecal extract on the expression of virulence genes, such as *tcdA*, *tcdB*, *tcdR*, *cdtA* and *cdtR* were analyzed using *rpoC* as an endogenous control. Preliminary results suggest that the expression of *tcdR* is repressed. Further tests are required to confirm this phenotype. We are currently determining the effect of the fecal extract on the global gene expression profile of *C. difficile*. Additionally, other tests, such as the cytotoxicity assay using Vero cells will be performed to further investigate the role of gut small molecules on virulence gene expression.

**Keywords**: *Clostridioides difficile*, microbiota, small molecules, virulence factors

**Development Agencies**: CAPES, FAPERJ