TITLE: Identification of *Clostridioides difficile* surface proteins responsible to Laminin-1 recognition

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ABSTRACT:

Clostridioides difficile is an anaerobic bacillus and the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis. While toxins are extensively studied, the contribution of surface proteins to the intestinal colonization is poorly understood, especially those involved in binding to extracellular matrix (ECM) components. Several adhesins have been reported and might contribute to *C. difficile* colonization and infection, but only a few studies about *C. difficile* binding to ECM components have been reported. Thus, the aim of this work was to identify the adhesive properties of different *C. difficile* ribotypes (027, 133, 135, 014, 012) towards to laminin-1 (LMN-1). These findings were validated by quantifying the adhesion with a direct binding assay by using coverslips coated with LMN-1 and bacterial strains grown in media containing different glucose concentrations (0.2, 0.5 and 1%). Samples were counted with a Live/dead bacterial kit and images acquired were transferred to ImageJ for quantification. Results have shown a greater adhesion of the RT012 and RT027 to LMN-1. While RT135 strains showed the strongest adhesion with 0.5% glucose. In the absence of glucose, the adhesion was poorly observed in all ribotypes, suggesting a biofilm involvement. Hence, a biofilm production test was conducted followed by transmission electron microscopy analyses showing that all ribotypes are biofilm producers when glucose is added. Although the immunoelectron microscopy showed *C. difficile* recognition to LMN-1, a fluorescence microscopy is been conducted for confirmation. Our results show that *C. difficile* ribotypes can recognize LMN-1 in a different way and raise the possibility of the biofilm proteins involvement of in ECM binding. We believe protein identification can lead to potential protein targets among ribotypes and possible elucidation of *C. difficile* colonization and inflammatory disease.

Keywords: *Clostridioides difficile,* extracellular matrix, Laminin

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