TITLE: BIOFILM FORMATION BY CLOSTRIDIUM DIFFICILE STRAIN FROM CEARÁ, BRAZIL (ICC-45)

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Clostridium difficile is a Gram positive anaerobic bacillus which can cause Clostridium difficile infection (CDI). The most difficult challenge of CDI treatment is the higher recurrence of this disease, which, among other factors may be associated with C. difficile ability to produced biofilm. In this study, the in vitro ability to form a biofilm by a strain isolated in a cancer hospital in Ceará (ICC-45) was characterized, and compared with biofilm formation by NAP1/027 and ATCC 70057 strains. We tested 4 strains: ATCC 70057, NAP1/027 (LIBA5756), ICC-45 and R20291 to assess their ability to form biofilm in vitro. The amount of total biofilm biomass at 24, 48, 72 and 120h was estimated by crystal violet staining methodology. The absorbance was measure by spectrophotometer reading at OD570nm. According to their ability to form a biofilm, strains were classified according Pantaléon et al. 2018 into three groups: the low-biofilm formers (OD570 < 1.1), the moderate-biofilm formers (1.1 < OD570 < 3.5) and the high biofilm formers (OD570 > 3.5). The visualization and evaluation of matrix thickness of biofilm was performed by confocal microscopy using the Film Tracer Ruby biofilm matrix stain (Invitrogen®). Excitation 488/561 nm, emission 500-550 / 570-620 nm. All statistical analyses were performed using GraphPad Prism. Differences were considered significant at $p < 0.05$. The different C. difficile strains exhibited biofilm formation at 24, 48 and 72h. In 48h, the strains ICC-45 and NAP1/027 (LIBA5756) grew as higher biofilm formers with a mean OD570 >3.5 and the strain ATCC 700057 grew as medium-biofilm former with a mean OD570=2.9. On the other hand, after 120h incubation, we observed a reduction of biofilm biomass, ICC-45 and NAP1/027 (LIBA5756) formed moderate biofilm with a mean OD570=2.08 and OD570=3.02, respectively. The microscopy confocal results demonstrate that in 48h the biofilm matrix thickness formed by R20291 was significantly greater than ICC-45 and NAP1/027(LIBA5756), however in 120h ICC-45 and NAP1/027(LIBA5756) produced a similar matrix, and both strains exhibite a matrix thickness significantly higher than R20291. We conclude that the strain ICC-45 is a competent biofilm forming strain. The biofilm formed by ICC-45 did not differ from the hypervirulent NAP1/027 (LIBA5756) indicating its potential to cause by Clostridium difficile infection (CDI) and induce recurrence.

Keywords: biofilm, Clostridium difficile, matrix

Development Agency: FUNCAP/CNPQ PR2-0101-00060.01.00/15 Pronex and National Council for the Improvement of Higher Education (CAPES).