TITLE: ISOLATION AND CHARACTERIZATION OF EXTRACELLULAR VESICLES OF *CLOSTRIDIOIDES DIFFICILE*

AUTHORS: LOPES, A.S.[1]; BOENTE, R.F.[1]; SILVA, R.C.[2]; DOMINGUES, R.M.C.P.[1]; MIRANDA, K.R.[3]; LOBO, L.A.[1]

INSTITUTIONS:

1. INSTITUTO DE MICROBIOLOGIA PROFESSOR PAULO DE GÓES, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, RJ (AV. CARLOS CHAGAS FILHO, 373, BLOCO I, CEP 21941-590, CIDADE UNIVERSITÁRIA, RIO DE JANEIRO - RJ, BRAZIL); 2. INMETRO – DIMAV LAB, XERÉM, RJ (AV. NOSSA SENHORA DAS GRAÇAS, 50, CEP 25250-020, XERÉM, DUQUE DE CAXIAS - RJ, BRAZIL); 3. UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, MACAÉ, RJ (AV. ALUIZIO DA SILVA GOMES, 50, CEP 27930-560, CIDADE UNIVERSITÁRIA, MACAÉ - RJ, BRAZIL)

ABSTRACT:

Bacteria are capable to produce and secrete extracellular vesicles (EV). These structures are commonly associated with Gram negatives, which were discovered in the 60' in *Escherichia coli* and originate from the outer membrane. Recently, EVs were described in Gram positive bacteria but since this group does not possess outer membranes, their local of origin remains elusive. These vesicles may carry several different molecules for long distances, including toxins and DNA, and keep these materials preserved. In *Clostridioides difficile*, these structures were just recently described. This study proposes to investigate these EVs and to characterize their molecular profile in four different strains of *C. difficile*: R20291 and ANA#2004016 (both designated as BI/NAP-1/027 with TcdA, TcdB and CDT) and two isolates from a hospital in RJ, HU29 (non-toxigenic) and CTI (TcdA and TcdB). The extraction and purification of the vesicles were initially made in the R20291 and the other NAP-1 strain. Supernatants from stationary phase cultures were obtained by centrifugation and ultra-filtration. Fractions containing EVs were separated by differential centrifugation in an Optiprep gradient. The fractions obtained were analyzed by transmission electron microscopy. The result shows the presence of high quantity of vesicles, with a diameter between 20 and 400 nm. SDS PAGE followed by silver staining showed a complex protein profile of the EV fractions. To detect the presence of toxins in the EVs, a commercial ELISA kit (r-biopharm) and cytotoxicity assays with Vero cells were performed. The result of the microscopy identified these vesicles in CTI, R20291 and the other NAP-1 strain, ANA#2004016. The ELISA test was positive with the ANA#2004016 strain, but the cytotoxicity assay was inconclusive in R20291. Further experiments are being conducted to determine the presence of DNA fragments in the EVs. This study reveals a new and important pathogenicity mechanism of *C. difficile*. Furthermore, vesicles and its products might be useful targets for vaccines since previously studies utilized that structure as a possible inductor of the immune response.

Keywords: *Clostridioides difficile*, Extracellular vesicles, Ribotype 027

Development Agencies: PIBIC-UFRJ/CNPq, CNPq, FAPERJ, CAPES