Título: EXOPROTEOME OF *Clostridium difficile* STRAINS TREATED WITH SUB INHIBITORY CONCENTRATIONS OF ANTIBIOTICS

Autores: Trindade, C.N.R.[1]; Moura, H.[2]; Barr, J.R.[2]; Miyajima, F.[4]; Ferreira, T.G.[1;] Domingues, R. M. C. P.[1]; Ferreira, E.O[1,3].

Instituição: [1]Laboratório de Biologia de Anaeróbios, Departamento de Microbiologia Médica, Instituto de Microbiologia Paulo de Góes, UFRJ/Brasil; [2]Division of Laboratory Sciences, NCEH, CDC/USA; [3]UFRJ-Polo Xerém/Brasil; [4] Medical Research Council University of Liverpool, Liverpool, UK.

Resumo:

Clostridium difficile is considered the major etiological agent of bacterial diarrhea associated with antibiotic use, thus being an important nosocomial pathogen and a significant cause of morbidity and mortality. The major virulence factors of *C. difficile* are two toxins - A and B, and several studies have examined the impact caused by sub inhibitory concentrations of antibiotics on these toxins. However, there are few studies about the interference of antibiotics on expression of secreted proteins. Thus, the aim of this work is to identify and compare the exoproteomes of two exclusive brazilian *C. difficile* ribotypes, RT133 and RT135, comparing with worldwide circulating ribotypes 014 and 027 (NAP1), when grown under sub lethal concentrations of clindamycin and levofloxacin. All strains were grown in 40 mL of BHI-PRAS and after the 18 h, the culture supernatants (CS) were concentrated and trypsinized using on-filter digestion (Spin filter Millipore). In addition, CS were concentrated and used for SDS-PAGE analysis. Selected bands that showed difference between the conditions were excised from the gel. All proteins were processed for analysis by nLC-ESI-MS/MS mass spectrometry/Orbitrap (MS). All these proteins will be identified by MS using a Dynabeads-proteinG system. So far, approximately 145 proteins were identified by MS for each strain and condition using the on-filter digestion. Lable-free quantification revealed variable amounts of a number of proteins, in special precursor S-layer, chaperonin, acyl-CoA dehydrogenase, cell surface, heat chock, rubrerythrin and molecular chaperone DnaK. The analysis suggests that clindamycin had a stronger effect in protein expression at least 10 folds for some proteins in most of the strains, such as, the S-layer precursor, chaperones, cell surface, cold shock and cell wall hydrolases proteins. Levofloxacin also induced the expression of ferrodoxin, aldolases, Co-A transferase, glyceraldeyde phosphate proteins. The 014 ribotype was the only strain, which showed an exclusive protein under the presence of clindamycin, a phospholipase C, which was previously described in most of virulent strains in *C. perfringes*. Future data analysis is underway by using Scaffold and Blas2Go analysis. We believe this work will provide new virulence factors that help to bring knowledge to *C. difficile* pathogenicity and also explain the epidemiology and involvement of RT133 and RT135 in clinical cases in brazilian hospitals.

Palavras-chaves: *Clostridium difficile*, exoproteome, mass spectrometry

Agência Fomento: Faperj, CNPq e CAPES