

Session: OS087 The role of the microbiome in pathogenesis

Category: 9b. Host-pathogen interaction

23 April 2017, 15:18 - 15:28
OS0455

How *Clostridium difficile* impacts gut microbiota in vitro

Sabina Zalig¹, Aleksander Mahnic², Maja Rupnik^{*3}

¹Faculty of Medicine, University of Maribor

²National Laboratory for Health, Environment and Food, Nizoh

³National Laboratory of Health, Environment and Food

Background: *Clostridium difficile* infection (CDI) generally occurs only in subjects whose normal gut microbiota has been disrupted, usually due to treatment with antibiotics. Mechanisms through which gut microbiota combats *C. difficile* are being partially elucidated, however not much is known about the possible effect of *C. difficile* on gut microbiota during colonization. It is very likely that expanded *C. difficile* population after antibiotic treatment affects the reconstitution of gut microbiota (e.g. via excreted metabolites or nutrient consumption). To explore this possibility we analyzed the differences in bacterial community structure of fecal microbiota after *in vitro* growth in spent medium from different *C. difficile* strains.

Material/methods: Six *C. difficile* strains, belonging to two PCR ribotypes (014/020, 027), were incubated anaerobically for 48 hours in two different culture media to obtain spent media for subsequent fecal microbiota culturing. Samples were taken after 3 and 5 day incubation period and total bacterial DNA was extracted. Bacterial community composition was determined by paired-end sequencing on Illumina MiSeq platform, targeting V3-V4 hypervariable region of the 16S rRNA gene. Mothur (version 1.36.1) was used to analyze raw sequence data and to calculate alpha and beta diversity indices. Hierarchical clustering and NMDS analysis were done in R (version 2.6.2) using pvclust and vegan package, respectively.

Results: A marked decrease of microbial diversity was observed in spent medium of both tested ribotypes compared to freshly prepared control medium. The majority of changes occurred within the phylum *Firmicutes*. Compared to the control, both ribotypes were associated with a decreased proportion of *Lactobacillus* and an increased proportion of *Enterococcus*. Despite distinction between different *C. difficile* ribotypes used to prepare spent medium, analysis of genera responsible for this

clustering did not show elevated deleterious effect of ribotype 027 in comparison to ribotype 014/020 as suggested previously.

Conclusions: Our findings suggest that *C. difficile* could modulate the composition of gut microbiota during its reestablishment with most prominent effect on decrease of genus related to probiotic strains (*Lactobacillus*) and increase of genus related to antibiotic resistant bacteria (i.e. vancomycin-resistant enterococci).