C. DIFFICILE GROWTH AND CYTOTOXICITY ASSOCIATED BACTERIAL SIGNATURES IN THE *IN VITRO* MODULATED GUT MICROBIOTA

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Introduction

The importance of gut microbiota in *C. difficile* infection is well established (Samarkos et al., 2018), nonetheless the exact mechanisms of colonization resistance against *C. difficile* overgrowth and factors influencing toxin activity remain largely unclear. Here we present microbial patterns associated with *C. difficile* growth and citotoxicity after *in vitro* fecal microbial community modulation in mini-bioreactor arrays (MBRA; Auchtung et al., 2015).

Results

Modulation with clindamycin and/or polyphenol extracts resulted in unique microbial communities (Fig 2 A, B).
However, all conditions except non-modulated control resulted in a successful overgrowth of *C. difficile* while cytotoxicity varied significantly among conditions (Fig 3). Only in a single condition we observed decreased cytotoxicity despite successful *C. difficile* growth.
Using 16S metagenomic analysis we were able to correlate three bacterial taxons (*C. sporogenes, C. oroticum* and *Blautia sp.*) with decreased cytotoxicity. Representatives were selectively isolated from bioreactor slurry and used in co-cultures with *C. difficile.* We showed decreased cytotoxicity in the presence of *C. sporogenes* without a large effect on *C. difficile* growth (Fig 4).

Conclusion

- In an i*n vitro gut* model both clindamycin as well as polyphenols modulate fecal microbial community towards the loss of resistance against *C. difficile* colonization.
- Modulation with clindamycin resulted in an overall higher *C. difficile* cell concentration and cytotoxicity.
- Microbial community modulated with pomegranate extract did not inhibit *C. difficile* growth but significantly decreased cytotoxicity.
- Among three differentially represented bacterial taxa coinciding with decreased citotoxicity only *C. sporogenes* reproduced comparable effect in an *in vitro* batch co-culture model *with C. difficile.*

Figures

Fig 1: Schematic presentation of experiment design and different tests that were performed.

Fig 2: 16S metagenomic analysis of bacterial communities in MBRA at different time points. NMDS plots show Bray-Curtis distances between samples highlighting combination of modulating factors (A) and changes in community structure by time (B). Abbreviations on identifiers are as followed: C – control, P – pomegranate, B – blueberry, Cli – clindamycin, 100 or 400 – polyphenol extract concentration in mg/L. Community richness (Chao1; C) and diversity (Shannon, D) are shown separately for each polyphenol condition.

Fig 3: *C. difficile* growth (A) and cytotoxicity test performed with HT-29 (B) and Vero cell line (C) are shown separately for each polyphenol condition.

Fig 4: Relative cytotoxicity per *C. difficile* CFU shown for *C. difficile* control and different combinations of co-culture between selected strains and *C. difficile*.

Methods

Antibiotic clindamycin and polyphenol extracts from pomegranate and blueberries were used alone and in combinations to modulate fecal microbial community in the MBRA (mini-bioreactor arrays). Modulated communities were then inoculated with *C. difficile* vegetative cells (ribotype 027) followed by a 7-day periodical monitoring of *C. difficile* growth (plating on selective media), cytotoxicity of toxin A and B (cytotoxicity assay with HT-29 and Vero cell line, respectively) and bacterial community composition. 16S metagenomes were acquired by paired-end sequencing on Illumina MiSeq platform targeting V3-V4 hypervariable region of the bacterial 16S rRNA gene. Sequence reads were analyzed in mothur (v 1.36.1) (Schloss et al., 2009). Co-culturing was performed in simple batch bioreactors (5 mL) by co-culturing *C. difficile* and selected strains in WCAB medium for 24 hours. *C. difficile* growth and cytotoxicity were tested as described above.

Literature

- Auchtung, J. M., Robinson, C. D. & Britton, R. A. Cultivation of stable, reproducible microbial communities from different fecal donors using minibioreactor arrays (MBRAs). Microbiome 3, (2015).
- Samarkos M, Mastrogianni E, Kampouropoulou O. The role of gut microbiota in Clostridium difficile infection. European Journal of Internal Medicine. 2018 Apr;50:28–32.
- Schloss, P.D., Westcott, S.L., Ryabin, et al. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541.

Fig 1

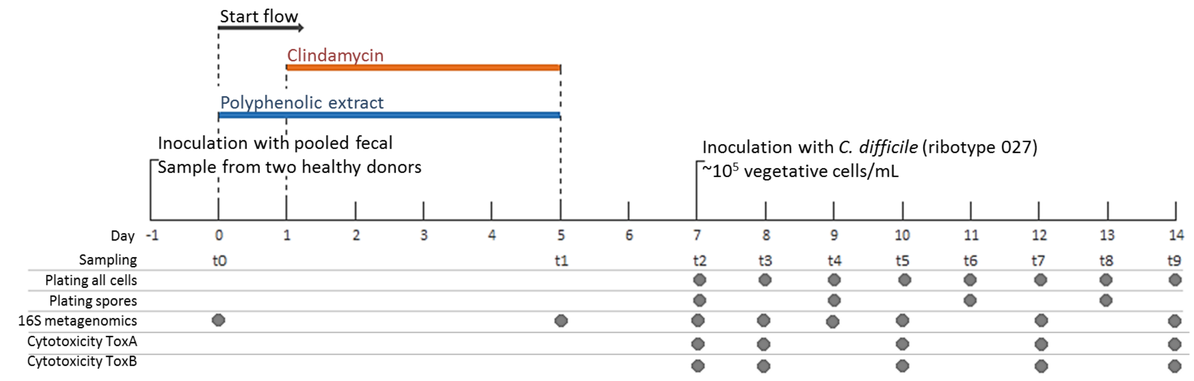

Fig 2

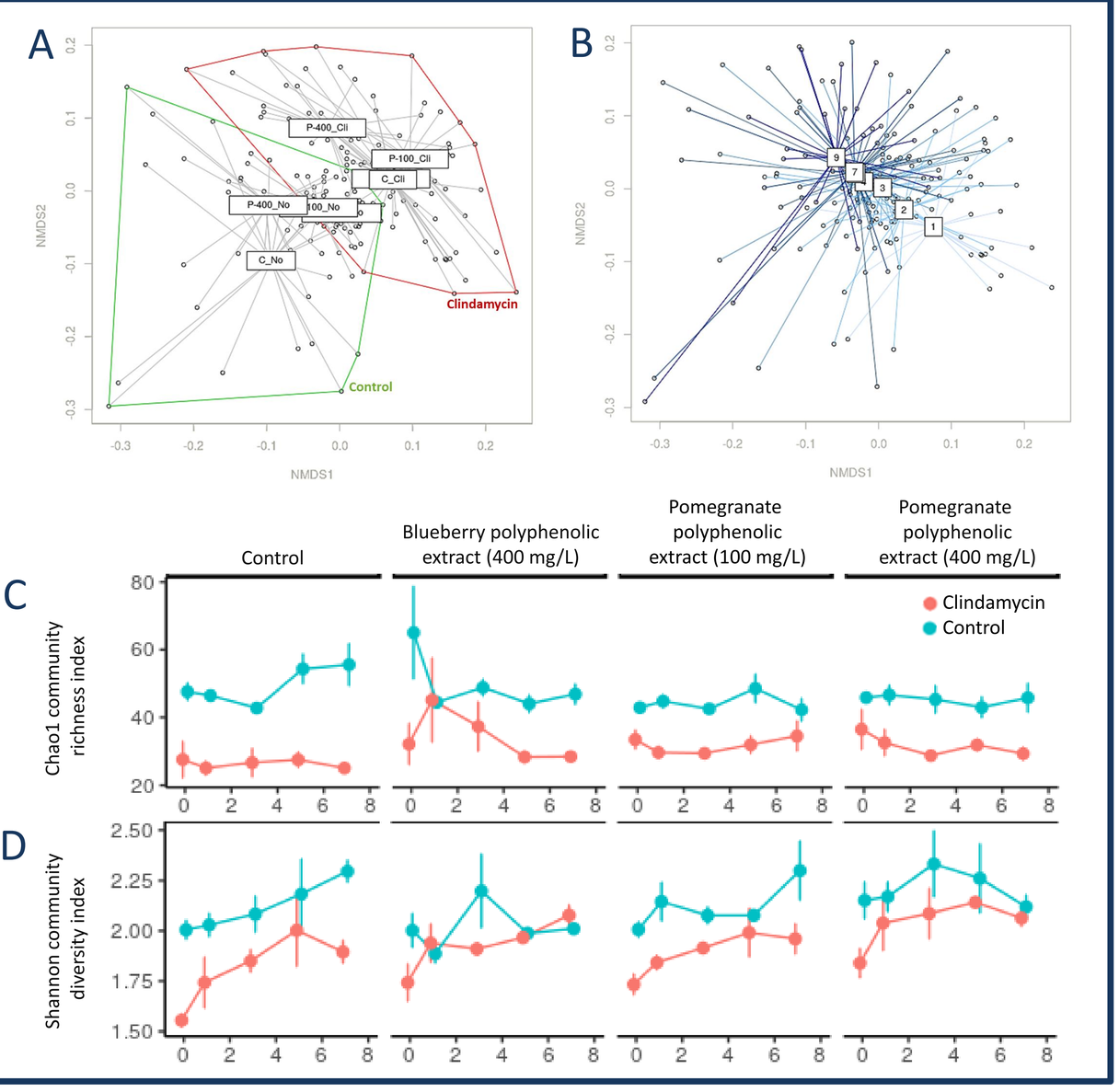

Fig 3

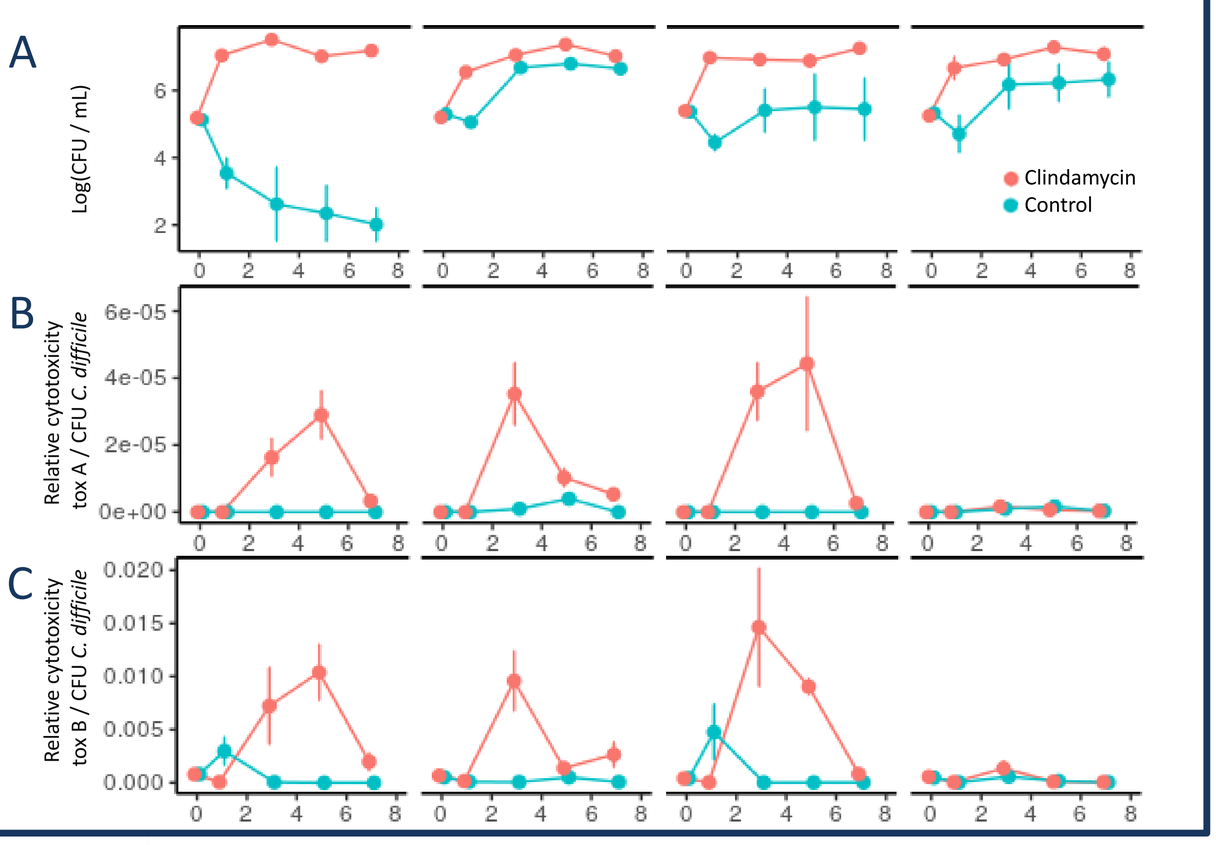

Fig 4