DIVERSE EFFECTS ON *in vitro* COLONIZATION RESISTANCE AGAINST *Clostridioides difficile* AFTER COMBINED ANTIBIOTIC/PROBIOTIC INTAKE

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BACKGROUND

Antibiotic therapy disrupts gut microbiota[1] and in our previous studies we have shown that dysbiotic fecal microbiota *in vitro* enhances *C. difficile* growth and sporulation.[2] However, in these studies the dysbiotic and healthy microbiota originated from different individuals and the reasons for dysbiosis were not known. In the present study we aimed to test paired samples collected before and after the prophylactic rifampicin + tetracycline therapy in combination with the commercially available probiotic (OMNiBiOTiC® 10 AAD, Institut Allergosan).

CONCLUSIONS

- Post-treatment (antibiotic/probiotic combination) fecal samples showed increased colonization resistance against *C. difficile in vitro*.
- Although further testing is required, we speculate that the increased colonization resistance against *C. difficile* in the post-treatment samples was the consequence of the consumed probiotic.

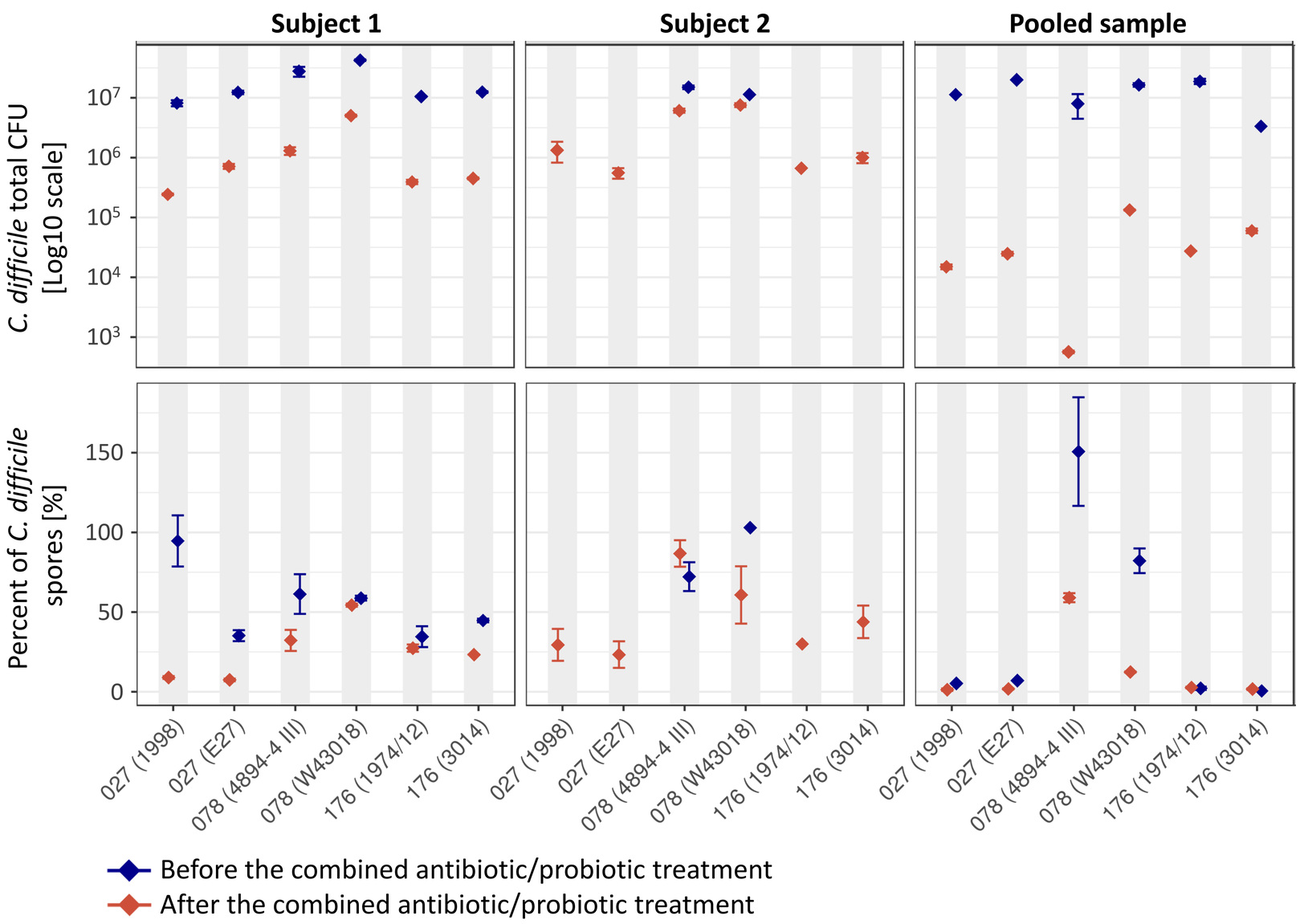

Figure 1: ***C. difficile in vitro* growth and sporulation in co-culture with fecal communities sampled before and after combined antibiotic/probiotic treatment**. Six *C. difficile* strains (three different ribotypes, denoted in the name of the strain) were cultivated in the batch model inoculated with fecal samples from subject before (blue) and after antibiotic/probiotic intake (orange). *C. difficile* CFU and percent of spores after 3-day cultivation are presented as mean value with standard error bars.

RESULTS

We hypothesized that the post-treatment fecal samples will show dysbiotic features because of the exposure to the antibiotic, and will consequently result in the increased *C. difficile* growth and increased sporulation. Surprisingly, many of the tested strains grew and sporulated at a higher rate in the pre-treatment samples with some differences between the individual strains (**Figure 1**).

Colonization resistance was significantly stronger when three fecal samples were pooled together (Pooled sample) compared to the treatments where a single stool samples was inoculated into the *in vitro* model.

Table 1: *C. difficile* strains used in the study.

Strain designation	PCR ribotype	Toksinotype	Source of isolation
1998	027	III (A+ B+ CDT+)	Human
E27	027	III (A+ B+ CDT+)	Human
W43018	078	V (A+ B+ CDT+)	Human
4894-4 III	078	V (A+ B+ CDT+)	Cattle
3014	176	IIIb (A+ B+ CDT+)	Human
1974/12	176	IIIb (A+ B+ CDT+)	Human

METHODS

Three volunteers donated fecal sample twice, ie. before combined antibiotic/probiotic intake and three days after the therapy. Both types of fecal microbiota were inoculated with six *C. difficile* strains belonging to three different ribotypes (027, 176 and 078) (**Table 1**). After 3-day incubation in an *in vitro* batch model we assessed *C. difficile* growth and sporulation frequency after plating on selective media (CHROM ID CDIFF, BioMerieux) with or without ethanol shock. Pooled sample consisted of all three samples pooled at the equal ratio. The results for the third individual sample were not yet available at the time of making this poster.

REFERENCES

1. Pilmis, B., Le Monnier, A., and Zahar, J.-R. (2020). Gut microbiota, antibiotic therapy and antimicrobial resistance: A narrative review. Microorganisms *8*.
2. Horvat, S., Rupnik, M. (2018). Interactions between *Clostridioides difficile* and fecal microbiota in *in vitro* batch model : growth, sporulation, and microbiota changes. Frontiers in microbiology, 9, 1-10.