

BACKGROUND

Clostridioides difficile is a microorganism responsible for potentially life-threatening diarrheal illness in individuals with an unhealthy mixture of gut bacteria, known as dysbiosis. Moreover, it causes recurrent infections (recurrent *C. difficile* Infection, rCDI) in nearly a third of infected individuals. The traditional treatment of rCDI was based on chemotherapy (Vancomycin, Metronidazole, Fidaxomicin). Besides these, a promising tool for remodulation of underlying dysbiosis in rCDI is represented by Fecal Microbiota Transplantation (FMT): a successful engraftment can be assessed using Next Generation Sequencing (NGS) approaches.

The aim of this work was a retrospective analysis of recipient's microbiota evolution from FMT procedure until 60 days, comparing them towards donor's microbial gut composition.

METHODS

59 stool samples collected for FMT procedure in the period April 2022 - October 2023 were analyzed in the present work. They were divided in the following subsets: donors (n=11), fecal material prepared for FMT infusion (n=6), recipients immediately before the transplant (n=17), recipients after 7 days (n=12), 30 days (n=8) and 60 days (n=5) from FMT procedure. Bacterial genomes were extracted with Qiaamp DNA mini kit (Qiagen, Germany) onto automatic instrument Qiacube (Qiagen, Germany). Then, NGS run was performed with AD4SEQ Microbiota solution B (Arrow Diagnostics, Italy) for evaluation of hypervariable regions V3, V4, V6, on Illumina Iseq100 platform (Illumina, USA). Finally, raw data were analyzed at phylum level with the MicrobAT stand-alone software (SmartSeq, Italy).

RESULTS

At phylum level the donor and the fecal material prepared for FMT infusion showed a prevalence of Bacteroidetes (47.55% ± 12.99 and 42.27% ± 8.48) and Firmicutes (42.28% ± 13.19 and 49.08% ± 10.33). Proteobacteria were respectively 4.24% ± 7.53 and 1.17% ± 0.72. Otherwise, the FMT recipient at before infusion showed a reduction of both Bacteroidetes (23.73% ± 24.81) and Firmicutes (33.16% ± 18.26), while Proteobacteria increased significantly (27.16% ± 23.23) (**Figure 1**).

After one week from FMT procedure, the average Bacteroidetes richness reached to 51.03% ± 11.07, remaining stable until 60 days (58.29% ± 17.67). At the same time Proteobacteria richness was reduced to 10.56% ± 11.56 at 7 days and 1.43% ± 1.13 at two months. Firmicutes showed a reduced increase during observational period (7 days: 34.5% ± 16.26; 60 days: 38.13% ± 16.96) (**Figure 1**).

Focusing on genus level, recipients showed a median reduction in the abundance of microorganisms with protective effects compared to donors (*Akkermansia* spp.: 0.06 vs 1.14%; *Blautia* spp.: 0.04 vs 0.67%; *Faecalibacterium* spp.: 0.14 vs 9.78%). After 60 days from FMT procedure, only *Faecalibacterium* spp. significantly increased (10.6%), while *Blautia* spp. and *Akkermansia* spp. showed a minimal or no variation in their abundance (0.38% and % and 0.05%, respectively) (**Figure 2**).

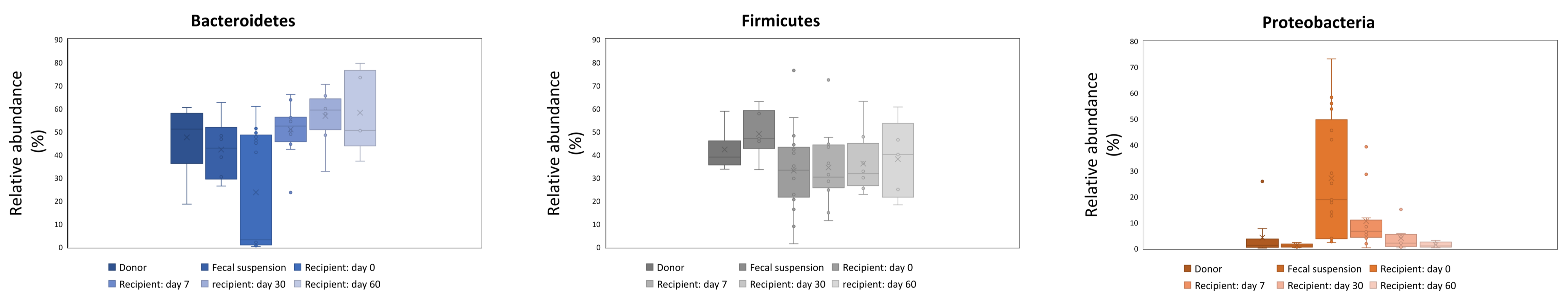


Figure 1. Relative abundance of the most represented phyla in donors and recipients.

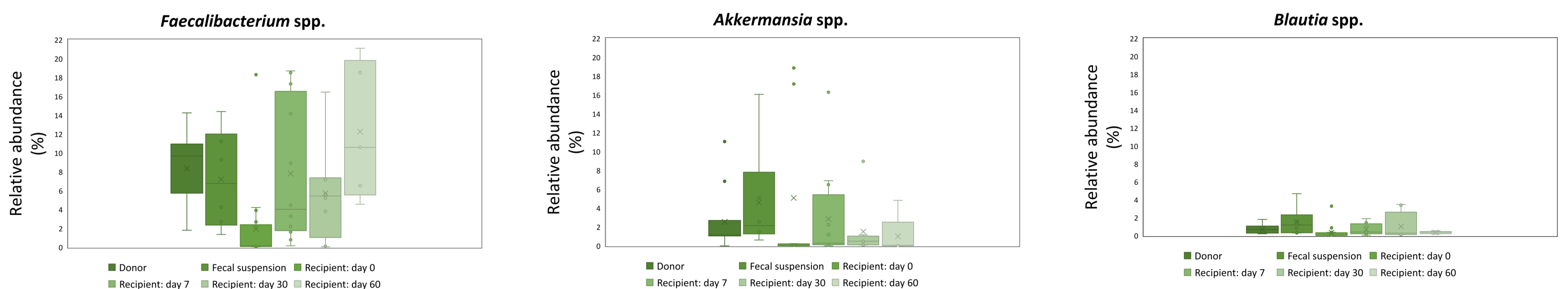


Figure 2. Relative abundance of microbial genus with potential protective effects in donors and recipients.

CONCLUSIONS

Until 60 days from FMT procedure, recipient gut microbiota showed a significant remodulation reaching a similar composition to those of donor at phylum level. Moreover, rCDI was resolved without need of antimicrobial therapies.