Decolonization of multi-drug resistant bacteria by fecal microbiota transplantation in five pediatric patients before allogeneic hematopoietic stem cell transplantation: gut microbiota profiling, infectious and clinical outcomes

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Supplementary Material

Post-HSCT anti-infectious prophylaxis/treatment strategy

As per institutional policy, broad-spectrum antibiotic prophylaxis with piperacilline/tazobactam was administered when neutrophils reached a value <500/µl and continued till sustained neutrophil engraftment after HSCT; anti-fungal agents was used, as well. In case of fever developing during aplasia, antibiotics active against bacteria isolated from stools were also administered.

MDR bacteria surveillance

Microbiological surveillance was performed through weekly cultures of stools inoculated on MacConkey medium (Biomerieux, Marcy l'Etolie, France) added by meropenem at 10 ug/ml. Identification of colonies was performed by MALDI-TOF MS.(1) Enterobacteriaceae with meropenem MIC >0.5 ug/ml were selected by Vitek 2 (Biomerieux) and confirmed by concentration gradient, according to EUCAST (www.eucast.org). Selected strains were subjected to synergy tests, modified Hodge tests (2) and real-time PCR Xpert Carba-R (Cepheid, Sunnyvale, CA, USA) to detect *bla* gene encoding for KP carbapenemase (bla_{KPC}), New Delhi metallo-beta-lactamase (bla_{NDM}), Verona imipenenase (bla_{VIM}), oxacillinase ($bla_{OXA\text{-}48\text{-}48\text{-}181\text{-}232}$) and imipenenase ($bla_{IMP\text{-}1}$) gene alleles.

Gut microbiota profiling

DNA from stools was manually extracted using QIAmp Fast DNA Stool mini kit (Qiagen, Germany). Bacterial libraries were obtained by amplifying V3-V4 region from 16S rRNA gene (~460 bp), following the MiSeq rRNA Amplicon Sequencing protocol (Illumina, San Diego, CA). Raw sequences were analyzed using QIIME 1.9.1 software pipeline (3) from quality check to computation of α and β diversity. Reads were clustered into Operational Taxonomic Units (OTUs) at 97% identity.

Taxonomy was assigned using UCLUST against the Greengenes 13.8 database at 97% sequence similarity.(4)

Stool samples of healthy children and adults were collected for age-matched microbiota profiling for comparison. Healthy children were recruited at primary and secondary schools through pediatricians and healthy adults were recruited among health workers and researchers of OPBG (EC Protocol N° 768.12).

Gut Microbiota ecology

Before FMT (T_0), the gut microbiota of Patient 1 was mainly composed by Enterobacteriaceae, Lachnospiraceae, *Blautia*, *Lactobacillus*, Enterococcaceae, and *Staphylococcus*. At day 1 T_1 after FMT, Enterobacteriaceae increased, colonizing up to 72% of the entire microbiota composition (**Figure 2**). At day 7 (T_3), donor species, such as *Parabacteroides*, *Dorea*, *Bacteroides*, Ruminococcaceae appeared in the microbiota of the recipient, while Enterobacteriaceae, *Lactobacillus* and Enterococcaceae were strongly reduced. Alpha diversity analysis confirmed a low bacterial richness at points T_0 and T_1 compared to the donor and to the T_3 point (**Figure 2**). In addition, beta diversity PCoA plot showed similarity between T_0 and T_1 and between UD and T_3, the latter resulting closer to the CTRL group (**supplementary Figure 1**).

The gut microbiota composition of Patient 2 at day 3 (T_2) from FMT resembled the microbiota of UD for the presence of Lachnospiraceae, Ruminococcaceae, *Faecalibacterium prausnitzii*, *Bacteroides*, and *Dorea*. Furthermore, the recipient sample was also composed of *Akkermansia muciniphila* and Enterobacteriaceae. At day 7 (T_3), a reduction of Enterobacteriaceae and *A. muciniphila* and an increment of *Bacteroides fragilis* were found, with a consequent reduction of microbiota richness compared to UD and T_3 recipient samples. At day 10 (T_4), Ruminococcaceae and *F. prausnitzii* increased to 20% and 38%, respectively, resulting in an increased bacterial richness. Beta diversity

analyses showed a microbiota similarity for samples of UD, T_2, T_3 and T_4 patient time points (**supplementary Figure 1**).

On subsequent follow-up, points T_5 (day 18), and T_6 (day 25) showed a marked distance compared to the donor and to T_2, T_3 and T_4 points, while T_7 appeared separated from the entire sample set. Alpha diversity analyses highlighted an increasing richness, starting from T_5 to T_7, the latter being the highest in the sample group.

At T_3 after FMT, more than 80% of the entire gut microbiota of Patient 3 was composed by Enterobacteriaceae, Lachnospiraceae, Ruminococcaceae, *Ruminococcus*, *Dorea*, *Collinsella aerofaciens*, *Dialister*, *Bifidobacterium longum*, Clostridiaceae, *Ruminococcus gnavus*, and *Eggerthella lenta*. The alpha diversity value was similar to the donor value (**Figure 2**). The beta diversity disclosed a short distance of the T_3 sample from UD and pediatric CTRL group (**supplementary Figure 1**). The absence of T_0 and following time-point samples does not allow to get more detailed information on the shaping of microbiota after FMT.

The gut microbiota of Patient 4, at T_0 was mainly composed of Lachnospiraceae, *Oscillospira*, *Bacteroides* and *B. ovatus*. After FMT (T_1), *Bacteroides*, *B. ovatus* and Enterobacteriaceae grew up. At T_3, Bacteroidetes phylum composed the 79% of the microbiota, while at T_4, the Bacteroidetes/Firmicutes ratio was inverted. At T_5, *A. muciniphila* increased, reaching the 20% of gut microbiota composition. Points T_6 and T_7 were proximate. Microbiota richness decreased from T_0 to T_1 and then slowly increased to T_7, the highest value observed within the entire set, a pattern similar to the UD value (**Figure 2**). Beta diversity showed short distance between samples, and a shorter distance among UD and T_3, T_4, T_5, T_6 and T_7, compared to T_0 and T_1 (**supplementary Figure 1**).

The 97% of gut microbiota of Patient 5 at T_0 was composed of Enterobacteriaceae. The day after FMT, Enterobacteriaceae lowered to the 86% of entire microbiota composition. After a month (T_7), Enterobacteriaceae increased to 99% of the microbiota. The Shannon indexes were lower compared to UD, decreasing over time (**Figure 2**). Beta diversity PCoA plot revealed a close distance between

T_0, T_1 and T_7 points, and a close distance between UD and adult CTRL group (**supplementary Figure 1**).

References

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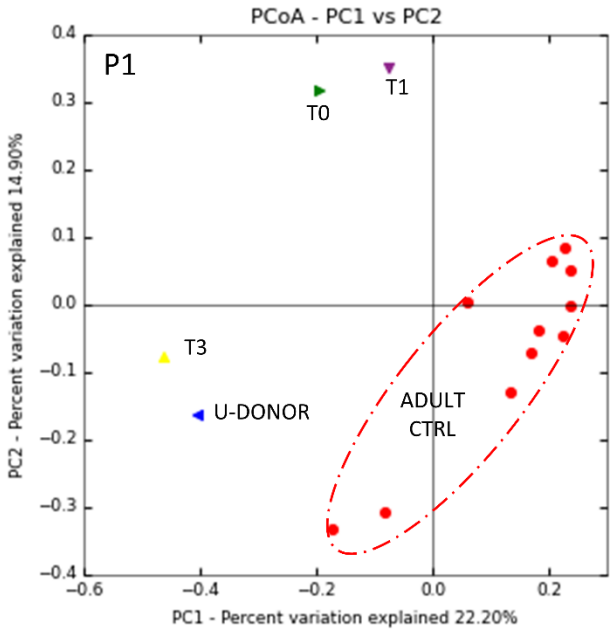
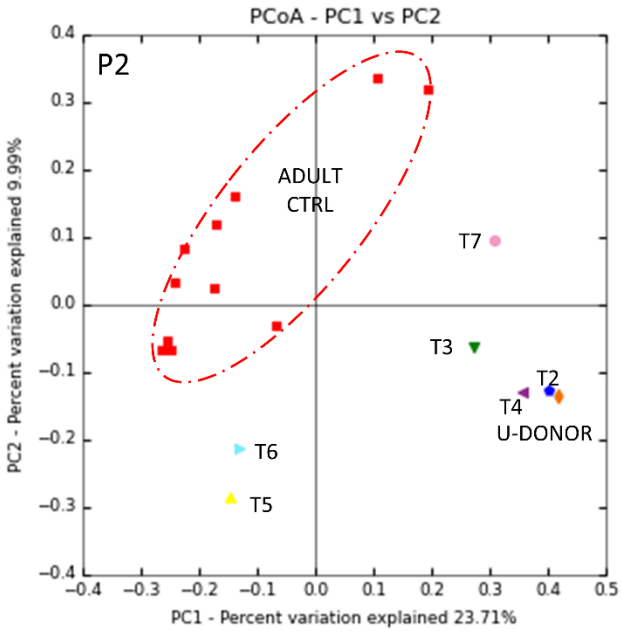
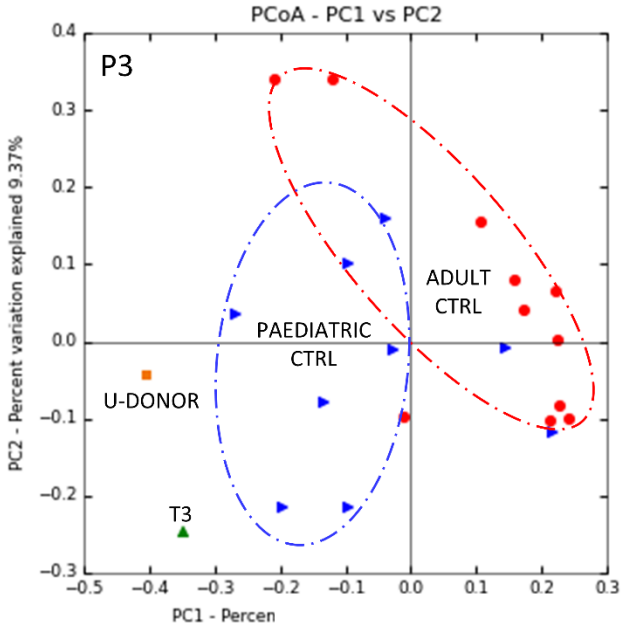
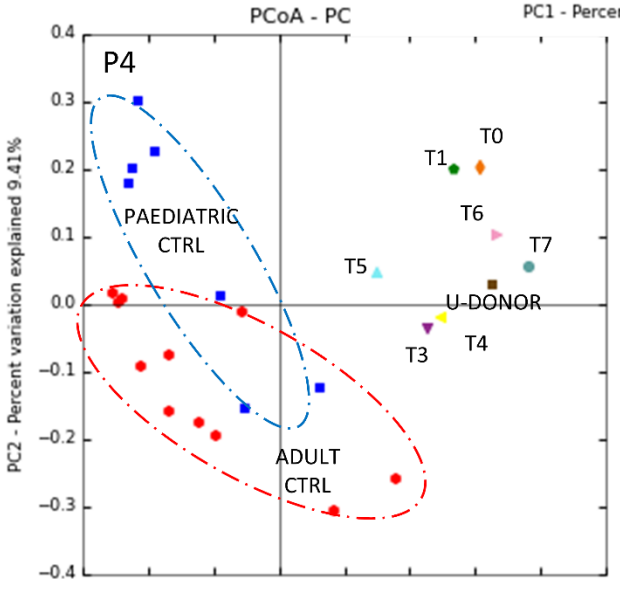
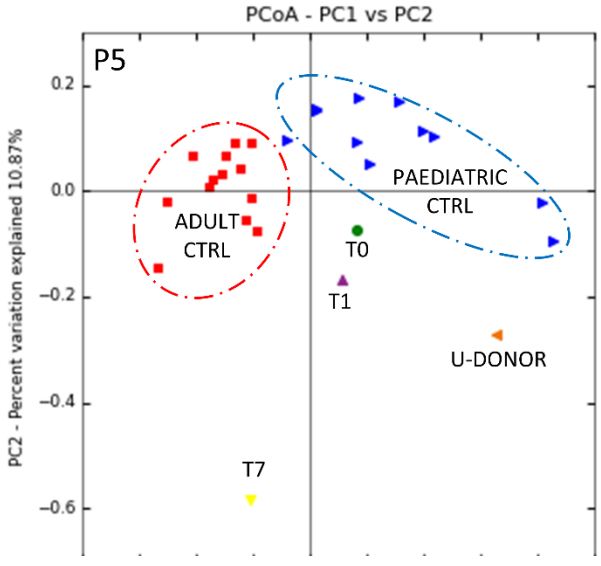

PCoA - PC1 vs PC2
P1
T0
T1
T3
U-DONOR
ADULT
CTRL
PC2 - Percent variation explained 14.90%
PC1 - Percent variation explained 22.20%


PCoA - PC1 vs PC2
P2
ADULT
CTRL
T7
T3
T2
T4
U-DONOR
T6
T5
PC2 - Percent variation explained 9.99%
PC1 - Percent variation explained 23.71%


PCoA - PC1 vs PC2
P3
ADULT
CTRL
PAEDIATRIC
CTRL
U-DONOR
T3
PC2 - Percent variation explained 9.37%
PC1 - Percen


PCoA - PC
P4
T0
T1
T6
T7
T5
U-DONOR
T3
T4
PAEDIATRIC
CTRL
ADULT
CTRL
PC2 - Percent variation explained 9.41%


PCoA - PC1 vs PC2
P5
PAEDIATRIC
CTRL
ADULT
CTRL
T0
T1
U-DONOR
T7
PC2 - Percent variation explained 10.87%

Supplementary Figure 1. Beta diversity of P1-P5 patients represented by PCoA plots. The PCoA plots report the beta diversity distant values for each patient time-point, UD and control groups (red circle, adult CTRL, blue circle, paediatric CTRL), calculated by Unweighted Unfrac metric.