**Supplementary appendix**

**Fecal Microbiota Transplantation in Patients with Blood Disorders Inhibits Gut Colonization with Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study**

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**SUPPLEMENTARY METHODS**

**STUDY POPULATION**

**Inclusion criteria:**

1. Aged > 18 years.
2. Colonization of the GI tract with ARB, particularly CPE (including those with genetic resistance mechanisms against carbapenem), extended-spectrum -lactamase positive (ESBL+) *Enterobacteriaceae*, vancomycin-resistant *Enterococcus faecalis* and *Enterococcus* *faecium* (VRE), and other bacteria with documented resistance to at least two classes of antibiotics. These ARB must be documented by at least two positive cultures of material from rectal swabs taken <2 weeks before FMT.
3. Absolute neutrophil count on the day of FMT >5 × 108 neutrophils/L.

**Exclusion criteria:**

1. Lack of consent or lack of logical contact.
2. An absolute neutrophil count on the day of FMT < 5 × 108 neutrophils/L or an expected decrease in the count within the following 2 days.
3. Planned use of strong myelosuppressive chemotherapy (e.g., dexamethasone, high-dose arabinofuranosyl cytidine and platinol [DHAP]; ifosfamide, carboplatin and etoposide [ICE]; etoposide, solumedrol, high-dose arabinofuranosyl cytidine and platinol [ESHAP]; high-dose cyclophosphamide [H-Cy]; high-dose arabinofuranosyl cytidine [HD Ara-C]; daunorubicin and arabinofuranosyl cytidine [DA]; bleomycin, etoposide, adriamycin, cyclophosphamide, oncovin, procarbazine and prednisone [BEACOPP]; or chemotherapy prior to receiving hematopoietic cell transplantation) <2 days after FMT.
4. Underwent hematopoietic cell transplantation <one month ago.
5. Clinical signs of mucositis (excluding symptoms of graft-versus-host disease).
6. Incapacitation, conscription into the military, imprisonment, or dependent on business with or any other form of dependency on the researchers.
7. Severe hepatic impairment.
8. Requirement for intensive antimicrobial therapy.

**MICROBIOLOGICAL ASSESSMENT**

The potential participants were routinely screened for gut colonization by the following ARB: methicillin-resistant *S. aureus* (MRSA), VRE, ESBL+ *Enterobacteriaceae* and CPE. Colonization status was ascertained by the microbiological examination of rectal swabs cultured on one of the following types of chromogenic media: MRSA Agar (Graso, Gdańsk, Poland), VRE Agar (Graso), chromID®ESBL (bioMérieux S.A., Marcy l’Etoile, France), and CHROMAgar KPC (Graso). Pathogens were inoculated on Columbia Agar plates that contained 5% sheep blood (Becton, Dickinson, Franklin Lakes, NJ, USA), and identified using matrix-assisted laser desorption/ionization (MALDI) (Microflex; Bruker Daltonik GmvH, Bremen, Germany). The presence of MRSA, VRE, ESBL+ *Enterobacteriaceae* and CPE was verified using phenotypic methods, in accordance with the national recommendations in Poland [1-3].

Additionally, the ability of isolates to produce carbapenemases (metallo*-*β-lactamase [MBL], K. *pneumoniae* carbapenemase [KPC] and extended-spectrum oxacillinase-48 [OXA-48]) was investigated using the Rapidec® Carba NP biochemical assay (bioMérieux S.A.) and/or the GeneXpert® quantitative real-time PCR method (Cepheid, Sunnyvale, CA, USA). The identified pathogens were designated as ARB [4]. Colonization was defined as the detection of an ARB in at least two consecutive rectal swabs.

**DONOR AND FECAL MATERIAL ASSESSMENT**

Fecal material from highly selected donors who were unrelated to the recipient was used.

**Donor recruitment**

Each donor signed an informed written consent form that stated that he or she agreed to undergo clinical checkups and to provide fecal samples for a particular recipient. The donors’ personal data were generally unavailable to the recipient, except when the donors were willing to disclose their data. In these cases, the donors signed an informed consent form and the data were only exchanged after the end of the treatment.

Potential donors were excluded from donating if the clinician who carried out the donor screening procedure obtained information that indicated that the donor had an increased risk of infectious diseases in the phase between screening and the donation of feces (such as a recent visit to a tropical area in the last three months, risky sexual behavior defined as a new sexual contact in the last six months, recent needle stick accident, receiving blood products, or getting a tattoo); any GI condition or symptoms (abdominal discomfort, frequent loose stools, or constipation); a family history of intestinal cancer or inflammatory bowel disease; any other condition that required the use of medication that could be excreted in feces and subsequently pose a potential risk to the participants.

The donors underwent extensive testing as follows:

A. Blood tests:

* Hepatitis A virus (HAV; anti-HAV IgM and IgG);
* Hepatitis B virus (HBV; HBsAg, anti-hepatitis B core antigen [HBc], HBV DNA);
* Hepatitis C virus (HCV; anti-HCV antibodies, HCV RNA);
* Human immunodeficiency virus (HIV; anti-HIV antibodies, HIV RNA, HIV proviral DNA);
* Syphilis (serology);
* Cytomegalovirus (CMV; anti-CVM IgM and IgG);
* Epstein–Barr virus (EBV; anti-EBV IgM and IgG).

B. Fecal sample tests:

* Parasites (microscopic examination);
* *C. difficile* toxin A/B (ELISA or equivalent);
* Enteropathogenic microflora (classical culture).

The donor also had to meet additional requirements:

* three months without antibiotic treatment;
* generally good health, with no autoimmune or metabolic diseases (based on an interview and clinical examination);
* ordinary diet;
* no relationship (or any permanent contact) with the recipient.

**Preparation of FMT product**

Each donor donated a fecal sample up to 2 h prior to FMT or 1 h prior to the sample being processed and frozen:

* Each donor placed a 10-cm3 fecal sample into a sterile disposable container in sterile conditions (at home or in a medical facility) after being given precise instructions by medical personnel on personal hygiene before defecation and during the handling of the sample;
* The sample was immediately forwarded to the Department of Microbiology;
* Only samples of formed stool (not loose) were processed further.

Aseptic procedures were carried out at the Department of Microbiology:

* to prepare each FMT product, 100 g feces was suspended in 100 ml sterile saline in a sterile test tube;
* the suspension was homogenized for 2–4 min;
* slow filtration through a sterile sieve, double gauze, or another suitable sterile filter was carried out;
* the filtrate was diluted with sterile saline to a volume of 100 ml in a sterile tube, homogenized slowly, and filtered as before;
* the filtrate was diluted with sterile saline to a final volume of 200 ml and it was divided into two 100 ml syringes (each syringe contained 50 g feces diluted to a total volume of 100 ml with sterile saline – total 100g/200ml);
* FMT products were then immediately transferred to the treatment room to administer them to the recipient (fresh material was preferred on the purposes of our study).

**FMT**

**Preparation of the recipient prior to FMT**

Informed written consent was obtained. A proton pump inhibitor (PPI) was administered orally or intravenously at the standard dose. The PPI was taken in the evening before FMT and twice daily on the day of FMT (no later than 1 h before the procedure). PPI treatment was maintained for at least three–five days after FMT.

Prior to FMT, standard bowel cleansing (identical to that carried out prior to colonoscopies to decontaminate the GI tract) was performed. For this purpose, a single oral dose of a conventional phosphate macrogol preparation (Fortrans®, Ipsen Pharma) was used.

An intraduodenal tube was inserted >2 h prior to FMT using a radiological method to control its placement. The recipient was not allowed to ingest any food from the afternoon before FMT to 2 h after (but ingestion of fluids and medications was allowed). If possible, the recipient discontinued antibiotics at least one day before FMT and did not take antibiotics for at least one week after (a clinician assessed the risk to the recipient of not taking antibiotics and allowed antibiotic use when it was deemed necessary).

**FMT**

The fecal microbiota suspension was administered to the first three participants in a one-day FMT due to safety concerns (two syringes that each contained 50 g feces in 100 ml sterile saline were used on a single day [100g/200ml in total]). As there were no adverse effects, the subsequent participants underwent a two-day FMT (two syringes that each contained 50 g feces in 100 ml sterile saline [100g/200ml in total] were used, one on the first day and one on the next day).

The fecal microbiota suspension was administered *via* an intraduodenal tube.

After placement of the nasoduodenal tube, the recipient was advised not to drink anything or take any medications for 1–2 h.

Patients who received FMT on two consecutive days, on day 2nd received fresh fecal material obtained from the same donor, except patient No. 3.

**Monitoring of the recipients**

Prior to the initial administration of the fecal material, assessments of the recipient’s vital signs, abdominal symptoms, complete blood count, parameters of inflammation (C-reactive protein [CRP]), procalcitonin, and parameters that demonstrate organ capacity (as set out in the Results section of the manuscript and Table 1s) were carried out.

Vital signs were monitored immediately after FMT, in the evening and on the next day.

After FMT, complete blood count, CRP, procalcitonin, and parameters that demonstrate organ capacity were assessed in the evening or on the next day.

Assessments of adverse events were carried out continuously (as all the recipients stayed in the hospital after FMT).

Toxicity of the adverse events was graded using the toxicity criteria set out in the *Common Terminology Criteria for Adverse Events* version 4.0 [5].

**Assessment of efficacy of FMT**

The efficacy of FMT was assessed for the first time approximately one week after the procedure, followed by assessments after one and six months. Additionally, if the participants were readmitted to hospital, the assessments were repeated and, for those who stayed in hospital for an extended period of time, the assessments were repeated at weekly intervals (in accordance with standard procedure). Even in cases in which the treatment was judged to be a success (no ARB in two consecutive rectal swabs or, in the case of CPE, no ARB in two consecutive rectal swabs and a negative qPCR test) at one week after FMT, rectal swab culture was performed at each subsequent readmission to hospital and one and six months after FMT. These subsequent assessments were carried out to evaluate the maintenance of the successful response. Follow-up visits took place based on the participants’ clinical need. Additionally, fecal samples were collected from the participants one week, one month and six months after FMT and stored in liquid nitrogen for subsequent use in next-generation sequencing (NGS).

**Statistical analysis**

The statistical analysis was conducted using a licensed version of STATISTICA 10 for Windows and R Statistics (http://www.r-project.org/foundation). The normality of the distribution of the variables was checked using the Shapiro–Wilk test. The results were largely presented as the mean or median ± standard deviation (SD)/range, or the number (n) and percentage (%). Differences in the parameter estimates were assessed using Student’s *t* test for quantitative unpaired data and the χ2 test or Fisher’s exact test for qualitative variables in multi-divided tables. When using *t* tests, the assumption regarding the homogeneity of the variance was tested using an *F* test. If this assumption was not met, the Welch modification was used.

**MICROBIOTA COMPOSITION ANALYSES USING NEXT GENERATION SEQUENCING (NGS)**

Three fecal samples collected for each participant who was originally colonized with *K. pneumoniae* NDM1 were used for NGS procedure; samples from seven participants were used, so these seven sample sets were labeled A–G. The samples collected from each participant before FMT were labeled 1; the samples from the transplanted fecal material were labeled 2; and the samples collected from each participant one week after FMT were labeled 3. Thus, for example, the sample from participant A taken one week after FMT was labeled A3. Of the seven participants, four (A–D) became decolonized.

***DNA extraction***

DNA was extracted from the fecal samples using PowerSoil DNA Isolation Kits (MO-BIO), which are well suited for use with fecal samples [6]. Negative controls were included [7] and a mock community served as an internal control for calibration of the bioinformatical pipeline.

***qPCR***

qPCR was carried out using the forward primer S-D-Bact-0341-b-S-17 and the reverse primer S-D-Bact-0785-a-A-21, which target the V3–V4 region of the 16S rRNA gene [8]. Illumina adapters were attached as follows: Illumina adapter plus S-D-Bact-0341-b-S-17: 5'-TCGTCGGCAGCGTCAGATGTG TATAAGAGACAGCCTACGGGNGGCWGCAG-3' and Illumina adapter plus S-D-Bact-0785-a-A-21: 5'-GTCTCGTGGGCTCGGAGATGTGTA TAAGAGACAGGACTACH VGGGTATCTAATCC-3'. The following qPCR schedule was used: 98°C for 30 s; 25 rounds of 98°C for 10 s, 55°C for 20 s and 72°C for 20 s; and 72°C for 5 min. Amplification was verified by running the products on an agarose gel. Barcodes were added in a subsequent qPCR using the Nextera Index Kit V2 (Illumina) with the following qPCR schedule: 98°C for 30 s; eight rounds of 98°C for 10 s, 55°C for 20 s and 72°C for 20 s; and 72°C for 5 min. Attachment of the barcodes was verified by running the products on an agarose gel. Both the initial and barcoding qPCR procedures included negative controls.

***Normalization and sequencing***

The products from the nested qPCR were normalized, pooled and cleaned using a Mag-Bind® EquiPure gDNA Normalization Kit (Omega Bio-tek). The concentration of DNA in the pooled libraries was measured using a Qubit fluorometer and a Qubit High Sensitivity Assay Kit (Thermo Fisher Scientific). Sequencing was carried out using an Illumina MiSeq Desktop Sequencer and a MiSeq Reagent Kit V3 (Illumina) that is designed for 2 × 300 bp paired-end sequencing.

***Bioinformatic analysis***

The 64-bit versions of USEARCH [9] and mothur [10] were used in combination with several in-house programs for the bioinformatical analysis of the microbiota composition sequence data. Following tag identification and trimming, the sequence data from all the samples were pooled. Paired-end reads were merged, truncating reads at a quality score of 4, and requiring at least 100 bp overlap and a merged read length of 300–600 bp. Sequences with ambiguous bases, those that did not match the primers perfectly, and those that had a homopolymer length >8 were discarded and the primer sequences were trimmed. The reads were quality filtered, and reads with >1 expected error were discarded. The sequences were strictly dereplicated, discarding clusters <5. The sequences were clustered based on 97% sequence similarity with the “cluster\_otus” command in USEARCH, using the most abundant strictly dereplicated reads as centroids and discarding suspected chimeras (based on internal comparison). Additional suspected chimeric OTUs were discarded based on comparisons with data in the Ribosomal Database Project Classifier training set version 9 [11] using UCHIME [12]. Taxonomic assignment of the OTUs was carried out using the method devised by Wang et al. [13] with the mothur PDS version of the Ribosomal Database Project training database version 14.

**SUPPLEMENTARY RESULTS**

**Next Generation Sequencing**

Sequencing yielded a total of 9,935,430 paired-end reads. After quality-filtering and removing the chimeras, this was rarefied to 10,736 sequences per sample.

A non-metric multidimensional scaling approach was used to analyze the generalized UniFrac distances (α = 0.5) [14] (Figure 1s). The fecal samples that were donated to the responders (i.e., recipients who became decolonized after FMT; A2–D2) appeared to cluster separately from those that were donated to the non-responders (E2–G2). This was not the case for the fecal samples collected from the participants before FMT (A1–G1), nor for those collected one week after FMT (A3–G3). That is, the microbiomes of the participants did not appear to exhibit a pattern that allowed discrimination between the responders and non-responders. However, it appears to be possible to distinguish between them based on the microbiota composition of the transplanted fecal material.

When comparing responders with non-responders, there were no significant differences in the abundance of *Barnesiella* spp., *Bacteroides*, *Butyricimonas*, number of OTUs or Shannon index for samples collected before and one week after FMT (Figure 2s in Supplement and Figure 1 in main manuscript).

We also tested several obligate anaerobes which, according to published data [15-18], may be relevant to the development of colonization resistance and may be able to inhibit pathogens and reverse gut dysbiosis. We analyzed the differences between responders and non-responders in terms of the abundance of these genera (*Barnesiella*, *Bacteroides, Butyricimonas, Clostridium IV, XIVa* and *XIVb* and *Lactobacillus*) as well as potentially pathogenic genera (*Klebsiella* and *Enterococcus*) in fecal samples collected from the participants before and one week after FMT and from transplanted fecal material (Figure 2s). Comparing microbiota composition after FMT we found the significantly lower abundance of *Clostridium* *XIVa* (0.7 vs. 1%; p=0.03), *XIVb* (0.06 vs. 0.2%; p=0.01), and *Enterococcus* (0.007 vs. 0.02%; p=0.01), one week after FMT in responders compared with non-responders (Fig. 2s).

**SAFETY ASSESSMENT**

See Table 1s.

**CASE-BY-CASE FMT RESULTS**

The case-by-case details of each of the FMTs are presented in Table 2s.

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**Table 1s.** Safety assessment of the FMT based on infectious, immunological and biochemical parameters of the fecal samples collected from participants before and after FMT.

**Table 2s.** Case-by case descriptions of the FMTs

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Before FMT** | **After FMT** |
| Temperature (oC) | 36.8 (36.4–37.2) | 36.7 (36.2–37) |
| Blood pressure (mmHg) | 116/72 (100/60–140/80) | 120/74 (100/60–145/90) |
| Heart rate (beats/minute) | 83 (65–96) | 83 (66–96) |
| IgA (mg/dL) | 137 (4–330) | 147 (4–350) |
| IgG (mg/dL) | 847 (207–1571) | 864 (18–1679) |
| IgM (mg/dL) | 70 (5–180) | 79 (5–194) |
| CRP (mg/L) | 26 (1.2–217.9) | 35 (2.4–156.6) |
| Procalcitonin (ng/ml) | 0.12 (0–0.46) | 0.12 (0–0.61) |
| LDH (U/L) | 264 (37–532) | 317 (139–636) |
| AST (U/L) | 32 (15–69) | 31 (11–70) |
| ALT (U/L) | 39 (7–101) | 35 (7–86) |
| GGTP (U/L) | 189 (12–1419) | 217 (14–1834) |
| ALP (U/L) | 123 (48–451) | 137 (42–648) |
| Bilirubin total (mg/dL) | 1.19 (0.21–6.67) | 1.1 (0.1–5.11) |
| Bilirubin indirect (mg/dL) | 0.7 (0.11–5.82) | 0.7 (0.17–3.84) |
| Bilirubin direct (mg/dL) | 0.5 (0.09–3.76) | 0.6 (0.08–4.86) |
| Total protein (g/dL) | 5.9 (4.51–7.4) | 6.2 (4.1–8.2) |
| Albumin (g/dL) | 3.4 (2.04–3.94) | 3.5 (2.1–4.57) |
| Alpha-1 globulin (g/dL) | 0.3 (0.17–0.49) | 0.3 (0.2–0.44) |
| Alpha-2 globulin (g/dL) | 0.7 (0.41–0.93) | 0.7 (0.44–1.01) |
| Beta-1 globulin (g/dL) | 0.4 (0.23–0.44) | 0.4 (0.25–0.48) |
| Beta-2 globulin (g/dL) | 0.3 (0.18–0.45) | 0.3 (0.18–0.46) |
| Gamma globulin (g/dL) | 0.8 (0.25–1.61) | 0.9 (0.22–1.73) |

None of the differences were significant. ALP - alkaline phosphatase; ALT - alanine aminotransferase; AST - aspartate aminotransferase; CRP - C-reactive protein; FMT - fecal microbiota transplantation; GGTP- gamma-glutamyl transpeptidase; Ig - immunoglobulin; LDH - lactate dehydrogenase

**Table 2s.** Case-by case descriptions of the FMTs.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient number** | **Age (years)/ sex** | **Diagnosis** | **Before FMT** | **1 week after FMT** | **1 month after FMT** | **6 months after FMT** | **Use of antimicrobials during the week after FMT** | **Donor** | **Comments** |
| **Gut colonization (rectal swab culture)** | **Gut colonization (rectal swab culture)**  | **Gut colonization** | **Decolonization** | **Gut colonization** |
|  **rectal swab culture** |  **qPCR**  | **partial** | **complete** | **rectal swab culture** | **qPCR** |
| **1\*** | 44/ male | sPCL | *K. pneumoniae* NDM1+; *E. coli* ESBL+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | Yes | No | Death | Death | Penicillin | 1 | Death due to disease progression |
| **2\*** | 52/ male | MM | *K. pneumoniae* NDM1+; *E. coli* ESBL+ | Negative | Negative | *K. pneumoniae* NDM1+ | Yes | No | Lost to follow-up | Lost to follow-up | No | 3 | Lost to follow-up after 187 days |
| **3\*****1st FMT** | 55/ female | AML | Carbapenem-resistant *E. cloacae*; *Stenotrophomonas maltophilia*; second recurrence of *C. difficile* infection | Carbapenem-resistant *E. cloacae* | Negative | NA | Yes | No | Negative | NA | No | 1 |  First and second FMT (performed 4 weeks after first procedure) for participant number 3; second and third recurrence of very severe *C. difficile* infections after the reintroduction of chemotherapy. Resolved with FMT (eradicated ARB). Second FMT performed with material from different donor . |
| **3****2nd FMT** | 55/ female | AML | Carbapenem-resistant *E. cloacae*; third recurrence of *C. difficile infection* | Negative | Negative | NA | Yes | Yes | Negative | NA | No | 2 |
| **4** | 59/ male | DLBCL | *K. pneumoniae* NDM1+; *E. coli* ESBL+ | Negative | Negative | Negative | Yes | Yes | Negative | Negative | No | 2 | – |
| **5** | 71/ male | MM | *P. aeruginosa* MBL+; carbapenem-resistant *K. pneumoniae* | Negative | Negative | Negative | Yes | Yes | Negative | Negative | No | 1 |  – |
| **6** | 38/ female | AML | Carbapenem-resistant *K. pneumoniae*; *Stenotrophomonas maltophilia* | Carbapenem-resistant *K. pneumoniae* | Negative | NA | Yes | Yes | Negative | NA | Amoxicillin; cotrimoxazole; azithromycin | 1 | – |
| **7** | 78/ female | DLBCL | *Acinetobacter ursingii* MBL+; *K. pneumoniae* ESBL+; *E. coli* ESBL+; carbapenem-resistant *K. pneumoniae* | Negative | Negative | Negative | Yes | Yes | Negative | Negative | Meropenem; colistin; micafungin | 2 | Colonized by new strain of *K. pneumoniae* with decreased susceptibility to carbapenems 2 weeks after FMT; spontaneous decolonization after 4 weeks; subsequently colonized by *P. rhodesiae* MBL+ |
| **8****1st FMT** | 58/ male | cGvHD | *K. pneumoniae* NDM1+; *E. coli* ESBL+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | Yes | No | Death | Death | Penicillin; azithromycin; cotrimoxazole | 1 | First and second FMT (performed 8 weeks after first procedure) for participant number 9; death due to progression of pulmonary cGvHD and fungal infection |
| **8****2nd FMT** | 58/ male | cGvHD | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | No | No | Death | Death | Penicillin; azithromycin; cotrimoxazole | 1 |
| **9** | 70/ male | Lung cancer | *K. pneumoniae* NDM1+; *E. coli* ESBL+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | Yes | No | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | Voriconazole | 2 | Colonized by new strain of VRE 2 weeks after FMT |
| **10** | 50/ male | AML | *P. aeruginosa* MBL+ | Negative | Negative | Negative | Yes | Yes | Negative | Negative | No | 1 | Recolonized by *P. aeruginosa* MBL+ from axillary abscess (persistent axillary skin colonization); sepsis involving this pathogen 16 days after FMT |
| **11****1st FMT** | 63/ female | MDS RAEB2 | Carbapenem-resistant *P. aeruginosa*; *K. pneumoniae* NDM1+; VRE; *E. coli* ESBL+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | Yes | No | Negative | Negative | Cotrimoxazole; fluconazole | 2 | First, second (performed 4 weeks after the first procedure) and third FMT (performed 22 weeks after the second procedure) for participant number 13; decolonized for 6 months (after two FMTs), then re-colonized with *K. pneumoniae* NDM1+ |
| **11****2nd FMT** | 63/ female | MDS RAEB2 | *K. pneumoniae* NDM1+ | Negative | Negative | Negative | Yes | Yes | Negative | Negative | No | 1 |
| **11****3rd FMT** | 63/ female | MDS RAEB2 | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | No | No | Time point not reached | Time point not reached | Ampicillin; cotrimoxazole | 1 |
| **12** | 24/ female | TTP | *K. pneumoniae* NDM1+ | Negative (RSC + qPCR) | Negative | Negative | Yes | Yes | Negative | Negative | No | 2 | Very fast decolonization (4 days after FMT, confirmed by rectal swab culture and qPCR test) |
| **13** | 29/ male | cGvHD | *K. pneumoniae* NDM1+ | negative | Negative | Negative | Yes | Yes | Negative | Negative | No | 2 |   |
| **14****1st FMT** | 38/ male | aGvHD; alloHCT due to MM | *K. pneumoniae* ESBL+; *E. coli* ESBL+ | *K. pneumoniae* ESBL+ | Negative | NA | Yes | Yes | Negative | NA | Meropenem; amikacin; linezolid | 3 | First and second FMT (performed 26 weeks after the first procedure) for participant number 18;after the first FMT, marked improvement in severe GI aGvHD resulting in discontinuation of immunosuppressants  |
| **14****2nd FMT** | 38/ male | aGvHD;alloHCT due to MM | *K. pneumoniae* ESBL+ | *K. pneumoniae* ESBL+ | *K. pneumoniae* ESBL+ | NA | No | No | Time point not reached | Time point not reached | No | 1 |
| **15** | 29/ male | aGvHD | *K. pneumoniae* NDM1+; *E. coli* ESBL+ | Negative | *K. pneumoniae* NDM1+ | *K. pneumoniae* NDM1+ | Yes | No | Death | Death | Ciprofloxacin | 1 | Death due to aGvHD progression |
| **16** | 77/ female | Immunosuppression; kidney Tx | *E. coli* ESBL+ | Negative | Negative | NA | Yes | Yes | Negative | NA | No | 1 | –  |
| **17** | 27/ male | aGvHD | *E. coli* OXA-48+ | Negative | Negative | NA | Yes | Yes | Death | Death | No | 2 | Death due to aGvHD progression; sepsis involving *E. coli* OXA-48+ despite previous decolonization |
| **18** | 23/ male | aGvHD | Carbapenem-resistant *P. aeruginosa* | Carbapenem-resistant *P. aeruginosa* | Negative | NA | No | Yes | Time point not reached | Time point not reached | No | 1 | Ileus before and after FMT; improvement in severe aGvHD;recolonized with carbapenem-resistant *P. aeruginosa*, then, after 1 month, spontaneous decolonization |
| **19** | 61/ male | AML | *K. pneumoniae* NDM1+; VRE; *E. coli* ESBL+ | Negative | Negative | Negative | Yes | Yes | Time point not reached | Time point not reached | No | 3 | – |
| **20** | 61/ male | AML | *K. pneumoniae* NDM1+ | Negative (RSC + qPCR) | Time point not reached | Negative after 1 week | Yes | Yes | Time point not reached | Time point not reached | Meropenem; colistin | 2 | Death due to AML  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |

\* One-day FMT (all other participants had 2-day FMTs on two consecutive days); \*\* At least two negative rectal swab cultures or, in the case of carbapenemase-producing ARBs two negative rectal swabs and a negative qPCR test result. aGvHD – acute graft-versus-host disease; AML – acute myeloblastic leukemia; ARB – antibiotic-resistant bacteria; cGvHD – chronic graft-versus-host disease; DLBCL – diffuse large B-cell lymphoma; ESBL – extended-spectrum β-lactamase; FMT – fecal microbiota transplantation; GI – gastrointestinal tract; MBL – metallo-β-lactamase; MDS – myelodysplastic syndrome; MM – multiple myeloma; NA – not applicable; NDM1 – New Delhi metallo-β-lactamase 1; OXA-48 – expanded-spectrum oxacillinase; RAEB 2 – refractory anemia with excess blast type 2; RSC – rectal swab culture; qPCR – quantitative real-time polymerase chain reaction; sPCL – secondary plasma cell leukemia; TTP – thrombotic thrombocytopenic purpura; Tx – transplantation;VRE – vancomycin-resistant enterococcus

**Figure 1s.** UniFrac distances between fecal sample microbiota compositions. Black is used to represent the responders (i.e, participants who became decolonized after FMT) and gray to represent the non-responders. The sample labels represent the participants’ letter (A–G) and sample number (1­–3, where 1 represents samples collected before FMT, 2 represents samples from transplanted fecal material, and 3 represent samples collected one week after FMT). Solid lines connect each sample collected before FMT with that collected one week after FMT. Dotted lines connect each sample from the transplanted fecal material with the sample collected one week after FMT.

**Figure 2s.** Differences in abundance of selected genera between responders and non-responders in samples collected from participants before FMT, samples taken from transplanted fecal material, and samples collected from participants one week after FMT. We found a significantly higher abundance of *Bacteroides* (12% vs. 0.7%, p=0.008) and *Butyricimonas* (0.1% vs. 0%, p=0.016) in fecal material and a significantly lower abundance of *Clostridium* *XIVa* (0.7% vs. 1%; p=0.03), *XIVb* (0.06% vs. 0.2%; p=0.01), and *Enterococcus* (0.007% vs. 0.02%; p=0.01), one week after FMT in responders compared with non-responders \* - p<0.05. The box excludes the upper and lower 25% (quartiles) of data, while the lines go to maximum and minimum excluding outliers. Outliers are defined as more/less than 3/2 of the upper/lower quartile. The line is the median. All data points are marked with “x” in the boxplots.