Fecal microbiota transplantation for decolonization from multidrug-resistant bacteria in pediatric allogeneic hematopoietic stem cell transplantation recipients: a retrospective real-world data study

Gut colonization by multidrug resistance (MDR) bacteria is a major concern in allogeneic hematopoietic stem cell transplantation (allo-HCT) recipients, being associated with a very high mortality rate.[1] Despite this clinical challenge, strategies to effectively decolonize from MDR strains and prevent translocation from the gut to the bloodstream are lacking.

Fecal microbiota transplantation (FMT) consists of the infusion of fecal matter from a healthy donor into the gastrointestinal tract of the recipient.[2] Based on the relevance of the gut microbiota (GM) configuration on clinical outcomes after allo-HCT,[3] several studies observed how FMT can ameliorate intestinal dysbiosis and improve clinical outcomes in adult HCT recipients, with a promising safety profile.[4] The results of FMT in decolonizing patients from MRD strains are also promising, but the rates of decolonization vary from 20% to 70% in a published study.[5]

The bulk of the current data on the use of FMT in the allo-HCT setting has been generated from the adult population, with pediatric studies significantly lacking.[6] To date, only data of 16 pediatric HCT patients <18 years receiving FMT have been published in the literature, and in only five patients the indication was MDR decolonization.[7] For this reason, we aimed to retrospectively assess the safety and efficacy of the use of FMT for MDR decolonization in pediatric allo-HCT recipients, and to analyze the factors associated with decolonization efficacy.

We retrospectively enrolled pediatric patients aged <21 years, colonized by MDR bacteria and who underwent FMT before or after allo-HCT between November 2018 and November 2024 in the pediatric stem cell transplantation units of Rome, Bologna and Padua, of whom five have been previously reported.[7] Screening for MDR colonization was performed periodically in the three centers prior and after allo-HCT based on local policies, employing culture from rectal swab or stools. Swabs or stool cultures were not performed after a washout from antibiotics, but in no case an antibiotic active against the MDR bacteria was given at sampling.

In this Ethical Committee-approved study, MDR bacteria was defined according to Magiorakos *et al.*[8] Patients were considered candidates for FMT if HCT was planned, or after HCT if immune recovery remained inadequate (e.g., due to the need for prolonged immunosuppressive therapy), with consequent increased infectious risk.

FMT was performed on a compassionate use basis after local ethical committee approval, permission from the national transplant center of the Italian minister of health and informed consent of parents/legal guardians. Unrelated adult healthy volunteer donors were employed for the different infusions, with ethical consent for using the biological material according to national regulation. Donor screening was performed according to the European consensus guidelines on FMT[2] and Italian recommendations of the National Health Authority. FMT emulsion was prepared under aerobic conditions, from either frozen or fresh preparation, according to stool bank availability and clinical need. FMT infusion was performed via the upper gastrointestinal system, either via esophagogastroduodenoscopy (EGDS) in the duodenum, naso-jejunal tube (NJT) or percutaneous endoscopic jejunostomy (PEJ). Severe adverse events and adverse events were reported and graded according to CTCAE v5.0 and related to FMT based on attending physician opinion. Decolonization rates were estimated at 1 and 6 weeks after the first FMT, as previously reported.[7]

Twenty-two patients were evaluated for this study, 16 of 22 (73%) of whom were male, with a median age at FMT of 4.2 years (range, 0.8-18.5). Of these, 14 were treated in Rome, six in Bologna, and two in Padua. The indication for allo-HSCT was a malignant or non-malignant disease in 15 and seven patients, respectively. The donor was HLA-identical sibling in six, haploidentical family member in nine, and matched unrelated donors in seven cases (Table 1).

The MDR bacteria identified through screening are reported in *Online Supplementary Table S1*, with 14 of 22 (64%) of patients being colonized by Verona integron-encoded metallo-β-lactamase (VIM) producing strains. Moreover, five of 22 (23%) patients were colonized by two concomitants different MDR strains. In three of 22 (14%) a previous sepsis by the MDR bacteria occurred, with two cases of multiorgan failure.

A total of 31 infusions of FMT were performed (median of 1/patient; range, 1-3) via upper digestive tract, using EGDS in 14 patients, NJT in seven and PEJ in one patient. In all cases, an unrelated fecal donor was selected, with frozen material employed in 21 of 22 (96%) patients. In 16 of 22 (73%) patients FMT was performed right before allo-HCT (median 15 days prior to HCT; range, 8-74), while the remaining six underwent the first FMT a median of 65 days post-transplant (range, 55-487). Antibiotic preparation prior to FMT was administered in nine of 22 (41%), with

oral colistin administered in seven patients and oral gentamycin in the other two. Median volume of fecal material infused was 150 mL per infusion (range, 60-240), equal to a median of 6,5 mL/kg (range, 3-13.3).

The procedure was generally well tolerated, and no severe adverse events related to FMT were reported. A total of five of 22 (23%) patients reported an adverse event related to FMT, with two cases of grade 2 abdominal pain and three cases of grade 1 nausea. All five events underwent complete resolution. Hematological parameters dynamics at time of and after the procedure are reported in *Online Supplementary Table S2*.

At 1- and 6-week follow-up, 22 of 22 (100%) and 21 of 22 (96%) patients were alive. One patient died due to systemic adenovirus infection not related to FMT infusion. The decolonization rate was 77% and 52% at 1- and 6-weeks post FMT, respectively. All patients decolonized at 6 weeks had at least three subsequent negative consecutive samples, 1 week apart from each other.

Among the five patients with two concomitant different colonizing strains, two remained colonized, two were decolonized from one strain and one was de-colonized from both bacteria at 6 weeks. Therefore, among the ten not completely decolonized patients, two were decolonized from at least one strain. After FMT, no blood stream infection (BSI) was observed in the six patients who received it after allo-HCT, and four BSI occurred during the early phase post allo-HCT in patients receiving FMT prior to transplantation, due to VIM-positive *P. aeruginosa* in three cases and *E. coli* in the other case.

Table 1. Characteristics of included patients.

Patient characteristics	Study cohort, N=22
Age at FMT, years, median (range)	4.2 (0.8-18.5)
Sex, male, N (%)	16 (73)
Indication for allo-HCT, N (%) ALL AML MDS Inborn error of immunity Thalassemia major	 6 (27) 9 (41) 1 (4.5) 5 (23) 1 (4.5)
Stem cell donor, N (%) Sibling MUD Haplo	 6 (27) 7 (32) 9 (41)
Stem cell source, N (%) BM PBSC	 11 (50) 11 (50)
Conditioning regimen, N (%) MAC RIC	 20 (91) 2 (9)
MDR pathogen, VIM$^+$, N (%)	14 (64)
Patients colonized by 2 concomitants different MDR strains, N (%)	5 (23)
FMT timing, N (%) Pre-HCT Post-HCT	 16 (73) 6 (27)
Route of FMT administration, N (%) NJT PEJ EGDS	 7 (32) 1 (4) 14 (64)
N of infusions per patient, median (range)	1 (1-3)
Patients receiving multiple infusions, N (%)	8 (36)
Antibiotic preparation, N (%)	9 (41)

ALL: acute lymphoblastic leukemia; allo-HCT: allogeneic hematopoietic stem cell transplantation; AML: acute myeloid leukemia; BM: bone marrow; Haplo: haploidentical; EGDS: esophagogastroduodenoscopy; FMT: fecal microbiota transplantation; MAC: myeloablative conditioning; MDR: multidrug resistant; MDS: myelodysplastic syndrome; MUD: matched unrelated donor; NJT: naso-jejunal tube; PBSC: peripheral blood stem cell; PEJ: percutaneous endoscopic jejunostomy; RIC: reduced-intensity conditioning; VIM$^+$: Verona integron-encoded metallo-β-lactamase producing strains.

No patient who was decolonized at 6 weeks experienced an MDR-BSI, while in two of ten patients still colonized, a bacteremia from the same VIM-producing strain occurred. We then studied factors associated with decolonization efficacy. Patients receiving >1 FMT infusions yielded higher decolonization rates at 6 weeks, compared to patients receiving only one infusion (88% *vs*. 44%; *P*=0.024). Among the eight patients receiving multiple infusion, the only one that did not get completely decolonized from MDR was colonized by two different strains, and one of them was absent at the 6-week evaluation. Moreover, a trend towards lower efficacy was observed in FMT performed via EGDS, compared to patients receiving it from NJT or PEJ (36% *vs*. 86%; *P*=0.063). No associations with center, age, antibiotic preparation, timing of FMT (prior *vs*. post allo-HCT), type of colonizing pathogens and use of antibiotic within 30 days post FMT were observed (*Online Supplementary Table S3*). Herein, we reported, to the best of our knowledge, the biggest cohort of pediatric allo-HCT recipients in which FMT was performed for MDR decolonization through the upper gastrointestinal tract.

In the present study, FMT has been a generally well-tolerated procedure with only minor adverse events described, mainly concerning the gastrointestinal tract, such as nausea and abdominal pain, and no serious adverse events. A major concern in this setting has always been the risk of infectious complications, due to the administration of living bacteria to an immune-compromised host with impaired gut permeability.[5] Indeed, a case of a fatal bacteremia in the neutropenic period caused by a MDR *E. coli* transmitted through FMT administered on day 4 and on day 3 before HCT has been previously reported in an adult patient.[9] Subsequent adult studies observed that FMT-derived bacteremia is a possible though rare event.[10] To minimize this risk and ensure the presence of neutrophils in the event of bacterial translocation, we administered FMT before conditioning or after neutrophil engraftment. In fact, no infectious complications related to FMT have been reported in our cohort.

We observed a promising rate of decolonization after 6 weeks from transplantation, particularly high in patients receiving multiple infusion (88%). This may be related to the observation of Zhang and colleagues, that not all donor microorganisms could establish colonization in the recipient after the first FMT. However, colonization is improved after subsequent infusions, possibly due to a priming effect on the patient's gut community of the first FMT, allowing donor microorganisms from a second FMT to better engraft.[11] Moreover, the administration of FMT via a NJT seemed associated with better results and could possibly ease the delivery of multiple infusions compared to the more invasive EGDS procedure. Indeed, in our experience only one patient undergoing FMT via EGDS was able to receive more than one infusion.

Interestingly, decolonization by MDR protected from MDR-related BSI, while in 20% of patients in which FMT failed to decolonize at 6-week, a BSI from the same strain occurred. This observation may be consistent with the protective effect of FMT on translocation of MDR bacteria from the gut to the bloodstream, even when decolonization is not fully achieved,[12] potentially contributing to reducing non-relapse mortality in allo-HCT recipients.

The present study has several limitations. First, the retrospective nature of the study and the lack of a control group limited the external validation of these data. Moreover, there was some heterogeneity in MDR colonization assessment and FMT procedure, including methods for MDR detection.

In conclusion, our findings highlight the safety and potential efficacy of FMT as a decolonization strategy for reducing infectious complications associated with MDR bacteria in pediatric allo-HCT. Notably, multiple infusions may enhance effectiveness in this setting. Further perspective studies are needed to determine the clinical usefulness and optimal clinical protocol for this procedure.

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RM and PM conceptualized the study. EM, MG, PM, GM, DL and RM collected the data. EM performed the statistical analysis and prepared the tables. EM, RM, PM, GM, and MG wrote the paper. All other authors critically reviewed the manuscript.

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Original data and protocols are available on reasonable request addressed to the corresponding author.

References

1. Caselli D, Cesaro S, Fagioli F, et al. Infectious Diseases Study Group of the Italian Association of Pediatric Hematology and Oncology (AIEOP). Incidence of colonization and bloodstream infection with carbapenem-resistant Enterobacteriaceae in children receiving antineoplastic chemotherapy in Italy. Infect Dis (Lond). 2016;48(2):152-155.
2. Cammarota G, Ianiro G, Tilg H, et al. European FMT Working Group. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017;66(4):569-580.
3. Masetti R, Leardini D, Muratore E, et al. Gut microbiota diversity before allogeneic hematopoietic stem cell transplantation as a predictor of mortality in children. Blood. 2023;142(16):1387-1398.
4. DeFilipp Z, Peled JU, Li S, et al. Third-party fecal microbiota transplantation following allo-HCT reconstitutes microbiome diversity. Blood Adv. 2018;2(7):745-753.
5. Pession A, Zama D, Muratore E, et al. Fecal microbiota transplantation in allogeneic hematopoietic stem cell transplantation recipients: a systematic review. J Pers Med. 2021;11(2):100.
6. Gray AN, DeFilipp Z. Fecal Microbiota Transplantation for acute graft-versus-host disease after allogeneic hematopoietic cell transplantation: expanding the horizon into pediatrics. Transplant Cell Ther. 2023;29(8):484-491.
7. Merli P, Putignani L, Ruggeri A, et al. 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. Haematologica. 2020;105(11):2686-2690.
8. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-281.
9. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. N Engl J Med. 2019;381(21):2043-2050.
10. Eshel A, Sharon I, Nagler A, et al. Origins of bloodstream infections following fecal microbiota transplantation: a strain-level analysis. Blood Adv. 2022;6(2):568-573.
11. Zhang F, Zuo T, Yeoh YK, at al. Longitudinal dynamics of gut bacteriome, mycobiome and virome after fecal microbiota transplantation in graft-versus-host disease. Nat Commun. 2021;12(1):65.
12. Ghani R, Mullish BH, McDonald JAK, et al. Disease prevention not decolonization: a model for fecal microbiota transplantation in patients colonized with multidrug-resistant organisms. Clin Infect Dis. 2021;72(8):1444-1447.