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Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematological malignancies carrying multidrug-resistance bacteria

Short title: FMT in carriers of MDRB undergoing allo-HSCT

Scientific category: transplantation

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Abstract

Fecal microbiota transplantation is an effective treatment in recurrent Clostridium difficile infection. Promising results to eradicate multidrug-resistant bacteria have also been reported with this procedure, but there are safety concerns in immunocompromised patients. We report results in 10 adult patients colonized with multidrug-resistant bacteria, undergoing fecal microbiota transplantation before (n=4) or after (n=6) allogeneic hematopoietic stem cell transplantation for hematologic malignancies.

Stools were obtained from healthy related or unrelated donors. Fecal material was delivered either by enema or via nasogastric tube. Patients were colonized or had infections from either carbapenemase-producing bacteria (n=8) or vancomycin-resistant enterococci (n=2). The median age at fecal microbiota transplantation was 48 (range 16-64) years. Three patients needed a second transplant from the same donor, due to initial failure of the procedure.

With a median follow-up of 13 (range 4-40) months, decolonization was achieved in seven out of ten patients. In all patients, fecal microbiota transplantation was safe: one patient presented with constipation during the first 5 days after FMT and 2 patients had grade I diarrhea. One case of gut grade III acute graft-versus-host disease occurred after fecal microbiota transplantation. In patients carrying or infected by multidrug-resistant bacteria, fecal microbiota transplantation is an effective and safe decolonization strategy, even in those with hematologic malignancies undergoing hematopoietic stem cell transplantation.

Introduction

During the last decades, the prevalence of multidrug resistant bacteria (MDRB), has largely increased, becoming a serious worldwide problem[1]. Under physiological conditions, commensal microbiota prevents gut colonization from MDRB. However, in particular conditions, such as in patients with hematologic malignancies, use of chemotherapeutic agents and broad spectrum antibiotics may favor selection of resistant pathogens through the alterations of the gastrointestinal barrier and the consequent dysbiosis[2]. Patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) are at even higher risk of dysbiosis due to their profound immune depression[3]. In case of bloodstream infections from MDRB, outcomes are even poorer, with consequently increased mortality[4]. An Italian study, for example, showed that carbapenemase producing (CP-) bacteria, including *Pseudomonas aeruginosa*, were independent predictors of death in patients diagnosed with acute leukemia, while this was not observed in case of extended-spectrum β-lactamase (ESBL-) Enterobacteriaceae[5].

In order to prevent bacteria spreading to other patients, preventive measures are warranted, including patient isolation, limitations of transfer to other healthcare centers and management by dedicated staff, with consequent related increased healthcare costs, which are not easily affordable in most centers[6]. According to French recommendations[7], for example, patients colonized with MDRB are not easily admitted in healthcare facilities not disposing of dedicated staff [7].

New classes of antibiotics are under study to treat infections related to MDRB, and active research is ongoing to find effective decolonization strategies[8]. The use of oral gentamicin had been initially proposed in some MDR-gram negative strains, but failure is common, and the risk of selecting gentamicin-resistant strains may also be present[9,10].

Fecal microbiota transplantation (FMT) is a procedure that has proven to be effective and safe in the treatment of recurrent *Clostridium difficile* infection (CDI), and it is now recommended as a therapeutic in this setting[11]. Use of FMT in patients carrying MDRB is still investigational, but there

are reports and case series showing its efficacy in this setting[12,13]. Many concerns were initially raised about the feasibility of FMT in immunocompromised patients, such as those affected by hematologic malignancies, because of the theoretic potential for local and bloodstream infections. However, recent case reports revealed the efficacy and safety in this particular population[14,15,16]. Recently, Bilinski et al. reported the results of a prospective study evaluating FMT in 20 patients with MDRB gut colonization and contemporarily affected by hematologic malignancies. Overall 25 FMT were performed and 15/20 patients experienced complete MDRB decolonization[17], including some of them with graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

In the current retrospective study, we report our experience with FMT in patients diagnosed with hematologic malignancies and undergoing FMT either before or after allo-HSCT.

Methods

In this single-center study, we retrospectively analyzed data on all consecutive adult patients diagnosed with hematologic malignancies who underwent FMT before or after allo-HSCT due to MDRB colonization. In our center microbiological screening is performed weekly in all inpatients, with consequent preventive measures in positive patients in order to limit MDRB spread, according to national guidelines[7], as better detailed in online Supplemental material.

This study was approved by the Ethic Committee. The treatment plan was discussed in advance by a multidisciplinary team (hematologist, gastroenterologist, pharmacist) in order to approve the procedure. The decision was made on a patient to patient basis. All patients signed an informed consent mentioning the theoretical risks of the procedure, due to the actual investigational use of FMT in the field of MDRB and in patients with hematologic malignancies.

We considered eligibility to FMT in case of asymptomatic carriers or systemic infections from VRE, carbapenemase-producing Enterobacteriaceae (CPE) or CP-*Pseudomonas aeruginosa*. Rationales for

FMT and MDRB decolonization were mainly to limit infectious complications related to these bacteria and to facilitate patients transfer in other departments such as intensive care units or rehabilitating centers.

Contemporary colonization from ESBL-producing bacteria was also registered in patients undergoing FMT. We therefore subsequently evaluated if FMT also allowed decolonization from these MDRB.

For the purpose of this retrospective analysis, we also classified MDRB as multi-drug (MDR), extensive-drug (XDR) and pan-drug-resistant (PDR) according to the definition proposed by Magiorakos et al. [18]: MDR was defined as the presence of acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR as non-susceptibility to all agents in all antimicrobial categories.. Details on donor selection, microbiological testing, fecal material preparation and delivery are available on online Supplemental material.

Decolonization from VRE, CPE or CP-*Pseudomonas aeruginosa* after negative results on a minimum of three consecutive microbiological cultures (performed weekly) was defined as “major decolonization” while “persistent decolonization” was defined as the persistence of negative rectal swab until last follow-up after a first or second FMT, whenever this was feasible. In patients concomitantly colonized by ESBL-producing Enterobacteriaceae, “concomitant decolonization” was defined as negative results on at least three consecutive rectal swabs after FMT. The safety of the procedure was also registered. For all patients, data on significant infections, defined as bacteriemia or sepsis occurring during the first 90 days after FMT were also collected. Febrile neutropenia or fever of unknown origin was not considered as significant infectious episodes, but they were also recorded. In patients presenting either a relapse of MDRB colonization or experiencing FMT failure, a second attempt could be proposed.

Results

During the period between 2014 and 2017, 10 patients underwent FMT, 7 due to gut colonization without systemic infection by either CPE (*Escherichia coli*, n=1; *Citrobacter freundii*, n=2; *Klebsiella pneumoniae*, n=1), or CP-*Pseudomonas aeruginosa* (n=1) or VRE (n=2) and 3 after having experienced systemic infections from CP-*Pseudomonas aeruginosa*. The median age at FMT was 48 (range 16-64) years. Four patients underwent FMT as a decolonization strategy before allo-HSCT, with a median interval from FMT to transplant of 28 (range 9-46) days. Of note, one patient was contemporarily colonized by three different CPE. Two patients started conditioning regimen 3 days after FMT and the other two after a month. Six patients underwent FMT after allo-HSCT, with a median time from allo-HSCT to FMT of 163 (range 98-344) days. Of note, all patients undergoing FMT after allo-HSCT were still on immunosuppressive therapy at the time of FMT, with only one out of six presenting active grade IV steroid-dependent gut graft-versus-host disease (GVHD). Overall, six patients were also colonized by ESBL-producing Enterobacteriaceae. All ESBL-producing bacteria were classified as being MDR.

A frozen product was used in eight out of ten patients and enema was the preferred way of administration in all but one patient. This patient, indeed, presented a compromised neurological status due to a cerebral toxoplasmosis and she was not considered eligible for enema. Median quantity of donor stools was 84 g (range 43-104). At the time of FMT patients neutrophil count was > 1 x 10^9/L in all patients but one that had a neutrophil count of 0.17 x 10^9/L (the one with steroid-resistant GVHD). Platelet count was count > 20 x 10^9/L in all patients.

Three patients required a second FMT: in one patient, after initial efficacy, VRE was again detectable 2 months after the first FMT. Of note, this patient developed multiple infectious episodes (particularly sinusitis and pneumonia), prompting to the frequent use of large spectrum antibiotics, thus probably leading to recurrence of VRE colonization. In the other two patients a second attempt was done due to the failure of the first procedure. In one patient this was mainly attributable to incorrect preparation with PEG (insufficient intake). After a second attempt with a correct

preparation, indeed, VRE eradication was achieved and persisted until 20 months after FMT. At that time, indeed, VRE was detectable contemporarily to hematologic disease recurrence. In the last patient, first and second FMT mainly had a compassionate aim in order to treat active grade IV gut GVHD and contemporarily multiple infectious episodes rendering impossible antibiotics withdrawal, even during the 72 hours following FMT, as detailed below.

Globally, major decolonization (three consecutive negative microbiological cultures) was achieved in 7 out of 10 patients, including two patients after a second FMT (Figure 1). Persistent decolonization (negative microbiological cultures at last follow-up) was achieved in 6 out of ten patients after a median follow-up of 13 (range 4-40) months from FMT. As already mentioned, indeed, one patient presented a positive rectal swab for ERV 20 months after FMT meanwhile to disease relapse. She finally died due to hematological progression.

Failure occurred in the remaining three patients. The patient undergoing FMT with a compassionate aim had presented multiple infectious episodes from CP-*Pseudomonas aeruginosa*, rendering it impossible to stop antibiotics during the 72 hours after FMT. Moreover, grade IV gut GVHD was associated to intestinal occlusion, with need for an aspirating nasogastric tube, at time of FMT. Despite two attempts with FMT, the procedure was a failure and the patient finally died. In the second patient, due to the difficulties encountered in the positioning of a nasogastric tube, FMT was administered by enema and the patient was not able to retain the product for the advised 2-3 hours. She then refused a second attempt. The third patient underwent FMT by enema from an unrelated donor and the hypothesis for FMT failure was that she received an insufficient quantity of stools (43 g), but what seems discordant with this hypothesis is that partial decolonization from concomitant ESBL-producing Enterobacteriaceae was achieved. A second attempt in this patient was not possible due to the unavailability of additional material.

Among the six patients concomitantly colonized from ESBL-producing Enterobacteriaceae, three obtained concomitant decolonization.

Details on FMT performed before or after allo-HSCT are reported in Table 1. As an example of successful FMT, Figure 2 shows the case of the patient undergoing FMT from nasogastric tube, after experiencing breakthrough infectious episodes related to colonization from CP-*Pseudomonas aeruginosa,* with need for continuous in hospital care during the first year after allo-HSCT. After FMT, this patient did not experience any other infectious episode and outpatient care was finally possible.

According to the safety of FMT procedure, one patient presented constipation during the first 5 days after FMT which was favorably resolved after the use of laxatives, while two patients presented grade I diarrhea the day after FMT. No other major adverse events were observed.

Only one patient undergoing FMT before allo-HSCT developed a grade III acute gut graft-versus host disease at day +30 after allo-HSCT and at day +51 after FMT. Differential diagnosis with CMV colitis was evoked, and she favorably evolved after both antiviral and steroid treatment.

When looking at severe infectious episodes during the 90 days following FMT, in two of those patients undergoing FMT before allo-HSCT, documented bacteriemia without sepsis occurred early after allo-HSCT, favorably evolving after the introduction of large-spectrum antibiotics. In particular, one patient experienced a documented bacteriemia from multi-sensible *Pseudomonas aeruginosa* at day +80 after allo-HSCT while the other patient experienced a documented bacteriemia from an ESBL-producing Escherichia Coli at day 60 after allo-HSCT. The additional two patients undergoing FMT before allo-HSCT also received large spectrum antibiotic such as piperacillin-tazobactam or cephalosporins for febrile neutropenia without documentation. Interestingly, despite the use of large spectrum antibiotics, no cases of MDRB recurrence were observed in those four patients.

Of note, fungal and viral infections were observed in only one patient more than 6 months after FMT but these were not considered in relation to FMT because this patient was under systemic immunosuppressive treatments for a cortico-resistant extensive GVHD (lung, skin, mucosal) and infectious episodes exacerbated during immunosuppressive treatment. Among the other patients, neither fungal nor viral infections were observed.

Discussion

Increasing emergence and diffusion of MDRB represents a major public health problem, with higher mortality in patients experiencing infections, and high costs of prolonged in-hospital care and preventive measures used to limit diffusion to other patients[6,19].

Human gut microbiota, also named as "gut resistome", is the primary site for MDRB acquisition and colonization, being an important reservoir of antibiotic resistance genes[20]. Patients diagnosed with hematological malignancies are at high risk of colonization from MDRB: conditioning regimens for allo-HSCT and intensive chemotherapy, indeed, significantly alter the gastrointestinal barrier and, subsequently, the composition of intestinal microbiota is largely modified. Moreover, patients affected by hematologic malignancies or undergoing allo-HSCT are at particular risk for MDRB colonization or infection due to the large, prolonged and, sometimes, improper use of large spectrum antibiotics[2]. Of note, most bloodstream infections in hematological patients derive from the gut, and infections are even more severe in those patients undergoing allo-HSCT, with high mortality rates of 36-95%[3,4].

It has been largely reported that microbioma modifications are associated to worse survival, higher risk of infections and GVHD in patients undergoing allo-HSCT[21,22]. Therefore, efficacious decolonization strategies in this particular setting of patients are urgently needed.

Fecal microbiota transplantation is a fascinating decolonization strategy, that has been proven to be efficacious in patients with recurrent CDI[23,24]. On the other hand, concerns were initially raised for the use of FMT as a decolonization strategy in immunocompromised patients, due to the possible risk of local or systemic infections after the inoculum of microbiota pathogens.

Recently, DeFilipp et al. investigated the use of third-party FMT with the use of oral capsules, as a strategy to restore microbioma diversity in patients undergoing allo-HSCT. They support the safety

and feasibility of this procedure underlying the possibility that microbiome restauration early after allo-HSCT may be of benefit[25].

Herein, we describe the results of FMT in 10 patients diagnosed with hematologic malignancies and undergoing FMT for MDRB colonization, namely CPE, CP-*Pseudomonas aeruginosa* or VRE, either before or after allo-HSCT. Decolonization was achieved in 7 out of 10 patients, this being persistent at last follow-up in 6 out 10 patients.

Our retrospective study not only suggests the efficacy of this procedure, but also its safety in patients with hematologic malignancies and undergoing allo-HSCT.

Of note, despite not being a selection criterion for FMT, we also registered patients concomitantly colonized from ESBL-producing enterobacteriaceae, with decolonization in 3 out of 6 cases.

We also showed that, in patients experiencing failure or relapse of MDRB colonization, a second FMT is feasible and efficacious. Interestingly, only three patients experienced significant infections after FMT.

Moreover, it is worth underlying the significant benefit of major decolonization in the patient who had experienced multiple infectious episodes due to a CP-*Pseudomonas aeruginosa*, limiting breakthrough infections.

Our results also highlight that despite administration of large spectrum antibiotics may hypothetically represent a risk of decolonization failure, the procedure remained effective in the majority of patients, without recurrence of MDRB in the majority of them despite use of broad spectrum antibiotics early after FMT.

Of note, in one patient VRE was detectable again at the time of disease relapse, despite no use of large-spectrum antibiotics just before this detection. One can speculate that disease relapse may

have probably been associated to dysbiosis favoring selection of VRE, but conclusions cannot be drawn on one case.

Despite the initial aforementioned concerns in immunocompromised patients, results of FMT in this setting are promising in terms of both efficacy and safety[4,15,16]. A recent prospective study showed, indeed, that FMT allowed total eradication of MDRB in 60% of cases, without any significant adverse event after the procedure[17]. The latter is the only prospective study published to date using FMT in 20 patients with blood disorders and colonized with MDRB. Differently from our series, in this study all types of MDRB were included and only a few patients underwent allo-HSCT.

In our Center, we only chose patients colonized with highly resistant bacteria and in particular those classified as eXDR according to French guidelines or those known to cause a significant higher risk of systemic infection with very poor prognosis (i.e. CP-*Pseudomonas aeruginosa*).

To date, there are no specific guidelines on the ideal timing, the best preparation of stools for FMT, and the best way of administration. In our experience, FMT was successfully undertaken either before or after allo-HSCT and, interestingly, it was also successful in two patients starting conditioning regimen for allo-HSCT 3 days after FMT.

As for stool preparation, frozen material was preferred in our Center particularly due to logistic reasons, although in two cases fresh stools were used, but this did not modify the results of FMT. It has recently been reported in a meta-analysis of patients receiving FMT for CDI, that the success rate of FMT was similar when using frozen or fresh stools[31]. Differently from most of the reported series of FMT for MDRB decolonization, we preferred enema as a way of administration, as this is associated with lower risk of inhalation as compared to nasogastric administration.

The mechanisms underlying the efficacy of FMT for MDRB decolonization are still not clear. ~~In mice,~~

Recent studies showed that recipient stool assumed donor-like taxonomic and functional composition immediately following FMT[34]. Therefore we hypothesize that FMT for MDRB

decolonization works through the restoration of a more physiological microbiome thus increasing the ecological pressure on MDRB. However, given the absence of translational studies on antibiotic resistance genes and microbiota composition on patient's stool after FMT, we cannot exclude that FMT works through lowering of MDRB below the threshold of detection rather than through true elimination.

In our series, after FMT, almost all patients did not experience major infectious complications during the first 3 months after FMT and, of note, in those patients subsequently undergoing allo-HSCT, no severe infectious bacterial complications occurred during the early transplant phase.

Regarding the impact of FMT on GVHD, only one of our patients had a grade IV acute gut GVHD concomitant to a carbapenemase-producing *Pseudomonas aeruginosa* at the time of FMT. In this specific case, the procedure was not efficacious neither for MDRB nor for GVHD. However it is worth underlying that FMT was performed at a very late stage ("compassionate" use), that may also explain the failure of the procedure. Importantly, among the nine remaining patients, only one experienced grade III acute gut GVHD after FMT (with a possible differential diagnosis with CMV colitis). A role of FMT in causing GVHD in this patient cannot formally be excluded and this point may be addressed in a prospective clinical trial.

Early studies in mice and humans suggested a link between gut microbiota and propensity to GVHD, with mice treated with gut-decontaminating antibiotics developing GVHD less often [35,36]. Recent results of a pilot study also highlight the possible advantage of microbiota modulation with FMT in patients affected by steroid-refractory or steroid-dependent GVHD [37].

With regards to donor choice, people living in the same household of the patient were preferred, when available, as they widely share the same pathogens and environment exposure, thus reducing the risk of transferring additional infectious agents from the donor to the recipient.

In line with previous reports, we consider that targeting gut microbiota in patients with impaired immune reconstitution in an attempt to reinstate a more equilibrated flora may favor stable eradication of the carrier status and prevent subsequent life threatening infections.

We are well aware of the limits of our study, being a retrospective one, including a low-number of patients, with non-homogeneous inclusion criteria and differences in FMT procedure according to patients, so that definitive conclusions cannot be drawn.

However, we consider that our results support the use of FMT as a promising strategy to manage the considerable potential risks associated with the MDRB carrier status in immunocompromised patients with intestinal dysbiosis and in those patients having experienced single or multiple systemic infections, with absence of breakthrough infections after decolonization and absence of MDRB recurrence despite the use of broad spectrum antibiotics in the majority of them. Furthermore, our results support again the safety of the procedure in this population, despite previous concerns in immunocompromised patients. These preliminary results underline the need for further prospective studies on the safety and efficacy of FMT.

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Autorship contributions:

HS and MM designed the study.

GB collected data.

GB, FM, HS and MM wrote or revised the manuscript and all authors reviewed its final version.

JT was in charge of microbiological cultures.

ACJ and MTB were in charge of product preparation.

GB, FM, MTR, AR, ACM, EB, FG, RD were in charge of patients.

Conflict of interest statement: The authors declare no conflicts of interest in preparing this article.

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Table 1. Characteristics of patients undergoing fecal microbiota transplantation before (a) or after (b) hematopoietic stem cell transplantation.

a)

	1	2	3	4
Patient sex	M	M	F	M
Age at time of FMT, years	64	42	45	47
Hematologic malignancy	AML	AML	AML	BPDCN
Identified MDRB	CP- *Pseudomonas aeruginosa*	CP-*Pseudomonas aeruginosa*	CPE	CPE°
Antimicrobial resistance category	XDR	MDR	MDR	MDR
Concomitant MDR-ESBL-producing bacteria colonization, bacteria	Y	N	Y	N
Systemic infections due to MDRB before FMT	Y	N	N	N
Time from FMT to allo-HSCT (days)	41	46	16	9
FMT donor	Daughter	Sister	Husband	Sister
Way of administration	Enema	Enema	Enema	Enema
Major decolonization	Y	Y	Y	Y
Persistent decolonization	Y	Y	Y	Y
Concomitant ESBL-producing bacteria decolonization	Y	N/A	N	N/A
Follow-up after FMT, days	820	368	148	399
Follow-up after allo-HSCT, days	779	322	132	390
Status	Alive	Dead	Alive	Alive

Cause of death	N/A	Disease progression	N/A	N/A	

b)

	5	6	7	8	9	10
Patient sex	F	M	F	F	F	F
Age at time of FMT, years	50	54	16	19	62	54
Hematologic malignancy	MPN	MPN	AML	ALL	MPN	ALL
Identified MDRB	CP- *Pseudomonas aeruginosa*	CP- *Pseudomonas aeruginosa*	VRE	VRE	CPE	CPE
Antimicrobial resistance category	PDR	XDR	XDR	XDR	MDR	XDR
Concomitant MDR-ESBL-producing bacteria colonization	N	Y	Y	Y	N	Y
Systemic infections due to MDRB before FMT	Y	Y	N	N	N	N
Time from allo-HSCT to FMT	324	344	98	160	123	167
FMT donor	Husband	Unrelated	Mother	Mother	Brother	Unrelated
Way of administration	Nasogastric tube	Nasogastric tube	Enema	Enema	Enema	Enema
Second FMT	N	Y	Y	Y	N	N
Time from first to second FMT, days	N/A	27	118	84	N/A	N/A

Major decolonization	Y	N	Y	Y	N	N
Persistent decolonization	Y	N/A	Y	N	N/A	N/A
Concomitant ESBL-producing bacteria decolonization	N/A	N	N	Y	N/A	Y
Colonization relapse	N	N/A	N	Y	N/A	N/A
Follow-up after FMT, days	678	33	1220	595	184	307
Follow-up after allo-HSCT, days	1002	404	1436	839	307	474
Status	Alive	Dead	Alive	Dead	Alive	Alive
Cause of death	N/A	Uncontrolled GVHD and infection	N/A	Disease progression	N/A	N/A

° 3 different types: *Citrobacter freundii, Klebsiella Pneumoniae, Enterobacter Cloacae*

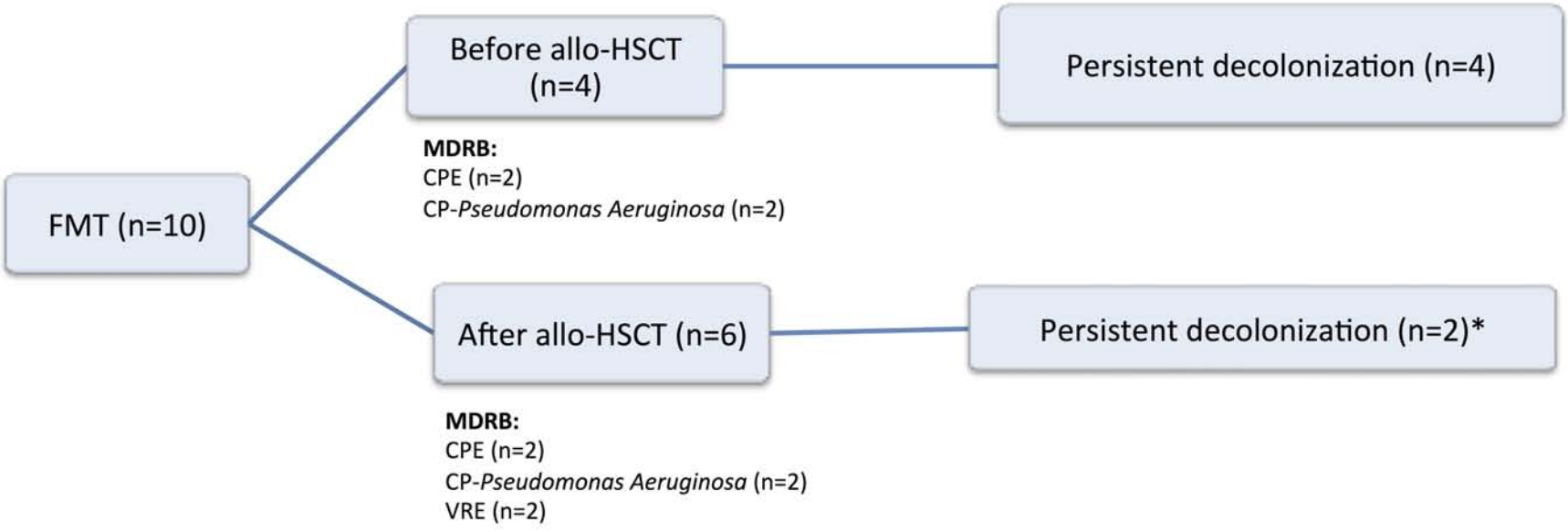
Abbreviations: F, female; M, male; FMT, fecal microbiota transplantation; AML, acute myeloid leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasm; MDRB, multidrug-resistant bacteria; CP, carbapenemase-producing; CPE, carbapenemase-producing *Enterobacteriaceae*; XDR, extensively-drug resistant; MDR, multi-drug resistant; ESBL, extended-spectrum beta-lactamase; Y, yes, N, no; allo-HSCT, allogeneic hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; N/A, not applicable; MPN, myeloproliferative neoplasm; ALL, acute lymphoblastic leukemia; VRE, vancomycin-resistant enterococci; PDR, pan-drug resistant.

Figure legends

Figure 1. Results of fecal microbiota transplantation

Figure 2. Evolution of patient 5 as model of successful fecal microbiota transplantation.

Figure legend: CP, carbapenemase-producing; FMT, fecal microbiota transplantation; HSCT, hematopoietic stem cell transplantation.



*A third patient obtained decolonization lasting 20 months and the ERV recurrence occurred after

Figure legend: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CPE, carbapenemase-producing enterobacteriaceae; CP, carbapenemase-producing; VRE, vancomycin resistant enterococci.

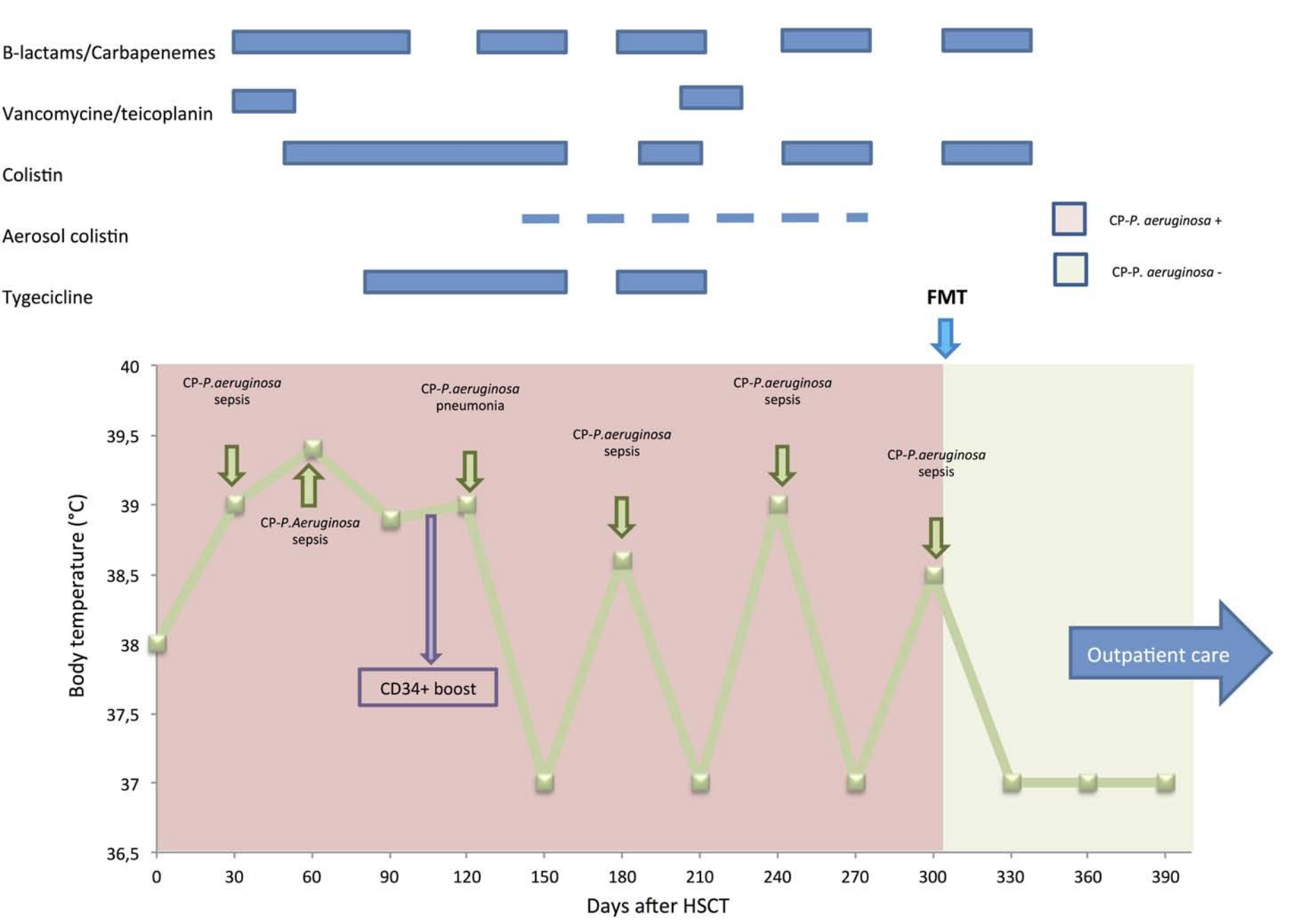


Figure legend: CP-P. aeruginosa, carbapenemase-producing Pseudomonas Aeruginosa; FMT, fecal microbiota transplantation; HSCT, hematopoietic stem cell transplantation

Supplemental materials

Patient screening and preventive measures

In our center weekly screening for MDRB is performed in order to identify asymptomatic carriers with high risk of spreading MDRB to other patients. Screening modalities consist of weekly rectal swab. After MDRB identification, patients colonized with vancomycin-resistant (VRE) or carbapenemase-producing enterobacteriaceae (CPE) are cohorted and cared for by dedicated staff, as these two classes of bacteria are classified as emerging XDR (eXDR), i.e. bacteria that present an emerging infection control challenge widely in France. Of note, when those patients are candidates to rehabilitation centers before being discharged at home, they cannot be easily admitted to other healthcare facilities that often do not dispose of dedicated staff [7].

Furthermore, in contact patients, defined as those patients having shared paramedical and/or medical healthcare workers with one or more patients colonized with VRE or CPE, cohorting is also warranted, with initial caring by another dedicated staff until three negative screening tests.

It is worth underlying that opportunistic saprophytic bacteria, such as CP-*Pseudomonas aeruginosa,* have not been considered as eXDR in national guidelines. However, it has been already reported that patients experiencing systemic infections from CP-*Pseudomonas aeruginosa* have a high risk of death[5], and in our Center three consecutive patients (data not published) died during the aplastic phase of allo-HSCT due to bloodstream fatal infections from CP-*Pseudomonas aeruginosa*.

For these reasons, patients colonized with CP- *Pseudomonas aeruginosa* were considered at high risk of fatal complications and, despite not needing isolation and caring by dedicated staff, FMT was proposed to patients experiencing systemic infections or in those colonized in order to limit systemic infections.

A minimal platelet count of 20 x 10^9/L was preferred in order to proceed to the FMT and use of platelet transfusion to reach that threshold before FMT was allowed.

Microbiological testing

For each patient, one rectal swab specimen was plated onto selective media: a screening medium designed to detect ESBL-producing enterobacteriaceae, ChromID ESBL (bioMérieux) and another designed to detect CP-bacteria, ChromID CARBA SMART (bioMérieux). A second rectal swab was used in an enrichment procedure, consisting of an overnight culture at 37°C in a specific broth before plating onto a screening medium designed to detect VRE, ChromID VRE (bioMérieux). All plates were incubated overnight at 37°C. Colonies growing on these selective media were identified at the species level by MALDI-TOF spectrometry. The production of ESBL was determined by an antibiogram and visualization of the characteristic “champagne cork” synergy between amoxicillin-clavulanate and third-generation cephalosporins disks. Carbapenemase production was determined by molecular analysis using the GeneXpert technology (Cepheid) and the Xpert Carba-R kit version 2 (detecting the most prevalent carbapenemases in France, OXA-48 and OXA-48-like enzymes, as well as NDM enzymes). Furthermore, VRE were also identified using the GeneXpert technology (Cepheid) and the Xpert VanA/VanB kit.

In patients achieving decolonization, rectal swabs and/or stool cultures were initially performed weekly and then at each follow-up visit. In patients considered as having achieved total and persistent decolonization, last follow-up for decolonization was considered as the date of the last available negative microbiological culture.

Patients and donors characteristics

The current study was approved by the Ethic Committee. Each patient signed an informed consent mentioning all potential risks of the procedure as described in the paper. According to French regulations in such cases, each patient case was extensively discussed and approved as part of an "RCP" (Réunion de Concertation Pluridisciplinaire") which is a sort of large multidisciplinary meeting aimed to discuss difficult cases and approve unusual therapeutic procedures. The minutes and

decisions of the RCP are recorded in writing, including the names of the participants and their feedback. Patients are informed about this discussion prior to signing the informed consent.

Large spectrum antibiotics were discontinued in the recipients 48-72 hours prior to the procedure and, when possible, use of antibiotics was avoided during at least 72 hours after the procedure.

Stools were preferentially obtained from healthy related or unrelated donors. Of note, related donors not necessarily coincided with allo-HSCT donors. According to regulatory recommendations, potential donors were selected after a previous questionnaire. Donor age was preferentially between 18 and 65 years. Excluded were people who had presented digestive disorders (i.e. diarrhea) within the 3 months prior to donation or having a chronic disease and/or chronic treatments, cases with antibiotic intake within 3 months before the donation, people having been living in the tropics during the three months prior to donation or having been hospitalized abroad for more than 24 hours in the 12 months prior to donation. History of typhoid fever was also considered as exclusion criteria. In people fulfilling inclusion criteria, a complete biological and microbiological assessment was then performed including: serology for *Treponema pallidum*, human immunodeficiency virus, Human T-Lymphotropic Virus, Hepatitis A, B and C, cytomegalovirus, Epstein-Barr virus, amebiasis, *Strongyloides strecoralis* ; stool examination for standard culture, *Clostridium difficile*, multi-resistant bacteria, norovirus, Cryposporidium, parasites. If the biological and microbiological panel was negative, a minimum of 50 g of stools were collected.

Fecal material, prepared as described below, was delivered either by enema or via nasogastric tube. A bowel preparation was performed the day before the FMT by administration of 4 liters of polyethylene glycol (PEG) based solution. For nasogastric administration, patients had to fast for at least 12 hours before transplantation and they received proton pump inhibitors the day before and the morning of the FMT. In the case of enema administration, patients were asked to retain the product for at least 2-3 hours.

Product preparation

Transplants were prepared in the Saint Antoine Hospital pharmacy. In case of freezing, the stool preparation is usually performed in two steps. In the first, preparation and freezing, the stools are manipulated in an extractor hood dedicated to this activity, in the 6 hours following emission. A total of 50-100g stools are weighted and mixed with a sterile cryopreservative saline solution (300mL glycerol+ saline solution 0.9% 10/90 V/V) using sterile blender, containers and medical devices (syringes, filters). The suspension is filtrated through sterile gauze compresses mounted in a funnel to remove solid residues, before freezing at -80°C. If in screening tests an exclusion criterion is fulfilled, the suspension is destroyed. The second step of the preparation procedure starts the day before FMT, when the frozen microbiota solution is placed in a refrigerator (between 4 and 8°C) for an overnight thawing. The thawed suspension is then transferred either to an enema bag (lower gastro intestinal tract delivery) to which 200mL of sterile saline solution are added, or to 50-mL syringes (colonoscopy or nasoduodenal delivery) as ready to be used. . On the other hand, when FMT is performed with fresh stools, fecal materials need to be prepared the day of FMT within the 6 hours following stools emission. In this case stool preparation is performed in a single step, without freezing.

Safety testing of the fecal product was done according to French recommendations[1].

1) Sokol H, Galperine T, Kapel N, et al. Groupe Français de Transplantation Fecale (GFTF). Transplantation de microbiote fecal dans le cadre des infections a Clostridium difficile recidivantes : recommandations pour la pratique clinique courante. Hepato Gastro 2015; 22: 278-290.