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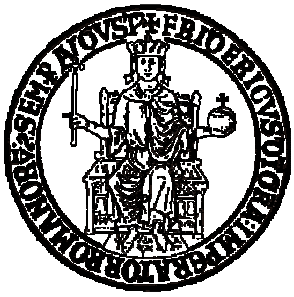

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TESI DI DOTTORATO

FECAL MICROBIOTA TRANSPLANTATION BEFORE OR AFTER ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES CARRYING MULTIDRUG-RESISTANCE BACTERIA

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1. INTRODUCTION

1.1. HUMAN GUT MICROBIOTA PATHOPHYSIOLOGY

A large microbial ecosystem, housing several trillion microbial cells, is harboured in human intestinal tract, collectively constituting gut microbiota. Data from more than one thousand persons from United States, China and Europe, identified nearly 10 million microbial genes in the fecal microbioma, including harmless symbionts, commensals and opportunistic pathogens[1].

Number of bacteria varies among the length of the gastrointestinal tract, reaching the highest load in the colon (Figure 1).

Interestingly, it has been shown that gut microbiota has several essential functions in humans such as in the host immune response[2], protection against pathogens overgrowth[3], regulation of intestinal endocrine functions[4], metabolic and excretion functions[5].

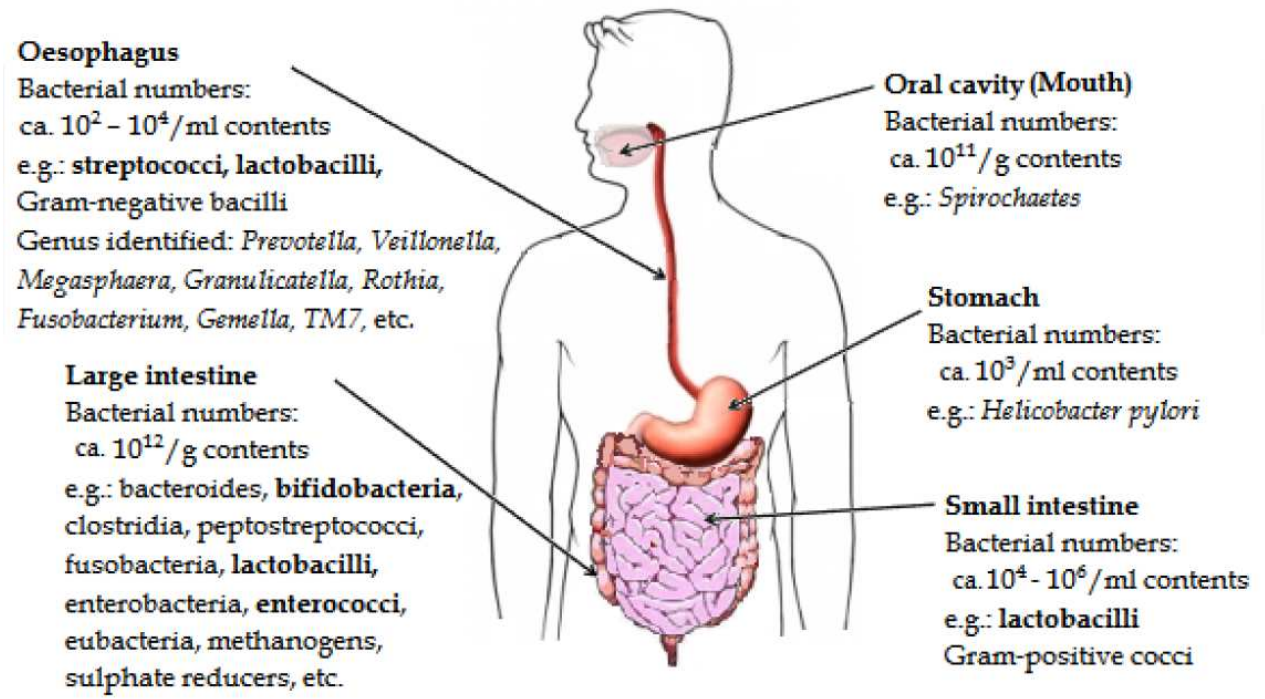


Figure 1. ***The human gastrointestinal tract and its microbiota***

Concerning gut microbiota composition, a significant interindividual variation has been reported, with each person having a unique gut microbioma[6]. However, despite differences among individuals, functional capacities are similar in healthy persons[7]. Moreover, significant changes occur in the same individual from infancy to elderly, with age-differentiated microbiologically communities, with different bacteria strains and proportions[8,9]. In the elderly, the gut microbiota become compositionally unstable and less diverse, events that are associated with coexisting conditions and age-related declines in immunocompetence (Figure 2)[10].

Different exogenous and endogenous factors may significantly modify microbiota composition, thus leading to dysbiosis. These factors include

mode of delivery of a neonate[11], host genetic features[12], host immune response[13], drugs[14], diet[15], etc.

Dietary habits, indeed, strongly influence the selection of gut microbiota, with studies showing that meat consumption favors bilemetabolizing expansion (which may be associated to the development of inflammatory bowel disease), while vegetable consumption favors plant polysaccharide-fermenting organisms. It has also been reported that persons have very different metabolic responses to identical meals[14].

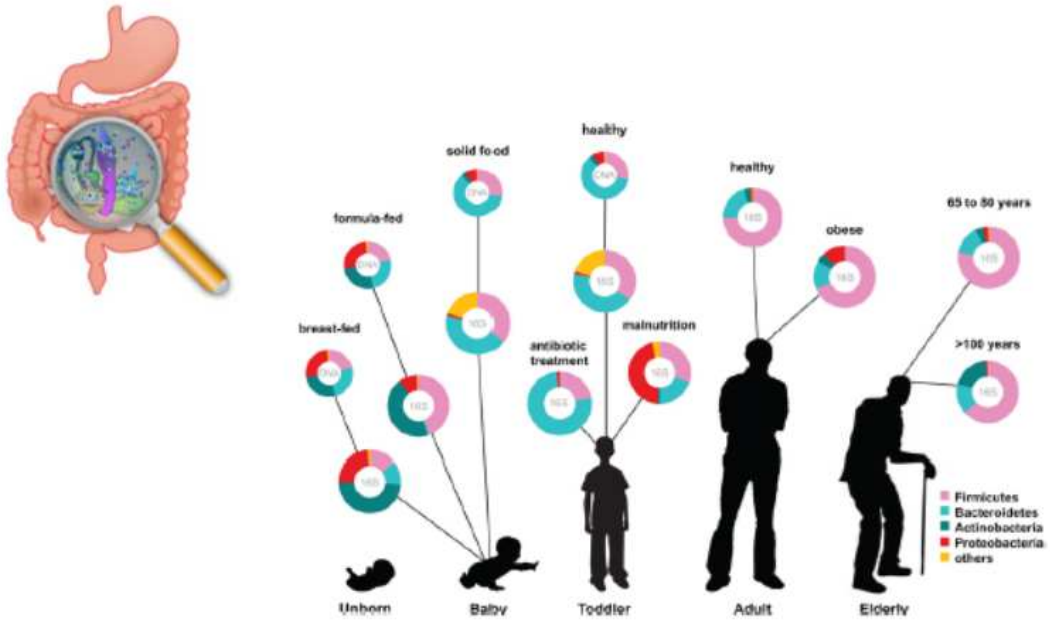


Figure 2. ***Changes in microbiota composition according to ages.***

Considering those several physiological functions of gut microbiota in human health, it is not surprising that dysbiosis has been also associated in multiple studies to a wide spectrum of common chronic disorders,

including atherosclerosis[16], metabolic disorders[17], asthma[18] and autism spectrum disorders[19].

The "common ground" hypothesis provides a possible pathogenetic explication on the relation between dysbiosis and chronic diseases. Increase in gut permeability, for example due to mucosal inflammation, diet or chronic infections, may favor modification and development of an aberrant gut microbiota[20]. In persons genetically predisposed to one or more chronic disorders, these modifications may favor the expansion of opportunistic and dysbiotic pathobionts, contributing to elicit specific disorders in predisposed individuals.

Interestingly, transplantation of the dysbiotic disease-associated gut microbiota to a genetically susceptible rodent host, allowed reproduction of disease phenotype, thus supporting this hypothesis and showing that transfer of gut microbiota from one to another is possible. Therefore, much effort is currently concentrated on exploring potential causality and related microbiota-mediated disease mechanisms, with the hope that an improved understanding will fuel the conception and realization of novel therapeutic and preventive strategies.

1.2. DYSBIOSIS IN HEMATOLOGICAL DISEASES

Important dysbiosis is observed in patients diagnosed with hematological malignancies needing treatments with intensive chemotherapies or in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT).

One of the most frequent side effects of chemotherapy is, indeed, mucositis, also referred as mucosal barrier injury. It is characterized by both inflammation and cell loss in the epithelial barrier lining the gastrointestinal tract. Clinically, mucositis is associated with bacteremia, malnutrition, use of total parenteral nutrition, and an increment in the use of intravenous analgesics.

Historically, research has focused on oral mucositis. More recently, attention has been drawn towards the pathophysiology and clinical symptoms of intestinal mucositis, which is characterized by symptoms like nausea, bloating, vomiting, abdominal pain and severe diarrhea.

Recent studies have shown that chemotherapeutic agents have an effect on gut microbiota composition inducing significant changes in commensal intestinal bacteria, favoring mucositis and its severity[21]. It has also been shown that some intestinal bacteria play a role in the metabolism of certain chemotherapeutic agents. The outgrowth of these bacteria might lead to the formation of active toxic metabolites of the chemotherapeutic drug, which directly affects the progression of intestinal mucositis[22]. However, the commensal intestinal microbiota might also have beneficial effects on the development of intestinal mucositis, as the mere presence of resident intestinal bacteria might offer protection against its development.

The epithelial barrier lining the gastrointestinal tract is composed of a single layer of epithelial cells intertwined by tight junctions, thus functioning as a mechanical barrier whose action is further increased by a

mucus layer. When inflammation of gut mucosa occurs (i.e. after chemotherapy), Toll-like receptors that are present at the outer membrane of the epithelial cells may interact with intestinal bacteria. This interaction favors the activation of nuclear factor kappa B (NFkB) which results in the development of an inflammatory response. However, multiple intestinal bacteria are capable of decreasing NFkB activation, resulting in a diminished production of inflammatory cytokines.

Intestinal permeability increases after chemotherapy treatment, probably subsequent to intestinal villous atrophy after epithelial loss. However, the resident intestinal microbiota has also been proposed to directly influence intestinal permeability, with some bacteria and their products increasing permeability and others attenuating cellular atrophy and strengthening tight junctions, thus highlighting different roles of gut microbiota in mucositis pathophysiology and severity.

Therefore, chemotherapy-induced dysbiosis may finally also be explained by the fact that it induces a deregulation in intestinal microbial homeostasis, with disappearance of bacteria implicated in the protection of enterocytes against harmful stimuli. The exact nature and relevance of the relationship between chemotherapy-induced mucositis, inflammation and intestinal microbiota is subject to ongoing research.

In patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) for hematologic malignancies, use of conditioning regimens and wide-spectrum antibiotics, may similarly impact on gut microbiota composition, inducing significant dysbiosis. During the aplastic phase

following allo-HSCT, for example, the diversity of gut ecosystem decreases of nearly 30%, with invasion by new species and loss of commensal bacteria (Figure 3).

Furthermore, in this particular population, development of gut graft-versus-host disease (GVHD) may also result in significant damage to gut epithelial cells and modifications in pathogen flora, with consequent promotion of proliferation of alloctonous opportunistic bacteria.

However, this condition does not seem irreversible, with reconstruction of a "healthy" microbiota (in the absence of significant complications favoring and perpetuating dysbiosis) during the 60-100 days following allo-HSCT.

Studies showed that gut microbiota composition is predictive of the overall mortality in transplanted patients. Indeed, in a cohort of 80 transplanted adults, low-, intermediate- and high-intestinal diversity of the gut microbiota at the engraftment defined three groups with different overall survival at 3 years, 36, 60, and 67%, respectively. The increase of the mortality rate in this study was primarily due to transplant-related causes, suggesting a link between that lack of microbial diversity and infection and/or GvHD[23].

According to GVHD, indeed, it has been largely demonstrated that certain bacteria species are more frequently found in patients developing GVHD, with ecosystems that significantly differ from those of patients not developing GVHD. Similarities with gut microbiota of patients diagnosed with intestinal bowel disease with those of patients developing GVHD have also been reported, including reduced microbial richness, relative

abundance of Gram-positive bacteria that were associated in both situations to increased intestinal inflammation[24].

Although associations between microbiome diversity and outcomes of allo-HSCT do not demonstrate causality, they provide data to support clinical evaluation as to whether these relationships can be modified to influence outcomes for patients.

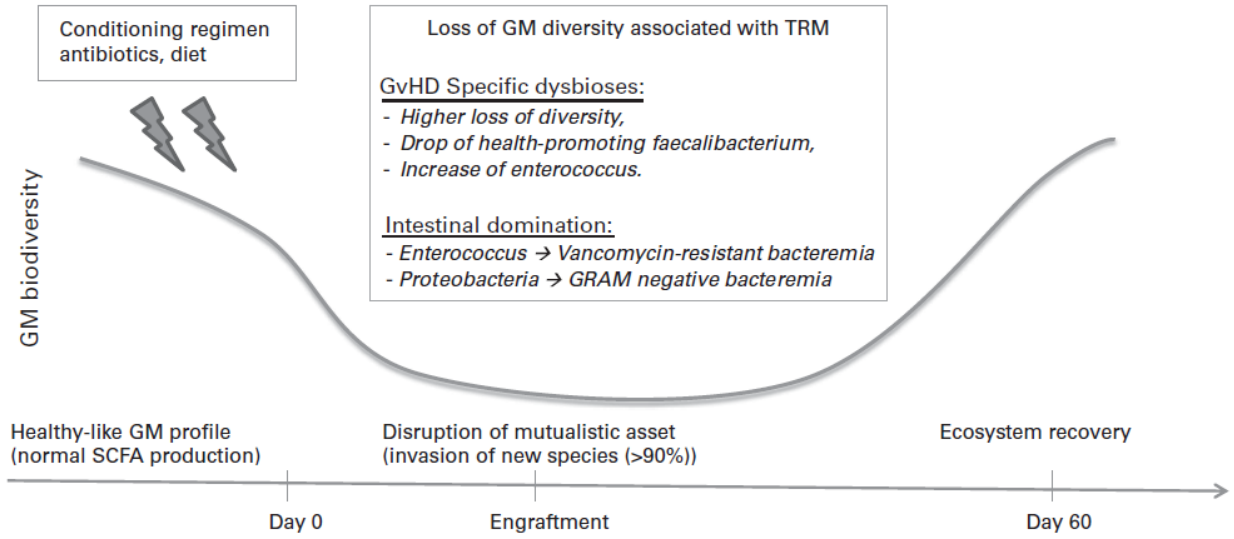


Figure 3. ***Gut microbiota modifications after allogeneic hematopoietic stem cell transplantation.***

1.3. DEVELOPING STRATEGIES TO ADDRESS MICROBIOTA: FECAL MICROBIOTA TRANSPLANTATION AND THE EXAMPLE OF CLOSTRIDIUM DIFFICILE *INFECTION*

Active research on efficacious strategies to modulate and correct dysbiosis is currently ongoing. A promising strategy is represented by fecal microbiota transplantation (FMT) which consists in transfer of stools and bacteria from a healthy individual to another experiencing dysbiosis, with

the rationale to allow gut microbiota modulation with subsequent reconstitution of a healthy microbiota[25].

The use of FMT for human disease goes back many centuries. Two chinese doctors firstly described the use of human fecal suspension in different preparations to successfully treat patients who had food poisoning or severe diarrhea. During World War II, German soldiers in Africa were recommended by Bedouins to treat bacterial dysentery with "consumption of fresh, warm camel feces"[26]. In 1958, an American surgeon used fecal enemas to treat four patients who had developed fulminant pseudomembranous enterocolitis after antibiotic use; the treatment resulted in a rapid resolution of symptoms[27].

In 2013, the first randomized controlled trial using FMT in *Clostridium difficile* infection (CDI) was published[28]. Since then, FMT has been investigated as a possible therapy in a variety of diseases with the largest experience reported in cases of severe recurrent diarrhea caused by antibiotic-resistant CDI, favored by the use of antibiotics[25].

Clostridium difficile is a ubiquitous, anaerobic, Grampositive, cytotoxin-producing bacillus which exists in both a vegetative form (the toxin-producing replicative state) and a spore form. The latter is particularly resistant to destruction even with antiseptics. This allows the organism to persist in the gut and the environment. If the gut microbiota remains disturbed after CDI therapy, the spores can germinate to the vegetative form, resulting in recurrent disease.

With the rationale of restoring gut microbiota in order to protect the intestinal lining and help eliminate or prevent residual spores from causing recurrent disease, use of FMT allowed infection eradication in >90% of patients, also decreasing the economic burden of this infection on healthcare systems[28,29].

In two randomized controlled trials, FMT was associated to a success rate of > 90% as compared to nearly 30% with the use of oral vancomycine in patients with recurrent CDI[28,30]. Despite these studies have small samples as they were early stopped, further studies, also grouped in metanalysis and systematic reviews, confirmed resolution rates of recurrent CDI with FMT of nearly 90%[31].

Since the introduction and success of FMT in CDI, its use has been subsequently investigated in other chronic diseases, such as inflammatory bowel disease (IBD) or decolonization from multidrug-resistant bacteria (MDRB) [32,33].

However, the dissemination of FMT in clinical practice is actually restricted by regulatory and bureaucratic issues (mainly related to costs, donor programme, safety control). To date FMT has not yet undergone the traditional regulatory approval process of pharmaceutical products with sequential testing leading to large phase 3 trials assessing efficacy and safety prior to clinical utilization[34,35].

Authoritative published guidelines and recommendations have been released as expert opinions rather than evidence-based consensus reports. Despite the existence of European consensus report on clinical

indications, methodological aspects and donor selection, there is still no real consensus, with large differences existing among centers worldwide[36]. For all these reasons, in all cases of planned FMT, local ethical approval and patient and donor informed consent should be obtained and FMT should be done in specialized centers. A multidisciplinary team, including gastroenterologist, microbiologist and infectious diseases physicians, should be encouraged in centers performing FMT.

The only clinical indication with sufficient evidence of benefit from the implementation of FMT in clinical practice to date is CDI, while its use in all other situations remains still investigational.

1.3.1. Safety of FMT

Despite initial concerns about the safety of FMT, most studies finally highlighted this procedure as being globally safe. To date, no cases of transmitted infectious diseases due to FMT have been reported. Minor short-term adverse events such as diarrhea, flatulence, abdominal discomfort, and cramping are common after FMT. Most of these adverse events are self-limiting and disappear within 2 days after FMT.

However, cases of fever as well as Gram-negative bacteraemia and perforation have been described. A case of death after regurgitation of faecal material infused into the duodenum under general anaesthesia has also been reported. After delivering donor stool via a pre-existing nasogastric tube, another patient developed septic shock and toxic megacolon and died after colonic resection. Finally, a patient had a severe

septic shock due to the faecal aspiration following endoscopic peroral jejunal FMT[37,38,39].

A systematic review analysing the clinical efficacy and safety of FMT identified 45 studies[40]. Adverse events were rare, being reported in 34 out of 45 studies. In total, 35 out of 1029 patients, were reported to have died and 10 patients were hospitalised during follow-up. One patient died from aspiration during sedation for FMT administered via colonoscopy, which was considered to be related to the FMT procedure. Four patients were reported to have died from complicated CDI with small bowel involvement confirmed at autopsy, a toxic megacolon due to persistent CDI one month after FMT, and complicated CDI not further specified. A severely ill patient treated with FMT for CDI, died of a peritonitis which could be related to treatment. Reported adverse events associated with FMT were mostly self-limiting and occurred frequently within hours after infusion. Intestinal reported symptoms were: bloating, flatulence, belching and abdominal cramps, abdominal discomfort, irregularity of bowel movements and vomiting. In 11 patients fever, without other clinical symptoms or signs of sepsis, was reported during and up to one day after FMT. No causative agents were identified by blood culture, but a rise in C-reactive protein was measured in some of these patients. Fever disappeared within 3 days in all patients. Overall, based on the systematic review, the safety profile of FMT proved to be excellent.

However, it is worth underlying that route of fecal infusion may influence appearance of adverse events after FMT. In another systematic review,

indeed, patients receiving infusion via upper gastrointestinal routes were more likely to develop adverse events as compared to those via lower gastrointestinal routes (44% versus 21%)[41].

Interestingly, more recent use of frozen capsules FMT for the treatment of CDI showed efficacy with a favorable safety profile consisting of few mild abdominal cramping and bloating, and may therefore represent in the future a safer and more easily available strategy for FMT, but more studies are needed[42].

Regarding very-long-term risks (more than 5 years), little evidence is available[43]. Despite anecdotal reports of "FMT-related diseases", with transmission of malignant, autoimmune, metabolic or neuropsychiatric diseases shown in animal models, causality to FMT remains unclear.

1.3.2. Use of FMT in hematologic patients

Many concerns were initially raised about the feasibility of FMT in immunocompromised patients, such as those affected by hematological malignancies, because of the theoretic potential for local and bloodstream infections but recent case reports revealed the efficacy and safety in this particular population[44,45,39]. However, to date use of FMT in this setting still remains investigational.

One case-series study reported on FMT use in 80 immunocompromised patients[39]. Although serious adverse events (2 deaths and 10 hospitalizations) occurred in 12 patients (15%), none seemed to be directly related to FMT. Non-serious adverse events were reported for 12

patients (15%); four were considered related to FMT, five were possibly related and three were unrelated to FMT.

A retrospective study done on immunocompromised and non-immunocompromised patients who received FMT for recurrent CDI concludes that response to FMT is equivalent in the two populations, with similar safety[46].

Heterologous FMT has been evaluated in 14 immunocompromised patients after allo-HSCT[47,48] without any severe adverse effect reported.

Webb et al. reported the use of heterologous FMT in 7 patients with recurrent CDI after allo-HSCT. FMT was administered via the naso-jejunal route in 6 of 7 patients. No serious adverse events were noted in these immunocompromised patients. Diarrhea was improved in all patients, with no recurrence in most of the patients. Therefore, FMT appears to be safe for recurrent CDI also in immunocompromised allo-HSCT patients[49].

More recently, due to the aforementioned dysbiosis related to GVHD in transplanted patients, use of FMT has also been explored as a treatment of patients presenting steroid refractory GVHD. A pilot study, indeed, investigated whether empiric third-party frozen FMT capsules would be safe and feasible after allo-HSCT, and would be able to restore recipient microbiome diversity[50], with promising results prompting to the activation of a prospective European study further exploring this issue (NCT03359980).

1.3.3. Use of fecal microbiota transplantation for the eradication of multidrug-resistant bacteria in hematological patients

Pretransplant conditioning and intensive chemotherapies for acute leukemias, indeed, induce aplasia causing important predisposition to disseminated infections, also favored by the aforementioned modifications in epithelial cells and by mucositis. These modifications render easier that commensal bacteria may invade underlying tissues and the bloodstream. This prompts the immediate introduction of large spectrum antibiotics in order to treat the infection. Use of antibiotics, not only in the hematologic field, is a well-known cause of dysbiosis, contributing to the selection and persistent colonization from multidrug resistant bacteria (MDRB) [51].

During the last decades, the prevalence of MDRB has largely increased, becoming a serious worldwide problem, significantly impacting on the healthcare system[52].

In order to prevent spreading of these bacteria 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[53]. According to French recommendations[54], for example, patients colonized with MDRB are not easily admitted in healthcare facilities not disposing of dedicated staff.

Under physiological conditions, commensal microbiota prevents gut colonization from MDRB. Patients undergoing allo-HSCT are at even higher risk of dysbiosis due to their profound immune depression[55]. In case of

bloodstream infections from MDRB, outcomes are even poorer, with consequent increased mortality[56]. 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[57].

Spontaneous decolonization is a long process, with variable durations according to the healthcare system where the patient is hospitalized and also depending on associated disease and concomitant treatments. In patients treated in long-term care facilities and intensive care units, for example, it has been reported a median duration of colonization with MDRB of 144 days, with spontaneous clearance in only 9% of patients[58]. In the hematological setting, an even longer time of 387 days was required to obtain negative rectal swab cultures from individuals who were originally colonized with MDRB[59].

New classes of antibiotics are under study to treat infections related to MDRB, and active research is ongoing to find effective decolonization strategies[60]. The use of oral gentamicin or colistin had been initially proposed in some MDR-gram negative strains, but failure is common, and the risk of selecting gentamicin- or colistin-resistant strains may also be present[61,62,63]. Due to the emergence of new resistant strains, use of oral decontaminating agents is not adviced in clinical practice and other decolonizating strategies have been explored, with promising results with the use of FMT for eradication of MDRB[56].

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[64], including some of them with GVHD after allo-HSCT.

One can speculate that early initiation of FMT therapy could possibly spare infectious complications from MDRB and decrease the economic burden related to these bacteria.

With this rationale, we retrospectively collected data on our experience with FMT in patients diagnosed with hematologic malignancies and colonized or experiencing systemic infections of MDRB.

2. MATERIALS AND 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, in order to identify asymptomatic carriers with high risk of spreading MDRB to other patients, with consequent preventive measures in positive patients in order to limit MDRB spread.

Screening modalities consisted of weekly rectal swab. After MDRB identification, patients colonized with vancomycin-resistant (VRE) or carbapenemase-producing enterobacteriaceae (CPE) were 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.

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.

According to French regulations, 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 (hematologist, gastroenterologist, pharmacist) 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, which mentioned 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.

2.1. INCLUSION CRITERIA

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.

It is worth underlying that opportunistic saprophytic bacteria, such as CP-*Pseudomonas aeruginosa*, have not been considered as eXDR according to French guidelines. However, it has been already reported that patients experiencing systemic infections from CP-*Pseudomonas aeruginosa* have a high risk of death, 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 also proposed to those patients experiencing systemic infections or in those colonized from CP-*Pseudomonas Aeruginosa*, in order to limit systemic infections.

On the other hand, we did not consider colonization from ESBL-producing enterobacteriaceae as an indication for FMT as those patients do not need isolation according to French guidelines. However, for the purpose of the current report, we retrospectively registered in patients undergoing FMT for the aforementioned indications, if they were also colonized with ESBL-producing bacteria in order to look if FMT also allowed decolonization from these bacteria.

Timing for FMT was either before allo-HSCT in patients having undergone induction and consolidation chemotherapies, or after allo-HSCT.

In patients initially achieving decolonization and then experiencing MDRB recurrence or in those patients for whom FMT was a failure, a second attempt could be proposed.

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.

2.2. DEFINITIONS

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. [65]: 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.

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.

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.

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.

2.3. 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.

2.4. DONOR SCREENING

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.

Chronic treatments not considered as a contraindication for stools donation, were stopped one week before FMT was performed due to the absence of certain knowledge on their impact on gut microbiota.

2.5. PRODUCT PREPARATION

Transplants are prepared in the Saint Antoine Hospital pharmacy according to recommendations from the French Group of FMT[66]. In case of freezing, the stool preparation are 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.

2.6. ADMINISTRATION OF THE FINAL PRODUCT

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 (2 liters the night before and 2 liters the day of the procedure).

In the case of enema administration, patients were positioned in lateral decubitus. A probe connected to the enema bag was then introduced up to the first 10-15 cm of the rectal ampulla. Duration of the administration varied from 15 to 30 minutes according to the final volume of the enema bag. After the procedure, patients were asked to hold the infused material for at least 2 hours. During this period, they were also asked to remain supine in order to minimize the urge to defecate and to periodically change position (i.e. each 30 minutes) in order to allow product diffusion in the colon.

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. Positioning of a nasogastric tube was done the day before FMT and radiological check of correct positioning was mandatory. Once patients conditions were considered favorable for the product administration (i.e. in the absence of gastrointestinal symptoms such as nausea or vomiting), this was made by 50 ml syringes. Once the

administration was completed, patients were kept in a 45° upright position for 4 hours after infusion in order to prevent material aspiration.

2.7. SAFETY

The safety of the procedure was also registered. For all patients, data on significant infections, defined as bacteriemias or sepsis occurring during the first 90 days after FMT were also collected. Febrile neutropenia or fever of unknown origin were not considered as significant infectious episodes but, when occurring during the first 90 days after FMT, they were also recorded.

Adverse events were graded according to Common Terminology Criteria for Adverse Events (CTCAE).

3. 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.

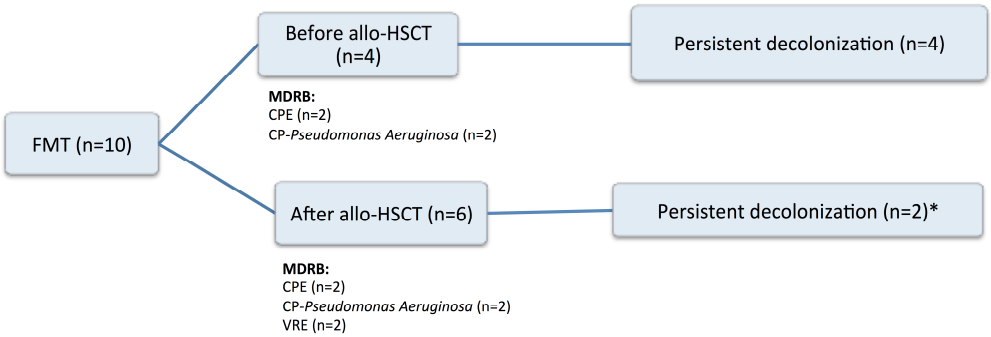
A frozen product was used in eight out of ten patients and enema was the preferred way of administration in all but one patient. This patient, indeed, presented a compromised neurological status due to a cerebral toxoplasmosis and she was not considered eligible for enema. Median

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. 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 4). 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.



*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 4. ***Results of fecal microbiota transplantation***

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 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 entrobacteriaceae 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 entrobacteriaceae, three obtained concomitant decolonization.

Details on FMT performed before or after allo-HSCT are reported in Table 1.

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

Table 2. ***Characteristics of patients undergoing fecal microbiota transplantation before (a) or after (b) hematopoietic stem cell transplantation.*** ***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,	N/A	27	118	84	N/A	N/A

days						
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.

As an example of successful FMT, Figure 5 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.

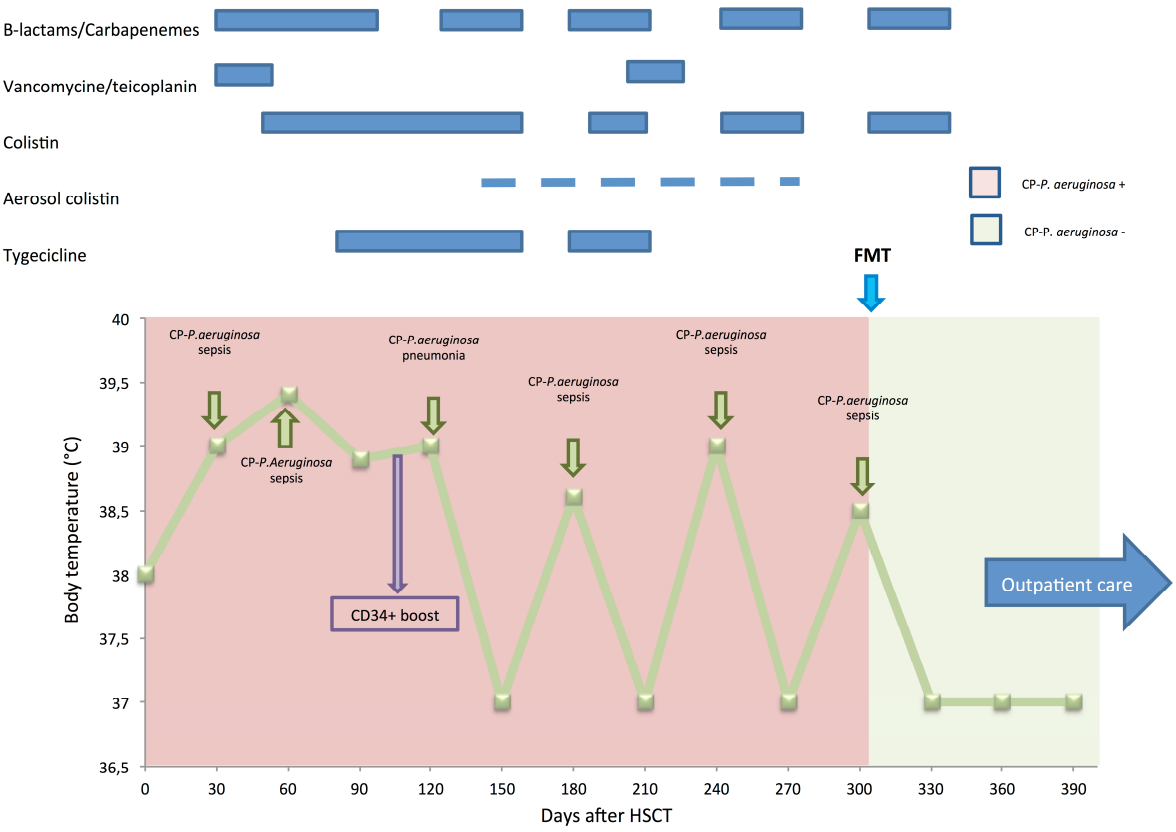


Figure legend: CP-P. aeruginosa, carbapenemase-producing Pseudomonas Aeruginosa; FMT, fecal microbiota transplantation; HSCT, hematopoietic stem cell transplantation

Figure 5 ***Example of a successful fecal microbiota transplantation in patient 5.***

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. Six patients were discharged from the hospital 24 hours after FMT was performed.

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.

4. 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[53,67].

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 (ARB)[68]. 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[51]. 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%[55,56].

It has been largely reported that microbioma modifications are associated to worse survival, higher risk of infections and GVHD in patients undergoing allo-HSCT[69,70]. 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[71]. 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[50].

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 entreobacteriaceae, 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.

The use of FMT represents an attractive strategy for MDRB decolonization, allowing intestinal microbiota modulation with

subsequent reconstitution of a healthy microbiota. Historically, FMT was first used in recurrent and severe CDI, and it has also shown promising activity in bowel disease[40,36,72]. Infusion of donor faeces in patients with CDI allowed improvement in gut microbial diversity, which persisted over time. Experience in the MDRB field is more recent, with several case reports and small series in different subsets of patients[73,74,75].

Despite the initial aforementioned concerns in immunocompromised patients, results of FMT in this setting are promising in terms of both efficacy and safety[56,45,39]. 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[64]. 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[76]. 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, antibiotic treatment allows intestinal colonization by VRE, and FMT is able to eradicate it [77].

In humans, initial lessons from FMT in CDI may help understand the pathophysiology of MDRB decolonization. One study showed that patients experiencing recurrent CDI present a significant dysbiosis, as they harbor a high number and a wide variety of ARB, as compared to healthy stool donors [78]. It seems that use of FMT may restore a more physiological microbioma by lowering the load of ARB, thus highlighting the possibility that FMT may eradicate MDRB and allow restoration of antibiotic susceptibility.

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. This suggests a possible protective role of the restoration of a healthy microbiota in preventing severe infections, but numbers are too low to draw significant conclusions.

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 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 [79,80]. 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 [47].

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.

5. REFERENCES

1. Li J, Jia H, Cai X, *et al. An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol. 2014; 32(8): 834-41.*

2. Fulde M, Hornef MW. Maturation of the enteric mucosal innate immune system during the postnatal period. *Immunol Rev.* 2014 Jul; 260(1) : 21-34.

3. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. *Nat Immunol* 2013; 14(7): 685-90.

4. Neuman H, Debelius JW, Knight R, Koren O. Microbial endocrinology : the interplay between the microbiota and the endocrine system. *FEMS Microbiol Rev* 2015; 39(4): 509-21.

5. Heiser HJ, Gootenberg DB, Chatman K, *et al*. Predicting and Manipulating Cardiac

   Drug Inactivation by the Human Gut Bacterium Eggerthella lenta. *Science* 2013; 341: 295-299.

6. Yatsunenko T, Rey FE, Manary MJ, *et al*. Human gut microbiome viewed across age and geography. *Nature* 2012; 486(7402): 222-7.

7. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012; 486(7402):

207-14.

8. Cheng J, Ringel-Kulka T, Heikamp-de Jong I *et al*. Discordant temporal development of bacterial phyla and the emergence of core in the fecal microbiota of young children. *ISME J*. 2016; 10(4): 1002-14.

9. Qin J, Li R, Raes J *et al*. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature.* 2010; 464(7285): 59-65.

10. Claesson MJ, Jeffery IB, Conde S *et al*. Gut microbiota composition correlates with diet and health in the elderly. *Nature.* 2012; 488(7410): 178-84.

11. Bäckhed F, Roswall J, Peng Y *et al*. Erratum Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe.* 2015; 17(6): 852.

12. Goodrich JK, Waters JL, Poole AC *et al*. Human genetics shape the gut microbiome. Cell. 2014; 159(4): 789-99.

13. Wang S, Charbonnier LM, Noval Rivas M *et al*. MyD88 Adaptor-Dependent Microbial Sensing by Regulatory T Cells Promotes Mucosal Tolerance and Enforces Commensalism. *Immunity*. 2015; 43(2):289-303.

14. Maurice CF, Haiser HJ, Turnbaugh PJ. Xenobiotics Shape the Physiology and Gene Expression of the Active Human Gut Microbiome. *Cell*. 2013; 152(1-2): 39-50.

15. David LA, Maurice CF, Carmody RN *et al*. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; 505(7484): 559-63.

16. Tang WH, Wang Z, Levison BS, *et al.* Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. 2013; 368(17): 1575-84.

17. Le Chatelier E, Nielsen T, Qin J *et al*. Richness of human gut microbiome correlates with metabolic markers. *Nature.* 2013; 500(7464): 541-6.

18. Fujimura KE, Sitarik AR, Havstad S, *et al*. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med*. 2016; 22(10): 1187-1191.

19. Hsiao EY, McBride SW, Hsien S, *et al*. Microbiota Modulate Behavioral and physiological Abnormalities Associated with Neurodevelopmental Disorders. *Cell.* 2013; 155(7): 1451-63.

20. Hansen TH, Gøbel RJ, Hansen T, Pedersen O. The gut microbiome in cardio-metabolic health. *Genome Med*. 2015; 7(1):33.

21. Touchefeu Y, Montassier E, Nieman K, *et al.* Alimentary Pharmacology and Therapeutics Systematic review : the role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis – current evidence and potential clinical applications. *Aliment Pharmacol Ther.* 2014; 40(5): 409-421.

22. Stringer AM, Gibson RJ, Bowen JM *et al*. Irinotecan-induced mucositis manifesting as diarrhoea corresponds with an amended intestinal flora and mucin profile. *Int J Exp Pathol*. 2009; 90(5): 489-99.

23. Taur Y, Jenq RR, Perales MA *et al*. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood.* 2014; 124(7): 1174-82.

24. Kolho KL, Korpela K, Jaakkola T *et al*. Fecal Microbiota in Pediatric Inflammatory Bowel Disease and Its Relation to Inflammation. *Am J Gastroenterol*. 2015; 110(6):921-30.

25. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal Microbiota Transplantation for Clostridium difficile Infection : Systematic Review and Meta-Analysis. *Am J Gastroenterol.* 2013 Apr;108(4):500-8.

26. Lewin RA. 1999. Merde: excursions in scientific, cultural, and

sociohistorical Coprology). Random House Inc, New York, NY.

27. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* 1958; 44: 854–859.

28. van Nood E, Vrieze A, Nieuwdorp M *et al*. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med*. 2013; 368(5): 407-15.

29. McGlone SM, Bailey RR, Zimmer SM *et al*. The economic burden of Clostridium difficile. *Clin Microbiol Infect.* 2012; 18(3): 282-9.

30. Cammarota G, Masucci L, Ianiro G. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. *Aliment Pharmacol Ther*. 2015; 41(9): 835-43.

31. Drekonja D, Reich J, Gezahegn S *et al.* Fecal Microbiota Transplantation for Clostridium difficile Infection: A Systematic Review. *Ann Intern Med*. 2015; 162(9):630-8.

32. Moayyedi P, Surette MG, Kim PT *et al*. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. *Gastroenterology* 2015; 149(1): 102-109.

33. Manges AR, Steiner TS, Wright AJ. Fecal microbiota transplantation for the intestinal decolonization of extensively antimicrobial-resistant opportunistic pathogens: a review. *Infect Dis (Lond).* 2016; 48(8): 587-92.

34. Jiang ZD, Hoang LN, Lasco TM, Garey KW, Dupont HL. Physician attitudes toward the use of fecal transplantation for recurrent Clostridium difficile infection in a metropolitan area. *Clin Infect Dis.* 2013; 56(7): 1059-60.

35. Zipursky JS, Sidorsky TI, Freedman CA, *et al.* Patient Attitudes Toward the Use of Fecal Microbiota Transplantation in the Treatment of Recurrent Clostridium difficile Infection. *Clin Infect Dis*. 2012; 55(12): 1652-8.

36. Cammarota G, Ianiro G, Tilg H *et al*. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut.* 2017; 66(4): 569-580.

37. Quera R, Espinoza R, Estay C, Rivera D. Bacteremia as an adverse event of fecal microbiota transplantation in a patient with Crohn's disease and recurrent Clostridium difficile infection. *J Crohns Colitis*. 2014; 8(3):252-3.

38. MacConnachie AA, Fox R, Kennedy DR, Seaton RA. Faecal

transplant for recurrent Clostridium difficile-associated diarrhoea: a UK case series. *QJM*. 2009; 102(11): 781-4.

39. Kelly CR, Ihunnah C, Fischer M *et al.* Fecal Microbiota Transplant for Treatment of Clostridium diffi cile Infection in Immunocompromised Patients. *Am J Gastroenterol*. 2014; 109(7): 1065-71.

40. Rossen NG, MacDonald JK, de Vries EM *et al*. Fecal microbiota transplantation as novel therapy in gastroenterology: A systematic review. *World J Gastroenterol.* 2015; 21(17): 5359-71.

41. Wang S, Xu M, Wang W *et al.* Systematic Review : Adverse Events of Fecal Microbiota Transplantation. *PLoS One.* 2016; 11(8): e0161174.

42. Hirsch BE, Saraiya N, Poeth K *et al.* Effectiveness of fecal-derived microbiota transfer using orally administered capsules for recurrent Clostridium difficile infection. *BMC Infect Dis.* 2015; 15: 191.

43. Brandt LJ, Aroniadis OC, Mellow M *et al*. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. *Am J Gastroenterol.* 2012; 107(7): 1079-87.

44. Biliński J, Grzesiowski P, Muszyński J, *et al.* Fecal Microbiota Transplantation Inhibits Multidrug-Resistant Gut Pathogens :

Preliminary Report Performed in an Immunocompromised Host. Arch Immunol Ther Exp (Warsz). 2016; 64(3): 255-8.

45. de Castro CG Jr, Ganc AJ, Ganc RL, Petrolli MS, Hamerschlack N. Fecal microbiota transplant after hematopoietic SCT: report of a successful case. *Bone Marrow Transplant*. 2015; 50(1): 145.

46. Mandalia A, Ward A, Tauxe W, Kraft CS, Dhere T. Fecal transplant is as effective and safe in immunocompromised as non-immunocompromised patients for Clostridium difficile *Int J Colorectal Dis*. 2016; 31(5): 1059–1060.

47. Kakihana K, Fujioka Y, Suda W *et al*. Fecal microbiota transplantation for patients with steroid-resistant acute graft-versus-host disease of the gut. *Blood*. 2016; 128(16): 2083-2088.

48. Spindelboeck W, Schulz E, Uhl B, Repeated fecal microbiota transplantations attenuate diarrhea and lead to sustained changes in the fecal microbiota in acute, refractory gastrointestinal graft-versus-host-disease. *Haematologica*. 2017; 102(5): e210-e213.

49. Webb BJ, Brunner A, Ford CD, Gazdik MA, Petersen FB, Hoda D. Fecal microbiota transplantation for recurrent Clostridium difficile infection in hematopoietic stem cell transplant recipients. *Transpl Infect Dis.* 2016; 18(4): 628-33.

50. 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 .

51. Montassier E, Gastinne T, Vangay P, *et al.* Chemotherapy-driven dysbiosis in the intestinal microbiome. *Aliment Pharmacol Ther.* 2015; 42(5): 515-28.

52. Spellberg B, Guidos R, Gilbert D, et al. T The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin Infect Dis*. 2008; 46(2): 155-164.

53. Birgand G, Leroy C, Nerome S, *et al*. Costs associated with implementation of a strict policy for controlling spread of highly resistant microorganisms in France. *BMJ Open*. 2016; 6(1): 1-9.

54. Lepelletier D, Berthelot P, Lucet JC, *et al*. French recommendations for the prevention of " emerging extensively drug-resistant bacteria " (eXDR) cross-transmission. *J Hosp Infect*. 2015; 90(3): 186-195.

55. Samet A, Śledzińska A, Krawczyk B, *et al*. Leukemia and risk of recurrent Escherichia coli bacteremia: Genotyping implicates E. Coli translocation from the colon to the bloodstream. *Eur J Clin Microbiol Infect Dis*. 2013; 32(11): 1393-1400.

56. Bilinski J, Robak K, Peric Z, *et al*. Impact of Gut Colonization by Antibiotic-Resistant Bacteria on the Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective, Single-Center Study. *Biol Blood Marrow Transplant*. 2016; 22(6): 1087-1093.

57. Cattaneo C, Zappasodi P, Mancini V, *et al*. Emerging resistant bacteria strains in bloodstream infections of acute leukaemia patients: results of a prospective study by the Rete Ematologica Lombarda (Rel). *Ann Hematol*. 2016; 95(12): 1955-1963.

58. O'Fallon E, Gautam S, D'Agata EM. Colonization with Multidrug-Resistant Gram-Negative Bacteria : Prolonged Duration and Frequent Cocolonization. *Clin Infect Dis*. 2009; 48(10): 1375-81.

59. Zimmerman FS, Assous MV, Bdolah-Abram T, Lachish T, Yinnon AM, Wiener-Well Y. Duration of carriage of carbapenem-resistant Enterobacteriaceae following hospital discharge. *Am J Infect Control*. 2013; 41(3): 190-4.

60. Bassetti M, Giacobbe DR, Giamarellou H, *et al.* Management of KPC-producing Klebsiella pneumoniae infections. *Clin Microbiol Infect.* 2018; 24(2): 133-144.

61. Tascini C, Sbrana F, Flammini S, *et al*. Oral gentamicin gut

decontamination for prevention of KPC-producing Klebsiella pneumoniae infections: Relevance of concomitant systemic antibiotic therapy. *Antimicrob Agents Chemother*. 2014; 58(4):1972-1976.

62. Lübbert C, Faucheux S, Becker-Rux D, et al. Rapid emergence of secondary resistance to gentamicin and colistin following selective digestive decontamination in patients with KPC-2-producing Klebsiella pneumoniae: A single-centre experience. *Int J Antimicrob Agents*. 2013; 42(6): 565-570.

63. Halaby T, Al Naiemi N, Kluytmans J, van der Palen J, Vandenbroucke-Grauls CM. Emergence of Colistin Resistance in Enterobacteriaceae after the Introduction of Selective Digestive Tract Decontamination in an intensive care uint. *Antimicrob Agents Chemother*. 2013 Jul;57(7):3224-9..

64. Bilinski J, Grzesiowski P, Sorensen N, et al. Fecal Microbiota Transplantation in Patients With Blood Disorders Inhibits Gut Colonization With Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clin Infect Dis*. 2017; 65(3): 364-370.

65. Magiorakos A, 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): 268-81.

66. Trang G, Scanzi J, Galperine T, *et al*. Transplantation de microbiote fe dans le cadre des infections a ` Clostridium difficile recidivantes : actualisation des recommandations pour la pratique clinique courante. Hepato-gatro et oncologie digestive 2017; 24:319-325.

67. WHO. Antimicrobial resistance. Global Report on Surveillance. *Bull World Health Organ*. 2014;61(3):383-394.

68. van Schaik W. The human gut resistome. *Philos Trans R Soc Lond B Biol Sci*. 2015; 370(1670): 20140087.

69. Taur Y, Jenq RR, Perales MA, *et al*. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood*. 2014; 124(7): 1174-82.

70. Jenq RR, Taur Y, Devlin SM, *et al*. Intestinal Blautia Is Associated with Reduced Death from Graft-versus-Host Disease. *Biol Blood Marrow Transplant*. 2015; 21(8): 1373-1383.

71. Austin M, Mellow M, Tierney WM. Fecal microbiota transplantation in the treatment of clostridium difficile infections. *Am J Med*. 2014; 127(6): 479-483.

72. Millan B, Laffin M, Madsen K. Fecal Microbiota Transplantation:

Beyond Clostridium difficile. *Curr Infect Dis Rep*. 2017; 19(9):31.

73. Crum-Cianflone NF, Sullivan E, Ballon-Landa G. Fecal microbiota transplantation and successful resolution of multidrug-resistant-organism colonization. *J Clin Microbiol*. 2015; 53(6): 1986-1989.

74. Dubberke ER, Mullane KM, Gerding DN, *et al*. Clearance of vancomycin-resistant Enterococcus concomitant with administration of a microbiota-based drug targeted at recurrent Clostridium difficile infection. *Open Forum Infect Dis*. 2016; 3(3): 1-6.

75. García-Fernández S, Morosini MI, Cobo M, *et al*. Gut eradication of VIM-1 producing ST9 Klebsiella oxytoca after fecal microbiota transplantation for diarrhea caused by a Clostridium difficile hypervirulent R027 strain. *Diagn Microbiol Infect Dis*. 2016; 86(4): 470-471.

76. Tang G, Yin W, Liu W. Is frozen fecal microbiota transplantation as effective as fresh fecal microbiota transplantation in patients with recurrent or refractory Clostridium difficile infection: A meta-analysis? *Diagn Microbiol Infect Dis*. 2017; 88(4): 322-329.

77. Ubeda C, Taur Y, Jenq RR, *et al.* Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest.*

2010; 120(12): 4332-41.

78. Millan B, Park H, Hotte N, *et al.* Fecal Microbial Transplants Reduce Antibiotic-resistant Genes in Patients with Recurrent Clostridium difficile Infection. *Clin Infect Dis*. 2016; 62(12): 1479-1486.

79. Jones JM, Wilson R, Bealmear PM. Mortality and gross pathology of secondary disease in germfree mouse radiation chimeras. *Radiat Res*. 1971; 45(3): 577-588.

80. Shono Y, Docampo MD, Peled JU, *et al*. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. *Sci Transl Med*. 2016; 8(339): 1-16.