Microb Health Dis 2019; 1: e182

European Helicobacter & Microbiota Study Group

Advances in stool banking

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Abstract: Transplantation of fecal microbiota (FMT) is now standard of care in treatment of recurrent *Clostridioides* difficile infections (rCDI). Research addresses whether FMT is also effective for other disorders associated with dysbiosis of the gut. To facilitate safe and cost-effective FMT, stool banks have been founded to provide ready-to-use FMT suspensions and to ensure quality assurance. Stool banks can operate at an institutional or regional/national level. Stool banks prepare feces suspensions of stool(s) from extensively screened healthy donors. Suspensions are quarantined at -80°C and only used for FMT after retesting of the donor. Standardization of stool banking is required because it enables stool banks to cooperate and compare their products and performance, which will provide important information for quality improvement. Ongoing and future studies with FMT should finally result in the development of standardized bacterial mixtures as microbiota modulating therapies for (r)CDI and other potential indications. Stool banks may help to facilitate these developments.

Keywords: Stool bank, Clostridioides difficile infection, Fecal Microbiota Transplantation, Quality assurance.

INTRODUCTION

Fecal microbiota transplantation (FMT) is a novel therapeutic strategy in medicine targeting a disturbed microbiome. It has become standard of care for patients with recurrent *Clostridioides difficile* infection (rCDI)[1,2], but is also promising for other disorders in which microbiota changes seem to contribute to development and progression of the disease[3,4]. The human gut microbiota consists of trillions of microbes living in symbiosis in the gastrointestinal tract and is responsible for many important gut-functions like contributing to digestion and training of the immune system, maintaining its barrier function and protection against pathogens[5-8]. Since recent innovations in the field of gene sequence technology and analysis, the microbiome can be analysed more precisely and leads to more fundamental knowledge of the human microbiome itself[9-11]. Thereby more insight is gained in the role of the microbiome on the pathogenesis of various diseases and their subsequent potential treatment options[12-16].

Failure rates of antibiotic therapy are relatively high in rCDI (40-65%)[17-19]. A single FMT either delivered by naso-duodenal tube, colonoscopy or even capsules display high cure rates of more than 80%[1,20,21]. Limited availability of donor feces suspensions has hampered the rapid implementation of FMT in daily practice. To overcome this hurdle, stool banks were founded to provide ready-to-use FMT suspensions. In addition, centres of excellence were established to treat patients with FMT[22].

In this selective review we describe requirements for stool banking and current practice of stool banks. In addition, we outline some challenges and future developments.

STOOL BANKS

The aim of a stool bank is to provide standardized screened donor feces suspensions, enabling the accessibility and safe use of FMT for patients. Stool banks can be institution-based (an expert center) or provide suspensions that can be used for treatment of patients in other hospitals by a local physician. Furthermore, stool banks strive to optimize the existing production protocol and facilitate FMT-research for other diseases in which the dysbiosis of the gut microbiota appears to play a role. The working process of stool banks is outlined in Figure 1.

In fact, large scale stool banking has only become possible because of the observations that frozen suspensions are non-inferior as compared to fresh FMT suspensions to treat patients with rCDI[23]. Whether this is also the case for other potential indications remains to be established.

Facility, storage conditions, and data management

At initiation a stool bank may be part of a microbiology, clinical or research laboratory. However, considering requirements for standardization and quality assurance, a stool bank should have a dedicated laboratory. It is questionable whether a true GMP facility is required for preparation of safe and high quality FMT solutions. Although many GMP requirements seem appropriate, true standardization of the end product (processed stool) is not possible given donor-to-donor variation. However, national regulatory requirements may dictate a GMP facility for stool banks and other biologically-derived products (e.g., plasma, IV Ig) have adopted product-appropriate GMP standards. A laboratory for stool banking should at minimum consist of a laminar flow cabinet to prevent cross contamination. A -80°C freezer is recommended for long-term storage to minimise sample degradation. A date of expiration should be registered on the product. Respectively, OpenBiome and the Netherlands Donor Feces Bank (NDFB) have positive experiences with storage up to 1 year and two years (unpublished). Freezers should have a connected alarm notification to guarantee continuous registration of the storage temperature. A biobanking information and management system for coding, registrations and track and tracing of the samples is also required in the context of biovigilance. Separate databases are needed to collect and store data of donors and patients, and for research purposes.

The role of anaerobic stool processing (using an anaerobic cabinet) remains unknown given the paucity of data. Aerobic processing suffices for treatment of patients with rCDI, but for other conditions, such as ulcerative colitis, anaerobic processing may be of benefit.

Processing of donor suspensions

Stool should be collected in a clean and single-use container and stored at room temperature for no longer than 6 hours[22,24]. Processing of donor feces suspensions immediately after defaecation is preferable if logistically possible.

Many stool banks and expert centers use > 50 gram of feces because systematic reviews suggest that the use of ≥ 50 gram of donor stool per suspension is more effective although the strength of this observation is limited by a paucity of comparative studies, low sample size, and confounding by various delivery routes[20,22]. However, a large US stool bank (OpenBiome) has reported similar rates of efficacy with 25 grams of feces in 250 ml lower GI preparation and 12.5 grams of feces in a 30 ml upper GI preparation[25].

While processing donor feces to suspensions, sterile or clean material should be used, which implies that all equipment should be autoclavable or disposable. Initially, sterile 0,9% saline is added as diluent to prepare the fresh donor feces[26-28]. The use of a bag mixer is easy and efficient, combining the steps of homogenization and filtering of the suspension.

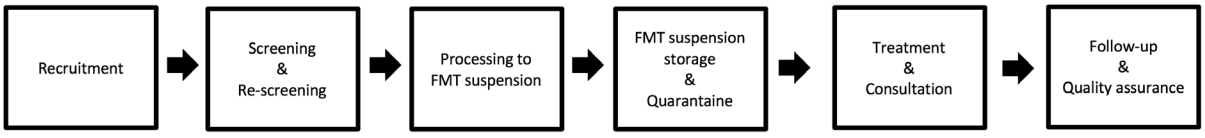

Figure 1. Working process of stool banks.

However, many alternative methods for homogenization and filtering have been reported. For example, a blender or a wooden spatula can be used for homogenization and a gauze or metal sieve can be used for filtration[1,28,29]. Concentration by centrifugation can be added as additional step[30]. As a cryopreservant, glycerol (provided by the hospital pharmacy) may be added to an end concentration of 10%. However, there is limited data to determine the ideal concentration[31].

To date, several stool banks use donor feces suspensions to treat patients. Capsules appear promising with comparable success rates as suspensions. However, each capsule formulation may be variable[32]. Drawbacks of capsules are the amount of capsules to be swallowed and the difficult handling of frozen capsules. The results of freeze drying (lyophilization) appear promising[33] and this capsule formulation may become the standard for FMT treatment of rCDI in the future[33-35]. Some key studies reporting FMT with capsules are listed in Table 1.

Quality assurance

A recently published consensus meeting report had a strong focus on safety and quality assurance[22]. Recommendations addressing quality assurance are listed in Table 2. Importantly, stool banks need a written organisational plan focussed on quality and safety.

Standard operating procedures (SOPs) should be implemented to guarantee the quality of the screening, processing, storage, labelling, packaging, and distribution of the feces suspensions. Aliquots of all suspensions that are used to treat patients should be stored for future investigation in case of (unpredicted) adverse events or quality control. In addition, all data about the screening of donors should be stored.

Outcome data (both efficacy and adverse events) should be collected and reported by stool banks for quality assurance. In case of adverse events, traceability (biovigilance) is required to enable retrospective analysis of the used donor feces.

To further improve the safety and quality of FMT, auditing of stool banks might become required to check the working processes.

TABLE 1. OVERVIEW FECAL MICROBIOTA TRANSPLANTATION (FMT) BY CAPSULES FOR TREATMENT OF RECURRENT CLOSTRIDIOIDES DIFFICILE INFECTION.

Study	Design	Processing	Patients (N)	Capsules (N)	Amount feces (g)	Primary success rate (%)[1]	Secondary success rate (%)[2]
Hirsch et al (2015)	Prospective	Frozen	20	8-12	2.3	68.0	89.0
Youngster et al (2016)	Prospective	Frozen	180	30	48	82.0	91.0
Youngster et al (2014)	Prospective	Frozen	20	30	48	70.0	90.0
Kao et al (2017)	RCT	Frozen	57	40	80 to 100	96.2	x
Chechri et al (2018)	Case-serie	Frozen	9	75	85	100.0	x
Staley et al (2017)	Prospective	Freeze-dried	49	2 to 3	x	87.8	x
Jiang et al (2018)	RCT	Freeze-dried	31	± 27	100	63	x
					200	91	x
Allegretti et al (2019)	Prospective	x	51	40 to 903	30 to 67.54	75 to 80.65	x

RCT= Randomized Controlled Trial, N= number, G= Gram, %= Percent, [1]Success rate after one course of FMT, [2]Total cure rate after repeated FMT's with capsules, x= not mentioned, not performed.
[3]Two cohorts receiving different doses, [4]Due to number of capsules, each capsule contained 0.75 gram, [5]Clinical cure rate for gastric route as well as colonoscopic route

TABLE 2. H. MEASURES FOR QUALITY ASSURENCE AND FMT*.
*Standardized protocols for donor screening
*Standard Operating Procedures (SOP) for all processes
*Rescreening and quarantaine
*Storage of aliquots of each donated stool
*Secured databases for storage of donor and patient data
*Follow-up and reporting of outcome data
*Consultation
*Auditing

*Rome II consensus report, Cammarota et al. (2019)

Donor Recruitment

In general, donors should live or work in the near proximity of the stool bank to enable fast delivery of stool after defecation.

Potential donors are extensively screened before they can become stool donor.

First, they receive an extensive questionnaire addressing general health, risk factors for potential transmittable diseases and risk factors for disorders associated with a perturbed microbiota. This questionnaire also includes family-history, medication use, and previous medical history. The NDFB selects donors with a maximum age of 60 years old and a Body Mass Index (BMI) not exceeding 25 kg/m^2. The chronic use of medication is a reason for exclusion of a donor, because it may have a negative impact on the microbiota or reflect a less healthy state. For example, individuals using proton pump inhibitors, statins, selective serotonin reuptake inhibitors (SSRI's), and nonsteroidal anti-inflammatory drugs (NSAID's) are excluded as donors. Health care workers are probably more frequently exposed to pathogens in their work-environment and could have transient carriage or passage of pathogenic microbes. However, there is no evidence showing that carefully screened health care workers are more likely to transmit (antibiotic resistant) pathogens if they serve as stool donors. Some stool banks exclude health care workers as donors while others do permit them to become stool donors.

If an individual has passed the first selection round by interview, microbiological testing is applied. A feces sample is checked for pathogenic bacteria, viruses, and parasites. Blood is tested for the presence of blood transmissible diseases like hepatitis, syphilis or HIV. Table 3 lists the panel of screening tests that are used by the NDFB[36].

Overall, less than 3 percent of all individuals that apply to become a stool donor are found to be suitable[30,37]. If a donor is suitable for donation, before every donation a questionnaire about the recent health status should be filled in. All actively donating donors undergo rescreening after at least 3 months.

TREATMENT OF PATIENTS

Since 2016 FMT is an approved treatment option for rCDI. For many other disorders showing disease associated dysbiosis, the clinical efficacy of FMT is currently investigated[38,39]. Because many case reports and some clinical studies suggest some benefit in a variety of disorders, FMT may be considered as compassionate use treatment if all other standard or reasonable therapeutic options are unsuccessful.

Although diagnosis and treatment of rCDI appears straightforward, there are potential diagnostic pitfalls in discerning colonization from true infection, particularly given the high prevalence of post-infection irritable bowel syndrome post-CDI[40,41]. Also the comorbidity of patients with rCDI may be substantial[42]. Therefore, stool banks may benefit from an expert panel to advise physicians on the clinical indication and eventual treatment with FMT. This expert panel should also be available for consultation in the case of a serious adverse event (SAE), although SAEs directly

TABLE 3. PANEL OF SCREENINGSTESTS APPLIED BY NDFB.

Questionnaire	Screeningstest serum	Screeningstest feces
Age >18 years	Hepatitis A (IgM + IgG)	*Clostridium difficile*
BMI <25	Hepatitis B (HBsAg + anti-Hb)	Helicobacter pylori
Recent use of antibiotics	Hepatitis C (anti-HCV)	Bacterial gastro-enteritis[1]
Gastro-intestinal complaints	Hepatitis E (IgM + IgG)	Antibiotic resistant bacteria[2]
Recent travel to endemic areas	HIV (anti-HIV, type 1 and 2)	Viral pathogens[3]
History of cancer	Lues	Parasites[4]
Chronic medication use	Cytomegalovirus*	Microscopy for ova, cysts etc.
Auto-immune disorders	Eppstein-Barr virus*	*Dientamoeba fragilis*
Atopic diseases	Human T-lympotrophic virus	
General comorbidity	Strongyloïdes	

[1]*Salmonella* spp., *Campylobacter spp.*, *Campylobacter jejuni, C. coli Shigella spp., Yersinia enterocolitica* and *Y. pseudotuberculosis, Aeromonas spp., Plesiomonas shigelloides*, Shiga toxin producing *E. coli.*
[2]ESBL and/or carbapenemase producing bacteria, Aminoglycoside AND quinolone resistant Enterobacteriacese, vancomycin resistant enterococci and methicillin resistant *Staphylococcus aureus*.
[3]Norovirus serotype I+II, Astrovirus, Sapovirus, Rotavirus, Adenovirus 40/41, Adenovirus non-40/41, Enterovirus, Parechovirus.
[4]*Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum* and *C. hominis, Microsporidium spp, Cystoisospora belli* and *Cyclospora cayetanensis. Strongyloïdes d*.
*Results are used to select donors for treatment of immunocompromised patients

attributable to FMT are rare. It is undecided whether consultation is required for all FMT requests or only on request of the treating physician. This can be performed according to local practices.

The NDFB evaluates all requests for FMT by an expert panel of at least three physicians who participate in the working group of the NDFB. The indication is checked with a focus on appropriate diagnostics and treatment of previous episodes of CDI. In addition, an advice treatment plan is made based on the comorbidity of the patient. This results in appropriate and safe use of FMT. As a consequence, some of the requests for FMT are rejected or postponed[30].

FUTURE PERSPECTIVE

Recently, a report[22] of an international consensus meeting about FMT and stool banking was published. Further attempts to standardize the process of stool banking are undertaken. Standardization of stool banking is required because it enables stool banks to undergo peer audits to compare and benchmark their performance (outcome of treatment and side effects) to that of other stool banks. This will provide important information for quality improvement. In addition, standardization may facilitate joint efforts to assess long-term safety of FMT and microbiota research. Therefore, all related serious adverse event should be registered[43]. In this regard, there is a need for registries for follow-up of patients who undergo FMT, also because the long-term consequences are unknown. In the future an increasing number of patients will be treated with FMT for other indications than rCDI. It is of importance that those patients are also prospectively included in registries to evaluate the effects in patients with various diseases.

Increasing insights in the mechanism underlying the effect of FMT in various dysbiosis associated diseases will enable the development of standardized bacterial mixtures that may replace FMT in the future. Although more difficult than initially expected, many groups are working on microbiome ther-

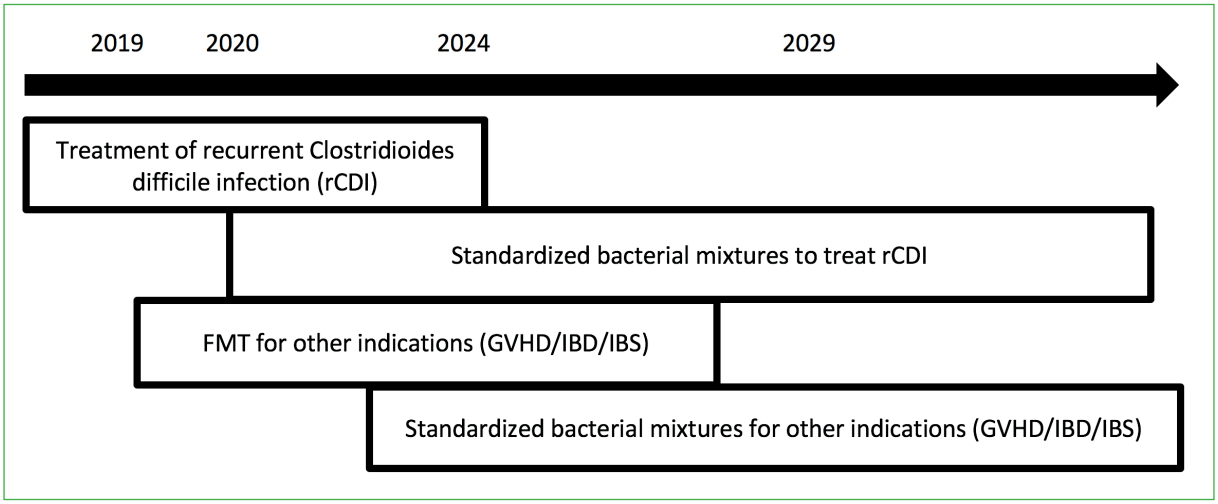

Figure 2. Perspective of stool banks for Fecal Microbiota Transplantation (FMT).

apeutics for rCDI[43,44]. For other conditions, FMT is important in a research context and may ultimately also lead to the development of microbiome therapeutics. Given the more complex pathogenesis of diseases, such as inflammatory bowel disease, the role of FMT has still to be elucidated and replacement of FMT by standardised microbiome therapeutics is not foreseen in the near future. Stool banks may have an important role in supporting this research and development of new therapies. The perspective of stool banks for Fecal Microbiota Transplantation is outlined in Figure 2.

CONCLUSIONS

Stool banks are a novel entity in medicine with their aim to facilitate FMT for daily practice and clinical research purposes. As described above, demands for standardization will improve the quality and result in a more advanced working process. Attempts to standardize these processes in Europe are currently undertaken. Ongoing and future studies with FMT should finally result in the development of standardized bacterial mixtures that can replace FMT as microbiota modifying treatment for (r)CDI and other potential indications.

Acknowledgements

JJK and EJK have received research grant from Vedanta Biosciences. ZK is an employee and shareholder at Finch Therapeutics and an unpaid Special Advisor at OpenBiome. The Netherlands Donor Feces Bank was founded with a grant from ZonMW (VIMP number 1708810011) and was supported with a grant from the Dutch Society of Gastroenterology (NVGE).

Conflict of Interest

The authors declare that they have no conflict of interest.

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