AP&T Alimentary Pharmacology and Therapeutics

Consensus report: faecal microbiota transfer – clinical applications and procedures

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Publication data
Submitted 4 July 2016
First decision 10 August 2016
Resubmitted 5 October 2016
Resubmitted 28 October 2016
Accepted 28 October 2016
EV Pub Online 27 November 2016

The Handling Editor for this article was Professor Jonathan Rhodes, and this uncommissioned review was accepted for publication after full peer-review.

SUMMARY

Background
Faecal microbiota transplantation or transfer (FMT) aims at replacing or reinforcing the gut microbiota of a patient with the microbiota from a healthy donor. Not many controlled or randomised studies have been published evaluating the use of FMT for other diseases than *Clostridium difficile* infection, making it difficult for clinicians to decide on a suitable indication.

Aim
To provide an expert consensus on current clinical indications, applications and methodological aspects of FMT.

Methods
Well-acknowledged experts from various countries in Europe have contributed to this article. After literature review, consensus has been achieved by repetitive circulation of the statements and the full manuscript among all authors with intermittent adaptation to comments (using a modified Delphi process). Levels of evidence and agreement were rated according to the GRADE system. Consensus was defined *a priori* as agreement by at least 75% of the authors.

Results
Key recommendations include the use of FMT in recurrent *C. difficile* infection characterised by at least two previous standard treatments without persistent cure, as well as its consideration in severe and severe-complicated *C. difficile* infection as an alternative to total colectomy in case of early failure of antimicrobial therapy. FMT in inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS) and metabolic syndrome should only be performed in research settings.

Conclusions
Faecal microbiota transplantation or transfer is a promising treatment for a variety of diseases in which the intestinal microbiota is disturbed. For indications other than *C. difficile* infection, more evidence is needed before more concrete recommendations can be made.

Aliment Pharmacol Ther 2017; **45:** 222–239

doi:10.1111/apt.13868

INTRODUCTION

Recent advances in culture-independent sequencing and other high-throughput techniques have increased our understanding of the role of the gastrointestinal microbiota in health and disease. An increasing number of diseases are being linked to a disturbed intestinal microbiota composition, including metabolic syndrome, irritable bowel syndrome (IBS), inflammatory bowel diseases (IBD) and extraintestinal disorders such as neuropsychiatric diseases.[1–3] As a consequence, therapeutic options are being tested which aim at restoring a disturbed microbiota towards a healthy, beneficial one. In general, moderate effects can be achieved by probiotic and prebiotic products, which increase the number of beneficial bacteria directly (probiotics) or indirectly by providing substrate for residing beneficial bacteria (prebiotics).[4–6] For more severe disturbances, however, these measures are not sufficient. Here, faecal microbiota transfer or transplantation (FMT) provides more powerful means to modify microbiota. It aims at replacing or reinforcing the gut microbiota of a patient with the microbiota from a healthy donor. As this procedure is not an actual transplantation, the term 'faecal microbial transfer' is preferable. FMT is used for the treatment of recurrent *Clostridium difficile* infection with efficacy rates of 90% or even higher,[7–11] giving reason for hope that it might also be successful in other diseases where disturbances of the intestinal microbiota are involved.

There is an increasing demand from patients for access to FMT as a treatment for various disorders and diseases. However, not many controlled or randomised studies have been published using FMT for other indications than *C. difficile* infection, which makes it difficult for clinicians to decide on a suitable indication. The aim of this article is to provide an expert consensus on current indications and methodological aspects of FMT for clinical application and to provide recommendations on how to conduct clinical studies using FMT. We will first discuss applicable indications of FMT, which is then followed by a section on practicalities of the FMT procedure. As well-conducted clinical studies are still missing in most fields, it is important to note that most recommendations, especially in the indications section, are not evidence-based yet. In addition, country-specific legal regulations need to be considered when working with FMT. Safety issues have been specifically discussed in the sections on FMT in IBD (with or without concurrent *C. difficile*) and in the section on route of administration. We further provide information on the assessment of safety in the section on study design. For a systematic review of adverse events associated with FMT please refer to Baxter and Colville, 2016.[12]

METHODS

A literature review was performed by Robert-Jan Brummer (RB), Jutta Keller (JKe), Julia König (JK) and Arno Siebenhaar using PubMed and MEDLINE using search terms based on 'Faecal microbiota transplantation' (Data S1) to identify studies that assessed the effect of FMT on various indications and/or practicalities of the FMT procedure. Based on these, the first draft of statements and comments was developed. The draft was then circulated among all co-authors for a first round of editing. Next, the level of evidence was agreed upon by JK, JKe and RB applying the GRADE system.[7] Evidence levels could be either rated as high, moderate, low or not applicable (NA). For the statements on the *General recommendations and considerations on clinical study design*, 'Level of evidence' was replaced with 'Grade of recommendation' (rated with either high, moderate or low). Levels of evidence were not applicable regarding the exclusion criteria. Co-authors were then asked to state their level of agreement according to the GRADE system in a modified Delphi process.[8, 9] The levels of agreement ranged from 1 to 6 (1: strongly disagree, 2: disagree with major reservation, 3: disagree with minor reservation, 4: agree with major reservation, 5: agree with minor reservation, 6: strongly agree). Authors were asked to provide comments if they disagreed with a statement (rating 3 or lower).

The answers were treated with confidentiality and only seen by JK, who did not participate in the voting. Consensus was defined *a priori* as agreement (rating 4 or higher) by at least 75% of the authors. Statements that achieved more than 75% but less than 90% agreement were discussed and/or adapted, and rated again in a second round. Statements that reached less than 75% consensus after the first round were omitted from the manuscript.

The voting team consisted of those with expertise in clinical gastroenterology and/or clinical microbiology with expertise in FMT.

RECOMMENDATIONS ON CLINICAL APPLICATIONS (INDICATIONS)

FMT in recurrent *C. difficile* infection

Key recommendations on FMT in recurrent *C. difficile* infection

	Level of evidence	Level of agreement
Patients with recurrent *C. difficile* infection with at least two previous standard treatments without persistent cure should be considered for FMT	HIGH	100%
Both standard or family/same household donors are applicable	HIGH	100%
If possible, patients should be included in a national registry	NA	100%
Faecal samples from patients before and after the treatment, if possible, as well as a donor sample, should be collected for follow-up in case of adverse events	NA	100%
So far, it is still unclear if antibiotic treatment and/or bowel cleansing before FMT is beneficial or if it could negatively affect the outcome (see also 'Recommendations on preparation of recipient')	NA	90%
No specific route of administration seems to be preferable regarding efficacy. However, preliminary data suggests that application by lower gastrointestinal tract may be safer (see also 'Recommendations on routes of administration')	LOW	90%

NA, not applicable.

Nowadays, FMT is established as a highly efficient treatment for recurrent *C. difficile* infection. FMT is included in the treatment guidelines for *C. difficile* infection provided by the European Society of Clinical Microbiology and Infectious Diseases, in which it is strongly recommended for multiple recurrent *C. difficile* infection unresponsive to repeated antibiotic treatment.[13]

Systematic reviews show that FMT treatment for recurrent *C. difficile* infection results in cure rates of about 90%.[8, 9, 14–16] So far, the method appears to be safe and acceptable to the patients.[14, 17] The microbiota changes after FMT in *C. difficile* infection can persist over several months. However, more studies evaluating long-term effects on the microbiota as well as investigating possible long-term adverse events are needed.[14, 18] Currently, studies are trying to identify factors determining treatment efficacy and risk of recurrence.[19]

The current literature on FMT in *C. difficile* infection is mostly based on case reports, case studies and a number of retrospective studies.[15] A randomised clinical trial comparing FMT for treatment of *C. difficile* infection to the use of vancomycin alone and to vancomycin plus bowel lavage was stopped after an interim analysis, as FMT proved to be much more effective.[20] 13 of 16 patients (81%) achieved resolution of *C. difficile* infection after FMT via a nasoduodenal tube, and two of the remaining three achieved resolution after a second infusion, resulting in an overall success rate of 94%. In the patients treated with vancomycin alone, four of 13 were cured (31%), and when receiving vancomycin in combination with bowel lavage, three of 13 (23%) achieved resolution of *C. difficile* infection.[20] A recent double-blinded, placebo-controlled clinical study found that the administration of stool from a donor was more efficient than the administration from the patient's own stool in achieving resolution (91% vs. 63%) in *C. difficile* patients (FMT administered by colonoscopy).[21]

As the efficacy of FMT in *C. difficile* infection is widely accepted nowadays, patients do not necessarily need to be included in clinical studies. It is advisable though to include them in a registry and to accurately report adverse events. If possible, faecal samples from the patient taken before and after treatment, as well as a donor sample, should be stored to allow follow-up in case of adverse events. As such, the 'American Gastroenterological Association (AGA) Fecal Microbiota Transplantation National Registry' from the AGA Center for Gut Microbiome Research and Education in the USA will be launched in the beginning of 2017. Its aim is to follow-up both short-term as well as long-term adverse events and safety concerns. A similar registry is already available in Germany under the name MicroTrans Registry.[22]

To avoid the potential risks associated with FMT, more selective applications of particular forms of microbes are investigated as potential alternative treatments for FMT in *C. difficile*. Preliminary results showed that the oral intake of nontoxigenic *C. difficile* spores could reduce *C. difficile* recurrence.[23] In addition, stool treated with ethanol to eliminate pathogens and to obtain a purified fraction of spores was shown to be effective in preventing *C. difficile* recurrence.[24]

FMT in severe and severe-complicated *C. difficile* infection

Key recommendations on FMT in severe and severe-complicated *C. difficile* infection

	Level of evidence	Level of agreement
FMT in severe and severe-complicated *C. difficile* infection should be considered as an alternative to surgery with total colectomy in case of early failure of antimicrobial therapy	MODERATE	100%
FMT could be considered already after one treatment failure	MODERATE	80%

Increasing clinical challenges in the treatment of *C. difficile* infection are severe courses with a considerable mortality and recurrence of the infection after successful antibiotic treatment. Different definitions of severe *C. difficile* infection are used, and some authors distinguish fulminant *C. difficile* infection or severe-complicated *C. difficile* infection from severe *C. difficile* infection as the most serious form of this infection.[13, 25, 26] If antibiotic therapy fails in severe *C. difficile* infection emergency total colectomy may be required. Mortality in this situation is very high and varies between 10% and 80%, mainly depending on the point of time in the course of disease when surgery is performed.[26, 27]

Besides a number of case reports[28, 29] smaller, retrospective, uncontrolled studies have been published showing that FMT is also effective in treating *C. difficile* infection in this clinical situation.[30–32] The cure rate was 66–88% with a single FMT and 89–94% if FMT was repeated in these patients.[30–32] Patients with severe-complicated *C. difficile* infection seem to have a lower primary response rate to FMT compared to severe *C. difficile* infection.[30, 32] FMT was applied by different administration routes, however, colonoscopic FMT was used most commonly. Some authors continued antimicrobial therapy against *C. difficile* infection with vancomycin during and after FMT in this patient group.[32] A recent report suggests that FMT might be used earlier in patients with the risk of a severe course. During a ribotype O27 (a *C. difficile* strain associated to a more severe disease course) outbreak in France, the treating physicians changed their treatment algorithm from use of FMT after at least three relapses to FMT application in addition to antimicrobial therapy during the first *C. difficile* infection episode. Mortality of the patients dropped from 64% to 19% with the use of the early FMT treatment approach.[33]

FMT in inflammatory bowel diseases (IBD)

Key recommendations on FMT in IBD

	Level of evidence	Level of agreement
FMT in IBD should only be performed in a research setting	NA	100%
If patients with a severely compromised colon are considered for FMT, FMT should be performed with caution as the risk for side effects is higher and systematic studies in severe disease are lacking	LOW	100%
Repeated transfers (possibly via enemas or capsules) over a longer time period seem to be preferable	LOW	90%
Possible donor variations should be considered	MODERATE	100%
Regarding efficacy, no evidence for a preferable route is available for the first administration; it should be chosen according to the location of the disease and/or the expertise of the physician. However, preliminary data suggests that application by lower gastrointestinal tract may be safer (see also 'Recommendations on routes of administration'). For repeated administrations, enemas are recommended.	LOW	90%

There is increasing evidence that the intestinal microbiota play a key role in the aetiopathology of IBD.[34] IBD mouse models do not develop IBD when kept germ-free,[35] and both an abnormal, excessive reaction to the commensal gut microbiota or components thereof, or the presence of one or several pathogens in the intestinal microbiota evoking an immune reaction are discussed as possible pathophysiologic mechanisms in IBD.

The intestinal microbiota composition has been shown to be different in both ulcerative colitis (UC) and Crohn's disease (CD), and is generally characterised by a lower diversity and a decreased stability compared to healthy controls, which may lead to the loss of normal, regulatory immune effects of the microbiota.[34, 36, 37] It is

still unclear if alterations in microbiota composition are a cause or consequence of the ongoing inflammatory processes, or a combination of both.[38–41]

A number of case studies showing positive effects of FMT in IBD have been published.[42–49] However, most of these studies involve low patient numbers, are mostly published in abstract form, and might be subjected to publication bias. Results from two controlled clinical studies have been published to date in full article form[50, 51] and the results of a third one were presented at the European Crohn's and Colitis (ECCO) meeting 2016.[52]

Moayyedi *et al.* investigated the efficacy of FMT in active UC (Mayo score ≥4 with an endoscopic Mayo score ≥1, patients with a disease severity requiring hospitalisation were excluded).[50] The transplant was provided as 50 mL enemas once a week for 6 weeks (n = 38). Water enemas served as placebo (n = 37). At week 7, remission was achieved in 24% (n = 9) of the patients that received the microbiota transfer compared to 5% (n = 2) of the patients that received the placebo. Interestingly, seven of the nine patients in remission after FMT received stool from the same donor, indicating that the success rate of stool transfer could be donor-specific. In addition, patients with a recent diagnosis of UC (≤1 year, three of four patients) seemed to respond better to the FMT compared to those treated later in the course of the disease (six of 34 patients). There was a trend towards patients on immunosuppressant therapy being more likely to respond to the FMT (5/11, 46%) than patients without immunosuppressant therapy (4/27 15%, P = 0.09). It needs to be mentioned that in this study most patients were on TNFα blockers, which might have introduced a potential bias. In the clinical study by Rossen *et al.*, the use of TNFα blockers was prohibited. The authors did not find a significant difference for achieving clinical remission between UC patients receiving faecal transfer (7/23, 30.4%) or their own stool as a placebo (5/25, 20%).[51] The effect of the placebo (autologous transplant) in this study was relatively high compared to the study by Moayyedi *et al.* in which water was used as a placebo. Rossen *et al.* included patients with mild to moderate active UC, and the FMT was performed twice via a nasoduodenal tube with a 3 weeks interval.

These studies suggest that FMT might be more effective in mild to moderate IBD, and that repeated treatments over a longer period of time could be superior. However, more evidence is needed, and data on microbiota composition before and after FMT may help to select patients that could benefit from this treatment. This is also important for the selection of suitable donors, as the treatment effect might be donor-dependent.[50, 53]

Available evidence suggests that FMT seems to be safe in IBD patients. However, especially treatment of patients with a severely compromised colon needs to be considered carefully, as the risk for side effects is higher in those patients, and systematic studies in severe disease are lacking. In addition, case reports have been published that demand caution in IBD patients. For example, an altered intestinal permeability could have been the cause for the onset of bacteraemia with fever after FMT in a Crohn's disease patient[54] or the new onset of microscopic colitis in an ulcerative colitis patients.[55] In one case, FMT might have induced a severe IBD flare with extraintestinal manifestations occurring for the first time in a Crohn's disease patient.[56]

FMT in IBD with recurrent *C. difficile* infection

Key recommendations on FMT in IBD with recurrent *C. difficile* infection

	Level of evidence	Level of agreement
Patients with mild to moderate active IBD with recurrent or refractory *C. difficile* infection should be considered for FMT	MODERATE	100%
Indication needs to be considered carefully, as flares of IBD activity have been reported after FMT in IBD with concurrent *C. difficile* infection, as well as a transition from inactive to active disease in one case	MODERATE	100%

C. difficile infection is a common comorbidity of IBD. It is assumed that IBD patients are at higher risk to develop *C. difficile* infection compared to non-IBD patients, and that *C. difficile* infection is associated with a higher mortality and more severe outcomes in IBD.[57] A decreased microbiota diversity and thereby probably lower colonisation resistance would explain the higher risk of *C. difficile* infection in IBD patients. However, so far, these assumptions are based on retrospective epidemiologic studies prone to selection bias and thus still remain to be proven.[58] To date, prospective studies evaluating specific treatments for *C. difficile* infection in IBD have not been published.

A recent retrospective study by Khoruts *et al.* compared the use of FMT in patients with *C. difficile*

infection and IBD to those without IBD, and found a lower efficacy in clearing the infection in those with IBD after one FMT (74.4% vs. 92.1%).[59] Moreover, minor IBD flares after FMT for *C. difficile* infection have been reported in up to 25% of those patients.[59]

In a retrospective study by Kelly *et al.*, FMT was investigated as a treatment for *C. difficile* infection in immunocompromised patients, which also included 36 IBD patients on immunosuppressive medication.[17] In this study, resolution of *C. difficile* infection in the IBD patients was comparable to previous studies, with resolution in 86% after a single FMT and 94% including repeated FMT. IBD flares were experienced by 14% of the IBD patients after the FMT.

Hamilton *et al.* performed a study in which the efficacy of standardised frozen compared to fresh FMT preparations was evaluated.[60] They included 14 *C. difficile* infection patients, of which $n = 4$ also suffered from UC, $n = 6$ from Crohn's disease and $n = 4$ from lymphocytic colitis. Independent of using fresh or frozen preparations, all of these patients achieved resolution of *C. difficile* infection after FMT, and none of these patients experienced IBD flares after the FMT.

Anderson *et al.* systematically reviewed case studies, most of which have been published in abstract form, which applied FMT for the treatment of recurrent or refractory *C. difficile* infection in IBD.[61] Outcome data, which could be obtained for 12 patients, showed a resolution of *C. difficile* infection in 11 of 12 patients, and improved response to IBD medication in six of seven patients. Reported adverse events included high fever in the majority of the patients following FMT, and one study reported deterioration of UC in two of five (moderate to severe chronic active UC, refractory to standard therapy).[62] One case study even reported the transition from inactive to active UC after FMT in one patient.[63]

In conclusion, efficacy of FMT in IBD with *C. difficile* infection for resolution of *C. difficile* infection seems to be high, but possibly lower than in non-IBD patients. While most studies conclude that FMT in IBD with *C. difficile* infection is safe, some have associated FMT with deterioration of IBD, particularly in patients with moderate to severe colitis, but also in patients with previously quiescent disease. Moreover, side effects have appeared to be more severe, as seen by the high fever in some patients, which could be due to impaired barrier function. Thus, indication needs to be considered carefully.

FMT in irritable bowel syndrome (IBS)

Key recommendations on FMT in IBS

	Level of evidence	Level of agreement
FMT in IBS should only be performed in a research setting	NA	100%
Primarily IBS patients in whom a disturbed microbiota seems to be present should be included in studies, i.e. patients who: (i) developed IBS or experienced deteriorated IBS symptoms after a gastrointestinal infection (post-infectious IBS) (ii) developed IBS or experienced deteriorated IBS symptoms after antibiotic treatment	LOW	90%
Preferably IBS patients in whom standard treatments (such as dietary changes, smooth muscle relaxants and reassurance therapy) have failed should be selected for studies	LOW	90%
No evidence for a specific route is available so far, but administration of the transplant in the prepared, right colon, by colonoscopic procedure, seems preferable	LOW	80%
Placebo-controlled study design is important as placebo response in IBS is known to be high (40%)	HIGH	100%

Irritable bowel syndrome (IBS) is a chronic, functional gastrointestinal disorder. Although the underlying pathophysiology is complex and still incompletely understood, aberrations in the bidirectional gut–brain axis signalling are generally considered to be a key factor.[64] A growing number of studies demonstrate an aberrant intestinal microbiota composition in IBS,[65–67] and treatments targeting the gut microbiota such as antibiotics, probiotics and prebiotics can improve IBS symptoms.[66, 68–71] A clear causal link between an aberrant gut ecosystem and IBS is the development of chronic IBS symptoms after an enteric infection, the so-called post-infectious IBS.[72]

Faecal transfer aiming at re-establishing a healthy intestinal microbiota could be a promising novel treatment option for IBS. To date, only a small number of clinical trials are registered under clinicaltrials.gov, and no randomised clinical trials investigating the impact of faecal transfer on IBS have been published so far. Only one study, published as an abstract, reported possible positive effects in patients with IBS symptoms.[73] The same group

later applied a mixture of 18 cultured, nonpathogenic bacteria resembling normal gut microbiota into the caecum of IBS patients and reported improved symptoms in 25 of 33 (published as an abstract[74]).

As the use of FMT in IBS is not evidence-based, it is important to treat IBS patients with FMT only as part of clinical studies. In these studies, accurate inclusion criteria, careful characterisation and recording of medical history of the patients are essential to evaluate which patients benefit from the offered treatment. In addition, we recommend to include only patients in cases where standard treatments (such as dietary changes, smooth muscle relaxants and reassurance) have failed. Ideally, an intestinal microbiota analysis should be performed before and after the treatment, as it may enable the identification of responders on basis of microbiota composition in combination with other clinical parameters, rather than on basis of symptom-related sub-classification of IBS. If possible, also mucosal microbiota should be analysed, as it is likely to have a large effect on the host due to its close proximity to the epithelium. In addition, studies have shown that faecal and mucosal microbiota differ, suggesting that the faecal microbiota might not be a good surrogate for the mucosal one.[75, 76]

Although there is no evidence available for a specific route, delivery via colonoscopy, after prior bowel cleansing, to administer the transplant in the right colon seems recommendable. The bowel cleansing will eliminate as much as possible of the present microbiota.[77–79]

FMT in metabolic syndrome

Key recommendations on FMT in metabolic syndrome

	Level of evidence	Level of agreement
FMT in metabolic syndrome should only be performed in a research setting	NA	100%
Patients with well-characterised metabolic syndrome should be chosen for participation in studies	NA	100%
Regarding the outcome of studies, it is recommended to not only evaluate weight loss, but also parameters of metabolic regulation, such as insulin sensitivity and satiety hormones	LOW	100%
In studies, careful donor selection based on predisposition to low body weight is essential (include family history)	LOW	100%

The intestinal microbiota plays an important role in metabolising otherwise indigestible food components. Nonfermentable, complex carbohydrates from plants reach the colon where they are metabolised by the resident intestinal microbiota. The main end products are short-chain fatty acids, mainly butyrate, acetate and propionate, providing both the microbes themselves as well as the host with energy.[80] It is hypothesised that the composition of the gut microbiota plays an important role in the amount of energy harvested from otherwise undigested food components, and in this way contributes to the susceptibility for obesity and metabolic syndrome. An increased capacity for energy harvest has been demonstrated in studies with germ-free mice transplanted with intestinal microbiota from obese mice, resulting in an increase in total body fat compared to germ-free mice transplanted with microbiota from lean mice.[81] When fed the same diet, germ-free mice have 40% less total body fat than conventional mice.[82]

Studies in humans are rare, but there is evidence suggesting an abnormal intestinal microbiota composition in overweight and obese subjects compared to lean people.[83–85] Vrieze *et al.* performed an important study in which faecal microbiota from healthy lean donors was transferred into patients with metabolic syndrome.[85] The placebo treatment was an autologous transplant prepared from the recipient's own stool. The authors chose to infuse the transplant into the small intestine, as this is where most of the carbohydrate and fat uptake occurs, and due to small-intestinal sensing mechanisms involved in insulin sensitivity. They found a significant increase in peripheral insulin sensitivity in the recipients of the donor transplant ($n = 9$) compared to those of the autologous transplant ($n = 9$) which was related to an increase in butyrate-producing bacteria both in the small intestine and faeces. Further evaluation of the bacterial strain populations in this study suggested that the success of colonisation of newly introduced strains by FMT was higher if the species were already present in the recipients' intestinal microbiota.[86]

Although this study showed promising results, more trials are needed and FMT in metabolic syndrome should only be performed in a research setting. Depending on the disease state, FMT can be hazardous if performed without adequate precautions, and strict indication and donor selection needs to be applied. More studies are currently ongoing and can be found on clinicaltrials.gov. Regarding the outcome, it is recommended to not only evaluate weight loss, but also to consider parameters of metabolic regulation, such as insulin sensitivity and satiety

hormones. In addition, monitoring of systemic and local inflammatory activity, e.g. via faecal calprotectin and serum highly sensitive C-reactive protein (CRP), is recommended.

General recommendations and considerations on clinical study design

Important points to consider when planning clinical studies using FMT are listed in the following:

Recommendations on clinical study design

	Grade of recommendation	Level of agreement
Especially in case of non-evidence based indications, faecal and, if possible, mucosal samples should be collected from the patients before and after the treatment for follow-up of adverse events and for identification of responders based on microbiota composition (i) Those samples should be collected before preparing the colonoscopy with bowel cleansing and/or antibiotic treatment	STRONG	100%
Faecal, and, if possible, mucosal samples from the donors should be collected to allow comparison of microbiota composition between patients and donors after the FMT and for follow-up in case of severe side effects	STRONG	90%
Research on the specific selection of a donor on basis of individual treatment cases (personalised medicine) is encouraged[86]	STRONG	100%
'Professional' donors instead of family/same household donors might be a better choice regarding standardisation and reproducibility as well as safety screening and cost efficiency	WEAK	90%
The delivery route should be selected based on the disease and the location where the effect is to be achieved (i.e. immune effect in the small bowel, microbiota composition effect in the colon)	STRONG	90%

METHODOLOGICAL RECOMMENDATIONS

Donor selection

Recommendations on donor selection

STATEMENT	Level of evidence	Level of agreement
Only healthy adults without acute or chronic diseases qualify as stool donors	NA	100%
Donor selection requires exclusion of diseases and unfavourable conditions (i) which have been shown to be transmitted via blood and possibly also via FMT (ii) for which a reasonable possibility exists that such a transmission can occur (iii) in which microbiota is considered to play a role or which have been associated with microbiota dysbiosis (iv) which increase probability of transmission of infections/multiresistant bacteria and parasites	NA	100%
Donors may be partners, relatives, friends or unrelated and previously unknown healthy subjects (the latter are preferred for indications in which genetics play a role, e.g. IBD)	MODERATE	100%

The diseases and conditions that should lead to transient or permanent exclusion of a potential donor are detailed in Table 1. Tables 2 and 3 show serological and stool markers that need to be tested to exclude clinically non-apparent infections.

Potential donors should be screened for infections and for conditions that may confer an increased risk of acquisition of infections. Current guidelines recommend protocols for screening that are similar to those for blood donors.[87–89] Experts agree that potential donors need to be excluded if they have known HIV infection, hepatitis B or C virus infection, or known exposure to these viruses within the previous year.[8, 87–90] Hepatitis E is supposed to be rare and a chronic course of disease is limited to immunocompromised patients who do not qualify as stool donors anyway.[91] Therefore, hepatitis E screening is usually not recommended, but subjects with a known diagnosis should be excluded. Also, subjects

Table 1 | Diseases and circumstances that should lead to (transient or permanent) exclusion of a potential donor for FMT

Positive history/clinical evidence for	Level of evidence
IBD or other chronic gastrointestinal diseases including IBS, chronic diarrhoea and chronic constipation	100%
History of or present malignant disease and/or patients who are receiving systemic anti-neoplastic agents	100%
Psychiatric disease (depression, schizophrenia, autism, Asperger's syndrome)	100%
Chronic neurological/neurodegenerative disease (e.g. Parkinson's disease; multiple sclerosis)	100%
Autoimmune disease and/or patients receiving immunosuppressive medications	100%
HIV, hepatitis A, B, C or E or known exposure within the recent 12 months	100%
Chronic pain syndromes (e.g. fibromyalgia)	90%
Obesity (BMI > 30), metabolic syndrome	100%
Major relevant allergies (e.g. food allergy, multiple allergies)	90%
Recent (gastrointestinal) infection (within last 6 months)	100%
Travelling in countries with low hygiene or high infection risk for endemic diarrhoea or acquisition of multiresistant bacteria within the last 6 months	100%
Tattoo or body piercing placement within the last 6 months	100%
Promiscuity	90%
Drug abuse	100%
Antibiotic therapy within the last 3 months	100%
Other chronic use of drugs that may affect the microbiome, e.g. proton pump inhibitors	90%

Table 2 | Serological parameters for infection that should be tested and lead to exclusion if positive

	Level of agreement
HIV-1 and -2	100%
Hepatitis A, B, C	90%
Human T-lymphotropic virus (HTLV)*	100%
Syphilis (TPHA)	100%
CMV and EBV (especially if recipient is negative)*	100%

CMV, cytomegalovirus; EBV, Epstein-Barr virus, HIV, human immunodeficiency virus.

* Optional, in alignment with the regional and legal situation.

Table 3 | Stool parameters (anal swab) for infection that should be tested and lead to exclusion if positive

	Level of agreement
Microscopic examination for ova and parasites (e.g. amoeba)	100%
Infectious bacteria (including enterohaemorrhagic *E. coli*, salmonella, shigella, yersinia, campylobacter)	100%
Clostridium difficile (GDH screening or PCR)	100%
Multiresistant bacteria (e.g. ESBL producing organisms, MRGN 3 und 4, VRE, MRSA)	100%
Helicobacter pylori (if nasogastric or oral capsules are used for FMT)	90%
Calprotectin >50 mg/kg	80%

ESBL, extended spectrum beta-lactamas; GDH, Glutamate dehydrogenase; MRGN, multiresistant gram-negative bacilli; VRE, vancomycin-resistant enterococci; MRSA, methicillin-resistant Staphylococcus aureus.

with Epstein-Barr virus (EBV) and cytomegalovirus (CMV) should be excluded, especially if the recipients are negative for those viruses. Some groups also screen for human T-lymphotropic virus (HTLV). If easily available, a multiplex PCR detecting further viruses could be performed (rotavirus, norovirus, enterovirus parechovirus, sapovirus, adenovirus 40/41/52, astrovirus). Some investigators also perform basic blood analyses

(full blood count, C-reactive protein, creatinine, liver enzyme levels) to further exclude undiagnosed relevant diseases.

Potential donors are also excluded if they participate in high-risk sexual behaviours, have sexual transmittable disease, in particular syphilis, use illicit drugs or have obtained a tattoo or body piercing within the previous 6 months.[8] Several studies also exclude subjects who have been incarcerated. Exclusion of clinically non-apparent infections requires additional evaluation of appropriate serological and/or stool parameters (Tables 1 and 2).

Recurrent *C. difficile* colitis is undoubtedly the main indication for FMT at present (compare below). Thus, it is self-evident that toxin producing *C. difficile* should not be transferred from the donor and its presence needs to be excluded (Table 2). Other viral, bacterial or parasitic gastrointestinal infections that may be associated with prolonged persistence of the pathogen in the gut of asymptomatic subjects need to be excluded by laboratory investigations (Tables 1–3).[8, 87–89] Moreover, gastrointestinal infections may lead to transient unfavourable alterations of the microbiota even after elimination of the pathogen.[92] Therefore, stool donation should not be performed within 6 months after a gastrointestinal infection.

The same applies to recent antibiotic therapy. Profound alterations of the microbiome occur in response to antibiotics. Overall normalisation could be seen within 3 months after the end of treatment although subtle disturbances were shown to persist much longer, even after 2 years.[93] A high-dose combination therapy, comprising amoxicillin, tetracycline and metronidazole, induced profound changes of the mucosa-associated microbiota in patients with ulcerative colitis after 3 months.[94] Later investigations were not performed making it unclear whether or when "normalisation" of the microbiota occurs.

There are other drugs that have been shown to affect the microbiome, in particular proton pump inhibitors (PPIs).[95] PPIs are associated with an increased risk of small bowel bacterial overgrowth.[96] It is less well known but highly likely that they also have an impact on the colonic microbiota. Several reports describe an association between PPI administration and *C. difficile* infection. However, this was not confirmed in a recent systematic review.[97] Thus, it could be discussed whether chronic use of PPIs should be an exclusion criterion for stool donors, and, if so, how long potential donors should abstain from PPI intake before donation.

Multiresistant bacteria have become a major health challenge for affected individuals and for the community,[98] and transmission of multiresistant gut bacteria during FMT needs to be avoided. The risk of acquiring multiresistant enterobacteria is particularly high if travelling to the Indian subcontinent (OR 24.8), Asia (OR 8.63) or Africa north of the equator (OR 4.94).[99] However, also in Europe, the prevalence of multiresistant gut bacteria may reach more than 50% and the EU/EEA population-weighted mean MRSA (Methicillin-resistant Staphylococcus aureus) percentage remains high at 18% with seven of 30 reporting countries reporting MRSA percentages above 25%, mainly in southern and eastern Europe.[98] Depending on the country where the FMT is to be performed it is more or less feasible to exclude donors who have visited these countries within the recent 6 months. However, it is preferable to screen for multiresistant bacteria in stool and to exclude donors in case of positive findings for ESBL (extended spectrum beta-lactamase) producing organisms, MRGN (multiresistant Gram-negative bacilli) 3 and 4, VRE (vancomycin-resistant enterococci) or MRSA. Remarkably, FMT could reduce the number of antibiotic-resistant genes in *C. difficile* patients.[100]

During recent years, an increasing number of diseases and health disorders have been shown to be associated with the composition of the intestinal microbiota. For most of these conditions causal relationship is largely unclear. However, given the potential detrimental long-term effects of FMT, experts agree that not only subjects with known infections or increased risks of transmitting infections should be excluded as stool donors, but also subjects with non-infectious diseases that have been shown to be transmitted via FMT or for which a reasonable possibility exists that such a transmission may occur.[8, 88–90] Hence, the following diseases and conditions are also generally regarded as exclusion criteria for potential stool donors: IBD or other chronic gastrointestinal disorders including IBS, chronic diarrhoea and chronic constipation, malignant disease/use of cytostatic drugs, autoimmune disease or use of systemic immunosuppressive medication, major allergies, pain syndromes, obesity (BMI > 30)/metabolic syndrome, psychiatric disease (e.g. affective disorders, schizophrenia, autistic spectrum disorder) and chronic neurological/

neurodegenerative disease (e.g. Parkinson's disease, multiple sclerosis).

Given the rigorous exclusion protocol, recruitment of donors for FMT may be challenging with only a small proportion of potential donors fulfilling all requirements. Of an unselected group of healthy volunteers replying to email, newspaper or online advertisements related to an FMT study and who were able to comply with the required frequency and duration of donation, approximately 40% failed to pass the stool screening.[101] Thus, patients who require FMT may not be able to name a suitable donor, although many physicians and patients prefer the patients' own partners, family members and friends. Intimate contacts (e.g. spouse) have the advantage of shared environmental risk factors minimising the risk of transmitting an infectious agent.[88] Moreover, maternal-line first-degree relatives may have a theoretical advantage of sharing many microbial species in their intestinal microbiota with that of the recipient. Therefore, it is conceivable that the recipient may obtain a more compatible microbiota derived from such donors.[88] On the other hand, there are no studies showing that material from related donors is better engrafted than material from unrelated donors, and there is evidence from blood safety analyses that donors identified by patients are more likely to test positive for infectious disease markers than unrelated volunteer donors.[102] Moreover, when FMT is considered to treat diseases in which genetics play a contributing role, such as IBD, unrelated donors who do not share the genetic risk may be preferred.

Many aspects argue in favour of building up a pool of volunteer donors (or noncommercial stool banks) which are regularly and thoroughly screened. The optimal screening frequency is not defined, but should be about every 3–6 months or more frequently in case of symptoms or if changes in risk factors occur, for example, travelling.

For the purposes of informed consent and a stable microbiota composition, donors should be at least 18 years of age. There are no data showing that men should be preferred over women (because of the higher prevalence of autoimmune disease and IBS in women) or that it is advantageous to select age- and sex-matched donors.[88] Donors should be asked to avoid potentially allergic foods such as nuts one week before donation.

Currently, regulatory authorities in several European countries[103] and the USA[104] treat human faeces as a drug. Exact regulations vary, but the general classification is criticised by several experts as creating a disincentive for research, restricting access to care, and failing to evaluate the long-term risks associated with the process. Instead, classifying stool as a body tissue would be preferable to address these issues.[104]

Preparation of stool

Key recommendations on preparation of stool

	Level of evidence	Level of agreement
Fresh stool should be used that has been evacuated within the preceding 6 h, in single cases stool that has been evacuated within up to 24 h before transfer may be accepted	LOW	90%
Until further preparation the evacuated stool should be stored at 2–8 °C in a hermetically sealed container	LOW	90%
Alternatively, frozen stool transplants can be prepared	MODERATE	100%
Depending on the administration route chosen, the stool needs to be diluted, homogenised and filtrated	NA	90%
To avoid vast overgrowth with aerobic bacteria, the preparation should be as brief as possible	MODERATE	100%
Stool should be handled according to legal requirements (e.g. biosafety level 2 measures) and adequate gloves and adequate protective gear should be used (e.g. facial shields, hood)	NA	100%
The facilities used need to be cleaned and disinfected using standards that also effectively eliminate pathogenic bacteria (e.g. *C. difficile*)	NA	100%

Studies show that storage conditions of faecal samples affect the composition of the microbial community, although major alterations only occurred after storage at room temperature for more than 24 h.[105, 106] Faecal samples exposed to −80 °C for up to 6 months also had a stable microbial composition.[106, 107] However, these studies aimed at determining stability of the microbiome over prolonged periods of time for subsequent DNA- and RNA-analysis and this does not necessarily translate into unaltered viability of all components. Still, high-throughput DNA sequence analysis has revealed stable engraftment of

gut microbiota following transfer of previously frozen faecal bacteria,[108] and frozen stool preparations containing glycerol as a preservative, partly encapsulated for oral application, have been used successfully for treatment of *C. difficile* infection.[10, 60, 107, 109, 110] These studies suggest that preparation of frozen transplants may simplify microbiota transfer without loss of efficacy or safety. On the other hand, commercial distribution of standardised stool preparations could be subject to legal regulations which could be difficult to fulfil.

Regarding fresh stool, which has been used in the vast majority of cases, experts recommend to transfer the sample within 6 h after evacuation.[8, 89] Until further preparation the evacuated stool should be stored at 2–8 °C in a hermetically sealed container,[89] although it is unclear whether this is absolutely necessary to maintain high clinical response rates.[15]

There is no consensus/evidence about the amount of stool to be used. While smaller amounts (30 g) seem to be sufficient for treatment of *C. difficile* infection,[10, 60] other researchers/physicians prefer a 'the more the better approach' with up to 200 g of stool per instillation.

Normal saline was used to prepare most FMT suspensions, followed by water and milk.[8] Again, there is no evidence favouring any of the dilutants. On the one hand, sterile saline is well standardised and less likely to affect the graft, on the other hand normal stool contains a very low sodium chloride concentration which might not be physiological for intestinal bacteria. The required amount varies and depends on stool consistency. Viscosity of the stool suspension should remain as high as possible to prolong residence time in the gut of the recipient.[89] The donor specimen is homogenised (using a blender, manual effort or other method) and usually filtered (e.g. gauze, coffee filter, strainer). This processed specimen is then either directly infused into the gastrointestinal tract or further processed for freezing or production of encapsulated preparations. Procedures are almost always performed under aerobic conditions although this may affect composition of the microbiota. To avoid vast overgrowth with aerobic bacteria, the preparation should be as brief as possible and/or the specimen should be cooled (2–8 °C) until application.[89] The use of carbon dioxide during colonoscopy might reduce the risk of disrupting the anaerobic environment of the intestine,[111] however, no studies have investigated if using carbon dioxide is more effective than oxygen.

Route of administration

There is no clear evidence favouring one of the potential routes of administration and decisions should be based on the location of the disease in the intestine and the expertise of the physician. However, upper gastrointestinal tract application of FMT has been associated with more severe side effects than lower gastrointestinal tract application.

Potential routes of administration are:

(i) Administration into the proximal gastrointestinal tract via nasogastric, nasoduodenal tube or by endoscopic procedure

(ii) Instillation into the proximal colon by endoscopic procedure

(iii) Rectal or distal colonic administration via enema

(iv) Combined approaches as well as oral application of encapsulated preparations

A recent systematic review has shown that the majority of adult patients received FMT therapy by colonoscopy (42.0%), followed by gastric or duodenal application via a nasogastric or nasoduodenal tube, gastroscopy or percutaneous endoscopic gastrostomy (PEG) (22.7%), enema or retention enemas (12.4%) or combination of two or more of these methods (11.8%).[8] In 8.6%, administration procedure was not reported, and nasojejunal application was performed in only 2.5% of patients. Paediatric patients were treated mainly by enema (45.5%), by nasogastric tube or PEG tube (36.4%) and less frequently by colonoscopy (18.2%). Colonic application may appear more physiological and appealing to the patient and includes prior cleansing of the bowel which helps to eliminate some of the present intestinal microbiota.

All routes are highly effective for treatment of recurrent *C. difficile* colitis with a numerical but statistically nonsignificant advantage for the colonic application.[22, 112, 113] Administration via enema is inexpensive and less invasive than colonic instillation but may be less effective because enemas usually do not spread beyond the splenic flexure. Moreover, it may be difficult for some patients to retain the donor material and commonly requires multiple treatments.[88] For other indications, the administration route should be selected based on the disease and on where in the intestine the transplant effect is to be achieved. For example, in immunological and metabolic diseases nasogastric or naosointestinal routes might be preferred, as the small intestine is much more involved in dietary glucose and

lipid uptake as well as in training of the innate immune system.[114]

However, one needs to be aware of that most severe side effects associated to the FMT procedure have been reported with upper gastrointestinal tract application, which carries some risk of vomiting and aspiration and is not feasible in patients with severe motility disorders. Three of four deaths related to FMT occurred after upper gastrointestinal tract application (two cases of aspiration pneumonia, one case with sepsis and pneumoperitoneum[12, 115, 116]). In addition, other cases of nonlethal aspiration pneumonia after vomiting of the faecal suspension[53] and a small bowel abscess have been reported with nasojejunal application of FMT.[51] Patients with IBD frequently show high fever if FMT is applied to the small bowel.[42, 51] In contrast, the one death during lower gastrointestinal tract application was caused by aspiration during sedation for endoscopy which was more related to the anaesthesia rather than to the FMT itself.[17]

Whether application by the upper gastrointestinal tract may also cause small bowel bacterial overgrowth has not been investigated until now.

Preparation of recipient

Key recommendation on preparation of recipient

	Level of evidence	Level of agreement
Any treatment with antibiotics in patients who are going to receive FMT should be stopped in due time according to the respective pharmacokinetic properties so that intracolonic antibiotic concentration is negligible	LOW	90%

In case of caecal administration patients should undergo normal colonic lavage or other cleansing procedures as routinely used prior to colonoscopy. In case of oral, transnasal tube placement or gastroscopic procedure, subjects need to be fasted.

Patients undergoing FMT for *C. difficile* infection typically receive antibiotic therapy until 1–3 days prior to the procedure, although it is unclear whether prior antibiotic therapy to minimise colonisation with pathogenic *C. difficile* is effective. If FMT is performed for non-infectious indications antibiotic therapy is usually omitted.[43] A bowel preparation is usually administered to patients the day before the transfer to further reduce *C. difficile* concentration in the bowel and/or to improve conditions for colonisation of the donor microbiota in general. One possible protocol for patient preparation is an abbreviated regimen of vancomycin (500 mg orally four times per day for 4 or 5 days), followed by bowel lavage with 4 litres of macrogol solution on the last day of antibiotic treatment and the infusion of a suspension of donor faeces through a nasoduodenal tube.[20] Whether this kind of pre-treatment is required for successful FMT in *C. difficile* infection or other potential indications has not been investigated in controlled trials.

Patients with upper gastrointestinal application, in particular gastric administration, frequently receive proton pump inhibitor therapy[117] to reduce gastric acidity that otherwise would compromise the viability of the transplant. In patients with colonic instillation loperamid (usually 2–4 mg) may be used to slow down intestinal transit and to enhance the time for colonisation of donor bacteria.[89]

CONCLUSION

FMT is a promising treatment for a variety of diseases in which the intestinal microbiota is disturbed and it is currently used as a routine treatment in recurrent *C. difficile* infection. For other indications not much evidence from clinical studies for its efficacy has been obtained so far, and further investigations are needed before more concrete recommendations can be made.

Although FMT has been shown to be a rather safe method, some severe side effects have been reported, and its application, especially in diseases with impaired intestinal barrier functions, can carry risks. These risks can be limited by systematically and carefully screening the donor, as described above. In addition, thorough follow-ups are essential to find out more about potential long-term risks and the maintenance of a national register is recommended.

An alternative for FMT in the future could be the so-called synthetic stool that consists of a mixture of selected beneficial microbial strains.[118, 119] It needs to be kept in mind though that the intestinal microbiota communities form a very complex ecosystem. The microbe–microbe interactions and networking play a pivotal role in the microbe–host interactions, and our knowledge on this networking is limited. This makes it difficult to find well-defined bacterial strains that are responsible for specific effects. Furthermore, the careful handling of strictly anaerobic microbial strains is very cumbersome yet at the same time a critical factor with respect to the production of resilient spores.[120] Hence, much research remains before an optimal

composition of a synthetic stool can be established, which could circumvent the potential risks associated with FMT.

There are further indications of FMT that we did not discuss in this article, as there is no data available, but might be of interest in the future. One is the preservation of an individual's healthy stool before a planned multiple antibiotic and/or cytostatic treatment. For example, in certain orthopaedic and urologic cases in which patients are treated with high doses of antibiotics over a long period of time, stool could be collected before, stored frozen and applied by FMT later on in case problems related to the intestinal microbiota occur. This could also be an option for multi-antibiotic treatment in infants, which is associated with many risks such as an increased disposition to develop autoimmune diseases.[121] Exposing infants delivered by caesarean section to maternal vaginal fluids and faecal matter to provide them with a more natural microbiota composition is also being discussed.

Future studies will hopefully assist in the challenge to identify patients that benefit from FMT based on their microbiota composition, as well as to find suitable donors. It seems likely that also immune effects play an important role in finding the 'perfect match'.

In addition, the impact of diet on improving the donor's microbiota as well as maintaining the new composition in the recipient needs to be investigated. Diet has clear short-term and long-term effects on the intestinal microbiota, as shown by comparison between Western and African populations, human intervention studies and animal models.[80, 122] Future FMT studies need to take these effects into consideration.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Search terms.

AUTHORSHIP

Guarantor of the article: Julia König.

Author contributions: Consensus has been achieved by repetitive circulation of the statements and the full manuscript among all authors with intermittent adaptation to comments.

All authors have reviewed and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

Declaration of personal interests: MN is in the SAB of Seres Therapeuticals and Caelus Health; none of these liasions has direct effects on the current content of this paper.

Declaration of funding interests: CH receives funding from Seres Therapeutics. ASt has received consulting fees from AbbVie, Astellas, Biogen, Janssen, MSD, Mundipharma, Takeda Summit Therapeutics, as well as lecture fees and travel accommodations from AbbVie, Astellas, FalkFoundation, Janssen, MSD, Mundipharma, Takeda.

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