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Feasibility, Acceptability, and Safety of Faecal Microbiota Transplantation in the Treatment of Major Depressive Disorder: A Pilot Randomized Controlled Trial

Faisabilité, acceptabilité et sécurité de la transplantation du microbiote fécal dans le traitement du trouble dépressif majeur : un essai pilote randomisé contrôlé

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Abstract

Objectives: Perturbations of the intestinal microbiota have been associated with mental health disorders, including major depressive disorder (MDD). Therefore, faecal microbiota transplantation (FMT) holds promise as a microbiota-modulating

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treatment for MDD. Yet, to date, there are no published controlled studies evaluating the use of FMT for MDD. This study aimed to address this gap by evaluating the feasibility, acceptability, and safety of FMT for MDD.

Methods: The study was an 8-week, double-blind, 2:1 parallel group, randomized controlled pilot trial ($n = 15$) of enema-delivered FMT ($n = 10$) compared with a placebo enema ($n = 5$) in adults with moderate-to-severe MDD.

Results: Recruitment was completed within 2 months, with 0% attrition and 100% attendance at key study appointments. There were no major protocol deviations. The placebo and blinding strategies were considered successful; nurses and participants correctly guessing their treatment allocation at a rate similar to that anticipated by chance. No serious or severe adverse events were reported in either group, and there were no significant differences in mild-to-moderate adverse events between groups (median of 2 adverse events per participant reported in both groups). Furthermore, the 12/15 participants who completed the Week 2 participant satisfaction survey agreed or strongly agreed that the enema delivery was tolerable and that they would have the treatment again if required. Whilst the study was not designed to measure clinical outcomes, exploratory data also suggested that the active FMT treatment may lead to improvements in gastrointestinal symptoms and quality of life in this population, noting that irritable bowel syndrome is commonly comorbid with MDD.

Conclusions: All feasibility targets were met or exceeded. This study found that enema-delivered FMT is feasible, acceptable, well-tolerated, and safe in patients with MDD. The findings of this study support further research to evaluate clinical efficacy, and the use of this protocol is supported.

RÉSUMÉ

Objectif : Les perturbations du microbiote intestinal ont été associées aux troubles de santé mentale, notamment le trouble dépressif majeur (TDM). Par conséquent, la transplantation du microbiote fécal (TMF) est prometteuse à titre de traitement de modulation du microbiote pour le TDM. Pourtant, à ce jour il n'y a pas d'études contrôlées publiées qui évaluent l'utilisation de la TMF pour le TDM. La présente étude visait à corriger cet écart en évaluant la faisabilité, l'acceptabilité et la sécurité de la TMF pour le TDM.
Méthodes : L'étude était un essai pilote randomisé contrôlé (n = 15), de 8 semaines, à double insu, en groupes parallèles 2:1, d'une TMF administrée par lavement (n = 10) comparé à un lavement placebo (n = 5) chez des adultes souffrant d'un TDM modéré à grave.
Résultats : Le recrutement s'est fait en 2 mois, avec 0 % d'attrition et 100 % de participation aux principaux rendez-vous de l'étude. Il n'y a pas eu de déviation majeure du protocole. Les stratégies du placebo et du double insu ont été estimées réussies; les infirmières et les participants ont correctement deviné leur affectation de traitement à un rythme semblable à celui anticipé par hasard. Aucun événement indésirable sérieux ou grave n'a été déclaré dans aucun groupe et il n'y a pas eu de différences significatives entre les événements indésirables bénins à modérés entre les groupes (la médiane de deux événements indésirables par participant rapportée dans les deux groupes). En outre, les 12/15 participants qui ont rempli le sondage sur la satisfaction du participant à la 2e semaine étaient d'accord ou fortement d'accord que l'administration du lavement était tolérable, et qu'ils suivraient le traitement de nouveau, si nécessaire. Bien que l'étude ne soit pas conçue pour mesurer les résultats cliniques, les données exploratoires ont aussi suggéré que le traitement actif de la TMF peut entraîner des améliorations des symptômes gastro-intestinaux et de la qualité de vie dans cette population, en faisant remarquer que le syndrome du côlon irritable est communément comorbide avec le TDM.
Conclusions : Toutes les cibles de faisabilité ont été satisfaites ou excédées. Cette étude a constaté que la TMF administrée par lavement est faisable, acceptable, bien tolérée et sécuritaire chez les patients souffrant du TDM. Les résultats de cette étude soutiennent plus de recherche pour évaluer l'efficacité clinique, et l'usage de ce protocole est soutenu.

Keywords
faecal microbiota transplantation, FMT, microbiome, psychiatry, mental disorder, depression, MDD, major depressive disorder, mood disorders, mental health

Background

There is an association between mental health disorders and perturbations in intestinal microbiota composition.[1,2] Various biological variables mediated by intestinal microbiota are associated with the aetiology and maintenance of major depressive disorder (MDD).[1,3] A recently published systematic review described differences in the intestinal microbiota profiles of depressed compared with non-depressed humans.[4] Interventions that modify gut microbiota, such as probiotics,[5–7] antibiotics,[8–12] and diet[13] may also affect depression and anxiety symptoms. These data suggest the intestinal microbiota might be a modifiable target in the treatment of depression.[1]

Faecal microbiota transplantation (FMT) holds promise as a potential microbiota-modulating treatment for diverse conditions[14] including depression, where current evidence-based treatments (antidepressants and psychotherapy) are ineffective in more

than one-third of individuals[15,16] and have limitations including access, side effects, and cost. Whilst probiotics are limited to a small number of mostly food-derived strains of bacteria, FMT encompasses the broader bacterial diversity found within the human gastrointestinal tract, including those not available in probiotics.[17] Therefore, FMT has the potential to alter the composition of the intestinal microbiota more effectively and deliver a broader range of microbial functions.

There is evidence supporting the use of FMT for MDD.[18,19] FMT-induced changes in intestinal microbiota composition can influence rodent models of psychiatric disorders and behaviour.[20–22] Compellingly, 4 studies have now demonstrated that FMT from depressed humans into rodents induces depression-like behaviours in the rodents.[23–26] Similar outcomes have been observed in the rodent models of schizophrenia.[27] In humans, FMT has shown promise in a range of other disorders,[14] including autism spectrum disorder.[28] Case studies also suggest promising outcomes with respect to the use of FMT for the treatment of depressive symptoms [29–31] and bipolar disorder.[32,33] Studies of FMT for irritable bowel syndrome (IBS), which is commonly comorbid with MDD, have shown concurrent improvements in mental health symptomatology.[14,34–36]

The acceptability and feasibility of FMT have been evaluated across a range of disorders and vary widely depending on the disorder. One study surveyed 887 patients in which 50% of patients with ulcerative colitis and Crohn's disease stated they would be interested or very interested in undergoing this type of procedure, but this figure was 25% for patients with psoriasis.[37]

To our knowledge, there are no published controlled studies specifically evaluating the use of FMT for mental illness in humans. Current evidence is limited to uncontrolled open-label studies and case reports. Thus, the safety, tolerability, acceptability, and feasibility of FMT for mental illnesses, including MDD, is yet to be established. The study presented here represents the first reported trial of FMT for MDD using a double-blind randomized controlled trial (RCT) design. The primary objective of this study (The Moving Moods Pilot Study) was to establish the feasibility and safety of FMT as an adjunctive treatment for MDD in adults. The secondary aim of the Moving Moods Pilot Study was to assess the degree of microbial engraftment of donor stool in recipients, and changes in intestinal microbiota composition following FMT (these data will be published separately). Further exploratory aims included assessing changes in depressive and other mental health symptoms, gut symptoms, sleep quality, quality of life, and cardiometabolic risk factors. The outcomes of this pilot study will help inform and support further trials in this emerging field.

Methods

Trial Design and Setting

The Moving Moods Pilot Study was an 8-week, double-blind, 2:1 parallel group, randomized controlled pilot trial (n = 15)

Table 1. Baseline Characteristics.

Characteristic		FMT (n = 10)	Placebo (n = 5)	Total (n = 15)
Age, mean (s.d.)		47.23 (±6.51)	38.44 (±2.14)	44.09 (±6.83)
Sex, *n* (%)	Female	6 (60)	3 (60)	9 (60)
Marital status, *n* (%)	Divorced/separated/widowed	3 (30)	0	3 (20)
	Married/de facto relationship	3 (30)	3 (60)	6 (40)
	Single (never married)	4 (40)	2 (40)	6 (40)
Highest level of education, *n* (%)	Completed secondary school/some secondary school	2 (20)	0	2 (13.33)
	TAFE/trade/apprenticeship/other post-secondary training	3 (30)	0	3 (20)
	University	5 (50)	5 (100)	10 (66.67)
Employment status, *n* (%)	Casual	2 (20)	1 (20)	3 (20)
	Part-time or full time work/student	6 (60)	4 (80)	10 (66.67)
	Unemployed/unable to work	2 (20)	0	2 (13.33)
Household income, *n* (%)	<$25,000	3 (30)	1 (20)	4 (26.67)
	$25,000–$100,000	2 (20)	3 (60)	5 (33.33)
	>$100,000	5 (50)	0	5 (33.33)
Medical history, *n* (%)	Current smoker	1 (10)	1 (20)	2 (13.33)
	Atopic condition	8 (80)	4 (80)	12 (80)
	Food intolerances	5 (50)	3 (60)	8 (53.33)
	IBD	2 (20)	1 (20)	3 (20)
	IBS	4 (40)	3 (60)	7 (46.67)
Psychiatric history, *n* (%)	Treatment-resistant depression	7 (70)	4 (80)	11 (73.33)
	Dysthymia	5 (50)	4 (80)	9 (60)
	Family history (psychiatric), *n* (%)	8/8 (100)	5 (100)	13 (100)
SAPAS score, mean (s.d.)		2.70 (±1.77)	2.60 (±0.89)	2.67 (±1.50)
Treatment, *n* (%)	Antidepressant use (current)	5 (50)	5 (100)	10 (66.67)
	Psychologist (current)	5 (50)	2 (40)	7 (46.67)
	TMS/ECT (current or past)	2 (20)	1 (20)	3 (20)

Note. ECT = electroconvulsive therapy; FMT = faecal microbiota transplantation; SAPAS = standardized assessment of personality abbreviated assessment; TAFE = technical and further education; TMS = transcranial magnetic stimulation.

of enema-delivered FMT (henceforward referred to as "active") ($n = 10$) compared with a placebo enema ($n = 5$) in adults with moderate-to-severe MDD, with a 26-week follow-up for safety data. Participants, investigators, raters, and staff delivering the intervention were blinded to allocation. The study was conducted at University Hospital Geelong (Barwon Health Research Ethics, Governance and Integrity Number: 21/56). The full protocol is submitted for publication (currently under review) and registered with the Australian and New Zealand Clinical Trials Register (ANZCTR): ACTRN12621000932864. Results are reported in accordance with the CONSORT randomized pilot and feasibility trials extension.[38]

One amendment was made to the eligibility criteria following the commencement of recruitment. Our original protocol excluded those with any previous history of inflammatory bowel disease (IBD)—which is commonly comorbid with MDD.[39] However, this was changed to only exclude those with "active" IBD. This was done because FMT is considered safe in individuals with IBD,[14] but also due to an open-label study showing FMT improved depression symptoms in some IBD patients.[40]

Participants

Details of eligibility criteria are available in the registered protocol. To summarize, participants were considered eligible if they were adults (age 18–65 years), with a diagnosis of MDD according to the Structured Clinical Interview for DSM-5 (SCID-5) MDD module[41]; had moderate-to-severe depressive symptomatology (Montgomery Asberg Depression Rating Scale [MADRS][42] score ≥20); and had been on stable treatment for their mental health (pharmacotherapy and psychotherapy) for at least 1 month prior to commencing the trial. Exclusion criteria were: active suicidality; use of probiotics, antibiotics, or any experimental drug in the 1 month prior to study entry; serious gastrointestinal conditions (including active IBD, bowel cancer, or a history of major bowel surgery, but not including IBS, chronic diarrhoea, or constipation); pregnancy or breastfeeding; major comorbid psychiatric disorders (including bipolar disorder, a primary psychotic disorder, obsessive compulsive disorder, bulimia nervosa, or anorexia nervosa); active substance-use disorder; inability to read and understand the participant information and informed consent form (PICF); and a history of severe anaphylactic or anaphylactoid food allergy.

Study Intervention and Procedures

Regarding recruitment, advertisements were placed on social media, which generated media attention. Further planned recruitment strategies outlined in the registered study protocol were not required due to sizeable interest in the study arising from the media exposure. Some participants learned of the study through the media attention and social media advertisements, whilst others heard about it from their treating doctor (e.g., gastroenterologist or psychiatrist).

Potential participants registered an expression of interest via the study website and were then contacted by a member of the study team. Potential participants were provided with a PICF and booked for a screening assessment. A signed copy of the PICF and verbal consent were required prior to screening and study enrolment. Consent was again verbally confirmed at the initial screening appointment. Participants were made aware that they could withdraw consent at any time.

Given the extensive screening requirements, screening for eligibility was conducted in a 2-part assessment to reduce participant burden. Appointment 1A was conducted by a research assistant and covered general eligibility criteria and informed consent. Appointment 1B was conducted by the study psychiatrist and involved confirmation of MDD diagnosis (using SCID 5-MDD module), and assessment of severity (using MADRS). Following Appointment 1B, eligible participants provided further baseline data including medical history, medication history, family history, and psychiatric history, which was also collected separately by the study psychiatrist within the diagnostic interview.

Participants were randomly assigned to receive a total of 4 doses of active or placebo FMT via enema over 4 consecutive days as an adjunct to treatment as usual, delivered by a qualified nurse in a hospital setting. The active FMT enema comprised of syringes supplied by BiomeBank (Adelaide, Australia) containing a 50 mL total volume of donor faeces, normal saline, and 10% glycerol. The placebo consisted of a visually identical placebo product containing 50 mL total of normal saline, 10% glycerol, and brown dye. In Australia, the manufacture of FMT is regulated by the Therapeutic Goods Association (TGA), ensuring compliance with strict, comprehensive, and detailed donor screening and FMT manufacturing requirements.[43] The FMT product utilized in the Moving Moods Pilot Study met these requirements. As required by the TGA, each participant allocated to active FMT received all 4 enemas from a single donor.

There is currently a lack of data and thus consensus regarding a suitable dosing strategy for health conditions other than *Clostridioides difficile*.[14] Our 4-enema dosing strategy, delivering a total of 50 g stool, was based on the knowledge that while 30–50g of stool is a widely accepted dose in the treatment of *C. difficile*,[44] the broader data on FMT for use in other conditions suggests that a higher dose is likely required for non-*C. difficile* health conditions conditions.[14] Hence, we selected the upper limit of the dosing strategy.

Following the intervention, participants were assessed every 2 weeks until the Week 8 primary endpoint. A final follow-up phone call assessment will be conducted at Week 26 to assess long-term safety.

Participants provided stool samples and blood samples at baseline, Week 2, and Week 8. Participants self-collected stool samples using a Microba stool collection kit[45] according to manufacturer's protocol. Venipuncture was completed by research nurses and processed by Australian Clinical Labs

Table 2. Feasibility Targets and Outcomes.

Target area	Feasibility target	Outcome achieved	Target achieved (Yes/no)
Recruitment	Successful enrolment of $n = 15$ participants across a 6-month active recruitment period	15 participants were enrolled in a 2-month period	Yes
Retention	A minimum of $n = 10$ participants to complete study until the 8-week primary endpoint (i.e., a 33% attrition rate).	All 15 participants completed the study until the 8-week primary endpoint	Yes
Adherence to protocol	Participants should: 1. Receive 2 of the total 4 enemas 2. Attend their baseline appointments (in-person) and Week 2 and Week 8 appointments (via telehealth or in-person) 3. Provide baseline and Week 2 stool samples	1. All 15 participants received all 4 enemas 2. Attendance rate of 100% at key appointments. 3. All participants provided baseline stool samples. 14/15 Week-2 stool samples were returned.	1. Yes 2. Yes 3. Yes
Safety	Nil severe and/or serious AEs rated as likely due to study intervention in the active FMT group.	No serious or severe AEs were reported in either group during the 8-week study period.	Yes
Adequacy of blinding	The best achievable outcome for adequacy blinding is participants and researchers correctly guessing allocation at a rate of 50% (the rate due to chance). Whilst we are not likely to be statistically powered to measure this outcome, we aim to reach an outcome approaching 50%.	35.7% of participants correctly guessed treatment allocation (Fisher's Exact $P = 0.45$).	Yes

(Geelong, Australia). Additional blood samples were collected and retained for future analyses.

Outcomes

Primary, secondary, and exploratory outcomes are described below. A more detailed description of the study outcome measures is available in the registered protocol.

Primary Outcome Measures

The primary outcome of this study was the feasibility and safety of FMT as an adjunctive treatment for MDD in adults.

Feasibility was measured by the ability to meet recruitment targets, participant retention and completion rates, adherence to the intended study protocol, participant acceptability, and robustness of study methodology including effectiveness of blinding. Acceptability was measured using a modified version of the Study Participant Feedback Questionnaire (SPFQ).[46] Data are reported descriptively. Participants completed the SPFQ 2, 4, and 8 weeks following the intervention.

Safety of FMT was measured by assessment of adverse events (AE). Feasibility targets, together with the feasibility outcomes, are summarized in Table 2. If satisfied, these criteria would suggest it is feasible to proceed with a future efficacy trial.

Secondary Outcome Measures

The secondary outcome measures were changes in intestinal microbiota and associated biomarkers. The results of these analyses will be published separately.

Exploratory Outcome Measures

The following data were collected; however, given the modest sample size, the study was expected to be underpowered to measure significant changes in any of these outcome measures. As such, these outcomes were considered exploratory. The exploratory outcome measures included between-groups differential changes post-intervention compared with baseline in the following outcome measures: (a) mental health symptoms as measured by the MADRS and the Depression-Anxiety Stress Scale (DASS)[47]; (b) quality of life as measured by the Assessment of Quality of Life-8 Dimension (AQoL-8D) with unweighted scoring[48]; (c) sleep, as measured by the Pittsburgh Sleep Quality Index[49]; (d) level of function as measured by the Sheehan Disability Scale[50]; (e) gut symptomatology as measured by the Gastrointestinal Symptom Rating Scale (GSRS)[51]; (f) cardiometabolic blood parameters (random lipid profile, random blood sugar levels, and HbA1c); (g) metabolic and cardiovascular risk factors assessed via physical exam, including heart rate, blood pressure, height, and weight; and (h) overall improvement assessed using the Patient Global Impression of Change.[52] Outcomes of exploratory blood biomarkers, such as brain-derived neurotrophic factor, markers of inflammation (e.g., macrophage inhibitory factor, interleukins 1b, 1ra, 6, and 10, soluble CD14, and high-sensitivity C-reactive protein), and markers gut permeability (lipopolysaccharide binding protein and zonulin) will be published separately along with the intestinal microbiota data.

Statistical Analyses

Feasibility Analysis. As this was a feasibility study, a power calculation was not performed, the sample size was

normative for a study of this type and available budget.[53] Feasibility data were reported descriptively. A qualitative synthesis of feasibility data was reported in accordance with the pre-determined feasibility targets provided in Table 2.

Safety was reported as the number of AEs of each type and severity experienced per participant.

Exploratory Outcomes Analyses

The exploratory outcome data are summarized in Table 4. Between-groups differential changes from baseline to Week 2 and Week 8 using generalized estimation equation (GEE) technique were estimated. To avoid Type I error inflation, all *p*-values reflect the overall comparisons for time points and group allocation from the 2-way interaction between time point and group allocation.

Trial Allocation, Sequence Generation, and Blinding. Allocation to treatment arms was randomly assigned by an independent statistician external to the study in a 2:1 ratio using a simple randomization method. Unblinded researchers independent to the study team allocated and packaged enema kits sequentially, using identical packaging to conceal treatment allocation and blinding. The study staff, investigators, nurses delivering the enemas, and participants were blinded to group allocations. Given the unequal treatment group sizes, it was not possible to blind the study statistician.

Table 3. Mild-to-Moderate Adverse Events.

Symptom	FMT ($n = 10$)	Placebo ($n = 5$)	Total ($n = 15$)
Number of participants who reported mild AE, *n* (%)			
Abdominal pain	2 (20)	0	2 (13.33)
Belching	0	1 (20)	1 (6.67)
Cramping	3 (30)	2 (40)	5 (33.33)
Constipation	3 (30)	0	3 (20)
Diarrhoea	1 (10)	1 (20)	2 (13.33)
Distension/abdominal discomfort	2 (20)	1 (20)	3 (20)
Excessive flatulence	2 (20)	2 (40)	4 (26.67)
Nausea	2 (20)	3 (60)	5 (33.33)
Vomiting	1 (10)	0	1 (6.67)
Other gastrointestinal	1 (10)	0	1 (6.67)
Headache	1 (10)	0	1 (6.67)
Rectal bleeding	1 (10)	0	1 (6.67)
Fatigue/malaise	3 (30)	1 (20)	4 (26.67)
Other systemic	3 (30)	1 (20)	4 (26.67)
Mild AE subtotal, *n*	25	12	37
Median mild AEs reported per participant [IQR]	2 [2]	2 [3]	2 [3]
Number of participants who reported moderate AE, *n* (%)			
Cramping	1 (10)	1 (20)	2 (13.33)
Other gastrointestinal	0	1 (20)	1 (6.67)
Infection (including COVID-19)	1 (10)	1 (20)	2 (13.33)
Moderate AE subtotal, *n*	2	3	5
Median moderate AEs reported per participant [IQR]	1 [1]	1.5 [1]	1 [0.5]
Total AEs, *n*	27	14	41
Median total AEs reported per participant [IQR]	2 [2]	2 [3]	2 [2]

Note. AE = adverse events; FMT = faecal microbiota transplantation.

Results

Baseline Demographics

Table 1 summarizes baseline demographic data.

Feasibility Outcomes

Table 2 presents the results of pre-specified targets considered as a minimum standard that would indicate that the study design was feasible. All targets were met or exceeded, and as such, the study was considered feasible.

Recruitment and Enrolment. The recruitment target of 15 participants in 6 months was met within a 2-month time frame, including completion of the 2-stage screening protocol requiring approximately a 2–2.5-h time commitment per participant. Expressions of interest ($n = 164$) greatly exceeded the 15-participant requirement, and additional participants were placed on a waitlist.

Eligibility. Eligibility is summarized in the CONSORT Flow diagram (see Figure 1).

Retention Rates. No participants were lost to follow-up or withdrew from the study following enrolment.

Adherence to Study Methodology. Regarding delivery of the intervention, no major protocol deviations were reported. All participants received 4 enemas as intended. Of the 60 enemas delivered to the 15 participants, 57 were retained for 30 min or longer, and the remaining 3 were retained for 26–30 min, representing a minor protocol deviation, which was not considered clinically significant.

With respect to attendance at key appointments, feasibility targets were met. There was an overall 93% in-person attendance rate; all participants attended their baseline appointments (15/15; 100%), and majority attended their Week 2 (13/15; 87%) and Week 8 (14/15; 93%) appointments. In-person attendance was necessary to conduct a physical examination.

All participants provided baseline stool samples (15/15; 100%), and majority provided Week 2 (14/15; 93%) and Week 8 (14/15; 93%) stool samples. A total of 58/60 (97%) stool samples across the 3 timepoints were returned, which was considered adequate adherence to the study protocol.

Table 4. Exploratory Outcomes Including Mental Health Outcome Measures, Other Questionnaire Data, and Physical Examination Data.

Group allocation	FMT	Placebo	FMT	Placebo	FMT	Placebo	FMT	Placebo	FMT	Placebo	FMT	Placebo	FMT	Placebo	FMT	Placebo	FMT	Placebo	FMT	Placebo
Outcome measure	MADRS		DASS		GSRS		PGI		SDS		PSQI		AQoL-8D		Weight (kg)		Systolic BP		Diastolic BP	
Week 0 (baseline), mean (s.d.)	29.5 (±5.8)	30.0 (±4.1)	34.3 (±11.1)	38.2 (±9.2)	2.2 (±0.9)	2.3 (±0.7)			22.3 (±6.3)	18.4 (±5.9)	13.5 (±3.8)	11.2 (±1.9)	115.0 (±14.2)	101.6 (±7.8)	87.4 (±23.4)	87.5 (±26.2)	126.2 (±17.4)	132.6 (±12.1)	75.6 (±11.2)	80.8 (±6.6)
Week 0, *n*	10	5	10	5	10	5			10	5	10	5	10	5	10	5	10	5	10	5
Week 2, mean (s.d.)	21.3 (±10.7)	22.0(±9.8)	21.5 (±12.5)	27.5 (±16.4)	2.0 (±0.5)	2.4 (±0.8)	2.4 (±0.7)	3.5 (±1.3)			11.3 (±1.9)	9.0 (±0.0)	95.9 (±20.4)	88.8 (±16.4)	87.8 (±23.2)	83.3 (±23.7)	131.2 (±22.7)	132.7 (±11.2)	75.7 (±8.7)	87.7 (±6.0)
Week 2, *n*	10	5	10	4	10	4	8	4			9	4	8	4	10	3	10	3	10	3
Between group differential change from baseline to Week 2, Hedges g effect size (CI)	-0.02 (-1.1-1.1)		-0.2 (-1.3-1.0)		-0.2 (-1.4-1.0)		-1.1 (-2.5-0.1)				0.2 (-0.9-1.4)		-0.3 (-1.6-0.9)		1.0 (-1.2-1.4)		0.4 (-0.9-1.7)		-1.4 (-2.9- -0.05)	
Week 8, mean (s.d.)	16.6 (±11.8)	16.6 (±6.9)	18.8 (±12.8)	19.3 (±16.4)	1.6 (±0.4)	2.6 (±0.3)	2.9 (±1.5)	2.8 (±1.0)	17.1 (±9.6)	14.0 (±8.3)	9.6 (±3.5)	9.3 (±2.2)	91.6 (±22.7)	81.8 (±24.2)	83.5 (±19.4)	88.6 (±27.0)	126.9 (±18.7)	134.4 (±8.9)	79.1 (±14.4)	86.2 (±6.1)
Week 8, *n*	10	5	9	4	10	4	9	4	9	4	8	4	8	4	9	5	9	5	9	5
Between group differential change from baseline to Week 8, Hedges g effect size (CI)	0.05 (-1.0-1.1)		0.2 (-1.0-1.4)		-0.8 (-2.0-0.4)		0.1 (-1.1-1.3)		-0.3 (-1.5-0.9)		-0.2 (-1.4-1.0)		-0.3 (-1.5-0.9)		-0.2 (-1.3-1.0)		-0.07 (-1.2-1.0)		-0.07 (-1.2-1.0)	
P-value*	0.67		0.19		0.001		0.87		0.86		0.592		0.068		0.476		0.527		0.253	

Note. FMT = faecal microbiota transplantation.

*All *P*-values reflect the overall values for time points and group allocation 2-way interaction.

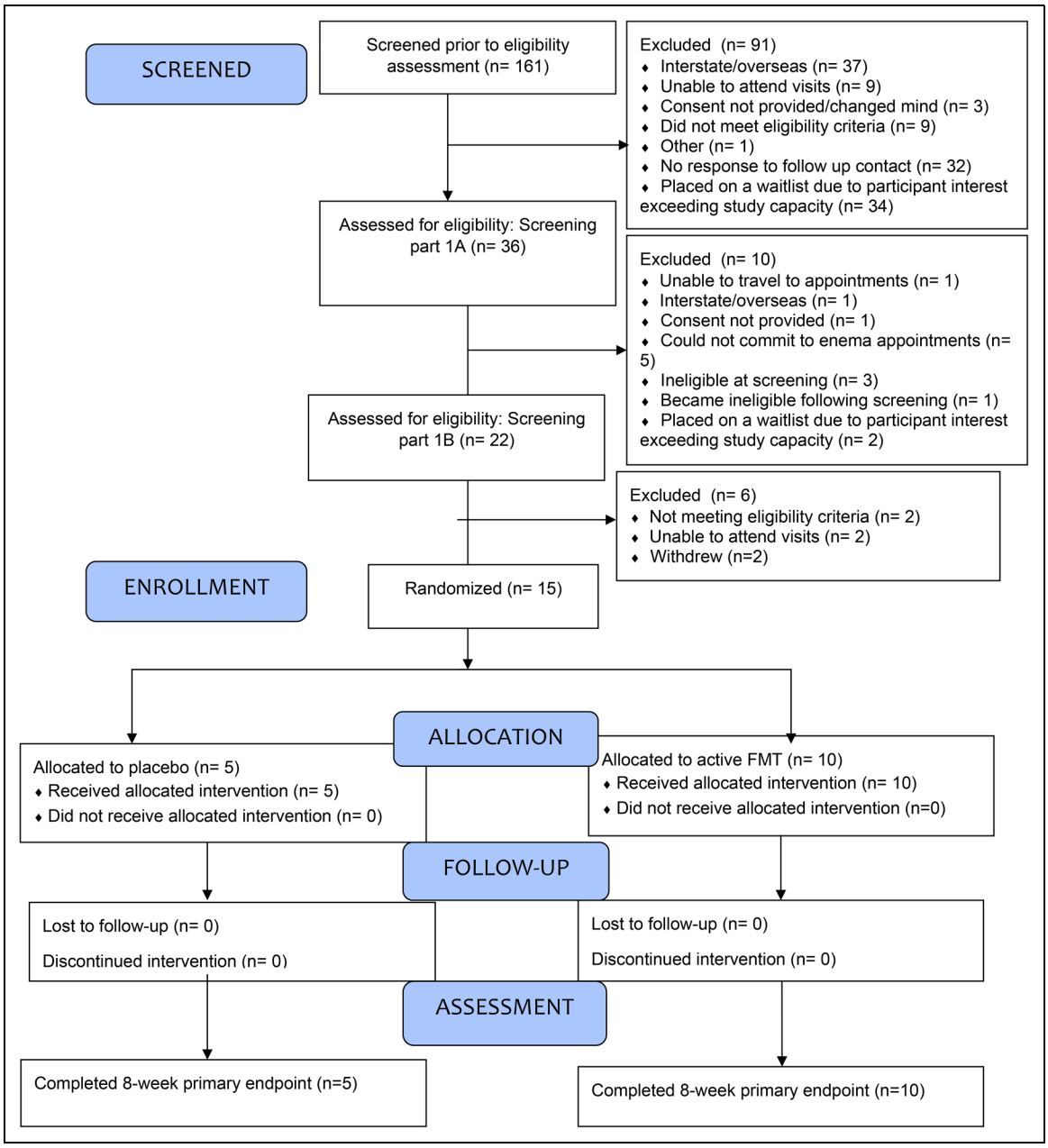

Figure 1. Consort flow diagram.

Adequacy of Blinding. Adequacy of blinding was assessed in both participants and nursing staff administering the enemas. Data were missing for 1 participant. Of the remaining 14 participants, 28.6% were unsure of their allocation, 35.7% believed they were allocated to placebo, and 35.7% believed they were allocated to active treatment.

With respect to maintaining blindness, 5 (35.7%) participants correctly guessed allocation, 5 (35.7%) guessed incorrectly, and 4 (28.6%) were unsure (i.e., they did not guess) (Fisher's exact test $P = 0.45$). Participants who correctly guessed allocation were, on average, 80% confident in their decision, whilst those who were incorrect were on average 69.8% confident.

Acceptability

Week 2 Survey (1 Week Following Intervention). Of the 12/15 (80%) participants who completed the Week 2 survey, 8 (66%) agreed or strongly agreed that they would prefer to receive enema-delivered FMT over orally delivered encapsulated FMT, and 6 (50%) agreed or strongly agreed that they would prefer enema-delivered FMT over medication. Regarding the number of treatments, 10 respondents (83%)

agreed or strongly agreed that 4 enema appointments was not too many. Nine respondents (75%) agreed or strongly agreed that they would recommend the intervention to others.

Week 4 Survey (Mid-point). Of the 14/15 (93%) participants who completed the mid-point survey, 12 (87%) agreed or strongly agreed that the amount of time spent on data collection was acceptable.

Week 8 Survey (End-point). At the conclusion of the study, 9 (67%) of the 12/15 (80%) participants that completed the final survey agreed or strongly agreed that overall they were satisfied with their trial experience. With respect to the time commitment, 9 respondents (67%) reported this was the same as expected. Participants were also given the opportunity to provide written feedback, and this was mainly positive. One participant emailed separately to the SPFQ providing feedback that the number of appointments and surveys was too burdensome and time consuming.

Safety and Tolerability

The intervention was considered safe, as no serious or severe AEs were reported in either group. According to the Week 2 SPFQ, all 12 respondents agreed or strongly agreed that the enema delivery was tolerable, and that they would have the treatment again if required.

The median number of mild-moderate AEs reported per participant was 2 in both the placebo and active FMT groups (see Table 3).

Exploratory Outcomes

The following outcomes are considered exploratory and were included to help inform the design of a full-scale RCT, albeit 2 outcomes met or approached statistical significance. The active FMT group had a greater improvement in average gastrointestinal symptom scores (measured by GSRS) from baseline to Week 8 (Hedges g = −0.77; 95%CI: −2.0, −0.4, $P < 0.001$). The active FMT group also reported a greater improvement in quality of life compared with the placebo group at both the Week 2 and Week 8 timepoints (Hedges g = −0.3 (95%CI: −1.6–0.9) and −0.3 (95% CI: −1.5–0.9), respectively, $P = 0.068$). The remaining exploratory outcomes are reported in Table 4. The blood biomarkers including lipid profile and blood glucose levels are not included in this table, as no significant within- or between-group changes were observed in either group. CRP results are not reported due to missing data arising from a lab error.

Discussion

To our knowledge, this study represents the first published study of FMT for MDD using a randomized, double-blinded, placebo-controlled design. We found that enema-delivered FMT is a feasible, acceptable, well tolerated, and safe treatment strategy for patients with MDD. A priori stated targets for feasibility outcomes were all met.

We note the high proportion of participants reporting a history of IBS (50%) and IBD (20%) in our study population. There are several possible explanations. Firstly, several participants were encouraged to participate in the study by local gastroenterologists, who were aware of the study. Gastroenterologists have greater awareness and acceptance of FMT, as it is a mainstay treatment for *C. difficile*.[54] Secondly, the acceptability of FMT has been reported to vary widely by indication, with highest acceptability reported in gastrointestinal disorders such as IBD.[37] As such, participants with gastrointestinal disorders may have been more interested in participating in FMT research. Thirdly, depression is highly comorbid with both IBS[55] and IBD.[39] However, whilst the prevalence of MDD in an IBS/IBD population has been widely reported, there are sparse literature around the prevalence of IBS/IBD in a population with MDD. It is unclear if our study cohort represents something close to a typical population prevalence of IBS/IBD in MDD. The population prevalence of IBS is reported to be 10–20%,[56] and 1 small study ($n = 40$) reported a prevalence of IBS in 27% of participants with MDD.[56] Given the prevalence of IBS/IBD in a MDD population is not well documented, but probably higher than the population average, it is also possible that the figures observed in our study are within typical limits for this cohort.

Whilst this study was not designed to measure efficacy, an improvement in gastrointestinal symptoms (large effect) was observed. This finding of significant improvement in gastrointestinal symptoms in our study population is also of importance given our cohort had a high proportion of gastrointestinal disorders at baseline.

A trend toward improvement in quality of life (small effect) was also observed in the active FMT group, compared with the placebo group. The improvement in quality of life may reflect the improvement in gastrointestinal symptoms reported in the active FMT group.

Whilst this study had significant strengths as described above, the following limitations should also be noted. A small sample size meant that exploratory outcomes were overtly underpowered to demonstrate efficacy. Therefore, at this stage it is not be possible to draw any conclusions with respect to the efficacy of FMT for MDD. With respect to the mental health outcomes (MADRS and DASS), these were no significant differences between groups. We also note that the mental health outcomes might have been strongly influenced by factors unrelated to the intervention such as participants changing their psychotropic treatments during the study or contracting COVID, making the results impossible to interpret. A larger, fully powered RCT would be expected to control for such problems.

In addition, the primary statistician was unavoidably unblinded due to the unequal group allocations. The study was therefore only double blinded, rather than triple

blinded as intended. A 1:1 allocation ratio in future studies would allow for triple blinding.

With respect to the design of a larger RCT, the question remains of whether the dosing strategy used in this study was sufficient or optimal. The intestinal microbiota analysis results from this study are still pending at the time of publication and will be published separately. It is expected that these data may help to inform dosing strategy. In addition, participant feedback suggested that, whilst the dosing strategy (i.e., 4 enemas over 4 days) was acceptable, the subsequent appointment burden should be reduced. In future studies, we would recommend removal of the 4- and 6-week follow-up appointments as these did not add significant value to the study outcomes, and were burdensome to participants.

Conclusions

This study was considered successful in that all feasibility targets were exceeded, and acceptability was high across all measured domains. In addition, the intervention appeared safe and well tolerated, with no serious or severe AEs recorded. The rates of mild-to-moderate AEs including constipation and nausea were similar in both treatment groups, and in-keeping with those reported with FMT in general.[14,57] Blinding by placebo was also successful. Exploratory data also suggested that the treatment may lead to improvements in gastrointestinal symptoms and quality of life in this population. The findings of this study support further research to evaluate clinical efficacy, and the use of this protocol is recommended given its established safety, acceptability, tolerability, and feasibility.

Contributors

JG was responsible for study design, writing and registering the protocol, psychiatric clinical oversight, rating, and review of psychiatric AEs, writing, editing the manuscript, and data analysis. FJ was the initiator and primary investigator for the study. MB, FJ, DC, EA, PS, and CH provided oversight and advice around study design. MB, MM, AL, and FJ contributed to data interpretation. MM was the study statistician and oversaw JG in performing the statistical analyses. CH provided gastrointestinal clinical oversight and rating of gastrointestinal AEs. MLC and JP performed scoring and analysis of the AQoL-8D. EA, MB, CH, and JG were additional investigators for the study. AM was the study coordinator. All authors contributed to and approved the final manuscript.

Conflict of Interest

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Supplemental Material

Supplemental material for this article is available online.

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