Effects of lyophilised faecal filtrate compared with lyophilised donor stool on *Clostridioides difficile* recurrence: a multicentre, randomised, double-blinded, non-inferiority trial

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Summary

Background Faecal microbiota transplantation (FMT) is highly effective in preventing recurrent *Clostridioides difficile* infection. However, it is not known whether live microbes are necessary in mediating FMT efficacy. This study aims to determine whether lyophilised sterile faecal filtrate (LSFF), free of live bacteria, is non-inferior to lyophilised donor stool (LFMT) in efficacy.

Methods This multicentre, randomised, double-blinded, non-inferiority trial was done at four academic centres in Canada. Eligible patients were adults aged 18 years or older with recurrent *C difficile* infection (at least two recurrences). Eligible patients were randomly assigned (1:1 using a prespecified computer-generated randomisation list with permutation blocks of 2 and 4, stratified by age >65 years or <65 years) to receive oral LSFF or LFMT. Each treatment dose consisted of 15 capsules that appeared identical. Participants and investigators were masked to treatment allocation. The primary outcome was the proportion of participants without recurrent *C difficile* infection (absence of more than three Bristol type 6 or 7 bowel movements per 24 h persisting more than 2 consecutive days) at 8 weeks. Analysis was done in the per protocol population, in which participants with unknown outcome status at 8 weeks due to death or loss to follow-up were excluded. Non-inferiority was established if the lower bound of the one-sided 95% CI for the difference in proportions of participants without recurrent *C difficile* between the LSFF and LFMT groups was above the non-inferiority margin of –10%. This trial was registered at ClinicalTrials.gov, NCT03806803, and is complete.

Findings Between March 27, 2019, and Nov 6, 2023, we assessed 409 patients for eligibility. 271 were excluded and the remaining 138 were enrolled and randomly assigned to receive LSFF (n=72) or LFMT (n=66). Participants' mean age was 61·2 years (SD 18·6); 91 (66%) of 138 patients were women and 47 (34%) were male. 127 participants (92%) were White. 130 (94%) of 138 participants completed the trial. At the planned interim analysis, 47 (65%) of 72 participants in the LSFF group and 57 (88%) of 65 participants in the LFMT group did not have *C difficile* recurrence at 8 weeks (difference –23%, one-sided 95% CI –33·8% to infinity; p=0·96). Given the pre-specified non-inferiority margin of –10%, non-inferiority of LSFF to LFMT could not be established and the study was terminated at the recommendation of the data safety monitoring board. Serious adverse events included one death (LFMT group) and five hospitalisations (four unrelated, one possibly related to interventions [LSFF group]). One event occurred before treatment and all others 2–20 weeks after study intervention. The most common adverse events were abdominal discomfort (48 [67%] of 72 patients in the LSFF group and 36 (55%) of 66 patients in the LFMT group) and nausea (13 [18%] in the LSFF group and 21 [32%] in LFMT group).

Interpretation Among adults with recurrent *C difficile* infection, non-inferiority of LSFF to LFMT was not established for the prevention of recurrent *C difficile* infection over 8 weeks, supporting the crucial role of live microbes in mediating clinical efficacy.

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Introduction

Faecal microbiota transplantation (FMT) is highly effective in preventing recurrent *Clostridioides difficile* infection and is recommended after the second recurrence by practice guidelines.[1] FMT has also been shown to significantly improve health-related quality-of-life measures in patients

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Research in context

Evidence before this study

Faecal microbiota transplantation (FMT) is a highly effective and guideline recommended therapy for preventing recurrence of *Clostridioides difficile* infection. Although the short-term safety profile remains good, rare transmission of serious bacterial infections, including multidrug-resistant organisms, has been reported. Furthermore, the mechanisms underpinning its efficacy are incompletely understood, limiting the advances in precision therapy. Bacterial engraftment, bacterially derived metabolites, and bacteriophages are thought to have a role in re-establishing microbial ecology following successful FMT. A small open-label trial using faecal filtrate devoid of live bacteria successfully prevented *C difficile* infection recurrence in five patients. Faecal filtrate has the advantage of eliminating transmission of pathogenic bacteria, but its efficacy at preventing *C difficile* infection recurrence has not been compared with FMT, the current standard-of-care therapy.

Added value of this study

We compared lyophilised FMT (LFMT) with lyophilised sterile faecal filtrate (LSFF) in adults with recurrent *C difficile* infection. Non-inferiority of LSFF to LFMT was not established and post-hoc superiority analysis showed LSFF was less efficacious than LFMT. No significant between-group differences occurred at week 24 in minor adverse events, quality of life improvement, or work productivity and activity impairment. Stool microbial compositions followed distinct trajectories after successful interventions. Although both groups had reduced Enterobacteriaceae, depleted commensal groups (eg, Ruminococcaceae, Oscillospiraceae, and Lachnospiraceae) recovered to healthy donor values only with LFMT. Recovery was less pronounced or absent (eg, Rikenellaceae or Coriobacteriaceae) in LSFF. Collectively, these findings establish that whole-community live microbes optimise efficacy in FMT, challenging bacteria-free filtrate as a therapeutic equivalent.

Implications of all the available evidence

LSFF is less efficacious than LFMT and should not be used in the treatment of recurrent *C difficile* infection. Live bacteria are important in mediating FMT efficacy, providing key information in clinical practice and microbial therapeutics development.

with recurrent *C difficile* infection.[2] The short-term safety profile of FMT is good, but rare transmission of serious bacterial infections, including multi drug-resistant organisms, has been reported.[3] The US Food and Drug Administration approved two microbial therapeutics to prevent further recurrence in those who have failed antibacterial treatment for recurrent *Clostridioides difficile* infection, Rebyota (also known as live-jslm) and Vowst (also known as live-brpk).[4,5] However, these products remain donor derived and therefore cannot be completely free from risk of transmitting an infection or an organism with undesirable characteristics.

The mechanisms underpinning FMT efficacy are incompletely understood. The gut microbiota in recurrent *C difficile* infection is characterised by altered composition and reduced diversity compared with that of healthy individuals, leading to reduced colonisation resistance against *C difficile*. Bacterial engraftment is thought to be important in correcting this dysbiosis, because stool bacterial compositions of successfully treated recipients resemble those of their donors.[6] The presence of bacterially derived metabolites, including secondary bile acids and short chain fatty acids, also correlate with efficacy.[7] Emerging evidence suggests that bacteriophages are transferred during FMT and might modulate bacterial ecology. A small, open-label trial using faecal filtrate devoid of live bacteria successfully prevented *C difficile* infection recurrence in five patients.[8] The main advantage of faecal filtrate over FMT is that it eliminates the risk of transmitting pathogenic bacteria. However, it remains unclear whether, and to what degree, the effects of FMT are mediated by live microbes, their metabolites, or bacteriophages.

The objective of this multi-centre study was to establish whether lyophilised sterile faecal filtrate (LSFF) is non-inferior to lyophilised FMT (LFMT) in efficacy for preventing subsequent *C difficile* infection. Additionally, we evaluated safety and changes in patient-reported health-related quality-of-life (HRQOL), work productivity and activity impairment, and faecal microbiota composition.

Methods

Study design and participants

This multicentre, randomised, double-blinded, non-inferiority trial was done at four Canadian academic centres (University of Alberta; University of Calgary; University of British Columbia; and Island Health, Victoria. The McGill site detailed in the protocol did not recruit any participants). The study was designed by the investigators with input from patient advisors. Study oversight was provided by the study steering committee and monitored by the Clinical Trials Office at University of Alberta and by an independent data safety monitoring board.

Eligible patients were outpatients aged 18 years or older with at least three episodes of *C difficile* infection. In the absence of an alternative cause of diarrhoea, each episode was defined as recurrence of diarrhoea (more than three unformed bowel movements every 24 h persisting for at least 2 days) within 12 weeks of completing a previous course of treatment (metronidazole, vancomycin, or fidaxomicin), with stool positive for *C difficile* by glutamate dehydrogenase and *C difficile* toxin A and B assay (C. diff QuikChek Complete; Techlab) or by detection of glutamate dehydrogenase and *C difficile* toxin B gene (Cepheid), plus resolution of

diarrhoea with *C difficile* infection-directed therapy. Severe *C difficile* infection (white blood cells >15 000/µL or serum creatinine >1·5 mg/dL) or fulminant *C difficile* infection (shock, ileus, or toxic megacolon),[9] chronic diarrhoeal illness, dysphagia, severe immunosuppression, pregnancy or breastfeeding, active infection requiring antibiotics, and life expectancy less than 6 months were key exclusion criteria. Written informed consent was obtained before screening. This study was approved by the local health research ethics board at each participating centre and Health Canada (Control No. 264376).

Randomisation and masking

Eligible participants were randomly assigned (1:1) to LSFF or LFMT ratio using a centralised, pre-established computer-generated randomisation list created by an online list generator with permutation blocks of 2 and 4, and stratified by age (>65 years *vs* <65 years). The individual who performed the randomisation (CM) was only responsible for manufacturing and distribution of the investigational product, but not in other clinical aspects of this trial. To ensure masking, LSFF and LFMT were encapsulated in gelatin capsules and appeared indistinguishable, and each treatment dose was stored in identical coded packaging. Each assigned treatment was dispensed to participants by the investigators who were not aware of treatment allocation. The only other individuals who were aware of treatment allocation were the biostatisticians (HX and MY).

Procedures

Following at least 10 days of oral vancomycin 125 mg four times a day or oral fidaxomicin 200 mg twice a day to attain symptom resolution, participants were treated with vancomycin at 125 mg by mouth twice a day or fidaxomicin at 200 mg by mouth once daily until 24 h before assigned treatment. Participants received bowel preparation (polyethylene glycol or picosulfate sodium) the night before and fasted until the scheduled treatment. Treatment consisted of a single dose of 15 capsules of LSFF or LFMT based on group assignment, taken orally in clinic under direct observation.

Participants were followed up in clinic at 1, 4, 8, and 24 weeks after treatment and were instructed to call the study team with suspected *C difficile* infection recurrence. Participants were also instructed to keep a stool diary, reviewed by the study team at follow-up visits, and to contact the study team with concern at any time. During the COVID-19 pandemic, follow-up visits were done by phone if participants were unable to or did not wish to attend in person. In the event of diarrhoea recurrence, *C difficile* testing was repeated. If test results were positive, either by *C difficile* toxin A or B assay (C diff QuikChek Complete; Techlab) alone or the combination of glutamate dehydrogenase and *C difficile* toxin B gene (Cepheid), participants were treated with vancomycin before receiving open-label LFMT capsules with identical follow-up. Participants were asked to collect a stool sample at home (storing in a freezer) within 24 h before each follow-up visit, which was immediately aliquoted and frozen at –80°C once received by the study team. Patient-reported outcomes questionnaires were completed in clinic (or at home with a mail-in option during the COVID pandemic) at screening, and at weeks 4, 8, and 24.

Serious adverse events were captured throughout the study. Minor adverse events were captured at week 1 by asking study participants if each symptom was present and to rate the severity of each present symptom from 1 (minimal) to 10 (worst). Optional stool samples were collected and frozen at –80°C before treatment and at 1, 4, 8, and 24 weeks after treatment. Participants responded to several validated questionnaires at screening and at 8 and 24 weeks after treatment.

Manufacturing of investigational products

Four volunteer stool donors, registered with the Edmonton FMT programme, provided stool. Donor inclusion and exclusion criteria, screening, and testing have been published previously (appendix pp 1–2).[10] Additional testing required by Health Canada included methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, carbapenem-resistant *Enterobacterales*, extended spectrum beta-lactamase-producing bacteria, Shiga toxin-producing *Escherichia coli* (after July 22, 2019), SARS-CoV-2 (after April 1, 2020), mpox (after Jan 30, 2023), and enteropathogenic *E coli* (after April 24, 2023).

See Online for appendix

Both LFMT and LSFF capsules contained trehalose (as a cryoprotectant[11]) and Neusilin (as a desiccant and dispersion aid). 100 g donor stool produced 40–60 LFMT capsules (size 0 gelatin capsules) stored at –80°C for up to 12 months. LSFF capsules were prepared by passing the faecal suspension through a series of progressively smaller filters (down to 0·2 µm), before lyophilisation and encapsulation. 100 g of donor stool yielded 15–20 LSFF capsules, stored at –80°C for up to 12 months (appendix pp 3–5). One LFMT treatment dose (15 capsules) was equivalent to approximately 25–37·5 g of stool, while one LSFF dose (15 capsules) was equivalent to from approximately 100 g of stool. Gelatin capsules were not acid resistant and were used in our previous clinical trial comparing frozen FMT delivered by oral capsules or colonoscopy, which reported 96% efficacy in both groups. In the same study, we also found significantly increased microbial diversity and similarity to donor's composition after FMT.[10]

Quality control was done on investigational products. LSFF was plated on growth media (de Man, Rogosa, and Sharpe agar; reinforced clostridial media; and brain heart infusion agar) and incubated aerobically and anaerobically at 37°C for 5–7 days to confirm no growth. Microscopy of Gram-stained LSFF products did not show microorganisms. By contrast, LFMT produced

10^8–10^9 colony forming units per capsule on these growth media.

Outcomes

The primary outcome was the proportion of participants in each group without recurrent *C difficile* infection, defined as the absence of more than three Bristol type 6 or 7 bowel movements per 24 h persisting more than 2 consecutive days, 8 weeks after treatment. Secondary outcomes were the proportion of participants without recurrent *C difficile* infection 24 weeks after treatment, and serious adverse events (infection, mortality, or hospitalisation considered directly attributable [by investigator assessment] to treatment or *C difficile* infection) and minor adverse events (fevers >37·8°C, nausea, vomiting, abdominal discomfort, and inability to retain at least 50% of ingested capsules). Minor adverse events were not captured beyond 1 week as these were expected to be rare. Exploratory outcomes included changes in HRQOL (EuroQol-5 Dimensions-5 levels [EQ-5D-5L] and *C difficile* Quality-of-Life Questionnaire [CDIFF32]) and work productivity and activity impairment (Work Productivity and Activity Impairment Questionnaire: Specific Health Problem [WPAI:SHP])[12–17] between baseline and week 24, and faecal microbial composition. Untargeted metabolomic analysis of LFMT and LSFF prepared treatments and raw stool were ad hoc analyses.

Statistical analysis

The sample size was determined to provide sufficient power to establish the non-inferiority of LSFF to LFMT in the primary outcome, assuming approximately 90% efficacy of LFMT at preventing *C difficile* infection recurrence at week 8.[11] With a non-inferiority margin of –10% (based on the consensus of the study team with expertise in FMT), power of 80%, type I error rate of 5%, no difference in efficacy between these two treatments, and allowing for 10% attrition), we required a sample size of 124 per group, or 248 participants in total.

The primary outcome and all secondary outcomes except for safety were analysed in the per-protocol population, in which participants with unknown outcome status at 8 weeks due to death or loss to follow-up were excluded. Safety outcomes were analysed in the intention to treat population (ie, all participants who were randomly assigned, regardless of treatment received). The primary outcome was analysed using a two-sample binomial non-inferiority test with one-sided 95% CI. Non-inferiority was established if the lower bound of the CI was above the non-inferiority margin of –10%.

Three sensitivity analyses were performed. In the first sensitivity analysis, we adjusted for the age stratification per the randomisation protocol. We first evaluated whether the difference in efficacy between LSFF and LFMT was homogeneous across the age groups (≥65 years *vs* <65 years) using logistic regression with Taylor approximation. If the difference was homogeneous, we estimated the common efficacy difference across age groups using Cochran-Mantel-Haenszel weights.[18] In the second sensitivity analysis, we took into consideration of donor effect. Due to the small number of donors, we again employed the stratification approach to estimate the age- and donor-adjusted difference in efficacy using Cochran-Mantel-Haenszel weights (random assignment based on availability of donor products). The third sensitivity analysis was done in the intention to treat population, and outcomes for patients with unknown status at 8 weeks were imputed (as non-recurrence for the LSFF group and recurrence for the LFMT group) based on extreme case imputation.

An interim analysis was planned when 50% of participants were recruited, with a stopping rule allowing early termination if the primary outcome for LSFF (point estimate for risk difference) was more than 10% less than that of LFMT. This rule provides a 50% chance of stopping enrolment to ineffective treatment with a slight loss of power (<2%).[19] Based on our simulations, 51% of the simulation runs were stopped early, with type I error rate decreased from 5·1% without early stopping to 5% with early stopping, when the null hypothesis that LSFF is inferior to LFMT is true (90% and 80% efficacy for LFMT and LSFF, respectively). When the alternative hypothesis that LSFF is not inferior to LFMT is true (90% efficacy for both LFMT and LSFF), 4% of the simulated runs were stopped early, with power decreasing from 80·1% without early stopping to 79·4% with early stopping. Due to the small decrease of type I error rate and in power, we did not increase the sample size to account for the interim analysis. If the non-inferiority failed to be established, a post-hoc analysis was performed to evaluate the superiority of LFMT relative to LSFF using the two-sided z-test for proportions.

The secondary outcome of efficacy at 24 weeks was analysed similarly to the primary outcome. Any patients with missing outcome at 24 weeks were excluded, and the same non-inferiority margin (–10%) was used. The same three sensitivity analyses were performed, where in sensitivity analysis #3, missing values were imputed so that all participants with missing outcome at 24 weeks were considered as having recurrence if they were in the LFMT group and as having non-recurrence if they were in the LSFF group. Safety outcomes were summarised using proportions and compared using Fisher's exact test.

Patient-reported outcomes (EQ-5D-5L, CDIFF32, and WPAI:SHP) were scored according to established guidelines.[12–17] HRQOL (EQ-5D-5L visual analog scale [VAS], CDIFF32) values range from 0 (worst HRQOL) to 100 (best HRQOL); EQ-5D-5L index value is calculated using the Canadian values set ranging from 0 (worst, being dead) to 1 (best, full health).[13] WPAI:SHP values range from

0% to 100% impairment.[17] Between-group (LFMT *vs* LSFF) and within-group differences (baseline *vs* week 24; for each group and for the overall study sample) were tested using non-parametric statistical tests (two-tailed Mann-Whitney U test and paired sign test, respectively; appendix p 6). Only completed instruments were included in the analysis. Study participants were grouped by the final treatment received. Secondary outcomes and exploratory outcomes were evaluated without multiple comparison adjustment, with safety and exploratory measures analysed based on two-sided tests at the 5% significance level. Statistical analyses were performed using SAS version 9.4.

A subset of 40 LSFF and 43 LFMT recipients were chosen for microbiome profile analysis based on stool sample availability. Stool microbial DNA was extracted using the Fast DNA Stool Mini Kit for 16S ribosomal DNA sequencing. Libraries were constructed using primers for the V3–V4 regions and sequenced on an Illumina platform using a 600-cycle protocol. Raw reads were trimmed, filtered denoised into amplicon sequence variants (ASVs), and taxonomically classified against the Genome Taxonomy Database r220[20–21] using DADA2[22] via the command line wrapper dadaist2.[23] Per sample local community diversities were calculated as Hill diversities[24] with coefficients q=0 (ASV richness), q=1 (exponential of Shannon entropy) and q=2 (inverse Simpson index) at the ASV level and summarised at the species level. Between-sample community dissimilarities were quantified as Bray–Curtis dissimilarity and taxa-interaction adjusted community distance,[25] and analysed using the PERmutational Multivatiate ANalysis of VAriance (PERMANOVA)[24] as implemented in the "adonis2" function of the vegan package version 2.6–8 in R.

The polar and volatile metabolome of raw stool, fecal suspension, lyophilised fecal suspension or lyophilised LFMT treatment, fecal filtrate, and lyophilised fecal filtrate or LSFF treatment from a single donor was assessed with untargeted analysis by comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GC×GC-TOFMS) (appendix pp 6–7). This study is registered at ClinicalTrials.gov, NCT03806803.

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between March 27, 2019, and Nov 6, 2023, we assessed 409 patients for eligibility. 271 were excluded and the remaining 138 were enrolled and randomly assigned to receive LSFF (n=72) or LFMT (n=66; figure 1). Participants' mean age was 61·2 years (SD 18·6). 91 (66%) of 138 patients were women and 47 (34%) were male. 127 (92%) were White, five (4%) were south Asian, three (2%) were southeast Asian or east Asian, one (1%) was Black or African American and two (1%) were Indigenous Canadian or Aboriginal. Baseline characteristics were similar between the groups (table 1). 137 participants were included in the per-protocol analysis for the primary outcome (one participant in the LFMT withdrew). 134 participants were included in the per-protocol 24-week efficacy analysis (three lost to follow-up [two in the LFMT group and one in the LSFF group] and one withdrawal in the LFMT group). 138 patients were included in the intention to treat safety analysis (figure 1). 121 participants were included in the patient-reported outcome analysis (based on data availability; appendix pp 7–8) and 83 were included in the microbial analysis (based on sample availability).

At the planned interim analysis, 47 (65%) of 72 participants in the LSFF group and 57 (88%) of 65 participants in the LFMT group did not have *C difficile*

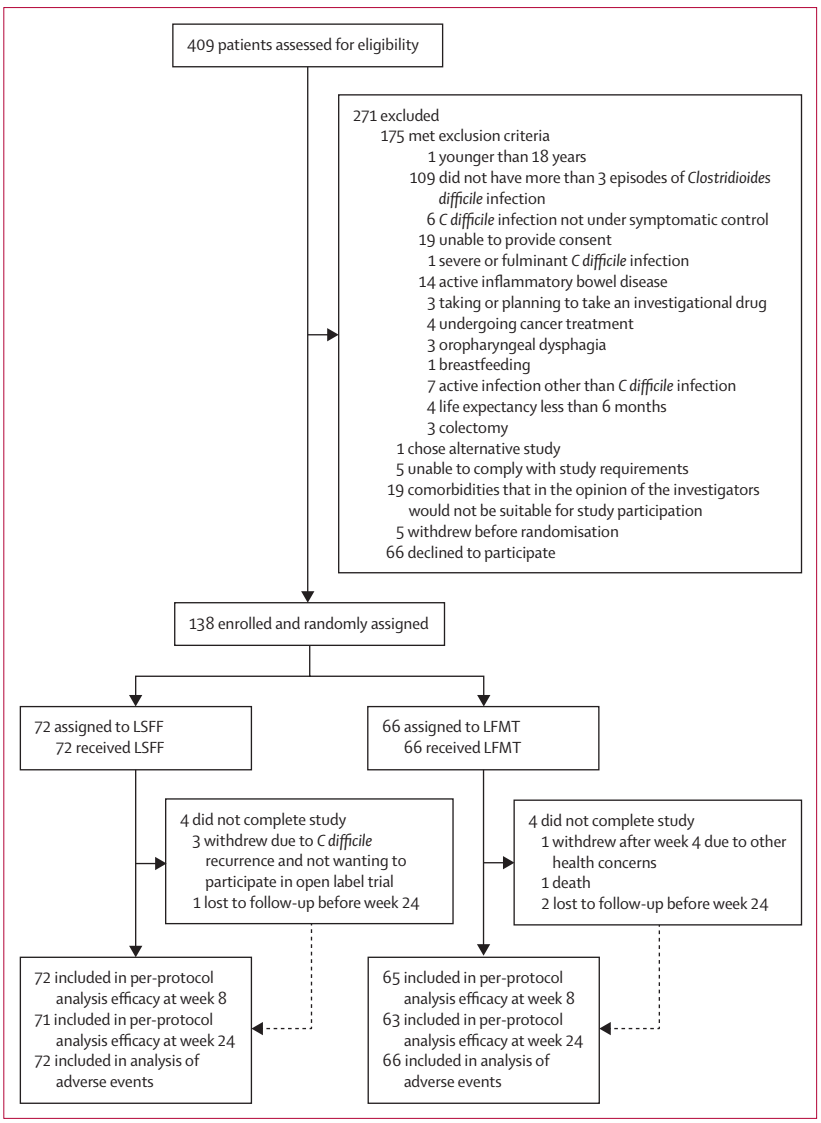

Figure 1: **Trial profile**
LSFF=lyophilised sterile faecal filtrate. LFMT=lyophilised faecal microbiota for transplantation.

	Lyophilised sterile faecal filtrate (N=72)	Lyophilised faecal microbiota for transplantation (N=66)
Sex		
Female	49 (68%)	42 (64%)
Male	23 (32%)	24 (36%)
Age, years, mean (SD)	62·5 (18·7)	60·4 (18·6)
Ethnicity		
Southeast Asian or east Asian	0	3 (5%)
White	69 (96%)	58 (88%)
South Asian	2 (3%)	3 (5%)
Black or African American	0	1 (2%)
Indigenous Canadian or Aboriginal	1 (1%)	1 (2%)
History of inflammatory bowel disease	3 (4%)	6 (9%)
Chronic proton pump inhibitor use	10 (14%)	13 (20%)
Total number of episodes of rCDI before enrolment	4 (3–4)	4 (3–4)
Number of participants receiving fidaxomicin instead of vancomycin for qualifying episode of CDI	7 (10%)	6 (9%)
Duration of CDI directed therapy for qualifying episode before investigational treatment, days	32 (19·0–54·5)	27 (17·5–57·0)
Number of emergency department visits or hospitalisation before enrolment	1 (0–2)	1 (0–3)
Duration of rCDI before enrolment (interval between first antibiotic use and enrolment), months	4·7 (3·8–6·1)	4·2 (3·1–6·2)
Haemoglobin, g/dL	13·5 (12·8–14·7)	13·7 (12·8–14·7)
White blood cell count per µL	7200 (5800–9050)	6150 (5200–8125)
Albumin, median (IQR), g/dL	4·1 (3·7–4·3)	4·0 (3·8–4·4)
C-reactive protein, mg/dL	0·2 (0·1–0·5)	0·3 (0·1–0·6)
Creatinine, mg/dL	0·9 (0·7–1·0)	0·8 (0·7–1·0)

Data are n (%) or median (IQR) unless otherwise specified. rCDI=recurrent *Clostridioides difficile* infection.

Table 1: **Baseline characteristics**

	Lyophilised sterile faecal filtrate (N=72)		Lyophilised faecal microbiota for transplantation (N=66)		p value
	n (%)	Severity*	n (%)	Severity*	
Nausea	13 (18%)	4 (3·0–5·0)	21 (32%)	4 (3·0–6·0)	p=0·24
Vomiting†	2 (3%)	..	6 (9%)	..	p=0·15
Abdominal discomfort	48 (67%)	3 (2·5–5·0)	36 (55%)	5 (3·0–7·0)	p=0·16
Fever‡	2 (3%)	..	0	..	p=0·50

Data are n (%) or median (IQR). No participants were unable to retain at least 50% of ingested capsules. *Participants were asked to rate symptom severity on a scale of 1–10. †Captured only yes or no without rating severity. ‡Highest fever reported was 39·1°C.

Table 2: **Minor adverse events reported 1 week after treatment**

recurrence at 8 weeks. The rate difference was –23%, (one-sided 95% CI –33·8% to infinity; p=0·96), which exceeded the pre-specified non-inferiority margin. The study was therefore terminated at the recommendation of the data safety monitoring board due to inefficacy of LSFF relative to LFMT.

In the first sensitivity analysis with age stratification, in participants younger than 65 years, 23 (66%) of 35 participants in the LSFF group and 29 (83%) of 35 participants in the LFMT group did not have *C difficile* recurrence at 8 weeks. These rates were 24 (65%) of 37 participants in the LSFF group and 28 (93%) of 30 participants in the LFMT group among participants aged 65 years or older. The rate difference was –17·1% (95% CI –37·2% to infinity) in participants younger than 65 years and –28·5% (–43·4% to infinity) in participants 65 years or older. These two rate differences were not significantly different from each other (p=0·41). The common risk difference after adjusting for age in the sensitivity analysis was –22·7% (one-sided 95% CI –34·1% to infinity; p=0·96). In the second sensitivity analysis that took into consideration the donor effect, the risk difference was –23·6% (one-sided 95% CI –35·3% to infinity; p=0·97). In the third sensitivity analysis, 47 (65%) of 72 participants in the LSFF group and 57 (86%) of 66 participants in the LFMT group did not have *C difficile* recurrence at 8 weeks, leading to similar results (difference –21·1%, one-sided 95% CI –32·6% to infinity; p=0·94). This assumed that the participants who were in the LFMT group and not assessed for the primary 8-week outcome due to withdrawal from the study before week 8 follow-up had *C difficile* infection recurrence at week 8. These results did not provide sufficient evidence to conclude that LSFF was non-inferior to LFMT within the 10% margin in preventing recurrent *C difficile* infection. Post-hoc analysis showed that the LMFT group had a significantly greater proportion of participants who did not recurrent *C difficile* infection at 8 weeks than the LSFF group in both the per protocol population (p=0·002) and intention to treat population (p=0·004).

Absence of recurrent *C difficile* infection 24 weeks after treatment was maintained in 44 (62%) of 71 participants in the LSFF group and 54 (86%) of 63 participants in the LFMT group in the per-protocol analysis (rate difference –23·7%, one-sided 95% CI –35·7% to infinity, p=0·97)). In the intention to treat analysis, absence of recurrent infection at 24 weeks was maintained in 45 (63%) of 72 participants in the LSFF group and 54 (82%) of 66 participants in the LFMT group (rate difference –19·3%, –31·5% to infinity, p=0·90). Both analyses produced insufficient evidence to conclude that LSFF was non-inferior to LFMT. Among the 36 participants who had recurrent *C difficile* infection after treatment, 31 (23 in the LSFF group and eight in the LFMT group) chose open-label LFMT; 26 of 31 (19 in LSFF and seven in LFMT) were free of recurrent *C difficile* infection at week 8 after treatment. Of the remaining five participants with recurrent *C difficile* infection (four in the LSFF group and one in the LFMT group), two participants (one in each group) received a third LFMT dose with no further recurrence at week 8. At week 24, 23 of 31 participants (18 in the LSFF group and five in the LFMT group) were free of recurrence. Two of the 31 participants were lost to follow-up before the open-label week 24 visit.

Serious adverse events were infrequent. There was one death from COVID-19 pneumonia in the LFMT group (20 weeks after intervention) and five hospitalisations. Four hospitalisations were deemed unrelated to study treatment (one in the LSFF group [third degree heart block requiring pacemaker insertion (before treatment)] and three in the LFMT group [one appendicitis requiring appendectomy, (9 weeks after intervention) one pulmonary embolism (13 weeks after intervention), and one COVID-19 pneumonia (5 weeks after intervention)]). One hospitalisation (LSFF group) 2 weeks after receiving open-label LFMT was deemed possibly related to study intervention (severe abdominal bloating and constipation with normal investigation including CT abdomen and colonoscopy, and symptom resolution within 48 h of conservative management). Minor adverse events were transient and not different between groups (table 2).

121 participants had baseline and week 24 patient-reported outcomes data (44 in the LSFF group and 77 in the LFMT group [participants who received LFMT and open-label LFMT (n=23) regardless of original blinded treatment group]; appendix p 8).

There were no statistically significant between-group differences in any of the patient-reported outcomes

	N	Baseline			Week 24			Baseline *vs* week 24		
		Median	IQR	p value	Median	IQR	p value	Median	95% CI	p value
EQ-5D-5L										
Visual analog scale*										
Overall	117	70·0	50·0–80·0	..	80·0	70·0–90·0	..	10·0	10·0 to 15·0	p<0·0001
LSFF	42	70·0	60·0–80·0	..	82·5	75·0–90·0	..	11·5	10·0 to 20·0	p<0·0001
LFMT	75	70·0	50·0–80·0	..	80·0	70·0–91·0	..	10·0	10·0 to 20·0	p<0·0001
Missing†	4	..	..	..	..	..	..	..	..	..
p value	..	..	..	p=0·51	..	..	p=0·71	..	..	..
Index value‡										
Overall	117	0·85	0·75–0·90	..	0·90	0·81–0·95	..	0·04	0·04 to 0·06	p<0·0001
LSFF	42	0·85	0·76–0·90	..	0·90	0·81–0·95	..	0·04	0·02 to 0·07	p<0·0001
LFMT	75	0·85	0·74–0·91	..	0·90	0·81–0·95	..	0·04	0·04 to 0·08	p<0·0001
Missing†	4	..	..	..	..	..	..	..	..	..
p value	..	..	..	p=0·91	..	..	p=0·80	..	..	
***Clostridium difficile* Quality of Life Questionnaire§**										
Overall score										
Overall	113	53·1	35·9–63·3	..	82·8	70·3–91·4	..	27·3	21·9 to 32·0	p<0·0001
LSFF	41	57·0	34·4–60·9	..	82·8	64·8–90·6	..	24·2	16·4 to 30·5	p<0·0001
LFMT	72	51·6	36·3–63·7	..	83·2	72·7–92·6	..	31·3	22·7 to 33·6	p<0·0001
Missing†	8	..	..	..	..	..	..	..	..	..
p value	..	..	..	p=0·82	..	..	p=0·28	..	..	..
Physical domain										
Overall	113	66·1	44·6–80·4	..	91·1	78·6–96·4	..	19·6	14·3 to 26·8	p<0·0001
LSFF	41	71·4	44·6–82·1	..	91·1	82·1–96·4	..	14·3	8·9 to 30·4	p<0·0001
LFMT	72	64·3	44·6–79·5	..	92·9	78·6–98·2	..	23·2	14·3 to 30·4	p<0·0001
Missing†	8	..	..	..	..	..	..	..	..	..
p value	..	..	..	p=0·56	..	..	p=0·18	..	..	..
Mental domain										
Overall	113	33·9	25·0–48·2	..	75·0	60·7–85·7	..	33·9	25·0–41·1	p<0·0001
LSFF	41	33·9	25·0–46·4	..	75·0	48·2–83·9	..	26·8	19·6–42·9	p<0·0001
LFMT	72	33·9	25·0–50·9	..	75·0	62·5–88·4	..	35·7	26·8–42·9	p<0·0001
Missing†	8	..	..	..	..	..	..	..	..	..
p value	..	..	..	p=0·80	..	..	p=0·40	..	..	..
Social relationships domain										
Overall	113	56·3	37·5–75·0	NA	93·8	75·0–93·8	NA	25·0	18·8–31·3	p<0·0001
LSFF	41	56·3	37·5–75·0	..	93·8	75·0–93·8	..	25·0	12·5–31·3	p<0·0001
LFMT	72	56·3	40·6–78·1	..	93·8	75·0–93·8	..	25·0	18·8–31·3	p<0·0001
Missing†	8	..	..	..	..	..	..	..	..	..
p value	..	..	..	p=0·82	..	..	p=0·66	..	..	..

(Table 3 continues on next page)

	N	Baseline			Week 24			Baseline *vs* week 24		
		Median	IQR	p value	Median	IQR	p value	Median	95% CI	p value
(Continued from previous page)										
WPAI:SHP										
Percent overall work impairment due to *C difficile* infection¶‖										
Overall	23	40·0	0·0–64·0	..	0·0	0·0–20·0	..	–10·0	–40·0 to 0·0	p=0·0080
LSFF	13	20·0	0·0–64·4	..	0·0	0·0–20·0	..	–10·0	–50·0 to 0·0	p=0·11
LFMT	10	40·0	10·0–62·5	..	0·0	0·0–10·0	..	–14·7	–64·0 to 15·0	p=0·070
p value	..	..	..	p=0·77	..	..	p=0·58	..	..	..
Percent activity impairment due to *C difficile* infection**										
Overall	118	30·0	10·0–70·0	..	0·0	0·0–10·0	NA	–30·0	–30·0 to –20·0	p<0·0001
LSFF	43	30·0	10·0–70·0	..	0·0	0·0–10·0	..	–20·0	–40·0 to –10·0	p<0·0001
LFMT	75	30·0	10·0–70·0	..	0·0	0·0–10·0	..	–30·0	–30·0 to –20·0	p<0·0001
Missing†	3	..	..	..	..	..	..	..	..	..
p value	..	..	..	p=0·90	..	..	p=0·45	..	..	..

Between-group differences in medians (LFMT *vs* LSFF) and within-group differences in medians (baseline *vs* week 24 for each group and for overall study sample) were tested using non-parametric statistical tests (two-tailed Mann–Whitney *U* test and paired sign test, respectively). LFMT=lyophilised faecal microbiota for transplantation. Confidence intervals for median within-group differences were obtained using the distribution-free method based on order statistics LSFF=lyophilised sterile faecal filtrate. HRQOL=health-related quality of life. WPAI:SHP=Work Productivity and Activity Impairment Questionnaire: Specific Health Problem. NA=not applicable. *EQ-5D-5L visual analog scale values range from 0 (lowest or worst HRQOL) to 100 (highest or best HRQOL). †Based on the full patient-reported outcomes sample of N=121 at week 24; only participants who fully completed the instrument at both baseline and week 24 are reported. ‡EQ-5D-5L index value based on Canadian value set. §Overall and domain scores range from 0 (lowest or worst HRQOL) to 100 (highest or best HRQOL). ¶Overall work impairment values range from 0% to 100% impairment, with higher values indicating greater overall work impairment or less productivity. ‖Participants only completed WPAI:SHP questions related to work if they were employed at the time of the survey; N reported is only those who were employed and fully completed the WPAI:SHP at both baseline and week 24. **Percent activity impairment values range from 0% to 100% impairment with higher values indicating greater activity impairment.

Table 3: **Changes in patient-reported HRQOL and WPAI between baseline and week 24**

measures at baseline or week 24. However, at week 24, both groups had statistically significant within-group improvements in generic and disease-specific HRQOL from baseline (EQ-5D-5L VAS and index scores p<0·0001 in both groups at week 24; table 3). Median CDIFF32 overall score increased from baseline to week 24 in both groups (p<0·0001). Both groups also had significant improvements in all CDIFF32 domain scores (all p<0·0001).

Few participants in either group reported being employed, and there were no statistically significant within-group changes in median overall work impairment. However, when participants from both groups were combined (N=23), there was a statistically significant decrease in median overall work impairment from baseline to week 24 (table 3). Activity impairment due to *C difficile* infection decreased from baseline to week 24 in both the LSFF group and the LFMT group (table 3).

24 weeks after intervention, the LSFF group had a loss in species diversity (Wilcoxon test on rarefied richness[26] p=0·016 at week 8 and p=0·0060 at week 24; Hill diversity[27] with q=1 p=0·091 at week 8 and p=0·042 at week 24), whereas LFMT community richness remained similar to baseline values (figure 2A). Treatment success was not associated with statistically significant shifts in species diversity in either group at baseline or weeks 1 or 8, although uneven group sizes with few failure cases limited statistical power in the LFMT group.

Community composition (Bray–Curtis dissimilarity;[28] figure 2B) was indistinguishable between LSFF and LFMT groups at week 0, but followed distinct trajectories after intervention: the community composition of participants who received LFMT significantly and consistently gravitated towards that of healthy donors, whereas participants who received LSFF shifted more slowly and less directly towards donor composition and remained more heterogeneous (PERMANOVA[28] p≤0·001, R^2 3·7–6·3%). This donor-directed compositional shift was more prominent in the LFMT group than in the LSFF group at every timepoint (Wilcoxon p≤10^{-7} unpaired), while values in patients who received LSFF remained more similar to baseline than LFMT (p<10^{-6}) before drifting off to a similar degree as patients who received LFMT (figure 2C). Treatment success at week 8 was not associated (per PERMANOVA) with the strength of the shift towards donor composition or away from baseline composition in either group at any timepoint.

We pinpointed these compositional changes to biologically relevant bacteria: both LFMT and LSFF induced a loss of Enterobacteriaceae relative abundance (enriched at pre-treatment baseline) towards donor values (low relative abundance or below detection limits; figure 3). However, this shift was quicker and more pronounced in the LFMT group (Wilcoxon test p<0·0001 at week 1 and p=0·030 at week 8). Commensal groups that were depleted in pre-treatment participant samples (eg, *Ruminococcaceae* spp, *Oscillospiraceae* spp, *Lachnospiraceae* spp, *Rikenellaceae* spp including genus *Allistipes*, and *Coriobacteriaceae* spp

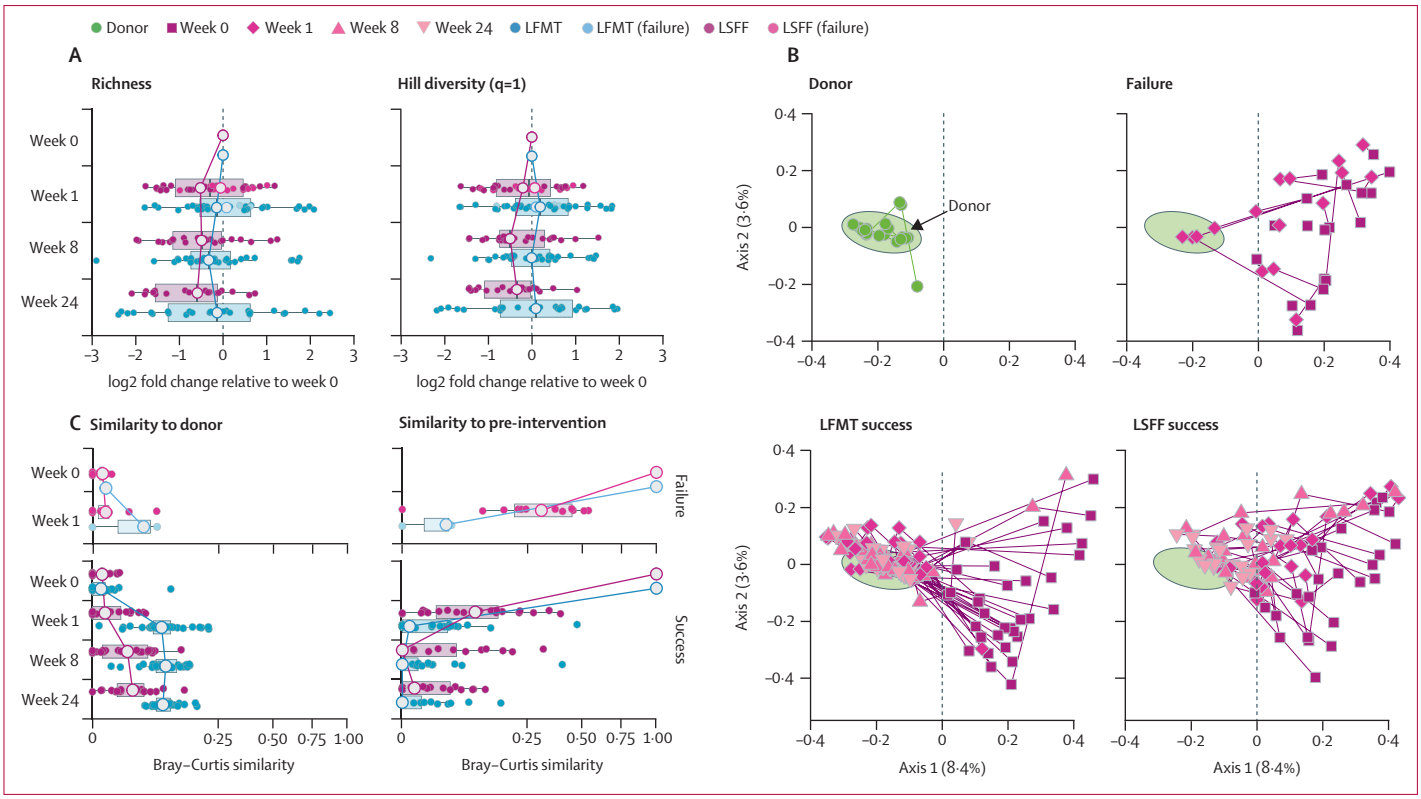

Figure 2: **Comparison of changes in microbial compositions at baseline and 8 weeks and 24 weeks following interventions with LFMT or LSFF**
(A) Richness and Hill diversity (q=1). (B) Principal coordinate analysis plot of microbial community similarity. (C) Bray–Curtis similarity. LSFF=lyophilised sterile faecal filtrate. LFMT=lyophilised faecal microbiota for transplantation.

including genus *Collinsella*) reliably and persistently recovered to healthy donor values in LFMT recipients. However, recovery was delayed, less pronounced, or completely absent (Rikenellaceae or Coriobacteriaceae) in the LSFF group. Physiologically relevant commensal Bacteroidaceae species (*Bacteroides uniformis* and *Phocaeicola vulgatus*) only partially recovered towards donor values in patients who received LFMT, but did not change significantly in the LSFF group. Neither overall community compositional shifts nor the recovery of individual clades was associated with treatment success (outcome at week 8).

In the untargeted analysis of polar metabolites in samples processed from one donor, raw stool had the highest number of analytes (582 metabolites) and non-lyophilised faecal filtrate had the fewest analytes (161 metabolites; appendix pp 9–10). Similarly, faecal filtrate had the fewest features on chromatograms targeting common ions for fatty acids (appendix p 13). A similar trend was also observed with volatile metabolites: raw stool had the highest number of peaks (525 peaks) and faecal filtrate had the fewest peaks (286 peaks; appendix pp 9, 13). Raw stool had the most amino acids detected and had the greatest abundance in total amino acids (appendix p 8). Raw stool and LFMT had considerably higher abundances of organic acids than did LSFF (appendix p 8). All short chain fatty acids and medium chain fatty acids were detected in the faecal filtrate, however, in much lower relative abundances than all other samples. The biologically relevant short chain fatty acids butanoic acid and pentanoic acid were detected in the highest abundances in LFMT samples (appendix pp 9, 14).

Discussion

In this multi-centre, randomised, non-inferiority trial, the non-inferiority of LSFF to LFMT in preventing *C difficile* infection recurrence in those with at least two recurrences was not established, contrary to results of a small preliminary study by Ott and colleagues.[8] Post hoc analysis based on two-sided comparison showed that LFMT had greater efficacy than LSFF. Once the absence of recurrent *C difficile* infection was achieved at week 8, recurrence was rare at week 24. The treatments were well tolerated with few safety concerns. Significant improvement in patient-reported outcomes was also reported 24 weeks after resolution of recurrent *C difficile* infection. Fundamentally, our study establishes that live microbes are crucial for the efficacy of FMT for this indication. Once absence of recurrent *C difficile* infection was reported at week 8, most participants had a sustained response to week 24. The safety profile of both treatments was good, with only one hospitalisation for severe

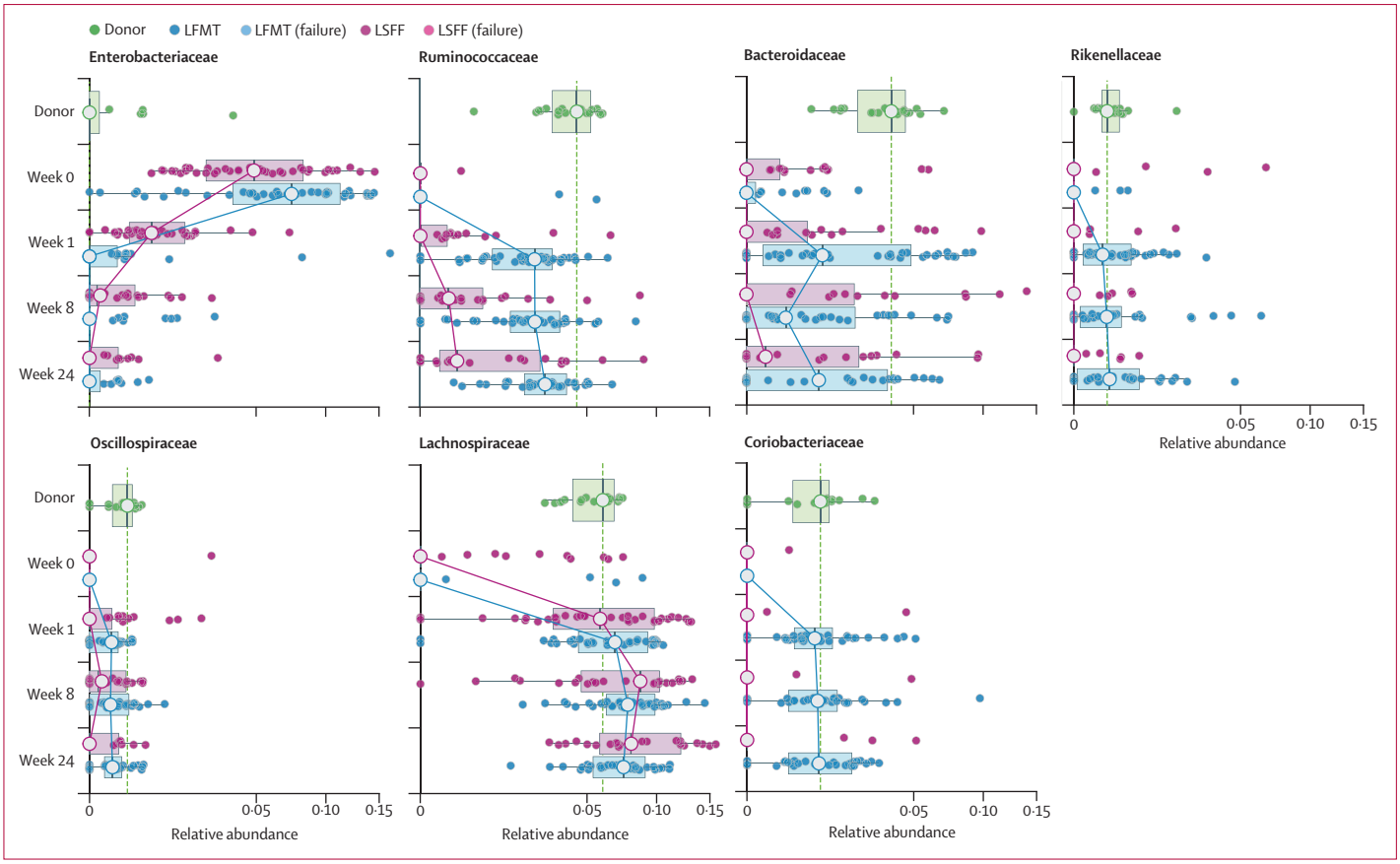

Figure 3: **Similarities and differences in microbial compositions after intervention in LFMT and LSFF recipients**
LSFF=lyophilised sterile faecal filtrate. LFMT=lyophilised faecal microbiota for transplantation.

abdominal pain possibly attributable to LFMT. The higher rate of abdominal discomfort (54·6% in LFMT and 66·7% in LSFF) than previously reported in systematic reviews (20–30%)[1] could be a result of our structured assessment of symptoms at week 1 rather than self-reporting at later timepoints, which is susceptible to recall bias. Patient-reported HRQOL and work productivity significantly improved in both groups from baseline to week 24 with no significant between-group differences. LFMT was more efficient in correcting the gut microbiome alterations observed in recurrent *C difficile* infection, shifting microbiomes and key taxa involved in bile acid metabolism and short chain fatty acid production towards that of healthy donors. These results establish the causal effect of live bacterial transfer on microbial community assembly after FMT.

This study was done as a non-inferiority trial without a placebo group. We could not justify including a placebo group given the availability of standard-of-care FMT at each participating centre. Instead, we used LFMT as an active comparator to LSFF, because LSFF eliminates the risk of transmitting a bacterial infection. We confirmed the absence of microbes in the filtrate, and found fewer metabolomic features, including short chain fatty acids, in LSFF than in LFMT, which could also affect efficacy.

We analysed the bacterial community over time and observed temporal shifts in microbial communities and relative importance of bacterial taxa. Discontinuing antibiotic suppression against *C difficile* infection might have caused these changes. For example, oral vancomycin treatment in the absence of *C difficile* infection reduces Lachnospiraceae and increases Enterobacteriaceae.[29] Thus, the recovery of Lachnospiraceae and loss of Enterobacteriaceae, albeit slower in LSFF, observed in both LFMT and LSFF recipients could reflect the effect of vancomycin discontinuation alone. Even if LSFF is assumed to have properties—mediated through antimicrobial peptides,[30] microRNAs,[31] or even phages[32]—that reduce *C difficile* infection recurrence, our study clearly showed that LSFF is inferior to stool preparations containing live bacteria, which was not addressed by Ott and colleagues.[8] On the other hand, partial recovery (Ruminococcaceae) or lack of recovery (Coriobacteriaceae, Bacteroidaceae, Rikenellaceae, and Oscillospiraceae) of bacterial taxa in LSFF recipients who did not have recurrence suggests alternative pathways of microbiome recovery. The beneficial effect of defined microbial

consortia also advances a concept of who rather than how many as a means of establishing colonisation resistance against *C difficile* infection.[33,34] It is unclear which taxa and in what abundance are required. In our preparation of LSFF, the lowest filter size (0·2 µm) will remove live bacteria while other constituents such as metabolites, cellular components, or even phages could be retained, but may be further reduced or modified through lyophilisation.

Our study has several strengths. To our knowledge, this is the first time a large, multi-centre, double blinded, adequately powered, randomised trial was used to compare LSFF to LFMT for recurrent *C difficile* infection in a non-inferiority design to elucidate the causal contribution of live microbes. The overall success rate of LFMT in our study was consistent with the success rate of FMT reported in other studies.[1] We included patients with a propensity for recurrent *C difficile* infection, with a median of four episodes over a 5-month period. Few studies have captured patient-reported outcomes using validated instruments,[10,16] and none have evaluated effects beyond 8 weeks. Activity impairment and work productivity have not been previously reported for recurrent *C difficile* infection. Longitudinal stool sampling and analysis revealed temporal microbial changes and important bacterial taxa. A small number of stool donors minimised risk of disease transmission, screening costs, and variables in microbial composition analysis.

This study has several limitations. Without a placebo group, we cannot determine if, or how, non-viable components in LSFF contribute to efficacy. In placebo-controlled FMT studies recruiting participants with three or more previous occurrences, the risk of recurrence in the control group was approximately 50%.[1] Placebo effect cannot be ruled out but would have existed in both groups and was minimised by our double blinded trial design. Furthermore, the LSFF used in our trial was lyophilised, rather than the fresh faecal filtrate suspension used by Ott and colleagues.[8] It is not known how this difference might have affected the results, since the lyophilisation process can potentially alter metabolite composition, as seen in our ad hoc analysis, or inactivate phages.[35,36] Evaluating HRQOL at week 24 instead of at week 8 could have missed the differences between the two intervention groups, since HRQOL may have stabilised between week 8 and 24 without *C difficile* infection recurrence. We did not characterise changes in the virome or phageome in our treatments or study participants. Participants with severe or fulminant *C difficile* infection were excluded because these patients usually require more than one FMT to report disease resolution.[37] As such, the results of our study might not be applicable to this patient population. Microbial profiling was done by 16S rRNA sequencing, which has significant limitations and biases. The metabolomics analysis was limited in statistical power (N=1; ie, samples from one donor), therefore the absence of particular metabolites might not be true for all LSFF products. Normalising and comparing metabolite profiles in different sample matrices is challenging without sufficient statistical power. Lastly, participants were mostly White, and did not provide dietary information, limiting generalisability.

In conclusion, bacteria-free LSFF is less efficacious than LFMT in preventing *C difficile* infection recurrence and in inducing an ecological shift toward donor composition and should not be used in clinical practice. Live microbes are essential in microbial-based therapeutics to maintain treatment efficacy.

Contributors

Study conceptualisation: DK, TMM, DAM, HX, and TL. Funding acquisition: DK, KW, CL, TS, JW, HX, TMM, and TL. Investigation: DK, KW, CL, TS, RF, CM, MS, TL, RL, RTG, and JJH. Supervision: DK, CL, TS, TL, and JJH. Formal analysis: HX, MY, DAM, KVM, and TSBS. Original draft: DK, KVM, DAM, TSBS, and JW. Reviewing and editing: all authors. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. DK, KW, CL, TS, RF, MS, and TL have directly accessed and verified the data reported in the manuscript.

Declaration of interests

DK consulted for Ferring and Vedanta. CL served as a member of advisory board for Ferring. TS consulted for or received clinical trial funding from Merck, Rebiotix/Ferring, Seres, and Vedanta and consulted for Biologics. TL consulted for Seres, Vedanta, Crestone, and Adiso. The other authors report no competing interests.

Data sharing

Deidentified participant data will be shared upon request from the corresponding author at the time of publication. Raw sequencing data will be deposited into the Sequence Read Archive of NCBI (http://www.ncbi.nlm.nih.gov/sra) and will be available at the time of publication.

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