# Faecal microbiota transplantation for first or second *Clostridioides difficile* infection (EarlyFMT): a randomised, double-blind, placebo-controlled trial

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## Summary

**Background** ***Clostridioides difficile*** **infection is an urgent antibiotic-associated health threat with few treatment options. Microbiota restoration with faecal microbiota transplantation is an effective treatment option for patients with multiple recurring episodes of *C difficile*. We compared the efficacy and safety of faecal microbiota transplantation compared with placebo after vancomycin for first or second *C difficile* infection.**

**Methods** **We did a randomised, double-blind, placebo-controlled trial (EarlyFMT) at a university hospital in Aarhus, Denmark. Eligible patients were aged 18 years or older with first or second *C difficile* infection (defined as ≥3 watery stools [Bristol stool chart score 6–7] per day and a positive *C difficile* PCR test). Patients were randomly assigned (1:1) to faecal microbiota transplantation or placebo administered on day 1 and between day 3 and 7, after they had received 125 mg oral vancomycin four times daily for 10 days. Randomisation was done by investigators using a computer-generated randomisation list provided by independent staff. Patients and investigators were masked to the treatment group. The primary endpoint was resolution of *C difficile*-associated diarrhoea (CDAD) 8 weeks after treatment. We followed up patients for 8 weeks or until recurrence. We planned to enrol 84 patients with a prespecified interim analysis after 42 patients. The primary outcome and safety outcomes were analysed in the intention-to-treat population, which included all randomly assigned patients. The trial is registered with ClinicalTrials.gov, NCT04885946.**

**Findings** **Between June 21, 2021, and April 1, 2022, we consecutively screened 86 patients, of whom 42 were randomly assigned to faecal microbiota transplantation (n=21) or placebo (n=21). The trial was stopped after the interim analysis done on April 7, 2022 for ethical reasons because a significantly lower rate of resolution was identified in the placebo group compared with the faecal microbiota transplantation group (Haybittle-Peto boundary limit p<0·001). 19 (90%; 95% CI 70–99) of 21 patients in the faecal microbiota transplantation group and seven (33%, 95% CI 15–57) of 21 patients in the placebo group had resolution of CDAD at week 8 (p=0·0003). The absolute risk reduction was 57% (95% CI 33–81). Overall, 204 adverse events occurred, with one or more adverse events being reported in 20 of 21 patients in the faecal microbiota transplantation group and all 21 patients in the placebo group. Diarrhoea (n=23 in the faecal microbiota transplantation group; n=14 in the placebo group) and abdominal pain (n=14 in the faecal microbiota transplantation group; n=11 in the placebo group) were the most common adverse events. Three serious adverse events possibly related to study treatment occurred (n=1 in the faecal microbiota transplantation group; n=2 in the placebo group ), but no deaths or colectomies during the 8-week follow-up.**

**Interpretation** **In patients with first or second *C difficile* infection, first-line faecal microbiota transplantation is highly effective and superior to the standard of care vancomycin alone in achieving sustained resolution from *C difficile*.**

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## Introduction

*Clostridioides difficile* infection is a leading cause of antibiotic-associated diarrhoea globally and an urgent health threat.[1–3] Annually, 700 000 patients in the USA and Europe contract *C difficile*, with a predominance among older patients (aged >65 years) or patients with comorbidities who are exposed to antibiotics.[1,4] Symptoms range from diarrhoea to pseudomembranous colitis and death.[5] Subsequently, *C difficile* infection has a high mortality rate and recurs in up to 40% of patients.[6,7] Vancomycin is the recommended first-line treatment followed by fidaxomicin, but antibiotic resistance is increasing and effective non-antibiotic alternatives are sparse.[8–10]

Targeting the intestinal microbiota has proven an effective approach for managing *C difficile*. The use of

## Research in context

### Evidence before this study

Effective treatment strategies for antibiotics-associated *Clostridioides difficile* infection are needed. Antibiotic treatment is the recommended first-line therapy for *C difficile*, but a high proportion of patients do not achieve a response or have recurrence of infection after stopping antibiotics. Microbiota restoration with faecal microbiota transplantation has proven effective as a treatment option for patients with three or more infections with *C difficile*. We searched PubMed, Embase, and Scopus from database inception until May 13, 2021, with no language restrictions using the search terms "*Clostridioides*/*Clostridium difficile* infection" and "Faecal/Fecal microbiota transplantation/installation". We found two small proof-of concept studies with faecal microbiota transplantation versus metronidazole or vancomycin for primary *C difficile* infection, but no formal trials of faecal microbiota transplantation for first or second *C difficile* infections.

### Added value of this study

This is the first randomised, double-blind, placebo-controlled trial to compare faecal microbiota transplantation following vancomycin with vancomycin alone in patients with first or second *C difficile* infection. The trial had broad inclusion criteria to represent real-world patients with *C difficile* infection. We found that faecal microbiota transplantation to supplement standard-of-care antibiotics was highly effective and superior to standard-of-care antibiotics alone in achieving sustained resolution of *C difficile* infection at week 8. Faecal microbiota transplantation was safe, and the trial was ended early because it was deemed unethical that all patients did not receive vancomycin followed by faecal microbiota transplantation on the basis of the poor efficacy of vancomycin alone at interim analysis.

### Implications of all the available evidence

Our study corroborates the poor efficacy of the current standard of care antibiotics alone for the treatment of first or second infection with *C difficile* and proposes microbiota restoration with faecal microbiota transplantation as a necessary, effective first-line option.

faecal microbiota transplantation for microbiota restoration has substantially changed the management of patients with recurrent *C difficile* infection (ie, more than three episodes) and is recommended as a supplement to antibiotics.[9,10] Previous studies have demonstrated clinical cure rates of up to 92%, and higher survival rates, and a number needed to treat of 1·5 compared with vancomycin monotherapy.[11–14] Early use of faecal microbiota transplantation for first or second *C difficile* infection has therapeutic potential,[15,16] but no formal randomised trials to support use of the approach as a first-line therapy have been done.

In this study, we aimed to assess the efficacy and safety of faecal microbiota transplantation compared with placebo after treatment with vancomycin in patients with first or second *C difficile* infection.

## Methods

### Study design

The faecal microbiota transplantation for early *Clostridioides difficile* infection (EarlyFMT) trial was a randomised, double-blind, placebo-controlled, clinical trial done at a tertiary gastroenterology referral centre at Aarhus University Hospital (Aarhus, Denmark). The trial was conducted and monitored in accordance with the principles for good clinical practice and local regulation.[17] An independent committee monitored the trial for safety and validity throughout its conduct. The trial protocol with amendments was approved by the Central Denmark Region Ethics Committee (j.no. 1-10-72-254-20) and the Regional Data Protection Agency (j.no. 1-16-02-10-21). The approved study protocol is available online.

For the **study protocol** see www.cefta.com/EarlyFMT

### Patients

Eligible patients were adults (aged ≥18 years) with a first or second *C difficile* infection, defined as three watery stools or more (Bristol stool chart [BSC] score 6–7) per day and a positive *C difficile* PCR test (for toxins A and B, binary toxin, and ribotype 027). There were no restrictions regarding comorbidities. Exclusion criteria were pregnancy or lactation, concomitant antibiotic use other than vancomycin, any medication with vancomycin interaction, allergy to vancomycin or previous anaphylactic reactions to any food sources, continuous need for proton pump inhibitors, gastroparesis, and life-threatening fulminant *C difficile* infection.

Patients were recruited from regional referrals made by the treating physicians at all hospitals in the region. Infections with *C difficile* within the region were monitored and treating physicians were contacted by study organisers to facilitate possible recruitment. At baseline, the severity of *C difficile* infection was graded according to guideline definitions, whereby the presence of hypoalbuminaemia (serum albumin <30 g/L) in addition to leukocytosis (white blood cell count >15 billion cells per L), leukopenia (white blood cell count <2 billion cells per L), or abdominal pain indicated severe infection.[9,18,19] All patients provided written informed consent and were screened for fulminant, life-threatening *C difficile* (indicated by fever [>38·5°C], cognitive impairment, hypotension, ileus, toxic megacolon, pseudomembranous colitis, signs of multiorgan failure, or admittance to intensive care) at the time of severity grading.

### Randomisation and masking

Patients were randomly assigned (1:1) to encapsulated faecal microbiota transplantation or encapsulated placebo after standard treatment with vancomycin. Patients, investigators, and study personnel with patient contact were masked to the allocated treatment group. The allocation table was generated from a simple computerised randomisation procedure to ensure equal, unrestricted distribution in each of the two treatment groups. The list was generated by independent staff without patient access and remained concealed from all study personnel. Investigators enrolled and randomly assigned patients using an online electronic case record form that assigned each patient a unique randomisation number. The allocation sequence was concealed from all study personnel with patient contact who requested the study treatment on the basis of randomisation numbers. Independent personnel prepared and released the study treatment in packages of sequentially labelled containers according to the generated randomisation list. A thorough documentation log was kept for each study treatment. A double-provision principle similar to the strict safety standards applicable to blood products and human tissues was applied to secure correct treatment allocation and handling. All evaluations of effect and safety outcomes were done by masked study personnel. The appearance of the placebo and the encapsulated faecal microbiota transplantation capsules was indistinguishable (appendix p 1).

To assess masking, both investigators and patients were asked at study visits one and two what allocated study treatment they believed the patient had received.

### Procedures

All patients received 125 mg oral vancomycin four times daily for a minimum of 10 days after diagnosis of *C difficile* infection. Patients then received their allocated study treatment twice after stopping vancomycin; the first dose was administered at day 1 and the second dose between day 3 and 7. Patients were followed up for 8 weeks after the final study treatment or until *C difficile* recurrence. All patients were tested for *Salmonella, Campylobacter, Yersinia, Shigella,* and enteropathogenic *Escherichia coli* using PCR assays. All patients with recurrence of *C difficile* infection were offered open-label rescue faecal microbiota transplantation.

Four study visits with clinical attendance were scheduled at day 1, days 3–7, 1 week, and 8 weeks after the second study treatment. At each visit, patients provided stool samples for testing and venous blood samples were taken. Most patients were treated during outpatient care visits or during hospital stays. Older patients deemed too weak for transportation were offered treatment and follow-up at nursing home visits by an investigator.

Patients fasted for at least 6 h before administration of faecal microbiota transplantation capsules or placebo capsules. Patients were prescribed 10 mg oral metoclopramide 10 min before ingesting the capsules with low-pH beverages; either apple juice or diet cola. Following administration, all patients were observed for 30 min.

Encapsulated faecal microbiota transplantation was processed from 50 g of crude faeces from five thoroughly screened healthy blood donors, according to international guidelines.[20,21] For each patient, faecal microbiota transplantation was done using faeces obtained from the same donor. Crude stool was homogenised in sterile saline and mixed with 85% glycerol followed by a series of centrifugation steps, which yielded a highly dense concentrate that was mixed 1:0·1 with glycerol and suspended in size 0 acid-resistant enterocapsules (Capsugel Vcaps capsules; Lonza, Colmar, France) and double-coated in size 00. This yielded a median of 25 capsules (range 12–30).

Encapsulated placebo was prepared from a concentration of 49·5% glycerol, 49·5% sterile saline, and 1% food colouring. Placebo concentrate was aliquoted to capsules identical to those used for faecal microbiota transplantation. The total capsule count was produced to match the varying number of faecal microbiota transplantation capsules.

All study treatments were sealed and stored at –80°C until use. Study treatments were produced in a dedicated routine facility by independent, trained personnel at the public Blood Center at Aarhus University Hospital in accordance with international guidelines and the National Danish Tissue Act.[19,21] Safety samples from all study treatments were stored.

See Online for appendix

Patients with recurrence were offered encapsulated first-line faecal microbiota transplantation according to the protocol, but were also offered faecal microbiota transplantation by colonoscopy or nasojejunal tube insertion, if deemed necessary according to the clinical state of the patients. Colonoscopy administration was used if additional diagnostics were required. Repeat transplantations were spaced in time to be adminstered only if the patient developed recurrence. If patients had recurrence after the rescue faecal microbiota transplantation, patients started vancomycin and were scheduled for faecal microbiota transplantation with a new donor.

### Outcomes

The primary outcome was resolution of *C difficile*-associated diarrhoea (CDAD) 8 weeks after the second study treatment, defined as either a daily stool frequency of less than three for a minimum of 2 days regardless of *C difficile* faecal PCR test result, or persistent diarrhoea (three or more watery stools daily [BSC score 6–7]) and a negative *C difficile* PCR test.

Study treatment failure was *C difficile* recurrence, defined as diarrhoea (≥3 watery stools daily [BSC score 6–7] for 2 days) and a positive faecal *C difficile* PCR

test during follow up; or death or colectomy during the study period with no history of new onset *C difficile*.

Secondary outcomes were resolution of CDAD at week 1 (defined as <3 daily bowel movements and BSC score <5), negative *C difficile* test at week 1 and 8, and death and colectomy rates at week 8, and health-related quality of life at week 8. Since patients with *C difficile* recurrence as a result of treatment failure were offered rescue faecal microbiota transplantation for ethical reasons, the primary trial follow-up for the secondary outcomes, death, and colectomy, were censored at the time of *C difficile* recurrence. The prespecified secondary outcome for the quality of life was not assessed because the study was stopped prematurely.

Adverse events and serious adverse events were graded according to the Common Terminology Criteria for Adverse Events (version 5.0).[22] We included all safety data recorded during the study follow-up. Serious adverse reactions and suspected unexpected serious adverse reactions were reported to the competent authorities. Adverse events were grouped into immediate complications during administration, events occurring within the first 24 h, and events occurring more than 24 h after administration.

### Statistical analysis

On the basis of an estimated drop-out rate of 10% during the 8-week follow-up period, we calculated that a sample size of 84 patients would provide 80% power to detect a 26% difference between an expected 92% effect in the faecal microbiota transplantation group and a 66% effect in the placebo, at a two-sided α level of 0·05.[6,12] We prespecified an interim analysis after 42 patients had been randomly assigned and had completed follow-up, with stopping rules according to the Haybittle-Peto limit of statistical significance of p<0·001 for the treatment effect on resolution of *C difficile*-associated diarrhoea at week 8.[23] Patient characteristics were compared using $\chi^2$ test or Fisher's exact tests for categorical variables and Wilcoxon or Student's *t* test for continuous variables, as appropriate. Primary outcome analyses were done according to the intention-to-treat principle, which included all patients who were randomly assigned, and tested for significance with Fisher's exact test. Secondary outcomes were analysed in the intention-to-treat population. Safety was assessed in all randomly assigned patients. Patients who were randomly assigned to a study group and did not receive any of the two study allocated treatments were excluded from the study as dropouts. Estimates were

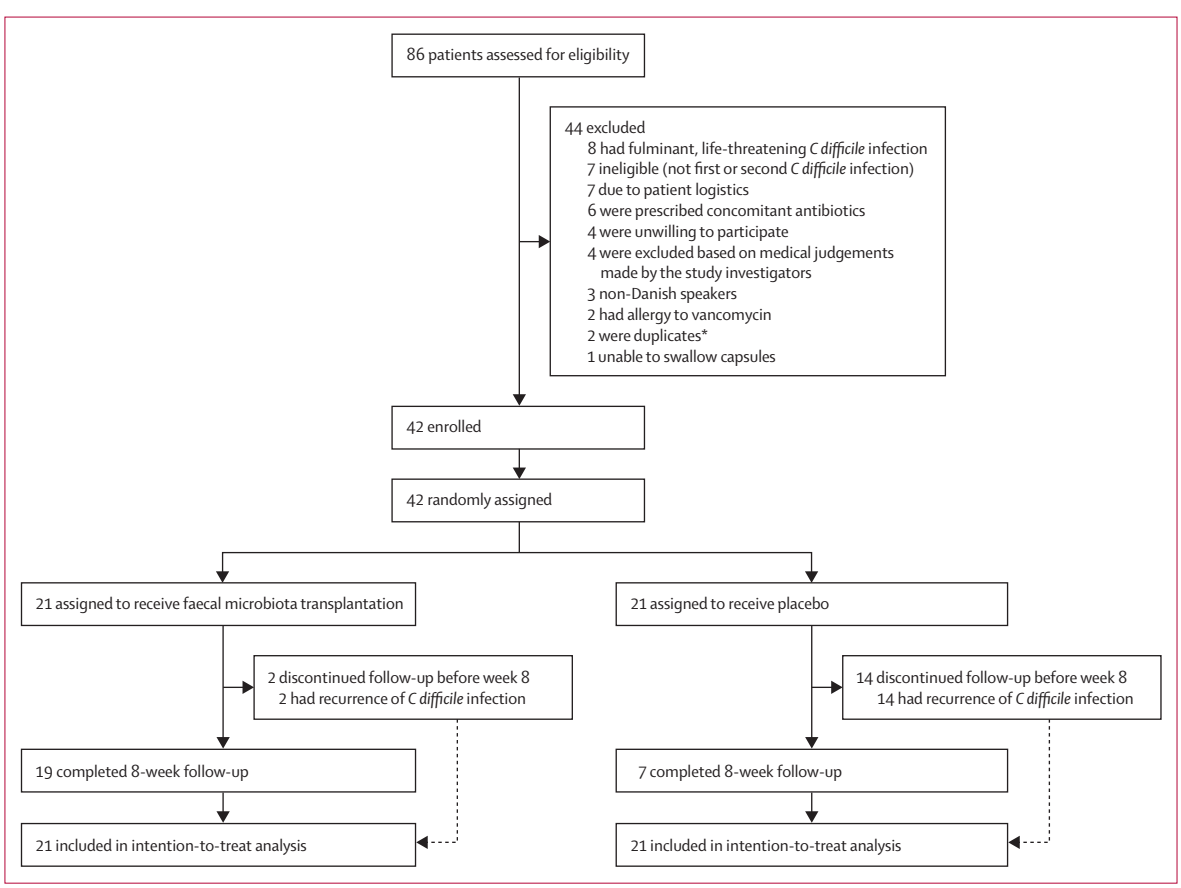


***Figure:*** **Trial profile**
*C difficile=Clostridioides difficile*. *Two patients were referred twice (ie, for first and second *C difficile* infection).

provided with 95% CI and medians with IQR or ranges, as applicable. Adverse events were stratified according to treatment group and summarised descriptively. Masking was evaluated by the James Blinding Index and the Bang Blinding Index.[24,25]

All analyses were done in R (version 3.6.1). The data were monitored by an independent external data monitoring committee. The trial is registered with ClinicalTrials.gov, NCT04885946.

### Role of the funding source

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

## Results

Between June 21, 2021, and April 1, 2022, we consecutively screened 86 patients (figure), of whom 42 were randomly assigned to the faecal microbiota transplantation group (n=21) or the placebo group (n=21). All 42 randomly assigned patients adhered to the study protocol and were included in the intention-to-treat analysis population. No patients dropped out of the study. The median age of patients was 59 years (range 25–89; IQR 40–73), 32 (76%) of 42 had severe *C difficile*, and 22 (52%) had been admitted to hospital during their course of infection. All patients received 125 mg oral vancomycin four times daily for at least 10 days, with a median treatment duration of 12 days (IQR 10–13). Among the 42 randomly assigned patients, 25 patients had a first *C difficile* infection and 17 patients had a second *C difficile* infection (table 1). Among patients with second *C difficile* infection, the median time from primary *C difficile* infection was 41 days (IQR 25–82).

The trial was stopped prematurely at the prespecified interim analysis due to a significantly lower rate of resolution of *C difficile* infection in the placebo group than the faecal microbiota transplantation group, thus continued randomisation was deemed unethical (Haybittle-Peto boundary limit less than p<0·001).

Resolution of CDAD at week 8 was achieved in 19 (90%; 95% CI 70–99) of 21 patients in the faecal microbiota transplantation group and seven (33%; 15–57) of 21 patients in the placebo group (p=0·00031). The absolute risk reduction for recurrence following faecal microbiota transplantation compared with placebo was 57% (95% CI 33–81). A graphical summary of the primary outcome is included in the appendix (p 2).

Stratified by *C difficile* episode, 13 (93%) of 14 patients with first *C difficile* infection in the faecal microbiota transplantation group and four (36%) of 11 patients with first infection in the placebo group had resolution of CDAD at week 8. Among patients with second *C difficile* infection, six (86%) of seven patients in the faecal microbiota transplantation group and three (30%) of ten patients in the placebo group had CDAD resolution at week 8.

Before random assignment, 24 (57%; 95% CI 41–72) of 42 patients had clinical resolution onset during vancomycin therapy alone, ten (48%; 26–70%) of 21 patients in the faecal microbiota transplantation group and 14 (67%; 43–85) of 21 patients in the placebo group (p=0·30; table 2). In patients with persistent diarrhoea after stopping vancomycin, clinical diarrhoea resolution occurred in nine (82%) of 11 patients in the faecal microbiota transplantation group within a median of 2 days (IQR 1–5) after the allocated study treatment and six (86%) of seven in the placebo group within a median of 1·5 days (1–2) after allocated study treatment. Overall,

| | Faecal microbiota transplantation (n=21) | Placebo (n=21) |
|---|---|---|
| **Patient characteristics** | | |
| Sex | | |
| Male | 6 (29%) | 5 (24%) |
| Female | 15 (71%) | 16 (76%) |
| Age, years | 58 (50–68) | 60 (37–73) |
| BMI | 25 (23–28) | 26 (23–33) |
| Charlson Comorbidity Index score | 2 (0–4) | 2 (0–3) |
| Immunosuppressed | 8 (38%) | 5 (24%) |
| Inflammatory bowel disease | | |
| Active disease | 3 (14%) | 4 (19%) |
| In remission | 2 (10%) | 4 (19%) |
| Proton pump inhibitor treatment | 6 (29%) | 7 (33%) |
| **Blood chemistry** | | |
| Albumin, g/L | 39 (4) | 37 (5) |
| C-reactive protein median, mg/L | 4 (4–8) | 4 (4–12) |
| Leucocyte count, cells per $10^9$/L | 8·0 (3·5) | 9·2 (3·6) |
| Creatinine, µmol/L | 74 (34) | 75 (39) |
| Haemoglobin, mmol/L | 8 (8–9) | 8 (8–10)* |
| ***C difficile* characteristics** | | |
| First infection | 14 (67%) | 11 (52%) |
| Second infection | 7 (33%) | 10 (48%) |
| *C difficile* severity | | |
| Mild to moderate | 5 (24%) | 5 (24%) |
| Severe | 16 (76%) | 16 (76%) |
| Hospital admission during infection | 13 (62%) | 9 (43%) |
| *C difficile* profile | | |
| Toxin A | 17 (81%) | 19 (90%) |
| Toxin B | 21 (100%) | 21 (100%) |
| Binary toxin | 6 (29%) | 1 (5%) |
| Ribotype 027 | 0 | 0 |
| *C difficile* case definition | | |
| Health-care facility-onset | 8 (38%) | 7 (33%) |
| Community-onset, health-care facility-associated | 1 (5%) | 0 |
| Community-onset | 12 (57%) | 14 (67%) |

Data are n (%), median (IQR), or mean (SD). *C difficile=Clostridioides difficile* infection. *Data missing for one patient.

***Table 1:*** **Baseline characteristics of the intention-to-treat population (n=42)**

16 patients had *C difficile* recurrence (14 patients in the placebo group and two patients faecal microbiota transplantation group) that occurred within a median of 13 days (10–17). 13 patients in the placebo group with recurrence had initial relief of diarrhoea, followed by recurrence. Three patients (two in the faecal microbiota transplantation group and one in the placebo group), had *C difficile* recurrence and persistent diarrhoea since their *C difficile* diagnosis.

At week 1, 19 (90%; 95% CI 70–99) of 21 patients in the faecal microbiota transplantation group and 16 (76%; 53–92) of 21 patients in the placebo group had CDAD resolution (p=0·40). All patients without stool *C difficile* PCR tests had a clinical effect. Among all patients who had a *C difficile* PCR test at week 1, 14 (78%; 52–94) of 18 patients in the faecal microbiota transplantation group and six (32%; 13–57) of 19 patients in the placebo group had negative *C difficile* PCR tests (p=0·008). Among all patients who had a *C difficile* PCR test at week 8, 17 (85%; 62–97) of 20 patients in the faecal microbiota transplantation group and four (19%; 4–42) of 21 patients in the placebo group had negative *C difficile* PCR tests (p<0·0001). No deaths or colectomies occurred during follow-up.

A total of 204 adverse events were recorded during follow-up (table 3; appendix pp 3–4). One or more adverse events were reported in 20 of 21 patients in the faecal microbiota transplantation group and in all 21 patients in the placebo group (p=1·00). Most commonly reported adverse events were transient diarrhoea (n=23 in the faecal microbiota transplantation group; n=14 in the placebo group), abdominal pain (n=14 in the faecal microbiota transplantation group; n=11 in the placebo group), and nausea (n=12 in the faecal microbiota transplantation group; n=5 in the placebo group; table 3).

During the 8-week follow-up, three serious adverse events occurred that were possibly related to study treatment, one in the faecal microbiota transplantation group and two in the placebo group. In the faecal microbiota transplantation group, one patient was briefly admitted 9 days after the last study treatment with severe, acute abdominal pain and vomiting consistent with constipation. The symptoms ceased within 24 h. In the placebo group, a patient who had been admitted to hospital before trial enrolment developed bacteremia and confusion after the second study treatment. The symptoms resolved following systemic antibiotics. A second patient in the placebo group was admitted to hospital for pneumonia 4 days after the last study treatment.

After stopping the primary study follow-up at the time of *C difficile* recurrence, rescue faecal microbiota transplantation was performed in two patients in the faecal microbiota transplantation group and 13 patients in the placebo group who had *C difficile* recurrence. One patient in the placebo group died before receiving rescue faecal microbiota transplantation and of the 15 patients who received rescue faecal microbiota transplantation due to recurrence, two of 15 patients (from the original placebo group) died during the 8-week follow-up. Both patients who died following the rescue faecal microbiota transplantation had clinical response at week 1 and died due to worsening of pre-existing comorbidities unrelated to *C difficile* or faecal microbiota transplantation. In total, 11 (85%; 95% CI 55–98) of 13 patients with *C difficile* recurrence after study treatment had resolution of CDAD at week 8 after rescue faecal microbiota transplantation. Three patients required repeated rescue faecal microbiota transplantations to achieve a sustained clinical response: one patient required two, one patient required three, and one patient required four.

The masking of patients and investigators was assessed at administration on the two treatment days. As evaluated by the blinding indexes, masking was successfully maintained (appendix p 5). The Bang Blinding Index indicated that, regardless of randomisation group, both patients and investigators had a high degree of wishful thinking, favouring beliefs that the treatment allocation was faecal microbiota transplantation.

| | Faecal microbiota transplantation (n=21) | Placebo (n=21) | p value |
|---|---|---|---|
| **Primary outcome** | | | |
| CDAD resolution at week 8 | 19 (90%; 70–99) | 7 (33%; 15–57) | 0·00031 |
| Clinical effect week 8 | 19 (90%; 70–99) | 7 (33%; 15–57) | 0·00031 |
| Negative *C difficile* PCR test week 8 | 17/20* (85%; 62–97) | 4 (19%; 5–42) | 0·00003 |
| **Secondary outcomes** | | | |
| CDAD resolution at week 1 | 19 (90%; 70–99) | 16 (76%; 53–92) | 0·40 |
| Clinical effect at week 1 | 18 (86%; 64–97) | 15 (71%; 48–89) | 0·50 |
| Negative *C difficile* PCR test at week 1 | 14/18† (78%; 52–94) | 6/19‡ (32%; 13–57) | 0·008 |
| Colectomy | 0 | 0 | NA |
| Death | 0 | 0 | NA |
| Clinical response to study treatment | | | |
| Patients with clinical resolution of diarrhoea after vancomycin therapy alone | 10 (48%; 26–70) | 14 (67%; 43–85) | 0·53 |
| Patients unresponsive to vancomycin with clinical resolution of diarrhoea after study treatment | 9/11 (82%; 48–98) | 6/7 (86%; 42–99) | 1·00 |
| Patients without clinical response to vancomycin nor study treatment | 2 (10%; 1–30) | 1 (5%; 0–24) | 1·00 |

Data are n (%; 95% CI) or n/N (%; 95% CI). CDAD=*Clostridioides difficile*-associated diarrhoea. *C difficile*=*Clostridioides difficile*. NA=not available. *Data missing for one patient. †Data missing for three patients with clinical effect. ‡Data missing for two patients with clinical effect.

***Table 2:*** **Primary and secondary outcomes in the intention-to-treat population**

## Discussion

In this randomised, double-blind, placebo-controlled clinical trial, faecal microbiota transplantation after vancomycin was superior to vancomycin alone for patients with first or second *C difficile* infection. The trial was stopped prematurely at the prespecified interim analysis, according to established stopping rules, due to a significantly lower rate of resolution in the placebo group

than the faecal microbiota transplantation group at interim analysis (Haybittle-Peto boundary limit p<0·001), thus the trial was stopped for ethical reasons. The effect of faecal microbiota transplantation for microbiota restoration demonstrates its potential to effectively manage early *C difficile* and suggests significant clinical benefits of faecal microbiota transplantation as a first-line treatment.

The broad inclusion criteria applied might explain the lower-than-expected effect rate in the placebo group. We designed the trial to reflect real-world patients with *C difficile*, and our inclusion criteria differ from those used in previous trials from which we made our power calculation.[6,12] Importantly, we included frail patients in nursing homes, patients with multimorbidities, immunocompromised patients, and patients with inflammatory bowel disease. Although this difference might explain the low efficacy of vancomycin alone, faecal microbiota transplantation maintained its anticipated high efficacy.

This is the first formal randomised trial of faecal microbiota transplantation for first or second *C difficile* infection, and the high efficacy of faecal microbiota transplantation was consistent with that observed in patients with recurrent *C difficile*.[11–14] In this trial, patients were treated with two sequential faecal microbiota transplantation procedures on separate days. This practice might have overtreated some patients and differs from previous trials. It remains unknown whether optimal effect is achieved by one or two treatments. Most patients in both groups had clinical effect onset before or within days following the first study treatment. In the placebo group, the effect was transient and the patients' wishful thinking might have influenced relief of symptoms at week 1, since a high proportion of patients had positive *C difficile* PCR tests at this timepoint and subsequently developed recurrence. Most of these patients had sustained resolution following rescue faecal microbiota transplantation.

The low efficacy in the placebo group illustrates the challenges in managing *C difficile* with antibiotics only.[9,10] Whereas antibiotics might effectively suppress *C difficile* during treatment, use of antibiotics alone to treat an antibiotic-related infection originating from intestinal microbiota disruption poses an ecological paradox. Patients in this trial were treated in a double-blind setting according to best care standards with concomitant optimisation of comorbid conditions. The gap in treatment effect underpins the need for non-antibiotic treatment alternatives, and microbiota restoration with faecal microbiota transplantation represents such an approach.

Faecal microbiota transplantation is currently recommended by the European Society of Clinical Microbiology and Infectious Diseases, the Infectious Diseases Society of America, and Society for Healthcare Epidemiology of America after three or more *C difficile* episodes refractory to standard recommended antibiotics, vancomycin or fidaxomicin as an alternative option.[9,10] The results of this trial highlight how the use

| | Faecal microbiota transplantation (n=21) | Placebo (n=21) |
|---|---|---|
| Any adverse event | 20 (95%) | 21 (100%) |
| Total adverse events | 112 | 92 |
| Adverse events possibly related to faecal microbiota transplantation or placebo | 85 | 68 |
| Serious adverse events not related to faecal microbiota transplantation or placebo | 2 | 2 |
| Serious adverse events possibly related to faecal microbiota transplantation or placebo | 1 | 2 |
| Serious unsuspected adverse events possibly related to faecal microbiota transplantation or placebo | 0 | 0 |
| Adverse event leading to withdrawal or unmasking | 0 | 0 |
| Final outcome of adverse events during trial follow-up | | |
| Adverse event leading to death | 0 | 0 |
| Resolved or improved | 84/112 (75%) | 70/92 (76%) |
| Resolved with sequelae | 0 | 0 |
| Not resolved during follow-up or unknown course | 28/112 (25%) | 22/92 (24%) |
| Adverse events reported ≥4 times | | |
| Diarrhoea | 23 | 14 |
| Abdominal pain | 14 | 11 |
| Nausea | 12 | 5 |
| Bloating | 6 | 7 |
| Malaise | 6 | 4 |
| Bowel sounds | 6 | 2 |
| Urgency to defecate | 4 | 3 |
| Fatigue | 1 | 5 |
| Fever | 4 | 2 |
| Vomiting | 4 | 1 |
| Flank pain | 2 | 3 |
| Hot flushes | 4 | 0 |
| Constipation | 2 | 2 |
| Organ-specific and timing of adverse events | | |
| Gastrointestinal adverse events* | 80 | 57 |
| During the administration | 7 | 0 |
| During follow-up within 24 h | 40 | 26 |
| During follow-up >24 h | 33 | 31 |
| Systemic adverse events† | 11 | 10 |
| During follow-up within 24 h | 5 | 3 |
| During follow-up >24 h | 6 | 7 |

*Gastrointestinal adverse events included diarrhoea, abdominal pain, nausea, bloating, malaise, bowel sounds, vomiting, constipation, bloody stool, increased defecation frequency, mucous stools, liquid stool, faecal incontinence, incomplete evacuation of stool, gastro-oesophageal reflux, and bacterial overgrowth.
†Systemic adverse events include fatigue, fever, hot flushes, headache, confusion, and insomnia.

***Table 3:*** **Adverse events in the intention-to-treat population**

of faecal microbiota transplantation as a first-line treatment can effectively prevent *C difficile* recurrence and suggests that microbiota restoration might be necessary to obtain sustained resolution.

The early use of faecal microbiota transplantation for all patients with *C difficile* might raise safety concerns because unidentified donor faeces constituents are transferred during the faecal microbiota transplantation. Accordingly, the risk of adverse reactions needs to be outweighed by the benefit of resolution of *C difficile* infection. In the present trial, adverse events were frequent and equally distributed in the two groups, illustrating the severe disease burden of patients with *C difficile*. No deaths nor colectomies occurred during primary follow-up. Immediate and self-limiting gastrointestinal symptoms were the most frequently reported and are comparable with the experiences of adverse events reported after faecal microbiota transplantation for recurrent *C difficile*.[12] Few serious adverse events were potentially related to the experimental procedures, albeit without clear causality. The trial was not designed for long-term follow-up, but long-term experiences with faecal microbiota transplantation for recurrent *C difficile* show low procedure-related risks if strict safety standards similar to those used for blood products and human tissues are applied.[26,27] In the present trial, the most common severe medical complication was *C difficile* recurrence, primarily occurring in the patients randomly assigned to the placebo group, highlighting the favourable risk to benefit ratio of faecal microbiota transplantation.

The exact mechanism of action for faecal microbiota transplantation is unknown but is thought to relate to restoration of the recipient's gut microbiota. Several attempts have been made to standardise faecal microbiota transplantation for restoration of the intestinal microbiome, including predefined bacterial consortia and refined donor-derived spores from donor feces.[28,29] Current microbiota-based treatments still involve an element of donor dependency, with faecal microbiota transplantation representing the first step in demonstrating the potential for microbiota-based therapeutics. Providing faecal microbiota transplantation or donor faeces derivatives at the scale needed to meet the full demand for all patients with *C difficile* requires considerable infrastructures. Access to, and clinical knowledge of, faecal microbiota transplantation might also limit its use. At present, only 10% of patients with multiple, recurrent *C difficile* infection and indication for faecal microbiota transplantation receive it.[30] International initiatives address the unmet need, but logistic and regulatory obstacles remain unsolved.[20]

Our trial has important limitations. This was a single-centre study with regional uptake, and despite having high statistical power for the clinical effect, the premature termination and low patient number prevent inference of mortality, time to effect, and cost. Because future placebo-controlled trials or the omission to offer faecal microbiota transplantation might be considered unethical, early faecal microbiota transplantation use should be tested observationally in real-life cohorts to confirm its effect and generalisability. Our study examined the additive effect of faecal microbiota transplantation after vancomycin. The results should therefore not be interpreted as evidence for faecal microbiota transplantation alone but for its use in combination with antibiotics. We treated patients according to existing guidelines and did not use fidaxomicin pre-treatment or other refined treatment strategies. Initial treatment with vancomycin might prevent clinical deterioration or recurrence before faecal microbiota transplantation.

In conclusion, faecal microbiota transplantation after vancomycin is superior to standard-of-care vancomycin alone in achieving sustained cure of first or second *C difficile* infection. The results provide evidence for first-line faecal microbiota transplantation after vancomycin as an effective treatment for early *C difficile* infection.

For the **study protocol and statistical analysis plan** see www.cefta.com/EarlyFMT

#### Contributors

All authors met the International Committee of Medical Journal Editors criteria for authorship, take integrity for the work, were involved in the critical review and drafting of the manuscript, and approved the final version. SMDB, JFD, CLH, MMH, and SEA designed and conceived the study. SMDB wrote the first draft with inputs from JFD and CLH. SMDB and JFD did the statistical analysis. SMDB, JFD, and CLH have directly accessed and verified the underlying data reported in the manuscript. SMDB, SEA, JK, KLH, SS, TMMR, EMSD, JFD, and CLH recruited and treated patients. SMDB, MMH, NR, LLE, SM, CE, JFD, and CLH contributed important developments necessary for the trial conduct and validity. All authors contributed important intellectual content and data interpretation had access to the study data and carried final responsibility for the decision to submit for publication.

#### Declarations of interests

We declare no competing interests.

#### Data sharing

Data will be made available upon request in an anonymised form compliant with European data legislation, the General Data Protection Regulation. To gain access to pseudonymised participant data, a formal data access agreement is required and, if necessary, a formal ethics committee approval. Data dictionary forms will be made available following publication. The study protocol and statistical analysis plan are available online. All proposals should be directed to simjor@rm.dk.

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