Original Investigation

Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent *Clostridium difficile* Infection
A Randomized Clinical Trial

Christine H. Lee, MD; Theodore Steiner, MD; Elaine O. Petrof, MD; Marek Smieja, MD, PhD; Diane Roscoe, MD; Anouf Nematallah, MD; J. Scott Weese, DVM; Stephen Collins, MBBS; Paul Moayyedi, MB; Mark Crowther, MD; Mark J. Ropeleski, MD; Padman Jayaratne, PhD; David Higgins, MB; Yingfu Li, PhD; Neil V. Rau, MD; Peter T. Kim, PhD

IMPORTANCE *Clostridium difficile* infection (CDI) is a major burden in health care and community settings. CDI recurrence is of particular concern because of limited treatment options and associated clinical and infection control issues. Fecal microbiota transplantation (FMT) is a promising, but not readily available, intervention.

OBJECTIVE To determine whether frozen-and-thawed (frozen, experimental) FMT is noninferior to fresh (standard) FMT in terms of clinical efficacy among patients with recurrent or refractory CDI and to assess the safety of both types of FMT.

DESIGN, SETTING, AND PARTICIPANTS Randomized, double-blind, noninferiority trial enrolling 232 adults with recurrent or refractory CDI, conducted between July 2012 and September 2014 at 6 academic medical centers in Canada.

INTERVENTIONS Patients were randomly allocated to receive frozen (n = 114) or fresh (n = 118) FMT via enema.

MAIN OUTCOMES AND MEASURES The primary outcome measures were clinical resolution of diarrhea without relapse at 13 weeks and adverse events. Noninferiority margin was set at 15%.

RESULTS A total of 219 patients (n = 108 in the frozen FMT group and n = 111 in the fresh FMT group) were included in the modified intention-to-treat (mITT) population and 178 (frozen FMT: n = 91, fresh FMT: n = 87) in the per-protocol population. In the per-protocol population, the proportion of patients with clinical resolution was 83.5% for the frozen FMT group and 85.1% for the fresh FMT group (difference, −1.6% [95% CI, −10.5% to ∞]; $P = .01$ for noninferiority). In the mITT population the clinical resolution was 75.0% for the frozen FMT group and 70.3% for the fresh FMT group (difference, 4.7% [95% CI, −5.2% to ∞]; $P < .001$ for noninferiority). There were no differences in the proportion of adverse or serious adverse events between the treatment groups.

CONCLUSIONS AND RELEVANCE Among adults with recurrent or refractory CDI, the use of frozen compared with fresh FMT did not result in worse proportion of clinical resolution of diarrhea. Given the potential advantages of providing frozen FMT, its use is a reasonable option in this setting.

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Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Christine H. Lee, MD, St Joseph's Healthcare–Hamilton, 50 Charlton Ave E, 424 Luke Wing, Hamilton, ON, Canada L8N 4A6 (clee@mcmaster.ca).

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Clostridium difficile infection (CDI), in health care settings and in the community, has become a major clinical and economic concern.[1-3] Increases in failure rates with conventional treatment, and recurrences following initial cure, present significant challenges to health care systems: more than 60% of patients experience further episodes after a first recurrence.[3] Treatment options for recurrent CDI are limited, because metronidazole and vancomycin exhibit suboptimal efficacy in this setting. A number of potential approaches have been studied, which include tapered or pulsed regimens of oral vancomycin[4] and administration of spores of nontoxigenic *C difficile*.[5] In addition, adjunctive vaccine or monoclonal antibodies against *C difficile* toxins are currently in development.[6,7]

Molecular typing studies have demonstrated that 10% to 50% of recurrent CDI cases may be attributable to reinfections rather than recurrence of the initial infection, suggesting that perturbed microbiota may play a role in facilitating reinfection.[8,9] Restoration of protective colonic microbiota by fecal microbiota transplantation (FMT) has shown promising results: there is evidence that FMT is an effective treatment for recurrent CDI.[10,11] High cure rates have been achieved with FMT given by enema, an administration method that is much more convenient than alternative reported methods, such as nasogastric tube or colonoscopy.[12] However, the usefulness of this approach may be limited by logistic difficulties in preparing fresh material. By contrast, the use of frozen-and-thawed (frozen) FMT offers a number of advantages: less cost with reduction in number and frequency of donor screenings; immediate availability of FMT; and the possibility of delivering FMT at centers that do not have on-site laboratory facilities. Previous studies have supported the use of frozen FMT for management of recurrent CDI but have not directly compared frozen with fresh FMT.[13-15] Hence, we performed a double-blind randomized clinical trial to determine whether frozen FMT is noninferior to fresh FMT for patients with recurrent or refractory CDI.

Methods

Study Population

Patients 18 years or older with a history of recurrent or refractory CDI were enrolled in the study. CDI was defined by a positive result for *C difficile* toxins by enzyme immunoassay or by polymerase chain reaction targeting the *C difficile* toxin B gene (*tcdB*) and 3 or more unformed stools within 24 hours, for a minimum of 48 hours. Sixty-nine isolates were typed for presence of binary toxin, as previously described.[16] Recurrent CDI was defined as recurrence of CDI symptoms for 48 hours or longer within 8 weeks after the completion of at least 10 days of CDI treatment. Refractory CDI was defined as persistent or worsening of diarrhea characteristic of CDI and 1 of the following: ongoing abdominal pain, fever (temperature >38.0°C), or peripheral white blood cell (WBC) counts greater than 15.0×10^9/L despite treatment with oral vancomycin at a dose of 500 mg 4 times daily for at least 5 days. Patients with only a single recurrence of CDI were not included unless the most recent episode became refractory to treatment.

Key exclusion criteria included neutropenia ($<0.5 \times 10^9$/L), peripheral WBC counts greater than 30.0×10^9/L, or toxic megacolon (defined as radiographic evidence, combined with fever >38°C, systolic blood pressure <90 mm Hg, peripheral WBC count $>15.0 \times 10^9$/L, or heart rate >120/min). Patients who remained symptom-free for 3 or more weeks after the completion of the CDI treatment were considered cured and were not enrolled in the study.

Donor volunteers were prospectively screened and rescreened every 6 months according to recommendations by Bakken et al[17]; the majority of stools were obtained from 3 donors. Health Canada and the institutional research ethics boards at each participating center approved the study protocol (study protocol and study procedures are available in Supplement 1), and all patients provided written informed consent. A data and safety monitoring board monitored the trial.

Study Design and Treatment

This was a randomized, double-blind, noninferiority clinical trial, conducted between July 2012 and September 2014 at 6 academic medical centers in Canada. Patients were assigned (1:1) to frozen or fresh FMT delivered by enema according to a computer-generated random number; randomization was performed in blocks (eAppendix in Supplement 2), with stratification according to major risk factors for recurrent CDI[9,18,19]: age (≥65 vs <65 years), setting (community- vs health care–associated CDI), and the number of recurrent CDI episodes (≥2 vs <2).

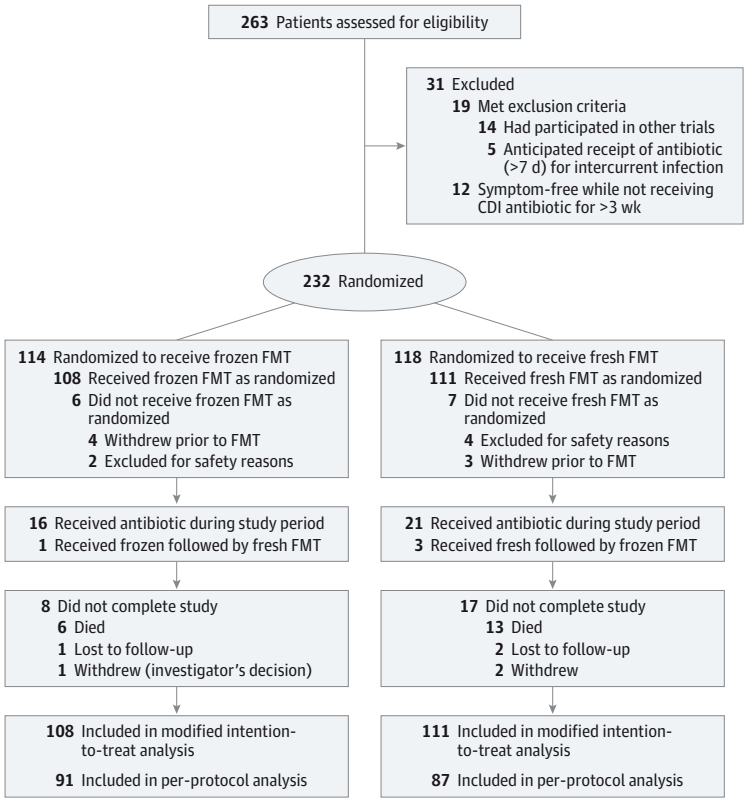
An unblinded laboratory technician prepared the first 2 enemas according to the assigned allocation. In situations in which a related donor's sample was used, the eligible relative donor collected the stool and submitted the stool at least 48 hours prior to the FMT to allow freezing and also in the morning of the scheduled FMT to the laboratory to ensure that the participant received the allocated FMT and the designated donor's stool. This also ensured the maintenance of blinding of the patient and the investigator.

All patients received suppressive antibiotics for their most recent episode of CDI, which were discontinued 24 to 48 hours prior to FMT. On day 1, patients received 50 mL of frozen or fresh FMT by enema. Patients who showed no improvement of CDI symptoms by day 4 received an additional FMT with the same donor and allocation as the original FMT between days 5 and 8. Patients not responding to 2 FMTs were offered repeat FMT or antibiotic therapy. No bowel preparation was performed prior to FMT because lavage is uncomfortable for the patient, and there was no published evidence that it improved the outcome of FMT.

FMT Preparation

Fresh stool samples from healthy donors were transported to the processing laboratories within 5 hours of collection and stored at 5°C until frozen or used for FMT. Approximately 100 g of stool sample was diluted with 300 mL of

Figure. Flow of Participants in Double-Blind Randomized Clinical Trial of Frozen or Fresh Fecal Microbiota Transplantation



CDI indicates *Clostridium difficile* infection; FMT, fecal microbiota transplantation.

commercially bottled water and emulsified using a sterile wooden spatula. Gauze was placed on top of an empty container to strain the solids, and the suspension in the container was aspirated into 60-mL syringes, which were also used to administer the enemas. Patients randomized to receive fresh FMT received the suspension within 24 hours of collection; those randomized to receive the frozen FMT received the suspension within 24 hours of thawing. Frozen suspensions were kept at −20°C for a maximum of 30 days and thawed overnight at 25°C; anaerobic bacteria counts have been found to remain stable for at least 30 days when stored at −20°C.[20]

Study End Points

The primary end points were no recurrence of CDI-related diarrhea at 13 weeks after receiving up to 2 FMTs without the need for antibiotics specifically for recurrence and safety. Safety was assessed by monitoring of adverse events at the time of FMT and up to 13 weeks after the last FMT. Serious adverse events (SAEs) were defined as death, a life-threatening event, new hospitalization or prolongation of current hospitalization, or development of a new significant incapacity to conduct regular daily activities.

Secondary end points included treatment failure rates in each group and assessment of the functional health and well-being of patients prior to and up to 1 year after FMT as measured by standardized questionnaire (RAND 36-Item Health Survey).[21] Treatment failure was defined as persistence of diarrhea and a positive *C difficile* toxin assay or *tcdB* polymerase chain reaction assay within 5 days of the last FMT or the need for additional therapy for CDI; colectomy; or death directly attributable to CDI at 13 weeks after the last FMT.

Statistical Analysis

Statistical analyses were performed in both the per-protocol and modified intention-to-treat (mITT) populations. The per-protocol population comprised patients who received up to 2 same-modality FMTs, did not require antibiotic for CDI between the first 2 FMTs, and did not receive systemic antibiotic for intercurrent infection during the study period. The mITT population comprised all randomized patients who received at least 1 FMT but required antibiotic for CDI between the FMTs or who received a type of FMT different from the first FMT, who were lost to follow-up, or who required systemic antibiotic therapy for other infections.

The study was designed as a noninferiority trial, with 5% level of significance and 80% power. Both the per-protocol and the mITT analyses were conducted according to the methods of Kaji and Lewis.[22] Noninferiority was confirmed if the lower limit of the 1-sided 95% CI for the between-group difference in the primary end point was not lower than −15%. Based on the literature review and previ-

Table 1. Demographics and Baseline Clinical Data (Qualifying CDI Episode) of Patients in the Modified Intention-to-Treat and Per-Protocol Populations

Clinical Characteristics	No./Total (%)			
	mITT Population		Per-Protocol Population	
	Frozen FMT (n = 108)	Fresh FMT (n = 111)	Frozen FMT (n = 91)	Fresh FMT (n = 87)
Age, mean (SD), y	73.0 (16.4)	72.5 (16.2)	72.2 (15.9)	72.9 (15.4)
<65	27 (25.0)	27 (24.3)	24 (26.4)	21 (24.1)
≥65	81 (75.0)	84 (75.7)	67 (73.6)	66 (75.9)
Women	72/108 (66.7)	74/111 (66.7)	58/91 (63.7)	54/87 (62.1)
Inpatient at time of FMT	51/107 (47.7)	60/111 (54.1)	41/90 (45.6)	46/87 (52.9)
Severity of CDI at baseline[a,b]				
Mild	41/108 (38.0)	33/111 (29.7)	37/91 (40.7)	31/87 (35.6)
Moderate	49/108 (45.4)	51/111 (46.0)	41/91 (45.1)	35/87 (40.2)
Severe	18/108 (16.7)	27/111 (24.3)	13/91 (14.3)	21/87 (24.1)
Presence of abdominal pain	63/108 (58.3)	69/109 (63.3)	52/90 (57.8)	52/85 (61.2)
Fever (temperature >38.3°C)	35/108 (32.4)	36/111 (32.4)	27/91 (29.7)	28/87 (32.2)
CDI				
Health care–associated	51/107 (47.7)	60/111 (54.1)	41/90 (45.6)	46/87 (52.9)
Community-associated	56/107 (52.3)	51/111 (45.9)	49/90 (54.4)	41/87 (47.1)
Refractory	6/108 (5.6)	9/111 (8.1)	4/91 (4.4)	6/87 (7.9)
Patients with recurrent	102/108 (94.4)	102/111 (92)	87/91 (95.6)	81/87 (93.1)
No. of CDI recurrences per patient, mean (SD)	2.7 (1.7)	2.5 (1.5)	2.8 (1.7)	2.5 (1.4)
<2	100 (92.6)	94 (84.7)	84 (92.3)	73 (83.9)
≥2	8 (7.4)	17 (15.3)	7 (7.7)	14 (16.1)
Duration of CDI, median (range), d				
From initial diagnosis to first FMT	91 (18-842)	82 (6-1351)	103.5 (18-842)	84.5 (14-870)
Antibiotic use prior to first FMT	58 (13-645)	43.5 (6-811)	60 (13-645)	45 (11-811)
Total white blood cell count, median (range), $\times 10^9$/L[c]	10.15 (4.0-45.0)	11.70 (4.1-78.1)	9.70 (4.0-45.0)	11.30 (4.1-78.1)
Neutrophil count, median (range), $\times 10^9$/L[c]	6.4 (2.2-36.7)	8.0 (1.5-45.7)	6.2 (2.2-23.6)	7.6 (1.5-44.7)
Serum creatinine, median (range), mg/dL	0.87 (0.35-5.81)	0.83 (0.32-8.08)	0.84 (0.35-5.81)	0.85 (0.32-8.08)
Albumin, mean (SD), g/L	30.5 (8.2)	30.7 (7.2)	31.6 (7.7)	31.1 (6.6)
Strain type BI/027	15/35 (42.9)	14/34 (41.2)	12/29 (41.4)	11/28 (39.3)
Proton pump inhibitor use	54/87 (62.1)	61/88 (69.3)	47/83 (56.6)	49/78 (62.8)
Positive *tcdB* assay at time of initial FMT	43/105 (40.1)	44/106 (41.5)	36/88 (40.9)	34/83 (41.0)
Combination of metronidazole and vancomycin, pre-FMT	37/108 (34.3)	35/107 (32.7)	27/91 (30.0)	25/83 (30.1)
Treated with ≥1 vancomycin taper regimen, pre-FMT	100/106 (94.3)	97/109 (90.0)	83/89 (93.3)	75/85 (88.2)

Abbreviations: CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; mITT, modified intention-to-treat; *tcdB*, *Clostridium difficile* toxin B gene; WBC, white blood cell.

SI conversion factor: To convert creatinine values to μmol/L, multiply by 88.4.

[a] Severity of illness was assessed at initial screening according to Zar et al.[25]

[b] Mild CDI defined as temperature 38°C or lower, WBC count 11.0 × 10^9/L or lower, and maintenance of baseline serum creatinine level. Moderate CDI defined as temperature 38°C or lower, WBC count greater than 11.0 × 10^9/L and less than 15.0 × 10^9/L, and maintenance of baseline serum creatinine level. Severe CDI defined as temperature 38°C or higher or WBC count 15.0 × 10^9/L or greater or increase in serum creatinine level more than 1.5 times baseline.

[c] WBC count greater than 30 × 10^9/L reflects CDI episodes associated with FMT but requiring antibiotics to control severe CDI. All patients had WBC counts less than 30.0 × 10^9/L at tim e of FMT.

ous experience, the efficacy of fresh FMT was determined to be 85% for recurrent CDI. The 15% margin difference between the treatment groups was established by the investigators' judgment of the advantages of frozen FMT compared with fresh FMT. This was set a priori and was based on the principles described by Schumi and Wittes[23] and the noninferiority trial guideline issued by the US Food and Drug Administration.[24]

A sample size of 156 (78 in each group) was calculated based on the assumed 85% efficacy of FMT[12,13] and the attrition rate of 10%. Between-group comparisons were performed using Fisher exact test, proportional test, *t* test, and Wilcoxon test. All analyses were performed using R version 3.1.1 software.

Results

Patients

A total of 232 patients were enrolled and randomized to receive fresh (n = 118) or frozen (n = 114) FMT. The numbers of patients included in the mITT and per-protocol populations are shown in the **Figure**.

Patients' demographics and clinical data are summarized in **Table 1**. There were no differences between the 2 treatment groups with respect to the baseline characteristics or the severity of CDI as defined by Zar et al.[25] Despite the fact that all patients had received antibiotics to control their most recent episode of CDI, stool samples from 87 patients

Table 2. Number of Fecal Microbiota Transplantations and the Proportion With Clinical Resolution at 13 Weeks After Last Transplantation

	No. (%) With Clinical Resolution			
	mITT Population		Per-Protocol Population	
No. of FMTs	Frozen (n = 108)	Fresh (n = 111)	Frozen (n = 91)	Fresh (n = 87)
1	57 (52.8)	56 (50.5)	57 (62.7)	54 (62.1)
2	24 (75.0)	22 (70.3)	19 (83.5)	20 (85.1)
3-5	13 (87.0)	12 (81.1	9 (93.4)	9 (95.4)
>5	4 (90.7)	5 (85.6)	2 (95.6)	1 (96.6)
Total	**98**/108 (90.7)	**95**/111 (85.6)	**87**/91 (95.6)	**84**/87 (96.6)

Abbreviations: FMT, fecal microbiota transplantation; mITT, modified intention-to-treat.

Table 3. Primary Efficacy Outcome in the Modified Intention-to-Treat and Per-Protocol Populations According to Subgroup at 13 Weeks After Last Fecal Microbiota Transplantation

	mITT			Per-Protocol		
	Proportion With Clinical Resolution, No./Total (%)			Proportion With Clinical Resolution, No./Total (%)		
	Frozen	Fresh	Difference (95% CI), %	Frozen	Fresh	Difference (95% CI), %
Overall Population						
Primary efficacy outcome[a]	81/108 (75.0)	78/111 (70.3)	4.7 (−5.2 to ∞) (P < .001)	76/91 (83.5)	74/87 (85.1)	−1.6 (−10.5 to ∞) (P = .01)
Subgroups						
Age, y						
<65	22/27 (81.5)	17/27 (63.0)	18.5 (−1.1 to ∞)	21/24 (87.5)	17/21 (81.0)	6.5 (−11.4 to ∞)
≥65	59/81 (72.8)	61/84 (72.6)	0.2 (−11.2 to ∞)	55/67 (82.1)	57/66 (86.4)	−4.3 (−14.7 to ∞)
Admission status at time of FMT						
Inpatient	35/51 (68.6)	45/60 (75.0)	−6.4 (−20.5 to ∞)	32/41 (78.1)	41/46 (89.1)	−11.0 (−24.1 to ∞)
Outpatient	46/56 (82.1)	33/51 (64.7)	17.4 (3.6 to ∞)	44/49 (89.8)	33/41 (80.5)	9.3 (−3.1 to ∞)
Severity of CDI at baseline[b,c]						
Mild	33/41 (80.5)	28/33 (84.9)	−4.4 (−18.8 to ∞)	31/37 (83.8)	28/31 (90.3)	−6.5 (−19.8 to ∞)
Moderate	34/49 (69.4)	29/51 (56.9)	12.5 (−3.2 to ∞)	32/41 (78.1)	26/35 (74.3)	3.8 (−12.4 to ∞)
Severe	14/18 (77.8)	21/27 (77.8)	0.0 (−20.8 to ∞)	13/13 (100.0)	20/21 (95.2)	4.8 (−2.9 to ∞)
Setting of primary episode of CDI						
Health care	49/65 (75.4)	53/74 (71.6)	3.8 (−8.6 to ∞)	46/55 (83.6)	50/58 (86.2)	−2.6 (−13.7 to ∞)
Community	29/39 (74.4)	23/35 (65.7)	8.7 (−8.9 to ∞)	27/33 (81.8)	22/27 (81.5)	0.3 (−16.2 to ∞)
Refractory CDI only	5/6 (83.3)	4/9 (44.4)	38.9 (1.9 to ∞)	4/4 (100.0)	4/6 (66.7)	33.3 (1.7 to ∞)
CDI recurrence ≥2 only	70/94 (74.5)	59/85 (69.4)	5.1 (−6.0 to ∞)	66/80 (82.5)	57/67 (85.1)	−2.6 (−12.6 to ∞)
Both recurrent and refractory CDI	6/8 (75.0)	15/17 (88.2)	−13.2 (−41.5 to ∞)	6/7 (85.7)	13/14 (92.9)	−7.2 (−31.7 to ∞)
Strain type						
Non-BI/027	15/20 (75.0)	18/20 (90.0)	−15.0 (−34.4 to ∞)	15/17 (88.2)	16/17 (94.1)	−5.9 (−21.8 to ∞)
BI/027	10/15 (66.7)	10/14 (71.4)	−4.7 (−33.0 to ∞)	10/12 (83.3)	9/11 (81.1)	2.2 (−24.5 to ∞)
Not tested	56/73 (76.7)	50/77 (64.9)	11.8 (−0.3 to ∞)	51/62 (82.3)	49/59 (83.1)	−0.8 (−12.1 to ∞)
CD toxin *tcdB* at baseline						
Positive	34/43 (79.1)	34/44 (77.3)	1.8 (−12.8 to ∞)	33/36 (91.7)	31/34 (91.2)	0.5 (−10.5 to ∞)
Negative	44/62 (71.0)	40/62 (64.5)	6.5 (−7.3 to ∞)	40/52 (76.9)	39/48 (81.3)	−4.4 (−17.7 to ∞)
Immunocompromised[d]	14/18 (77.8)	14/17 (82.3)	−4.5 (−26.7 to ∞)	14/15 (93.3)	13/14 (92.9)	0.4 (−15.0 to ∞)
Inflammatory bowel diseases	5/10 (50.0)	5/7 (71.4)	−21.4 (−59.7 to ∞)	5/6 (83.3)	5/6 (83.3)	0.0 (−35.4 to ∞)

Abbreviations: CDI, *Clostridium difficile* infection; FMT, fecal microbiota transplantation; mITT, modified intention-to-treat; *tcdB*, *Clostridium difficile* toxin B gene; WBC, white blood cell.

[a] Defined as clinical resolution of diarrhea without relapse or the need for antibiotics for CDI recurrence at 13 weeks after the last transplant.

[b] Severity of illness was assessed at initial screening according to Zar et al[2] and defined as temperature 38°C or higher or WBC count 15.0 × 10^9/L or greater or increase in serum creatinine level greater than 1.5 times baseline.

[c] Mild defined as temperature 38°C or lower, WBC count 11.0 × 10^9/L or lower, and maintenance of baseline serum creatinine level. Moderate defined as temperature 38°C or lower, WBC count greater than 11.0 × 10^9/L and less than 15.0 × 10^9/L, and maintenance of baseline serum creatinine level. Severe defined as temperature 38°C or higher or WBC count 15.0 × 10^9/L or greater or increase in serum creatinine level more than 1.5 times baseline.

[d] Defined as patients who had received at least 1 significant immunosuppressant (azathioprine, cyclosporine, infliximab, methotrexate alone or with corticosteroids) (n = 18), were postrenal transplant (n = 5), had undergone chronic hemodialysis (n = 5), had metastatic solid tumors (n = 3), and/or had hematologic malignancy (n = 4) at the time of FMT.

tested positive for *C difficile* toxin or *tcdB* at the time of FMT, as did the stool samples collected at the time of CDI relapse after FMT. The median duration of CDI from the initial diagnosis of CDI that led to the first FMT was 85 days (range, 6-1351 days) in the mITT population and 91 days (range, 14-870 days) in the per-protocol population. The median duration of antibiotic usage for CDI treatment prior to the first FMT was 48 days (range, 6-811 days) in the mITT population and 52 days (range, 11-811 days) in the per-protocol population. (The wide ranges associated with these medians were attributable to the fact that the study included both refractory and recurrent cases.)

Efficacy

The per-protocol population comprised 91 patients in the frozen FMT group and 87 in the fresh FMT group. The mITT population comprised 108 patients in the frozen FMT group and 111 in the fresh FMT group. The response rates following FMTs are shown in **Table 2** and in eTable 1 in Supplement 2.

The proportions of primary clinical resolution in the per-protocol population were 76 of 91 (83.5%) in the frozen FMT group and 74 of 87 (85.1%) in the fresh FMT group (difference, −1.6% [95% 1-sided CI, −10.5% to ∞]; $P = .01$ for noninferiority). In the mITT population, the corresponding proportions were 81 of 108 (75.0%) in the frozen FMT group and 78 of 111 (70.3%) in the fresh FMT group, after up to 2 FMTs (difference, 4.7% [95% 1-sided CI, −5.2% to ∞]; $P < .001$ for noninferiority). Since the lower confidence limits were above the noninferiority margin of −15% in both populations, frozen FMT was determined to be noninferior to fresh FMT. The proportions of response after each FMT are shown in Table 2. Whenever patients received CDI antibiotic in between FMTs or were lost to follow-up, they were considered as having experienced treatment failure and placed in the mITT population; therefore, the proportions of response in the mITT groups were lower than in the per-protocol population. Differences between the 2 treatment groups in primary cure rates in patient subgroups are reported in **Table 3**.

Six patients who did not respond to multiple FMTs remained symptom-free while receiving once-daily oral vancomycin at 12 to 18 months; 4 of these patients were refractory to vancomycin prior to FMT. The patients who did not respond after up to 2 FMTs were deemed to have experienced treatment failure with regard to the primary end point.

Safety

There were no observed differences in the proportion of adverse events or SAEs between the 2 treatment groups. The most common adverse events considered at least possibly related to FMT were transient diarrhea (70%), abdominal cramps (10%), or nausea (<5%) during the 24 hours following an FMT and constipation (20%) and excess flatulence (25%) during the follow-up period. All were mild to moderate. Other adverse events observed were urinary tract infections, which occurred several weeks after FMT in patients with a previous history of recurrent urinary tract infections (<5%), respiratory tract infection, blood in stool, and exacerbation of preexisting rheumatoid arthritis after discontinuation of immunosuppressants (all <1%). These episodes were assessed by blinded investigators and deemed unlikely to be related to FMT. The patients who developed urinary tract and respiratory tract infections received antibiotics for these infections but did not develop subsequent CDI.

There were 29 SAEs during the 13-week follow-up period from the last FMT. Twelve patients (8 in the frozen FMT group and 4 in the fresh FMT group) required hospitalization because of illnesses unrelated to FMT following clinical resolution of CDI after FMT. A total of 19 patients (6 in the frozen FMT group and 13 in the fresh FMT group) died during the 13-week study period; none of these deaths were considered directly attributable to FMT (**Table 4**; eTable 2 in Supplement 2). Four patients (2 in the frozen FMT group and 2 in the fresh FMT group) died with unresolved CDI: of these, 1 had received 3 FMTs, 2 had received 2 FMTs, and the other had received a single FMT.

Table 4. Death Following Fecal Microbiota Transplantation

	FMT Type	
	Frozen (n = 108)	Fresh (n = 111)
Deaths, No. (%)	6 (5.6)	13 (11.7)
Age, range, y	79-100	59-95
Women, No. (%)	54 (50.0)	85 (76.9)
Time to death from last FMT, median (range), d	16.5 (7-58)	31 (3-83)
No. of deaths attributable to CDI[a]	2	2

Abbreviation: FTM, fecal microbiota transplantation.

[a] See eTable 2 in Supplement 2 for other causes and time to death from last FMT.

Discussion

In this clinical trial, the use of frozen FMT compared with fresh FMT for the treatment of recurrent or refractory CDI was noninferior in terms of efficacy; findings for frozen FMT and fresh FMT were similar in terms of safety. The proportion of clinical resolution with up to 2 FMTs in this study is similar to proportions achieved with FMTs (70%-91%) in previous FMT trials.[10,14] Although some systematic reviews[11];have reported higher cure rates, it should be noted that the data included in these reviews were derived from uncontrolled, retrospective case reports and case series, and that there was substantial variability in the patient populations studied and the delivery of FMT. A recently published study of oral administration of capsules containing frozen FMT achieved clinical resolution of 70% following the primary treatment and greater than 90% with subsequent treatments, demonstrating that frozen FMT is safe and effective when administered orally.[14,15] We chose to use retention enemas in this study because a previous study indicated that FMT delivered by enema was safe and effective.[12,26]

In this study, as in others,[10,13,14,27,28] there was evidence that the proportion of clinical resolution increased with the

number of FMTs. The primary end point was the rate of clinical resolution achieved with up to 2 FMTs; the aim of the study was to compare the efficacy of frozen and fresh FMTs, rather than to assess the efficacy of repeated FMTs.

Patients in this study had an extensive burden of morbidity: 111 of 218 (51%) were inpatients at the time of FMT, 145 of 219 (66%) had moderate to severe CDI, 29 of 69 (42%) were infected with 027 ribotype strain, and 24 were immunocompromised. Nevertheless, the proportions of clinical resolution after receipt of up to 2 FMTs were high (27/29 [93%] for immunocompromised patients and 5/6 [83%] for patients with inflammatory bowel disease). Furthermore, the number of adverse events in all subgroups was low, similar to previous studies.[12-15]

A total of 19 patients (8.7%) (6 in the frozen FMT group and 13 in the fresh FMT group) died during the follow-up period, of whom 4 (1.8%) died with unresolved CDI. None of these deaths were considered directly related to FMT. A systematic review reported an all-cause 30-day mortality rate among patients with CDI of 15% or greater and 30-day CDI-attributable mortality of 5.7% to 6.9%.[29]

The FMT preparation technique used in this study confers a number of advantages. First, the stool preparation protocol uses disposable equipment and does not require specialized, costly devices. Second, administration by enema is significantly less invasive than colonoscopy or nasojejunal/gastric administration and can be performed outside an acute care facility. Third, frozen FMT reduces the number and frequency of donor screenings compared with fresh FMT, potentially reducing the costs. This approach also has the added value of wide applicability in diverse health care settings. Last, concern about potential transmission of pathogens from the donor to the recipient with fresh FMT can be ameliorated by quarantining (and freezing) the collected stool sample until screening results are available.

This study has several limitations. First, the follow-up was limited to 13 weeks after the last FMT, which is insufficient to evaluate the long-term safety of the treatment. However, this time frame was longer than the majority of CDI therapeutic trials, which have followed up patients for up to 40 days. Second, this study showed a lower proportion of clinical response in the per-protocol population following a single FMT (62%) than a previous study and the published case series, in which the cumulative cure rate was greater than 90%.[10-15,26-28] Although this raises the possibility that small-volume enemas may be slightly less effective than other delivery methods, a direct comparative trial is needed to examine this. It is also likely that the larger sample size of the study, and its prospective, randomized design, allowed more sensitive detection of clinical failures; in addition, 51% of the patients were hospitalized at the time of FMT. The low number of stool donors may also be considered a study limitation; however, the aim was to compare fresh and frozen FMTs, rather than potential donor effects. Although it would have been useful to determine the efficacy of FMT according to the total number of recurrences, this was not feasible because of the prolonged duration of recurrence and the history of multiple recurrences in the majority of patients.

There is a need to determine the long-term safety of FMT. Extended (10-year) follow-up of the patients in this trial is under way to investigate the long-term positive (eg, improvement of the metabolic syndrome, diabetes, autoimmune disease) or negative (eg, development of metabolic or autoimmune diseases, cancer) outcomes.

Conclusions

Among adults with recurrent or refractory CDI, the use of frozen compared with fresh FMT did not result in worse proportion of clinical resolution of diarrhea. Given the potential advantages of providing frozen FMT, its use is a reasonable option in this setting.

ARTICLE INFORMATION

Author Affiliations: Hamilton Regional Laboratory Medicine Program, Department of Pathology & Molecular Medicine, McMaster University, Hamilton, Ontario, Canada (Lee, Smieja, Jayaratne, Kim); Department of Medicine, McMaster University, Hamilton, Ontario, Canada (Lee, Smieja, Crowther); St Joseph's Healthcare, Hamilton, Ontario, Canada (Lee, Smieja, Crowther, Jayaratne, Higgins); Division of Infectious Diseases and Immunity and Infection Research Centre, Vancouver Hospital and University of British Columbia, Vancouver, British Columbia, Canada (Steiner, Nematallah); Gastrointestinal Diseases Research Unit, Department of Medicine, Queen's University, Kingston, Ontario, Canada (Petrof, Ropeleski); Division of Medical Microbiology and Infection Control, Vancouver General Hospital and University of British Columbia, Vancouver, British Columbia, Canada (Roscoe); Centre for Public Health and Zoonoses, Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada (Weese); Farncombe Family Digestive Health Research Institute, Division of Gastroenterology, Department of Medicine, McMaster University, Hamilton, Ontario, Canada (Collins, Moayyedi); Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada (Li); Department of Mathematics and Statistics, University of Guelph, Guelph, Ontario, Canada (Kim); Division of Infectious Diseases, Department of Medicine, University of Toronto, Toronto, Ontario, Canada (Rau); Halton Healthcare Services, Oakville, Ontario, Canada (Rau).

Author Contributions: Drs Lee and Kim had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Lee, Petrof, Smieja, Roscoe, Collins, Moayyedi, Higgins, Kim.
Acquisition, analysis, or interpretation of data: Lee, Steiner, Petrof, Smieja, Nematallah, Weese, Moayyedi, Crowther, Ropeleski, Jayaratne, Li, Rau, Kim.
Drafting of the manuscript: Lee, Steiner, Petrof, Smieja, Higgins, Kim.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Smieja, Kim.
Obtained funding: Lee, Petrof, Crowther, Higgins, Kim.
Administrative, technical, or material support: Lee, Petrof, Smieja, Roscoe, Moayyedi, Crowther, Ropeleski, Jayaratne, Higgins, Li, Kim.
Study supervision: Lee, Petrof, Smieja, Roscoe, Higgins, Kim.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Lee reported participating in clinical trials for ViroPharma, Actelion, Cubist, and Merck and serving as a member of the advisory boards for Rebiotix and Merck. Dr Steiner reported receiving consulting fees and an unrestricted grant from Cubist, consulting fees and a phase 3 trial contract from Merck Canada, and a phase 3 trial contract from Sanofi Pasteur; additionally, his institution was recently approved as a site for a phase 2b randomized clinical trial of frozen stool product with Rebiotix. Dr Petrof reported holding a patent for synthetic stool formation. Dr Crowther reported receiving grants from the Heart and Stroke

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