Supplementary Online Content

Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium difficile* infection. *JAMA*. doi:10.1001/jama.2014.13875

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Inclusion/Exclusion Criteria for FMT Recipients

Inclusion criteria

- Patients with refractory, recurrent or relapsing CDI defined as EITHER(13):
    - At least three episodes of mild-to-moderate CDI.
    - At least two episodes of severe CDI resulting in hospitalization and associated with significant morbidity.
    - One protracted episode of CDI, defined as at least 3 weeks of ongoing Grade 3 severe symptoms of CDI despite standard antimicrobial therapy for CDI.
    - We expect that most, but not all, subjects will have tried and failed a taper of vancomycin.
- Willingness to accept risk of unrelated donor stool.
- Age 7 and above. Seven is chosen as a lower limit based upon the legal age of assent. Most children aged 7 and above can be taught to swallow even large capsules through simple coaching techniques. If they cannot, they can be offered FMT through our expanded access IND using a liquid frozen inoculum.
- Able to consent for self, or parental assent/child assent as age appropriate.

Exclusion criteria

- Delayed gastric emptying syndrome
- Known chronic aspiration
- Swallowing dysfunction or oral-motor dyscoordination.
- Inability or unwillingness to swallow multiple large capsules
- Pregnant women
- Patients with an acute illness unrelated to CDI or an acute exacerbation of underlying comorbid condition
- Patients with comorbidities associated with increased risk of serious infection following bacterial translocation, including but not limited to:
    - subjects on major immunosuppressive agents including high dose corticosteroids, calcineurin inhibitors, mTOR inhibitors, lymphocyte depleting biologic agents, anti-TNF agents, and others; chemotherapeutic anti-neoplastic agents*
    - Patients with decompensated liver cirrhosis, advanced HIV/AIDS, recent bone marrow transplant, hypoglobulinemia or other cause of severe immunodeficiency*
- Patients with a history of significant allergy to foods not excluded from the donor diet

*In some instances, possible restoration of normal flora is likely more beneficial than risky, so this will be discussed carefully and individualized. We will consider patients on stable or decreasing doses of biological or chemical immunomodulators like infliximab, etanercept, or adalimumab, azathioprine and methotrexate (e.g. for Crohns, dermatological or rheumatological disease), with the requirement that the treating physician prescribing these drugs agrees that the individual is not a high risk of infectious complications of FMT or other medical interventions at the time of FMT. Indeed, in patients with Inflammatory Bowel Disease (IBD) or rheumatologic joint disease and recurrent/refractory CDI on stable doses of immunomodulator remission therapy, the ongoing presence of CDl mimics and exacerbates the disease process and makes it difficult to clinically assess need for further management of underlying autoimmune disease. Patients who are on triple immunosuppression (e.g. organ transplant regimens) will only be allowed with the written agreement of transplant physicians or transplant ID consultants.

eAppendix 2. Inclusion/Exclusion Criteria for Healthy Volunteer Stool Donors

Inclusion Criteria

Healthy donors must be healthy, non-pregnant adults 18-50 years of age, on no medications, with a normal Body Mass Index (BMI 18.5-25). Volunteers must pass the American Association of Blood Banks (AABB) Donor questionnaire for exposure to infectious agents, have a normal physical exam (including fecal occult blood testing negative), and general laboratory screening tests within the normal range (CBC with differential, BUN/Cr, complete liver function tests, normal glucose, lipids, and C-Reactive Protein within normal range, and negative anti-nuclear antibody). Volunteers must have no significant past medical history with the exception of past resolved traumatic injury or routine surgery (e.g., wisdom teeth extraction, appendectomy, cosmetic dentistry or plastic surgery).

Exclusion Criteria (in addition to passing AABB questionnaire for blood donors)

These are virtually verbatim from Hamilton et al (18) and Bakken et al (13) or more stringent

- Any past or current malignancy including GI malignancy or polyposis
- Personal or Family History of inflammatory bowel disease or unexplained GI illness
- History of irritable bowel syndrome, excessive gas, bloating, lymphocytic colitis, idiopathic chronic constipation, chronic use of laxatives or chronic diarrhea
- Any chronic medications
- Use of probiotics or any OTC aids for regulating digestion
- Antibiotics within the preceding 6 months
- Major immunosuppressive medications, e.g., calcineurin inhibitors, exogenous glucocorticoids, biologic agents, etc.
- Systemic anti-neoplastic agents
- Recent ingestion of a potential allergen (e.g., nuts, shellfish, eggs, peanuts)
- History of gastrointestinal surgery (e.g., gastric bypass) or endoscopy
- Metabolic syndrome
- Neurological, neurodevelopmental disorder e.g. Parkinson's disease, autism, etc.
- Systemic autoimmunity, e.g., multiple sclerosis, psoriasis, vasculitis, connective tissue disease, any rheumatological or inflammatory condition
- Atopic diseases, e.g. asthma and eczema, food allergies, eosinophilic disorders of the gastrointestinal tract
- Chronic pain syndromes, e.g., chronic fatigue syndrome, fibromyalgia

eAppendix 3. Laboratory Testing of Healthy Volunteer Stool Donors

General health laboratory testing:

- CBC and differential WNL ranges for gender at MGH clinical labs. Minor excursions of the % of differential for leukocyte subsets of monocytes, lymphocytes and PMNs, or red cell indices are allowed if deemed not clinically significant by the reviewing physician. No elevation of eosinophils is allowed. Total WBC as low as 4,000 is allowed given that many healthy young males often display this finding
- ALT, AST, Bilirubin, alkaline phosphatase WNL ranges at MGH clinical labs.
- Electrolytes and fasting glucose WNL ranges at MGH clinical laboratories(minor excursions related to intake, for example K+ slightly below normal may be allowed at the discretion of the investigator)
- BUN/Cr within normal range
- Serum triglycerides, HDL cholesterol within normal range for gender
- Fluorescent ANA negative (18)
- High sensitivity CRP WNL (<2.4 mg/dL)

Stool testing for donors (at MGH clinical laboratories unless specified below, all results must be NEGATIVE)

- *Clostridium difficile* toxin by PCR
- Routine bacterial culture for enteric bacterial pathogens (with enrichment broth)
    - Include Salmonella, Shigella, Yersinia, Campylobacter, E.coli 0157.
- Specialized media culture for Vibrio and Listeria
- Fecal Giardia antigen (DFA)
- Fecal Cryptosporidium antigen (DFA)
- Acid-fast stain for Cyclospora, Isospora and Cryptosporidium
- Ova and parasites (microscopy)
- Helicobacter pylori fecal antigen (send out to: Mayo Laboratories, Rochester MN)
- Rotavirus Enzyme Immunoassay

Serologic testing for donors (at MGH clinical laboratories, all must be NEGATIVE).

- *HIV, type 1 and 2*
- *HAV IgM*
- *HBsAg, anti-HBc (both IgG and IgM), and anti-HBs.*
- *HCV Ab*
- *Treponemal test for syphilis (Trep-Sure); if positive; RPR is done subsequently*

Healthy volunteer donors will have all tests performed within one month of donation, and viral and syphilis testing (underlined and italicized above) within 2 weeks of the donation. Volunteers will be asked to refrain from eating common allergens within 5 days of the donation (*tree nuts, eggs, peanuts, shellfish*) but otherwise not alter their diets. Volunteers will have an interim health query for febrile, system, and GI symptoms at the time of donation and deferred for any change in health status. Donors with acute gastrointestinal symptoms, including diarrhea, vomiting, fever and abdominal pain, will be excluded from stool donation until symptoms have resolved. All tests will be performed at CLIA certified clinical laboratories, placed in medical records, and made available to volunteers for their personal physician's records if desired.

eAppendix 4. Early and Late Follow Up Questionnaires

Early Case Report Form

Subject Number: _____ Age_____

Date Form Completed: _____ by: _____

FMT Date_____

Post procedure day _____

Weight: _____ Temperature: _____

# of Bowel Movements:_____
Diarrheal episodes past 24 hr? _____

New Medications _____

Overall Health Scale: WORST = 1 2 3 4 5 6 7 8 9 10 = BEST

GI Health Scale: WORST = 1 2 3 4 5 6 7 8 9 10 = BEST

Other comments: _____
_____
_____
_____
_____
_____
_____
_____

Please refer to and complete attached Adverse Event Grading Chart (FMT) which delineates additional symptoms being monitored.

Study Physician Review: _____Date_____

eAppendix 4. Early and Late Follow Up Questionnaires (continued)

Late POST Procedure Questionnaire (6 months)

Subject Number: _____

Date Completed: _____ by_____

After the fecal transplant

(1) Regarding the following symptoms, check which applies to you:

Diarrhea: Resolved ___, Improved ___, Did not improve ___

Number of Bowel Movements per day: _____

Abdominal pain: Resolved ___, Improved ___, Did not improve ___, Was not present before fecal transplant ___

General well-being (strength, fatigue, etc.): Resolved ___, Improved ___, Did not improve ___, Was not impaired before fecal transplant ___

Overall Health Scale: WORST = 1 2 3 4 5 6 7 8 9 10 = BEST (circle one)

GI Health Scale: WORST = 1 2 3 4 5 6 7 8 9 10 = BEST (circle one)

Weight: Increased ___, Stayed same ___, Decreased ___ Current Weight:_____

(2) How soon after the transplant did diarrhea stop or improve?

_____

_____

(3) How soon after fecal transplant did your abdominal pain stop or resolve?

_____

_____

(4) How soon after fecal transplant did your well-being improve?

_____

_____

eAppendix 4. Early and Late Follow Up Questionnaires (continued)

(5) Have you had a recurrence of diarrhea after the fecal transplant?

a. Yes

b. No

If yes, how long after fecal transplant did it recur? _____

Was this recurrence associated with antibiotic use? _____

List antibiotic, if known _____

Was recurrence associated with return of C diff? _____

If yes, how was it treated? _____

Was treatment successful? _____

(6) Have you required antibiotics since the fecal transplant?

a. Yes

b. No

(8) Did you eat yogurt or take other probiotics while taking the antibiotics?

a. Yes

b. No

(7) Have you taken acid suppressing medications since your fecal transplant?

a. Yes; Specify: _____

b. No

(8) Do you have kidney disease requiring dialysis?

a. No

b. Yes

(9) Did any medical condition you had *before* your fecal transplant go away after your fecal transplant (for example, arthritis and chronic skin rash)?

a. No

b. Yes; Specify: _____

(12) Have you developed any *NEW* medical conditions since your fecal transplant?

a. No

b. Yes; Specify: _____

(13) Please list all medications you take on a regular basis (dosage not necessary) include chemotherapy, if applicable

_____

_____

_____

_____

(14) If your diarrhea did not improve after your fecal transplant, were other treatments tried?

a. No

b. Yes; Specify: _____

c. If yes, were they successful?

i. Yes

ii. No

(15) If you were to develop C. Diff again, would you have another fecal transplant?

a. Yes

b. No

If yes, would you have it after trying antibiotic treatment once? ____, Twice? ____ , More than twice? ____, Fecal transplant instead of any antibiotic treatment? _____

Additional Comments:_____

_____
_____
_____
_____
_____

Completed by_____Date_____

Study physician _____Date_____

eAppendix 5. Adverse Event Grading Chart

Patient Name:_____ Date:_____ Date of Procedure:_____

**Patient's responses will be marked on grading sheet by circling the appropriate grade or indicating no symptoms.*

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
ESTIMATING SEVERITY GRADE				
	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYMPTOM SPECIFIC SEVERITY GRADE				
Fever (oral)	(99.9-100.5°F)	(100.6-102.5°F)	(102.6-104°F)	> 104°F
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to perform basic self-care functions
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Headache with significant impairment of alertness or other neurologic function

eAppendix 5. Adverse Event Grading Chart (continued)

Distension/bloating, abdominal discomfort	Asymptomatic	Symptomatic, but not interfering with GI function	Symptomatic, interfering with GI function	---
Abdominal Pain	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Life threatening consequences (i.e. acute peritonitis)
Dehydration	Increased oral fluids indicated; dry mucous membranes; diminished skin turgor	IV fluids indicated <24 hours	IV fluids indicated >24 hours	Life-threatening consequences (e.g. hemodynamic collapse)
Dizziness	With head movements or nystagmus only; not interfering with function	Interfering with function, but not interfering with ADL	Interfering with ADL	Disabling
Colitis Symptoms	Asymptomatic, pathologic or radiographic findings only	Abdominal pain; mucus or blood in stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal	Life-threatening consequences (e.g. perforation, bleeding, ischemia, necrosis, toxic megacolon)
Weight Loss	5 to <10% from baseline; intervention not indicated	10-<20% from baseline; nutritional support indicated	≥20% of baseline	---
Rash	Macular or popular eruption or erythema without associated symptoms	Macular or popular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of body surface area (BSA)	Severe, generalized erythroderma or macular, popular, or vesicular eruption; desquamation covering ≥50% BSA	Generalized exfoliative, ulcerative, or bullous dermatitis
Dysphagia	Symptomatic; able to eat regular diet	Symptomatic and altered eating/swallowing (e.g., altered dietary habits, oral supplements); IV fluids indicated <24hrs	Symptomatic and severely altered eating/swallowing (e.g. inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ≥24hrs	Life-threatening consequences (e.g. obstruction, perforation)

Physician Review:_____Date:_____

eTable 1. Factors Associated With Diarrhea Resolution After a Single Dose - Logistic Regression With Mixed Effect Controlling for Donor Clustering

	Cure after 1 dose		
	Odds Ratio	95% CI	P-value
Age (years)	1.03	(0.98-1.08)	0.265
Male Gender	3.6	(0.47-27.11)	0.214
Number of prior recurrences	0.74	(0.36-1.54)	0.424
Maximal number of daily BM	0.96	(0.8-1.16)	0.686
Weight Loss (pounds)	1.12	(0.97-1.29)	0.127
Previous Fidaxomicin Treatment	0.75	(0.11-5.11)	0.769
Patient on ASM	1.36	(0.11-16.58)	0.808
Pre-treatment overall health score	3.18	(0.99-10.31)	0.050
Pre-Treatment GI Health Score	1.57	(0.85-2.92)	0.149
Pre-treatment BM per day	0.56	(0.3-1.06)	0.074
Storage of capsules prior to use (days)	0.98	0.62-1.54	0.919

Abbreviations: ASM-acid suppressing medication; BM-bowel movements; GI-gastrointestinal. Overall and gastrointestinal specific health score – self reported health ranking on a scale from 1 to 10, with 1 being the lowest and 10 being “best possible health for you”.

eTable 2. Linear Mixed Model Controlling for Donor Clustering

	Bowel Movements per day			GI Health Score			Overall Health Score		
	B	(95% CI)	p	B	(95% CI)	p	B	(95% CI)	p
Age	0.04	(-0.11-0.19)	0.561	-0.08	(-0.34-0.16)	0.482	-0.13	(-0.33-0.07)	0.199
Gender (male)	0.26	(-0.38-0.9)	0.407	-0.13	(-1.21-0.96)	0.808	0.03	(-0.86-0.93)	0.942
Number of Prior Recurrences	-0.07	(-0.32-0.17)	0.534	0.1	(-0.31-0.51)	0.604	0.1	(-0.23-0.43)	0.525
Previous Vancomycin Taper	0.3	(-1.18-1.79)	0.672	-0.49	(-2.98-1.99)	0.678	-0.69	(-2.7-1.32)	0.481
Previous Fidaxomicin Treatment	-0.49	(-1.09-0.12)	0.107	0.69	(-0.35-1.73)	0.177	0.42	(-0.45-1.29)	0.320
Maximal number of BM	0.02	(-0.04-0.08)	0.496	-0.01	(-0.11-0.1)	0.866	-0.04	(-0.12-0.04)	0.296
Patient on ASM	0.39	(-0.39-1.18)	0.307	-0.3	(-1.65-1.04)	0.633	-0.79	(-1.83-0.25)	0.182
Storage time	0.39	(-0.39-1.18)	0.307	-0.3	(-1.65-1.04)	0.633	-0.79	(-1.83-0.25)	0.182

Abbreviations: ASM-acid suppressing medication; BM-bowel movements; GI-gastrointestinal. Overall and gastrointestinal specific health score – self reported health ranking on a scale from 1 to 10, with 1 being the lowest and 10 being “best possible health for you”.

eTable 3. Clinical Information About Study Participants

Patient No.	Diarrhea Resolution After 1 Dose	Overall Diarrhea Resolution	Predisposing Factors or Inciting Event	Comments
1	No	Yes	Contracted CDI during hospital admission for pneumonia and treatment with piperacillin/tazobactam.	Failed to respond to first dose. Immediate symptomatic improvement after re-treatment.
2	Yes	Yes	No significant past medical history.	
3	Yes	Yes	End-stage renal disease.	No relapse in spite of azithromycin treatment during follow up.
4	Yes	Yes	No significant past medical history.	
5	No	Yes	Lung cancer, recent chemotherapy.	Failed to respond to first dose. Immediate symptomatic improvement after re-treatment.
6	Yes	Yes	Chronic renal failure.	Diarrhea resolution, no relapse in spite of chronic nitrofurantoin prophylaxis and two courses of ciprofloxacin during follow up.
7	Yes	Yes	Inflammatory bowel disease. Not on active immunosuppression.	
8	Yes	Yes	No significant past medical history.	
9	Yes	Yes	No significant past medical history.	
10	No	No	Hepatitis C, progressive liver disease.	Diarrhea resolution after 2 doses, relapsed at 7 weeks post treatment. Was subsequently re-treated and is currently asymptomatic.
11	Yes	Yes	Breast cancer. Previous chemotherapy and radiation.	
12	No	Yes	Clindamycin treatment for periorbital cellulitis.	Symptomatic improvement after first dose, relapsed two weeks later. Diarrhea resolution after second dose. No relapse at 6 month follow up.
13	Yes	Yes	Hepatitis C, ethanol abuse, liver cirrhosis.	
14	Yes	Yes	Contracted CDI during routine post-partum antibiotic administration.	
15	Yes	Yes	Hepatitis C, ethanol abuse, known liver cirrhosis.	
16	No	No	Admitted to the intensive care unit twice in the previous six months for CDI-related illness.	Failed to respond to two doses.
17	Yes	Yes	Breast cancer. Previous chemotherapy and radiation.	Relapsed 4 months out when treated with ciprofloxacin.
18	Yes	Yes	No significant past medical history.	
19	No	Yes	Inflammatory bowel disease. Low-dose steroid treatment. Contracted CDI when treated with amoxicillin/clavulonate after a cat bite.	Amoxicillin treatment during follow up, no relapse.
20	Yes	Yes	Breast cancer. Previous chemotherapy	