Fecal microbiota transplantation capsules with targeted colonic versus gastric delivery in recurrent *Clostridium difficile* infection: A comparative cohort analysis of high and low dose

Authors: Jessica R. Allegretti[1,2], Monika Fischer[3], Sashidhar V. Sagi[3], Matthew E. Bohm[3], Hala M. Fadda[4], Sejal R. Ranmal[8], Shrish Budree[5,6], Abdul W. Basit[8,9], Dean L. Glettig[7], Eva L. de la Serna[7], Amanda Gentile[7], Ylaine Gerardin[7], Sonia Timberlake[7], Rotem Sadovsky[7], Mark Smith[7] and Zain Kassam[7]

1. Division of Gastroenterology, Hepatology and Endoscopy, Brigham and Women's Hospital, Boston MA
2. Harvard Medical School, Boston, MA
3. Division of Gastroenterology, Indiana University, Indianapolis, IN
4. College of Pharmacy and Health Sciences, Butler University, Indianapolis, IN
5. OpenBiome, Somerville, MA
6. University of Cape Town, Cape Town, South Africa
7. Finch Therapeutics, Somerville, MA
8. Intract Pharma, London, United Kingdom
9. School of Pharmacy, University College London, London, United Kingdom

Corresponding Author:
Jessica R. Allegretti, MD, MPH
Brigham and Women's Hospital, Division of Gastroenterology
75 Francis Street, Boston, MA 02115, USA
+1-617-732-6389
jallegretti@partners.org

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Abstract

Background
Fecal microbiota transplantation (FMT) is an effective therapy for recurrent *Clostridium. difficile* infection (rCDI). FMT capsules have emerged and, it is unknown if delivery location and dose impacts efficacy.

Methods
We compared two cohorts of patients receiving two capsule formulations: gastric release (FMTgr) and targeted colonic release (FMTcr) at two different sites. **Cohort A** received FMTgr at 1*) high dose*: 60 capsules and *low dose*: 30 capsules. Patients in **Cohort B** received FMTcr at 1) *high dose*: 30 capsules 2) *low dose*: 10 capsules. Clinical cure rates and adverse events were monitored through week 8. Paired t-tests were used to compare diversity pre- and post- FMT.

Results
51 rCDI patients were enrolled. Cohort A contained n=20 and cohort B contained n=31. Overall cure at week 8 for FMTgr was 75% (15/20) compared to 80.6% for FMTcr, (25/31), p=0.63. Both formulations were safe with no serious adverse events. FMTcr were superior at increasing gut microbial diversity.

Discussion:
To our knowledge, this is the first study to compare targeted delivery of FMT capsules. While both capsules were safe and efficacious, microbial engraftment patterns were superior in FMTcr.

Key Words: Fecal microbiota transplantation, microbiome, Clostridium difficile infection, fecal capsule

Background:

Clostridium difficile infection (CDI) continues to be a very significant health threat, with the incidence of recurrent infection also increasing[1, 2]. Fecal microbiota transplantation (FMT) is an effective therapy for recurrent *C. difficile* infection (CDI) [3, 4]. FMT can be delivered through several different modalities. Routes of administration include the lower gastrointestinal (GI) tract (with instillation into the right colon or terminal ileum [TI] via colonoscopy or instillation into the distal colon via enema or flexible sigmoidoscopy) or the upper GI tract (any delivery site proximal to the colon) via upper endoscopy, nasoenteric tubes, or capsules[5]. Data suggests lower GI delivery (colonoscopy) is a more effective treatment modality than upper GI delivery (nasogastric tube)[5]. However, there are confounding factors including dose and colonoscopy prep that have not been controlled in these studies.

More recently, FMT capsules have emerged as a novel route of delivery; however, to date capsule studies have had varying luminal targeting[6, 7]. Currently, it is unknown if delivery location impacts the efficacy. Additionally, the optimal dose remains unknown; and among the several proof-of-concept studies with varying doses there have been varying efficacy rates reported of ranging from 70-96%[8-10].

To better understand issues surrounding both dose and luminal targeting, we present a comparison of two cohorts examining the safety, efficacy and engraftment profiles of two capsule formulations and varying dosing regimens: FMT capsules with gastric release (FMTgr) and FMT capsules with targeted colonic release using Phloral® technology (FMTcr). Phloral® is a clinically validated coating technology that protects the FMT capsule through the stomach and small intestine and releases its content in the lower GI tract[11, 12]. This unique dual-trigger delivery system exploits both the presence of the microbiota and changes in pH along the GI tract, with each component acting as a fail-safe to guarantee reliable and consistent delivery to the colon[12, 13].

Methods:

We compared two cohorts of patients that underwent FMT for clinical care at two large FMT referral centers. The protocol was approved by the institutional review boards at both medical centers.

Study Population:

Eligible patients at each site were adults (18 years or older) who had recurrent CDI, defined as 3 or more laboratory-confirmed episodes, within the prior 12 months who were eligible for a clinically indicated FMT. Patients were required to be an outpatient at the time of capsule administration and required a demonstrated clinical response to standard antibiotic therapy for CDI. Relevant exclusion criteria included patients with the following: unable to swallow an inert test capsule, pregnant or nursing, inflammatory bowel disease, severely immunocompromised, and those unable to provide consent were excluded. Additionally, patients. Patients who met eligibility criteria also needed to have demonstrated a clinical response to standard antibiotic therapy for CDI.

FMT Capsule Preparation:

Screening of Donors: Donor material for the both capsules was produced at a large stool bank based on a previously described protocol (OpenBiome, Somerville) [14]. Briefly, donors were subjected to rigorous health and infection screening processes. Potential donors underwent an on-site clinical assessment and a 240-point health questionnaire, which collects information about the current health status and comprehensively screens for infections (current and past), chronic diseases, risky health behavior and conditions that are known to be associated with intestinal microbial dysbiosis such as autoimmune, neuropsychiatric and chronic inflammatory diseases. Thereafter, the potential donor undergoes a battery of stool and serological tests, aimed at screening for bacterial, viral and fungal infectious diseases [15]. In addition, a liver function panel and complete blood count are conducted. Abnormalities in any of the health screening questions or infection screens led to disqualification from the donor program. Those donors that successfully pass these check-points were eligible to donate stool for a period of 60 days. Subsequently, all material is placed into quarantine pending a physician review and release after repeat clinical assessment and infection screen at the end of the 60-day period, with a built in seroconversion window period. If both the day 0 and day 60 screening check-points are negative, the material was released for use. In addition, for safety and quality assurance purposes, all donors underwent regular brief health screens at each sample donation and repeat infection screens every 60 days. All active donors also underwent random health checks by a registered nurse.

FMTgr (upper GI delivery): These FMT capsules contain encapsulated minimally processed donor material in lipid carrier within a gelatin capsule. Previous stability work suggests that FMTgr capsules release the drug substance within minutes *in vitro* in simulated gastric fluid. Each capsule contained 0.75 grams of stool suspension.

FMTcr (lower GI delivery): These FMT capsules contain encapsulated minimally processed donor material. The capsules are film coated externally with Phloral®, a blend of bacterially-triggered polysaccharide and pH-responsive polymer. A dual-release mechanism takes place which utilizes pH and enzymes produced by the colonic microbiota; thus enabling colon specific delivery. Each capsule contained 0.75 grams of stool suspension.

Treatment Protocol

Patients referred for FMT who met the above criteria were eligible for capsule administration. Patients consumed one inert test capsule (identical to FMT capsule but without active drug substance) under direct observation to ensure there were no aspiration risk concerns. Oral vancomycin, metronidazole or fidaxomicin was discontinued 48 hours prior to capsule administration.

Each site was utilizing a different capsule formulation based on availability and were therefor considered two different cohorts. **Cohort A** received FMTgr at 1*) high dose*: 60

capsules (dose of 30 capsules on Day 0 and Day 1) or 2) *low dose*: 30 capsules (Day 0). Patients in this cohort received proton pump inhibitors for 2 consecutive days prior to capsule administration. Patients in **Cohort B** received FMTcr at 1) *high dose*: 30 capsules (Day 0) or 2) *low dose*: 10 capsules (Day 0). Patients within each cohort were enrolled consecutively with the higher dose followed by the lower dose with the goal of utilizing all available capsule doses to maximize access to patients.

Patients were assessed with phone calls at 72 hours, 1 week, and 4 weeks and with in clinic visits at 8 weeks post administration to assess for diarrheal symptoms as well as adverse events. If diarrheal symptoms recurred stool was sent for CDI testing by either enzyme immunoassay (EIA) for toxin and polymerase chain reaction (PCR) for presence of the *C. difficile* toxin gene or PCR alone (due to hospital availability/local standard of care) and if positive, patients were eligible for a second dose. Those in any cohort were offered retreatment with capsules for the second 'rescue' dose if needed. If the patients received a lower dose of capsules the rescue dose was the higher dose within that cohort. Patients were followed similarly after the second dose, if given. Stool samples were collected at baseline and the 8-week clinic visit.

Outcomes: The primary clinical outcome was clinical cure at 8 weeks after capsule administration. This was defined as the absence of diarrhea or a negative test for CDI if diarrhea was present. CDI recurrence at any time point during the follow up period was defined as diarrhea (3 or more unformed stools in a 24 hour period over 2 or more days) and a confirmed laboratory test for CDI. Adverse events were assessed via patient interviews, laboratory assessments and physical exams through week 8.

Microbiome Analysis

16S sequencing: Samples were collected for sequencing from donor stool and from patient stool at baseline and 8 weeks post-FMT. Samples were stored by flash freezing at -80C. DNA extraction, PCR amplification of the 16S rDNA V4 region, and Illumina paired-end sequencing was performed at the University of Michigan core facility, as described previously [16].

16S processing
Primers were trimmed, paired ends merged, and operational taxonomic units (OTUs) identified with a custom pipeline. In order to have maximum resolution for engraftment analysis, OTUs were defined by unique 16S sequences. OTUs represented in fewer than two unique samples and samples with fewer than 100 remaining reads were discarded. Taxonomic assignments for each OTU were called using UTAX trained on the RDP database.

Microbial community analysis.
For alpha-diversity calculations, samples were rarefied to the lowest sample read count (923 reads) and the Shannon diversity index was calculated for each sequenced stool sample. For engraftment analysis, samples from recurrent patients and with sequencing depth lower than 1000 reads were excluded, and the remaining samples were rarefied to

the lowest sample read count (13,022 reads). For each patient, engrafting OTUs were identified based on presence in both the FMT donor and the patient's post-FMT stool sample, as well as depletion in the patient's pre-FMT stool sample. For each unique genus, probability of engraftment was calculated as the fraction of patients where any OTU from that genus engrafted, conditioned on the presence of the genus in the donor; these probabilities were averaged across 20 independent rarefactions of the read count data. Engraftment bias was calculated as the difference in engraftment probability between the FMTcr and FMTgr cohorts. Paired t-tests were used to compare diversity before and after FMT, and independent t-tests were used for all other comparisons; all reported p-values are two-sided.

Results:

A total of 51 recurrent CDI patients were enrolled (Fig. 1). Baseline characteristics were similar between the sites: FMTgr [mean age: 57.1 years (SD=17.1), 70% female, and mean number of recurrences =3.9] and FMTcr groups [mean age: 63.3 years (SD=15.4), 67.8% female, and mean number of recurrence =3.6] (Table 1).

Patients at site 1 or Cohort A received FMTgr (n=20) and were divided into *high dose* (n=10) and *low dose* (n=10) arms and those at site 2 or Cohort B received FMTcr (n=31), and were divided into *high dose* (n=15) and *low dose* (n=16) arms.

Overall, the clinical cure rate at week 8 for FMTgr was 75% (15/20) [*high dose* 80% (8/10) and *low dose* 70% (7/10), p=.60]. In comparison, overall clinical cure rate for FMTcr was 80.6% (25/31) [*high dose* 80% (12/15) and *low dose* 81% (13/16), p=0.92]. The overall difference between FMTgr and FMTcr was not significant (75% vs 80.6%, p=0.63).

Three patients from Cohort B (FMTcr) received non-*C. difficile* antibiotics prior to the 8 week follow-up visit. No patients in Cohort A received non-*C. difficile* antibiotics. Given that exposure to antibiotics within 8 week of FMT increase FMT failure [17], we did assess cohort B without these patients as well. Removing patients who received antibiotics, Cohort B contained 27 participants and this was divided into *high dose* (n=13) and *low dose* (n=14) arms. Overall, clinical cure at week 8 for FMTcr was higher after removal of these three patients at 89% (24/27) [*high dose* 92% (12/13) and *low dose* 86% (12/14), p=0.58], though overall differences between FMTcr and FMTgr were still not significant (75% vs 89%, p=0.21) even after removal of the patient who had early exposure to non-*C. difficile* antibiotics.

Safety

Both capsule formulations were safe and well tolerated. All patients completed their full dose regardless of group assignment, and took no more than 30 minutes (average ~20 minutes). No serious adverse events attributed to the capsules occurred in either group. Mild adverse events were observed in both groups (Table 2). The most common events reported in both groups were bloating, flatulence, abdominal pain and constipation.

Microbiome Analysis:
FMTcr capsules increased diversity via superior engraftment compared to FMTgr. Prior to FMT, rCDI patient microbial communities in both FMTgr and FMTcr cohorts had a low community diversity (Figure 2a), and though patients across both cohorts significantly increased diversity from baseline ($p<0.01$), FMTcr capsules resulted in larger increases in gut microbial diversity than FMTgr ($p<0.01$), even when restricting our analysis to patients achieving clinical cure at the endpoint. As previously reported, rCDI patients have a dysbiotic gut community, with a relatively high abundance of *Proteobacteria* and *Fusobacteria* phyla (Fig 2b). Following treatment with FMTcr capsules, the taxonomic profile of patients looked more similar to the healthy donor profiles, whereas patients treated with FMTgr on average lacked reconstitution of the phylum *Bacteroidetes* (Fig 2b). We used exact 16S-matching to identify strains transferring from the donor and engrafting in the patient (see Methods). Importantly, even *10-capsule* FMTcr resulted in higher number of engrafter strains than either the 30-capsule or 60-capsule FMTgr (Fig. 2c, $p<0.02$), directionally consistent with the PP clinical cure rates. Examining the taxonomic identities of these engrafters, we found that FMTcr delivered individual genera with a higher probability (Fig 2d), across multiple phyla. In particular, *Bacteroidetes* were transferred better with FMTcr than FMTgr release capsules, consistent with their better representation in the communities of post-FMTcr -treated patients.

Discussion:

FMT has emerged as standard therapy for recurrent *C. difficile* infection [18]; however, the mode of delivery can differ by provider or healthcare center. The most common mode of delivery is either nasogastric tube or lower endoscopy. These methods can be uncomfortable and associated with additional procedure-related risks. Capsules appear to have a tolerable safety profile, preferred by patients and no bowel preparation is required making the administration easier for patients. Furthermore, capsules are accessible to more patients and treatment is not confined to tertiary centers. Here we compared two capsule preparations, upper GI release as well as targeted colonic release. While both capsules were safe and well tolerated and had similar clinical sure rates, FMTcr capsules did have high efficacy rates (though non-significant) and the microbial engraftment patterns were equivalent to FMT by colonoscopy, the most common current delivery modality.

In addition to delivery location, varying doses were also tested. Within both cohorts, the lower dosed treatments were equally as effective as the higher dosed treatments. Interestingly, the FMTcr was consistently more efficacious than the FMTgr, though not significantly so. When assessed in the context of the engraftment data, we saw that FMTcr at either dose resulted in superior engraftment compared to FMTgr. Following treatment with FMTcr capsules, the taxonomic profile of patients looked more similar to healthy donor profiles, whereas patients treated with FMTgr on average lacked reconstitution of the phylum *Bacteroidetes* (Fig 2b). It is possible that targeted delivery to the cecum, achieved by FMTcr but not FMTgr capsules, is required for colonization by these taxa. This is critical because the ability to provide the lowest most efficacious dose

will ultimately improve patient experience. At this point it remains unclear if engraftment parallels efficacy.

This study has several limitations. First, this study compared two separate cohorts of patients and in addition lacked a placebo arm, which hinders the ability to judge efficacy. Second, the study was not powered to compare proportions across 4 groups. Third, PCR was used as the standard CDI laboratory at one site and has been recognized to be problematic due to false positives [19, 20]; however, this may lead to an underestimate of the clinical cure rate.

Overall, targeted colonic administration appears result in engraftment profiles similar to that of colonoscopy even at lower doses. This may lead to high efficacy rates and larger studies are needed to assess this. To our knowledge, this is the first study to compare targeted delivery of FMT capsules. While both capsules were safe and efficacious, FMTcr had a higher microbial engraftment patterns superior to FMTgr.

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