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**Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhoea in Patients with Recurrent Clostridium difficile Infection**

JAMA. 2016;315(2):142-149. doi:10.1001/jama.2015.18098.

**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.

**Method:** 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 diarrhoea 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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**Clostridium difficile infection: a review of current and emerging therapies**

Annals of Gastroenterology (2016) 29, 147-154 (Review article)

Clostridium difficile (C. difficile) infection (CDI) is the most common cause of healthcare-associated infections in US hospitals. The epidemic strain NAP1/BI/ribotype  027 accounts for outbreaks worldwide, with increasing mortality and severity. CDI is acquired from an endogenous source or from spores in the environment, most easily acquired during the hospital stay. The use of antimicrobials disrupts the intestinal microflora enabling C. difficile to proliferate in the colon and produce toxins. Clinical diagnosis in symptomatic patients requires toxin detection from stool specimens and rarely in combination with stool culture to increase sensitivity. However, stool culture is essential for epidemiological studies. Oral metronidazole is the recommended therapy for milder cases of CDI and oral vancomycin or fidaxomicin for more severe cases. Treatment of first recurrence involves the use of the same therapy used in the initial CDI. In the event of a second recurrence oral vancomycin often given in a tapered dose or intermittently, or fidaxomicin may be used. Fecal transplantation is playing an immense role in therapy of recurrent CDI with remarkable results. Fulminant colitis and toxic megacolon warrant surgical intervention. Novel approaches including new antibiotics and immunotherapy against CDI or its toxins appear to be of potential value.

[*References studies that have compared the efficacy of fresh versus frozen samples*]

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Kellermayer R.,Hollister E.B.,Nagy-Szakal D.,Ihekweazu F.D.,Haynes A.,Pitashny M.,Bocchini C.E.,Luna R.A.,Versalovic J.

**Special considerations for fecal microbiota transplantation in pediatric recurrent clostridium difficile infection**

Gastroenterology, April 2015, vol./is. 148/4 SUPPL. 1(S961-S962), 0016-5085 (April 2015)

**BACKGROUND:** Fecal microbiota transplantation (FMT) is the most effective treatment for recurrent (antibiotic refractory) Clostridium difficile infection (rCDI). Higher asymptomatic carriage rates and more commonly existing underlying clinical conditions compared to adults provide special considerations for FMT in pediatric rCDI. We performed metagenomic analyses to evaluate the microbial mechanism of action of FMT with the incorporation of these pediatric considerations in a cohort of rCDI patients.

**METHODS:** Nine rCDI patients (1.5-16 year old) received filtered, frozen-thawed fecal preparation from screened, selfdesignated or universal donors through colonoscopy under an IRB approved protocol, followed by enema FMT, if clinically indicated. Two patients had inflammatory bowel disease (1 ulcerative colitis [UC], 1 Crohn disease [CD]), 1 had heart transplant, and 2 had significant neurologic impairment as underlying conditions. All patients provided a stool sample 1 day prior and 8 weeks after the first FMT. Select patients gave additional samples to be analyzed. The fecal microbiomes were studied by 454 pyrosequencing of the bacterial 16S rRNA gene.

**RESULTS:** All 4 patients without underlying disease had resolution of their symptoms for more than 2 months following a single FMT. One of these patients was found to be positive for C. difficile 4 months after FMT during an upper respiratory infection, but without gastrointestinal complaints. He was considered as an asymptomatic carrier. Metagenomic analyses indicated that FMT induced convergence of the recipient microbiomes to the donor's microbiome. Bacteroidetes specifically increased in abundance following FMT. Interestingly, asymptomatic carriage of C. difficile did not significantly modify microbiome composition. Out of the 5 patients with complicating clinical conditions, only 2 had obvious clinical benefit from C. difficile directed antibiotic therapies prior to FMT. These patients responded well to a single FMT. The UC patient required FMT 3 times to clear CDI, but still received colectomy during his subsequent clinical course. The CD patient's diarrhea did not respond to either antibiotics or FMT. One patient was diagnosed with poorly managed constipation and C. difficile carriage during her 2nd enema FMT.

**CONCLUSIONS:** FMT is highly effective for the treatment of pediatric rCDI in patients without complicating clinical conditions. Clinical improvement during C. difficile directed antibiotic therapy in the course of rCDI indicates a favorable outcome following FMT. FMT results in engraftment of donor bacterial species and reconstitution of recipient microbiomes. Asymptomatic C. difficile carriage may predispose to CDI. A special approach, incorporating these findings, should be made towards pediatric rCDI patients with underlying disorders when considering FMT.

Satokari R, Mattila E, Kainulainen V, Arkkila PE.

**Simple faecal preparation and efﬁcacy of frozen inoculum in faecal microbiota transplantation for recurrent Clostridium difﬁcile infection – an observational cohort study**

Aliment Pharmacol Ther. 2015 Jan;41(1):46-53. doi: 10.1111/apt.13009

**Background:** Faecal microbiota transplantation (FMT) is an effective treatment for recurrent Clostridium difﬁcile infection (rCDI). The ﬁnding of suitable donor, donor screening and preparation of faecal transplants are challenging inclinical work.

**Aim:** To develop a practical protocol for preparing frozen transplants and to compare the efﬁcacy of previously frozen and fresh faeces in treating rCDI.

**Methods:** Two healthy volunteers acted as universal donors for the frozen faecal preparations, which were prepared by suspending faeces into physiological saline, adding glycerol to a ﬁnal concentration of 10% and storing at -80°C. We compared the outcomes of patients with rCDI who had undergone FMT at colonoscopy and received infusion of previously pre-pared, freeze-stored faeces (n = 23) or fresh faeces from individual(n = 15) or universal donors (n = 11) (total n = 49). Clinical failure was deﬁned as persistent or recurrent symptoms with a positive C. difﬁcile toxins tool test, and a need for new therapy.

**Results:** At 12 weeks post-FMT, symptoms were resolved in 22 of 23 patients receiving previously frozen faeces, and in all 11 or 14 of 15 patients receiving fresh faeces from the universal or individual donors respectively (totally 25 of 26; P = ns, success rate 96%). Mild transient fever appeared for two patients receiving frozen faeces, but no other signiﬁcant side effects were observed. 42 patients were followed up for a year post-FMT and the success rate was 88% in both fresh and frozen faeces groups.

**Conclusions**: Preparation of frozen transplants simpliﬁes the practical aspects of faecal microbiota transplantation without loss of efﬁcacy or safety.

Costello S., La Brooy J., Tucker E., Holloway R., Schoeman M., Andrews J.M.

**Establishment of a fecal microbiota transplant service for the treatment of recurrent Clostridium difficile colitis in the Australian public hospital setting**

Journal of Gastroenterology and Hepatology (Australia), October 2014, vol./is. 29/(134), 0815-9319 (October 2014)

**Background:** Faecal microbiota transplantation (FMT) has previously been performed only outside mainstream gastroenterological care, but has recently been proven to be highly efficacious for recurrent Clostridium difficile colitis (CDC) refractory to antimicrobial therapy. The increasing prevalence and severity of CDC as well as the new randomized control trial data supporting the use of FMT in this setting has made the prompt establishment of well-structured hospital FMT services mandatory. From a large institutional viewpoint, there are technical and logistical issues in establishing such a non-standardized, non-drug therapy with due safety and governance. We describe our approach to establishing a FMT service in South Australia and the results of its first year.

**Methods:** An evidence based protocol for FMT was developed and submitted to a newly formed clinical procedures committee in the central Adelaide health network. The submission to the committee emphasized the strong randomized control evidence for effectiveness of FMT for CDC, the limited efficacy of current therapies for recurrent CDC and the favourable safety profile of FMT. Healthy donors were sought by advertisement, screened with a medical history, blood and stool tested focused for infection, autoimmune and metabolic diseases. A frozen stool bank was established by blending 50 g of donor stool with 130ml of saline and adding 20ml of pharmaceutical grade glycerol packaging these under anaerobic conditions into 200 ml aliquots. These were immediately frozen at -80 degrees Celsius and logged into a donor register. Recipient information sheets were developed. An FMT for CDC patient register was created that contained patient demographic details, date of first CDC episode, prior number of relapses, previous CDC therapy, date of FMT, donor sample used and method of FMT delivery. Patients were reviewed in clinic or contacted via telephone to confirm resolution of symptoms and report side effects 2 to 11 months after FMT. In those without complete resolution of diarrhoea a stool sample for CD toxin PCR was sent. Positive results were treated with repeat FMT.

**Results:** 7 potential donors were screened, 4 were excluded; 2 due to significant contraindications in their past medical history and 2 had detectable Dientamoeba fragilis DNA on stool PCR. 3 stool donors contributed to the frozen stool bank. From June 2013 to April 2014 we have successfully treated 9 patients with FMT for recurrent CDC. The average number of relapses prior to FMT was 3.4. Eight patients had FMT via colonoscopy into the caecum and 1 patient had FMT via enteroscopy into the jejunum. Seven of 9 achieved cure of CDC after a single FMT (follow up duration 2-11 months). Two patients had CDC relapse and had a second FMT, with cure in both. Five of the 9 patients received an antimicrobial during follow up with one suffering CDC relapse. There have been no complications from the FMT to date.

**Conclusion:** An FMT service with frozen stool bank has been successfully established in South Australia for patients with recurrent CDC. This was achieved by collaboration between the departments of Gastroenterology and Infectious Diseases and has resulted in the cure of all 9 patients treated thus far. We have shown this to be an effective approach to service implementation and confirmed this as an effective therapy in line with published data.

Perttu E. Arkkila, Eero Mattila, Veera Kainulainen, Reetta Satokari

**Simple and Practical Frozen Preparation for Transplantation of Fecal Microbiota for Recurrent Clostridium difficile Infection**

Gastroenterology (May 2014) Volume 146, Issue 5, Supplement 1, Pages S-193–S-194

**Background:** Transplantation of fecal microbiota (FMT) for recurrent Clostridium difficile infection (CDI) has been shown to be an effective treatment in over 90% of CDI patients. Colonoscopy seems to be the most useful route for FMT. The donor testing and the stool preparation has been a challenging task in clinical work.

**Methods:** We report clinical experience with 55 consecutive patients who were treated with FMT for recurrent CDI at Helsinki University Central Hospital. All the patients had got at least two antibiotic treatments for CDI prior the FMT. All the donors were equally tested, and FMT was performed by colonoscopy by standardized method. The patients received fecal transplant prepared either from fresh faeces or previously prepared, frozen faeces. The donor for fresh stool was the family members of the recipient or healthy volunteers and the fecal transplant was prepared as described previously (Mattila et al. Gas-troenterology. 2012;142(3):490-6). Frozen stool specimen was prepared by homogenizing 30g of stool into 0,9 % NaCl and adding glycerol to the final concentration of 10% and storing at − 80 ° C. The preparation was fast and the fecal suspensions were frozen within 1.5 hours from defecation Thawing was done over 4-5 hours in room temperature or water bath. The result of FMT was tested by control Clostridium difficile culture and toxins after one month, and by symptom follow-up up to one year.

**Results:** CDI was cured in 26 of 27 (96%; 95%CI 81-100%) (Mean age 53 years, range 22-81 years) of patients who got FMT by using fresh faeces, and in 16 of 17 patients (95%; 95%CI 71-100%) (mean age 61, range 20-88 years) receiving the frozen stool (p=

ns). Mild transient fever appeared for two patients receiving frozen stool, but no other significant side effects were found.

**Conclusions:** FMT is effective and safe treatment for CDI. Standardization of fecal material preparation by freezing the donated stool simplified the practical aspects of FMT without loss of efficacy.

Youngster, Sauk, Pindar, Wilson, Kaplan, Smith, Alm, Gevers, Russell, Hohmann

**Fecal Microbiota Transplant for Relapsing Clostridium difficile Infection Using a Frozen Inoculum From Unrelated Donors: A Randomized, Open-Label, Controlled Pilot Study**

Clin Infect Dis. (2014) doi: 10.1093/cid/ciu135

**Background:** Recurrent Clostridium difficile infection (CDI) with poor response to standard antimicrobial therapy is a growing medical concern. We aimed to investigate the outcomes of fecal microbiota transplant (FMT) for relapsing CDI using a frozen suspension from unrelated donors, comparing colonoscopic and nasogastric tube (NGT) administration.

**Methods:** Healthy volunteer donors were screened and a frozen fecal suspension was generated. Patients with relapsing/refractory CDI were randomized to receive an infusion of donor stools by colonoscopy or NGT. The primary endpoint was clinical resolution of diarrhea without relapse after 8 weeks. The secondary endpoint was self-reported health score using standardized questionnaires.

**Results:** A total of 20 patients were enrolled, 10 in each treatment arm. Patients had a median of 4 (range, 2–16) relapses prior to study enrolment, with 5 (range, 3–15) antibiotic treatment failures. Resolution of diarrhea was achieved in 14 patients (70%) after a single FMT (8 of 10 in the colonoscopy group and 6 of 10 in the NGT group). Five patients were retreated, with 4 obtaining cure, resulting in an overall cure rate of 90%. Daily number of bowel movements changed from a median of 7 (interquartile range [IQR], 5–10) the day prior to FMT to 2 (IQR, 1–2) after the infusion. Self-ranked health score improved significantly, from a median of 4 (IQR, 2–6) before transplant to 8 (IQR, 5–9) after transplant. No serious or unexpected adverse events occurred.

**Conclusions:** In our initial feasibility study, FMT using a frozen inoculum from unrelated donors is effective in treating relapsing CDI. NGT administration appears to be as effective as colonoscopic administration.

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**Standardized Frozen Preparation for Transplantation of Fecal Microbiota for Recurrent Clostridium difficile Infection**

The American Journal of Gastroenterology 107, 761-767 (May 2012) doi:10.1038/ajg.2011.482

**Objectives**: While fecal microbiota transplantation (FMT) is historically known to be an effective means to treat recurrent Clostridium difficile infection (CDI) refractory to standard antibiotic therapies, the procedure is rarely performed. At least some of the reasons for limited availability are those of practicality, including aesthetic concerns and costs of donor screening. The objective of this study was to overcome these barriers in our clinical FMT program.

**Methods:** We report clinical experience with 43 consecutive patients who were treated with FMT for recurrent CDI since inception of this program at the University of Minnesota. During this time, we simplified donor identification and screening by moving from patient-identified individual donors to standard volunteer donors. Material preparation shifted from the endoscopy suite to a standardized process in the laboratory, and ultimately to banking frozen processed fecal material that is ready to use when needed.

**Results:** Standardization of material preparation significantly simplified the practical aspects of FMT without loss of apparent efficacy in clearing recurrent CDI. Approximately 30% of the patients had underlying inflammatory bowel disease, and FMT was equally effective in this group.

**Conclusions**: Several key steps in the standardization of donor material preparation significantly simplified the clinical practice of FMT for recurrent CDI in patients failing antibiotic therapy.

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