Fecal Microbiota Transplantation for *Clostridium difficile*-Associated Diarrhea

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ABSTRACT: *Clostridium difficile*-associated diarrhea is a problem most hospital-based physicians will face in their career. This review aims to refresh current knowledge with regard to *Clostridium difficile* infection and bring physicians up to date with the latest developments in the growing field of fecal microbiota transplantation, the benefits it offers, and the promise this and other developments hold for the future.

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Antibiotic-associated colitis caused by *C. difficile* is the most common cause of hospital-acquired diarrhea and a major cause of morbidity and mortality within the elderly hospitalized patient population. In the United States the number of cases doubled during the years 2000 to 2009. This condition is the result of the widespread use of antibiotic therapy, which disrupts the normal gut flora, causing dysbiosis (a change of the normal gut bacterial population) that enables colonization of the intestinal tract by *C. difficile* [1].

Clostridium difficile infection is the most common hospital-acquired infection, placing an increasing burden on the health care system

Since 2000, there have been significant increases in the incidence and severity of health care-associated *C. difficile* infection (CDI), particularly in patients over 65 years old. It was found that about 20% of patients with negative stool culture on admission become infected during their hospitalization. These patients, although mostly asymptomatic, then become reservoirs for environmental contamination to other hospitalized patients as they shed spores of *C. difficile* [1].

Due to the dramatic increase in CDI rates, many efforts are invested in improving treatment. However, until recently, none of the regimen protocols succeeded in eradicating this bacterium, and a sizable number of patients suffer from recurrent CDIs that result in severe morbidity and sometimes death. In addition, the burden of this infection on patients' families and on the health care system are also significant owing to the cost of antibiotics, multiple hospitalization days, complications, and the need to isolate these patients to prevent transmission to others.

Recently, it was shown that a simple and cheap procedure, fecal microbial transplantation (FMT), can eradicate *C. difficile* in up to 90% of cases. In this review, we discuss the risk factors for CDI and the current treatment approach, describe the FMT procedure, list its indications, and present evidence supporting the use of FMT for CDI.

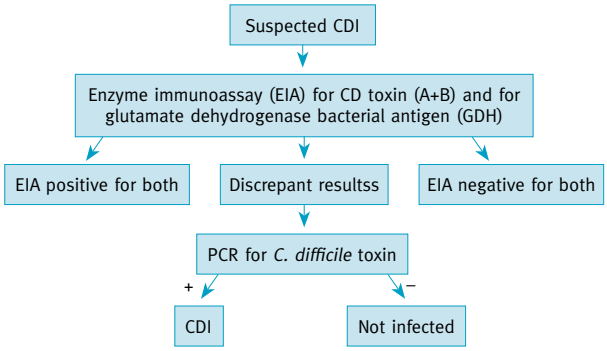
RISK FACTORS ASSOCIATED WITH CDI

Identification of risk factors for *C. difficile* infection in patients with diarrhea is an important step in early diagnosis and treatment in these patients. Antibiotic usage is the greatest risk factor for CDI, increasing the risk eight- to tenfold during and for one month after usage and threefold for the subsequent 2 months [2]. The most commonly implicated antibiotics are ampicillin, clindamycin and third-generation cephalosporins [3]. Other risk factors include anti-neoplastic agents, corticosteroids, increasing age, use of stool softeners and gastrointestinal stimulants, enteral feeding, recent hospitalization, chronic dialysis, and residency in long-term care facilities. Whether the use of proton pump inhibitors is a risk factor remains controversial, but a meta-analysis by Janarthanan et al. [4] found them to be a significant risk factor in CDI. It is currently thought that apart from an impaired immune response, most of these risk factors alter the enteric microbial population which allows *C. difficile* colonization.

PATHOGENESIS

Clostridium difficile is an anaerobic, gram-positive, spore-forming and toxin-producing bacillus. *C. difficile* can exist in spore and vegetative forms and these spores are resistant to heat, acid and antibiotics. Once these spores enter the small intestine they convert to fully functional vegetative forms and start producing toxins [1]. *C. difficile* releases two potent exotoxins: an enterotoxin (Toxin A) and a cytotoxin (Toxin B). These toxins lead to intestinal fluid secretion, mucosal injury and inflammation

Figure 1. Flow chart for the diagnosis of *Clostridium difficile* infection (CDI)



PCR = polymerase chain reaction

[5]. Toxin A can directly activate neutrophils, while both toxins can promote neutrophil chemotaxis to localize in pseudomembranes [6]. It was found that Toxin B is essential for the virulence of *C. difficile*, and strains lacking Toxin A can be just as virulent as strains with both toxins [7].

DIAGNOSIS

The diagnosis of CDI is based on the presence of the following factors: moderate to severe diarrhea, ileus, and stool positive for either *C. difficile* toxins or toxigenic *C. difficile* [Figure 1]. Endoscopy with biopsy can be an adjunctive tool for diagnosis in patients with a high clinical suspicion and negative laboratory tests, when a prompt diagnosis is required, when there is no response to treatment, or when the presentation is atypical [8]. Additional tools for the diagnosis of CDI were recently described, including a new immunochromatography test that has proven to be quicker, cheaper and more accurate than regular tests [9].

CURRENT TREATMENT PRACTICES

Current treatment guidelines categorize patients into those with mild/moderate, severe, severe-complicated (based on leukocyte counts and renal function), and recurrent infection. As shown in Table 1, initial treatment is usually oral metronidazole for mild/moderate disease, oral vancomycin for severe disease, and a combination of oral vancomycin and intravenous metronidazole for severe-complicated disease [10]. However, evidence to support these recommendations is controversial [3].

Fecal microbiota transplantation is a safe, effective and durable treatment for recurrent *C. difficile* infection, proving more effective than standard medical therapy

Up to 20% of patients suffer from CDI recurrence after the initial treatment and 40%–65% after treatment for a second episode [11]. Newer treatments that were recently approved for recurrent CDI and experimental treatments currently being tested are delineated in Table 2.

Table 1. Standard treatment of *Clostridium difficile* infection [10]

	Mild/Moderate*	Severe*	Severe-complicated*
Initial treatment and first recurrence	Metronidazole 500 mg orally 3 times daily	Vancomycin 125 mg orally 4 times daily	Vancomycin 500 mg orally 4 times daily and metronidazole 500 mg intravenously 3 times daily
Second recurrence	Vancomycin orally with tapering dosage		

*Determined by leukocyte count and renal function

Table 2. Treatment options investigated for use in CDI

Treatment	Detail	Evidence
Rifaximin	Semi-synthetic, non-absorbable antibiotic	Has shown benefit in preventing recurrence when administered immediately after a course of vancomycin [34]
Fidaxomicin	Narrow-spectrum macrocyclic antibiotic	8 times more active in vitro than vancomycin [35]. Decreased recurrence of CDI compared to vancomycin [36]
Intravenous immune globulin (IVIG)	Administered in addition to antibiotics	Mixed results, requires further investigation [37]
Vaccination against Toxins A + B	Chimeric toxin vaccine	Conferred protection against numerous strains of *C. difficile* and prevented relapse [38]
Sanofi Pasteur [40] anti-toxin vaccine	Bivalent formalin-inactivated vaccine against Toxins A + B	Seroconversion in 75% of patients [39]

One of the most promising treatments is fecal microbiota transplantation (FMT), which has demonstrated up to 90% efficacy in eradication of CDI and was recently approved by the U.S. Food and Drug Administration as well as by the Ministry of Health in Israel. The aim of FMT is to correct the gut dysbiosis and reestablish a normal and functional intestinal microbiota in patients with CDI.

FECAL MICROBIOTA TRANSPLANTATION

The use of fecal content in medicine is not new. The first evidence of its usage comes from China. During the Dong Jin dynasty in the 4th century AD, Ge Hong, a well-known Chinese traditional medicine doctor, described the use of human fecal suspension by mouth for patients who had food poisoning or severe diarrhea. Astonishingly, this yielded positive results [12]. During the Ming dynasty in the 16th century, herb doctors used a fecal suspension called "yellow soup" to treat abdominal pain and severe diarrhea [13]. Later, in the 17th century, Italian anatomist Fabricius Aquapendente used FMT in veterinary medicine [14]. Its first application in more recent times was in 1958 in the USA. In that study, Eiseman et al. [15] reported four patients with pseudomembranous colitis (before identification of *C. difficile*) who were treated with fecal

enemas and all four had prompt resolution of their symptoms. The first report of FMT for an established CDI infection was published in 1983 [16].

DONOR SELECTION

FMT transplant material is medically classified as human tissue [17]. Potential donors should be screened for risk of infectious agents such as human immunodeficiency virus, hepatitis B or C infections, high risk sexual behavior, use of illicit drugs, tattoo or body piercing within 6 months, known current communicable disease, or travel within the previous 6 months to high risk areas. Donors are similarly excluded if they have a history of inflammatory bowel disease, irritable bowel syndrome, constipation or diarrhea, gastrointestinal malignancy, or polyposis. Potential donors should not have used antibiotics or immunosuppressive agents for 3 months prior to donation. Other exclusion criteria include a history of gastrointestinal surgery, metabolic syndrome, or systemic immune mediated diseases [18].

Future research will lead to a standardized and more acceptable method of transplanting the gut microbiome to make this therapy available to a greater number of people and perhaps turn it into first-line treatment for CDI

There is a debate whether or not a donor should be a family relative. Using donors who live in the same household may minimize the risk of transmitting infectious disease since, theoretically, they already share most of the microbial species in their intestinal microbiota with the recipient [18]. However, in support of using rigorously screened, healthy, unrelated and young donors is the fact that intestinal microbiota are potentially involved in a number of systemic diseases, such as metabolic syndrome, diabetes, and inflammatory bowel disease [19]. There are other benefits to using anonymous donors: the burden of finding a donor is removed from the patient; and donors with a proven cure history can be used, reducing the risk of similar susceptible microbiota in related donors [17]. It has also been suggested that men are preferred donors over women as the microbiota that females carry might result in a higher risk of irritable bowel syndrome in recipients [18].

THE FMT PROCEDURE

When fresh samples are used, donor stool is delivered to the institution within a few hours of passing; saline solution or water is added to the sample which is blended to achieve a homogenous liquid. This liquid is then filtered to remove particulate matter. Bowel preparation is required in order to clear the gut and reduce the abnormal host microbiota and allow for the more effective implantation of donor microbiota [17]. With regard to administration of the fecal solution, various routes are possible: nasoduodenal, transgastroscopic, transcolonoscopic, or enema based. The limitations of nasoduodenal infusion include patient reluctance, the risk of vomiting, and the introduction of lower gastrointestinal (GI) microbiota into the upper GI which may not be physiologic. In contrast, colonoscopic administration allows for the assessment of disease severity at the time of transplantation, the bowel preparation having removed abnormal microbiota which allows the new "healthy" microbiota to have greater effect. The benefit of enema administration is its cost-effectiveness and safety. In terms of results, one systematic review reported a success rate of 95% for enemas, 89% for colonoscopy and 76% for nasoduodenal administration [20]. The latest work on the topic of FMT, by Dutta and co-authors [21] who used a combined jejunal and colonic transplantation approach, showed a 100% response rate to FMT. This is the first indication that combined transplantation from the upper and lower GI tract, despite incurring increasing cost, may provide the best and most lasting results.

Another option for FMT currently under study is the use of frozen stool samples. Research by Borody and Khoruts [22] described the successful use of this technique. The benefits of using frozen stool preparation include the ability to standardize fecal samples for donation, and the establishment of a stool bank to ensure availability of material for transplantation.

EFFECTIVENESS OF FMT

Table 3 shows the results of numerous studies investigating the effectiveness of FMT in recurrent CDI. These data show FMT to be a highly effective and durable treatment for recurrent CDI, proving more effective than standard treatment using vancomycin in patients with CDI regardless of age, disease severity or comorbidities.

Perhaps as important as its success in the treatment of CDI was the finding that FMT is a safe and durable mode of treatment [23-27]. The procedure is also acceptable to patients, with one study noting that 97% of patients who underwent FMT

Table 3. Studies of fecal microbiota transplantation (FMT) for CD

Authors [ref]	Study design	Result
Garborg et al. [23]	Retrospective study	40 patients with recurrent CDI previously treated with vancomycin had 83% success rate with FMT
Mattila et al. [25]	Multicenter retrospective study	70 patients treated with FMT had 94% success rate
Brandt et al. [24]	Multicenter long-term follow-up study	Long-term durability of FMT success, with 77 patients (› 90%) remaining recurrence free post-FMT
Van Nood et al. [26]	Randomized controlled trial	FMT was more effective than vancomycin for recurrent CDI. Of 16 patients in the FMT group 81% were cured compared to 31% in the vamcomycin group
Cammarota et al. [27]	Systematic review	536 patients with CDI treated with FMT had a cure rate of 87%

would undergo another FMT if they had recurrence of disease and 53% stating they would choose FMT as first-line treatment over antibiotics [24].

MECHANISMS OF ACTION

In gastrointestinal homeostasis, the diverse commensal microbiota prevent the colonization and overgrowth of pathogenic microbes. Antibiotic therapy damages this balanced commensal gut microbiome, allowing *C. difficile* to dominate and cause disease. FMT reintroduces normal microbiota and restores this balance, allowing the suppression of *C. difficile* and rebuilding colonization resistance [10]. It was shown that in mice chronically infected with CDI, there was decreased microbial diversity, upregulated pro-inflammatory cytokines, and reduced butyrate level. In these mice, vancomycin reduced the shedding of *C. difficile*; however, the bacteria shedding returned to pretreatment values following cessation of therapy. In contrast, FMT and targeted treatment with six specifically identified, phylogenetically diverse species of obligate and facultative anaerobes suppressed the shedding and resolved the CDI and its contagiousness, partly by restoring gut diversity [28].

Another presumed mechanism involves gut permeability. It is thought that FMT may decrease gut permeability through increased production of short chain fatty acids, thereby decreasing CDI severity. Increased production of short chain fatty acids, particularly butyrate (which is the main energy source of epithelial cells in the colon), helps to maintain epithelial barrier integrity through reducing intestinal permeability [29]. Recently, a study by Dutta and team [21] found that FMT increased fecal microbiota diversity and increased the relative proportions of butyrate-producing bacteria, namely, Lachnospiraceae. These findings not only support the mechanism of action presumed above but indicate that Lachnospiraceae may be the key bacterial family responsible for the success of FMT. This finding may lead to even more targeted and standardized fecal microbiota transplantation.

PROCEDURAL SAFETY AND COMPLICATIONS

FMT is a safe procedure in most patients and was recently demonstrated to be safe in immune compromised patients as well [30]. Following the procedure, transient GI symptoms or altered bowel habits commonly occur – such as altered bowel habits, abdominal cramping, increased gaseousness or bloating – which resolve within several days.

The usual post-endoscopic complications of perforation and hemorrhage may also occur. Other concerns about FMT include the transfer of metabolic and immune mediated diseases such as obesity, diabetes, inflammatory bowel disease and irritable bowel syndrome, particularly in light of current research on the causal relationship that the gut microbiota has with these diseases. While FMT appears to be a safe procedure, further studies with large numbers of patients and long-term follow-up are needed to assess additional risks that may be mediated by the transplanted microbiota.

OUR EXPERIENCE

We recently established the Bacteriotherapy Service at the Tel Aviv Medical Center. We perform FMT by means of a colonoscopy or gastroscopy and maintain a bank of frozen stool preparations donated by meticulously screened donors. These preparations are ready to be used for CDI patients as well as for research purposes. CDI patients who suffer from a recurrent proven CDI and have failed vancomycin treatment are eligible for FMT. A summary report of our experience will be published in the near future.

QUESTIONS FOR THE FUTURE

FMT has proven to be a safe and effective treatment for recurrent CDI. However, some questions have yet to be resolved, such as whether this therapy should be used as first-line treatment at the initial presentation of CDI and, if not, how to risk stratify patients accordingly. The answers to these questions will probably be determined by the procedure's safety, cost-effectiveness and patients' acceptance. Studies are also required to investigate the most effective mode of transplantation (duodenal or colonic), whether donors should be relatives or not, and whether material should be frozen or fresh.

While the above questions are being investigated, it is obvious that FMT will not maintain its current form for long and that eventually we will be able to administer a probiotic pill that will contain the beneficial bacteria required to eliminate *C. difficile*. A significant innovation in this field has already occurred. A group in the USA was able to develop multilayered capsules containing concentrated donor fecal matter [31] in order to eliminate the need for endoscopy. Twenty-seven CDI patients with more than three episodes of recurrent CDI swallowed up to 34 capsules and achieved 100% cure rate. A study by Khanna et al. [32] has proven the success of an orally delivered community of microbes, namely, a 100% cure rate in treating recurrent CDI and an excellent safety profile. A novel formula for FMT is described by Petrof and collaborators [33], where purified intestinal bacterial cultures consisting of 33 isolates from a single healthy donor were used to treat CDI in two patients who had failed standard treatment. CDI was resolved in both patients and normal bowel behavior, which lasted through 6 months of follow-up, was restored. Although these studies show promising results, there are still potential obstacles to overcome, such as demonstrating efficacy in severely ill patients (who were not included in most of those studies), and preventing industrially cultured bacteria (as opposed to stool-isolated bacteria) from undergoing mutations that will prevent their efficacy.

Currently, the use of FMT for CDI is becoming increasingly popular worldwide and demonstrates impressive and consistent results. Once we learn which specific bacteria are required

to eradicate CDI, this therapy will likely become the first-line therapeutic intervention for this disease.

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"Men never do evil so completely and cheerfully as when they do it from religious conviction"

Blaise Pascal (1623-1662), French mathematician, physicist, inventor, writer and Christian philosopher