Fecal Transplant for Recurrent *Clostridium difficile* Infection

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ABSTRACT

Clostridium difficile infection (CDI) results in clinical manifestations ranging from mild diarrhea to life-threatening pseudomembranous colitis. Infection is most often initiated by antimicrobial therapy which causes an imbalance in normal colonic microflora. The pathogenesis of *C. difficile* is predominantly controlled by the production of its two cytotoxins, A and B, which damage the intestinal mucosa. In recent years a nationwide increase in the rate of CDI has been noted as well as an increase in mortality, reduced initial response to antimicrobials, extended resolution time, and increased rates of recurrence. Traditional treatment includes administration of antimicrobials. Fecal microbiota transplant (FMT) is an alternative therapy for CDI that is effective and promising in multiple CDI relapse patients. This paper will provide an overview of CDI epidemiology, pathogenesis, diagnosis, and treatment, and explore the case of a 53-year-old woman suffering from her sixth episode of CDI.

ABBREVIATIONS: CDI - *Clostridium difficile* infection, PMC - pseudomembranous colitis, FMT = Fecal microbiota transplant, GTPases - guanosine triphosphatases, GDH - glutamate dehydrogenase, EIA -= enzyme immunoassay, NAAT - nucleic acid amplification test

INDEX TERMS: *Clostridium difficile*, enterocolitis, *Clostridium difficile* toxin, fecal transplant

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Epidemiology

Clostridium difficile was discovered by Hall and O'Toole in 1935 who initially described the organism as a normal constituent of colonic flora in newborn infants.[1] It wasn't until the late seventies that *C. difficile* was recognized as the major cause of antibiotic-associated diarrhea and pseudomembranous colitis (PMC). Today, use of broad-spectrum antimicrobial agents continues to escalate the occurrence of *C. difficile* infection (CDI). It has become a prevalent nosocomial pathogen, causing approximately one quarter of antibiotic-associated diarrhea cases, two-thirds of antibiotic-associated colitis cases, and nearly all PMC cases.[2]

C. difficile has been isolated from the intestines of healthy individuals as well as from environmental sources. Risk factors include antimicrobial therapy, advanced age, underlying illness, gastrointestinal surgery, hospitalization or admission to a long-term care facility.[3,4]

There was a substantial increase in discharge rates for *C. difficile* infections in the 2000's and it is currently estimated that more than 500,000 cases of CDI are diagnosed each year.[5,6] The highest incidence is noted among elderly patients, in hospitals and long-term care facilities, after administration of antibacterial therapy. A lower but increasing rate has been reported in other populations: outpatients receiving antibiotics or proton-pump inhibitors, children with an immunological disadvantage or prior gastric surgery, and post-partum women.[6,7]

The increasing incidence is attributed to the spread of a

particularly virulent *C. difficile* strain. Molecular analysis was used to classified this strain as belonging to PCR ribotype 027, restriction endonuclease analysis group BI and North American pulse-field type 1 (027/NAP1/B1).[8,13] It is primarily associated with nosocomial epidemics and outbreaks but has also been detected in the community setting.[7,8,13]

CDI is typically treated with antimicrobial agents but approximately 24% of patients develop a second episode of *C. difficile* disease. Once a patient has had a single relapse of CDI the chance of developing another case becomes significantly higher.[9] Moreover, the wide spread use of antimicrobials has contributed to the increase in resistance among *C. difficile* isolates. A recent, clinically proven alternative to conventional therapy is fecal microbiota transplant (FMT) which involves repopulation of the gut with normal fecal flora.[2,10]

Pathogenesis

Administration of antibacterial agents kills many constituents of normal fecal flora. This allows overgrowth of *C. difficile* with subsequent toxin production. The effects of CDI range from self-limiting diarrhea to life-threatening inflammation of the large intestine. When antibiotic-associated diarrhea persists it may lead to colitis, an inflammation of the colon. PMC results when the inflammation is severe and pseudomembranous plaques form in the gut. Other indicators of infection are fever, loss of appetite, nausea, and abdominal pain.

C. difficile is a Gram-positive, anaerobic, spore-forming bacillus. However, its spores do not need anaerobic conditions to survive and may be recovered from a variety of surfaces for more than three months after exposure. The spores are characteristically durable and strong. As a result, they are difficult to eradicate with disinfectants and are often resistant to even the strongest antimicrobial agents. The ability of spores to remain on surfaces for extended periods of time is a major factor contributing to reoccurrence of infection.[11]

During CDI, spores germinate and rapidly outgrow normal flora.[11] As *C. difficile* proliferates in the large bowel, it utilizes multiple virulence factors to establish infection.[4,11,12,13] Proteases and flagella allow *C. difficile* to penetrate the mucous layer and multiple adhesins are utilized to allow the organism to coat the intestinal epithelial cells.[4,11] The second phase of CDI results in production of two primary protein toxins, TcdA (toxin A) and TcdB (toxin B). These cytotoxins elicit inflammation and mucosal damage by inactivating the host's Rho and Ras family guanosine triphosphatases (GTPases) through glucosylation. The Rho and Ras family GTPases control an array of biochemical mechanisms in cells that include: cycle progression, cell to cell adhesion, cytokinesis, and maintenance of the cytoskeleton. The loss of ability to maintain the cell's cytoskeleton induces cell rounding and apoptosis. These are the two key features of cell intoxication and lead to the symptoms associated with CDI. [11,12,13]

Diagnosis

C. difficile is an obligate anaerobe producing large, flat colonies on blood agar. The colonies produce a "horse stable" odor and fluoresce chartreuse under ultraviolet light. The organism is isolated from stool samples by the characteristic appearance of yellow, ground-glass colonies on cycloserine-cefoxitin-fructose agar.

Screening tests utilize the detection of the *C. difficile* antigen, glutamate dehydrogenase (GDH). Since GDH is produced by toxin and non-toxin producing strains, this test may only be used to rule out infection. Positive tests should be followed by toxin or gene detection assays. Enzyme immunoassays (EIA) are commercially available, detect toxin A and toxin B in stool sample, and provide results in a few hours. Cytotoxin neutralization assays examine the cytopathic effect produced by toxin and the reversal of this effect by antitoxin. However, both EIA and cytotoxin neutralization assays lack sensitivity and negative results should be confirmed using toxigenic culture or nucleic acid amplification tests (NAAT).

Toxigenic culture involves culturing stool and identifying suspicious colonies followed by tissue culture cytotoxicity testing. This is the current gold standard method, however, it requires several days to deliver results. NAAT are more costly but provide sensitivity, specificity, and rapid detection of the toxin gene in stool samples.[14,15,16,19,20] See Table 1 for *C. difficile* testing.

Treatment

Recommended treatments for *C. difficile* colitis typically include oral antimicrobial therapy of metronidazole or

Table 1. Clostridium difficile Testing[14,15,16,19,20]

Method	Test Type	Sensitivity*	Specificity*	TAT† (hours)
Stool Culture	screening	high	low	24-48
Glutamate Dehydrogenase	screening	high	low	<1
Toxin A/B EIA	screening	low	high	1-4
Cytotoxin Neutralization	screening	low	high	24-48
Nucleic Acid Amplification Test	confirmatory	high	high	<1
Toxigenic Culture	confirmatory	high	high	48-72

*high, greater than 90%; low, less than 85%
†Turnaround time

vancomycin.[9] This course of treatment is clinically effective in the majority of CDI cases with no further incidence of reoccurrence. Failure to respond to therapy or recurrence of CDI is noted in 8 – 36 % of patients with primary CDI.[4] Reoccurrence after initial infection typically occurs within 7-10 days after treatment.[1,17] Additionally, the likelihood of reoccurrence increases with each reinfection of *C. difficile*. In recent years, CDI has become more frequent, severe, and more resistant to standard treatments. Successful treatment of relentless CDI continues to be clinically challenging. FMT is an emerging technique in the field of medicine for treatment of reoccurring CDI cases and has gained popularity due to the growing CDI epidemic and the emergence of the hypervirulent *C. difficile* strain, 027/NAP1/B1.[13,18]

FMT, also known as fecal bacteriotherapy, is the process of delivering a saline stool suspension from a healthy, preapproved donor into the patient's upper gastrointestinal tract. Delivery is achieved with a nasogastric tube or gastroscopy, or directly into the colon by colonoscopy or a rectal catheter. No studies have suggested that either of the two methods of stool transplantation has substantial clinical advantages over the other.[2,9]

FMT aids in the restoration of bacterial homeostasis in the intestinal environment to resolve the diarrhea and colitis that is attributed to overgrowth of *C. difficile*.[1,10] Studies have shown that the transplanted bacteria remain in the host's intestines and exert protective effects to reverse gastrointestinal infection by durably altering the colonic environment.[18] In cases where conventional therapies have failed, stool transplantation is advantageous over repetitious treatment attempts with antibiotics. Aas et al., highlights that most importantly, FMT breaks the continuous cycle of antimicrobial use, which may perpetuate or renew the disruption of the intestinal flora. Additionally, stool transplantation decreases the risk of problems associated with antimicrobial use, such as the development of antimicrobial-resistant enteric bacteria and allergic reactions. Candidates for FMT are patients who have undergone the routine regimen of antimicrobial therapy but continue to have CDI reoccurrence, or those who do not respond to antimicrobial therapy.[9]

Optimal donors for the procedure include spouses or close relatives. A close relationship is preferred as they are more likely to possess similar gastrointestinal flora. This decreases the risk of introducing new pathogens to the patient. Donors must undergo a regimen of laboratory screening to ensure that they themselves are not asymptomatic *C. difficile* carriers and do not have any infectious diseases that could be transmitted to the recipient. All symptoms generally improve immediately after FMT and cure rates as high as 94% have been reported.[18]

Conclusion

The treatment of recurrent CDI continues to be a challenge to healthcare professionals and clinicians. FMT is a promising technique that offers an alternative to traditional antimicrobial treatment of recurring and severe *C. difficile* colitis. Through FMT, normal enteric flora can be restored with resolution of gastrointestinal infection. Additional trials and further research need to be done to definitively mark this as an effective measure against CDI but, to date, it has proven advantageous for the majority of the patients who have undergone the treatment.

Case Study

A 53-year-old woman with a past medical history of depression, hypertension, and coronary artery disease, suffers from recurrent CDI with symptoms including: abdominal pain, diarrhea up to 14 times per day, and fever. She reported a 40-pound weight loss over the past year as a result of decreased appetite. She presents with her sixth episode of CDI in the past 13 months. Her first CDI episode occurred after the completion of a 7-day regimen of levofloxacin for treatment of a urinary tract infection. After her initial episode of CDI, she

experienced 4 relapses within 9 months. These relapses were treated with several courses of conventional antimicrobials, but the attempts to rid the patient of CDI proved to be unsuccessful. Following her initial relapse, she received an oral treatment of metronidazole. The 2nd and 3rd relapses were treated with a combination of oral vancomycin therapy followed by rifaximin. Her 4th relapse was treated with a course of oral vancomycin in addition to 8 weeks of probiotics. She experienced no symptoms for approximately 4 weeks following the completion of her vancomycin and probiotic therapy. The patient reported that she has not taken any recent antibiotics or medications, and has not eaten any unusual food.

Examination of the patient showed a temperature 38.3 °C. Her abdomen was tender to touch. Bowel sounds were hyperactive. The patient's initial laboratory assays showed an elevated white blood cell count of 33.3K/μL with 93% neutrophils. Her liver enzymes and basic metabolic panel were normal except for a decreased serum potassium level of 2.7 mmol/L. A stool sample was positive for *C. difficile* toxin B by PCR but was negative for the more virulent 027-NAP1-BI C strain. Additional tests for other disease states that often result in diarrhea, including HIV-1/2 antibody, stool for ova and parasite, *Giardia* antigen, celiac disease serology, and thyroid hormone, were negative.

On the patient's first day in the hospital, she was put into contact isolation to prevent exposing others to *C. difficile*. She was placed on fidaxomicin, an oral antibiotic, twice a day and *Lactobacillus* therapy for 10 days. On day seven, her condition was re-evaluated. A colonoscopy of the patient showed mild colitis without PMC. The patient's symptoms improved slightly, but she still suffered from continuous diarrhea. Due to her multi-recurrent *C. difficile* colitis even after repeated rounds of antimicrobial therapy, it was concluded that FMT was the best treatment. The patient's two daughters were screened as viable donors. A laboratory microbiologist and an infectious diseases pharmacist prepared a saline stool sample for transplant. The patient received the stool solution via colonoscopy. This method was selected because it allowed for instillation of stool throughout the entire colon as well as thorough cleaning and direct inspection of the colon at the time of transplantation. No adverse side effects were noted from the procedure. The patient was instructed to have her home bleached to remove *C. difficile* spores before her return. The patient remained symptom free on her follow-up visits at 2, 8 and 12 weeks post-procedure and continues to show no symptoms of CDI.

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