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Fecal Bacteriotherapy for the Treatment of Recurrent Clostridium Difficile Infection in Adults

Julie L. Cramer
Pacific University

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Abstract

Background: Clostridium difficile infection (CDI) has emerged as a rapidly growing problem associated with the widespread use of broad-spectrum antibiotics. These antibiotics disrupt the natural bacterial flora in the colon and create an opportunity for C. difficile to invade and multiply. Current treatment with oral vancomycin and metronidazole is effective in many cases, however up to 35% of patients experience a relapse of CDI after completion of treatment, placing them at increased risk for recurrent C. difficile infections (RCDIs). Alternative therapies consist of probiotic and immunotherapy treatments, which are helpful in preventing CDI, but not at stopping the infection once it exists. Surgery is also an option, however it has many serious implications. Recently, fecal bacteriotherapy, or stool transplantation, has been successful at eradicating RCDI. Fecal bacteriotherapy uses the complete normal human flora as a therapeutic probiotic mixture of living organisms, transporting the healthy flora from a donor to a recipient with RCDI. The therapeutic use of fecal bacteriotherapy in the treatment of RCDI is reviewed here.

Methods: A systematic review was conducted using Medline, EBSCOhost and Web of Science, utilizing the keywords Clostridium difficile, bacteriotherapy and pseudomembranous colitis. Relevant articles were found and cross-referenced; references were reviewed for additional pertinent materials. At the time of this review, there are no RCTs published, therefore this review focuses on case series and case reports.

Results: Four case reports and case studies were reviewed and include a total of 26 patients. Each patient had at least two recurrences of CDI prior to receiving fecal bacteriotherapy treatment. Eighteen patients received treatment through the upper GI tract via nasogastric tube, while eight patients received therapy through the lower GI tract via colonoscope or fecal enema. Most patients were treated in the outpatient setting. Treatment response was similar in all methodologies, with patients reporting a decrease in diarrhea almost immediately. All patients, except one, remained free from any CDI recurrence through the follow-up period of 90 days to five years.

Conclusion: RCDI is an increasing problem in our medical community. Very difficult to treat, it takes a heavy toll on both patients and providers. Other treatment options, such as probiotics and immunotherapy, have had minimal successes. Fecal bacteriotherapy is a safe, rapid and highly effective option for the treatment of RCDI. Despite inadequacies in the current published literature, the studies appraised in this review support the therapeutic benefits of fecal bacteriotherapy for the treatment or RCDI and suggest potential for this inexpensive and minimally-risky treatment modality to undergo further investigations for clinical use.

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First Advisor
Mary Von PA-C, MS, DFAAPA

Second Advisor
Annjanette Sommers MS, PAC

Third Advisor
Rob Rosenow PharmD, OD

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Clostridium difficile, fecal bacteriotherapy, stool transplants, pseudomembranous colitis

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NOTICE TO READERS

Fecal Bacteriotherapy for the Treatment of Recurrent
Clostridium Difficile Infection in Adults

Julie L. Cramer

A Clinical Graduate Project Submitted to the Faculty of the

School of Physician Assistant Studies

Pacific University

Hillsboro, OR

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Faculty Advisor: Mary Von PA-C, MS, DFAAPA
Clinical Graduate Project Coordinators:
Annjanette Sommers PA-C, MS & Rob Rosenow PharmD, OD

Biography

Julie Cramer was born in San Leandro, California. She has lived in many places around the United States, including Arizona and Kentucky, but has spent the majority of her time in Boulder, Colorado. She graduated high school in Boulder and went on to earn a Bachelor of Arts in Environmental, Population and Organismic Biology from the University of Colorado at Boulder. Julie worked as an EMT and Paramedic in the Denver and Boulder areas for many years. She greatly enjoyed the diversity of the job and extended her responsibilities to include teaching with many fire departments in the area as well as being a lead Paramedic Field Instructor and Team Leader. In the spring of 2008, she moved to the Portland, Oregon area to attend Pacific University and obtain a Masters of Science in Physician Assistant Studies. After graduation, Julie looks forward to returning to the Denver area and serving the community as a health care professional. She also plans to volunteer on missions overseas, extending the quality of healthcare beyond our borders.

Abstract

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Keywords: *Clostridium difficile*, fecal bacteriotherapy, stool transplants, pseudomembranous colitis

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To *my parents*: Thank you for being so involved in my life and raising me with such high standards and expectations. I thank you for the work ethic you instilled in me and for giving me the courage and determination to do anything to which I put my mind. Your love and support keep me going. You are terrific parents and wonderful role-models; I am blessed to have you in my life.

To *my family and friends*: Thank you for your love and encouragement as I embarked down this crazy road. I am fortunate to be surrounded by such love and strength. Each of you holds a special place in my heart.

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AAD……………………………………………………….Antibiotic-Associated Diarrhea

CDAD…………………………………………….*Clostridium difficile*-Associated Diarrhea

CDI………………………………………………..……………...*Clostridium difficile* Infection

GI……………………………………………………………………..Gastrointestinal

IG…………………………………………………………...…….Immunoglobulin

IVIG………………………………………………....Intravenous Immunoglobulin

NG…………………………………………………………………..Nasogastric Tube

PMC………………………………………………………...Pseudomembranous Colitis

RCDI……………………………………………….Recurrent *Clostridium difficile* Infection

RCT…………………………………………………….…… Randomized Control Trial

ST………………………………………………………………………Stool Transplant

Fecal Bacteriotherapy for the Treatment of Recurrent *Clostridium Difficile* Infection in Adults

BACKGROUND

Diarrhea is a common side effect of the administration of antibiotics, with *Clostridium difficile (C. difficile)* being responsible for most of the severe cases of antibiotic-associated diarrhea, including the development of pseudomembranous colitis.[1-3] The incidence of infection with *C. difficile* is rapidly increasing worldwide consequent to the widespread use of broad-spectrum antibiotics. Population data from Quebec has shown the instance of *Clostridium difficile* –associated diarrhea (CDAD) has increased from 35.6 cases per 100 000 in 1991 to 156.3 per 100 000 in 2003.[4] In the United States, *C. difficile* infections (CDI) are projected to reach 450 000-750 000 cases per year by the end of 2010.[5,6] Infection severity has also increased, with the percentage of cases defined as complicated, increasing from 7.1% in 1991-92 to 18.2% in 2003. A ten-fold increase in incidence in patients older than 65 years has also occurred during this same time period.[4] In 2000, Kyne et al[7] found that 31% of patients who received antibiotics in an acute care setting were colonized with *C. difficile,* and 56% of these patients developed CDAD.[7] Furthermore, persons affected by CDAD are more likely to suffer recurrent *C. difficile* infections (RCDI).[8]

Conservative estimates suggest that patients with CDAD incur at least $3669 extra in hospital costs and spend at least 3.6 additional days hospitalized.[9] O'Brien et al[10] studied 1034 CDI cases in Massachusetts during 2000, concluding the average cost associated with each case of CDI was between $10 212 and $13 675 per instance, creating a national cost of $3.2 billion per year for the treatment of CDI alone. With

instance and cost associated with CDAD rising dramatically, a reliable treatment modality must be found.

Etiology

Clostridium difficile is an anaerobic, gram-positive, spore-forming, toxin-producing bacillus that was first described in 1935.[11] It was not until the late 1970s, however, that the *C. difficile* toxin was found in the stools of patients with antibiotic-associated diarrhea (AAD) and pseudomembranous colitis (PMC).[12] *Clostridium difficile* can exist in both spore and vegetative forms. Outside the colon, *C. difficile* lives in its spore form, which is commonly found in soil, the general environment, pets, untreated water, and in some raw vegetables.[13] These spores, which are resistant to heat, acid (including gastric acid) and chemical agents such as antibiotics and disinfectant, can survive for months on surfaces such as bed rails, stethoscopes, skin folds and the hands of caregivers.[14] Inside the colon, the spores convert to their fully functional vegetative, toxin-producing forms and can be destroyed by antimicrobial agents. Vegetative forms have been found in the intestinal tracts of animals, including household pets.[13]

Pathophysiology

Two potent exotoxins that mediate colitis and diarrhea are released by *Clostridium difficile*, toxin A (an enterotoxin) and a more potent toxin B (a cytotoxin).[15,16] Toxin A activates macrophages and mast cells, which release inflammatory mediators.[17] These mediators disrupt the cell wall, increase the permeability of the intestinal wall and lead to intestinal fluid secretion and diarrhea, which in itself causes mucosal injury and inflammation.[18] Toxin B is approximately ten times more potent than toxin A with respect to propagating colonic mucosal damage.[16] It

degrades epithelial cells in the colon, causing colitis. As the colitis worsens, purulent and necrotic debris accumulates and forms characteristic ulcers, the pseudomembranes.[15,17] A new, hypervirulent strain of *C. difficile*, NAPI/BI/027, has been implicated as the responsible pathogen in the dramatic increase of *C. difficile* infection (CDI) since the early 2000s.[4] It is more virulent and can produce 16 times greater quantities of toxin A and 23 times greater quantities of toxin B.[18] The epidemic strain also produces binary toxin, a toxin related to the iota-toxin found in *Clostridium perfringens*, however, its role in *C. difficile* is not fully understood.[4,18] This binary toxin is resistant to fluoroquinolones, a situation which did not occur in other *C. difficile* strains prior to 2001.[4]

Transmission of *C. difficile* occurs via the fecal-oral route. The infected human gut and the contaminated hospital environment remain the most important reservoir for the organism. *Clostridium difficile* has been isolated from health care workers' hands; in fact, hand transmission is thought to be the most important factor in the acquisition and spread of *C. difficile* in hospitalized patients.[14] In healthy individuals, the growth of *C. difficile* is kept in check by normal flora in the gut. The use of antibiotics and medications that decrease stomach acidity, such as proton pump inhibitors, can cause *C. difficile* bacteria to flourish.[19] The mechanisms by which antibiotic administration leads to the development of CDAD are not yet entirely clear. The human gut is colonized by a diverse community of microorganisms that exist in a complex symbiosis with its host.[20] Approximately 20% of hospitalized adults are *C. difficile* carriers; in long term care facilities, this number may approach 50%.[14,21] Although asymptomatic, these carriers shed pathogenic organisms and serve as a reservoir for environmental contamination.[14,21]

The native flora in the gut are a primary defense mechanism which prohibit the pathogen colonization. The resident flora produce antimicrobial factors which compete for nutrients in the matrix and binding sites on the epithelium.[22] The mechanisms by which antimicrobial agents induce intestinal disease associated with *C. difficile* are still not known. These drugs may alter the normal bacterial flora of the gastrointestinal (GI) tract so as to permit colonization and/or proliferation of *C. difficile* and toxin elaboration by the organism. *Clostridium difficile* appears to flourish when the competing bacterial flora are suppressed.[22] After an initial insult on gut flora by antibiotics, *C. difficile* and other pathogens have the ability to invade and multiply. Current treatment regimes for CDI involve stopping the offending antibiotic, eradicating the *C. difficile* spores to prohibit multiplication and ultimately replenishing the natural flora of the gut.

Clinical Manifestations

Clostridium difficile-associated diarrhea can be mild, severe or systemic. Mild disease is characterized by non-bloody diarrhea, occasionally accompanied by cramping in the lower part of the abdomen, but without systemic signs or symptoms. The diarrhea, often mucoid and foul smelling, can be accompanied by nausea, dehydration, low grade fever and possible leukocytosis.[18] Colitis occurs with severe forms of CDAD and causes profuse watery diarrhea up to 10 to 15 times daily, with lower abdominal cramping, dehydration, low grade fever and leukocytosis.[18] Characteristic raised white and yellow plaques may be visible during sigmoidoscopy.[3,18] The presence of pseudomembranes is considered sufficient to make a presumptive diagnosis of *C. difficile*.[23] Complications such as sepsis, volume depletion, electrolyte imbalances, hypotension, peritonitis, paralytic ileus and toxic megacolon may be present in systemic CDAD.[18,23]

Current Treatment Modalities

Standard treatment for CDAD involves the administration of antibiotics that suppress *C. difficile*, such as metronidazole or vancomycin. Although the majority of cases will respond initially to either drug, recurrence after the discontinuation of the medication can occur.[24] Furthermore, risk of CDI doubles after three days of antibiotic therapy. Recurrent *C. difficile* infection (RCDI) can be defined as the reappearance of symptoms within eight to ten weeks after the termination of specific antibiotic therapy and is best confirmed with a positive diagnostic test for CDI.[25] Between 20 to 25% of patients experience a recurrence of symptoms after the initial episode of CDI has resolved.[26] Up to 45% of those with one recurrence of CDI will go on to suffer further recurrences,[27] and up to 65% will relapse after two or more recurrences.[28]

The current antibiotic therapy protocol for the treatment of RCDI includes stopping the causative antibiotic, if possible, and placing the patient on vancomycin and metronidazole.[29,30] Although generally effective in achieving a clinical recovery, the use of an antibiotic, such as vancomycin, does not restore the gut microflora, nor does it reduce the exposure to *C. difficile* in the environment, the comorbidity, or other host risk factors. Up to 25% of patients develop another episode of diarrheal disease within two months.[1,30] Although some of these recurrent cases may be caused by ongoing exposure to *C. difficile* spores in the environment, most are due to the bacterial strain that caused the original episode, reflecting a lack of efficacy of antibiotic treatment in eliminating the *C. difficile* spores from the gut.[29,30] Kelly and LaMont[31] show the recurrence rates after treatment with vancomycin and metronidazole are similar, 18.4% and 20.8%, respectively. These numbers have increased, however, since the year 2000. Before 2000,

vancomycin had a recurrence rate of 17.9%, with a slight rise to 19.9% after 2000. Metronidazole has shown a much more dramatic increase of 6.7% prior to and 28.6% recurrence after the year 2000.[31] This raises the question concerning the role of alternative treatments for CDI, adjunctive to current standard antimicrobial treatment with vancomycin or metronidazole.

Probiotics have long been studied in the prevention and treatment of CDI. Administration of antibiotics disrupts the balance of microorganisms in the gut; patients with RCDI have shown markedly diminished bacterial diversity compared to controls.[32] Probiotics restore the gut's colonization resistance by providing a barrier of relatively low-virulence microorganisms – mainly yeasts or *Lactobacillus* species – that compete with *C. difficile* for essential nutrients and mucosal adherence sites. Limited data suggest that probiotics may play a useful role in treatment of CDAD, particularly in the setting of recurrent disease, although there is no data that probiotics have a role in the treatment of severe CDAD.[33,34] In a 2006 meta-analysis of probiotics for the treatment of CDAD, including six randomized controlled trials involving 354 patients, *Saccharomyces boulardii* was the only effective probiotic agent for reducing the recurrence of CDAD.[33] In a 2008 Cochrane review of probiotics for the treatment of CDAD, including four randomized trials, the authors concluded that there was insufficient evidence to recommend probiotics as an adjunct to antimicrobial therapy for treatment of CDAD.[34]

Recently, questions have been raised as to the safety associated with introducing live organisms into an immunocompromised gut. In 2009, McFarland[35] reviewed the use of probiotics for the treatment of AAD and CDI. Risks associated with probiotics include (1) the transfer of antibiotic-resistant genes (2) translocation of the living organism from

the intestine to other areas of the body and (3) persistence in the intestines or the development of adverse reactions relating to interactions with the host's microflora. Her extensive review purports most clinical trials of AAD and CDI have not detailed any serious adverse reactions that can be linked with the use of probiotics. Side effects of probiotics have been mild to moderate reactions, namely nausea, vomiting, abdominal cramping or pain, rash, diarrhea or constipation. Of note, frequencies of reported adverse reactions are rarely higher than those reported by the control groups. McFarland found 12 cases of *Lactobacillus* bacteremia and 24 cases of fungemia in patients taking *Lactobacillus* and *S. boulardii*, respectively, mostly in severely ill patients.[35] Translocation of the living organism has been found only in animal models if the animal has been immunocompromised.[36] Finally, many bacterial and yeast products do not contain the exact makeup stated on the outside of the bottle. There is a lack of stringent quality control regulations for food and dietary supplements. Many probiotic products are not regulated and are manufactured in uncontrolled environments, opening the possibility for other strains of bacteria and yeast to enter the product, or not enough bacteria being present to ensure the patient receives the proper effective dose.[35,37]

Another treatment option is the use of intravenous immunoglobulin (IVIG) for the treatment of RCDI. Although there is questionable evidence that immunity against *C. difficile* infection exists, there appears to be an association between a systemic response to the presence of toxin A and CDAD development. In an analysis of patients colonized with *C. difficile*, patients who remained asymptomatic had significantly larger increases in serum antitoxin A immunoglobulin (Ig) G than those who developed CDAD ($p<0.001$).[7] Therefore, increasing antitoxin A IgG levels may result in increased

asymptomatic *C. difficile* carriage and decreased progression to CDAD, although the mechanism leading to the different pathological courses is unknown. Even though favorable outcomes have been reported, no data from randomized, controlled trials are available. Additional trials, with greater power and therapy regime standardization, are needed to fully evaluate this treatment option.

Surgery, most often colectomy, is a last-resort treatment for severe to fulminant, life-threatening CDAD. Severe CDAD is defined as CDAD with any evidence of systemic inflammatory response syndrome indicated by the presence of one or more of the following: temperature $>38°C$ or $<36°C$, tachycardia of >90 beats per minute, tachypnea of >20 breaths per minute, pCO_2 of < 4.26 kPa (32 mmHg), leukocyte count of <4000 or $>12\,000$, or the presence of $>10\%$ immature neutrophils.[38] Fulminant CDAD is defined by the manifestation of any of the following: heart rate >120 beats/minute, $>30\%$ immature neutrophils, decline in respiratory status requiring mechanical ventilation, immunosupression, oliguria, prior bowel surgery unrelated to prior CDAD occurrence and hypotension requiring vasopressors.[39] Both severe and fulminant CDAD can result in bowel perforation, toxic megacolon and peritonitis, each carrying a high mortality rate.[23] In 2002, Dallal et al[39] found a death rate of 57% in patients with colitis status post colectomy. This high rate reflects a critically ill patient population and possible contribution to mortality from accompanying CDAD. Furthermore, no patient over the age of 80 years survived the surgery. Koss et al[23] found an overall mortality rate of 35.7% in their analysis of surgery in fulminant *C. difficile* colitis. While these mortality rates may seem rather high, the risk of bowel perforation and toxic megacolon are also associated with dramatic mortality rates. Early surgical intervention, and thorough

evaluation of the predictors of survival, is necessary for the best possible treatment outcomes.

Fecal Bacteriotherapy

An alternative approach to probiotics or surgery, fecal bacteriotherapy utilizes the complete normal human flora as a therapeutic probiotics mixture of living organisms. Fecal bacteriotherapy refers to the process of instilling a liquid suspension of stool from a healthy donor into the gastrointestinal (GI) tract of the recipient. The stool sample may be collected on the day of transplantation from a patient's spouse, household contact or an unrelated donor.[24,40-42] Prior to collection, the sample donor is typically screened for potentially contagious infectious agents.[24] After collection, the stool sample is processed in the clinical laboratory into a liquid suspension, and is subsequently instilled into the upper GI tract through a nasogastric (NG) tube or into the colon through a colonoscope or a retention enema catheter.[24,40-42]

Fecal bacteriotherapy has been shown to be quite effective. The first investigators to report the successful use of fecal enemas in the management of four patients with pseudomembranous colitis (PMC) occurred in 1953.[43] Since then, numerous articles have been published touting the almost instantaneous success of fecal bacteriotherapy in the treatment of RCDI. If this treatment modality has been shown to be so effective, why is its use not more prevalent? In 2009, Bakken[8] conducted a literature review on the use of fecal bacteriotherapy to treat RCDI. In his review, however, Bakken included all research available on the topic, including studies that examined the role of fecal bacteriotherapy as treatment for other diseases such as irritable bowel syndrome. This systematic review will examine the literature addressing fecal bacteriotherapy as

treatment of RCDI specifically, in adults who have failed antibiotic therapy, looking for the halt of diarrhea and the decreased instance of CDI recurrence.

METHODS

This systematic review appraises the current research and literature published on the treatment of recurrent *Clostridium difficile* infections (RCDI) with fecal bacteriotherapy. A comprehensive literature search was conducted utilizing the Medline (via OVID), EBSCOhost (via CINAHL) and Web of Science databases. The keywords *Clostridium difficile, bacteriotherapy* and *pseudomembranous colitis* yielded nine references from Medline, four from EBSCOhost and twenty-four from Web of Science. Relevant articles were selected and cross-referenced for additional materials. All references of pertinent literature were reviewed, with relevant articles being included in this review. At the time this review was conducted, there were no randomized control studies published; therefore this review will focus on case series and case reports.

All compiled references were analyzed for pertinence with the following criteria: articles evaluating adult patients with RCDI that were treated with fecal bacteriotherapy or stool transplant, as well as the instance of diarrhea and recurrence of CDI following treatment as outcomes were included in this review. Exclusion criteria included patients younger than 18 years old and studies that did not assess the effect of treatment on *C. difficile* directly. Materials unavailable in English were excluded. Articles published in non-peer-reviewed format, such as letters to the editor, were not included despite their relevancy to this topic, as it is difficult to evaluate their accuracy and accountability. Authors of such literature were contacted to determine if their material was available in

published format. Literature with only the abstract published and those which were unable to be obtained in full text were also excluded.

RESULTS

A total of thirty-seven articles were found using Medline, EBSCOhost and Web of Science. An additional five related studies were identified while reviewing the references of the original articles. After inclusion and exclusion criteria were applied, a total of five articles remained; however, one had to be excluded as only the abstract was published. The remaining four articles[24,40-42] were assessed to ensure their patient population, interventions and measured outcomes were all consistent with the goals of this review (See Table 1: Matrix of Reviewed Literature). The articles were then thoroughly reviewed and their validity was scored according to the criteria illustrated in Table 2: Validity Matrix. Prior to rating the articles, it was decided that articles with a validity of three or greater would be included; at the end of this exhaustive literature search and review process, four articles were included in this review.

In Sweden, Schwan et al,[41] published a case report of a 67 year old female who was successfully treated with fecal bacteriotherapy via enema. The patient had a lifelong history of irritable colon, developing into diverticulosis and subsequent diverticulitis which ultimately had to be treated with a sigmoid colectomy. The patient was treated prophylactically with antibiotics prior to the resection. One week after the surgery, the patient developed bilateral pneumonia and pleuritis, for which she was treated with trimethoprim/sulfamethoxazole and recovered without incident. Two weeks later, the patient began experiencing frequent diarrhea and febrile illness with a *C. difficile*-positive

stool sample. Oral vancomycin successfully treated this initial *C. difficile* infection

(CDI), however, two weeks after ending treatment, the patient experienced a recurrence

of CDI confirmed with a stool sample. The patient was again treated with vancomycin,

and the infection resolved. The patient had four more recurrent *C. difficile* infections

(RCDIs) over the next six months, even after prolonged (four week) treatment with

vancomycin. Probiotics were utilized in two separate instances, as adjuvant therapy,

without any obvious effect. The patient was finally treated with fecal bacteriotherapy.

Her husband donated his stool which was screened for salmonella, shigella, *Yersenia*

enterocolitica, Staphylococcus aureus, Campylobacter jejuni and *C. difficile*. The stool

was prepared as outlined in the original article. The patient then received the stool

mixture as an enema which was not evacuated for more than 24 hours after the procedure.

Three days later, a second enema was given, which was not evacuated for six hours. The

fecal bacteriotherapy treatment resulted in "prompt and complete normalization of the

bowel function".[41]   The patient was followed for more than a year, during which she had

no recurrence of CDI. She did experience slight GI disturbances related to her irritable

colon, however, not to the extent previously experienced. The patient also gained six

kilograms of weight.[41]

Tvede et al[42] conducted a case report of six patients in Denmark with chronic

RCDI who were treated with fecal bacteriotherapy. The patients, five females and one

male aged 59 to 83 years, had each had at least two diagnosed relapses of CDI after the

initial infection. A chart is included in the original source, detailing each patient's

specific preceding infection and treatment regimen. Initially, two patients were treated

with a fecal enema and four with bacteriotherapy. The bacteriotherapy treatment

consisted of rectal instillation of a mixture of ten different faculatative aerobic and

anaerobic bacteria species that had been cultured in the laboratory and diluted in sterile

saline. These bacterial species included *Streptococcus (Enterococcus) faecalis,*

Clostridium innoctutum, C. ramnosum, C. bifermentans, Bactroides ovatus, B. vulgatus,

B. thetaiotaomicro, Escherichia coli (two separate strains), and *Peptostreptococcus*

productus. On the day of treatment, two patients were given fecal enemas of stool

donated by family members. The other four patients were given rectal enema of the

bacterial mixture. One patient was not responsive to the fecal enema and was

consequently treated with bacteriotherapy. All cases of CDI were ultimately resolved.

Rapid resolution of diarrhea and normalization of serum albumin and serum orosomucoid

concentrations occurred. Stool cultures and toxin assays for *C. difficile* remained

negative for the year-long follow-up of all patients.[42]

In the United States, Persky et al[40] describe a case report of a 60 year old woman

who had been experiencing RCDIs for six months and was successfully treated with stool

transplantation via colonoscope. This previously healthy patient presented with severe

left lower quadrant pain and was found to have multiple sigmoid diverticula surrounded

by inflammatory changes. She was hospitalized and treated with intravenous ceftazidime

and metronidazole. While her abdominal pain resolved, she developed AAD while in the

hospital. Stool cultures were initially negative for *C. difficile,* and the patient was

discharged on oral cefaclor and metronidazole to finish the treatment for diverticulitis.

Her diarrhea persisted and the antibiotics were discontinued. Subsequent stool samples

were positive for *C. difficile.* The diarrhea resolved with metronidazole, but returned less

than one week after the cessation of treatment. Repeat CDI were resolved with courses

of vancomycin. A presumed recurrent episode of diverticulitis was treated with amoxicillin/clavulanate, sparking another CDI. The patient then endured several months of oral vancomycin therapy (over $17 000 worth) without improvement in her condition. The patient presented to Montefiore Medical Center and was treated with stool transplantation with stool donated by her husband. The donor was not tested for hepatitis A, B or C, or for HIV as the donor and patient had been in a monogamous relationship for over 30 years. The stool was not tested for any pathogen either as the donor did not have any GI complaints. The patient's colon was prepared with a cleansing lavage and a 500 ml infusion of the donor stool was distributed evenly every 10 cm throughout the colon utilizing a colonoscope. The patient had immediate and complete resolution of diarrhea and was negative for the *C. difficile* toxin on subsequent stool assays on follow up. The patient has remained free of diarrhea and CDI for five years.[40]

Aas et al[24] published a retrospective case series of 18 patients in Deluth, Minnesota who received donor stool transplants (ST) through a nasogastric (NG) tube for RCDI. These patients all received treatment from the same physician between June 1994 and August 2002. Study participants included 13 women and five men, ages 51 to 88 years, with a history of RCDI. Five of the patients were hospitalized at the time of ST, while the rest were treated in an outpatient gastroenterology clinic. The study's inclusion criteria consisted of a laboratory-confirmed diagnosis of *C. difficile* colitis, two or more laboratory-confirmed relapses of *C. difficile* colitis after treatment with a specific initial antimicrobial and sufficient clinical and laboratory documentation, by either telephone or chart review, of the patient's course of health after the stool transplantation. Stool donors had not received any antimicrobial therapy in the six months preceding donation.

Donors, in order of preference, were individuals who had intimate physical contact with the patient, such as a spouse or partner, family household members, or any other healthy donor. Donors' blood was screened for hepatitis A, B and C viruses; HIV-1 and HIV-2; and syphilis. Donor stool samples were screened for *C. difficile*, enteric bacterial pathogens, ova and parasites. Fifteen of the donors were members of the recipients' families, while the remaining three received stool from a healthy clinic staff volunteer. Preparation of stool transplant (ST) specimens is outlined in the original source. Prior to the ST, patients were prophylaxed with at least a four-day course of antibiotics to reduce the amount of *C. difficile* in the colon; treatment was discontinued the evening before the ST. Patients were also prophylaxed with omeprazole the evening before and day of the procedure. During the procedure, a NG tube was placed into the patient's stomach, with the tip position confirmed by abdominal radiography. Twenty-five milliliters of the transplant stool was infused and flushed with saline. Patients were able to resume normal diet and physical activity immediately. Two patients died shortly after the ST from diseases unrelated to the *C. difficile* or ST. One patient did have a recurrence of diarrhea 17 days after ST, which was negative for *C. difficile*. He was treated with vancomycin and all symptoms resolved. He had no further recurrences and continued to test negative for *C. difficile* over the next six months. Fourteen of the 16 surviving patients tested negative for *C. difficile* in stool samples taken within 30 days of the ST. The other two patients did not submit samples, but they were contacted and denied any return of diarrhea or other symptoms following the ST. All patients remained diarrhea free during the 90 day follow-up period.[24]

DISCUSSION

The primary goal of this review is to appraise the current research and literature available on the use of fecal bacteriotherapy in the treatment of recurrent *C. difficile* infection (RCDI). The four included studies[24,40-42] took place between 1984 and 2003 in Denmark, Sweden and the United States. A total of 26 patients were included, 20 females and five males, aged 51 to 88 years. Each patient had at least two recurrences of *C. difficile* infection (CDI) prior to receiving fecal bacteriotherapy treatment. Most patients received treatment in the outpatient setting. Eighteen patients were given stool transplants (ST) through the upper gastrointestinal (GI) tract via nasogastric (NG) tube,[24] while eight patients received therapy through the lower GI tract via colonoscope or fecal enema.[40-42] Treatment response was almost immediate, with patients reporting a decrease in diarrhea within 24 hours in most cases. All but one patient had success with the treatment, and that patient was quickly cured with one course of antimicrobial therapy without recurrence.[24] Two patients died soon after ST from unrelated causes.[24] A summation of these studies can be found in Table 3: Summary of Findings.

Overall, the success of treatment with fecal bacteriotherapy was remarkable. Including the two deceased patients in the patient total, but not as cured, 23 of the 26 patients were cured with fecal bacteriotherapy, a cure rate of 88.5%. If these two patients are excluded, 23 of the 24 patients had successful treatments, with 95.8% cured. Only one of the patients experienced a recurrence of CDI during of follow-up period as evidenced by a positive stool sample, and even that recurrence was quickly responsive to antibiotics and did not occur again.[24] None of the other patients experienced RCDI during the follow-up period of 90 days to five years. These numbers are quite dramatic

compared to the 65% relapse rate after being treated two or more times with antibiotics or a mortality rate of almost 36% following colectomy for treatment of RCDI, as discussed above. Probiotics have proven useful in the prevention of recurrence of CDI, however, they are quite ineffective once CDI has occurred. Immunoglobulin research remains in the preliminary phases, but looks somewhat promising. The exact mechanisms of fecal bacteriotherapy in the treatment of CDI are unclear, but probably involve the recolonization of colonic flora with missing components to regenerate colonization resistance.[44] Unlike the transient use of antibiotics and antimicrobials, directly implanting fecal flora provides a lengthy exposure of "antagonistic" activity that not only cures the current infection, but also prevents future colonization by *C. difficile,* presumably by restoring flora colonization resistance.[44] Fecal bacteriotherapy shows much potential in the treatment of RCDI, however, there are many refinements to the research, proven efficacy and procedures that need to take place before the therapy can become commonly used.

One major limitation to the validity of these studies was the size of the studies themselves. In this review, two of the included studies each had only one participant,[40,41] while the other two had six participants[42] and 18 participants.[24] This, in large part, is due to the fact that these were retrospective case reviews and therefore not pre-planned with actively recruited participants. Also, fecal bacteriotherapy remains a relatively unknown and underutilized treatment modality.   Interest in this area is growing, however. In December 2009, Rubin et al[45] submitted a letter to the editor detailing their successes in treating 61 patients with RCDI with fecal bacteriotherapy since 2003. Unfortunately,

their data has not been published formally, and was therefore ineligible for inclusion in this review. New studies are being devised and initiated with larger patient populations.

Although transmission of communicable disease has not been reported after fecal infusions, lingering concern remains as to the potential spread of infectious agents contained in the fecal sample from stool donors to recipients. The literature included in this review varied widely in how this risk was minimized. Aas et al[24] had the most stringent screening processes with a clearly stated donor screening protocol, as reviewed above. Schwan et al[41] tested the donor feces a total of eight different times in the three weeks preceding ST. The feces were negative for parasitic and bacterial pathogens as detailed above, but the donor's blood was not reported to have been tested. This may have been omitted as the donor was the patient's husband; however, the blood screening of donors was not addressed in the study.[41] Persky et al[40] openly stated their donor, the recipient's husband, was not tested for hepatitis or HIV as the couple had been in a monogamous relationship for 30 years, nor was the stool tested for common pathogens as the donor "did not have any GI complaints".[40] The authors do, however, strongly recommend the routine testing of all donors prior to the initiation of stool donation. Tvede et al[42] had a detailed report of bacterial strains cultured from study participants, but made no mention of screening either donor blood or fecal samples. The variance of screening protocols permits a substantial risk of the passage of infection during ST, even in donor/patient relationships in which it is likely any exposure has already taken place. This risk may be reduced by obtaining stool from healthy donors with normal bowel function and by testing both donor stool and blood for common viral and bacterial pathogens and parasites. In a 2004 review of fecal bacteriotherapy, Borody et al[2]

suggested donors should be treated much the same way as traditional organ donors. In

addition to being free of antimicrobial therapy for at least three to six months prior to

donation, donors should have a complete blood count and liver function tests performed.

Serological testing should be negative for hepatitis A, B and C; HIV-1 and HIV-2;

cytomegalovirus; Epstein-Barr virus and syphilis. Furthermore, stool should be negative

for any detectable parasites, ova or bacterial pathogens.[2] Selecting a patient's spouse or

significant other has the theoretical advantage that any transmissible disease should have

emerged well before treatment with ST. Regardless, the selection of relatives or spouses

as fecal donors should not preclude the necessity for thorough screening, just as blood

donations should not be transfused until properly screened for infectious disease.

Treatment with fecal bacteriotherapy has been hindered by a lack of

standardization in the preparation and administration of the actual fecal suspension. Even

among the four studies included in this review, significant variations exist in the

protocols for fecal infusion. Aas et al[24] clearly outline their methods for not only the

preparation of donor stool samples prior to ST, but also the preparation of the ST

recipient and description of the transplantation procedure. The donor stool sample was

obtained within six hours of the ST. Approximately 30 grams of the stool specimen were

blended with 50-70 milliliters of sterile 0.9% NaCl. The suspension was filtered, and

refiltered, through a paper coffee filter. A NG tube was placed in the recipient

immediately prior to ST, with confirmation of placement via radiography. Twenty-five

milliliters of the stool suspension was infused via the NG tube, the tube was flushed with

NaCl and then withdrawn.[24] Tvede et al[42] elaborately describe the preparation of the

bacterial mixture for fecal instillation in the original literature. The fecal enema was

prepared by mixing fifty grams of the donor stool in 500 milliliters of saline. The

procedure for fecal instillation was not described.[42] The stool sample for the patient

reported by Schwan et al[41] was transported to the laboratory in a sterile glass container

within 20 minutes after passing. The feces was suspended in approximately 100

milliliters of sterile 0.15 M NaCl and sieved through a strainer several times. A quantity

of 450 milliliters of the stool suspension was inserted into the patient as an enema.[41]

Persky et al[40] were not as diligent in relating their stool sample preparations and ST

procedure. The patient was prepped for colonoscopy with 1 gallon of Golytely as a

cleansing lavage. During the colonoscopy, a "500-ml infusion comprised of stool

(donated from the patient's husband) mixed with saline was uniformly injected every 10

cm throughout her colon".[40] In summary, stool samples varying in size from 30 to 50

grams, were mixed with 50 to 500 milliliters of various concentrations of saline, and

anywhere from 25 to 500 milliliters of stool suspension were inserted in the patient (see

Table 4: Fecal Infusion Constituents). In their review of fecal bacteriotherapy, Borody et

al[2] conclude low doses of fecal mass used in treatment may have less effect and that

severe disease may require a higher therapeutic dose to fully eradicate infection. Further

research is necessary to determine the ideal concentration of stool specimen to ensure

optimal results in the fewest number of therapy sessions necessary for resolution of CDI.

The efficacy of fecal bacteriotherapy appears to depend on a number of factors

including the freshness of the donated stool, the frequency and route of fecal

administration, and the repopulation of the entire colon. It is generally agreed upon that

the freshness of the stool is important; timely placement of the transplant reduces the

likelihood of foreign pathogens being introduced into the sample, as well as ensuring the

healthy flora do not expire outside their native environment. Neither the length of time

between the donation of the stool and the mixing of the stool infusion, nor the length of

time between the mixing of the stool infusion and instillation of the infusion has yet to be

studied. Current transplantation protocol calls for stool transplantation within six hours

of the passing of the donor stool; however, there is much variance in actual timeliness of

separate studies.[24,25] The number of fecal instillations necessary to cure a patient also

remains ambiguous at this time. As discussed above, this may correlate with the amount

of fecal mass transplanted in a single treatment. The method of placement may also

contribute to the success of the ST; instillation of donor feces can be accomplished from

either the proximal or the distal end of the GI tract. In this review, most ST were

performed via the upper GI tract, however, Bakken reports most occur via the lower GI

tract.[8] Donated stool administered by enema is not optimal as it only reaches the splenic

flexure, theoretically allowing proliferation of spores proximal to the splenic flexure.[40]

Colonoscope placement of donor stool suspension seems to allow the entire colon to be

treated and replenished, decreasing the recurrence of CDI after ST.[40] Fecal instillation

via colonoscope is associated with an increased risk of complications, such as colon

perforation, than other methods.[8] This is partly due to the inflamed, and therefore friable,

state of the colon. Preparation of the colon with a cleanser such as Golytely is preferable

to allow room for the movement of the colonoscope and direct instillation of the fecal

suspension. ST via the lower GI tract requires a larger volume of fecal suspension to

allow for seepage through the rectum and often requires multiple instillations over several

days to reach success.[8] One advantage of ST via colonoscope is the ability to fully

visualize the entire colon and to look at pathology for further clues as to the cause of

relapse.[40] The use of NG tubes for fecal instillation has several purported advantages. The placement of a NG tube is quite simple, requiring little, if any sedation. Unlike colonoscope instillation, there is no preparation needed for a NG tube, which is advantageous in dehydrated or severely ill patients. A smaller fecal sample is needed for NG placement as there will be no leakage of the sample. Associated complications include possible perforation of the esophagus, although less likely than a colonic perforation, and possible aspiration of fecal matter.[8] Finally, there is limited cost associated with NG assisted ST as the only radiology costs are to ensure the tip of the infusion catheter is in the correct position.[24] Future studies need to directly compare ST via NG tube, fecal enemas and colonoscope placement in the reduction of CDI incidence. Treatment protocol for the use of fecal bacteriotherapy for RCDI should be standardized.

Preparation of the colon prior to fecal bacteriotherapy with antimicrobials, acid reducers and/or cleansing of the colon may be helpful in increasing the efficacy of the procedure and reducing the need for multiple infusions. Pretreatment with oral antibiotics, such as vancomycin, prior to the ST reduce the number of vegetative *C. difficile* colonies.[8] Aas et al[24] reported success following pretreatment with oral vancomycin for four or more days before subsequent infusion of stool via a NG tube into the stomach in a series of patients with multiple relapses. A clinical response was observed within 12 to 24 hours. The pretreatment with antibiotics, however, makes it impossible to determine whether the antibiotics or the ST halted CDI and prevented recurrence. The fact that these patients have had RCDIs with the previous use of antibiotics may suggest the ST is more effective at long term cure rates.[24] Another aspect of protocol worthy of further research is the use of acid reducers, such as proton pump

inhibitors, just before the procedure to decrease gastric hydrochloric acid production and to create an optimal environment for the instilled fecal bacteria to implant.[8,24] Finally, colonic lavage with agents such as Golytely, has been suggested prior to ST via the lower GI tract. Cleansing may reduce the density of *C. difficile* organisms including the metabolically inactive spores, which could otherwise convert to vegetative (active) forms.[40] Colonic lavage is a risky procedure in a severely ill patient as the risk of dehydration is great in patients of suboptimal health. Research is needed to determine whether this step truly aids in the success of the fecal instillation, or whether it can be disregarded in severely ill patients, but still maintain success of the procedure.

The next step in solidifying the use of fecal bacteriotherapy in the treatment of RCDI is to conduct randomized control trials (RCT) to conclusively demonstrate the efficacy of the therapy. RCTs help reduce the instance of bias; for example, reporting bias can occur with overestimation of a treatment effect because of failure to randomize and recall bias cannot be accounted for when there is no control group of patients to compare the report of adverse events.[46] In a RCT, participants are randomly allocated to receive or not to receive an experimental therapeutic procedure and then followed to determine the effect of the intervention. A true RCT may be difficult to assemble as finding a placebo resembling fecal matter to blind both patients and clinicians may be quite challenging. No RCTs have been completed or published to date, however, several are ongoing[47] both in the United States and abroad. In the Netherlands, the FECAL (Faecal therapy to Eliminate *Clostridium difficile*-Associated Longstanding diarrhea) trial[25] is underway to assess whether restoring the intestinal flora in patients with recurrent CDAD by feces from a healthy donor can prevent future recurrences. This

RCT compares donor feces infusion to conventional antibiotic treatment with oral vancomycin. Initiated in January, 2008, this study was scheduled to close at the end of 2009. Results from this study have yet to be published, but look promising as the study appears to be a well-planned, protocol-driven, double-blind RCT.

CONCLUSION

Clostridium difficile infection (CDI) is a rapidly increasing problem worldwide, both in and out of the hospital setting. The instance of recurrence is growing as the organism becomes resistant to antibiotic treatment, which in itself provides the opportunity for *C. difficile* to inhabit the colon as the natural flora of the gut are disturbed. Recurrent *C. difficile* infection (RCDI) takes a heavy toll on the patients, hospitals and providers, insurance agencies and the economy as antimicrobial therapy is expensive and patients lose more time working from worsening, debilitating illness. Other treatment options, such as adjuvant therapy of probiotics and immunotherapy have had minimal successes – especially in moderate to severe CDI – and surgery remains a risky last-choice option. Fecal bacteriotherapy is a safe, rapid and highly effective option for the treatment of RCDI. Despite inadequacies in current study designs, the literature appraised in this review adds support to the therapeutic benefits of fecal bacteriotherapy for the treatment of RCDI and suggests potential for this inexpensive and minimally-risky treatment modality to undergo further investigations for clinical use.

REFERENCES

1. Bartlett JG. Clinical practice. antibiotic-associated diarrhea. *N Engl J Med*. 2002;346:334-339.

2. Borody TJ, Warren EF, Leis SM, Surace R, Ashman O, Siarakas S. Bacteriotherapy using fecal flora: Toying with human motions. *J Clin Gastroenterol*. 2004;38:475-483.

3. LaMont JT. Clinical manifestations and diagnosis of *Clostridium difficile* infection. Available at: www.uptodate.com. Accessed 03/02, 2010.

4. Pepin J, Saheb N, Coulombe MA, et al. Emergence of fluoroquinolones as the predominant risk factor for clostridium difficile-associated diarrhea: A cohort study during an epidemic in quebec. *Clin Infect Dis*. 2005;41:1254-1260.

5. Zilberberg MD, Shorr AF, Kollef MH. Increase in adult clostridium difficile-related hospitalizations and case-fatality rate, united states, 2000-2005. *Emerg Infect Dis*. 2008;14:929-931.

6. McFarland LV, Clarridge JE, Beneda HW, Raugi GJ. Fluoroquinolone use and risk factors for clostridium difficile-associated disease within a veterans administration health care system. *Clin Infect Dis*. 2007;45:1141-1151.

7. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of clostridium difficile and serum levels of IgG antibody against toxin A. *N Engl J Med*. 2000;342:390-397.

8. Bakken JS. Fecal bacteriotherapy for recurrent clostridium difficile infection. *Anaerobe*. 2009;15:285-289.

9. Kyne L, Hamel MB, Polavaram R, Kelly CP. Health care costs and mortality associated with nosocomial diarrhea due to clostridium difficile. *Clin Infect Dis*. 2002;34:346-353.

10. O'Brien JA, Lahue BJ, Caro JJ, Davidson DM. The emerging infectious challenge of clostridium difficile-associated disease in massachusetts hospitals: Clinical and economic consequences. *Infect Control Hosp Epidemiol*. 2007;28:1219-1227.

11. Hall IC, O'Toole E. Intestinal flora in newborn infants with a description of a new pathogenic anaerobe bacillus difficilis. *Am J Dis Child*. 1935;49:390.

12. Bartlett JG, Moon N, Chang TW, Taylor N, Onderdonk AB. Role of clostridium difficile in antibiotic-associated pseudomembranous colitis. *Gastroenterology*. 1978;75:778-782.

13. Williams OM, Spencer RC. The management of clostridium difficile infection. *Br Med Bull*. 2009;91:87-110.

14. McFarland LV, Mulligan ME, Kwok RY, Stamm WE. Nosocomial acquisition of clostridium difficile infection. *N Engl J Med*. 1989;320:204-210.

15. Riegler M, Sedivy R, Pothoulakis C, et al. Clostridium difficile toxin B is more potent than toxin A in damaging human colonic epithelium in vitro. *J Clin Invest*. 1995;95:2004-2011.

16. Alfa MJ, Kabani A, Lyerly D, et al. Characterization of a toxin A-negative, toxin B-positive strain of clostridium difficile responsible for a nosocomial outbreak of clostridium difficile-associated diarrhea. *J Clin Microbiol*. 2000;38:2706-2714.

17. Schroeder MS. Clostridium difficile--associated diarrhea. *Am Fam Physician*. 2005;71:921-928.

18. Sunenshine RH, McDonald LC. Clostridium difficile-associated disease: New challenges from an established pathogen. *Cleve Clin J Med*. 2006;73:187-197.

19. Dial S, Alrasadi K, Manoukian C, Huang A, Menzies D. Risk of clostridium difficile diarrhea among hospital inpatients prescribed proton pump inhibitors: Cohort and case-control studies. *CMAJ*. 2004;171:33-38.

20. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*. 2006;124:837-848.

21. Riggs MM, Sethi AK, Zabarsky TF, Eckstein EC, Jump RL, Donskey CJ. Asymptomatic carriers are a potential source for transmission of epidemic and nonepidemic clostridium difficile strains among long-term care facility residents. *Clin Infect Dis*. 2007;45:992-998.

22. Berg RD. The indigenous gastrointestinal microflora. *Trends Microbiol*. 1996;4:430-435.

23. Koss K, Clark MA, Sanders DS, Morton D, Keighley MR, Goh J. The outcome of surgery in fulminant clostridium difficile colitis. *Colorectal Dis*. 2006;8:149-154.

24. Aas J, Gessert CE, Bakken JS. Recurrent clostridium difficile colitis: Case series involving 18 patients treated with donor stool administered via a nasogastric tube. *Clin Infect Dis*. 2003;36:580-585.

25. van Nood E, Speelman P, Kuijper EJ, Keller JJ. Struggling with recurrent clostridium difficile infections: Is donor faeces the solution? *Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles = European Communicable Disease Bulletin*. 2009;14.

26. Bartlett JG. Management of clostridium difficile infection and other antibiotic-associated diarrhoeas. *Eur J Gastroenterol Hepatol*. 1996;8:1054-1061.

27. Surawicz CM, McFarland LV, Greenberg RN, et al. The search for a better treatment for recurrent clostridium difficile disease: Use of high-dose vancomycin combined with saccharomyces boulardii. *Clin Infect Dis*. 2000;31:1012-1017.

28. McFarland LV, Surawicz CM, Greenberg RN, et al. A randomized placebo-controlled trial of saccharomyces boulardii in combination with standard antibiotics for clostridium difficile disease. *JAMA*. 1994;271:1913-1918.

29. Gerding DN, Muto CA, Owens RC,Jr. Treatment of clostridium difficile infection. *Clin Infect Dis*. 2008;46:S32-42.

30. McFarland LV. Alternative treatments for clostridium difficile disease: What really works? *J Med Microbiol*. 2005;54:101-111.

31. Kelly CP, LaMont JT. Clostridium difficile -- more difficult than ever. *N Engl J Med*. 2008;359:1932-1940.

32. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent clostridium difficile-associated diarrhea. *J Infect Dis*. 2008;197:435-438.

33. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of clostridium difficile disease. *Am J Gastroenterol*. 2006;101:812-822.

34. Pillai A, Nelson RL. Probiotics for treatment of clostridium difficile-associated colitis in adults. *Cochrane Database Syst Rev*. 2008.

35. McFarland LV. Evidence-based review of probiotics for antibiotic-associated diarrhea and clostridium difficile infections. *Anaerobe*. 2009;15:274-280.

36. McCullough MJ, Clemons KV, McCusker JH, Stevens DA. Species identification and virulence attributes of saccharomyces boulardii (nom. inval.). *J Clin Microbiol*. 1998;36:2613-2617.

37. Miller M. The fascination with probiotics for clostridium difficile infection: Lack of evidence for prophylactic or therapeutic efficacy. *Anaerobe*. 2009;15:281-284.

38. Marra AR, Edmond MB, Wenzel RP, Bearman GM. Hospital-acquired clostridium difficile-associated disease in the intensive care unit setting: Epidemiology, clinical course and outcome. *BMC Infect Dis*. 2007;7:42.

39. Dallal RM, Harbrecht BG, Boujoukas AJ, et al. Fulminant clostridium difficile: An underappreciated and increasing cause of death and complications. *Ann Surg*. 2002;235:363-372.

40. Persky SE, Brandt LJ. Treatment of recurrent clostridium difficile-associated diarrhea by administration of donated stool directly through a colonoscope. *Am J Gastroenterol*. 2000;95:3283-3285.

41. Schwan A, Sjolin S, Trottestam U, Aronsson B. Relapsing clostridium difficile enterocolitis cured by rectal infusion of normal faeces. *Scand J Infect Dis*. 1984;16:211-215.

42. Tvede M, Rask-Madsen J. Bacteriotherapy for chronic relapsing clostridium difficile diarrhoea in six patients. *Lancet*. 1989;1:1156-1160.

43. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery*. 1958;44:854-859.

44. Wells CL, Maddaus MA, Jechorek RP, Simmons RL. Role of intestinal anaerobic bacteria in colonization resistance. *Eur J Clin Microbiol Infect Dis*. 1988;7:107-113.

45. Rubin TA, Gessert CE, Aas J. Stool transplantation for older patients with clostridium difficile infection. *J Am Geriatr Soc*. 2009;57:2386.

46. Guyatt G, Rennie D, Meade MO, Cook DJ. The philosophy of evidence-based medicine. In: *User's Guide to the Medical Literature: A Manual for Evidence-Based Clinical Practice*. Second ed. New York: McGraw-Hill; 2008:9.

47. McFarland LV. Renewed interest in a difficult disease: *Clostridium difficile* infections - epidemiology and current treatment strategies. *Curr Opin Gastroenterol*. 2008;25:24-35.

Tables

Table 1: Matrix of Reviewed Literature

Author/ Ref #	Year	Patients/ Population	Intervention	Comparison	Outcome(s)	Study Type	Validity Score (Out of 5)
Aas[24]	2003	Adults with recurrent CDI	Given donor stool by nasogastric tube	None	Prompt halt of diarrhea. No CDI recurrence.	Case Series	4
Persky[40]	2000	Adult with recurrent CDI	Donor stool via colonoscope	None	Prompt halt of diarrhea. No CDI recurrence.	Case Report	3
Schwan[41]	1984	Adult with recurrent CDI	Donor stool given via 2 enemas	None	Prompt halt of diarrhea. No CDI recurrence. Weight gain.	Case Report	4
Tvede[42]	1989	Adults with recurrent CDI	Donor stool via enema or bacteriotherapy	None	Prompt halt of diarrhea. No CDI recurrence. Weight gain.	Case Series	4

Table 2: Validity Matrix

Author/ Ref #	Lab-Confirmed Diagnosis of CDI	≥2 Lab-Confirmed Relapses of CDI After Initial Abx Treatment	Failed ≥2 Treatment Methods[a]	Fecal Testing Within 30 Days of ST	≥ 90 Day Follow-Up	Validity Score (Out of 5)	Comments
Aas[24]	1	1	0	1	1	4	Tx with abx
Persky[40]	1	1	0	0[b]	1	3	Tx with abx
Schwan[41]	1	1	1	0	1	4	Tx with abx, probiotics Serum testing, not fecal
Tvede[42]	1	1	0	1	1	4	Tx with abx

[a] Treatment methods to include antibiotics, probiotics, immunotherapy
[b] Had negative CD test, but unknown how long after ST

Abx – Antibiotics   ST – Stool Transplant
CD – *Clostridium difficile*   Tx - Treatment
CDI – *Clostridium difficile* Infection

Table 3: Summary of Findings

Author/ Ref. #	Year/ Location	Cases (*n*)	Sex of Patients	Age Range (Years)	# of Relapses Prior To ST	# of Relapses After ST	Inpatient/ Outpatient	Who Donated the Stool	Method of Delivery	Number of ST Received	Cure (%)	Follow -up Time	Comment[a]
Aas[24]	2003 US	18	13 F 5 M	51-88	2-7	17 = 0 1=1	3/15	15 – Family member 3 – Volunteer staff	Upper GI Tract	1	15 (83.3)	90 days	1 treatment failure 2 deaths from unrelated illnesses
Persky[40]	2000 US	1	1 F	60	4	0	Unknown	Husband	Lower GI Tract	1	1 (100)	5 years	Golytely lavage ST via colono-scope
Schwan[41]	1984 Sweden	1	1 F	67	5	0	1/0	Husband	Lower GI Tract	2	1 (100)	1 year	3 days between enemas
Tvede[42]	1989 Denmark	6[b]	5 F 1 M	59-83	2-4	0	Unknown	2 – Family 5 – Bacterial Suspension[c]	Lower GI Tract[a]	1 (2 [a])	6 (100)	6-12 months	See additional information below
Total	-	26	20 F 6 M	51-88	2-7	25=0 1=1	4/15 7 Unknown	19 – Family 3 – Volunteer 5 – Bacterial Suspension[c]	18-Upper 8-Lower	1-2	23 (88.5)[d] (99.5)[e]	90 days – 5 years	See additional information below

[a] Limitations include no randomization of patients, no blinding and no control groups in all studies

[b] Initially, two patients were treated with fecal enema and four with bacteriotherapy. One patient was not responsive to the fecal enema and was treated with bacteriotherapy at a later date.

[c] The bacteriotherapy treatment consisted of rectal instillation of a mixture of ten different faculatative aerobic and anaerobic bacteria species diluted in sterile saline. These bacterial species included *Streptococcus (Enterococcus) faecalis, Clostridium innoctutum, C. ramnosum, C. bifermentans, Bactroides ovatus, B. vulgatus, B. thetaiotaomicro, Escherichia coli* (two separate strains), and *Peptostreptococcus productus.*

[d] Percent cure including the two deaths from unrelated illnesses

[e] Percent cure excluding the two deaths from unrelated illnesses

Table 4: Fecal Infusion Constituents

Author/ Ref #	Stool Sample Size	Mixing Solution	Amount Infused
Aas[24]	30 g	50-75 ml 0.9% NaCl	25 ml
Persky[40]	Unknown	Unknown	500 ml
Schwan[41]	Unknown	100 ml 0.15 M NaCl	450 ml
Tvede[42]	50 g	500 ml Saline	Unknown
Total	30 – 50 g	50 – 500 ml	25-500 ml