Antibiotic Associated Diarrhea

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Background information

Soon after the widespread introduction of antibiotics, a syndrome of "antibiotic associated diarrhea and colitis" was recognized. In 1935, Hall and O'Toole isolated *Bacillus difficilis*, a gram-positive, cytotoxin producing anaerobic bacterium as the putative agent.[1] During the 1950s, nosocomial diarrhea and pseudomembranous colitis were erroneously attributed to staph aureus, though ultimately in 1978, Bartlett et al identified *Clostridium difficile* as the causative agent for pseudomembranous colitis.[2] It is estimated that *Clostridium difficile* accounts for approximately 20 percent of the cases of antibiotic associated diarrhea and 60 percent of the cases of antibiotic associated colitis.[3,4] The importance of C*lostridium difficile* in 2012 is due to its increasing incidence, increasing disease severity and higher recurrence rates then historic controls. In 1989, the J strain was implicated in large hospital outbreaks of diarrhea in the United States. In 2003, the hypervirulent strain NAP1/BI/027 was identified in Canada, and in 2005 *C. difficile* infection due to ribotype 078 was identified in the Netherlands.

Antibiotic use is the most widely recognized and modifiable risk factor for *Clostridium difficile* associated diarrhea. Clindamycin was the first antibiotic associated with *Clostridium difficile* associated diarrhea, [5] followed by the third generation cephalosporins and more recently fluoroquinolones. Other putative risk factors for C*. diff* have included hospitalization, advanced age, severe comorbidities, gastric acid suppression, enteral feeding, and immunosuppressive therapy. [3, 6, 7, 8, 9, 10] However, *C. difficile* associated diarrhea can also occur in the absence of any risk factors. [11.12.13.14]

Three percent of the healthy adult US population is colonized with *Clostridium difficile*. [3] While data on treatment of asymptomatic carriers is limited, routine treatment is not recommended. There is a high rate of colonization with *Clostridium difficile* in children younger than 2 years of age; however this does not typically lead to disease. It has been hypothesized that the immature epithelial lining in neonates lacks the necessary toxin receptors. [1, 3] In adults and in children older than 2 years, normal intestinal flora are the first line of defense against *Clostridium difficile* colonization. [15]The normal flora can be disrupted by antibiotic exposure and this allows for germination of the *Clostridium difficile* spores and subsequent toxin production. In certain individuals, this may then lead to *C difficile* associated disease, although it appears that host factors may be as important as bacterial virulence factors in defining disease activity. [6,7,16] Gastric acidity and intestinal motility constitute the second line of defense against *Clostridium difficile* colonization, and it appears that patients with an intact immune system are more likely to be able to mount an effective antibody response against toxin production, prevent disease and lessen recurrences.[16]

About 20 percent of hospitalized adults are *Clostridium difficile* carriers who harbor *Clostridium difficile* in their stools but do not have diarrhea. [17] This carriage rate may approach 50 percent in long-term care facilities. [17] About 20 percent of patients with negative admission stool cultures become infected during their hospitalization. [17] Although asymptomatic, these individuals are capable of shedding pathogenic organisms and serve as a reservoir for environmental contamination to other hospitalized patients. *Clostridium difficile* is highly transmissible via inanimate objects and can be cultured readily from nearly any surface including items in patient rooms as well as on the hands, clothing and stethoscopes of healthcare workers. [17] Infection can also be transmitted readily between hospital roommates. The rate at which hospitalized patients acquire C*lostridium difficile* increases linearly with the duration of the hospitalization (8% per week). [17, 18]

Over 20 different genetic variants of *Clostridium difficile* have been described. There are specific gene loci within the bacteria genome which are responsible for inducing toxin production. They are also able to produce gene products that have an inhibitory effect upon toxin production. It is differences within gene loci that has apparently resulted in hypervirulent strains. [19, 20, 21, 22]

The incidence of *Clostridium difficile* infection has risen greatly over the past 20-30 years. In the 1990s, the incidence was 30-40 cases per 100,000, but by 2001 the incidence had risen to 50 cases per 100,000 and in 2005 it was 84 cases per 100,000.[3] Of even greater concern has been the increase in severe or fatal infections. Approximately 3-8 percent of C*lostridium difficile* infected patients develop fulminant disease, defined as patients whose course is complicated by perforation, severe ileus with toxic megacolon, hypotension requiring pressors or refractory bacteremia. [4, 18, 23] A significant number of these will require emergency colectomy. [24, 25] Overall mortality within 30 days after diagnosis has also increased from 4.7 percent in 1991 to 14 percent in 2003. [3]

Characteristics of *Clostridia difficile* and its pathogenicity

Clostridium difficile is an anaerobic gram-positive, spore forming, toxin producing bacillus. It is a non-invasive organism which possesses multiple virulence factors that can aid in colonization and promote disease. It can exist in both a spore and vegetative form. Outside the colon, it survives in the spore form and these spores are resistant to heat, acid and antibiotics. Once the spores reach the colon, they convert to a fully functional vegetative, toxin producing form and only then become susceptible to killing by antimicrobial agents.

Clostridium difficile colonize the human intestinal tract after the normal gut flora has been altered by antibiotic therapy. It is not known why only a fraction of patients exposed to a given antibiotic become colonized with *Clostridium difficile* or why only a fraction (approximately 30 percent) of those colonized develop symptoms. [7]

Enterotoxin A. and cytotoxin B., produced by *Clostridium difficile*, induce an inflammatory process. Both toxins adhere to receptors on the human colonocyte border and cause necrosis and shedding of the cells into the lumen. [26, 27, 28, 29] Once intracellular, toxins A and B. inactivate regulatory pathways mediated by Rho family proteins that are involved in cytoskeleton structure and signal transduction via GTP. [29] This disruption leads to cell retraction and apoptosis. Both toxins also disrupt intracellular tight junctions. [30]

Toxin A causes inflammation leading to intestinal fluid secretion, mucosal injury and inflammation. [29] Mediators of this pathway include arachadonic acid metabolites, substance P., tumor necrosis factor and interleukin-8. [27, 29] Toxin A can also directly activate neutrophils, and can promote neutrophil chemotaxis to localize within the pseudomembrane.

Toxin B is essential for the virulence of *Clostridium difficile*, and is approximately 10 times more potent then toxin A on a molar basis for mediating colonic mucosal damage.[26, 29] Strains lacking toxin A can be as virulent as strains with both toxins. A minority of *Clostridium difficile* strains are non-toxigenic and do not produce toxins and are, therefore, not pathogenic.

Toxins A and B are transcribed from a pathogenicity locus that comprises 5 genes: 2 toxin genes, tcdA (toxin A), tcdB (toxin B) and 3 regulatory genes, one of which (tcdC) encodes a putative negative regulator of toxin transcription. [29] Beginning in 1989 with the identification of the J- strain, hypervirulent strains of *Clostridium difficile* have been implicated in large hospital outbreaks throughout the world. [3, 19] In 2003, several hospitals in Québec Canada noted a marked increase in the incidence of *Clostridium difficile* associated diarrhea. A predominant strain, resistant to fluoroquinolones (82 %) was identified and it was characterized by the production of a binary toxin and partial deletion in the tcdC gene. [22] The 18 base pair deletion in tcdC allows for the production of toxins A and B in far greater quantities than control isolates. [22] Two chromosomal genes (cdtA and cdtB) separate from the chromosomal pathogenicity locus, encode this binary toxin. The cdtB gene encodes the binding component of binary toxin, whereas cdtA encodes the enzymatic component of the binary toxin. It disrupts the assembly of the actin filament through ribosylation of adenosine diphosphate, causing cell death. [20, 22] The binary toxin is related to the iota-toxin found in Clostridium perfringens. This hypervirulent strain does produce substantially larger quantities of toxin A and toxin B, and a partial deletion of tcdC impairs the inhibitory effect on toxin production. [20, 22] Finally, there is resistance to fluoroquinolones which may provide a selective advantage to this organism. [20, 22]

Host factors in disease phenotype

The clinical outcome of infection with toxigenic *Clostridium difficile* ranges from asymptomatic carriage to mild diarrhea or fulminant pseudomembranous colitis. It is felt that host, rather than bacterial, factors determine these differences in clinical presentation. There is some evidence that the immune response to *Clostridium difficile* and its toxins contributes to the wide spectrum of disease presentation. Kyne,et al prospectively studied *Clostridium difficile* infection in hospitalized patients who were receiving antibiotics. [16] The baseline antibody levels were similar in the patients who later became colonized and those who did not. After colonization, those who became asymptomatic carriers had significantly greater increases in serum levels of IgG antibody against toxin A than did the patients in whom *Clostridium difficile* diarrhea developed. [16] A diminished immune response among older individuals may in part explain the increased frequency and severity of C*lostridium difficile* diarrhea in the elderly.

Some individuals may be colonized with non-toxigenic strains of *Clostridium difficile* and this could afford protection against disease. The initial nonpathogenic colonizing strain may occupy a microbial niche in the large intestine and by occupying colonic receptors be protective against superinfection with a new *Clostridium difficile* strain. [15, 30]

Elevated serum interleukin IL8 levels appear to correlate with impaired humoral immune response to *Clostridium difficile* toxin A, and enhanced susceptibility to *Clostridium difficile* associated diarrhea. [7]

Laboratory Diagnosis

There are 2 categories of laboratory tests for *Clostridium difficile*. [3] The toxin assays which evaluate for evidence of the toxins and, organism detection assays which evaluate for the presence of the organism. Anaerobic stool culture is the most sensitive test but is not practical due to the slow turnaround time (72 hours). The need to detect toxin production by the recovered isolate further slows down this approach, since not all strains are toxin-producing. However, the cytotoxicity assay should be the "gold standard" against which other clinical tests are compared. Because only a few toxin molecules per cell are sufficient to cause "rounding" on the plate, the cytotoxicity assay can detect as little as 10 picograms of toxin B., making it the most sensitive *Clostridium difficile* test (94-100 percent). [26, 31]

Many laboratories use enzyme immunoassay (EIA) testing for *Clostridium difficile* toxins A and B., which is rapid but less sensitive than the cytotoxicity assay. Commercially available agents should detect both toxins A and B, since some strains only produce one of the toxins. In one study of 276 stool samples positive for *Clostridium difficile* by EIA, 19 percent were positive for toxin A. alone, 48 percent for toxin B. alone, and 33 percent for both toxins. [31, 32, 33] Currently at University Hospital, EIA testing is used against both toxin A and B. There is a high false negative rate with EIA since 100 to

1000 picogram of toxin must be present for the test to be positive. This test will have a PPV of 50 to 80%, assuming a CDI prevalence of 10%.[32,33]

One strategy to overcome this problem is to test for glutamate dehydrogenase(GDH), an essential enzyme produced by all *Clostridium difficile* isolates. [34, 35] This enzyme will be positive in the presence or absence of toxin production, so it may be used as the initial screening test, followed by cytotoxicity testing of positive samples.

In 2009, the FDA approved PCR assays for the gene encoding toxin B. These are rapid and accurate diagnostic tools which are important for the prompt initiation of treatment and for infection control measures. [35]

Colonoscopy or sigmoidoscopy can be a useful adjunct to stool studies for the diagnosis of *Clostridium difficile* infection when the clinical suspicion is high for *Clostridium difficile* but there are negative laboratory assays, when prompt *Clostridium difficile* diagnosis is needed, when there is failure of *Clostridium difficile* infection to respond to appropriate therapy or when there is an atypical presentation with an ileus or minimal diarrhea. Up to 4% of patients do not have diarrhea as a major symptom. Care should be taken to introduce minimal amounts of air during sigmoidoscopy given the risk of perforation if fulminant colitis is present.

Nosocomial infection

The rate of nosocomial *Clostridium difficile* associated diarrhea in the United States has doubled between 1996 and 2003, from 31 per 100,000 to 61 per 100,000. [3, 17] The rate is substantially higher in patients older than 65 (228 per 100,000). [3, 17] Since *Clostridium difficile* carriage rates range up to 50 percent of adults in hospitals and long-term care facilities, it is important to identify those who might serve as a reservoir for infection of other patients, and to institute the measures that will control spread among healthcare workers. *Clostridium difficile* spores may survive 4 months on bed rails, thermometers, stethoscopes or other inanimate objects. Alcohol-based antimicrobial soaps are ineffective; surfaces need to be wiped with a chlorine based solution. [17, 36, 37] It is prudent for healthcare workers to wash their hands with soap and water, rather than with alcohol-based hand sanitizers.

New exposure to *Clostridium difficile* is more likely to lead to C*lostridium difficile* associated diarrhea, while patient's previously colonized with C*lostridium difficile* are more likely to remain asymptomatic during their hospitalization. Shim, et al prospectively studied 810 hospitalizations including 618 patients with new *Clostridium difficile* exposure and 192 patients with previous C*lostridium difficile* colonization. [38] Newly exposed patients developed C*lostridium difficile* associated diarrhea much more frequently than colonized patients (22 percent versus 1 percent). These phenomena have been observed even when carriers and symptomatic patients are colonized with identical strains. [38] The explanation for this difference is unclear although, again, host factors may play a role.

Best, et al studied the potential for aerosolization of C*lostridium difficile* after flushing toilets. *Clostridium difficile* was recoverable from air sampled at heights up to 25 cm above the toilet seat. The highest numbers of C*lostridium difficile* were recovered from air sampled immediately following flushing, and then declined 8- fold after 60 minutes and a further threefold after 90 minutes. [39]

Community-acquired infection

Khanna,et al conducted a population based study in Olmsted County, Minnesota to better understand the epidemiology of community-acquired *Clostridium difficile* infection.[14] Community-acquired *Clostridium difficile* infection cases accounted for 41 percent of the 385 cases that were analyzed. Patients with community-acquired infection with younger (50 years versus 72 years), more likely to be female (76 percent versus 60 percent), have fewer co-morbidities, and were less likely to have severe infection, or have been exposed to antibiotics (78 percent versus 94 percent). [14] Other studies of community-acquired *Clostridium difficile* infection have reported a similar lack of antibiotic and proton pump inhibitor exposure. These observations suggest that there may be additional or different risk factors for community-acquired *Clostridium difficile* infection. [11, 36]

Pepin,et al studied the risk of secondary cases of C*lostridium difficile* infection among household contacts of index cases in Québec Canada. [22] Their retrospective review of 1061 spouses and 501 children living in the same household as the index case discovered 5 infections among spouses and 3 infections among children. This slightly increased relative risk only persisted for the first 3 months after the diagnosis in the index case.

Disease Manifestations

Frequent, small volume watery diarrhea is the common symptom of *Clostridium difficile* colitis, although there can be a wide spectrum of disease manifestations. This could range from an asymptomatic carrier state to a severe form of colitis with toxic megacolon and perforation. Fever is associated with *Clostridium difficile* infection in about 15 percent of cases, and leukocytosis is common. In those with severe colitis, an ileus may develop and diarrhea may not be a predominant symptom. It is under these circumstances that sigmoidoscopy might be helpful. The finding of pseudomembranes is sufficient to make a presumptive diagnosis of *Clostridium difficile* infection. These raised yellow plaques are scattered over the mucosa with relatively normal-appearing intervening mucosa.

With fulminant colitis, there may be severe lower abdominal pain, abdominal distention, marked leukocytosis and lactic acidosis. One potential complication includes bowel perforation in the setting of toxic megacolon. KUB may show small bowel dilatation and "thumb printing" due to submucosal edema. The diagnosis of fulminant colitis needs to be established quickly, as it has been shown that colectomy may improve survival compared to conservative management. Jaber,et al noted that 3-8 percent of patients

with *Clostridium difficile* infection developed fulminant disease. [18] Features that defined the need for colectomy included age greater than 65 years, leukocyte count higher than 20,000, acquisition of infection in the hospital, renal failure and immunosuppression.

Immunocompromise appears to affect disease presentation, whether due to inherent immunodeficiency or induced by a drug or disease process.
Immunosuppressed patients with solid organ transplantation and those with an impaired antibody- mediated immune response to *Clostridium difficile* toxins, as well as patients with inflammatory bowel disease exacerbation appear to be at increased risk of fulminant *Clostridium difficile* infection. [39, 40, 41, 42, 43, 44] Boutros,et al reviewed 1331 solid organ transplant recipients, and found a incidence of 12.4 percent for *Clostridium difficile* associated diarrhea. [45] The peak frequency was between 6 and 10 days post transplantation, and was more likely in those older than 55 years, those who had received antithymocyte globulin, and those who had transplants other than lone kidney transplant. Pant et al analyzed 184,139 cases of end-stage renal disease and identified an incidence of *Clostridium difficile* infection of 2.8 percent. [43] Compared to patients with endstage renal disease alone, those with *Clostridium difficile* infection had a higher in-hospital mortality rate (13.2 percent versus 5.3 percent). [43] Ali, et al looked at 193,714 patients who had undergone liver transplantation between 2004 and 2008. The prevalence of *Clostridium difficile* infection was higher at 2.7 percent (compared to 0.9 percent in the non-liver transplant population). [42] *Clostridium difficile* infection in liver transplant patients was also associated with a higher mortality, 5.5 percent as compared to 2.3 percent in liver transplant -only population. [42] Current data suggests that 34 percent of patients who have undergone lung transplantation will develop symptomatic *Clostridium difficile* infection shortly after transplantation. [41] High incidence may be due to several factors including immunosuppressive therapy, the frequent use of antibiotics in the post lung transplant setting, and a disproportionate number of individuals who have hypo-gammaglobulinemia.[41]

HIV-seropositive patients, particularly with lower CD4 counts, are at increased risk of bacterial enteric infection including Escherichia coli, Salmonella, and Campylobacter. Presentation of C*lostridium difficile* in HIV is similar to that among non-HIV-infected individuals with diarrhea, abdominal pain and fever. Where HIV patients already have low CD4 counts, average leukocytosis may be significantly lower during the infection. One study involving HIV patients with advanced immunosuppression found that 25 percent had features of a protein-losing enteropathy. [40] *Clostridium difficile* strains isolated from the stool of HIV positive cases have been reported to be associated with higher rates of antimicrobial resistance than isolates from those without HIV infection, but this did not translate into reduced response rates. [40]

Alonso et al performed a retrospective nested case control study of *C. difficile* infection in bone marrow transplant recipients. [41] The overall one year incidence of *Clostridium difficile* infection was 9.2 percent among patients who had received stem cell transplantation. The median time to diagnosis was short among both autologous and allogenic transplant recipients (6.5 days and 33 days respectively). There was also a

strong relationship between early *Clostridium difficile* infection and the subsequent development of gastrointestinal tract graft versus host disease in the year following stem cell transplantation. [41] In addition, among allogenic HSCT recipients, GI GVHD conferred a nearly 5-fold higher risk of CDI recurrence. It is unclear if this is associated with impaired systemic immunity of local GI tract phenomenon. [41]

Current estimates suggest that 10 percent of patients with inflammatory bowel disease will develop symptomatic *Clostridium difficile* infection at some point during their lifetime. Enteric infections account for about 10 percent of symptomatic relapses in patients with IBD. [46] *Clostridium difficile* appears to account for at least one half of these infections. Immunosuppressive therapy with purine analogs, methotrexate, and steroid therapy appear to be strongly associated with the development of *Clostridium difficile* infection. Indeed, steroid exposure is linked to a threefold increase in the risk of Clostridium difficile infection during the following year. [46, 47, 48] Ananthakrishnan et al showed that IBD patients who are hospitalized with *Clostridium difficile* infection have a mortality rate of 4.2 percent which is significantly higher than the overall mortality rate for patients hospitalized with this infection. [48] There is also a high prevalence of *Clostridium difficile* carriage in patients with IBD. Clayton, et al demonstrated that patients with long-standing IBD had an 8 percent *C. difficile* carriage rate compared to healthy volunteers (1 percent). [49]

Jacobs, et al has reviewed the extracolonic manifestations of *Clostridium difficile* infection. [50] These include small bowel involvement, visceral abscess formation, prosthetic device infection, encephalopathy, reactive arthritis and osteo-myelitis.

Clostridium difficile is usually confined to the colon where receptors are present for the organism. Most of the reported patients with small bowel involvement had surgically altered intestinal anastomosis, and this may be a predisposing factor to small bowel colonization. It has been hypothesized that surgical alteration of the small bowel could lead to bacterial colonization resembling the colon, resulting in increased susceptibility to *Clostridium difficile* infection. Among a few reported cases in the literature, when the small bowel is involved with infection with *Clostridium difficile*, mortality rate is higher. [51, 52]

Since *Clostridium difficile* is a spore forming bacterium found in the environment, it would seem possible that it could cause cellulitis and then infect superficial wounds. Jacobs,et al identified 9 cases of *Clostridium difficile* bacteremia that had been reported in the literature. [50] It has been hypothesized that the organism can enter the blood stream via the inflamed gut mucosa which would normally have served as a barrier. Visceral abscess formation may similarly result from bacteremia, and both splenic abscess and pancreatic abscess formation have been reported.

In 1976, Putterman and Rubinow described the first case of pseudomembranous colitis associated with arthritis. According to Jacob et al, there are now 36 reported cases of reactive arthritis secondary to *Clostridium difficile* infection. The onset of arthritis appears to occur about 11.3 days after the onset of the colonic symptoms, and the

illness is frequently prolonged with recovery taking up to one year. Osteomyelitis and infection of implanted prosthetic devices has also been described.

Treatment

Shortly after the first description of *Clostridium difficile* infection in the late 1970s, effective therapy with either metronidazole or oral vancomycin was reported. Despite the dramatic increases in the incidence and severity of *Clostridium difficile* infection during the past decade, these 2 agents remain the treatment of choice for almost all patients with C*lostridium difficile* infection. Aslam et al, reviewed the treatment of *Clostridium difficile* associated infection, and a review of controlled trials conducted before 2000 indicates that the cumulative failure rates for treatment with metronidazole (2.5 percent) and vancomycin (3.5 percent) were virtually identical. [53] However, since 2000, substantially higher failure rates (18.2 percent) have been reported for metronidazole therapy. [53] Wilcox et al, in a retrospective study reported that the time to resolution of diarrhea in patients who were treated with metronidazole was significantly longer than those treated with vancomycin (4.6 days versus 3 days). [54] In mild infection, vancomycin and metronidazole showed general similar efficacy. However, among patients with severe infection, vancomycin appears to be significantly more effective. There is more prompt symptom resolution and a lower risk of treatment failure with vancomycin. However, oral vancomycin may not be suitable for some patients with severe or fulminant infection because of coexisting ileus or megacolon. In these circumstances, intravenous metronidazole should be used, though it may be supplemented with vancomycin administered through a nasogastric tube or by enema. [55] Among experts, it is felt that vancomycin should be reserved for patients who cannot tolerate metronidazole, or who do not respond to 2 courses of metronidazole, or who have more severe disease. This is in an effort to reduce the proliferation of vancomycin-resistant enterococcal infection. Despite a lack of significant clinical evidence, oral vancomycin may be advisable for immunosuppressed patients.

Adjunctive therapy with probiotics (Saccharomyces boulardii, for example) in combination with vancomycin or metronidazole appear to help restore normal flora. [56, 57, 58] McFarland et al, demonstrated a lower recurrence rate with the combination (65 percent versus 35 percent). [59] Bacteria probiotic preparations produce fatty acids that lower the pH of the local gut environment, as well as toxins that inhibit the growth of other bacteria. Saccharomyces has been shown in vitro to secrete a protease that inhibits binding of enterotoxin A. [58] Probiotics may also be capable of interfering with the binding of *Clostridium difficile* toxins A and B to intestinal epithelial cells. [56] For antibiotic associated diarrhea, a meta-analysis has shown that 3 types of probiotics (Saccharomyces boulardii, Lactobacillus rhamnosus GG and probiotic mixtures) will reduce symptoms. However, only Saccharomyces boulardii appears effective for C*lostridia difficile* diarrhea and colitis. [57]

Recently, the FDA approved a new drug, Dificid for the treatment of *Clostridium difficile* infection. Preliminary results indicate good efficacy. Fidaxomicin, which is narrow

spectrum and spares Bacteroides species was shown to reduce the initial relapse rate of *Clostridium difficile* infection by 50 percent compared with vancomycin treatment. [46]

Perhaps the most controversial treatment for *Clostridium difficile* has been fecal microbiota transplantation (FMT). [60, 61] Evidence suggests that normal gut microbiota positively affects immune and inflammatory responses by fermenting dietary fiber to produce short chain fatty acids which directly behind a receptor, GPR43, on immune cells. [62] Studies have shown that patients with initial or recurrent Clostridium difficile infection are deficient in Bacteroides and Firmicutes. Khoruts, et al showed that transplantation of fecal microbiota from a healthy individual into a recipient with recurrent *Clostridium difficile* infection resulted in a fecal bacteria composition dominated by Bacteroides strains similar to those in a healthy donor. [63] A review of published reports of fecal transplantation for the treatment of *Clostridium difficile* infection reported a response rate of 87 percent. [64] Acceptance of this form of therapy has largely been hindered by its lack of aesthetic appeal and by the cumbersome nature of screening donors in a timely fashion. Donors must have screening of both blood and stool and this may take several days. Initial therapy has been done via administration through the colonoscope, though protocols are also available where the bacteria may be delivered via a nasogastric tube into the distal small bowel. [59, 60, 61] The efficacy of fecal bacteriotherapy may depend upon the technique used to cleanse the colon before administration of the fecal enemas. Cleansing with a PEG type solution may reduce the density of *Clostridium difficile* organisms including the metabolically inactive spores that could otherwise convert to vegetative forms. The use of the colonoscope to deliver fecal bacteria has the theoretical advantage of delivering microbiota to the distal small bowel where *Clostridium difficile* can reside.

One protocol for administration by colonoscopy suggests that oral vancomycin should be given at a dose of 500 mg twice daily for 7 days, followed by a single oral lavage 4 L of GoLYTELY. 200-300 g of donor stool suspended in 300 mL of sterile normal saline can then be administered within 10 minutes of preparation. After the treatment, patients are advised to adhere to a high-fiber diet to assist in the support of infused bacteria. [59, 60, 61]

When administering via a nasojejunal tube, it is also been recommended that a proton pump inhibitor be given the evening before and the morning of the instillation in order to decrease gastric acid. A single instillation of 30 g of stool diluted in 50 mL of saline is typically sufficient in patients receiving therapy via the upper GI tract. [59, 60, 61] Recently, Hamilton et al, published a standardized frozen preparation for transplantation of fecal microbiota for recurrent C*lostridium difficile* infection. This allowed the process to move away from individual donors to standard volunteer donors. Material was able to be stored in a frozen manner and could be ready for use when needed. [61]

Recurrent Infection

The risk of recurrent *Clostridium difficile* infection is about 20 percent after an initial episode, but may increase up to 60 percent after 2 or more recurrences. [17] Most patients develop recurrences within the first 2 months after receiving oral therapy with

vancomycin or metronidazole. In a prospective, randomized double blind trial, Fekety et al identified 5 factors that were associated with a higher risk of recurrent C*lostridium difficile* diarrhea. [6] These included the number of previous *Clostridium difficile* episodes, onset of the initial disease in the spring time, exposure to additional antibiotics for treatment of other infections, infection with immunoblot type 1 or 2 strains of *Clostridium difficile*, and female gender. For patients with recurrent disease, the severity did not appear to become progressively worse as the number of episodes increased. However, the risk of having another recurrence was proportional to the number of previous episodes. The type of antibiotic therapy that preceded the first episode was not significantly different for patients with recurrent disease in general. Among the patients who submitted a stool sample 3 weeks after cessation of antibiotic therapy, carriage of *Clostridium difficile* was found to be significantly higher for those who ultimately developed a recurrence than for those who did not develop another recurrence. [6]

Kamboj et al used molecular typing to determine whether second episodes were related to relapse or reinfection. [36] They examined *Clostridium difficile* isolates from 102 patients with repeated episodes of *Clostridium difficile* infection. They found that for those with a second episode within 8 weeks of the index case, almost all second episodes were due to the same strain. However, even for episodes occurring more than 8 weeks after the initial episode, the majority (65 percent) was also due to the original infecting strain and this represented relapse and not a second new infection. [36]

Role of the appendix in *Clostridium difficile* infection

Although the function of the appendix has been debated, its active, gut associated lymphoid tissue and biofilm production suggest potential rolls in recovery from initial *Clostridium difficile* infection and protection against recurrent C*lostridium difficile* infection. Im et al published a retrospective review of the medical records of inpatients with *Clostridium difficile* infection who were admitted to a tertiary care teaching hospital from 2005-2007 to identify those with and without an appendix. The presence of an appendix was associated inversely with *Clostridium difficile* infection recurrence. [65] One hypothesis for this finding was that the appendix is a secondary lymphatic organ affecting pro- inflammatory and anti-inflammatory T cell responses. The lymphoid tissue of the human appendix has a higher density of IgA and IgG producing cells than any other sites in the colon. The biofilm, which contains microbe, was within the extracellular matrix and mucus lining. It was suggested that this biofilm may enhance the survival of commensal bacteria and permit the re-inoculation of the gut especially after an episode of intestinal injury. In this study, the *Clostridium difficile* recurrence rate for patients with an appendix was 18 percent, compared with 45 percent in those without an appendix. [65]

Summary

After years of stable C. *difficile* related morbidity and mortality, increases in both the rate and severity of CDAD began to be observed in the late 1990's and early 2000's in North

America and Europe. [3] Along with the emergence of more virulent strains, there have been some changes in antimicrobial resistance. The organism is now prevalent in both the community and health-care facilities, and with an aging population, host factors are allowing the disease to become more difficult to treat.

CDAD results from antibiotic exposure, in conjunction with colonization of the gut by one of the toxigenic strains of C. *difficile*. The most important way to control CDAD, and perhaps the most important "take home message" from this review, is to limit antibiotic exposure to appropriate circumstances.

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