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***Clostridium difficile* Infection**

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Abstract

Clostridium difficile is the leading cause of hospital-acquired diarrhea in Europe and North America and is a serious re-emerging pathogen. Recent outbreaks have led to increasing morbidity and mortality and have been associated with a new strain (BI/NAP1/027) of *C. difficile* that produces more toxin than historical strains. With the increasing incidence of *C. difficile* infection, clinicians have also seen a change in the epidemiology with increased infections in previously low-risk populations. This chapter highlights the current knowledge on *C. difficile* virulence, human disease, epidemic outbreaks, and optimal treatment strategies.

Keywords

Clostridium difficile; toxin

Clostridium difficile was first isolated from the stool of a healthy infant by Hall and O'Toole in 1935.¹ The species name was chosen to reflect the difficulty with its culture and isolation.² Pseudomembranous colitis was first described in 1893.³ It was not until 1978, however, that George and colleagues associated *C. difficile* with human disease and discovered that *C. difficile* was the organism responsible for the majority of cases of antibiotic-associated diarrhea.^{4, 5} *C. difficile* has become a leading cause of hospital-acquired illness in the United States and developed countries. Each case of *C. difficile*-associated disease has been estimated to result in more than \$3600 in excess health care costs, and these costs cumulatively may exceed \$1 billion annually in the United States.⁶ Data from the U.S. Center for Disease Control and Prevention show that the rate of the discharge diagnosis of *C. difficile* infection (CDI) has increased from 31 cases per 100,000 persons per year in 1996 to 61 per 100,000 in 2003.⁷ An estimated 500,000 cases per year occur in the United States.⁸ Not only has there been a dramatic increase in the number of cases of CDI, there has been a recent increase in the morbidity of CDI by as much as 25% per year⁷ as well as a 20-fold increase in mortality.^{9–11} An estimated 15,000–20,000 patients die annually from CDI in the United States.⁸

In this symposium article we provide updated information on pathogenesis, epidemiology, clinical presentations, and treatment of CDI. Major goals are to emphasize the shift in the epidemiology and virulence of CDI over the past decade and highlight how these changes have increased the complexity of clinical management of this illness.

Bacteriology

C. difficile is a Gram positive rod – shaped bacterium that can exist in a vegetative or spore form. In its spore form, the bacterium can survive harsh environments and common sterilization techniques. Spores of *C. difficile* are resistant to high temperatures, ultraviolet light, harsh chemicals, and antibiotics. Furthermore, because spores are resistant to antibiotics, they can remain in the gastrointestinal tract and potentially contribute to recurrent disease following treatment and eradication of vegetative *C. difficile*.⁹

Virulence factors

The primary virulence factors of *C. difficile* are 2 toxins, toxin A (TcdA) and toxin B (TcdB). TcdA (308 kD) and TcdB (270 kD) are 2 of the largest bacterial toxins known and are part of the Large Clostridial Toxin family that glucosylate small GTPases in the cytosol of targeted cells.¹² TcdA and TcdB are encoded within a 19.6 kB pathogenicity locus (PaLoc) which also contains genes encoding for negative and positive regulators of toxin expression and a holin thought to promote release of the toxins from the organism. TcdA induces a florid inflammatory response when injected in an intestinal loop of rodents.¹³ TcdB is devoid of enterotoxin activity in animals but is a potent cytotoxin. It has been shown to be 10 times more potent than toxin A in causing epithelial damage to colonic cells *in vivo*.¹⁴ TcdA and TcdB act by disrupting the actin cytoskeleton of fibroblasts in tissue culture cells by glycosylating the small GTPases Rho, Rac, and Cdc42, thereby preventing the activation of these proteins and their ability to regulate actin polymerization.^{15, 16}

The respective roles of TcdA and TcdB have been scrutinized recently. The traditional paradigm that TcdA is the major virulence factor has not been supported by several recent findings. It is known that TcdA-negative, TcdB-positive strains can be found in patients that clearly have CDI.¹⁷ Yet, reciprocal strains that are TcdA-positive and TcdB-negative have yet to be identified, despite identification of almost 2 dozen different toxinotypes of *C. difficile*. Without exception, all disease - causing strains of *C. difficile* examined to date have been found to express TcdB.

Recent work by Lyras *et al*¹⁸ provides important insight into the contributions of TcdA and TcdB to disease. These investigators reported the generation of isogenic strains of *C. difficile* lacking either TcdA or TcdB. Interestingly, when these strains were tested in a rodent model of CDI, only the strain lacking TcdB was found to be attenuated in virulence. These findings are further evidence that TcdB, but not TcdA, is the major virulence factor of *C. difficile*.

Immune response

Up to 60% of healthy adults have detectable serum IgG and IgA against TcdA and TcdB.¹⁹ *C. difficile* colonizes 60 – 70% of healthy newborns and infants up to 12 – 18 months of age when normal colonic microflora is established.²¹ These infants rarely develop colitis but have large amounts of toxins in stool, presumably establishing this humoral immunity. Most patients with acute infection therefore do not exhibit an IgM response, but rather a secondary IgG response.²⁰ In patients with acute CDI, higher levels of anti-TcdB IgG have been associated with milder disease.²² The development of high titers of anti-TcdA antibodies are associated with development of asymptomatic carriage, whereas low titers of anti-TcdA antibodies are associated with the subsequent development of CDI.²³ Low levels of antibodies against TcdA have also been associated with more severe disease.²⁴ These studies demonstrate that humoral immunity may be important in preventing disease. This has set the groundwork for therapeutic use of antitoxin antibodies and vaccine development, as will be discussed later.

Pathogenesis

A detailed model of *C. difficile* pathogenesis is emerging. The bacteria are known to colonize the colon after exposure; colonization in other areas of the human body including the small bowel is quite rare. This proclivity for the large bowel is probably dictated by the anaerobic environment and the absence of competitor flora depleted during antibiotic treatment.

Although toxin production is essential for damage to the host, there are several events that must take place before toxin production. One of the first events is the germination of *C. difficile* spores. Recent work by Sorg and Sonenshein²⁵ found that bile salts promoted spore germination. This finding suggests a model wherein germination is promoted once the spores have reached an appropriate location within the gastrointestinal tract. Following germination, *C. difficile* adheres to the intestinal epithelium by triggering the production of microtubule-based cell extensions that are induced by *C. difficile* toxin (CDT), a binary toxin that ADP-ribosylates actin.²⁶ However, only a small percentage of clinical *C. difficile* isolates carry the genes that encode for CDT, suggesting that other factors are involved in adherence. Following or during colonization, vegetative *C. difficile* releases TcdA and TcdB. The triggers and mechanisms of toxin secretion have not been defined in *C. difficile*, but changes in amino acid availability have been shown *in vitro* to induce toxin expression²⁵. Once released into the colon, TcdA acts primarily on the intestinal epithelium, causing fluid secretion, inflammation, and tissue necrosis, whereas TcdB with its broad cell tropism acts as a potent cytotoxin. Previous work from our group has shown that TcdB functions as a potent cardiotoxin in animal models of intoxication,²⁷ suggesting that this toxin could contribute to the complicated systemic damage observed in fulminant CDI.

Epidemiology

C. difficile is most commonly a nosocomial pathogen, with reservoirs commonly being patients, healthcare workers, and the contaminated hospital or other healthcare facility environment.²⁸ Studies have shown a 20% to 40% rate of colonization in hospitalized adults compared with 2% to 3% in healthy adults.^{29, 30} However, community-acquired infections have also been described.³¹ It has been estimated that approximately 28% of CDI cases are community-acquired.^{32, 33} Recent data suggest that community acquisition may be increasing,^{32, 33} which may be contributing to the increase in CDI cases. Possible community sources for CDI include soil, water, pets, meats, and vegetables.⁸

The major risks for CDI are antibiotic exposure within the preceding 2 – 3 months, advanced age, hospitalization, and long-term care facility residence.^{8, 34–36} A Swedish nationwide prospective population-based analysis showed that the rate of CDI was 10 times higher in persons older than 65 years compared to persons younger than 20 years of age.³²

Two case control studies from England found that treatment with gastric acid suppressants (especially proton pump inhibitors and H₂-blockers) increase the risk for community-acquired CDI.^{37, 38} Acid suppressants have also been associated with recurrent disease: patients on proton pump inhibitors are 4 times more likely to have a recurrence.³⁹ Multiple recent studies have shown an increased risk for CDI in hospitalized patients taking PPIs.^{40–42} Most recently, Aseeri *et al* published a retrospective case control study that showed a relative risk of 3.6 for patients using proton pump inhibitors.⁴² However, it has been hypothesized that acid suppression is merely a marker for multiple comorbidities that increase the risk of CDI.⁴³ The use of narcotics has not been shown to increase the risk of CDI; however, their use has traditionally been considered a risk factor for development of toxic megacolon owing to their antiperistaltic effects.⁴⁴

There were 4 CDI outbreaks in the United States reported between 1989 and 1992.^{45–49} Pooled case-control studies performed at 3 hospitals found that patients infected with the epidemic strain of *C. difficile* were significantly more likely to be treated with clindamycin than those patients with diarrhea due to the non-epidemic strain. These outbreaks were attributed to the frequent use of clindamycin because all isolates of the epidemic strain were highly resistant to clindamycin.⁴⁹

In 2001, a trend of increasing CDI rates was noticed at the University of Pittsburgh Medical Center. In this hospital the incidence of disease in 2000–2001 nearly doubled when compared to 1990–1999, and 10% of patients diagnosed with CDI required an emergency colectomy.⁵⁰ To further study this outbreak, McDonald *et al.* collected 187 *C. difficile* isolates from outbreaks between 2000 and 2003 from 8 health care facilities in 6 states. Isolates were characterized by toxinotyping, restriction endonuclease analysis, pulsed-field gel electrophoresis, and polymerase chain reaction for binary toxin and/or *tcdC* deletions. The results were compared to over 6000 isolates obtained before 2001, allowing characterization of the new strain responsible for the outbreaks. The outbreak strain (termed BI/NAP1/027) was positive for the binary toxin, contained an 18 base-pair deletion in the *tcdC* gene, and had increased resistance to fluoroquinolones.⁵¹ This strain has also been found in Canada and many European nations.⁸ The function of binary toxin has been discussed above, and TcdC is a negative regulator of TcdA and TcdB production.⁵² This epidemic *C. difficile* strain was found to be highly transmissible (including person-to-person) and thought to be more virulent.² The BI/NAP1/027 strain produces about 10-fold more toxin A and approximately 23-fold more toxin B than historical strains.⁵³

The significance of this new epidemic strain is as yet not completely defined. The BI/NAP1/027 strain appears to be associated with infection in individuals not previously considered at risk, including young and previously healthy persons not exposed to a healthcare environment or antimicrobial therapy.^{2, 54, 55} Severe infection leading to colectomy and/or death was also described in young women in the peripartum period.^{2, 54, 55} However, 2 recent studies have shown that in a nonepidemic setting the BI/NAP1/027 strain is not associated with more severe disease.^{56, 57} Finally, a study in Canada showed an increase in mortality in patients infected with BI/NAP1/027, particularly in patients between the ages of 60 and 90.⁵⁸

Spectrum of disease in humans

CDI presents as a wide range of clinical disease, ranging from asymptomatic colonization or trivial diarrhea to life threatening illness. Although disease begins with uncomplicated watery diarrhea, symptoms may progress to become more serious. In addition to massive colonic inflammation, lesions begin to form and coalesce within the colon, creating a pseudomembrane of immune cells, mucus, and necrotic tissue. Pseudomembranous colitis represents an advanced stage of disease and is virtually diagnostic of CDI. In some cases, colonic distension can occur, a condition known as toxic megacolon.¹⁰ Notably, in up to 20% of patients with advanced disease, diarrhea and fluid loss are minimal and patients present instead with abdominal distention and ileus,¹⁰ a condition which is in contrast to mild forms of CDI, often leading to misdiagnosis. Patients with severe or fulminant CDI also may exhibit signs of systemic toxicity and the systemic inflammatory response syndrome, including leukocytosis ($\geq 35.0 \times 10^4/\mu\text{l}$),^{59, 60} rising serum lactate levels ($\geq 5 \text{ mmol/L}$), hypotension requiring vasopressor therapy, acute renal failure, and respiratory distress, all indicators of poor prognosis and high mortality.⁶¹ Fulminant colitis can also lead to the need for total colectomy. Unfortunately, despite surgery and aggressive antibiotic therapy, the average mortality rate following colectomy is 67%⁶².

The progression to fulminant CDI requiring colectomy can occur in as little as hours to as long as weeks from the initial symptoms of disease, with the median time to colectomy in 1 study

7.5 days in patients who died after colectomy and 15 days in patients who survived after colectomy.¹⁰ Unfortunately, it is not clear what instigates the sometimes rapid transition from mild to fulminant disease nor is it known why or which patients will eventually develop severe or fulminant disease. In some cases, patients with benign symptoms will suddenly and rapidly progress to shock. The contributors to disease severity and patient death likely include factors from both the host, such as age, immune status, and co-morbidities, as well as the *C. difficile* organism, such as virulence and perhaps relative antimicrobial resistance.

Diagnosis

Strategies for the diagnosis of CDI have evolved over the past few years.⁶³ It is emphasized that patients at risk for CDI who develop severe disease should begin therapy empirically after appropriate stool samples have been collected but before the results of diagnostic studies become available.⁶⁴ Specific toxin identification is required for definitive diagnosis. Sigmoidoscopy in particular and even colonoscopy are insensitive tests because only about one-half of CDI cases are associated with visible pseudomembrane formation.⁶⁵ Furthermore, there is an increased risk of perforation during endoscopy in CDI. Radiographic studies are not required for diagnosis of CDI, but some characteristic features on cross-sectional imaging (colonic wall thickening, pericolonic stranding, the “accordion sign”, and the “double-halo sign”) are reported, although their diagnostic sensitivity and specificity are not known.⁶⁴

An algorithm for definitive diagnosis has been recently reported.⁶³ The initial screening test should be for *C. difficile* antigen in stool, specifically glutamate dehydrogenase, most commonly by enzyme-linked immunosorbent assay (ELISA) or immunochromatographic assay. Glutamate dehydrogenase is a cell-wall protein that is produced in much higher amounts than the toxins. This test is comparatively rapid, highly sensitive, and has a high negative predictive value, and thus can be used to eliminate the need for further testing or treatment. However, it is no more than 50% specific, because false positive tests can result from other gut flora and non-toxigenic *C. difficile*. In those with a positive glutamate dehydrogenase test, the next step is an assay for toxin A/B, again either by ELISA or immunochromatographic assay. If the GDH and toxin A/B assay are both positive, a definitive diagnosis of CDI is established. However, this test is not very sensitive for detection of toxin B, and as noted above, TcdA-negative and TcdB-positive disease does occur. Therefore, with a positive glutamate dehydrogenase test and a negative toxin A/B test, a more sensitive assay for presence of toxin B is required for diagnosis.

Current treatment

Recommendations for therapy of CDI vary and are based on the severity of the illness and whether it is an initial or recurrent infection. All agree that stopping the inciting antibiotic whenever possible is the most important first step in treatment of CDI and has historically resulted in cure. However, because of the presence of more virulent organisms currently, therapy is indicated for all. The efficacy of changing antimicrobial therapy to a more narrow spectrum agent less likely to provoke CDI is logical but unconfirmed.²¹ It has long been believed that anti-diarrheal agents should be avoided and narcotic use minimized, as the anti-peristaltic effects and toxin entrapment can predispose to toxic megacolon.⁶⁶ However, recent evidence challenges this view when such therapies are combined with therapies specific to *C. difficile*.⁶⁷ Replacing fluid and electrolyte losses are indicated as needed.

The antibiotic therapies for CDI are metronidazole and vancomycin. It is important to note that topical therapy in the colon with vancomycin delivered either orally or rectally is required, because intravenous vancomycin therapy is ineffective. Metronidazole achieves similar colonic and intraluminal levels given either orally or intravenously;²¹ when feasible the oral route is preferred. Metronidazole was initially thought to be equivalent to vancomycin.⁶⁸

however, failures to respond to metronidazole have been more common recently, although not related clearly to metronidazole-resistant *C. difficile*.^{69, 70} Therefore vancomycin is more commonly being used as first-line treatment, especially in patients with more severe disease. Initial concerns about development of vancomycin – resistant enterococci are not warranted because metronidazole is equally provocative.⁷¹ The superior efficacy of vancomycin compared to metronidazole has been confirmed in severe cases of CDI.⁷²

For mild to moderate CDI, treatment with either metronidazole (250 mg every 6 hours or 500 mg every 8 hours orally) or vancomycin (125 mg orally every 6 hours) for 10 – 14 days results in resolution of symptoms and cure rates in more than 90% of cases within 7 – 10 days.^{21, 73} In patients with moderate to severe disease, particularly those with ileus and/or toxic megacolon, other strategies should be employed. Combined therapy with metronidazole and vancomycin has been recommended despite a lack of clinical trial evidence of improved efficacy.²¹ Evaluation for colectomy should be performed by a gastrointestinal surgeon. In those with ileus, based on the hypothesis of inadequate delivery of oral vancomycin to the site of infection, consideration should be given to administering vancomycin 500 mg 4 times daily by nasogastric or small intestine intubation or by retention enema.² Other evolving therapies as discussed below may be considered.

Patients with a first relapse (estimated to occur in up to 30% of patients, usually within 14 days of cessation of therapy⁷⁴) should be retreated with vancomycin or metronidazole (including the same agent originally used). However in patients with multiple relapses (about one-half of those with a first relapse), pulse therapy or tapering therapy with vancomycin is generally recommended.^{2, 21, 73} The use of a course of rifaximin after previous treatments has also been recommended.⁷⁵ These patients may also benefit from other therapies such as probiotics and/or toxin - binding resins (see below).

Evolving therapies

Treating a disease due to disturbance of the normal colonic flora with antimicrobial agents seems to be an inherently flawed strategy. Nonetheless, additional antimicrobial agents are under investigation for treatment of CDI.^{21, 73} These include tinidazole (a 5-nitroimidazole similar to metronidazole), rifaximin⁷⁶ and rifalazil (rifamycin antimicrobials similar to rifampin), nitazoxanide (a thiazolide antimicrobial used primarily to treat giardiasis),⁷⁷ glycopeptide derivatives (teichoplanin, oritavancin, ramoplanin), fidoxamicin (a novel nonabsorbable macrocyclic antimicrobial),⁷⁸ and topical bacitracin.

Probiotics have been used with varying results based on the theory of replacing the normal flora of the colon and competing with *C. difficile* for nutrients and mucosal lining.^{79, 80} Probiotics are presumably non-pathogenic microorganisms; the most studied are *Saccharomyces* and *Lactobacillus*. Several randomized studies have examined the use of probiotics as an adjuvant to standard therapy and generally have demonstrated modestly improved outcomes compared to vancomycin or metronidazole therapy alone.^{81–84} There are safety concerns about the use of probiotics in immunocompromised patients because blood - borne infections have been reported.^{85, 86} In a conceptually appealing approach, concurrent administration of non-toxicogenic *C. difficile* with antimicrobial therapy reduced the incidence of CDI in hamster models.⁸⁷ Fecal bacteriotherapy (administration of strained feces, generally from a relative, by gastric intubation or enema) has been used for treatment of recurrent CDI, as reviewed.⁸⁸ Recently, concurrent administration of probiotics with antimicrobial therapy was effective in reducing the incidence of CDI in a single report.⁸⁹

Inert nonabsorbable anionic polymers, beginning with colestipol and cholestyramine, have been used in CDI, although data to support their use are largely lacking.^{21, 73} More recently, tolevamer was specifically developed for its ability to bind *C. difficile* toxins *in vitro*;⁹⁰

however more recent data using a human gut model did not confirm this.⁹¹ A randomized, double-blind trial with tolevamer therapy showed overall inferiority compared to vancomycin therapy (83% versus 91% with resolution of diarrhea); however the study claimed that tolevamer was non-inferior to vancomycin in mild cases.⁹² It is important to note that if the 2 drugs are used in combination they should not be given at the same time, because tolevamer binds vancomycin and prevents its antimicrobial activity.

Immunotherapy, based on the importance of antitoxin antibodies as discussed above, is the final evolving strategy to be discussed here. Pooled human immunoglobulin for intravenous administration neutralized TcdA and TcdB and has been studied in both recurrent and severe CDI with results that are inconsistent.² Recently, neutralizing human monoclonal antibodies to TcdA and TcdB, given concomitantly with vancomycin or metronidazole, significantly reduced the CDI relapse rate.⁹³

Prevention

The major step in prevention of CDI is appropriate use of antimicrobial therapy. The risks of CDI must be compared to the benefits of antimicrobial therapy whenever antimicrobial therapy is contemplated. In – hospital isolation of suspected or infected patients is essential.⁸ Thorough hand hygiene with chlorhexidine gluconate - containing soap (not ethanol solutions because these do not kill spores) and wearing protective gowns by all healthcare providers after every contact with a patient with suspected or known CDI are essential.⁹⁴ Appropriate cleaning of rooms vacated by patients with CDI, preferably employing sodium hypochlorite, is also essential.^{8, 21}

As with virtually all infectious diseases, the ultimate hope for prevention is the development of an effective vaccine. Work on such a vaccine is underway. As has been reviewed, antitoxin vaccine prevents animal infection, phase 1 trials have shown that intramuscular injection of toxoid vaccine is safe and immunogenic in healthy human volunteers, and a small case series suggested the efficacy of therapeutic vaccination in adults with multiply recurrent disease.²¹

Conclusion

CDI has become an increasingly common infection and has shown an increase in severity over the past several years. Our goal with this review is that physicians will be more aware of the seriousness of CDI and be vigilant about quick diagnosis and appropriate treatment.

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