Treatment of *Clostridium difficile*-Associated Diarrhea in the Era of Hypervirulence and Antibiotic Resistance

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Linda M. Mundy, M.D.**

ABSTRACT

Severe morbidities and fatalities due to *Clostridium difficile* are usually rare. The emergence of hypervirulent strains containing binary toxin (toxinotype III, ribotype 027), mutation in \textit{tcdC} variants, high-level toxin A and B production in clinical isolates together with the evolution of a *C. difficile* strain with high-level resistance to the newer quinolones (i.e. gatifloxacin and moxifloxacin) have contributed to the rise in *C. difficile*-associated diarrhea (CDAD) disease severity and mortality in recent years. Metronidazole or vancomycin is regarded as initial CDAD therapy, although several adjunctive strategies have been introduced to treat severe infection. Our current understanding of CDAD suggests that further characterization of virulence is imperative in order to effectively optimize CDAD treatment and prevention strategies. (*J Infect Dis Antimicrob Agents* 2007;24:151-62.)

INTRODUCTION

*Clostridium difficile* was first discovered by Hall and O’Toole in 1935 while examining the normal flora of newborn infants. They described an obligate anaerobic, Gram-positive, spore-producing bacillus that grew slowly in culture and was difficult to isolate, giving the organism its descriptive name. In 1974, Tedesco and colleagues were among the first researchers to establish a link between the use of antibiotics and colitis. Four years later, Bartlett and colleagues identified *C. difficile* and its toxins as the cause of antibiotic-associated pseudomembranous colitis (PMC).

*C. difficile* is responsible for up to 20 percent of cases of antibiotic-associated diarrhea and almost all cases of PMC. Penicillins, cephalosporins, and clindamycin are the most frequently associated antibiotics with *C. difficile* infection. Although most

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cases of *C. difficile*-associated diarrhea (CDAD) are associated with antibiotic use, CDAD without prior antibiotic exposure has been reported in patients with renal failure, diabetes mellitus, intestinal surgery, and those receiving antineoplastic chemotherapy.  

Approximately 25 percent of patients with CDAD will experience relapse of the disease. Although CDAD is a common problem, severe morbidity and fatality are rare. However, over the past 5 years a new hypervirulent, more drug-resistant strain has emerged in several countries resulting in large outbreaks and unusually high numbers of severe cases and deaths. In this review article, we describe the current understanding of the epidemiology, pathogenesis, and treatment options for CDAD, along with an update on this hypervirulent strain.

**EPIDEMIOLOGY**

Approximately 3 million cases of CDAD and colitis are reported among hospitalized patients in the United States (US) each year, compared to an estimated 20,000 cases from the community. These recent estimates reflect the importance of *C. difficile* as a major nosocomial pathogen and, furthermore, that the incidence of CDAD has doubled over the past 6 years in university-based US hospitals. About 1-3 percent of healthy adults carry *C. difficile* as part of the normal intestinal flora. The colonization rate among healthy individuals with previous antibiotic exposure is 5-15 percent. Historically, many studies have consistently identified that 20 percent of hospitalized adults are colonized or acquire *C. difficile* during the inpatient stay, with subsequent diarrhea in one-third while the majority (two-thirds) comprise a potential asymptomatic reservoir. Severe morbidity and fatality occur in approximately 3 percent of all cases. In contrast, a recent Canadian outbreak reported severe complications in 11 percent of patients infected with a hypervirulent *C. difficile* strain.

Transmission of *C. difficile* usually occurs through the oro-fecal route. Hardy *C. difficile* spores survive in the hospital environment for months. Previous studies showed a higher surface contamination rate in rooms of patients with CDAD, compared to asymptomatic carriers (49% versus 29%). Additional studies have also documented the presence of *C. difficile* and its spores on telephones, rectal thermometers, bath tubs, toilets, stethoscopes, and hands of hospital personnel. Patients in a double-bed room had a higher infection rate. Routine use of disposable gloves, single use thermometers, hand washing with chlorhexidine, and environmental cleaning with a 10 percent hypochlorite solution have been shown to significantly reduce the rate of CDAD. Exposure to antibiotics is the major risk factor for CDAD. Administration of multiple antibiotics increases the likelihood of development of CDAD. However, CDAD has been reported even after a single prophylactic dose of antibiotic prior to surgery. Other risk factors include advanced age, recent gastrointestinal surgery, prolonged length of hospital stay, use of antineoplastic agents, enteral feeding, and use of laxatives.

Between 1989 and 1992, several large outbreaks of CDAD caused by clindamycin-resistant, *ermB*-gene-positive strain of *C. difficile* (type J7 and J9) occurred in four US hospitals in four states, with an incidence ranging from 15.8 to 20 per 1,000 admissions. Subsequently, clinical isolates of *C. difficile* with toxin B and without toxin A production (A-B') were reported from several countries between 1997 and 2003. The prevalence of CDAD caused by this toxin variant varied from 0.2 percent to 37 percent.

The epidemiology, risk factors, and outcomes for patients with CDAD were compared between in the era before and during the occurrence of strains with hypervirulence and antibiotic resistance (Table 1).
### Table 1. Epidemiological features of *Clostridium difficile* colitis before and after detection of hypervirulent *C. difficile* strains.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Historical era</th>
<th>Hypervirulent era</th>
</tr>
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<tbody>
<tr>
<td>Geographic distribution</td>
<td>Worldwide</td>
<td>United States&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Canada&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>United Kingdom</td>
</tr>
<tr>
<td>Prevalence/incidence</td>
<td>0.1-30 case per 1,000 admissions*</td>
<td>22.5 cases per 1,000 admissions*</td>
</tr>
<tr>
<td></td>
<td>8-12 cases per 100,000 person-year**</td>
<td></td>
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<tr>
<td>Risk factors</td>
<td>Age, antibiotic exposure, severity of illness, length of hospital and ICU stay, prior disease, CRF, chemotherapy, recent gastrointestinal surgery, enteral feeding, laxatives and enemas</td>
<td>Receipt of fluoroquinolones and cephalosporins&lt;sup&gt;c&lt;/sup&gt;, proton pump inhibitor, advanced age, severity of illness, length of hospital ICU stay, presence of binary toxin and 18-bp deletion in the <em>tcdC</em> gene</td>
</tr>
<tr>
<td>Outcomes</td>
<td>Recurrence: 5 percent to 50 percent</td>
<td>Recurrence: 20 percent to 58 percent</td>
</tr>
<tr>
<td></td>
<td>Mortality: 0.1-3.2 percent</td>
<td>Mortality: 1.2 to 22 percent&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ICU: intensive care unit, CRF: chronic renal failure

*In nosocomial setting,  **In community setting,

<sup>a</sup>Including Georgia, Illinois, Maine, New Jersey, Oregon, and Pennsylvania,

<sup>b</sup>Including Quebec, British Columbia, and Ontario,

<sup>c</sup>Including gatifloxacin, levofloxacin, moxifloxacin and first; second; third-generation cephalosporins,

<sup>d</sup>Depends on age
Although several sporadic cases of toxin variant (A-B+) C. difficile diarrhea and PMC have been reported, to date there have been only two hospital outbreaks of CDAD caused by this strain.\textsuperscript{40-41} Al-barrak and colleagues were the first to report a toxin variant (A-B+) outbreak of CDAD in Canada in 1998.\textsuperscript{40} In this study, five patients had recurrent diarrhea (36%), three had severe colitis (20%), with the overall mortality of 66 percent.\textsuperscript{40} Kuijper and colleagues described an outbreak in the Netherlands where 24 patients were diagnosed with CDAD caused by toxin-variant C. difficile.\textsuperscript{41} Three patients (13%) had recurrent disease; there were seven cases of severe disease and one death (14%). The high mortality associated with this toxin-producing C. difficile strain may be attributable to an excess toxin B production. Toxin B is a more potent enterotoxin inducing ten-fold more damage in human colonic explants than toxin A.\textsuperscript{42-43} Between 2000 and 2003, the spread of a hypervirulent, fluoroquinolone-resistant, binary toxin-producing strain of C. difficile (BI/NAP1) with a partial deletion of the tcdC caused outbreaks in eight facilities in six US states. These epidemic strains reportedly produce 23 times more toxin B than wild type strains.\textsuperscript{44} The same strain was also responsible for large outbreaks of CDAD in Quebec, Canada in 2003 where the incidence was 22.5 per 1,000 admissions and the mortality was 6.9 percent.\textsuperscript{13-14}

**PATHOGENESIS**

Normal gut flora can inhibit growth of C. difficile in vitro and in vivo.\textsuperscript{45-46} The pathogenesis of CDAD involves alteration of the endogenous intestinal flora, usually due to antibiotic use and exposure to pathogenic strains of C. difficile, most often during hospitalization. Approximately 25 percent of C. difficile strains are not pathogenic.\textsuperscript{9,46} Development of disease depends on host defense and C. difficile virulence factors. Pathogenic strains of C. difficile produce two exotoxins, toxin A (enterotoxin) and toxin B (cytotoxin). Both bind to intraluminal colonic epithelial receptors.\textsuperscript{19,24,43} Toxin A causes a cytokine-mediated hypersecretion of fluids and a subsequent inflammatory hemorrhagic process. Toxin B causes cell death via alteration of the actin cytoskeleton.\textsuperscript{4,19} Other virulence factors include adhesion factor (binding to colonic mucosal cells) and hyaluronidase (hydrolytic activity). In addition, a formation of spore allows C. difficile to survive in the environment for months.\textsuperscript{4,47} Approximately, 6 percent of C. difficile isolates produce a binary toxin. Toxins A and B are encoded by the genes tcdA and tcdB. Together with two regulatory genes and a porin gene (tdcC, tcdD, and tcdE), they form the chromosomal pathogenicity locus (PaLoc).\textsuperscript{14,48} Polymorphism or partial deletion of tcdC has been associated with 6-20-fold increase in toxin A and B production. During recent outbreaks in Canada and the US, more than 50 percent of the epidemic strains produced the binary toxin and had the partial deletion of the tcdC gene.\textsuperscript{13-14} In a cross-sectional study of hospitalized patients with diarrhea, investigators developed a predictive model to identify C. difficile infection.\textsuperscript{13-14,49} The final model suggested that the presence of at least 2 of 3 variables (fecal leukocytes, peripheral leukocytosis, and antecedent hospitalization) were associated with a 77 percent probability of positive C. difficile toxin assay.

The clinical presentations range from mild diarrhea to severe colitis with pseudomembrane formation, ileus, perforation, or toxic megacolon. Non-immunological host defense mechanisms include inhibition of C. difficile by normal flora, reduction of the number of spores and toxins through gastric acid, and elimination of the organism and toxins via intestinal peristalsis.

The host immune response appears to play a major role in the development of CDAD, and several studies suggest that an inadequate immune response may result in severe or recurrent C. difficile
The presence of anti-toxin A IgG in adult sera has been associated with asymptomatic carriage and apparent neutralization of *C. difficile* toxins. In addition, children with recurrent disease have been found to have lower levels of circulating IgG to toxin A. Immunization to toxin A has been shown to prevent colitis in animal models and secretory IgA blocks the binding of toxin A to intestinal receptors.

**RISK FACTORS AND OUTCOMES (Table 1)**

The major risk factor for CDAD is antibiotic exposure. Although any antibiotic can cause CDAD, penicillins, cephalosporins, and clindamycin are most frequently associated with CDAD. Of interest, while fluoroquinolones have not been a risk factor in multivariate analyses, recent outbreaks of CDAD suggest an association with third-generation drugs in this class.

McDonald and colleagues characterized 187 *C. difficile* isolates from outbreaks occurring in eight health care facilities in six US states between 2000 and 2003. A molecular comparison with more than 6,000 isolates collected before 2001 revealed that the BI/NAP1, toxinotype III, binary-toxin-producing strain accounted for the majority (52%) of clinical isolates from the latter era. All recent epidemic and historic (14 cases) BI/NAP1 isolates had the 18-bp deletion in tcdC gene and resistance to gatifloxacin and moxifloxacin; most also had clindamycin and levofloxacin resistance. In contrast, none of the pre-2001 BI/NAP1 isolates showed resistance to third-generation fluoroquinolones. Patients infected with the BI/NAP1 strain tended to have more severe disease, pseudomembranous colitis, and higher peripheral leukocytosis. In several facilities, the outbreak was preceded by a change in the hospital formulary from levofloxacin to gatifloxacin or moxifloxacin. It is hypothesized that the broad anti-anaerobic activity of the new fluoroquinolones selected for this outbreak. The same strain was responsible for large outbreaks of CDAD among 1,703 patients in 12 Quebec hospitals in 2003 where the incidence of CDAD was 22.5 per 1,000 admissions with a 30-day mortality rate of 6.9 percent. The majority of the isolates (84.1%) produced binary toxin, had the tcdC gene deletion and third-generation fluoroquinolone resistance (82.2%). In contrast, clindamycin use was not an identified risk factor for CDAD in this population.

Pepin and colleagues studied 293 hospitalized patients with CDAD in Canada between 2003 and 2004. At least 50 percent of cases occurred in patients with more than 80 years of age, and the 30-day mortality was 21.8 percent. Although cephalosporins, clindamycin, macrolides, and penicillins shared similar adjusted hazard ratios (aHRs, 1.56-1.89) for causing CDAD, a use of fluoroquinolones (aHR, 3.44) was the most significant risk factor especially with longer duration.

Recent data suggest that BI/NAP1 isolates of *C. difficile* produce significantly higher concentrations of toxin A and B, compared to non-BI/NAP1 strains and may be responsible for more severe disease. Other risk factors for recent outbreaks may include the increasing hospitalized geriatric population with multiple co-morbidities and inadequate immune response, widespread use of alcohol-based hand-rubs, shortage of private rooms, and suboptimal response to metronidazole.

**TREATMENT OPTIONS**

One of the key steps in managing CDAD is to discontinue all current antimicrobial agents, if clinically plausible. Epidemiological studies in hospitalized patients indicate that the majority of people infected with *C. difficile* are asymptomatic. Treatment of these “healthy carriers” has no effect on toxin production. Patient with mild-to-moderate CDAD typically presents with diarrhea, abdominal pain, and peripheral leukocytosis. Oral therapy with vancomycin (125 mg
every six hours) or metronidazole (250 mg every six hours) is recommended for patients with mild-to-moderate CDAD and usually results in clinical improvement within 3-5 days. Intravenous metronidazole is reserved for patients who are unable to take oral medication because of ileus or abdominal surgery. Oral vancomycin is preferred to metronidazole among pregnant and breast-feeding women, individuals with known intolerance to metronidazole, or patients who fail to respond to metronidazole. Several comparative trials indicated that metronidazole and vancomycin were equally effective with the response rates of greater than 95 percent. However, the excessive cost of vancomycin and its potential for selection of enteric vancomycin-resistant enterococci has led the Centers for Disease Control and Prevention (CDC) to recommend metronidazole as the treatment of choice for CDAD.

A relapse is common and occurs in 25 percent of patients with CDAD. Relapses are usually successfully treated with another course of metronidazole or vancomycin. Switching between these two antibiotics, and longer courses, have not reduced the frequency of relapses. However, during a large outbreak with a hypervirulent strain of *C. difficile* in Canada, an unusually high rate (47.2%) of recurrent CDAD was observed in patients who were initially treated with metronidazole. Alternative oral treatment options for recurrent CDAD include pulsed doses of vancomycin, adjunctive cholestyramine or rifampin, and probiotics such as *Saccharomyces boulardii*. Patients with fulminant CDAD may present with hypotension, ileus, hypoalbuminemia, toxic megacolon, or bowel perforation. Among these cases, an abdominal X-ray may show “thumbprinting” of the colon, and on computed tomography colonic wall thickening will likely be evident. In severe CDAD, treatment with standard oral or intravenous drugs has been limited by inadequate achievement of intracolonic drug concentrations and on-going toxin production. When oral anti-*C. difficile* drug is not a therapeutic option, the evidence is limited for selecting a biologically plausible alternative. Parenteral metronidazole drug excretion occurs mainly in the upper gastrointestinal tract with less than 14 percent recovered in feces. Anecdotal reports suggest poor clinical responses of CDAD were noted in patients treated with intravenous metronidazole or infected with the strain with reduced metronidazole susceptibility. Parenteral vancomycin has a limited penetration of the bowel as well, with stool concentrations of only 6.4 to 10 µg/mL. Available adjunctive CDAD treatment options for severe *C. difficile* colitis include intravenous immunoglobulin (IVIG), intracolonic vancomycin (ICV), and pancolectomy, while donor stool enemas and *C. difficile* vaccination have been successfully used in recurrent CDAD. Adjunctive treatment alternatives for severe CDAD colitis are summarized in Table 2.

Several studies suggest a benefit of IVIG in severe or refractory CDAD. In one retrospective study, two patients with persistent CDAD after treatment with metronidazole and/or vancomycin were given pooled human IVIG (200-300 mg/kg). Both patients experienced prompt, dramatic resolution of diarrhea, abdominal distension and tenderness. Their sera subsequently were able to neutralize *C. difficile* toxin in cytotoxic assays. In a recent study, IVIG at 150-400 mg/kg was used to treat 14 patients with severe, refractory, recurrent CDAD. All patients tolerated IVIG without major side effects and nine (64%) had symptom resolution at a median of 10 days (range, 2-26); one patient had a partial response from two doses but died two months later after recurrent CDAD, and the other four died of non-CDAD-related comorbidities within three weeks of the IVIG treatment. Beales reported a successful treatment of four elderly patients with recurrent CDAD after two doses of IVIG, three weeks apart. A study by Leung
Table 2. Adjunctive treatment alternatives for severe *Clostridium difficile* colitis.

<table>
<thead>
<tr>
<th>Option</th>
<th>Indiation</th>
<th>Dose</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancolectomy</td>
<td>No clinical response to standard therapy, multiple organ failure, peritonitis, toxic megacolon, radiographic evidence of fulminant colitis</td>
<td>NA</td>
<td>Short bowel syndrome, require ostomy care, malnutrition; Despite surgery, the mortality has been reported to be as high as 38 percent-48 percent</td>
</tr>
<tr>
<td>Intracolonic vancomycin</td>
<td>No clinical response to standard therapy, impaired oral intake, severe ileus resulting in cessation of diarrhea, clinical evidence of fulminant colitis</td>
<td>2-3 g/day with intervals of 4-12 hours</td>
<td>Colonic perforation, require monitoring of vancomycin level in patients with renal insufficiency, potential development of VRE</td>
</tr>
<tr>
<td>Intravenous immunoglobulin</td>
<td>No clinical response to standard therapy, severe, refractory and recurrence CDAD</td>
<td>150-400 mg/day</td>
<td>Expensive</td>
</tr>
</tbody>
</table>

CDAD: *Clostridium difficile*-associated diarrhea, 
NA: not applicable, 
VRE: vancomycin-resistant *Enterococcus* spp.
and colleagues showed that IVIG was also effective in five pediatric patients with recurrent CDAD. Together, these data suggest that IVIG may be an effective alternative for severe, refractory, or recurrent CDAD after failing conventional treatment.

The administration of ICV as an adjunctive regimen, may occur via an 18-French Foley catheter or soft 6-French pigtail catheter as a 60-minute retention enema, lavage, or as a component of decompressive colonoscopy. In a review of the archived literature for ICV treatment of CDAD, 20 of 24 patients (83%) had clinical and microbiological response to adjunctive ICV therapy. As with most observational studies, the duration and dosing interval of ICV varied (2-3 g/day of ICV, with intervals of 4-12 hours), yet the observed findings suggested that ICV was safe and effective in the majority of ICV recipients. Lastly, pancolectomy is considered a treatment option for patients with multisystem organ failure, peritonitis, or radiographic evidence of fulminant colitis. The postoperative long-term sequelae include short gut syndrome, malnutrition, and ostomy care. And postoperative mortality is as high as 48 percent.

Donor stool via a nasogastric tube or enemas from healthy volunteers has been effective ways of treating recurrent CDAD. Several studies indicated that an adequate antibody (IgG and IgA) response to C. difficile may prevent CDAD and that an inadequate response is associated with recurrent disease. Therefore, a development of a C. difficile vaccine seems prudent. Different vaccines have been tested in animals with various results. In one study of 30 healthy volunteers, a highly immunogenic response was reported following the administration of an intravenous C. difficile toxoid vaccine. Recently, a C. difficile toxoid vaccine showed promising results in three patients with refractory CDAD.

**CONCLUSION**

The epidemiology of CDAD now encompasses hypervirulent and more fluoroquinolone-resistant strains, particularly among elderly patients. Recent outbreaks demonstrate the ability of C. difficile to adapt to an antibiotic-rich environment and for strains to emerge with toxin hyperproduction that are associated with higher morbidity and mortality than previously encountered. Any increased incidence of CDAD should be monitored while strict adherence to infection control measures as well as appropriate and judicious antibiotic therapy are employed. Recent outbreaks indicated that current management strategies have not prevented recurrent CDAD or reduced mortality especially in the elderly populations. The existing data suggests that adjunct use of ICV or IVIG should be vigorously studied and that the development of an effective C. difficile vaccine is warranted. Randomized, controlled trials with and without these adjunctive interventions will contribute to future streamlined treatment options of severe CDAD. Until such data are available, clinicians should carefully select the antimicrobial agent(s) for patients with CDAD and consider the potential benefits of adjunctive therapy.

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