Clostridium difficile–Associated Diarrhea

A Review

Eleftherios Mylonakis, MD; Edward T. Ryan, MD; Stephen B. Calderwood, MD

Clostridium difficile causes 300,000 to 3,000,000 cases of diarrhea and colitis in the United States every year. Antibiotics most frequently associated with the infection are clindamycin, ampicillin, amoxicillin, and cephalosporins, but all antibiotics may predispose patients to C difficile infection. The clinical presentation varies from asymptomatic colonization to mild diarrhea to severe debilitating disease, with high fever, severe abdominal pain, paralytic ileus, colonic dilation (or megacolon), or even perforation. The most sensitive and specific test available for diagnosis of C difficile infection is a tissue culture assay for the cytotoxicity of toxin B. However, this test takes 1 to 3 days to complete and requires tissue culture facilities. Detection of C difficile toxin by means of enzyme-linked immunoassay is more rapid and inexpensive. A minority of patients may require more than 1 stool assay to detect toxin. Oral metronidazole or oral vancomycin hydrochloride for 10 to 14 days are equally effective at resolving clinical symptoms; oral metronidazole is preferred in most cases because of lowered cost and less selective pressure for vancomycin-resistant organisms. Approximately 15% of patients experience relapse after initial therapy and require retreatment, sometimes with an extended, tapering regimen. Immunity appears to be incomplete and predominantly mediated by serum IgG to toxin A. Measures for preventing the spread of the pathogen, appropriate diagnostic testing, and treatment may avert morbidity and mortality due to C difficile–associated diarrhea.
Pathogenesis and Immunological Features

Infants and young children commonly harbor *C difficile* in fecal flora but have no symptoms related to production of toxin.\(^2\)\(^0\)\(^3\)\(^0\)\(^3\) For unclear reasons, the number of carriers quickly declines as children age, and toxigenic *C difficile* is isolated from stool specimens in only 0%-3% of healthy adults.\(^2\)\(^0\)\(^3\)\(^0\)\(^3\) During hospitalization, however, colonization frequently occurs (21% of patients in one study, for example).\(^1\)\(^3\)\(^0\)\(^3\)\(^0\)\(^3\) Although asymptomatic carriers are an important hidden reservoir of *C difficile*, clinical symptoms develop in only about one third of colonized patients, and asymptomatic colonization with *C difficile* may be associated with a decreased risk for development of CDAD.\(^3\)\(^1\)

*Clostridium difficile* forms spores that persist in the environment for years, and contamination by *C difficile* is common in hospitals and long-term care facilities, especially in rooms occupied by an infected individual.\(^1\)\(^3\)\(^2\)\(^3\)\(^2\)\(^3\)\(^3\) Patient-to-patient transmission of the organism occurs, and the organism can be cultured from many environmental surfaces in rooms of infected patients and on hands, clothing, and stethoscopes of health care workers. Hospital personnel may carry the bacteria from room to room and promote infection, but fecal carriage by staff is rare.\(^1\)\(^3\)\(^2\)\(^3\)\(^2\)\(^3\)\(^3\) Outbreaks can occur in hospitals, nursing homes, and other extended-care facilities.\(^2\)\(^2\)

The first step in development of *C difficile* colonization is disruption of the normal flora of the colon, usually caused by antibiotics or, in unusual cases, by antineoplastic or immunosuppressive drugs.\(^2\)\(^1\)\(^0\)\(^3\)\(^0\)\(^3\) colonization occurs by the fecal-oral route; ingested spores of *C difficile* survive the gastric acid barrier and germinate in the colon.\(^2\)\(^0\)\(^2\) Symptoms of CDAD may start on the first day of antibiotic therapy or 6 weeks or longer after antibiotic therapy is stopped.\(^6\)

The following 2 factors recently have been shown to increase the probability of symptomatic disease in patients who acquire *C difficile* colonization in hospital: the severity of other illnesses, and reduced levels of serum IgG antibody to toxin A.\(^3\)\(^7\) These results suggest that preexisting anti-toxin A antibody may ameliorate severity of disease and that immunization might be efficacious in preventing and controlling nosocomial CDAD.

Some strains of *C difficile* are nontoxinogenic; these strains do not cause disease. Clinically significant strains produce 2 protein exotoxins, toxin A, a 308-kd protein, and toxin B, an approximately 270-kd protein; these toxins are largely responsible for disease manifestations. However, rare cases of CDAD caused by toxin A-negative, toxin B-positive strains have been reported.\(^3\)\(^8\)\(^3\)\(^0\) The genes encoding both toxins are located very near each other on a 19-kilobase-pair pathogenicity island in toxigenic strains.\(^3\)\(^0\)\(^3\) Toxin A binds to a specific receptor on the brush border of the intestinal epithelium, a glycoprotein with an α-linked galactose; the intestinal receptor in humans for toxin B is less well characterized.\(^4\)\(^3\) After binding to appropriate receptors, the toxins are internalized and act within the eukaryotic cell to modify proteins covalently in the Rho subfamily, a group of low-molecular-weight GTP-binding proteins involved in regulation of the F-actin cytoskeleton.\(^4\)\(^5\) Both toxins modify Rho proteins at a specific threonine residue by addition of a glucose molecule, leading to inactivation of the protein.\(^4\)\(^3\)\(^4\) This is followed by disaggregation of polymerized actin, opening of tight junctions between cells, cell rounding, and subsequent cell death. The toxins also induce the release by various cells of proinflammatory mediators and cytokines, as well as activation of the enteric nervous system, leading to neutrophil chemotaxis and fluid secretion.\(^4\)\(^6\) Toxin B is not enterotoxic in animals (as is toxin A), but it is a much more potent cytotoxin in tissue culture than toxin A, and full tissue damage requires the action of both toxins.\(^2\)\(^2\)\(^4\)\(^7\)

Early histological changes due to *C difficile* toxins include patchy epithelial necrosis with an exudate of neutrophils and fibrin; the exudate
may appear to “erupt” into the colonic lumen and, if prominent, such a histological feature has been termed a summit or volcano lesion. More prominent histological changes are characterized by diffuse epithelial necrosis and ulceration with development of an overlying pseudomembrane containing cellular debris, leukocytes, fibrin, and mucin (Figure 1). 48

In animal models, antibodies directed against toxin A prevent toxin binding, neutralize secretory and inflammatory effects, and limit or prevent clinical disease. 49,50 The immune response to toxin B is less well understood, but anti–toxin B antibodies also appear to protect against C difficile–associated disease. 50,51,61

Clinical studies suggest that the level of anti–toxin antibody in serum is inversely associated with severity of disease and risk for relapse. 32,62–65 Serum levels of IgG antibody to toxin A are lower in children with recurrent CDAD than in age-matched controls, whereas rising levels of anti–toxin A antibodies have been associated with resolution of clinical disease. 63,66,67 Also, serum samples of convalescent patients contain IgG and IgA that can neutralize toxin A 67; immunization with a toxoid of toxin A has achieved active protection from clinical disease 32,68, and passive protection from clinical disease is achieved by the administration of anti–toxin A antibodies. 50,51,65 In humans, therefore, systemic anti–toxin IgG and IgA and mucosal IgA all appear to be involved in protective immunological responses to C difficile infection.

Antibodies against C difficile toxins are present in most adults and older children (>60%). 65,62 The presence of such antibodies may be due to repetitive, transient presence of toxigenic C difficile organisms in the normal intestinal flora. Cellular immunity appears to be of lesser import, but has not been studied in detail.

CLINICAL MANIFESTATIONS

The clinical presentation of CDAD is variable and includes diarrhea, colitis without pseudomembranes, pseudomembranous colitis, and fulminant colitis (Table 2). Some individuals with toxigenic strains in stool remain totally asymptomatic. 31 Mild to moderate CDAD is usually accompanied by lower abdominal cramping pain but no systemic symptoms or physical findings. 22 Moderate or severe colitis usually presents with diffuse diarrhea, abdominal distention with pain, and, in some cases, occult colonic bleeding. Also, systemic symptoms such as fever, nausea, anorexia, and malaise are usually present. 22 A minority of patients have disease primarily in the cecum and right colon, presenting with marked leukocytosis and abdominal pain but little or no diarrhea.

Fulminant colitis develops in approximately 1% to 3% of patients, with ileus, toxic megacolon, perforation, and death. 20 Clinicians should be aware that the development of these life-threatening complications may be accompanied by a decrease in diarrhea due to loss of colonic muscular tone and ileus.

Other complications of C difficile infection include hyperpyrexia, chronic diarrhea, and hypoalbuminemia with anasarca. 11 Clostridium difficile infection may occasionally complicate idiopathic inflammatory bowel disease. Also, a reactive

<table>
<thead>
<tr>
<th>Table 2. Common Signs, Symptoms, and Diagnostic Features of Clostridium difficile–Associated Diarrhea</th>
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<tbody>
<tr>
<td><strong>Mild disease</strong></td>
</tr>
<tr>
<td>Mild lower abdominal cramping pain</td>
</tr>
<tr>
<td>Endoscopy may reveal diffuse or patchy, nonspecific colitis*</td>
</tr>
<tr>
<td><strong>Moderate disease</strong></td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Volume depletion</td>
</tr>
<tr>
<td>Nausea, anorexia, malaise</td>
</tr>
<tr>
<td>Profuse diarrhea</td>
</tr>
<tr>
<td>Abdominal distention and cramps</td>
</tr>
<tr>
<td>Moderate leukocytosis</td>
</tr>
<tr>
<td>Fecal leukocytes</td>
</tr>
<tr>
<td>Endoscopy may reveal diffuse or patchy, nonspecific colitis*</td>
</tr>
<tr>
<td><strong>Severe disease</strong></td>
</tr>
<tr>
<td>Usually, profuse diarrhea; however, in some cases</td>
</tr>
<tr>
<td>there is little or no diarrhea</td>
</tr>
<tr>
<td>because of involvement of the cecum and right colon or</td>
</tr>
<tr>
<td>because of ileus</td>
</tr>
<tr>
<td>Usually severe abdominal pain</td>
</tr>
<tr>
<td>High fever and appearance of toxic effects</td>
</tr>
<tr>
<td>Volume depletion</td>
</tr>
<tr>
<td>Peritoneal signs</td>
</tr>
<tr>
<td>Fecal leukocytes</td>
</tr>
<tr>
<td>Radiographic findings can include paralytic ileus, dilated</td>
</tr>
<tr>
<td>colon (and even toxic megacolon)</td>
</tr>
<tr>
<td>“Thumbprinting” on abdominal plain films, and diffusely thickened or edematous colonic mucosa</td>
</tr>
<tr>
<td>Endoscopy may demonstrate adherent yellow plaques that vary in diameter and in some cases, may coalesce to cover large areas of the mucosa*</td>
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</tbody>
</table>

*Due to the increased risk for intestinal perforation, endoscopy should be used sparingly as a diagnostic tool in patients with suspected C difficile–associated diarrhea.
arthritis occurring 1 to 4 weeks after C difficile colitis develops in some patients, resembling that seen after other enteric infections such as Yersinia, Shigella, and Salmonella disease.69

**DIAGNOSIS**

Non-specific laboratory abnormalities in patients with CDAD include leukocytosis with a shift to the left and fecal leukocytes in about 50% to 60% of cases. The average peripheral white blood cell count is 12 × 10⁹/L to 20 × 10⁹/L, but occasionally the peripheral count is higher and cases of leukemoid reaction have been described.5,11,70

Even among patients with the most severe colitis, C difficile constitutes only a small part of the colonic flora, and its morphologic features are identical to that of other Clostridia species, making Gram staining of fecal specimens of no value in diagnosing CDAD. Clostridium difficile can be isolated by means of anaerobic culture of stool, but this test is seldom used for clinical diagnosis (it takes 2-3 days to complete and does not distinguish toxigenic from nontoxigenic strains; nontoxigenic strains are not associated with clinical illness).

The most sensitive and specific test available for diagnosis of C difficile infection is a tissue culture assay for the cytotoxicity of toxin B, using preincubation with neutralizing antibody to show the specificity of the cytotoxicity.22,71 This test can detect as little as 10 pg of toxin in stool and has a high sensitivity (94%-100%) and specificity (99%).50,22 However, the test takes 1 to 3 days to complete and requires tissue culture facilities.

More recently, enzyme-linked immunosorbent assays (ELISAs) have been developed to detect toxin A and/or B in stool. These assays detect 100 to 1000 pg of either toxin, and have a sensitivity of 71% to 94% and a specificity of 92% to 98%.71,72 Because of the rapidity of testing and ease of performance, ELISAs for toxins A and B are now used most frequently by clinical laboratories for diagnosis of C difficile infection. Approximately 5% to 20% of patients may require more than 1 stool assay to detect toxin.73 If CDAD is suspected, a single stool specimen should be sent. If the results are negative and diarrhea persists, 1 or 2 additional stool samples can be sent.73,74 The cytotoxicity assay for toxin B will detect an additional 5% to 10% of cases missed by ELISA techniques, and it may be useful to perform this test if the ELISA results are negative but clinical suspicion is high (Table 3).20 Diagnostic testing during or at the end of treatment or during follow-up is not needed, unless symptoms recur.75 Transmission of infection to other patients is associated with ongoing diarrhea and not with the presence of the toxin in stool. Enteric precautions can be removed when diarrhea subsides, without the need for repeated diagnostic testing.73

Stadium have also evaluated other laboratory methods for the diagnosis of CDAD. The polymerase chain reaction is extremely sensitive but requires technical expertise and is unable to distinguish between asymptomatic carriage and symptomatic infection.75 A dot-immunobinding assay yielded fair initial results several years ago, but this test has had very limited clinical experience since.76 Counterimmunolectrophoresis for detection of C difficile antigens has demonstrated poor positive predictive value, and a latex agglutination test is less sensitive than other available tests; newer forms of ELISAs are under evaluation.77,78

Radiographic imaging studies can be used to assist in making a diagnosis of CDAD. Abdominal imaging studies may reveal paralytic ileus and a dilated colon (Figure 2). Diffusely thickened or edematous colonic mucosa may sometimes be seen by means of abdominal computerized tomographic scans (Figure 3); such thickening can present as “thumbprinting” on abdominal plain films.

Endoscopy for CDAD is reserved for special situations, such as when other diseases need to be ruled out, rapid diagnosis is necessary, or a stool sample cannot be obtained because ileus develops.79 The results of sigmoidoscopy may be normal in patients with mild disease. Endoscopy in more severe colitis without pseudomembrane formation usually reveals diffuse or patchy, non-specific colitis.22 In more dramatic cases, endoscopy may demonstrate characteristic, adherent yellow plaques that vary in diameter from 2 to 10 mm and in some cases, coalesce to cover large areas of the mucosa (Figure 4). In most cases, the rectum and sigmoid are involved, but 10% of episodes of colitis involve only the right colon and may not be detected by flexible sigmoidoscopy.79 Due to the increased risk for intestinal perforation, endoscopy should be used sparingly as a diagnostic tool in patients with suspected CDAD.

**TREATMENT**

Practical guidelines for the treatment of CDAD are summarized in Table 4. The inciting antibiotic therapy should be discontinued if

Table 3. Diagnostic Tests for Detecting Clostridium difficile

<table>
<thead>
<tr>
<th>Enzyme-Linked Immunosorbent Assay</th>
<th>Cytotoxin Assay</th>
<th>Latex Particle Agglutination</th>
<th>Culture</th>
<th>Polymerase Chain Reaction†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>+++</td>
<td>+++</td>
<td>++/++</td>
<td>+++</td>
</tr>
<tr>
<td>Specificity</td>
<td>++/++++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ease of performance</td>
<td>++++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Time required for results</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

*Indicates fair; **, good; ***, very good; and ****, excellent.
†Initial results fair but still not widely used.
‡Allows strain typing in epidemics.
possible, and supportive therapy should be given with fluids and electrolytes as needed. Education of hospital personnel and infection control issues are of paramount importance in the management of CDAD. Education should reinforce the importance of handwashing between patients and the need for glove use for the handling of bodily substances of all patients. Hospital personnel should thoroughly disinfect objects contaminated with *C. difficile* and should be familiar with the disease and its epidemiological features. Enteric isolation precautions are recommended for patients with CDAD, and patients should be moved to a private room if possible.

Antiperistaltic and opiate drugs should be avoided in patients with CDAD, because they mask symptoms and may worsen the course of the disease. Diarrhea will resolve without specific antimicrobial therapy in up to one fourth of patients.

Antimicrobial therapy for CDAD is indicated for patients with moderate or severe disease or significant underlying conditions. There is no evidence that treatment of asymptomatic carriers of *C. difficile* provides any clinical benefit, and such therapy has been associated with a prolongation of the carrier state. In the appropriate clinical setting, antimicrobial therapy should be instituted even before the laboratory results for *C. difficile* are available.

Oral metronidazole and vancomycin hydrochloride are the antibiotics most commonly used, whereas bacitracin methylene disalicylate, teicoplanin, and fusidic acid also have some clinical efficacy. *Clostridium difficile* is uniformly susceptible to vancomycin, but occasional strains have been reported that are resistant to metronidazole. Two studies have directly compared initial therapy consisting of metronidazole (in an oral dose of 500 mg 3 times per day or 250 mg 4 times per day [qid]) with oral vancomycin hydrochloride (500 mg qid), each given for 10 days, and have shown that these therapies are equally efficacious in resolving symptoms as well as in the subsequent risk for relapse. Another study examined different doses of vancomycin hydrochloride and showed that an oral dose of 125 mg qid was equivalent to one of 500 mg qid for 10 days for symptomatic response and risk for subsequent relapse. In many series of patients, therapy with metronidazole or vancomycin was effective in
resolving symptoms in more than 95% of patients, although 10% to 20% of patients subsequently experienced relapse. 22,74

Because of lower cost and avoidance of selective pressure for vancomycin-resistant organisms such as vancomycin-resistant enterococci, initial therapy with oral metronidazole (either 250 mg qid or 500 mg 3 times per day) is currently the preferred initial therapy for C. difficile colitis. However, some authorities prefer initial therapy with vancomycin in the most severely ill patients, or in women who are pregnant. 74,81 The duration of initial therapy should be 10 to 14 days (or therapy may be continued until 1 week after completion of the inciting antibiotic therapy, if it cannot be stopped earlier).

Metronidazole is well absorbed from the small intestine and produces low or undetectable levels in the absence of diarrhea. 94,88 In the presence of severe ileus or toxic megacolon, intravenous metronidazole hydrochloride (given as 500 mg every 8 hours) produces fecal concentrations above the inhibitory concentration for C. difficile and may be used for initial therapy. 88 Patients receiving metronidazole should avoid alcohol, because it may result in disulfiramlike reactions. 74 Metronidazole resistance in C. difficile has been reported, but it is probably rare. 80 Oral bacitracin methylene disalicylate (20,000 to 25,000 U qid) has been shown to be as effective as vancomycin in ameliorating clinical symptoms of C. difficile disease, but it was less effective in eradicating the organism or toxin from stool; patients treated with bacitracin also had a higher risk for subsequent relapse. 90,91 Bacitracin should be reserved for unusual situations, in which metronidazole and vancomycin cannot be used.

In critically ill patients who are unable to take oral antimicrobials, treatment is empirical and may include intravenous metronidazole or administration of vancomycin by means of rectal enema or through long catheters in the small intestine. Intravenous treatment can be used with metronidazole but not vancomycin. Occasionally, surgery (usually subtotal colectomy) is required. 5,73

Despite the high initial response rate to therapy, recurrence of symptoms develops in 10% to 20% of patients, usually within the first 2 weeks after initial treatment is discontinued. 70 Two studies attempted to identify possible risk factors for recurrent CDAD. High-risk groups included patients with chronic renal failure, those with multiple previous episodes of CDAD, individuals who had to continue other antibiotic therapy, patients with community-acquired CDAD, those with high white blood cell counts (≥15 × 10^9/L), and those infected with certain strains of C. difficile. 92,93 About 50% of all recurrences of symptoms are actually due to reinfection and not to relapse of the primary infection. 93,94

The first relapse or recurrence of C. difficile colitis can be treated with another 10- to 14-day course of oral metronidazole or vancomycin, either of which produces a response rate of approximately 95%; mild disease can be managed without further antibiotic treatment. 20,71

A smaller number of patients have multiple relapses. One study showed a relapse rate of 24% after an initial episode of C. difficile colitis, but a relapse rate of 65% after treatment of recurrent disease. 85 Antimicrobial resistance as the mechanism for relapse has not been documented, and one hypothesis is that spores of C. difficile persist in the colon or the environment of the patient and that subsequent germination in the intestine produces vegetative forms and clinical illness. Therapy for multiple relapses of C. difficile colitis has not been examined by randomized, prospective, controlled clinical trials and the best therapeutic approach is currently uncertain. 74 A tapering course of metronidazole or vancomycin during a 4- to 6-week period has been used. A 6-week tapering course of vancomycin, as seen in the following tabulation, may allow spores to germinate during days between vancomycin therapy, with killing of vegetative forms on reexposure. 20,95:

<table>
<thead>
<tr>
<th>Week</th>
<th>Vancomycin Hydrochloride Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125 mg qid</td>
</tr>
<tr>
<td>2</td>
<td>125 mg twice a day</td>
</tr>
<tr>
<td>3</td>
<td>125 mg every day</td>
</tr>
<tr>
<td>4</td>
<td>125 mg every other day</td>
</tr>
<tr>
<td>5 and 6</td>
<td>125 mg every 3 days</td>
</tr>
</tbody>
</table>

In one study, such an approach cured all 22 patients who had had multiple previous relapses. 93

Another approach to relapsing CDAD is the use of vancomycin hydrochloride with oral cholestyramine resin (4 g twice daily). Cholestyramine binds C. difficile toxins and may assist in amelioration of CDAD. Since cholestyramine also binds vancomycin, patients should be instructed to stagger their doses of
cholestyramine and vancomycin (as well as other drugs) by at least 3 hours. Oral vancomycin hydrochloride, 125 mg qid, and oral rifampin, 600 mg twice daily for 7 days, may have some increased efficacy compared with vancomycin alone. Relapsing C difficile disease has also been treated with a combination of Saccharomyces boulardii, beginning 4 days before the end of a 10-day course of standard antimicrobial therapy such as vancomycin. In one study, this combination reduced the relapse rate in patients with previous relapses compared with use of vancomycin alone. However, results with this approach were not as good as published studies examining the tapering vancomycin regimen above. There is no evidence that the concomitant use of S boulardii with antibiotics alters diarrhea or prevents the appearance of C difficile toxin in the stool. Saccharomyces boulardii is not available in many countries, including the United States, but Saccharomyces cerevisiae (brewer’s yeast) is more widely available, and has been used successfully in patients with refractory CDAD. A small number of patients with refractory C difficile disease have responded to antimicrobial therapy in conjunction with an infusion of intravenous immunoglobulin at a dose of 200 to 300 mg/kg, or to orally administered bovine colostrom antibody harvested from cows immunized against C difficile or orally administered human IgA, but these are not yet accepted therapies. Similarly, reconstitution of intestinal flora through bacteriotherapy has occasionally been used in the management of individuals with refractory CDAD.

CONCLUSIONS

Clostridium difficile is the most common cause of nosocomial diarrhea and is a leading cause of diarrhea in the elderly. The antibiotics most frequently associated with infection are clindamycin, ampicillin and/or amoxicillin, and cephalosporins; however, all antibiotics have been associated with CDAD, and nonessential antibiotic prescription should be avoided. The clinical presentation may vary from mild diarrhea to severe debilitating infection. In some cases, life-threatening complications may be accompanied by a decrease in diarrhea due to ileus. Education of hospital personnel, appropriate diagnostic testing, early treatment, and measures for preventing spread of the pathogen may avert morbidity and mortality.

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