INTRODUCTION

*Clostridium difficile* is a gram-positive, spore-forming, anaerobic bacillus that was first isolated in 1935 from the stools of neonates. It was not until 1974 that this pathogen was identified as the major cause of pseudomembranous colitis. More than 400 strains have been identified, yet only toxin A–producing and toxin B–producing strains are known to cause disease. 

*Clostridium difficile* is responsible for approximately 20% of all cases of antibiotic-associated diarrhea, and the overall incidence of disease is 0.1 to 2% among hospitalized patients. Although systematic surveillance programs do not exist, the incidence of community-acquired disease has been approximated at eight per 100,000 population per year. 

*Clostridium difficile* infection also has profound consequences for health care expenditures. In 2002, a prospective, observational study by Kyne and colleagues found that hospital length of stay (LOS) increased by 3.6 days in those with *Clostridium difficile* infection, resulting in additional hospital costs of $3,669 per patient. 

The total estimated cost of treating *Clostridium difficile* infection in the U.S. exceeds $1.1 billion annually. Recent reports of an increased disease incidence and severity, hypervirulent strains of disease, and diminished response to metronidazole (Flagyl, Pfizer) justify institutional, local, and national surveillance of this pathogen.

PATHOGENESIS AND CLINICAL SYMPTOMS

*Clostridium difficile–associated diarrhea (CDAD)* occurs in patients after an alteration in the normal microflora of the colon and after exposure and ingestion of spores and vegetative cells. The organism multiplies in the colon, and toxins A and B are released. The production of toxins stimulates the release of tumor necrosis factor (TNF) and interleukins and the recruitment of neutrophils and monocytes. Colonic epithelial cell junctions widen, and cell death occurs. Additional production of hydrolytic enzymes leads to colitis and pseudomembrane formation in some patients. These changes in the colon cause watery diarrhea that is occasionally bloody (Figure 1).

The range of symptoms in CDAD varies from mild diarrhea to toxic megacolon and fulminant life-threatening colitis. Other systemic symptoms include malaise; abdominal cramps or pain; nausea and vomiting; fever; and leukocytosis, which can be seen in more severe disease. In the hospital setting, *Clostridium difficile* infection should be considered as a cause of unexplained elevated leukocyte count and fever. A leukemoid reaction has also been observed. Some patients may also have asymptomatic infection or colonization.

When colonoscopy is performed, patients with mild-to-moderate disease exhibit diffuse erythematous changes. Pseudomembranes, white-yellow plaques on the inner lining of the colon, are highly specific for the diagnosis of CDAD; however, they are not a sensitive marker for disease and are found in fewer than 25% of patients with CDAD.

DIAGNOSIS

Because colonoscopy is not routinely performed and is an insensitive test for *Clostridium difficile* infection, laboratory confirmation of disease is preferred for the definitive diagnosis. Laboratory methods for detection include culture, cytotoxin assay, polymerase chain reaction (PCR), and enzyme immunoassay (Table 1).

Culture

Stool culture to isolate *Clostridium difficile* organisms has greater than a 90% sensitivity for the detection of infection, although the process is labor-intensive; a tissue culture facility is needed, and at least 72 to 96 hours must elapse before results are available. In fact, this species derives the name “*difficile*” because specimens are notoriously difficult to culture. Another major disadvantage is that culture methods detect all strains of *Clostridium difficile*, including non-toxigenic strains that are not responsible for disease. Although their routine use and clinical utility are limited, cultures can be useful in outbreaks of disease for strain typing.

Cytotoxin Assay

A cytotoxin assay can be obtained directly from the stool sample or from stool culture, and it can detect the presence of toxin B. This method is highly specific for disease-causing strains, but its sensitivity is reduced. The assay is also technically difficult to accomplish, because a tissue culture facility is also required.

Disclosure. Dr. Martin has received financial support from Wyeth, Ortho-McNeil, and Cubist. He has also conducted research for the Levofloxacin Pharmacokinetics Study, sponsored by Ortho-McNeil.
needed, results take 48 hours, and only the presence of toxin B can be detected.\(^{11,12}\)

**Polymerase Chain Reaction**

Although PCR detects DNA targets and is a highly sensitive and specific test, cost and time limit its clinical utility.\(^{12}\)

**Immunoassay**

Enzyme immunoassay is commonly performed and detects the presence of toxin A or A + B. This assay is specific and rapid; however, because of reduced sensitivity, consecutive samples must be obtained before infection can be definitively excluded.\(^{11,12}\)

### RISK FACTORS

**Antimicrobial Therapy**

A number of risk factors for *C. difficile* infection have been described, primarily through case–control studies (Table 2). Exposure to antimicrobial agents has been cited as an independent risk factor for disease, with an increased duration of therapy and specific antibiotics increasing patients’ risk for CDAD. However, disease has been linked to virtually all antimicrobial agents, even after use of the offending agent has been discontinued.

**Cephalosporins**

In a prospective, randomized trial, Privitera and colleagues examined the risk of *C. difficile* colonization and the presence of toxin in the stool.\(^{14}\) They randomly assigned 108 volunteers who were undergoing elective surgical procedures to receive one of the following:

![](image)

**Figure 1** Pathogenesis of *Clostridium difficile*.
(Data from Poutanen J, et al.,\(^{11}\) Bouza E, et al.,\(^{12}\) Kelly CP, et al.,\(^{42}\) and Schroeder MS.\(^{43}\))
A single cephalosporin dose: cefazolin (Ancef, GlaxoSmithKline), cefoxitin (Mefoxin, Merck), cefotan (Cefotan, AstraZeneca), cefoperazone (Cefobid, Pfizer), or ceftriaxone (Rocephin, Roche).

A single dose of mezlocillin (Mezlin, Bayer), or

no perioperative antimicrobial prophylaxis.

Although symptomatic disease did not develop in any of the patients in the study, 17.3% of those receiving antimicrobial prophylaxis were found to have colonization by *C. difficile* and 14.4% were toxin-positive, compared with 0% in patients who received no prophylaxis. A statistically significant increase in colonization was observed in patients receiving cephalosporins compared with mezlocillin (23% vs. 3.3%; *P* = .02).

A single dose of antibiotics can cause asymptomatic infection; however, an increased duration of therapy is associated with higher rates of *C. difficile* infection. This is likely a result of more profound alterations in the normal colonic microflora. In a case–control study of 74 patients at a single tertiary-care center, patients who received antimicrobial therapy for more than 10 consecutive days were 16 times more likely to develop infection than those with a duration of therapy of less than 10 days. Differences in the antimicrobial spectrum and other factors increase the risk of *C. difficile* infection associated with specific agents. Clindamycin (Cleocin, Pfizer) was the first antibiotic associated with CDAD, and its product information contains specific warnings about the risk of infection. Its effects on the anaerobic colonic microflora are prolonged, thereby placing patients at greater risk for CDAD. Subsequently, cephalosporins increased the risk of colonization and infection compared with other agents.

In an open-label, prospective, crossover study, patients were randomly assigned by hospital ward to receive either cefotaxime or piperacillin–tazobactam (Zosyn, Wyeth) whenever broad-spectrum antibiotic therapy was prescribed. Of the 48 patients enrolled, 34 received cefotaxime (Claforan, Sanofi-Aventis). The remaining 14 patients received piperacillin–tazobactam. The subjects receiving cefotaxime had a higher rate of *C. difficile* colonization and active disease. In the cefotaxime group, *C. difficile* colonization occurred in 76.4% of subjects and disease developed in 53%. In the piperacillin-tazobactam group, colonization occurred in 21.4% (*P* = .001) and disease developed in 7.1% (*P* = .006). The study was terminated early because of hospital construction and ethical concerns.

### Fluoroquinolones

More recently, exposure to fluoroquinolones has been described as a risk factor for CDAD. In outbreaks, the fluoroquinolones were identified as the predominant antimicrobial agent associated with disease. In a retrospective cohort study conducted during an epidemic in Quebec, Canada, data from 5,619 patients were collected during a total of 7,451 episodes of care. In these subjects, a total of 293 cases of CDAD occurred.

After an adjustment for demographic and clinical characteristics, the use of fluoroquinolones was associated with an increase of about 3.5-fold in the rate of CDAD (adjusted hazard ratio [HR], 3.44). By comparison, the adjusted HRs for the development of CDAD for first-generation, second-generation, and third-generation cephalosporins ranged from 1.56 to 1.89. Although they were not specifically assessed, variations among specific fluoroquinolones and the rates of CDAD may exist. During the epidemic in Quebec, the risk of CDAD appeared higher in patients receiving gatifloxacin (Tequin, Bristol-Myers Squibb) (adjusted HR, 6.10; 95% confidence interval [CI], 2.22–16.74), compared with moxifloxacin (Avelox, Bayer) (adjusted HR, 2.04; 95% CI, 0.50–8.31).

The risk associated with levofloxacin (Levaquin, Ortho-McNeil) (adjusted HR, 2.52; 95% CI, 1.68–3.79) also appeared to be smaller than with ciprofloxacin (Cipro, Bayer) (adjusted HR, 3.74; 95% CI, 2.81–4.97). Gatifloxacin was also associated with an outbreak of *C. difficile* in a long-term-care facility. However, a clinical study comparing the safety of moxifloxacin with levofloxacin in hospitalized elderly patients found no statistical difference in the rates of CDAD between the two agents.

### Formulary Control

From a health system perspective, programs that optimize antimicrobial use through formulary control have had a positive impact on the rate of CDAD. In a systematic review, both programs that restrict antimicrobial use and those that educate physicians about prescribing were associated with reductions in rates of *C. difficile* infection. The introduction of antibiotics has had a profound positive impact on public health, but the “collateral damage” associated with their use must also be considered in determining which agents to use and the duration of therapy.

### Proton Pump Inhibitors

The use of proton pump inhibitors (PPIs) has been associated with bacterial colonization of the upper gastrointestinal tract, and the use of these agents is a known risk factor for the development of travelers’ diarrhea, salmonellosis, and cholera. In theory, the survival of *C. difficile* and its toxin is facilitated by higher gastric pH levels. Dial and associates reported the results of retrospective cohort and case–control studies of the relationship between the use of PPIs and *C. difficile* in hospitalized patients receiving concomitant antibiotics. In both studies, the use of PPIs, histamine-2 (H2) antagonists, and antimicrobial agents was compared with the rates of positive *C. difficile* toxin assays. After an

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**Table 2: Risk Factors for Clostridium difficile Infection**

<table>
<thead>
<tr>
<th>Risk Factor</th>
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<tr>
<td>Antimicrobial therapy</td>
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<tr>
<td>Hospitalization</td>
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<tr>
<td>Increased age (older than 65 years)*</td>
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<tr>
<td>Intensive-care unit stay*</td>
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<tr>
<td>Immunosuppression*</td>
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<td>Gastrointestinal procedures*</td>
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* Emerging risk factor or demonstrated through case–control or cohort studies.
adjustment for antibiotic use, the cohort study of 1,187 patients found that the rate of C. difficile infection among patients receiving PPIs was increased two-fold, compared with patients receiving no acid-suppressive therapy (adjusted odds ratio [OR], 2.1, 95% CI, 1.2–3.5). The risk of infection associated with H2 antagonists was not significant (adjusted OR, 1.1, 95% CI, 0.4–3.4).

The case–control study included 94 subjects per group. Using a logistic regression model (adjusted OR, 2.6, 95% CI, 1.3–5), the investigators found that the use of PPIs resulted in a 2.6-fold increase in C. difficile infection. In addition, 90% of the 21 subjects experiencing a relapse of C. difficile infection were receiving PPIs.

The association between PPI use and C. difficile infection is not limited to hospitalized patients. In a population-based case–control study by Dial et al., patients with the infection who had not been hospitalized within the previous year were analyzed for an association between infection and PPI use.23 Over the 10-year period studied (1994–2004), 1,233 cases were classified as community-acquired. The number of CDAD cases increased over the study period along with the number of PPI prescriptions, whereas the number of antibiotic prescriptions decreased over the study period. After the investigators controlled for age, sex, and comorbidities, the relative risk for C. difficile infection was 2.9% in patients receiving PPIs (95% CI, 2.4–3.4). The adjusted relative risk for infection in patients receiving H2 antagonists was 2% (95% CI, 1.6–2.7).

More prospective studies are needed to validate PPI use as a risk factor for CDAD, and experts recommend increased vigilance and formulary monitoring of the use of these agents.17

Hospitalization

Although CDAD can occur in the community, most infections are nosocomial. Hospitalization and an increased LOS have been identified as risk factors for disease.3 For patients hospitalized for two weeks or less, the rate of acquisition of C. difficile is approximately 13%. For patients with hospital stays of longer than four weeks, the acquisition rate is 50%.20

In a study examining nosocomial acquisition of C. difficile infection, specimens of surfaces of hospital rooms and hands of health care workers were obtained to detect evidence of contamination in the cultures.22 Almost half of the rooms of patients who had symptomatic disease showed environmental contamination by C. difficile. For patients with asymptomatic infections, the rate of contamination with C. difficile organisms in the room was 29%; for patients with no infection, the rate was 8%.

This high rate of infectivity in rooms housing patients with asymptomatic infections is especially alarming for hospitals, because these patients are more likely to be grouped with asymptomatic or uninfected patients than with patients with symptomatic infection. Such an arrangement increases the likelihood of horizontal transmission. In addition, C. difficile was detected on the hands of 59% of health care personnel caring for patients with infection.22

Miscellaneous Risk Factors

Other risk factors for C. difficile infection, elucidated from relatively small case-control or cohort studies, have been found to increase a host’s susceptibility to acquiring CDAD. Older age (over 65 years), a stay in the intensive care unit (ICU), immunosuppression, and gastrointestinal procedures are known to increase the risk of infection.3 Case reports have also implicated antineoplastic agents as an independent risk factor for CDAD.28

Even though it is difficult to eliminate risk factors for CDAD in patients, careful consideration of these factors on a systems level, as well as on the individual patient level, can often help minimize infection.

PREVENTION AND TREATMENT

Environmental Disinfection

High rates of C. difficile contamination on surfaces and hands highlight the need for infection-control practices to minimize transmission of infection. Patients with symptomatic infection should be placed in isolation. Commonly used quaternary ammonium compounds for environmental disinfection are not sporicidal and are therefore ineffective. In addition, alcohol-based hand sanitizers are not sporicidal and do not effectively remove C. difficile from the hands.

In outbreak situations, the use of 10% sodium hypochlorite has been shown to reduce the incidence of CDAD and environmental contamination.17 Hand-washing with chlorhexidine soap and water is an effective measure to reduce the spread of infection.17 Minimizing patients’ exposure to known risk factors can also help prevent the development of CDAD.

Antibiotic Therapy

Several antibiotics, including oral vancomycin (Vancocin, Baxter), metronidazole (Flagyl, Pfizer), bacitracin, fusidic acid, teicoplanin (Targocid, Sanofi-Aventis), and rifaximin (Xifaxan, Salix), have been studied for the treatment of CDAD.20 Other treatment modalities include probiotic agents such as Lactobacillus or Saccharomyces boulardii; resins such as cholestyramine (Questran, Par); and immune globulin.11 These treatments are generally reserved for refractory cases of disease.

The primary treatments used in the U.S. are oral vancomycin and metronidazole. Two major randomized, double-blind trials have been conducted to compare the use of these two agents.30,31

Teasley et al.30 In the first of these studies, Teasley and colleagues reported the results of a prospective, randomized trial that identified 149 cases of C. difficile using screening with endoscopy, culture, and cytotoxin assay.30 Ninety-four cases were included for analysis. Fifty-two subjects were selected to receive vancomycin 500 mg orally four times daily or metronidazole 250 mg orally four times daily for 10 days. Treatment failure, defined as persistent watery stools four times per day or more after six days of treatment, occurred in two subjects receiving metronidazole and in none of the subjects receiving vancomycin (P = nonsignificant).

Relapse of disease, defined as recurrence of diarrhea within 21 days of completed therapy in a patient having normal stools at the end of the treatment period, occurred in six patients treated with vancomycin and in two patients treated with metronidazole (P = nonsignificant). The authors concluded that vancomycin and metronidazole were equally effective for the treatment of C. difficile.
Those results were reaffirmed in a second prospective, randomized study comparing fusidic acid, metronidazole, teicoplanin, and vancomycin for the treatment of severe CDAD. CDAD was defined as diarrhea (more than three loose stools per day) in addition to a positive toxin assay and/or pseudomembranous colitis with fecal granulocytes. The patients were assigned to receive fusidic acid, metronidazole, and vancomycin at a dosage of 500 mg orally three times daily. Teicoplanin was administered as a 400-mg dose twice daily. Clinical failure was defined as persistent diarrhea after six days of treatment, and relapse was the reappearance of CDAD during the 25- to 30-day assessment period.

Vancomycin and metronidazole demonstrated similar response and relapse rates of 94% and 16%, respectively. For patients receiving fusidic acid, the response rate was 93% and the relapse rate was 28%.

For teicoplanin, the response rate was 96% and the relapse rate was 7%. The only statistically significant (P < .05) difference between the groups occurred in the relapse rates between fusidic acid and teicoplanin.

In an effort to reduce the development and spread of vancomycin-resistance, the Hospital Infection Control Practices Advisory Committee (HICPAC) of the Centers for Disease Control and Prevention (CDC) issued recommendations for the prudent use of vancomycin in 1995. The use of this agent as the primary treatment for antibiotic-associated colitis was discouraged in order to prevent the proliferation of vancomycin-resistant enterococcus (VRE). However, vancomycin was deemed appropriate for patients with colitis who were not responding to metronidazole therapy or patients with severe, life-threatening colitis.

With evidence supporting the equivalency of metronidazole and oral vancomycin, HICPAC recommendations to minimize vancomycin use, and the lower cost of metronidazole, many clinicians and hospitals use metronidazole as the first-line treatment for CDAD. However, since the 1995 recommendations, changes in C. difficile infection have prompted clinicians to re-evaluate their approaches to the management of CDAD.

Clostridium difficile infection is associated with high relapse rates of up to 20%. Most relapses are not associated with metronidazole resistance. Since re-infection or germination of remaining C. difficile spores is the cause of most relapses, metronidazole remains the initial treatment of choice.

### EMERGING CONCERNS

#### Incidence of Infection

Through CDC surveillance studies of nosocomial CDAD, an increased incidence of disease has been detected, with rates varying according to hospital size. Among hospitals with more than 500 beds, a statistically significant increase in disease rates among ICU patients was noted between 1987 and 2001. This increase was also associated with a longer LOS. Among smaller hospitals with fewer than 250 beds, the overall rate of disease increased significantly during the same period. These surveillance studies also noted an increase in disease rates in the winter months.

A 26% relative increase in rates of CDAD among U.S. hospitals, as measured by discharge diagnoses, was noted in 2001, compared with the period from 1998 to 2000. More recently, the CDC detected a doubling of the incidence of CDAD as a discharge diagnosis between 1996 and 2003. Although the exact epidemiology is unknown, the CDC also reported several cases of severe community-acquired disease in populations previously considered to be at low risk. Because of these changes, it would be prudent for institutions and local health agencies to monitor for an increased incidence of CDAD.

#### Increased Disease Severity

Beyond changes in disease incidence, a marked increase in disease virulence has been observed. In a survey of 447 physician members of the Infectious Diseases Society of America (with a response rate of 54%), 29% of respondents perceived an increase in the number of severe cases and 34% reported an increase in disease relapse. In a prospective study conducted during an outbreak of CDAD in Quebec, Canada, death attributed to C. difficile disease occurred in 6.9% of patients (117 of...
1,703).\textsuperscript{30} CDAD was identified as a contributing factor in the deaths of an additional 7.5% of patients (127 of 1,703).

From the Sherbrooke region of Canada, increases in severe disease and mortality have also been noted.\textsuperscript{8,30} Complicated cases, defined as those resulting in megacolon, perforation, shock, the need for colectomy, or deaths within 30 days, increased from six to 10 cases annually between 1991 and 1998 to 71 cases in 2003.\textsuperscript{8}

**Microbiologic Changes in the Organism**

Documented microbiologic changes in *C. difficile* can account for the changes in disease virulence.\textsuperscript{9} On the pathogenicity locus of the *C. difficile* genome, there are five major regulatory genes: tcdA, tcdB, tcdC, tcdD, and tcdE (Figure 2, page 518). Genes tcdA and tcdB encode for toxins A and B. One or both of these toxins are produced by all pathogenic strains of *C. difficile*. The tcdC portion of the genome acts as a negative regulator of toxin production, whereas tcdD acts to stimulate toxin production.

Gene tcdE is essential for the release of toxin from the cell. To detect the existence of genomic changes, McDonald et al. performed strain typing on isolates from outbreaks throughout institutions in the U.S.\textsuperscript{9} Via restriction endonuclease analysis and pulsed-field gel electrophoresis, these strains were found to have deletions of the tcdC gene. Deletions of the negative regulator of production resulted in increased toxin production. These strains were positive for binary toxin genes, cdtA and cdtB, which are suspected markers for increased disease virulence. These strains also demonstrated increased resistance to fluoroquinolones, as measured by E-test strips.

Another analysis of isolates characterized these epidemic strains as toxinoype III, North American PFGE type I, and PCR-ribotype 027, commonly referred to as NAP1/027.\textsuperscript{10} In vitro, this strain demonstrates increased production of both toxins A and B.

**Changes in Response to Metronidazole**

Clinical and microbiologic changes are also reflected in the response of CDAD to metronidazole, leaving institutions and professionals to re-evaluate the management of this disease. Musher et al. assessed responses to metronidazole in a prospective, observational study of 207 patients with *C. difficile* infection who had received oral metronidazole therapy for seven days or more.\textsuperscript{10} Only 50% of study patients achieved a complete clinical cure, defined as resolution of symptoms at 10 days without recurrence of disease. Twenty-two percent of patients either had persistent symptoms or signs of colitis at 10 days. The remaining 28% of patients had an initial response to treatment but experienced recurrence of disease, defined as a repeated positive toxin assay within 90 days.

In an additional retrospective review in the Sherbrooke region of Canada, this increased risk of disease relapse was described among 845 patients receiving metronidazole as monotherapy for CDAD.\textsuperscript{10} The disease recurrence rate, within 60 days of therapy completion between 1991 and 2002, remained approximately 20%. However, the rate of recurrence between 2003 and 2004 dramatically increased to 47.2% (P < .001). These changes are disturbing, because many institutional formularies include recommendations for metronidazole as a first-line treatment. In this new era of CDAD, randomized trials are needed to determine the best treatment option.

**CONCLUSION**

As with other pathogens, *C. difficile* has emerged as a virulent pathogen, creating challenges for health care providers and institutions. Some strategies should be implemented to help prevent outbreaks of disease:

- minimizing patients’ exposure to known and suspected risk factors
- using antimicrobial agents for the shortest possible duration
- following strict infection-control practices to prevent transmission of organisms from surfaces and health care workers
- using PPIs prudently to reduce the incidence of disease within an institution
- re-evaluating the use of metronidazole as a first-line treatment because of microbiologic changes in *C. difficile* that have led to an increased virulence of disease and to diminished responses to this therapy

Awareness of these concerns can be helpful in preventing and managing CDAD. On a systems level, surveillance for disease incidence and virulence, as well as institutional trends in patients’ responses to metronidazole, can be useful in developing policies and formulary restrictions for appropriate empirical therapy.

**REFERENCES**


