Clostridium difficile Infection

JAE HYUN SHIN,1 ESTEBAN CHAVES-OLARTE,2 and CIRLE A. WARREN1

1Department of Medicine, Division of Infectious Disease and International Health, University of Virginia, Charlottesville, VA 22908; 2Centro de Investigación en Enfermedades Tropiclas, Facultad de Microbiología, Universidad de Costa Rica, Costa Rica

ABSTRACT Clostridium difficile is an anaerobic, Gram-positive, spore-forming, toxin-secreting bacillus that has long been recognized to be the most common etiologic pathogen of antibiotic-associated diarrhea. C. difficile infection (CDI) is now the most common cause of health care–associated infections in the United States and accounts for 12% of these infections (Magill SS et al., N Engl J Med 370:1198–1208, 2014). Among emerging pathogens of public health importance in the United States, CDI has the highest population-based incidence, estimated at 147 per 100,000 (Lessa FC et al., N Engl J Med 372:825–834, 2015). In a report on antimicrobial resistance, C. difficile has been categorized by the Centers for Disease Control and Prevention as one of three “urgent” threats (http://www.cdc.gov/drugresistance/threat-report-2013/).

Although C. difficile was first described in the late 1970s, the past decade has seen the emergence of hypoxigenic strains that have caused increased morbidity and mortality worldwide. Pathogenic strains, host susceptibility, and other regional factors vary and may influence the clinical manifestation and approach to intervention. In this article, we describe the global epidemiology of CDI featuring the different strains in circulation outside of North America and Europe where strain NAP1/027/BI/III had originally gained prominence. The elderly population in health care settings has been disproportionately affected, but emergence of CDI in children and healthy young adults in community settings has, likewise, been reported. New approaches in management, including fecal microbiota transplantation, are discussed.

PATHOPHYSIOLOGY

The primary virulence factors that are known to cause clinical disease in Clostridium difficile infection (CDI) are the two large toxins: TcdA (toxin A; 308 kDa) and TcdB (toxin B; 270 kDa) (1). Both toxins are glucosyltransferases that inactivate Rho, Rac, and Cdc42 and result in actin condensation and subsequent cytoskeletal changes, apoptosis, and cell death of target cells. These toxins also induce an intense inflammatory response characterized by infiltration of inflammatory cells, especially neutrophils; activation of submucosal neurons; secretion of cytokines, chemokines, and arachidonic acid metabolites; and production of substance P and reactive oxygen intermediates (1, 2). We have published previously that mediators of inflammation such as COX2 and angiotensin II, inflammatory cytokines such as interleukin-8 (II–8), and immune cells such as neutrophils are significantly elevated in intestinal loops treated with TcdA (3–5). Similarly, we see in our patients’ histopathology mucosal disruption, intense inflammatory cell infiltration, and thick fibrous exudates of degenerating cells (pseudomembranes), which are the hallmarks of pseudomembranous colitis (PMC) (6) (Fig. 1). Increased fecal lactoferrin, II–1β, and II–8 were observed in patients with CDI (2). The link between glucosylation of the small GTPases and induction of the inflammatory cascade is unclear. This raises the possibility that the host inflammatory response is not a direct effect of the toxins on intracellular signaling but a concerted response by the cells surrounding the “intoxicated” cell to the changes that occurred in the latter.
In addition to TcdA and TcdB, some epidemic strains harbor an additional toxin known as binary toxin or CDT (8). However, the role of this toxin in the pathophysiology of CDI is still being debated. There is considerable variation in the sequence and activity of Tcds among different strains. For instance, the epidemic strain NAP1/027 encodes for a TcdB with an enhanced ability to penetrate, and thus intoxicate, target cells due to variations in the receptor binding and autoprocessing domains (9, 10). Furthermore, the presence of TcdA-negative strains in different locations has been consistently reported. Strikingly, all these strains encode for a variant TcdB with variations in the catalytic N-terminal domain that result in a different set of small GTPases being modified (11). The impact of this differential pattern of modified small GTPases in the pathophysiology of CDI is currently unknown, but it is certainly interesting that these TcdA-negative strains are fully virulent.

In humans, CDI is predominantly a localized disease in the colon, although the spectrum of disease may range from asymptomatic infection to severe diarrhea associated with PMC, sepsis, and even death. Even in severe disease, extracolonic infection and bacteremia are very rare (12). However, an intense systemic inflammatory response is observed where patients present with fever, leukocytosis, and even leukemoid reactions (13).

**EPIDEMIOLOGY**

CDIs have been extensively studied in high-income countries such as the United States and European countries such as the United Kingdom, France, and The Netherlands. In contrast, regions encompassing low- and middle-income countries such as Latin America in general show a lack of information on these diseases. Several reasons might account for this relatively small amount of knowledge on the impact of CDIs. These infectious diseases mainly affect a population that has access to good health care systems with the economic capability to support expensive treatments for chronic diseases (e.g., antibiotics, chemotherapy, and immunosuppressants, among others). The relationship between access to health care and CDI incidence has been recently put forward in an elegant study indicating that racial differences, directly relating to health care access, account for significant differences in CDI incidence rates (14). This study concluded that CDI represents a deviation from the paradigm that increased health care access is associated with less morbidity. If this concept is applied to a global scale, it can be hypothesized that low- and middle-income countries with budget limitations in their health care systems would generate a decreased number of *C. difficile* target populations in comparison to high-income countries. This would then result in less CDI incidence in low- and middle-income countries. In addition, the relatively expensive and technically challenging procedures required for the diagnosis of CDIs and the molecular characterization of circulating *C. difficile* would also play a role in the lack of information available about this nosocomial infectious disease in low- and middle-income countries.

**North America and Europe**

In the past decade, the incidence rates of CDI and fulminant *C. difficile* colitis have increased in the United States (15–17), Canada (18–20), and Europe (21, 22).

---

**FIGURE 1** (A) Gross pathology of pseudomembranous colitis. (B) Histopathology of pseudomembranous colitis.
The rising rates and severity of CDI were attributed to strains characterized as “group BI” by restriction endonuclease analysis, as North American pulse-field type “NAP1” by pulse-field gel electrophoresis, as ribotype 027 (BI/NAP1/027), and as toxinotype III (23). More recently, other strains such as PCR ribotypes 001, 017, and 078 were identified in certain outbreaks and severe cases of CDI (24–28). Although typically seen in older adults exposed to antibiotics in health care settings, community-associated CDI in younger populations—including children and pregnant women—and cases with no previous exposure to antibiotics have also now been noted (29–32). Advanced age remains the critical risk factor for severe disease, recurrence, and mortality (33, 34).

Latin America
Most of the studies in Argentina, Brazil, Mexico, Chile, and Costa Rica have been performed in hospitalized adult populations being treated for various chronic conditions. Whereas hospitalized populations have been the main objective of these studies, community-acquired CDI prevalence rates were found to range from 18.7 to 30% (24, 33). Several studies have focused on antibiotic-associated diarrhea (26, 27), whereas others have addressed the impact of CDIs in specific hospitalized populations, such as those with immunosuppressive disorders or hematologic and hematopoietic stem cell transplantation patients (28, 35). Several CDI diagnostic tests have been used, including technically challenging strategies such as toxigenic culture and cell cytotoxic assays (24, 36), but most of the studies were based on enzyme immunoassay (EIA) detection of C. difficile toxins (26–35). C. difficile has been found to cause antibiotic-associated diarrhea and diarrhea in nosocomial settings in as low as 8.3 to up to 38.5% of cases (26, 36, 37). Similar to North America and Europe, advanced age (mean age ranging from 65 to 72.9 years) appears to be an important risk factor for CDI in these regions (24, 38). A study conducted in Mexico identified H2 blockers, advanced age, prior hospitalization within 12 weeks of diagnosis, prior use of cephalosporins and fluoroquinolones, extended hospital stay, and antimicrobial use before diagnosis as significant risk factors for the development of CDI (39). The role of CDI in the community setting is less well documented. A case report of a community-acquired CDI caused by the epidemic NAP1/027 strain in an 18-month-old child has been published (40). The presence of toxigenic C. difficile has been reported in retail meats, dogs, and South American coati, suggesting these as sources and possible reservoirs for community-acquired CDI (41–43). However, these few studies do not shed light on the real impact of C. difficile in the induction of diarrhea in ambulatory patients.

Several attempts have been made to characterize the molecular feature of the strains circulating in the region and to define their virulence potential. Of particular interest due to its global circulation is the report of the presence of the epidemic NAP1/BI/027 strain. In Latin America, this strain was initially reported in Costa Rica and was described as a major participant during an outbreak in a tertiary hospital in 2009 (44). This epidemic strain harbored all the molecular determinants recognized in other reports such as resistance to fluoroquinolones, deletion in tcdC, and presence of the binary toxin. Recent analysis by whole-genome sequencing has indicated that the NAP1/BI/027 strain circulating in Costa Rica belongs to the FQR2 epidemic lineage, which is spread more widely and is associated with outbreaks in the United Kingdom, continental Europe, and Australia (45; C. Rodriguez, personal communication). In addition to the presence of the NAP1/BI/027 strain in Costa Rica, this epidemic variant has recently been reported in countries separated by thousands of kilometers such as Mexico, Panama, and Chile, demonstrating the distribution of this epidemic strain throughout the continent (46–48).

In all these studies, the molecular hallmarks of NAP1/BI/027 strains (tcdC deletion, presence of binary toxin, fluoroquinolone resistance) have been detected. In a study of 719 isolates of C. difficile from 45 hospitals in Chile, 79% of the strains were found to be of the epidemic variant, reinforcing the notion of the ability of this strain to have an epidemic character and potentially displace local circulating strains (47). Another interesting genotype reported was of the TcDA-negative, TcDB-positive C. difficile strain belonging to ribotype 017/toxinotype VIII. The annual percentage of this particular genotype went from 7.7% in 2000 to 92% in 2004 in an Argentinean general hospital (49). C. difficile strains belonging to ribotypes 014 and 106, the latter found previously in the United Kingdom, have also been reported (33, 43). Autochthonous C. difficile strains have also been described. Of particular interest is a variant described as responsible, together with an epidemic NAP1/BI/027 strain, of causing an outbreak in a tertiary hospital in Costa Rica (44). This strain belongs to a previously undescribed NAP type called NAPCR1 and had virulent behavior comparable to that of cocirculating NAP1/BI/027 epidemic strains. In addition, it shared with these epidemic strains molecular determinants such as deletions in tcdC, the presence of binary toxin, and resistance to fluoroquinolones due to muta-
tions in gyrA, but unlike the epidemic strains, it did not overproduce TcdA or TcdB (50).

Asia
A review of epidemiology of CDI in Asia is inevitably linked to discussion of TcdA-negative, TcdB-positive variant strains, the most commonly reported ribotypes being 017 and 018 (51).

East Asia
Despite interest in C. difficile and different molecular techniques to type them, there has been only one recent study on the epidemiology of CDI in Japan. Honda et al. found in a retrospective study of a tertiary regional referral center that the incidence at 3.31 cases per 10,000 patient-days is slightly lower than North America or Europe at 4.1 to 9.8 cases per 10,000 patient-days. However, 30-day all-cause mortality reached 15%, and 51.6% of patients met criteria for severe CDI, rates which were all higher than those seen in other countries (52). This may be due to a low index of suspicion leading to diagnosing only patients with severe illnesses. Multiple typing techniques have been employed to characterize the strains. Several ribotyping studies have indicated predominance of ribotype “smz” over the past decade (53, 54). Ribotype smz is recognized internationally as ribotype 018, which is one of the TcdA-negative, TcdB-positive strains (55). Ribotype 027, a recently epidemic strain in North America and Europe, has been reported only occasionally (54).

There have been multiple studies on the epidemiology and molecular characteristics of C. difficile in South Korea. A country-wide survey of 17 tertiary hospitals from 2004 to 2008 showed an incidence of CDI that increased from 1.7 cases per 1,000 adult admissions to 2.7 cases per 1,000 adult admissions (56). A large proportion of the CDI cases in South Korea are caused by TcdA-negative, TcdB-positive strains as demonstrated in multiple studies (57, 58). In a retrospective survey of six tertiary care hospitals in South Korea between 2000 and 2005, the prevalence of TcdA-negative, TcdB-positive strains grew from less than 7% before 2002 to a peak of 50.3% in 2004 (57). In another study evaluating the cause of PMC in a tertiary care hospital between 2004 and 2005 it was found that TcdA-negative, TcdB-positive strains caused 50.9% of PMC cases (58). Ribotypes 017 and 018 were predominantly TcdA-negative, TcdB-positive strains based on different molecular studies (51, 59). Ribotypes 027 and 078, more common in North America and Europe were also reported, with 078 being the most common binary-toxin positive strain (51).

CDI is not part of the diagnostic testing for diarrhea in hospitals in mainland China, and hence there is a paucity of epidemiologic studies looking at the incidence of CDI in the hospitalized patients in China (60, 61). In a study of diarrheal samples in hospitalized patients who were treated with antibiotics, 21/70 (30%) were positive for C. difficile by culture (60). Consistent with studies in Japan and Korea, the predominant PCR ribotype was 017 (48%), followed by 046 (14%) and 012 (14%); strains of the epidemic PCR ribotypes 027 and 078 were not observed (60). Another study looking at diarrheal stool in hospitalized patients showed 31/111 stools (28%) positive for C. difficile by PCR (61). The authors of this study noted that since CDI is not tested for as part of diagnostic work-ups, it is possible that CDI is an underrecognized problem in China.

In Taiwan, reports of CDI have appeared only quite recently. Of stools tested for CDI, 5.3% came back positive in one retrospective study, while another retrospective study showed an incidence of 42.6 cases per 100,000 patient-days, or 3.4 cases per 1,000 discharges, comparable to reports from North America (62, 63). An analysis of 110 isolates from 2002 to 2007 showed 70 isolates that were TcdA and TcdB positive, while 40 isolates were TcdA-negative, TcdB-positive (64). Interestingly, the TcdA-negative, TcdB-positive strains were initially higher, making up 73.3% of all isolates in 2004, but decreased to 23.9% in 2007, while TcdA-positive, TcdB-positive strains increased in numbers (64). Ribotype 027 strain was not found in Taiwan until recently, when three isolated cases were reported (65–67).

A retrospective study in Hong Kong showed that 12.5% of patients tested were positive for C. difficile, but only 5.9% were positive for toxin production (68). In terms of ribotyping, 70% of isolates were of a pattern not represented by 23 of the most internationally common ribotypes, with a further 11.6% being nontypable (55). Ribotype 002 was the predominant strain, representing 9.4% of the isolates (68). Another surveillance study showed 5.1% of the stools positive for C. difficile by cytotoxic assay (69). Cheng et al. also found that while testing for CDI increased, the prevalence of CDI-positive cases remained constant (23). The first and only case of CDI from a ribotype 027 strain was identified in the same study (69).

Southeast Asia
There are few reports from Southeast Asia on CDI. In the Philippines, a recent study demonstrated underestimation of CDI by showing that while historically patients with colitis were presumed to be infected with
Entamoeba histolytica, 43.6% of the stools were positive for C. difficile, compared to 25.6% which were positive for E. histolytica (70). Since the first-line treatment for both is metronidazole, the outcome would be indistinguishable between the two pathogens. Toxin assays on stool samples from patients with antibiotic-associated diarrheas at a tertiary hospital in northeastern Malaysia showed 13.7% prevalence (71). No ribotyping or other molecular analysis has been reported on Malaysian or Philippine C. difficile isolates. A study of diarrhea in children identified C. difficile in 1.3% of stool samples tested in both hospital and community settings in Jakarta, Indonesia (72). A molecular study of isolates from Indonesia showed that five of eight isolates were ribotype 017/toxinotype VIII (73).

There have been multiple studies evaluating the prevalence of C. difficile in the stool of pediatric and adult patients in Thailand, but the prevalence of positive stools varied widely from 14.3 to 52.2% between studies, which may be related to the differences in method of detection (cytotoxicity assay versus toxin A EIA) (74, 75). Since 2000, more studies have been performed on patients admitted with antibiotic-associated diarrhea. A retrospective study in 2003 showed C. difficile isolated in 18.6% of diarrheal stool specimens, all of which were positive for both tcdA and tcdB by PCR, with only one tcdA-negative, tcdB-negative result (76). A more recent study in 2012 showed even higher prevalence of 26.9% of stool specimens, with mortality due to C. difficile reported to be 6.4% (76).

There are few reports on the epidemiology and clinical behavior of C. difficile in Singapore. An earlier report indicated a percentage of isolation of this nosocomial pathogen of 9.6% in samples processed for its detection (77). Another study indicated an incidence of 3.2 cases per 1,000 admissions, occurring mainly in renal and hematological patients. From all the isolates, 11.8% corresponded to TcdA-negative strains (78). Interestingly, the decrease in the incidence of CDI was detected in the period of 2006–2008 despite an increase in the use of carbapenems and fluoroquinolones (79). The hyper-virulent strain NAP1/027 was detected and reported in 2011 (80). However, the antibiotic resistance pattern of this strain was more compatible with historic isolates, indicating that it probably does not correspond to the epidemic strain of global distribution.

South Asia
CDI was detected in India first in 1986 by toxin detection and culture from Nehru Hospital (81). A subsequent prospective study in a hospital in Delhi in 1999 showed CDI in 15.2% of hospitalized patients with antibiotic-associated diarrhea as detected by culture and toxin A EIA (82). Analysis of antibiotic-associated diarrhea in the same institution in 2009 revealed a similar incidence of 12.1% of patients being positive for C. difficile and deaths in 23% of the cases (83). Multiple studies have documented the presence of C. difficile in stool of patients with antibiotic-associated diarrhea, with incidences ranging between 11 to 17% (84, 85). Molecular epidemiology of C. difficile strains in India is not currently known. A study in the 1990s in Bangladesh found that only 13/814 children admitted to the hospital with diarrhea had stool positive for C. difficile by cell cytotoxin assay (86).

Australia
In Australia, CDIs have been associated with the use of cephalosporins since the 1980s. Indeed, a decrease in the use of third-generation cephalosporins has been observed to be followed by a concomitant decrease of CDIs (87). A survey in 48 microbiology laboratories carried out between 2009 and 2010 indicated that most laboratories screened stools by EIA. This study determined an overall mean incidence of 25.6 per 100,000 population (88). As in other parts of the world, CDI incidence is increasing in Australia and thus so is its impact on health care and the economy. A prospective study carried out between 2011 and 2012 in 450 public hospitals covering all the Australian states revealed that the increases in rates were notable in hospital-identified CDI, hospital-associated CDI, and community-associated CDI (89). C. difficile positivity in clinical samples increased from 5.9% in 2003 to 18.8% in 2012 in one study (90). In another study in 2011, CDIs were identified to be the third-most-costly complications in hospitalized patients in Australia, behind only complications associated with procedures related to endocrine or metabolic disorders and multidrug-resistant Staphylococcus aureus infections (91). A comprehensive survey in two tertiary care hospitals in Australia determined that 53.8% of CDIs were hospital onset disease, whereas 28.8% were community-onset and health care facility–associated disease, and only 7.5% were community-associated disease (92). In addition, severe disease was reported in 40% of the cases, but the 30-day mortality rate was low at only 2.5% (92).

A global study comparing the strains circulating in North America, Europe, and Australia as detected by restriction endonuclease analysis and PCR ribotyping was performed on 894 isolates (93). From those, ribotype 027 was the most common isolate in North America.
and some European countries but not in Australia. Instead, ribotypes circulating in Australia corresponded to strains rarely found in other developed countries. A study of 274 samples screened for toxigenic *C. difficile* determined a prevalence of the infection of 9.8%, of which the most frequent ribotypes were 014 and 052 (94). In other analyses the predominant ribotypes were 014 (24.3%), 020 (5.7%), 056 (5.7%), and 070 (5.7%) (92), while in yet another analysis of 474 isolates collected in 2013, the most frequent ribotypes were 014 (29.8%) and 002 (15.9%). Epidemic ribotype 027 was not identified, and small numbers of virulent ribotype 078 were found (95). In Queensland, the most common ribotypes were 002 (22.9%), 014 (13.3%), and 244 (8.4%) (96). The ribotype 244 strain had a single deletion in the tcdC gene and was positive for binary toxin and found to be closely related to ribotype 027 epidemic strains by whole-genome sequencing. This strain was associated with a more severe disease and a higher mortality rate (97, 98). Thus, strain 244 represents an autochthonous strain in Australia, with an increased virulence potential probably related to its genomic closeness to epidemic NAP1/027 strains.

TcdA-negative strains reported elsewhere have also been found in Australia. In a study of 817 human clinical isolates from all Australian states, 9 (1.1%) were found to be TcdA negative. Of those, six were positive for binary toxin. These TcdA-negative strains belonged to seven different ribotypes. Among these ribotypes, 017, a common genotype found in TcdA-negative strains, was seen in only two strains (99). The epidemic strain NAP1/BI/027 was not reported in Australia until 2009, when it was isolated from a patient who was infected in the United States (100). The first recognized case of the epidemic *C. difficile* strain acquired in Australia was not reported until 2011 (101). So far, the prevalence of the NAP1/BI/027 genotype is low in Australia and has not been able to displace other common ribotypes. Among 477 cases of *C. difficile* between 2010 and 2011 reported by the Victorian Health Care infection surveillance system, only 2.3% corresponded to the hyper-virulent strains (102).

**Africa**

South Africa has published the most reports on CDI. A random sampling of stool specimens at the University of Venda between 2004 and 2005 evaluating 322 stool specimens showed toxigenic *C. difficile* in 11.4% of all diarrheal stool samples (103). There was also an outbreak reported in 2008 in a tertiary hospital in Pretoria during which time 17.2% of all diarrheal stools were positive for *C. difficile* toxin (104). A more recent study reported *C. difficile* as the most frequently detected pathogen (16% of cases) in patients with diarrhea at a tertiary care center in the 21- to 87-year-old age range (105).

There are few published studies on CDI in other African countries, and the published studies have had a focus on evaluating patients with HIV infection. The earlier studies in the 1990s evaluating chronic diarrhea in HIV-infected patients in Zambia and Kenya showed that 0% of diarrheal stools were positive with *C. difficile* (106, 107). More recent studies evaluating HIV-infected patients with diarrhea suggest increasing prevalence of CDI in this population (108, 109). A study of HIV patients in Zimbabwe reported a prevalence of 8.6% in diarrheal stools (109). In Nigeria, CDI prevalence was 14% for hospitalized HIV-negative patients and 43% for hospitalized HIV-infected patients with diarrhea (108).

**CLINICAL PRESENTATION**

CDI may present without any symptoms, with varying degrees of diarrhea, or with sepsis to fulminant colitis leading to death. Severe disease is particularly notable in the elderly. Several factors have been reported to increase the risk of acquisition of CDI (Table 1).

**Risk Factors for Symptomatic Infection**

Antibiotic exposure is the most common predisposing factor in the development of CDI. Historically, clindamycin has been the first agent to be associated with severe diarrhea (21% of those receiving the antibiotic) and PMC (seen in 50% of those with diarrhea) (110). Most antibiotics have been implicated, but the most common agents include penicillins, cephalosporins, and fluoroquinolones. In a meta-analysis of antimicrobial usage in community-acquired CDI, clindamycin (odds ratio [OR] = 20.43) carried the greatest risk of community-acquired CDI, followed by fluoroquinolones (OR = 5.65), cepha-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Risk factors associated with acquisition of <em>Clostridium difficile</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colonization</strong></td>
<td><strong>Infection</strong></td>
</tr>
<tr>
<td>Recent hospitalization (within 2–3 months) (114, 212)</td>
<td>Advanced age (212)</td>
</tr>
<tr>
<td>Cancer chemotherapy (212)</td>
<td>Antibiotic use (111, 113, 212)</td>
</tr>
<tr>
<td>Colonization (114)</td>
<td>Proton pump inhibitor use (212)</td>
</tr>
<tr>
<td>Prokinetic feeding (213)</td>
<td>Gastrointestinal surgery (214)</td>
</tr>
<tr>
<td>Obesity (214)</td>
<td>Cancer chemotherapy (212)</td>
</tr>
</tbody>
</table>

ASMscience.org/MicrobiolSpectrum
Clostridium difficile Infection

In a more recent study of the burden of CDI in the United States in 2011, it was found that while people 65 and older made up 57% of estimated total CDI cases, the deaths from CDI in this age group made up 83% of the total estimated deaths from CDI (126). In a study of 336 patients with stool positive for C. difficile in Brigham and Women’s Hospital in 2004 and 2005, the odds ratio for severe disease was 3.35 for ages 70 and older (127). Increased risk for recurrence of CDI in people 65 and older was seen in multiple studies, with the chance of recurrence ranging from 2 to 10 times more likely in this age group, depending on the study (128, 129).

Symptoms and Signs

The clinical manifestations of infection with toxin-producing strains of C. difficile range from symptomless carriage to mild or moderate diarrhea to fulminant and sometimes fatal PMC (130–132). Symptoms of CDI usually begin soon after colonization, with a median time to onset of 2 to 3 days (119). C. difficile diarrhea may be associated with the passage of mucus or occult blood in the stool, but melena or hematochezia are rare. Fever, cramping, abdominal discomfort, and peripheral leukocytosis are common (131). In one study, 24.7% of patients had mild, self-limiting disease, 35.6% had moderately severe disease, and 39.7% had prolonged symptoms (130). In addition, 8.2% of patients had fulminant colitis with severe inflammation and pseudomembrane formation necessitating emergency colec- tomy in half of these patients (130). Extraintestinal manifestations, such as arthritis or bacteremia, are very rare (131). C. difficile ileitis or pouchitis has also been rarely recognized in patients who have previously undergone a total colectomy (for complicated CDI or some other indication) (133). Patients with severe disease may develop a colonic ileus or toxic dilatation and present with abdominal pain and distention but with minimal or no diarrhea (130–132). Complications of severe C. difficile colitis include dehydration, electrolyte disturbances, hypoalbuminemia, toxic megacolon, bowel perforation, hypotension, renal failure, systemic inflammatory response syndrome, sepsis, and death (130–132).

Severity of Disease: White Blood Cell Count (WBC), Creatinine, Complicated versus Uncomplicated

The following criteria for defining severity of CDI were adapted from the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America clinical practice guidelines (131). Mild

losporins (OR = 4.47), penicillins (OR = 3.25), mac- rolides (OR = 2.55), and sulfonamides/trimethoprim (OR = 1.84) (111). In health care settings, the role of antibiotics is difficult to ascertain because of confounding variables associated with patients in these settings (112). However, it appears that cephalosporins, clindamycin, carbapenems, trimethoprim/sulfonamides, fluoroquinolones, and penicillin combinations are associated with hospital-acquired CDI (113).

Initial acquisition of C. difficile spores invariably precedes infection. In a systematic review of 19 studies that examined colonization in patients on hospital admission, the pooled prevalence of toxigenic C. difficile carriage was about 8% (range 0 to 24%) (114). The risk of CDI for colonized patients was significantly higher at 21.8% compared to 3.4% in noncolonized patients. Hospitalization in the previous 3 months and no previous antibiotic exposure, proton pump inhibitor (PPI) use, or history of CDI was associated with higher risk of colonization. While health care facilities appear to be a major source of C. difficile transmission (presumably from asymptomatic and symptomatic patients) (115, 116), a recent study of symptomatic patients with CDI in health care settings in the community in the United Kingdom found that 45% of the isolates were genetically distinct from previous health care– or community-related cases, indicating the presence of genetically diverse sources, including asymptomatic carriers in both settings (117). C. difficile spores may be acquired through the contaminated skin or environment of both asymptomatic and symptomatic carriers (118–120). Skin contamination in infected patients persists even after resolution of diarrhea (121).

Advanced age increases the risk for developing CDI as documented in multiple studies. Studies in the 1990s and 2000s in North America when the dramatic increase in CDI cases occurred showed a significant increase in the age group 65 or older and a population incidence more than 5-fold higher than the other age groups (15, 20). In a more recent survey of hospital stays for CDI in U.S. hospitals in 2009, the rate of CDI was 1,554 per 100,000 population for patients 65 or older compared to 138 per 100,000 in the rest of the age groups (122).

Advanced age is also a risk factor for worse outcome from CDI. Mortality has been found to be significantly higher with older age (19, 123). Even when controlling for comorbidity burden, Pépin et al. demonstrated that 30-day and 1-year mortality increased with older age, especially above the age of 75 (124). The death registry from 2008 also showed that 92% of deaths from CDI occurred in people age 65 and over (125). In a more
or moderate CDI is defined by a WBC of 15,000 cells/ml or lower and serum creatinine level less than 1.5 times the premorbid level (131). Severe CDI is with WBC of 15,000 cells/ml or higher or a serum creatinine level greater than or equal to 1.5 times the premorbid level (131). Severe complicated CDI refers to cases with hypotension or shock, ileus, or megacolon (131). The distinctions are made to guide the choice of therapy. In a retrospective study evaluating patients with CDI in Brigham and Women’s Hospital, maximum WBC of >20,000 cells/ml, maximum serum creatinine level of >2 mg/dl, minimum serum albumin level of <2.5 g/dl, ileus or small bowel obstruction, and abnormal abdominal computed tomography image were all independent risk factors for severe outcome as defined by death within 30 days after onset, more than one ICU admission, colectomy, or intestinal perforation in which CDI was a major contributor (127). In other studies as well, the peak WBC and peak serum creatinine level are predictive of complicated CDI (20). Other experts and practice guidelines consider age, body temperature, comorbidities, serum albumin, serum lactate, and other factors in defining disease severity in CDI (134–136).

Mortality

In a review of patients admitted to a single tertiary center with an urban population in the United States from 2006 to 2011, 30-day overall mortality was 12.9% for hospital-associated CDI and 13.8% for community-associated CDI, which was a decrease from 2006 to 2008, when the mortality was 17.0% and 17.1%, respectively (137). Through multivariate analysis, age, WBC, and albumin level at the time of diagnosis were all associated with the 30-day mortality (137). For attributable mortality, a pooled mortality of 5.99% was calculated from the published literature up to 2010 (138). When studies before 2000 were excluded to reflect the recent trend since the emergence of the epidemic NAP1/BI/027 strain, the mortality went up to 8.03% (138).

Recurrence Disease

CDI is particularly difficult to treat due to its high rate of relapse. In a review of patients treated in Quebec, the proportion of patients who had at least one relapse was 28.8% in patients treated with metronidazole and 27.7% in patients treated with vancomycin (129). A more recent study in 2015 indicated a first relapse rate of 13.5% in community-associated CDI and 20.9% in health-care–acquired CDI (126). Risk factors for recurrence included age 65 or older, severe or fulminant underlying illness as determined by the Horn index, additional antibiotic use after discontinuation of metronidazole or vancomycin therapy for the initial CDI episode, and low serum antitoxin A IgG concentration (129, 139).

DIAGNOSIS

Diagnosis of CDI is a field with significant recent advances as reflected in the difference between the IDSA practice guidelines and current practice in many health care facilities. For example, when the guidelines were written in 2010, PCR was still considered not to have enough evidence to be included in the recommendations (131).

Diagnostic Methods

Two conventional microbiologic laboratory methods are considered the reference standards for testing of C. difficile: toxigenic culture and cell culture cytotoxicity assay. Toxigenic culture consists of culture on selective media followed by in vitro toxin detection to determine the toxigenicity of the isolated strain (140). In cell culture cytotoxicity assay, stool filtrates are inoculated onto a monolayer of a cell culture, which is then observed for a toxin-induced cytopathic effect (rounding of the cells) after 24 and 48 hours. To determine the specificity of the cytopathic effect, neutralization with an antiserum (Clostridium sordellii antitoxin or C. difficile antitoxin) is executed (140). These reference methods have a longer turnaround time, as well as requiring specific laboratory facilities and technical expertise, such as anaerobic cultures and cell cultures. As a result, many laboratories have started utilizing newer techniques.

The newer techniques are rapid and easy to perform. One technique is EIA, which can detect the presence of C. difficile by detecting the enzyme glutamate dehydrogenase (GDH), which is abundantly produced by C. difficile. EIA can also be used to detect the presence of C. difficile toxins A and B (140). Multiple assays are available, some of which combine both GDH and toxin EIA. Another technique is real-time PCR to detect the presence of C. difficile by detecting the toxin B gene (tcdB) (140).

All of the above techniques have limitations. According to the IDSA practice guidelines, culture is the gold standard but is not clinically practical because of its slow turnaround time, and while EIA is rapid, it is less sensitive and is thus a suboptimal approach for diagnosis (131). Limitations of the methods are closely related to their targets.

Toxigenic culture, GDH EIA, and PCR all detect the presence of C. difficile in the stool. These methods of
A testing strategy that is agreed upon by different guidelines is the need to test for CDI only in unformed stool (131, 136). This is due to the high rate of asymptomatic colonization of patients with C. difficile ranging as high as 59 to 94% on admission to the hospital based on some studies (115, 119). With such high rates of asymptomatic colonization, the question of testing and diagnosis in appropriate clinical settings becomes very important. Additionally, it has been found that restricting the samples tested to unformed stool does not decrease the sensitivity of the test (143).

In conclusion, with very high rates of asymptomatic colonization, only patients with an appropriate clinical picture and loose, unformed stool should be tested for CDI. Patients with ileus from toxic megacolon should be tested with rectal swab (144). For the laboratory testing strategy, there currently is not a consensus among different societies, but a two-step approach using a combination of GDH EIA, toxin EIA, or PCR may yield the best positive and negative predictive values.

**MANAGEMENT**

Management approaches for CDI may include targeting bacteria (antibiotics), toxins (antibodies, binders), host response (modulating inflammation), or microbiota (preservation or restoration), and in fulminant cases or where nonsurgical approaches fail, colectomy or other less invasive procedures. The choice of approach depends on the severity of illness, previous history of the disease, and host factors. Various international professional societies and experts have published management guidelines (131, 134–136). There have been significant advances in drug and biological research and developments in the treatment of CDI, although the current standard of care is still the administration of anti–C. difficile antibiotics (Table 2). The challenges continue to be disease

**TABLE 2** Emerging trends in the management of *Clostridium difficile* infection

<table>
<thead>
<tr>
<th>Goal</th>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention of CDI</td>
<td>Vaccines, Probiotics</td>
</tr>
<tr>
<td>Treatment of initial CDI</td>
<td>Anti–C. difficile antibiotics</td>
</tr>
<tr>
<td>Prevention of recurrence</td>
<td>Addition of antitoxin agent to anti–C. difficile antibiotics</td>
</tr>
<tr>
<td>Prevention or treatment of multiple recurrences</td>
<td>Nontoxicogenic C. difficile posttreatment with anti–C. difficile antibiotics</td>
</tr>
<tr>
<td>Management of fulminant colitis</td>
<td>Surgery: less invasive approach vs. colectomy</td>
</tr>
</tbody>
</table>
prevention in the susceptible host and prevention, and treatment of recurrences and management of fulminant cases.

**Antibacterial Agents**

Discontinuation of the offending antibiotics to allow the normal intestinal flora to recover is the ideal treatment for CDI. However, in cases where there is high infection burden and/or a need for continuing antibiotic treatment for another infection, an antimicrobial agent targeting *C. difficile* is often required. Unfortunately, nearly all antibiotics, including those specific for *C. difficile* have the potential to further disrupt the intestinal microbiota, delay recovery of colonization resistance, and predispose to another CDI.

**Metronidazole**

Oral preparations of metronidazole were widely used for CDI in the 1990s because of observed equivalence in efficacy with vancomycin, cost advantage, and concerns about the spread of vancomycin-resistant enterococci. Metronidazole was the recommended drug for CDI, with vancomycin reserved for severe and potentially life-threatening infection, unresponsiveness to metronidazole, or when oral metronidazole could not be used (145, 146). With the emergence of the epidemic strain of *C. difficile*, BI/NAP1/027/III, treatment failures with metronidazole have increasingly been reported (129, 147). Recent data from clinical trials have shown that vancomycin is superior to metronidazole for severe disease (148, 149). Intravenous (i.v.) metronidazole is usually empirically added to oral or rectal vancomycin for complicated severe disease. A recent retrospective observational study in a single institution has shown that in patients who were admitted to an intensive care unit, the receipt of combination treatment with i.v. metronidazole with either oral or rectal vancomycin was significantly associated with improved mortality (150). In this study, patients on combination therapy had more renal disease, hypoalbuminemia, leukocytosis, and fever. Administration of i.v. metronidazole alone is not recommended. In a prospective cohort study comparing oral metronidazole, i.v. metronidazole, and oral vancomycin for mild CDI, administration of i.v. metronidazole alone was observed to be associated with increased mortality (151).

**Vancomycin**

Vancomycin is the first FDA-approved drug for CDI. Soon after the identification of toxigenic *C. difficile* as the cause of antibiotic-associated PMC, the efficacy of oral vancomycin against PMC and postoperative diarrhea was tested in a small randomized controlled trial (152). Among 44 patients randomized to 5 days of either 125 mg of vancomycin or placebo taken orally, 16 patients had very high titers of neutralizable toxin and 12 had PMC by sigmoidoscopy and biopsy. After treatment, fecal toxin was undetectable and PMC was cured when treated with vancomycin in eight of nine patients with high toxin levels and six of seven patients with PMC compared to five of seven patients with high toxins and one of five patients with PMC in those who received placebo. A later study showed that oral vancomycin doses of 125 mg and 500 mg are equally efficacious in resolving diarrhea secondary to antibiotic-associated *C. difficile* colitis (153). Indeed, fecal levels of vancomycin at 125 mg every 6 hours are above the MIC$_{90}$ for *C. difficile* (152, 154). Although vancomycin quickly became the standard of treatment, metronidazole was noted to be as effective for CDI in the 1980s (155). Metronidazole was preferred by clinicians because it was cheaper and theoretically less likely to promote colonization with vancomycin-resistant enterococci (156). However, more recent randomized, controlled trials have shown that vancomycin is superior to metronidazole for severe disease (148, 149), and most experts now recommend it as the drug of choice for severe disease (131, 134, 135). Although there is not sufficient evidence of efficacy, vancomycin delivered by rectal enema or intracolonic administration has also been used in cases when oral administration is not possible and in the presence of complicated severe disease (157, 158). Vancomycin at pulsed or tapering doses has been recommended for treatment of recurrent episodes of CDI (159).

**Fidaxomicin**

Fidaxomicin is the only other FDA-approved drug for CDI. Two multicenter, randomized, noninferiority trials that enrolled a total of 1,164 patients from Europe, Canada, and the United States were conducted to compare the efficacy of fidaxomicin with vancomycin (160, 161). Both phase 3 trials revealed that fidaxomicin at 200 mg two times a day is noninferior in treating acute CDI and superior in preventing recurrent disease when compared to vancomycin at 125 mg four times a day, both given for 10 days. The lower recurrence rates noted in the fidaxomicin group may be secondary to the persistence of major microbiome components in patients treated with the new agent, compared to vancomycin, which was associated with 2- to 4-fold decreases of the *Bacteroides/Prevotella* groups (162). It appears
that protection from recurrence was conferred by fidaxomicin in both relapse and reinfection (163). However, decreased cure rates and increased recurrences were noted in both fidaxomicin- and vancomycin-treated participants who were infected with isolates of the BI strain by restriction endonuclease analysis (164). Indeed, the advantage of fidaxomicin in preventing recurrences is lost in infections caused by the prevalent epidemic strain, BI/NAP1/027. In animal studies, we have shown that pretreatment with fidaxomicin, just as with vancomycin, increases susceptibility to initial infection and is as likely to cause recurrent disease in mice (165). Although fidaxomicin is an alternative drug to vancomycin in patients with severe CDI, no data are available on its efficacy in severe complicated or life-threatening disease.

**Antitoxin**

The pathogenesis of CDI is predominantly toxin-mediated, and thus, strategies that neutralize or block toxins are logical approaches. Interestingly, although in animal models antitoxins appear to ameliorate disease, the benefits in humans only involve reduction of recurrent CDI.

**Monoclonal antibodies**

The results of a phase 2, randomized, double-blind, placebo-controlled study investigating two neutralizing, human monoclonal antibodies against *C. difficile* toxins A and B were promising (166). Both antibodies were given as a single infusion (each antibody at 10 mg per kg of body weight) in patients (still with diarrhea) receiving either metronidazole or vancomycin. Among 200 participants equally divided into antibody and placebo groups, the rate of recurrence of CDI was lower in the treatment group (7% versus 25%; *p* < 0.001). The recurrence rates were even lower in those participants with the epidemic BI/NAP1/027 (8% versus 29%; *p* = 0.06) or with a history of previous CDI (7% versus 38%; *p* = 0.006). However, the duration and severity of diarrhea and length of hospital stay were not reduced by the monoclonal antibodies. Subgroup analyses suggested that subjects who are hospitalized, are older, have severe underlying comorbidities, and have severe CDI may not respond as well to treatment. Two phase 3 studies were recently completed: one investigated the effect of each monoclonal antibody and their combination on CDI recurrence (MODIFY I), and the other investigated the effect of the monoclonal antibody against TcdB compared with the two monoclonal antibodies combined on CDI recurrence (MODIFY II). Preliminary results presented at two international conferences indicate that the monoclonal antibody to TcdB (bezlotoxumab), and not to TcdA (actoxumab), when given with standard of care, significantly decreased *C. difficile* recurrence at 12-week follow-up (16.5% versus placebo 26.6%; *p* < 0.0001). Combination treatment with both monoclonal antibodies did not confer additional benefit (215).

**Tolevamer**

Tolevamer is a high molecular weight, soluble polymer of styrenesulfonate that binds and neutralizes *C. difficile* toxins in vitro (167). A phase 2 study in patients with mild to moderate disease showed a dose response for tolevamer with 3- or 6-g daily doses, with the 6-g dose considered to be noninferior to vancomycin administered at 500 mg per day because of a <1-day difference in median time to resolution of diarrhea (tolevamer at 2.5 days compared to vancomycin at 2 days) (168). Because of these promising initial studies, 2 identical, phase 3, multicenter, randomized, double-blind, active-controlled studies in 91 sites in the United States and Canada and 109 sites in Europe, Australia, and Canada were conducted (149). Unfortunately, the clinical success of tolevamer (given as a loading dose of 9 g followed by 3 g every 8 hours) was found to be inferior to both metronidazole (375 mg every 6 hours) and vancomycin (125 mg every 6 hours). In the pooled analyses, 44.2% of 534 patients who received tolevamer, compared to 72.7% of 278 receiving metronidazole and 81% of 259 receiving vancomycin, attained clinical success. Clinical success appeared to decrease further with increasing severity of disease. Interestingly, recurrence rates were significantly higher in both the metronidazole and vancomycin groups compared to tolevamer (23% and 20.6%, respectively, compared to 4.5%), although the authors warned that this advantage may have been secondary to selection bias and the predominantly mild disease in clinical responders. The roles of pathogen clearance and perturbation of the microbiomes have been implicated to explain the outcomes of treatments in these subjects (169).

It is unclear if combination treatment with tolevamer and a *C. difficile* antibiotic may be beneficial in preventing recurrences. However, there may be issues about binding of the antibiotic, and in addition, the effect of the latter may abrogate the flora-preserving advantage of the toxin binder.

**Immunoglobulin (IG)**

The benefit of i.v. IG in the treatment of recurrent CDI or severe CDI has not been studied systematically.
Anecdotal evidence of efficacy has been reported in few case reports and case series (170). The doses, number of times administered, and intervals between doses have been variable. A retrospective study in Pittsburgh of 18 C. difficile–infected subjects receiving i.v. IG pair-matched by baseline characteristics and severity of CDI with 61 subjects not receiving i.v. IG did not show any differences in all-cause mortality, colectomies, and length of stay. Inconsistencies in the effect of i.v. IG on clinical outcomes may be secondary to the unpredictable presence of antitoxin antibodies in the general population (171).

**Bacteriotherapy**

Perturbation of the intestinal microbiota is the critical factor for the development of CDI. Vancomycin or metronidazole treatment of asymptomatic infection has only led to recurrent and prolonged clostridial shedding (172). Consistent with these findings is the observation that the risk of recurrence in humans increases from 24% in individuals with one episode of CDI to up to 64.7% in those with prior recurrences (and, therefore, consequent CDI treatments) (159). Restoration of the disrupted intestinal microbiota by either defined organisms or an undefined microbial community has reemerged as a strategy to break the cycle of CDI–antibiotics-CDI.

**Fecal microbiota transplant**

Transfer of fecal suspension from a “healthy” donor to a patient with CDI or fecal microbiota transplantation (FMT) is the most direct way of restoring the intestinal flora homeostasis. The earliest documentation of FMT use for gastrointestinal disease was reported to be in the fourth (Dong-jin dynasty) and sixth (Ming dynasty) centuries A.D. (173). FMT, mostly through rectal enema, has been used since the 1950s for PMC. Since toxigenic C. difficile was discovered to be the pathogen involved in PMC, succeeding reports of success were published in case reports, case series, and other observational studies in the 1980s and onward (174, 175). The overall rate of symptom resolution after FMT was around 90%, with a trend toward higher clinical resolution after lower gastrointestinal FMT route (174–176). A randomized controlled trial compared FMT using nasoduodenal tube with two control groups (177). FMT was performed after 4 to 5 days of oral vancomycin (500 mg four times daily), while control groups received the same amount of vancomycin for 14 days. In the FMT group, 81% of patients achieved resolution of symptoms at 3 months compared with 31% and 23% of the vancomycin and vancomycin plus bowel lavage control groups, respectively ($p < 0.001$ for FMT versus both control groups). The trial was prematurely terminated because of the unexpected extremely low response rates in the control groups.

Another randomized controlled trial comparing FMT via colonoscopy with oral vancomycin at 125 mg four times daily for 10 days followed by 125 to 500 mg/day every 2 to 3 days for at least 3 weeks showed similar findings. In the FMT group, 18 of 20 patients (90%) had symptom resolution compared with 5 of 19 (26%) patients in the vancomycin control ($p < 0.0001$). This study was terminated after a 1-year interim analysis. While most studies have administered fecal suspension via a nasogastric or nasoduodenal route, rectal enema, or colonoscopy, oral administration using encapsulated fecal material is emerging as an option for FMT. An open-label, single-arm study was conducted to determine the efficacy of oral, encapsulated, frozen feces among 20 patients with at least three episodes of mild to moderate CDI and failure of a prolonged tapering course of vancomycin or at least two episodes of severe CDI requiring hospitalization (178). Patients received 15 capsules on 2 consecutive days and were followed for up to 6 months. Resolution of diarrhea was achieved in 70% of patients after one FMT, and after another FMT for the nonresponders, in 90% of all patients. No serious adverse events attributed to the capsule-based FMT were observed. Regardless of route of administration, FMT appears to be effective in patients who have recurrent CDI.

Although FMT has been used for decades now, there remain concerns about its efficacy for severe, complicated CDI, safety in the immunocompromised hosts, and long-term safety of transfusing undefined donor feces to a recipient. An observational study examined the efficacy and safety of FMT in elderly patients with recurrent, severe and complicated CDI (179). In this study, patients were age 65 years or older. Severe CDI was defined by albumin <3 g/dl, WBC >15,000/μl, and/ or abdominal tenderness. Complicated CDI was defined by the occurrence of one or more of the following as a consequence of CDI: admission to the intensive care unit, altered mental status, hypotension, fever >38.5°C, ileus, WBC <2,000 or >30,000/μl, lactate >2.2 mmol/liter, or evidence of end-organ damage. Primary cure (resolution of CDI symptoms after initial FMT with no recurrence in the subsequent 12 weeks) in recurrent, severe, and complicated CDI was 82%, 91%, and 66%, respectively. In a small study of 17 patients, of whom 76.4% had severe and complicated CDI, primary and secondary cure rates were reported to be 88% and 94%, respectively.
Clostridium difficile Infection

respectively (179). In a retrospective study of 80 immunocompromised patients (75 adult and 5 pediatric), the primary and secondary cure rates were noted to be 78% and 89%, respectively (180). The immunocompromised state was reported as HIV/AIDS (3), solid organ transplant (19), malignancy (7), immunosuppressive therapy for inflammatory bowel disease (36), and other medical conditions (15). It appears that in select patients with either severe or complicated CDI or who are immunocompromised, FMT is effective, although some patients may require repeat FMT to achieve resolution of disease.

The efficacy of FMT, as well as potential long-term effects of other components of the donor stool, have driven interest in defining the microbial community that confers protection from recurrent CDI. Investigators from Ontario, Canada, have isolated 33 species from two patients with recurrent CDI to “RePOOPulate” their gut (181). Both patients achieved normal bowel pattern within 2 to 3 days after the procedure and remained symptom-free at 6-month follow-up. Bacterial 16S rRNA analyses of the recipient stools at 6 months revealed persistence of donor bacteria. Commercial products either for oral or rectal enema delivery of defined bacterial spores or microbiota suspension, respectively, are currently being tested in clinical trials.

Probiotics

Multiple small studies performed on various probiotics have been reported, and meta-analyses or systematic reviews of these studies have shown some association of probiotic use with prevention of C. difficile–associated diarrhea (182, 183). Two of the most common probiotic species studied in small clinical trials are Lactobacillus acidophilus and Bifidobacterium spp. In six previously published small studies (sample sizes ranged from 40 to 437) of L. acidophilus and/or Bifidobacterium bifidum containing probiotics, the relative risk ratio associated with treatment ranged from 0.21 to 0.40 (184–189). Recently, a multicenter, randomized, double-blind, placebo-controlled study tested the efficacy of a high-dose probiotic preparation containing two strains each of L. acidophilus and Bifidobacterium (B. bifidum and Bifidobacterium lactis) in patients age ≥65 years who were exposed to one or more oral or parenteral antibiotics (181, 190). One group of subjects (1,493) were randomly assigned to the treatment group, and 1,488 were assigned to the placebo group; 1,470 and 1,471, respectively, were included in the analyses of primary endpoints. Antibiotic-associated diarrhea (AAD) occurred in 10.8% of subjects in the probiotic group and 10.4% of the placebo group (p = 0.71), while CDI occurred in 0.8% and 1.2% of the probiotic and placebo groups, respectively (p = 0.35). It is unclear whether the low incidence of CDI in the European study (Wales and England) may have influenced the results, but based on this well-powered study, the lactobacilli- and bifidobacteria-containing preparations were not effective in the prevention of either AAD or CDI.

Nontoxigenic C. difficile (NTCD)

In hamsters, prior colonization with nontoxigenic C. difficile provided protection from subsequent colonization and disease with toxigenic strains (191). Following this observation in animals, two patients with relapsing CDI after treatment with metronidazole and vancomycin were reported to respond to a defined NTCD given orally in three doses (192). Indeed, NTCD has been shown to colonize asymptomatic patients in the hospital, suggesting that the presence of the bacteria may protect against development of symptomatic infection (193). Different strains of NTCD have been tested and have been shown to prevent mortality from CDI in hamsters (194, 195). Spores of one of the strains used in animal studies (VP20621/M3) were then tested in healthy subjects (196). VP20621 was found to be well tolerated and colonized the gastrointestinal tract of subjects pretreated with vancomycin. Persistent colonization was detected in stools of 44% of the subjects on days 21 to 28. A phase 2, randomized, double-blind, placebo-controlled, dose-ranging study was conducted among 173 participants age 18 years or older (at least one-third were ≥65 years old) with a diagnosis of CDI (first episode or first recurrence) successfully treated with metronidazole, oral vancomycin, or both at 44 centers in the United States, Canada, and Europe (197). Participants received NTCD-M3 at 10^4 spores daily for 7 days, 10^7 spores daily for 7 days, 10^7 spores daily for 14 days, or placebo for 14 days. Of the participants who received treatment, 69% had stools colonized with NTCD-M3. Recurrence of CDI occurred in 30% of the placebo group and 11% of the NTCD-M3 groups combined (odds ratio, 0.28; 95% CI, 0.11 to 0.69; p = 0.006). The lowest recurrence rate was 5%, which was noted in subjects who received 10^7 spores daily for 7 days. Recurrence was 2% versus 31% in subjects who were colonized versus those who were not colonized by NTCD-M3, respectively (p < 0.001). The results of this phase 2 study suggest that colonization with NTCD reduces CDI recurrence.
Surgical Interventions
Removal of the diseased intestinal tissue (colecctiony) is considered in those that have failed medical therapy or have fulminant colitis. The overall rates for colectomies remain low, but in some centers, the emergence of the epidemic strain of C. difficle has been associated with increased rates of colectomies for severe CDI (16, 198). In Pittsburgh, Pennsylvania, 1.1% of patients with nosocomial CDI from 1989 to 1999 and 10.3% from 2000 to 2001 underwent colecctionty. Because of the condition of the patients and the degree of disease severity, the procedure is usually associated with very high mortality.

Colectomy
Indications for surgery include fulminant colitis with shock and multiorgan failure, toxic megacolon, perforation, peritonitis, and failure of medical treatment (199). Partial (segmental) or total colecctionty with end ileostomy is the most common surgical procedure performed for fulminant colitis. Subtotal or total abdominal colecctionty with end ileostomy is the procedure of choice because other procedures (segmental resection, defunctioning stoma, nontherapeutic laparotomy) are associated with high rates of reoperation and mortality (200). When indicated, surgery appears to be most beneficial in patients who are 65 years or older, immunocompetent, with WBC of ≥20 x 10^9/liter and lactate level of 2.2 to 4.9 mmol/liter (199, 201). Risk factors for poor outcomes include age ≥75, immunosuppression, WBC of ≥50 x 10^9/liter, lactate level of ≥5 mmol/liter, mental status change, and delay in timing (201–203). Delayed surgical intervention has been associated with high mortality rates (33, 204), although a recent retrospective study suggested that a shorter time between diagnosis and surgery (median time 2 versus 3 days, p = 0.009) correlates with postsurgical mortality (205). The authors of the latter study mentioned improved medical treatment as the reason for their unexpected findings, although it is unclear whether patients who underwent early surgery were sicker than those who did not. Mortality rates from surgery for CDI were reported to range from 30 to 80% in the 1990s and 34 to 57% in the 2000s (199). Although deaths after surgery appear to be high, mortality from fulminant colitis without surgical intervention is almost 100%.

Diverting loop ileostomy with lavage
In an attempt to preserve the colon and avoid morbidity and mortality associated with aggressive surgical interventions, a minimally invasive procedure involving diverting loop ileostomy with colonic lavage has been described (206). In this procedure, an ileostomy is created, through which antegrade colonic lavage and colonic enemas with vancomycin are administered. Compared to a historical control of age-, sex-, and disease severity–matched patients who underwent colecctionty, postoperative death was lower (19%) in those who had diverting ileostomy, compared to 50% of the control group (p = 0.006). Recurrence rates were not reported in these patients.

Vaccine
Because of the increasing incidence, morbidity, mortality, and health care cost from CDI, immunization to prevent the disease itself would be ideal. Active immunization has been successful in controlling and even eradicating some infectious diseases. Human data suggest that an adequate humoral response to C. difficle toxins is associated with either asymptomatic colonization or decreased recurrence (128, 207). Several vaccine candidates—toxoid-based, recombinant toxin-based peptide, DNA-based, and surface protein antigens—are in development, but most are in the preclinical stage (208). A few vaccines are in varying stages of clinical studies.

A partially purified toxoid A and B vaccine has been found to be safe and immunogenic in 30 healthy adults (209). In this study, ≥90% of the subjects developed serum antibody responses to both toxins. In a follow-up pilot study, three patients with multiple episodes of CDI and on prolonged treatment with oral vancomycin were given the toxoid preparation intramuscularly on days 0, 7, 28, and 56 (210). Two of the three patients had increased serum IgG levels to both toxins (IgG against TcdB several-fold higher than IgG against TcdA). All three patients stopped vancomycin after the vaccination, and no recurrence was observed at 6-month follow-up. A highly purified version of the toxoid was tested in 50 healthy adults (18 to 55 years old) and 48 elderly (≥65 years old) volunteers in a phase 1 study (211). The vaccine (2 μg, 10 μg, or 50 μg) or placebo was given on days 0, 28, and 56. Seroconversion for TcdA was 100% in the younger age group, while 100% conversion was achieved only in the elderly group receiving the highest dose. Seroconversion for TcdB was lower in all groups. IgG levels remained high in the younger volunteers and was declining in the older volunteers at day 236. Two phase 2 trials recently completed testing the vaccine in middle-aged to elderly individuals at risk (NCT01230957) and subjects with first-episode CDI (NCT00772343), and a phase 3 trial is underway to evaluate the efficacy of...
the vaccine to prevent primary (first episode) CDI in up to 15,000 participants in 17 countries (NCT 01887912). Other vaccine candidates that have recently completed a phase 1 study include a genetically and chemically modified full-length TcdA and B (NCT01706367) and a recombinant fusion protein, IC84 (NCT01296386). No results have been published yet on these recently concluded phase 1 and 2 studies.

CONCLUSIONS

CDI continues to be a major health care problem in North America, Canada, and Europe and is now being recognized as a significant cause of morbidity in other parts of the world as well. Community-associated onset and infections in children and otherwise healthy adults are emerging, but advanced age remains a key risk factor in the acquisition of severe disease. Nonantimicrobial approaches such as use of monoclonal antibodies, nontoxigenic C. difficile, FMT, and less invasive surgery are novel interventions that may change management and outcomes of CDI.

REFERENCES


42. Silva ROS, Santos RLR, Pires PS, Pereira LC, Pereira ST, Duarte MC, de Assis RA, Lobato FCF. 2013. Detection of toxins A/B and isolation of *Clostridium difficile* and *Clostridium perfringens* from dogs in Minas Gerais, Brazil. Braz J Microbiol 44:133–137.


Clostridium difficile infection


20 ASMscience.org/MicrobiolSpectrum

Shin et al.


