REVIEW ARTICLE

CURRENT CONCEPTS

Clostridium difficile — More Difficult Than Ever

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In 1935, Hall and O'Toole first isolated a gram-positive, cytotoxin-producing anaerobic bacterium from the stool of healthy neonates.[1] They named it *Bacillus difficilis* to reflect the difficulties they encountered in its isolation and culture. We now face the opposite problem of being unable to contain the growth and spread of the same bacterium, now called *Clostridium difficile*, which is a frequent cause of infectious colitis, usually occurring as a complication of antibiotic therapy, in elderly hospitalized patients. In this article we review recent changes in the epidemiology of *C. difficile* infection, discuss changes in disease severity and response to therapy, and review new approaches to the management of this increasingly problematic infectious diarrhea.

INCIDENCE AND SEVERITY

During the mid- and late 1990s, the reported incidence of *C. difficile* infection in acute care hospitals in the United States remained stable at 30 to 40 cases per 100,000 population.[2] In 2001, this number rose to almost 50, with subsequent increases to the point that the number of cases of *C. difficile* infection that were reported in 2005 (84 per 100,000) was nearly three times the 1996 rate (31 per 100,000). Of even greater concern are the increases in severe or fatal infection.[3-5] In England, for example, *C. difficile* infection was listed as the primary cause of death for 499 patients in 1999, a number that rose to 1998 in 2005 and to 3393 in 2006.[6]

In addition to more prevalent endemic *C. difficile* infection, sporadic outbreaks have been reported in many hospitals nationwide and internationally. A 2003 outbreak in Quebec, Canada, was especially notable because of its scope and impact.[3,4] In the Estrie region of Quebec, the incidence of *C. difficile* infection was stable from 1991 through 2002 (22.2 and 25.2 per 100,000 population, respectively) but quadrupled in 2003 (92.2 per 100,000) (Fig. 1).[3] An exceptional feature of this outbreak was that all the major acute care hospitals in the region were simultaneously affected, causing substantial concern among the general population. As with endemic *C. difficile* infection, epidemic cases were most likely to afflict the elderly (867 per 100,000 over the age of 64 years). The major increase in the incidence of *C. difficile* infection in Quebec in 2003 was accompanied by a substantial increase in disease severity and mortality. In a study of 1703 patients, *C. difficile* infection was the attributable cause of death in 117 cases (6.9%) and a contributing factor in an additional 127 deaths (7.5%).[4]

EMERGENCE OF A VIRULENT STRAIN

Similar increases in the incidence, severity, and mortality associated with *C. difficile* infection have occurred in the United States. McDonald et al. examined *C. difficile* isolates collected from eight health care facilities in six states (Georgia, Illinois, Maine, New Jersey, Oregon, and Pennsylvania) during outbreaks of the infection between 2000 and 2003. Isolates of a single strain accounted for at least half the

isolates from five facilities, and 82% of stool samples from the Quebec outbreak were positive for the same strain.[4,7] This epidemic strain was initially identified in the 1980s by restriction endonuclease analysis and named BI, but is currently referred to as North American Pulsed Field type 1 (NAP1) and PCR ribotype 027 (i.e., BI/NAP1/027, or NAP-1/027).[7]

Three bacterial factors have been implicated in outbreaks of *C. difficile* infection caused by the virulent NAP-1/027 strain: increased production of toxins A and B, fluoroquinolone resistance, and production of binary toxin. Toxins A and B are the major virulence determinants of *C. difficile;* indeed, toxin-negative strains are nonpathogenic. Toxins A and B are transcribed from a pathogenicity locus that comprises five genes: two toxin genes, *tcdA* (toxin A) and *tcdB* (toxin B), and three regulatory genes, one of which (*tcdC*) encodes a putative negative regulator of toxin transcription (Fig. 2A and 2B).[8,9] TcdC protein appears to inhibit toxin transcription during the early, exponential-growth phase of the bacterial life cycle. NAP-1/027 isolates that were obtained from patients during recent outbreaks of *C. difficile* infection carry deletion mutations in the *tcdC* inhibitory gene that have been associated with an increase by more than a factor of 10 in the production of toxins that mediate colonic tissue injury and inflammation in *C. difficile* infection (Fig. 2C).[7,9,10] These toxins bind to the surface of intestinal epithelial cells, where they are internalized and catalyze the glucosylation of cytoplasmic rho proteins, leading to cell death (Fig. 2D).[11]

All NAP-1/027 isolates from the 1980s and 1990s, like those from recent outbreaks, carry *tcdC* mutations.[4,7] In contrast, high-level resistance to gatifloxacin and moxifloxacin is evident in recent isolates but not in historic NAP-1 strains. Resistant strains may have a competitive advantage in a hospital environment where fluoroquinolone use is widespread.[12] This theory is supported by the finding in the Quebec outbreak that the odds ratio for fluoroquinolone use in patients with *C. difficile* infection, as compared with control subjects, was 3.9 (95% confidence interval [CI], 2.3 to 6.6), which was virtually the same as the odds ratio (3.8) for the use of cephalosporin (95% CI, 2.2 to 6.6), a longtime leading antibiotic class predisposing to *C. difficile* infection.[4] This observation suggests that limiting fluoroquinolone use may help to contain outbreaks caused by NAP-1/027, as was reported earlier for the restriction of clindamycin in an outbreak caused by a clindamycin-resistant strain.[13]

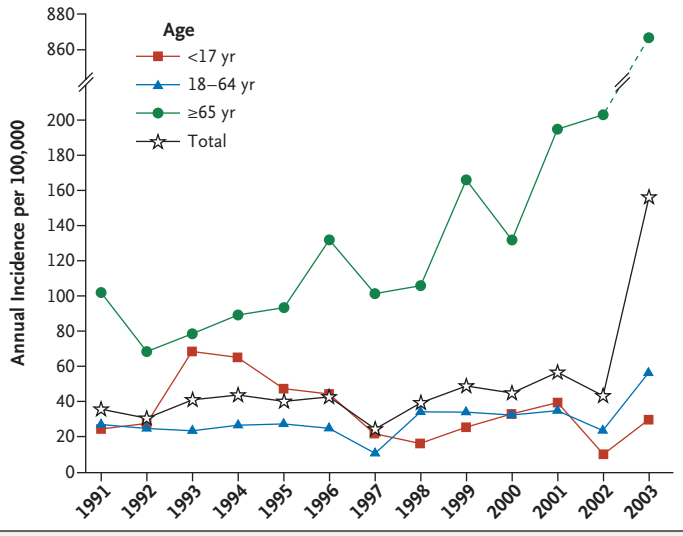

Figure 1. Annual Incidence (per 100,000 Population) of *C. difficile* Infection in Sherbrooke, Quebec, 1991–2003.

The overall incidence of *C. difficile* infection was relatively stable during the period from 1991 through 2002, although there was a gradual increase in the rate of infection among elderly patients (≥65 years). In 2003, the population incidence increased by a factor of 4, as compared with 2002. This increase was especially evident in the elderly. Data are from Pépin et al.[3]

Another potential virulence determinant of NAP-1/027 strains is the production of a third toxin, binary toxin, that is unrelated to the pathogenicity locus that encodes toxins A and B.[14] Previously, about 6% of *C. difficile* clinical isolates produced binary toxin, homologous to the iota toxin of *C. perfringens* and comprised of a 48-kD enzymatic component and a 99-kD binding component. Binary toxin has enterotoxic activity in vitro, but its role, if any, in the pathogenesis of *C. difficile* infection is not clear.[14-16] *C. difficile* strains that produce binary toxin in the absence of toxins A and B do not appear to be pathogenic. Nonetheless, the finding that NAP-1/027 epidemic strains produce binary toxin has raised renewed speculation that this toxin may act synergistically with toxins A and B in causing severe colitis.[4,5,7,14-16]

EXPANDING EPIDEMIOLOGY

C. difficile infection predominantly affects elderly and frail hospital and nursing home patients (Fig. 1).[2,3] However, a recent advisory from the Centers for Disease Control and Prevention warns of a risk of the infection in populations not previously considered at risk.[17] These include young

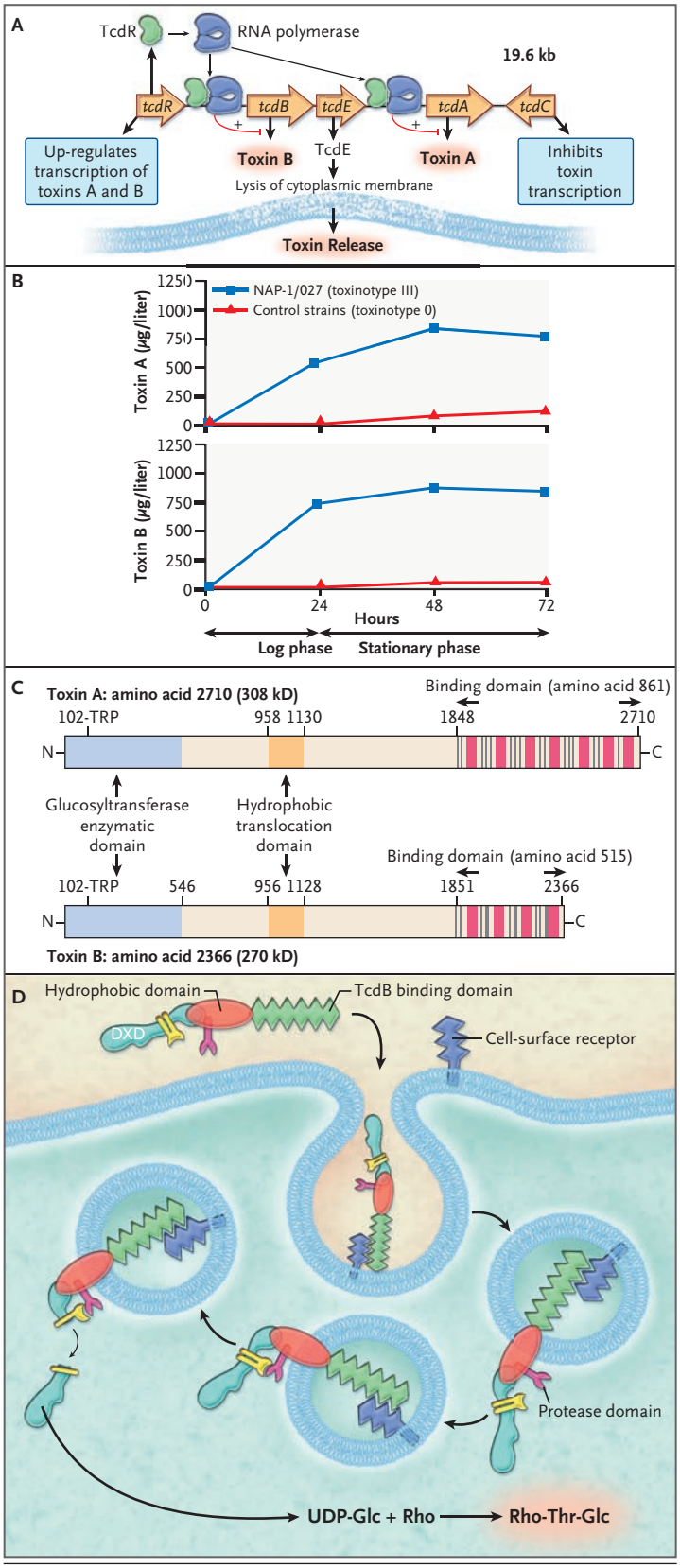

Figure 2. The Pathogenicity Locus of *C. difficile*; In Vitro Production and Structure of Toxins A and B; and Toxin Binding, Internalization, and Intracellular Actions.

In Panel A, the 19.6-kb pathogenicity locus encodes toxin A (*tcdA*), toxin B (*tcdB*), a positive regulator of toxin transcription (*tcdR*), and a putative negative regulator of transcription (*tcdC*). The function of the *tcdE* gene product is uncertain but may include the facilitation of toxin release by bacterial membrane lysis. The NAP-1/027 strain carries mutations in *tcdC* that prevent the expression of TcdC protein. Data are from Warny et al.[8] In Panel B, the median concentration of toxins A and B are shown in the log phase and stationary phase. *C. difficile* strains included 25 toxinotype 0 and 15 NAP-1/027 strains from various locations. Data are from Warny et al.[9] In Panel C, toxins A and B of *C. difficile* show considerable sequence and structural homology. Both have a C-terminal receptor-binding domain, a central hydrophobic domain that is believed to mediate the insertion of the toxin into the membrane of the endosome, thereby allowing the N-terminal glucosyltransferase enzymatic domain to enter the cytosol. Data are from Warny et al.[8] In Panel D, the interaction of the TcdB binding domain (green) with cell-surface receptors (dark blue) induces receptor-mediated endocytosis. The acidic pH of the endosome triggers the first conformational change and results in pore formation of the hydrophobic-translocation domain (red oval). Within the cytosol, a second conformational change activates intrinsic protease activity (pink). Autocatalytic cleavage of TcdB releases the catalytic-DXD glucosyltransferase domain (light blue) into the cytosol. Glucosylation of the cytosolic target Rho GTPases at a conserved threonine residue (Thr) leads to disaggregation of the cytoskeleton and cell death. Glc denotes D-glucose, and UDP uridine diphosphate. Data are from Reineke et al.[11]

and previously healthy persons who have not been exposed to a hospital or health care environment or antimicrobial therapy. Close contact with patients who have *C. difficile* infection was the only evident risk factor in some pediatric cases, indicating the importance of direct person-to-person spread.[18] Severe infection leading to colectomy and then death was also described in young women in the peripartum period, events that were devastating and unexpected.[17] Increased awareness of the possibility of fulminant *C. difficile* infection in atypical settings should facilitate earlier diagnosis and treatment.

METRONIDAZOLE VERSUS VANCOMYCIN

Shortly after the first descriptions of *C. difficile* infection in the late 1970s, effective therapy with

either metronidazole or oral vancomycin was reported. Despite the dramatic increases in the incidence and severity of *C. difficile* infection during the past decade, these same two agents remain the treatments of choice for almost all patients with *C. difficile* infection. A review of controlled trials of therapy for *C. difficile* infection conducted before the year 2000 indicates that the cumulative failure rates for treatment with metronidazole and vancomycin were virtually identical (2.5% and 3.5%, respectively). However, since 2000, substantially higher failure rates have been reported for metronidazole therapy (18.2%).[19-21] For example, in the outbreak of *C. difficile* infection in Quebec, 26% of patients did not have a response to metronidazole treatment.[20] A retrospective study also reported that the time to resolution of diarrhea in patients who were treated with metronidazole was significantly longer than in those treated with vancomycin (4.6 vs. 3.0 days, P<0.01).[22]

These data sustain an ongoing debate as to whether vancomycin is superior to metronidazole as initial therapy for *C. difficile* infection. Recommendations from a number of professional societies advocate vancomycin as the first-line agent for patients with severe infection, since a small increment in efficacy may be critical in patients with fulminant disease.[23] These recommendations are supported by the findings of a recent prospective, randomized, placebo-controlled trial that compared metronidazole (at a dose of 250 mg four times per day) with vancomycin (at a dose of 125 mg four times per day) in 172 patients stratified according to the severity of *C. difficile* infection (Fig. 3).[24] The two agents showed similar efficacy in mild infection, although the response rate with vancomycin (98%) was greater than that with metronidazole (90%, P=0.36). In patients with severe infection, vancomycin was significantly more effective (97% vs. 76%, P=0.02). Another recent prospective, randomized, controlled trial showed similar results.[25] Thus, metronidazole remains the first-line agent for treatment of mild infection because of its lower cost and concerns about the proliferation of vancomycin-resistant nosocomial bacteria. On the basis of recent prospective, controlled trials, vancomycin can now be recommended as the first-line agent in patients with severe infection because of more prompt symptom resolution and a significantly lower risk of treatment failure.

Markers of severe *C. difficile* infection include pseudomembranous colitis, a marked peripheral leukocytosis, acute renal failure, and hypotension. Despite its proven superiority, oral vancomycin may not be suitable for some patients with severe or fulminant infection because of coexisting ileus or toxic megacolon. Intravenous metronidazole (at a dose of 500 mg four times daily) is used in this situation and should, if possible, be supplemented with vancomycin administered through a nasogastric tube or by enema (500 mg four times daily).[26] Passive immunotherapy with the use of normal intravenous immunoglobulin (400 mg per kilogram of body weight) has been reported, but its efficacy is unproven.[27,28] Patients with severe or refractory disease should be evaluated early by a gastrointestinal surgeon, since timely subtotal colectomy can be lifesaving.

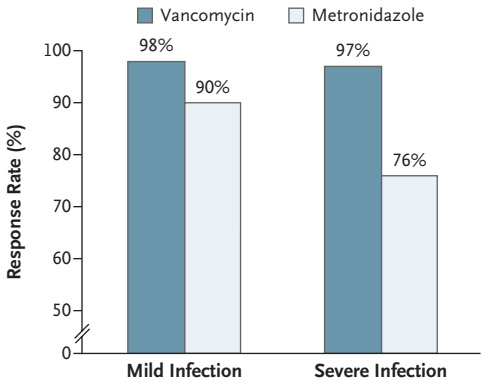

Figure 3. Response Rates to Vancomycin and Metronidazole Therapy, According to the Severity of *C. difficile* Infection.

Patients with *C. difficile* infection were randomly assigned to receive therapy with either oral vancomycin (at a dose of 125 mg four times daily) or metronidazole (at a dose of 250 mg four times daily) after stratification according to disease severity. Severe infection was defined according to the presence of pseudomembranous colitis on endoscopy, admission to an intensive care unit, or any two of the following factors: an age of more than 60 years, a temperature above 101°F (38.3°C), a serum albumin level of less than 2.5 g per deciliter, and a white-cell count of more than 15,000 cells per cubic millimeter. The difference in response rates between vancomycin and metronidazole was not significant in patients with mild infection (P=0.36), but was significant in those with severe infection (P=0.02). Data are from Zar et al.[24]

RECURRENT INFECTION

One of the most challenging aspects of caring for patients with *C. difficile* infection is the recurrence

of disease after successful initial therapy is completed. Recurrence rates after treatment with metronidazole or vancomycin are similar (20.2% and 18.4%, respectively) (Table 1). The use of either metronidazole or vancomycin impairs resistance to colonization, thereby facilitating recurrent infection, which typically occurs within 4 weeks after the completion of therapy. Antimicrobial resistance to vancomycin in patients with *C. difficile* infection has not been reported, and resistance to metronidazole is rare. Recurrence may result from reinfection with a different strain of *C. difficile* or persistence of the strain responsible for the initial episode.[29]

ROLE OF HOST IMMUNITY

The risk of recurrent *C. difficile* infection is increased in patients who have already had one recurrence, rising from about 20% after an initial episode to about 40% after a first recurrence and to more than 60% after two or more recurrences.[30,31] This dramatic escalation in the risk of recurrent *C. difficile* infection is probably caused in part by the selection of patients without protective immunity against *C. difficile*, which makes them vulnerable to repeated attacks. *C. difficile* infection develops in only half the hospitalized patients who become colonized with toxigenic *C. difficile* as a complication of antimicrobial therapy, whereas the remainder are symptomless carriers.[32] After colonization, symptomless carriers manifest an early increase in serum IgG antibodies against toxin A, whereas patients in whom *C. difficile* infection develops do not have such increased levels (Fig. 4A).[32] During an initial episode of infection, some patients manifest a primary immune response with an early rise in IgM antitoxin A, followed by an increase in IgG antitoxin (Fig. 4B).[33] In one study, patients with the highest titers of serum IgG antitoxin at the end of antimicrobial therapy were at a decreased risk for subsequent recurrence by a factor of 44, as compared with those with lower antitoxin titers.[33]

Table 1. Treatment Failures and Recurrences of *C. difficile* Infection with Metronidazole and Vancomycin Therapy.*

Variable	No. of Studies	Treatment Failure	Recurrence
		no./total no. (%)	
Metronidazole			
Year 2000 or before	4	18/718 (2.5)	48/715 (6.7)
After 2000	5	275/1508 (18.2)	332/1162 (28.6)
Combined periods	9	293/2226 (13.2)	380/1877 (20.2)
Vancomycin			
Year 2000 or before	11	22/637 (3.5)	112/624 (17.9)
After 2000	2	2/71 (2.8)	36/181 (19.9)
Combined periods	13	24/708 (3.4)	148/805 (18.4)

* Data are from Aslam et al.[21] and Zar et al.[24]

MANAGEMENT OF RECURRENCE

General Considerations

First, the ultimate goal of treatment is to discontinue all antibiotics and allow the normal bowel microflora to restore itself. Early studies of antibiotic-associated colitis (published before *C. difficile* was identified as the causative agent) reported complete recovery in most patients after the discontinuation of clindamycin.[34,35] Second, not all patients in whom recurrent diarrhea develops when they stop taking metronidazole or vancomycin have recurrent *C. difficile* infection. Other conditions, such as postinfectious irritable bowel syndrome, microscopic colitis, and inflammatory bowel disease, may be responsible. Third, a positive toxin assay in a patient with minimal or no symptoms should not prompt treatment. Repeated stool assays are not recommended after therapy, except in patients with moderate or severe diarrhea. Fourth, in patients with persistent diarrhea despite several weeks of treatment with metronidazole or vancomycin, another cause should be sought, since *C. difficile* is rarely if ever resistant to metronidazole or vancomycin.

Antibiotics and Probiotics

An approach to the management of recurrent *C. difficile* infection is presented in Table 2.[36] Since antimicrobial resistance is not clinically problematic, a first recurrence of *C. difficile* infection can be treated with the same agent used to treat the initial episode. There is no standard or proven therapy for multiple recurrences. However, in one study of 163 patients with recurrent infection, regimens that incorporated tapering or pulsed administration of vancomycin resulted in significantly fewer recurrences, with rates of 31.0% ($P=0.01$) for tapering and 14.3% ($P=0.02$) for pulsed administration, as compared with the rate for all other metronidazole or vancomycin treatments combined (49.6%).[31] Probiotics, such

as lactobacillus species and *Saccharomyces boulardii*, have shown efficacy in reducing the incidence of simple antibiotic-associated diarrhea, but their efficacy in preventing *C. difficile* infection is inconsistent.[37-39] Probiotics are not effective as solo therapy for active infection. Antibiotic combinations have been used to treat recurrent infection, including a recent report in which oral rifaximin (at a dose of 400 to 800 mg daily in two or three divided doses) was administered to patients with recurrent infection for 14 days after active infection had been controlled with the use of vancomycin.[40]

Immunotherapy

An inability to mount a protective immune response to *C. difficile* and its toxins appears to underlie susceptibility to recurrent infection (Fig. 4).[32,33] Accordingly, passive or active immunization against *C. difficile* toxins has been used to treat patients with multiple recurrences. More than half of all adults have circulating antibodies against *C. difficile* toxins, and normal pooled immunoglobulin can neutralize toxins A and B.[27,41] On the basis of these observations, intravenous immunoglobulin has been used to treat recurrent infection.[42] Although favorable outcomes have been reported, no data from randomized, controlled trials are available.[42,43] Less consistent results have been reported regarding the use of intravenous immunoglobulin to treat patients with severe, refractory infection who had not had a response to standard therapy and for whom colectomy was being considered.[27,44] The published data regarding the efficacy of active immunization against *C. difficile* are even more sparse. A *C. difficile* vaccine containing inactivated toxoids A and B was well tolerated and immunogenic in healthy volunteers.[45] Three patients with recurrent infection were vaccinated, and none had a subsequent relapse.[46] Thus, immunization (both active and passive) for recurrent infection appears promising, but prospective, controlled trials are needed to establish efficacy.

Bacteriotherapy

Recurrent *C. difficile* infection results from a disruption of the colonic microflora initiated by antibiotic therapy and perpetuated by metronidazole or vancomycin. Some imaginative treatments

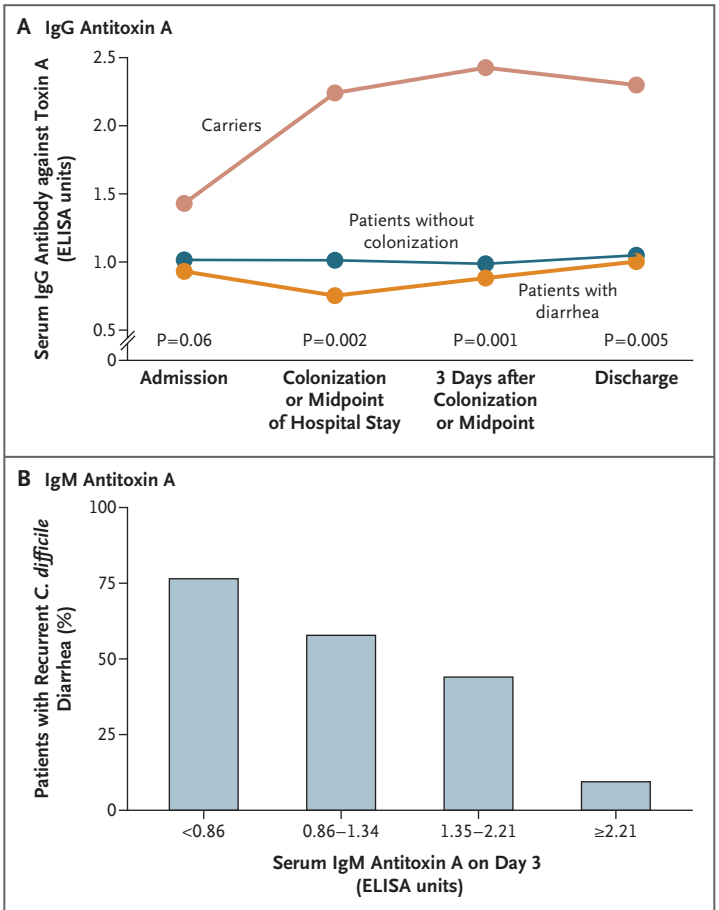

Figure 4. Levels of Serum IgG Antibody against Toxin A in Hospitalized Patients with Active *C. difficile* Infection, Symptomless Carriers of *C. difficile*, and Noncolonized Control Subjects and the Relationship between Levels of IgM against Toxin A and Recurrent Infection.

Panel A shows the median serum IgG antibody levels against *C. difficile* toxin A for 28 patients in whom *C. difficile* infection developed and in 19 asymptomatic carriers at the time of hospitalization, at the time of colonization by *C. difficile*, 3 days after colonization, and at discharge. The results are also shown for 187 patients without colonization by *C. difficile* at admission, at the midpoint of the hospital stay, 3 days after the midpoint, and at discharge. The P values refer to the comparison among the three groups (by the Kruskal–Wallis test). Data are from Kyne et al.[32] Panel B shows the relation between levels of IgM against toxin A that were measured in serum samples collected 3 days after the onset of infection and the subsequent development of recurrent infection. Serum levels of IgM against toxin A that were measured 3 days after the onset of diarrhea are shown, expressed in enzyme-linked immunosorbent assay (ELISA) units. Levels were categorized on the basis of quartile ranges. The percentage of patients in whom recurrent *C. difficile* diarrhea later developed are shown for each category of IgM antitoxin A antibody level. There was a significant trend in rates of subsequent recurrent diarrhea across the four quartiles of the serum levels of IgM against toxin A on day 3 (Mantel–Haenszel chi-square test, 10.0; P=0.002). Data are from Kyne et al.[33]

Table 2. Suggested Approaches to Therapy.*

Initial episode
Mild-to-moderate infection
Metronidazole at a dose of 500 mg orally 3 times daily for 10 to 14 days
Severe infection or unresponsiveness to or intolerance of metronidazole
Vancomycin at a dose of 125 mg orally 4 times daily for 10 to 14 days
First recurrence
Mild-to-moderate infection
Metronidazole at a dose of 500 mg orally 3 times daily for 10 to 14 days
Severe infection or unresponsiveness to or intolerance of metronidazole
Vancomycin at a dose of 125 mg orally 4 times daily for 10 to 14 days
Second recurrence†
Vancomycin in tapered and pulsed doses
125 mg 4 times daily for 14 days
125 mg 2 times daily for 7 days
125 mg once daily for 7 days
125 mg once every 2 days for 8 days (4 doses)
125 mg once every 3 days for 15 days (5 doses)
Third recurrence
Vancomycin at a dose of 125 mg orally 4 times daily for 14 days, followed by rifaximin at a dose of 400 mg twice daily for 14 days
Other options for recurrent infection
Intravenous immune globulin at a dose of 400 mg per kilogram of body weight once every 3 weeks for a total of 2 or 3 doses
Therapy with other microorganisms, including "fecal transplantation"

* Data are from Kelly and LaMont.[36]

† A probiotic such as *Saccharomyces boulardii* or lactobacillus species may be added during the final 2 weeks of the vancomycin taper and for at least 4 weeks thereafter (preferably 8 weeks). However, the efficacy of probiotics in preventing recurrent *C. difficile* infection is unclear because of inconsistent study results. Bacteremia or fungemia may rarely complicate the use of probiotics in immunocompromised, critically ill patients.

have been described to restore resistance to colonization. In 1987, Seal et al.[47] described the administration of a nontoxigenic strain of *C. difficile* with the goal of filling the environmental niche required for infection by toxigenic strains. This approach was effective in protecting against infection in animals and is now being developed for human use.[48] A filtrate of human feces, usually obtained from a family member, has also been administered either through a nasogastric tube or at colonoscopy. Several case series describe efficacy in preventing recurrent infection, but in the absence of controlled trials, fecal transplantation remains unpopular for practical and aesthetic reasons.[49]

New Antibiotics

The recent increase in the incidence and severity of *C. difficile* infection has spurred efforts to develop more effective treatments.[50] As in the past, antibiotic agents have attracted the greatest attention. Although vancomycin remains the only therapy for *C. difficile* infection that has been approved by the Food and Drug Administration, researchers are evaluating a variety of other antimicrobial agents, including some (e.g., nitazoxanide and rifaximin) that are approved for use in the United States for other indications and others (e.g., ramoplanin and Difimicin [PAR-101/OPT-80]) that do not have an approved indication. Tolevamer is a high-molecular-weight, inert polymer that binds toxins A and B and has shown promise in a phase 2 clinical trial.[51] However, in two subsequent phase 3 trials, tolevamer was inferior to vancomycin and metronidazole for initial therapy. Tolevamer has no direct antimicrobial activity, a feature that may facilitate restoration of resistance to colonization. Consistent with this theory, recurrent infection was far less common in subjects who had a response to tolevamer (3%), as compared with vancomycin and metronidazole (23% and 27%, respectively; $P<0.001$ for both comparisons). This finding highlights the intrinsic limitations of treating antibiotic-associated diarrhea with additional antibiotics and encourages the search for effective nonantibiotic alternatives for both treatment and prophylaxis.

SUMMARY

The difficult clostridium bacteria has gained a secure hold in hospitals and nursing homes and may now also be more common in the outpatient setting. Mutations that confer antibiotic resistance, increase toxin production, or facilitate sporulation have substantially increased the prevalence and virulence of this opportunistic pathogen. The effort to develop and refine new, more effective therapies, including nonantibiotic drugs, is ongoing and essential. However, since most cases of *C. difficile* infection are both iatrogenic and nosocomial, the careful selection of antibiotics and, whenever possible, the avoidance of their use remain the mainstay of primary prevention. Environmental decontamination (e.g., with cleaning agents containing at least 5000 ppm available chlorine) and the minimization of opportunities

for cross-infection by hand hygiene and barrier precautions are effective control measures. Ultimately, it is likely that a broadly based approach of responsible antibiotic use, infection-control measures, and the application of new nonantibiotic agents will be needed to turn the tide against this antibiotic-induced endemic disease.

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