REVIEW ARTICLE

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Clostridium difficile Infection

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CLOSTRIDIUM DIFFICILE IS AN ANAEROBIC GRAM-POSITIVE, SPORE-FORMING, toxin-producing bacillus that is transmitted among humans through the fecal–oral route. The relationship between the bacillus and humans was once thought to be commensal,[1] but *C. difficile* has emerged as a major enteric pathogen with worldwide distribution. In the United States, *C. difficile* is the most frequently reported nosocomial pathogen. A surveillance study in 2011 identified 453,000 cases of *C. difficile* infection and 29,000 deaths associated with *C. difficile* infection; approximately a quarter of those infections were community-acquired.[2] Nosocomial *C. difficile* infection more than quadruples the cost of hospitalizations,[3] increasing annual expenditures by approximately $1.5 billion in the United States.[4] In this article, we review the changing epidemiology of this infection, discuss risk factors and preventive strategies, outline current recommendations for treatment, and highlight developing strategies for disease control.

PATHOGENESIS AND EPIDEMIOLOGY

C. difficile colonizes the large intestine and releases two protein exotoxins (TcdA and TcdB) that cause colitis in susceptible persons. Infection is transmitted by spores that are resistant to heat, acid, and antibiotics. The spores are plentiful in health care facilities and are found in low levels in the environment and food supply, allowing for both nosocomial and community transmission.[5] Colonization is prevented by barrier properties of the fecal microbiota; weakening of this resistance by antibiotics is the major risk factor for disease (Fig. 1). Advanced age, antineoplastic chemotherapy, and severe underlying disease also contribute to susceptibility. Symptoms of colitis do not develop in all colonized persons. For example, the majority of infants are colonized with *C. difficile* but are asymptomatic,[6-8] possibly owing to the lack of toxin-binding receptors in the infant gut, as shown in animal models[9] and as suggested by the common development of antibodies to *C. difficile* toxins in infants without clinical infection.[7]

C. difficile diarrhea is mediated by TcdA and TcdB, which inactivate members of the Rho family of guanosine triphosphatases (Rho GTPases), leading to colonocyte death, loss of intestinal barrier function, and neutrophilic colitis. The organism itself is noninvasive, and infection outside the colon is extremely rare. The two factors that exert a major influence on clinical expression of disease are the virulence of the infecting strain and the host immune response. In the early 2000s, hospitals began reporting dramatic increases in severe *C. difficile* infection. Isolates were characterized by the Centers for Disease Control and Prevention as toxinotype III, restriction endonuclease analysis group BI, North American pulsed-field gel electrophoresis type NAP1, and polymerase-chain-reaction (PCR) type 027 and were subsequently known as BI/NAP1/027.[10] The BI/NAP1/027 strain is characterized by high-level fluoroquinolone resistance, efficient sporulation, markedly high

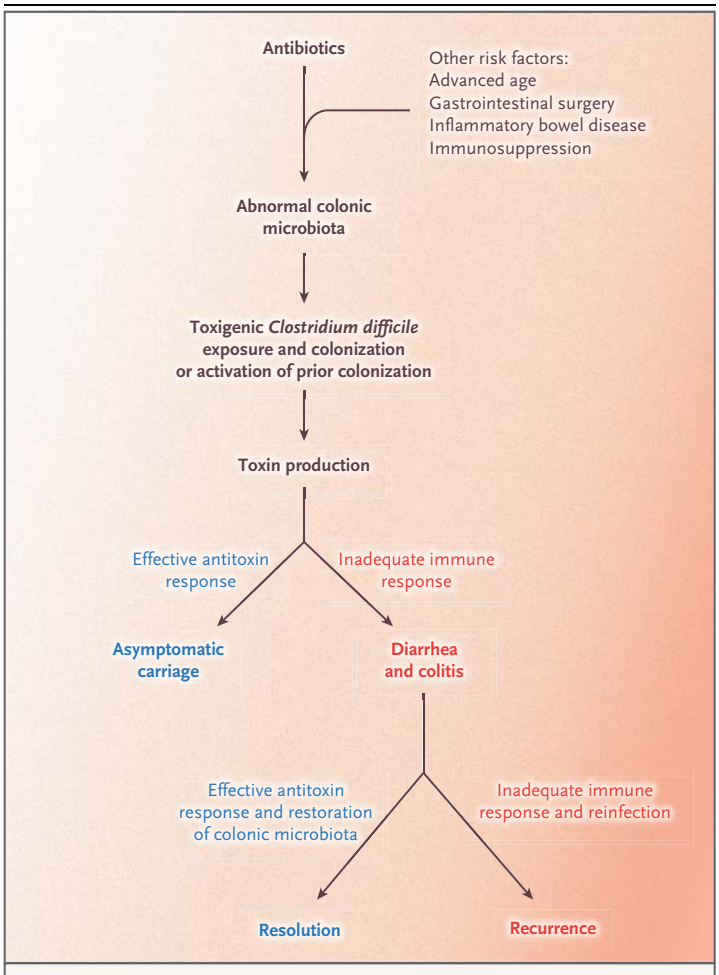

Figure 1. Pathogenesis of *Clostridium difficile* Infection.

toxin production,[11,12] and a mortality rate three times as high as that associated with less virulent strains, such as the 001 or 014 ribotypes.[13,14]

Asymptomatic colonization with toxigenic *C. difficile* in infants stimulates a durable immune response that appears to protect against symptomatic infection later in life.[7] For example, high titers of serum IgG antitoxins to TcdA and TcdB are associated with asymptomatic colonization in hospitalized patients exposed to antibiotics.[15] Immunization of experimental animals with TcdA is also protective,[16] and passive immunization with monoclonal antibodies directed at TcdA and TcdB in patients who have acute *C. difficile* infection reduces the overall recurrence rate.[17]

The incidence of *C. difficile* infection among hospitalized patients varies widely from year to year and in different locations but has generally been increasing, to almost 15 cases per 1000 hospital discharges[18] and approximately 20 cases per 100,000 person-years in the community[19] (Fig. 2). *C. difficile* infection was first recognized in Western Europe and North America, where the BI/NAP1/027 strain originated. However, *C. difficile* now has global reach, and epidemic strains can be found in diverse hospital settings.[20]

RISK FACTORS

The most important risk factor for *C. difficile* infection remains antibiotic use. Ampicillin, amoxicillin, cephalosporins, clindamycin, and fluoroquinolones are the antibiotics that are most frequently associated with the disease, but almost all antibiotics have been associated with infection (Table 1). Paradoxically, many predisposing antibiotics show at least some in vitro activity against *C. difficile*, and regimens including metronidazole can both incite the disease and provide effective treatment.[21] In hospitals and long-term care facilities, environmental contamination and frequent antibiotic usage are risk factors for infection.[22] The risk of *C. difficile* infection and the severity of infection increase as age increases[23,24] (Fig. 2). In one study, the risk of contracting *C. difficile* during an outbreak was 10 times as high among persons older than 65 years of age as among younger inpatients.[25] The majority of *C. difficile* infections are hospital-acquired, but community-acquired infection has increased dramatically in the past decade[26] and may now account for up to a third of new cases.[27] Community-acquired *C. difficile* is defined as disease onset in a person who had no overnight stay in a health care facility within 12 weeks before infection; the definition does not rule out acquisition in a health care facility. As compared with nosocomial infection, community-acquired *C. difficile* infection occurs in patients who are younger and more often have had no clear exposure to antibiotics or other known risk factors; major modes of acquisition of community-acquired infection remain to be elucidated. Furthermore, morbidity and mortality associated with community-acquired *C. difficile* infection are lower than those associated with nosocomial infection, because of the younger age and fewer coexisting conditions of the nonhospitalized population; however, up to 40% of patients with community-acquired infection require hospitalization, and

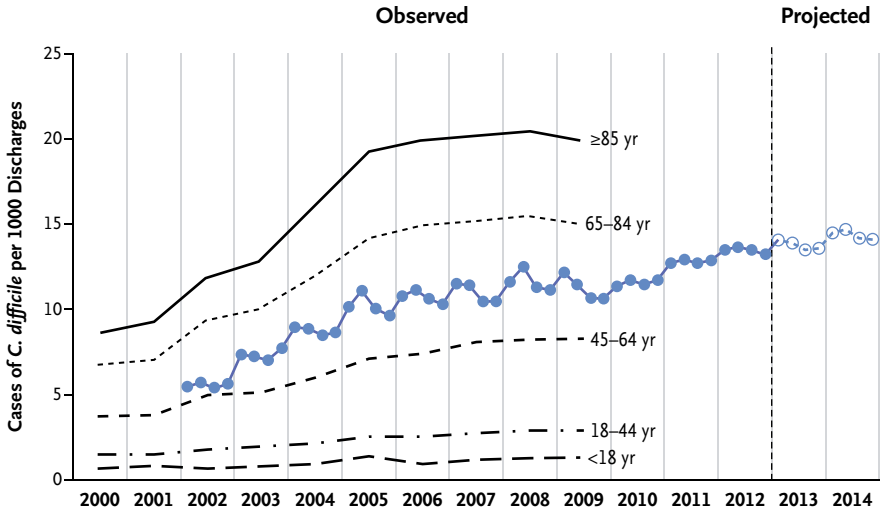

Figure 2. Incidence of Nosocomial *Clostridium difficile* Infection.

The overall incidence of nosocomial *C. difficile* infection is shown by year (blue), as is the incidence according to patient age (black). Data are from Steiner et al.[18] and Lessa et al.[24]

rates of recurrence are similar among the two populations.[19,27]

The influence of acid suppression in *C. difficile* infection remains uncertain. In theory, gastric acid suppression should allow more vegetative organisms to reach the colon; however, *C. difficile* spores, the vectors for infection, are acid-resistant and remain viable at gastric pH. Some investigators have reported an increased risk of infection in association with acid suppression,[28] whereas others, after adjusting for coexisting conditions, have not confirmed an increased risk.[22,29,30] Other documented risk factors for infection include advanced age, inflammatory bowel disease, organ transplantation, chemotherapy, chronic kidney disease, immunodeficiency, and exposure to an infant carrier or infected adult.[19,31]

C. difficile infection is associated with severe illness, infection-related mortality of 5%, and all-cause mortality of 15 to 20%.[3,32] Severe *C. difficile* infection, identified by a white-cell count greater than 15,000 per cubic millimeter, hypoalbuminemia, and acute kidney injury, is an independent predictor of urgent colectomy and death.[32,33] Risk factors are similar to those for recurrent *C. difficile* infection and include advanced age, a severe initial episode of *C. difficile* infection, and ongoing use of antibiotics not directed at *C. difficile*.[34,35]

DIAGNOSIS

C. difficile infection is currently diagnosed either by enzyme immunoassay for toxins in stool or by DNA-based tests that identify the microbial toxin genes in unformed stool. Stool culture for *C. difficile* requires anaerobic culture and is not widely available. Enzyme immunoassay used to be the mainstay of testing for *C. difficile* infection, since it is rapid and easily performed. Recently, many hospital laboratories have adopted DNA-based tests that detect toxigenic strains and provide higher sensitivity and specificity than does enzyme immunoassay. Some DNA-based tests also detect the presence of the BI/NAP1/027 strain, a finding that may influence the choice of therapy, since fidaxomicin is associated with a reduction in the risk of recurrence of non-BI/NAP1/027 strains only, as compared with vancomycin. DNA assays for *C. difficile* infection may appear to show a higher incidence of infection than earlier tests[36] because the high sensitivity of DNA assays allows for low levels of toxigenic organisms of uncertain clinical significance. The concern that DNA assays can detect clinically insignificant infections is supported by the results of recent studies that suggest that detection of toxigenic *C. difficile* by DNA testing in the absence of free toxin in the stool does not influ-

Table 1. Antibiotic Classes and Their Association with *Clostridium difficile* Infection.*

Class	Association with *C. difficile* Infection
Clindamycin	Very common
Ampicillin	Very common
Amoxicillin	Very common
Cephalosporins	Very common
Fluoroquinolones	Very common
Other penicillins	Somewhat common
Sulfonamides	Somewhat common
Trimethoprim	Somewhat common
Trimethoprim–sulfamethoxazole	Somewhat common
Macrolides	Somewhat common
Aminoglycosides	Uncommon
Bacitracin	Uncommon
Metronidazole	Uncommon
Teicoplanin	Uncommon
Rifampin	Uncommon
Chloramphenicol	Uncommon
Tetracyclines	Uncommon
Carbapenems	Uncommon
Daptomycin	Uncommon
Tigecycline	Uncommon

* Specific antibiotics are listed if their association with *C. difficile* infection differs from that of most other antibiotics in their class.

ence clinical outcomes.[33,37] In the future, highly sensitive quantitative toxin assays may also contribute to diagnostic algorithms.

Conversely, heterogeneous diagnostic tests and lack of clinical suspicion contribute to delayed diagnosis.[38] Sequential testing with the use of PCR and enzyme immunoassay has been advocated,[39] but in clinical practice, in a patient with diarrhea, positive results of either enzyme immunoassay or PCR assay should prompt treatment. Endoscopy is rarely required but may be helpful in patients with an overlapping condition such as inflammatory bowel disease. Conversely, the negative predictive value of PCR assay and enzyme immunoassay is more than 95% in average-risk groups, and negative results should prompt evaluation for other causes.[39]

Stool testing for *C. difficile* toxins should be confined to patients with diarrhea. Although a substantial proportion of at-risk, hospitalized patients may be colonized, the testing and treatment of persons with solid stools is not recommended. Similarly, posttreatment testing has no role in confirming eradication. Many successfully treated patients will continue to test positive for weeks or months after the resolution of symptoms; additional treatment is neither required nor effective.[40] More difficult is the decision of when to test and treat patients who have mild ongoing or recurrent diarrhea after initial treatment. In such patients, stool testing can be helpful in differentiating recurrent *C. difficile* infection from postinfectious irritable bowel syndrome or inflammatory bowel disease that can be triggered by acute enteric infections.

PREVENTION

In the absence of an effective vaccine, infection control has focused on antibiotic stewardship, prevention of spread in health care facilities, and probiotics. Minimizing antibiotic use has been successful in decreasing *C. difficile* infection in hospitalized patients.[41] The prohibiting of the routine use of ceftriaxone and ciprofloxacin accompanied by an educational campaign reduced the rate of *C. difficile* infection by 77% in a 450-bed hospital in Scotland.[42] However, strict stewardship of antibiotics is labor-intensive and may not be effective in all settings.[41]

C. difficile is nearly ubiquitous in health care facilities, and viable spores can be identified on the hands and stethoscopes of health care workers, on bedding, on telephones, in bathrooms, and on bedside furniture.[43] Using alcohol-based hand sanitizers does not reduce the number of viable *C. difficile* spores, whereas washing with soap and water does.[44] However, because the availability and convenience of hand-sanitizer solutions greatly increases overall adherence to hand hygiene,[45] alcohol-based preparations are likely to remain standard. Patients with known or suspected *C. difficile* infection should be isolated in a single room, and health care professionals should wear gloves and gowns and wash hands with soap and water; postdischarge disinfection of the room is also recommended.[46]

The use of probiotics to prevent *C. difficile* colonization could be a safe and easily adoptable control strategy. Various strains of probiotics are effective for the prevention of noninfectious,

antibiotic-associated diarrhea.[47] Initial studies evaluating the use of probiotics for control of antibiotic-associated diarrhea were underpowered for the detection of protection against *C. difficile* infection. More recent studies have shown mixed results, with a few studies showing that probiotics conferred significant protection in cohorts with unusually high rates of *C. difficile* infection[48,49] and another study showing no protection in hospital inpatients who had low rates of infection.[50] At present, probiotics have an uncertain effect on the prevention of *C. difficile* infection, and their routine use for the prevention or treatment of active infection is not recommended.

TREATMENT OF ACUTE INFECTION

Metronidazole and oral vancomycin have been the mainstays of treatment for *C. difficile* infection since the 1970s, and despite their use by millions of patients, clinically important resistance to either vancomycin or metronidazole has not been reported. For the treatment of severe disease, vancomycin is better than metronidazole, but for mild-to-moderate infection, the two antibiotics have been considered to be equivalent.[51] However, a marked rise in clinical failure associated with metronidazole, especially in patients with the BI/NAP1/027 strain, has been seen in the past decade.[52] Previous studies were underpowered to evaluate differences between metronidazole and vancomycin in cases of nonsevere infection, but recent data suggest an overall superiority of vancomycin. Studies of tolevamer, a toxin-binding polymer, showed that with respect to curing acute *C. difficile* infection, tolevamer was inferior to vancomycin and to metronidazole, but the studies also showed that clinical success, defined as resolution of diarrhea, was lower with metronidazole than with vancomycin (73% vs. 81%, $P=0.02$).[53] The superiority of vancomycin was observed in patients with mild disease, those with moderate disease, and those with severe disease.[53] These factors, along with the more frequent side effects associated with metronidazole and the decreasing cost of generic vancomycin, have led to increasing use of vancomycin.[54,55] (Table 2).

In 2011, fidaxomicin, a poorly absorbed, bactericidal, macrocyclic antibiotic with activity against specific anaerobic gram-positive bacteria, was approved by the Food and Drug Administration (FDA) for the treatment of *C. difficile* infection. In phase 3 clinical trials, the cure rate for acute infection was nearly equivalent among patients receiving fidaxomicin and those receiving vancomycin (approximately 90% for each), but the risk of recurrence was 15% among patients receiving fidaxomicin, as compared with 25% among those receiving vancomycin.[56,57] However, a reduced risk of recurrence was not seen among patients infected with the BI/NAP1/027 strain, which was found in 38% of isolates. The markedly higher cost of fidaxomicin has limited its use, despite its superiority to vancomycin in reducing the risk of recurrence (Table 1).

TREATMENT OF RECURRENT INFECTION

The risk of *C. difficile* recurrence ranges from 20% after an initial episode to 60% after multiple prior recurrences.[58,59] The costs associated with recurrent infection may exceed those associated with primary infection.[60] Recurrence is most often due to reexposure to or reactivation of spores in patients who have an impaired immune response to infection and weakened barrier function of the colonic microbiota.

ANTIBIOTIC TREATMENT

Treatment of a first episode of recurrent infection with a repeat course of either metronidazole or vancomycin for 10 to 14 days is successful in approximately 50% of patients.[31,34] Second and subsequent recurrences can be difficult to cure, primarily because of the persistence of spores in the bowel or environment and the inability of the patient to mount an effective immune response to *C. difficile* toxins, rather than to antibiotic resistance.[61] Second recurrences can be treated with fidaxomicin (200 mg twice a day for 10 days) or by a vancomycin regimen involving tapered (decreased over time) and pulsed (intermittent [i.e., every few days]) dosing (Table 2). Recent data suggest that fidaxomicin may be more effective than vancomycin at preventing further episodes of *C. difficile* after an initial recurrence.[62]

Options are limited for patients with severe colitis in whom vancomycin and fidaxomicin are ineffective. Emergency colectomy for fulminant *C. difficile* infection is associated with mortality as high as 80%, although a diverting ileostomy and a colonic lavage with vancomycin may be an

Table 2. Treatment of *Clostridium difficile* Infection.*

Severity	Clinical Manifestations	Treatment
Asymptomatic carrier	No symptoms or signs	No treatment indicated
Mild†	Mild diarrhea (3 to 5 unformed bowel movements per day), afebrile status, mild abdominal discomfort or tenderness, and no notable laboratory abnormalities	Predisposing antibiotic cessation, hydration, monitoring of clinical status, and either administration of metronidazole (500 mg three times per day) or close outpatient monitoring without the administration of antibiotics
Moderate	Moderate nonbloody diarrhea, moderate abdominal discomfort or tenderness, nausea with occasional vomiting, dehydration, white-cell count >15,000/mm^3, and blood urea nitrogen or creatinine levels above baseline	Consideration of hospitalization and cessation of predisposing antibiotics; hydration, monitoring of clinical status, and either administration of oral metronidazole (500 mg three times per day) or first-line therapy with oral vancomycin (125 mg four times per day for 14 days)
Severe	Severe or bloody diarrhea, pseudomembranous colitis, severe abdominal pain, vomiting, ileus, temperature >38.9°C, white-cell count >20,000/mm^3, albumin level <2.5 mg/dl, and acute kidney injury	Hospitalization; oral or nasogastric vancomycin (500 mg four times per day) with or without intravenous metronidazole (500 mg three times per day), or oral fidaxomicin (200 mg twice a day for 10 days) instead of vancomycin if the risk of recurrence is high
Complicated	Toxic megacolon, peritonitis, respiratory distress, and hemodynamic instability	Antibiotics as for severe infection, and surgical consultation for subtotal colectomy or a diverting ileostomy with vancomycin colonic lavage; consideration of fecal microbial transplantation or additional antibiotics
First recurrence		Oral vancomycin (125 mg four times per day for 14 days) or oral fidaxomicin (200 mg twice a day for 10 days)
Second or further recurrence		Vancomycin in a tapered and pulsed regimen‡, fecal microbial transplantation, or fidaxomicin (200 mg twice a day for 10 days)

* Some data are from Debast et al.[54] and Cohen et al.[55]
† *C. difficile* infection should be considered mild only if it occurs in outpatients.
‡ A tapered and pulsed regimen involves the administration of vancomycin as follows: 125 mg four times a day for 1 week, 125 mg three times a day for 1 week, 125 mg twice a day for 1 week, 125 mg daily for 1 week, 125 mg once every other day for 1 week, and 125 mg every 3 days for 1 week.

effective alternative.[63] Other antibiotics that have activity against *C. difficile* are rifaximin, nitazoxanide, ramoplanin, teicoplanin, and tigecycline. However, because of limited data, high cost, an unfavorable adverse-event profile, and resistance to *C. difficile* (associated with rifaximin in particular), the use of these agents is not recommended except in cases of unacceptable adverse effects associated with standard therapy, the need for salvage therapy for fulminant disease when surgery is not possible, and intractable recurrent infection (Table 2).

FECAL MICROBIAL TRANSPLANTATION

The human colonic microbiota, which provides colonization resistance against bacterial pathogens, is considered to be a key determinant in the pathogenesis of *C. difficile*. After a patient has had brief exposure to oral antibiotics, a rapid decline in fecal microbial diversity is common and may last many months.[64,65] Stopping the administration of all antibiotics is the best way to eliminate *C. difficile* from the colon and allow the fecal microbiota to recover spontaneously. However, recovery may take 12 weeks or longer, during which patients may have a relapse. Fecal microbial transplantation, a procedure that was first reported in 1958,[66] has recently emerged as an accepted, safe, and effective treatment for recurrent *C. difficile* infection. The FDA initially suggested that an investigational new drug (IND) application would be necessary before treatment

of *C. difficile* infection with fecal microbial transplantation but later ruled that it would allow fecal microbial transplantation for this indication without an IND application, although informed consent is still required.[67]

The precise components of the fecal microbiome that provide resistance against *C. difficile* are not known, but the phyla Bacteroidetes and Firmicutes are thought to comprise critical components of the material that needs to be transplanted.[68,69] The oral or rectal transplantation of feces from a healthy, pretested donor and the simultaneous cessation of all antibiotic use in the recipient are successful in treating more than 90% of patients with recurrent *C. difficile* infection.[70] Although the transmission of an undetected or unidentifiable pathogen from the donor is a possibility, there are no known reports of serious infectious complications resulting from fecal microbial transplantation that was performed with appropriate donor screening. In 2013, the results of a randomized, controlled trial of fecal microbial transplantation were reported.[71] The trial showed that the administration of vancomycin followed by an infusion of donor feces delivered by nasoduodenal tube was safe and superior to vancomycin alone for recurrent *C. difficile* infection (Fig. 3).

Given the efficacy of fecal microbial transplantation for recurrent infection, there has been growing interest in its use for severe primary disease.[72] To date, there are few studies about this treatment approach, and although case series are promising,[72,73] more work is needed to understand the possible role of fecal microbial transplantation in primary *C. difficile* infection. In addition, efforts to develop a suitable mixture of cultured fecal bacteria as a substitute for stool in fecal microbial transplantation are under way. Capsules administered orally that contain the spores of fecal bacteria have shown efficacy in treating recurrent disease and warrant further testing as a substitute.[74]

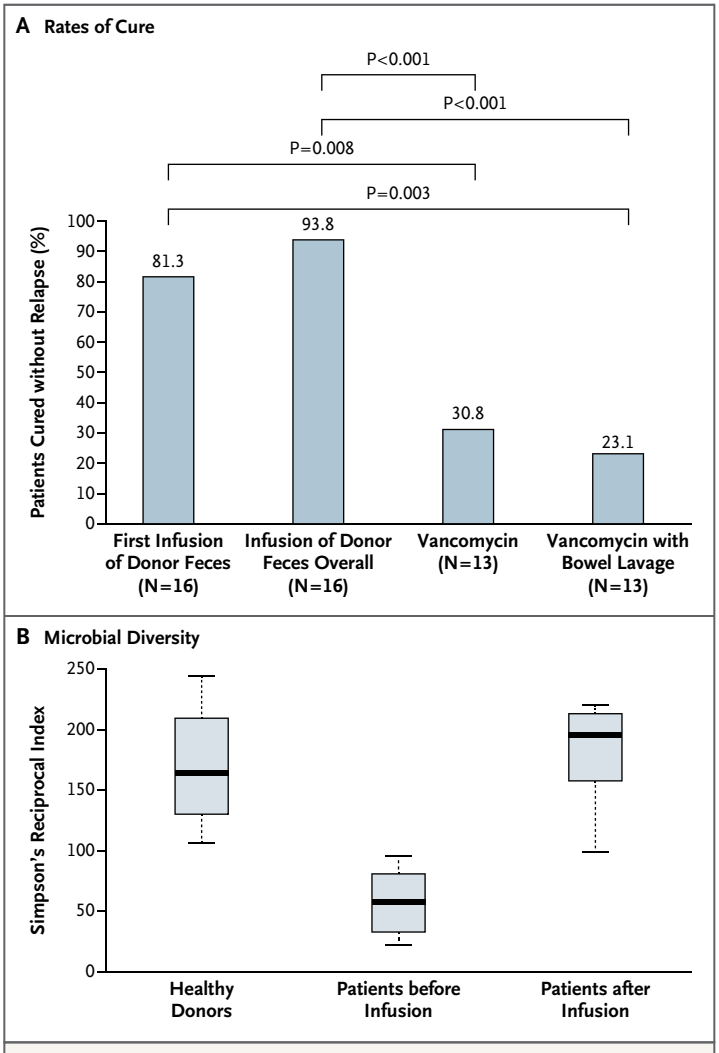

Figure 3. Rates of Cure and Changes to the Microbiota after Fecal Microbial Transplantation for Recurrent *Clostridium difficile* Infection.

Among patients with recurrent *C. difficile* infection, the rate of cure without relapse was higher among those who received an infusion of donor feces than among those who received vancomycin with or without bowel lavage (Panel A). Fecal microbial diversity in recipients before and after the infusion of donor feces is compared with the diversity in healthy donors (Panel B). Microbial diversity is expressed by Simpson's Reciprocal Index. The index ranges from 1 to 250, with higher values indicating more diversity. The box-and-whisker plots indicate interquartile ranges (boxes), medians (dark horizontal lines in the boxes), and highest and lowest values (whiskers above and below the boxes). Data are from van Nood et al.[71]

IMMUNIZATION

Results of the immunization of animals with toxoids TcdA and TcdB[75] and findings showing the protective effect of naturally acquired serum IgG antitoxins in patients colonized with *C. difficile* suggest the potential for vaccination of humans against *C. difficile* infection.[15,76] Passive immunization with monoclonal antibodies to *C. difficile* toxins also provides substantial protection from recurrence after acute infection and may be cost-effective in patients who are at high risk for recurrence.[17]

Vaccination against the toxins of *C. difficile* offers the possibility of an effective and rela-

tively inexpensive approach to prevention. Initial phase 1 studies have shown strong antitoxin responses in healthy volunteers immunized with toxoids of TcdA and TcdB.[77] At least two international, placebo-controlled studies are currently under way to test the immunogenicity, safety, and efficacy of vaccination for the prevention of nosocomial *C. difficile* infection (ClinicalTrials.gov numbers, NCT01887912 and NCT02117570). The larger of these trials involves the administration of three doses of toxoid vaccine or placebo in 15,000 study participants and an evaluation of the risk of acute disease over the course of 3 years. It is unclear whether vaccination will be used for primary or secondary prevention and whether vaccination will prevent or lessen the severity of clinical infection. Clinical use will depend on numerous variables, including safety, efficacy, and cost-effectiveness, as well as improved ability to predict the risk of *C. difficile* infection. In addition, neither vaccination nor the administration of monoclonal antibodies is likely to eliminate colonization, so isolation of patients will still be necessary to prevent transmission. Nevertheless, if studies are positive, it is likely that vaccination will become prevalent.

SUMMARY

Despite concerted efforts to improve the prevention and treatment of *C. difficile* infection, this infection remains common and serious in both hospitals and the community. In recent years, fecal microbial transplantation has emerged as a safe and very effective strategy for the treatment of recurrent infection. With further refinement, fecal microbial transplantation will most likely become the standard of care for recurrent infection. Newer antibiotics with clinical activity against *C. difficile* are now available, but widespread use has been limited by their cost, which is higher than the cost of vancomycin. Although antibiotic stewardship and decontamination in health care settings remain essential for infection control, effective probiotics and vaccination will most likely become important tools for the prevention of *C. difficile* infection in the future. Until such time, *C. difficile* infection will continue to be a common and highly morbid consequence of antibiotic use.

No potential conflict of interest relevant to this article was reported.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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