

Historical Perspectives on Studies of *Clostridium difficile* and *C. difficile* Infection

John G. Bartlett

Johns Hopkins University School of Medicine, Baltimore, Maryland

The initial period of studies on *Clostridium difficile* (published during 1978–1980) appeared to provide a nearly complete portfolio of criteria for diagnosing and treating *C. difficile* infection (CDI). The putative pathogenic role of *C. difficile* was established using Koch's postulates, risk factors were well-defined, use of a cell cytotoxicity assay as the diagnostic test provided accurate results, and treatment with oral vancomycin was highly effective and rapidly incorporated into practice. During the next 10 years, enzyme immunoassays (EIAs) were introduced as diagnostic tests and became the standard for most laboratories. This was not because EIAs were as good as the cell cytotoxicity assay; rather, EIAs were inexpensive and yielded results quickly. Similarly, metronidazole became the favored treatment because it was less expensive and quelled fears of colonization with vancomycin-resistant organisms, not because it was better than vancomycin therapy. Cephalosporins replaced clindamycin as the major inducers of CDI because they were so extensively used, rather than because they incurred the same risk. Some serious issues remained unresolved during this period: the major challenges were to determine ways to treat seriously ill patients for whom it was not possible to get vancomycin into the colon and to find methods that stop persistent relapses. These concerns persist today.

Antibiotic-associated colitis due to *Clostridium difficile* has been under intense investigation since 1974. This article reviews studies on *C. difficile* infection (CDI) that were published between 1974 and the mid-1990s and set the stage for more-recent work.

EARLY STUDIES

Three separate lines of study fostered initial knowledge of *C. difficile* and CDI: initial work on the organism, investigation of antibiotic-associated typhlitis in rodents, and anatomic studies of pseudomembranous colitis (PMC).

Initial work. *C. difficile* was originally described by Hall and O'Toole [1] in 1935 as a component of the normal intestinal flora of newborn infants. These investigators also showed that this organism produced a toxin that was highly lethal to mice. In fact, the toxin

was only 10–100-fold less toxic than botulinum toxin, but subsequent studies by the famous authority on clostridia, L. D. S. Smith, showed that *C. difficile* was not biologically important in extraintestinal infections. A review by Smith and King [2] indicated that *C. difficile* infection had no unique features that suggested a histotoxic clostridial syndrome.

The rodent model. Work on the rodent model was first performed by Hambre et al. [3] during World War II to investigate the potential benefit of penicillin for treatment of gas gangrene. They found that penicillin caused typhlitis, which actually proved to be more lethal than *Clostridium perfringens*-induced gas gangrene. Subsequent studies showed that multiple rodent species were susceptible to the development of typhlitis and that many types of antibiotics were associated with this complication; however, the cause was elusive. Relevance of this work to CDI in humans lies in the fact that the hamster model eventually proved to be the source of nearly all of the clinically important early data on this complication. Furthermore, Green et al. [4], who were studying penicillin-induced death in guinea pigs, found that stool specimens contained cytopathic changes, which they attributed to the activity of a latent

Reprints or correspondence: Dr. John G. Bartlett, Johns Hopkins University School of Medicine, 1830 Monument St. #437, Baltimore, MD 21205 (jb@jhmi.edu).

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virus. This, in retrospect, appears to be the first identification of *C. difficile* cytotoxin.

Anatomic studies of PMC. J. M. T. Finney performed the first anatomic studies of PMC. In 1893, Finney [5] reported pseudomembranous changes in the intestinal tract of a 22-year-old postoperative patient being treated by William Osler. PMC became a commonly recognized complication of antibiotic use in the early 1950s and was primarily encountered by surgeons, who reported rates as high as 14%–27% among postoperative patients [6, 7]. *Staphylococcus aureus* was the suspected pathogen, and vancomycin given orally became standard treatment for this condition [8].

The “*C. difficile* era” began in 1974, when Tedesco et al. [9] reported high rates of PMC among patients at Barnes Hospital (St. Louis, MO) who were receiving clindamycin. This study was the first in which endoscopy was a routine diagnostic procedure for patients with antibiotic-associated diarrhea. Of 200 patients given clindamycin, diarrhea developed in 42 (21%). Twenty clindamycin recipients (10%) had PMC at the time of endoscopy. Although not stated in the article, stool cultures were negative for *S. aureus*, despite the ease of growing this organism on selective media. This article crystallized interest in this adverse drug reaction and spawned studies to define the cause, pathophysiological characteristics, and management of “clindamycin colitis.”

Detection of *C. difficile*. The early work on *C. difficile* detection involved clinical specimens and hamster models and was performed primarily by Keighley et al. [10] at a hospital in Birmingham, England, where *C. difficile* was endemic on a surgical ward; by the group headed by Bob Fekety and Joe Silva in Ann Arbor, Michigan [11]; and by my group in Boston, Massachusetts [12]. In most of the early studies, the hamster model turned out to be pivotal in establishing methods for detection of *C. difficile* [13], confirming the diagnostic role of the “cell cytotoxicity assay” [13], detecting toxin B produced by *C. difficile* [14], confirming that antibiotics (including oral vancomycin and metronidazole) are inducers of PMC [15, 16], and confirming the efficacy of oral vancomycin for treatment of CDI [17].

The initial work on the cell cytotoxicity assay was by Te-Wen Chang, who demonstrated that stool specimens from hamsters with antibiotic-associated typhlitis and from patients with PMC contained a potent cytopathic toxin that was neutralized with *Clostridium sordellii* antitoxin [18]. However, because cultures of stool specimens from hamsters did not yield *C. sordellii*, my colleagues and I [12] analyzed other clostridial species recovered from hamster stool specimens to determine which species could produce the cytopathic toxin that was neutralized by *C. sordellii* antitoxin. *C. difficile* satisfied this criterion. *C. difficile* antitoxin was not available, so the standard test for detection of *C. difficile* was to demonstrate the presence of a cytotoxin

neutralized by *C. sordellii* antitoxin. The first person with a positive test result was a postoperative patient in California with cephalothin-induced “clindamycin colitis.” This patient had a cytotoxin titer of 10^6 , which remains the highest titer I have measured in a patient [13]. (Of note, Tedesco sent some stool specimens to Upjohn, which sponsored the study at Barnes Hospital [9]. In 1978, I received 6 stool specimens from Upjohn with a note indicating that they had “been stored in unspecified conditions for 4 to 5 years”; all of the specimens were positive for *C. difficile* cytotoxin.)

STUDIES FROM THE LATE 1970S THROUGH THE EARLY 1990S

Pathologic characteristics of PMC. Like most enteric bacterial pathogens, *C. difficile* causes disease with a wide spectrum of severity, ranging from mild “nuisance” diarrhea with a normal colonic mucosa to PMC, the most characteristic and severe form of CDI. PMC lesions are nearly always limited to the colon; through 1992 only 7 cases involving the small bowel had been reported [19]. The prior reports of *S. aureus* enterocolitis showed that the small bowel was commonly involved, suggesting a distinction between “*S. aureus* enterocolitis” and “*C. difficile* colitis” [6, 7]; recent work has supported this distinction [20]. Of practical importance, oral vancomycin would be appropriate treatment for both conditions, but metronidazole would not.

PMC in the great majority of patients seen since 1978 has been caused by *C. difficile*, and a subset of patients have histologic lesions most characteristic of PMC. Anatomic studies of these lesions revealed that the pseudomembrane is composed of fibrin, mucin, sloughed mucosal epithelial cells, and acute inflammatory cells. There are various differences between lesions in different persons, but lesions in the same person appear to be uniform [21, 22]. The initial lesion has focal necrosis and inflammation, as well as the characteristic “summit” (figure 1A and 1B). The most advanced disease involves complete structural necrosis with extensive involvement of the lamina propria, which is overlaid by a thick, confluent pseudomembrane. There are multiple other causes of PMC, including intestinal obstruction, colon cancer, leukemia, severe burns, shock, uremia, heavy metal poisoning, hemolytic-uremic syndrome, Crohn disease, shigellosis, neonatal necrotizing enterocolitis, ischemic colitis, and Hirschsprung disease. Nevertheless, the vast majority of PMC cases seen since 1978 have been attributed to *C. difficile*.

Anatomic lesions are best detected by colonoscopy; in 20%–30% of cases, PMC is limited to the proximal colon and therefore may be missed by sigmoidoscopy [23, 24]. Characteristic lesions may also be detected by CT scan [25]. Nevertheless, because of stool toxin assays, demonstration of anatomic findings is now seldom necessary, although imaging or endoscopy may sometimes be performed for other reasons.

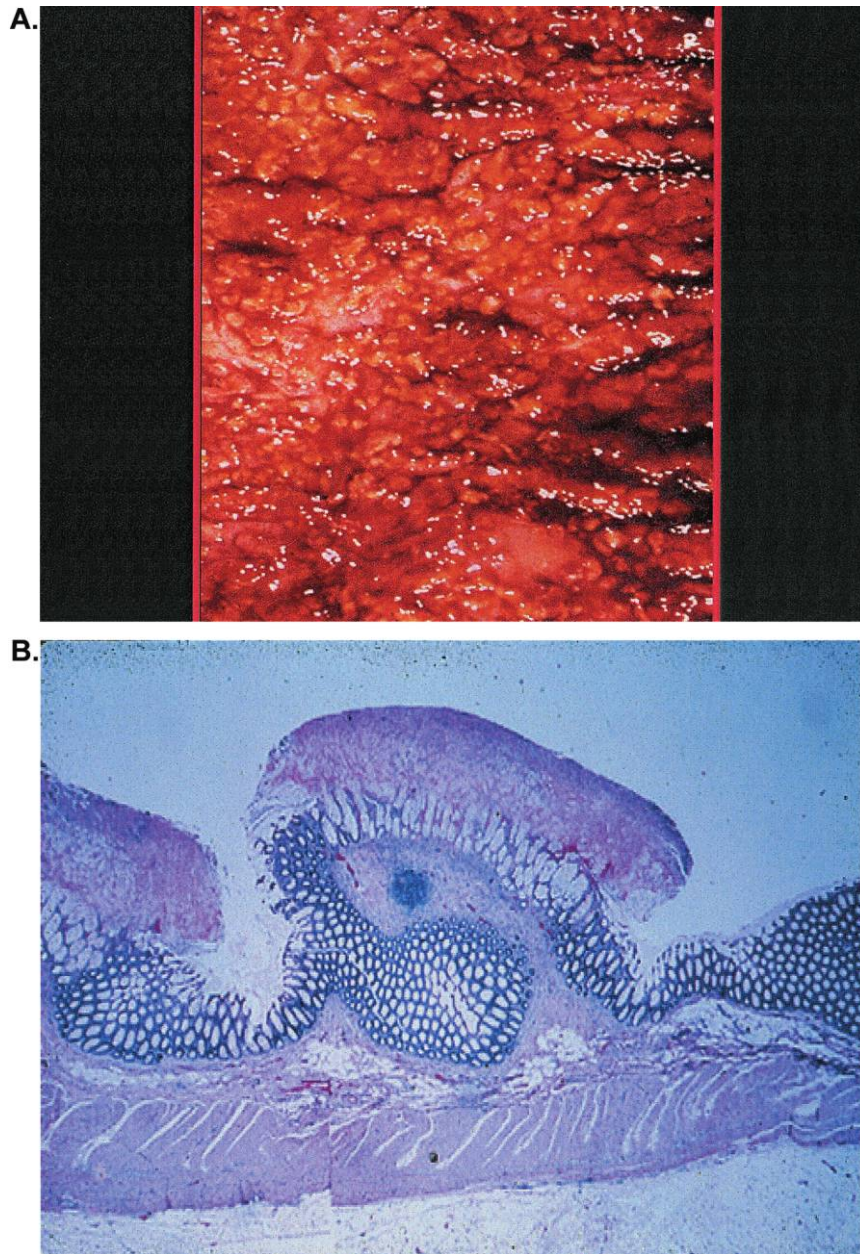


Figure 1. Typical summit lesions associated with pseudomembranous colitis. *A*, Pseudomembranous colitis with typical raised lesions (diameter, 0.2–1 cm). The appearance is light yellow against a hyperemic bowel mucosa. *B*, Microscopic pathologic characteristics of a mushroom-shaped pseudomembrane.

Risk factors for CDI. The major risk factors for CDI are hospitalization, older age (i.e., ≥ 65 years), and antibiotic exposure. Use of nearly any antibiotic with a spectrum of antibacterial activity has been implicated as a risk factor in people and hamsters. The initial attention was on clindamycin. The connection between this agent and PMC was the source of concern in the 1970s and was the subject of the report by Tedesco et al. [9], the findings of which showed that “clindamycin colitis” is synonymous with “antibiotic-associated colitis.” However, most of the studies done in the 1980s showed

that cephalosporins had become by far the most frequently implicated agents [26–29]. Broad-spectrum penicillins, including amoxicillin, were the second-most frequently implicated drugs.

Care in the interpretation of these data is important. Clindamycin was associated with the greatest risk of CDI; cephalosporins and broad-spectrum penicillins were associated with the greatest number of CDI cases because of their extensive use. Macrolides, trimethoprim-sulfamethoxazole, metronidazole, rifampin, and tetracyclines were far less commonly as-

sociated with CDI. Ciprofloxacin was the first fluoroquinolone created and was approved for human use in 1987, but fluoroquinolones did not seem to figure prominently in cases of CDI until recently [30]. The only other class of drugs recognized to induce CDI comprised antineoplastic agents, primarily methotrexate [31, 32]. There were additional cases of CDI that were not associated with antibiotic exposure, but these were described in anecdotal case reports. Attempts to identify *C. difficile* in other diseases, such as neonatal necrotizing enterocolitis, sudden infant death syndrome, Crohn disease, ulcerative colitis, and chronic enigmatic diarrhea, were uniformly unsuccessful (table 1) [33].

Early studies on the association between age and CDI showed that neonates had high rates of both *C. difficile* colonization and positive results of a stool toxin assay without clinical symptoms. This is the only age group with a high prevalence of *C. difficile* toxin and no clinical expression of disease [34]. The postulated but unproven mechanism for this finding is the lack of receptors for *C. difficile* toxins in the infant colon. The disease is recognized in children aged >1 year but is relatively rare considering the frequency of antibiotic exposure [35]. CDI is an occasional cause of diarrhea in older children, and a recent study observed that *C. difficile* toxin was detected in 46 (7%) of 688 patients with diarrhea who were seen in a pediatric emergency department. Vulnerability appears to increase with age, especially in elderly persons. Population-based studies in Sweden for the early 1980s showed that rates of CDI among persons >65 years of age were 20-fold higher than rates among persons aged ≤65 years [36, 37].

The role of the hospital in CDI was not clearly recognized in the early stages of *C. difficile* studies. It was noted that hamsters in a new facility would not get this disease unless they were fed *C. difficile*; however, once the pathogen was established in the animal facility, such feeding was not necessary. It was clearly logical to assume that the hospital was a nidus of infection, because of patient clustering, a greater likelihood of antibiotic use (30%–40% of patients), and the large proportion of patients who are elderly. The same logic applies to long-term care facilities (LTCFs), and both hospitals and LTCFs have been common sources of *C. difficile*: the pathogen was associated with an epidemic of antibiotic-associated colitis at the long-term care facility of Barnes Hospital in 1974 [9] and was found to be endemic in the surgical ward at General Hospital in Birmingham, England, in 1978 [38]. Particularly important and relevant to this issue are studies showing high rates of environmental contamination by *C. difficile* in hospitals [39, 40] and high rates of colonic colonization associated with hospitalization [41]. The rates of colonization are as high as 40%–60% for neonates but are usually only 2%–3% for individuals aged >1 year [34]. These rates increase to 20%–30% with hos-

Table 1. Results of *Clostridium difficile* cell cytotoxicity assays, 1978–1980.

Characteristic	Proportion (%) of patients
Antibiotic-associated diarrhea	
Confirmed PMC	136/141 (97)
No confirmed PMC ^a	193 ^a /710 (27)
Antibiotic use not associated with diarrhea	2/110 (2)
Diarrhea not associated with antibiotic use	0/562
Healthy adults	0/60
Healthy infants	12/45 (27)

NOTE. Data are number of patients with a positive test result/number tested (%). PMC, pseudomembranous colitis. Adapted with permission from the following article published by The American Journal of Clinical Nutrition: Bartlett JG, Taylor NS, Chang T, Dzink J. Clinical and laboratory observations in *Clostridium difficile* colitis. *Am J Clin Nutr* 1980;33(11 Suppl):2521–6.

^a Forty-six patients had colitis, 22 had erythema with or without edema, 36 had a normal colonic mucosa, and 89 did not undergo endoscopy.

pitalization and increased even further with the duration of hospital stay [42, 43].

Clinical expression of CDI. Symptoms of CDI are highly variable, ranging from nuisance diarrhea to life-threatening colitis. Typical features include watery diarrhea, with as many as 15–30 bowel movements per day [9, 44–47]. Most patients complain of abdominal pain or cramps, and they often have lower quadrant tenderness in association with fever and leukocytosis. Fever, when present, is usually low grade but may involve a temperature as high as 40.6°C. The peripheral leukocyte count is usually 10,000–20,000 leukocytes/mm³ but may be in the leukemoid range, a feature more commonly recognized now than in the past [48, 49]. A leukemoid reaction is also observed in patients with *C. sordellii* infection, which is of interest because the toxins of the 2 clostridial species are antigenically related [50–52]. Hypoalbuminemia is a common feature because CDI is a protein-losing enteropathy, and some patients present with anasarca during the late stages of disease [9]. Extraintestinal symptoms are rare, except for occasional cases of polyarthritis involving large joints [53, 54]. Some patients develop ileus or toxic megacolon; colonic perforation is rare.

Diagnosis of CDI. The first diagnostic test for *C. difficile* involved neutralization of the cytotoxin by *C. sordellii* or *C. difficile* antitoxin. Both toxin A and toxin B are cytopathic toxins, but toxin B is ~1000-fold more potent in nonintestinal cell lines [14]. This is probably the best test that is currently available in terms of sensitivity and specificity. However, it requires a 24–48-h turnaround time and is relatively work intensive and expensive, and many laboratories do not perform tissue cultures. These problems led to the development of multiple alternative tests including latex particle agglutination [55–57], dot immunoblot [58], PCR [59, 60], stool culture on selective media [61–64], and EIA [61, 62, 65–68]. The technique

Table 2. Efficacy of oral vancomycin (Van) in the treatment of *Clostridium difficile* infection (CDI).

Variable	Patients with PMC, % (n = 100)	Patients without PMC, % (n = 89)	All patients, % (n = 189)
Responded to Van therapy	97	96	97
Had a CDI relapse			
≥1 relapse	26	22	24
≥2 relapses	11	11	11
≥3 relapses	5	4	5
4–6 relapses	2	2	2

NOTE. PMC, pseudomembranous colitis.

that emerged as most commonly used in the United States was EIA. Studies in the early 1980s showed that this assay could detect both toxin A and toxin B but that for toxin B, it was at least 10-fold less sensitive than cell cytotoxicity assays [69]. Nevertheless, EIA was easy to perform, results were available in 4 h, and commercialization with multiple suppliers of reagents came rapidly. On the basis of results of ileal loop challenges in rodents, it was originally thought that toxin B was simply a marker of *C. difficile* toxin production and that only toxin A was important in human disease [14, 70]. Thus, early test development emphasized the detection of toxin A, with little regard for toxin B. More-recent studies with human intestinal cells (T84 cells) in Ussing chambers have shown that the potency of toxin B is actually 10 times that of toxin A in inducing increased permeability and morphologic changes [70]. The relevance of this observation has been the demonstration that occasional strains of *C. difficile* produce toxin B only and, consequently, give false-negative results of EIA tests that detect only toxin A [71–74]. To my knowledge, all toxigenic strains of *C. difficile* produce toxin B.

Perhaps more concerning was the observation by many that the sensitivity of the EIA was only ~75% for the first stool sample, compared with the cell cytotoxicity assay [75]. At Johns Hopkins Hospital (Baltimore, MD), the EIA was only 40% sensitive, compared with the cell cytotoxicity assay, leading to use of a screening test for *C. difficile* (i.e., EIA for detection of glutamate dehydrogenase or “common antigen”) followed by cell cytotoxicity assay for stool specimens that test positive for common antigen [76]. This method appears to have a good sensitivity and a specificity approaching 100% but requires 24–48 h for a final report [77]. An alternative to detection of common antigen is stool culture on selective media, which has the potential advantage of recovering the implicated strain for epidemiologic investigation or to detect the BI/NAP1 strain (restriction-endonuclease analysis group BI/North American PFGE type 1) [78].

The terms used to denote CDI have changed during 3 decades of study. In the initial period, endoscopy was often done

in addition to the cell cytotoxin assay, and commonly used terms, which included “*C. difficile*–associated diarrhea,” “*C. difficile* colitis,” and “PMC,” were derived from cell cytotoxicity assay results and endoscopy findings. Although there are at least 14 reported causes of PMC, the great majority involve *C. difficile*. As a result, many physicians came to view “PMC” as synonymous with “*C. difficile* infection,” although this is technically incorrect. Growth of *C. difficile* in stool culture or detection of common antigen via EIA indicates *C. difficile* colonization, whereas in vivo production of toxin indicates CDI. Nevertheless, laboratory findings need to be considered in light of symptoms, because some adults and many neonates harbor *C. difficile* toxins without clinical expression of disease. The term “*C. difficile*–associated disease” (often abbreviated as “CDAD”) emerged in the 1980s as a favored term for symptomatic patients who tested positive for *C. difficile* toxin. The term now favored is “*C. difficile* infection (CDI),” which includes enteric disease caused by *C. difficile* toxins.

Treatment of CDI. The initial use of oral vancomycin for treatment was based on favorable experience with *S. aureus* enterocolitis [8] and favorable experience in the hamster model [79]. Then came a highly successful experience in early clinical trials [80] and approval by the Food and Drug Administration (FDA) on the basis of results of a placebo-controlled trial for this indication in the early 1980s. This remains the only drug that is approved by the FDA for treatment of CDI. Oral vancomycin has ideal pharmacologic properties for treatment of a universally susceptible pathogen that is retained within the colonic lumen. It is not absorbed, so colon levels are usually >100-fold higher than the highest MIC ever recorded.

Subsequent studies indicated that metronidazole compared favorably with vancomycin as an effective treatment for CDI [81, 82], although metronidazole has the theoretic disadvantage that the colonic level of *C. difficile* is virtually nil except in the presence of diarrhea. Occasional strains are resistant in vitro, although the significance of this is questioned, because it is levels in the colonic lumen and not serum levels that count. Despite theoretic disadvantages, metronidazole is currently fa-

vored in guidelines from the Centers for Disease Control and Prevention, the Infectious Diseases Society of America, and the Society for Healthcare Epidemiology of America [83] on the basis of cost and the concern that oral vancomycin promotes colonization with vancomycin-resistant enterococcus [43]. Other drugs that also work well are fusidic acid, teicoplanin, and bacitracin, all of which are given orally [82, 84].

Metronidazole and vancomycin therapy have worked well in the majority of cases, but there are 2 problems with these treatments. First, some patients, particularly those with ileus or toxic megacolon, simply do not respond. This is presumably because of difficulty in getting the oral agent to the colonic lumen. Desperation measures of administering vancomycin involve delivery by retention enema or by a long tube via gravity. Alternative treatments have been tried, such as intravenous metronidazole and intravenous immunoglobulin (IVIG). The published experience of using these alternative methods has been anecdotal with variable success rates. For patients who are seriously ill, total colectomy may be required. The frequency of this intervention at Johns Hopkins Hospital in the early 1990s was 0.5% of all cases [85].

The second complication of treatment has been relapse following discontinuation of therapy. Relapse is reported in 22%–26% of cases (table 2) [82, 86–89]. The frequency of relapse after metronidazole appears to be nearly equal to frequency after initial treatment with vancomycin. Relapse is invariable after vancomycin administration to hamsters and to *C. difficile* growing in a chemostat [90]. Some patients have multiple relapses that are incredibly debilitating and extremely difficult to stop without continuous oral vancomycin therapy. Various treatments for such patients have included probiotics (i.e., *Saccharomyces boulardii*, *Lactobacillus rhamnosus* strain GG, and *Lactobacillus acidophilus*) [91–94], IVIG [95, 96], anion-exchange resins (i.e., cholestyramine and colestipol) that bind to *C. difficile* toxins [97–99], feces administered by nasogastric tube or by enema [100, 101], or vancomycin given by a tapered dose or a pulsed dose of 125 mg every other day for 6 weeks. All of these interventions work some of the time, and none work all of the time.

CONCLUSION

Clinicians working to prevent and treat CDI have made much progress over the past 30 years. However, continued research, improved preventive measures, and better treatments are still in great demand.

The articles that follow review and provide insight about the more recent experience with CDI, including the substantial concern about the BI/NAP1 strain [102–105]. These studies emphasize the factors that should alert physicians to the diagnosis of CDI. There are also treatment algorithms to help guide patient management. The ultimate goal is disease pre-

vention, which can be accomplished by means of rigorous antibiotic stewardship efforts, enhanced infection control, and improved education of health care workers. The goal for all of these treatments is to reestablish a normal colonic flora to control *C. difficile* growth dynamics, and the most predictably successful therapy is fecal replacement.

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