

Clinical update for the diagnosis and treatment of *Clostridium difficile* infection

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Abstract

Clostridium difficile infection (CDI) presents a rapidly evolving challenge in the battle against hospital-acquired infections. Recent advances in CDI diagnosis and management include rapid changes in diagnostic approach with the introduction of newer tests, such as detection of glutamate dehydrogenase in stool and polymerase chain reaction to detect the gene for toxin production, which will soon revolutionize the diagnostic approach to CDI. New medications and multiple medical society guidelines have introduced changing concepts in the definitions of severity of CDI and the choice of therapeutic agents, while rapid expansion of data on the efficacy of fecal microbiota transplantation heralds a revolutionary change in the management of patients suffering multiple relapses of CDI. Through a comprehensive review of current medical literature, this article aims to offer an intensive review of the current state of CDI diagnosis, discuss the strengths and limitations of available laboratory tests, compare both current and future treatments options and offer recom-

mendations for best practice strategies.

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Key words: *Clostridium difficile*; Antibiotic-associated diarrhea; Fidaxomicin; Rifaximin; Fecal transplantation; Probiotics

Core tip: This paper seeks to explore the treatment and diagnosis of *Clostridium difficile* infection (CDI) through an extensive literature review of available laboratory techniques and new treatment options. For diagnosis, this includes the glutamate dehydrogenase of stool and polymerase chain reaction for gene toxin. For treatment this includes guidelines based on severity, newer antibiotics for the treatment of CDI, fecal microbiota transplantation, and several new experimental treatment options. Finally, this manuscript offers suggested clinical guidelines for how to optimizing diagnosis and treatment of CDI.

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INTRODUCTION

Clostridium difficile infection (CDI) continues to be a significant and increasing problem. By far, CDI remains, the most common cause of hospital acquired diarrhea with the number of hospitalized patients with any CDI discharge diagnosis doubling from 139000 in 2000 to 336600 in 2009 at a cost of \$1 billion annually^[1]. In fact, recently CDI has surpassed methicillin-resistance *Staphylococcus aureus* (MRSA) as the most common hospital-onset, healthcare facility-associated infection^[2]. Despite

significantly trailing MRSA in nosocomial deaths^[3], the CDI death rate has dramatically increased from 3000 per year in 1999-2000 to 14000 per year in 2006-2007^[4].

One of the most important developments has been the emergence of a new epidemic strain, which is resistant to quinolone antibiotics, such as ciprofloxacin. The first noted in 2001, the epidemic strain produces 16-fold more toxin A and 23-fold more toxin B than other *C. difficile* strains^[5]. In addition, the organism produces more spores which results in contamination of the environment and the potential for further spread. The epidemic strain has been associated with an increased incidence of complicated cases and mortality compared to other strains^[6]. Confusingly, the epidemic strain is referred to as 027 by polymerase chain reaction (PCR)-ribotyping, B1 by restriction endonuclease analysis (REA), Type 1 by pulse field gel electrophoresis (PFGE) and toxin type III by restriction fragment length polymorphism PCR. For continuity throughout the article, the epidemic strain will be identified only as B1. The B1 epidemic stain has now spread widely throughout the United States, however, very few clinicians are aware of its presence in their hospital because culture and identification of *C. difficile* strains is rarely, if ever performed.

In this update, we will review recent advances in the diagnosis of CDI, with a focus on laboratory methods, and also new advances in the treatment of CDI and relapses, including the rapidly expanding area of fecal transplants.

DIAGNOSIS OF CDI

Risk factors for CDI

The first issue in a patient with suspected CDI is to determine if there are associated risk factors. Antibiotic use increases the risk of CDI by 8-10-fold during and for one month after administration and 3-fold for the next 2 mo^[7]. Numerous studies have looked at risk factors for CDI with a consistent implication of ampicillin (or amoxicillin), clindamycin and cephalosporins (in particular the third generation cephalosporins (TGC), such as cefotaxime, ceftriaxone and ceftazidime). For the TGCs, an almost perfect correlation has been noted between increasing use and rising incidence of CDI^[8] and conversely a decrease in CDI with decreased use of TGCs^[9]. Increasingly, quinolones have been shown to be a significant risk factor for CDI, especially with the epidemic B1 strain^[6]. The use of multiple antibiotics and > 10 d of antibiotics have also been associated with increased risk (suggesting that therapeutic use of antibiotics poses a greater use than prophylactic use)^[10,11]. Antibiotics which have been less commonly associated with CDI include aminoglycosides, macrolides, sulfonamides and tetracyclines. Although the correlation with CDI is highest with certain antibiotics, all antibiotics, even vancomycin and metronidazole on rare occasion, have been reported to cause CDI. However, exposure to antibiotics is not necessary for acquisition of CDI. In one study, 24% of patients with CDI had no antibiotic exposure and 9%

Table 1 Characteristics of tests for *Clostridium difficile* infections

	Sensit-ivity	Detects toxin in stool	Time to test completion	Cost	Availability	Stand alone test
EIA Toxin A/B	++	Yes	h	+	++++	Yes
GDH ¹	++++	No ⁵	h	+	++++	No
PCR ²	+++	No ⁶	h	++++	+++	Yes
TC ³	+++++	No ⁶	d	+++	+ ⁷	Yes
CCCNA ⁴	+++	Yes	d	+++	+ ⁷	Yes

¹Glutamate dehydrogenase; ²Polymerase chain reaction; ³Toxicogenic culture; ⁴Cell culture cytotoxicity neutralization assay; ⁵Detects presence of *Clostridium difficile* (*C. difficile* I) only, but not toxin producing capability, requires confirmatory testing; ⁶Detects toxin producing *C. difficile*, but not toxin in stool, false (+) in asymptomatic carriers; ⁷Only available in research laboratory. EIA: Enzyme immunoassay; GDH: Glutamate dehydrogenase; PCR: Polymerase chain reaction; TC: Toxicogenic culture; CCCNA: Cell culture cytotoxicity neutralization assay.

had received 3 d or less^[12]. Of the patients without any antibiotic exposure, however, 75% were either hospitalized or had close contact with a person with diarrheal illness.

Antineoplastic agents have also been associated with CDI, including doxorubicin, cisplatin, cyclophosphamide, fluorouracil and chlorambucil^[13], with methotrexate most commonly implicated. The proposed mechanism behind the pathogenesis of chemotherapy related CDI is two-fold. First, the antineoplastic agents have been shown to alter the gut microflora in a manner similar to antibiotics, acting as the primary predisposing factor for developing CDI^[14]. The second, these agents are capable of inducing mitotic arrest in intestinal epithelial cells, subsequently causing necrosis and desquamation of the mucosal membrane^[15].

Immunocompromised patients may represent a special subset of CDI for which the incidence and treatment may be more challenging to approach, in particular those with solid organ transplantation. The incidence of CDI in transplant patients has been estimated at 3%-7% for liver recipients, 3.5%-16% for kidney recipients, 1.5%-7.8% in pancreas-kidney recipients, 9% in intestinal recipients, 15% in heart recipients, and 7%-31% in lung recipients^[16]. Further fulminant colitis is noted to occur in up to 8% of immunocompromised patients and 13% of solid organ transplant recipients with the highest incidence within the first 3 mo^[17,18]. The treatment of CDI in immunosuppressed patients should follow the same guidelines based on disease severity as those outlined in this paper. One important caveat to consider is the potential for drug interactions with metronidazole, in particular the potential for alteration in levels of tacrolimus^[19] (Table 1).

Increasing age has been a consistently noted risk factor, with a > 10-fold increased risk for those 60-90 years old^[11,20]. In fact, 90% of all deaths are in persons 65 and older^[11]. Other associated risk factors have included enemas, stool softeners and gastrointestinal stimulants^[21], and also enteral feedings (especially postpyloric), which have been associated with an 11-fold increased risk of CDI^[22].

Although rates of non-CDI diarrhea with enteral feedings have been reported in up to 60% of patients^[23], the increased risk of CDI would suggest that CDI is a significant problem for enterally fed patients. The significant risk related to postpyloric tube feeding may be related to the fact that gastric acidity has been shown to eliminate 99% of vegetative *C. difficile* cells^[24]. Rates have also been noticed to be increased after gastrointestinal operations up to 25-fold compared to controls, probably related to impaired motility, nasogastric tubes and preoperative antibiotics^[11].

Recently, several studies have found a higher risk of *C. difficile* infection in proton pump inhibitor (PPI) users. In theory, PPIs may increase the risk of *C. difficile* infection by increasing the ability of the spore to convert to the vegetative form and to survive in the lumen of the gastrointestinal tract. Several meta-analysis have found a significant relationship between PPI use and CDI with odds ratios ranging from 1.69 (95%CI: 1.395-1.974)^[25] to 2.05 (95%CI: 1.47-2.85)^[26]. Despite these results, the most recent studies offer conflicting viewpoints as to the association between PPI use and increased risk of *C. difficile* infection. These studies showed that while univariate analysis may show a statistically significant relationship between PPI use and CDI, multivariable analysis reveals no significant relationship^[27-29]. Further, the most recent review on detection, prevention and treatment of *C. difficile* does not include restriction or avoidance of PPIs in the recommendations for prevention of *C. difficile* infection^[30], nor is this recommended by multi society clinical practice guidelines^[31].

Although CDI is commonly felt to be a hospital-acquired infection, with up to 87% of infections nosocomially acquired, a significant number of cases are community acquired^[10]. In a prospective study of diarrheal pathogens, 20% of infections were community acquired. For an additional 15% of patients, CDI was acquired in the hospital, but diarrhea began after discharge at home for a total of 43% of cases with onset of symptoms at home^[16]. As many as 25% of all cases of CDI develop in nursing home patients^[1]. Suspicion should always be high for CDI whenever there is diarrhea in a resident of a long term care facility where there is a concentration of elderly, high use of antibiotics, CDI infection in other residents, or frequent exposure to hospitals. This increased risk is bidirectional: 20% of CDI with onset in the hospital are in residents of a nursing home and 67% of CDI in nursing home residents occurs in patients recently discharged from an acute care hospital^[1].

Even among asymptomatic patients many of these risk factors appear to be the same. During a 2-mo period, researchers at a tertiary care hospital in Minnesota performed PCR for toxigenic *C. difficile* on all consenting asymptomatic patients, who had greater than a 24 h stay without any known or suspected CDI, diarrhea or colitis. Of the 320 stool samples collected, 31 samples (9.7%) were positive for toxigenic *C. difficile*^[29]. Multivariate analysis revealed three main risk factors for *C. difficile* colonization: recent hospitalization within 3 mo (OR = 2.45,

95%CI: 1.02-5.84), chronic dialysis (OR = 8.12, 95%CI: 1.80-36.65), and corticosteroid use (OR = 3.09, 95%CI: 1.24-7.73).

Clinical presentation of CDI

There is a broad range of clinical manifestations from asymptomatic carriage (20% of culture positive patients) to colitis with or without pseudomembranes to fulminating colitis and toxic megacolon. In one series, a two-year institutional study of CDI revealed that “acute abdomen” was the presenting feature in 5% of patients with CDI, with 2 of 5 having no diarrhea prior to emergency laparotomy^[32]. This acute abdomen presentation without diarrhea may be particularly confusing in the postoperative patient. Onset is usually 5-10 d after antibiotic use, but ranges from 1 d up to 10 d after antibiotics are stopped. Frankly bloody diarrhea is uncommon (5%-10%)^[33]. In fact, only 26% have occult blood^[10]. Fever is noted in 30%-50%, usually low grade, not to exceed 102°F^[28].

Leukocytosis, hypoalbuminemia and elevation of baseline serum creatinine are highly suggestive of CDI. Elevated white blood cell (WBC) count is common (50%-60%), as well as increased band forms (47%) and may be marked elevated^[34]. Wanahita found a mean WBC of 15800/mm³ with 26% of patients having a WBC > 20000/mm³ and 6% > 30000/mm³. In fact, for all patients without a hematologic malignancy who had a WBC > 30000/mm³, 25% were found to have CDI^[34]. The elevation of WBC may even precede the onset of diarrhea or abdominal discomfort^[35] and may be responsible for up to 58% of cases of unexplained leukocytosis in hospitalized patients^[36]. In a series of patient with leukocytosis who were *C. difficile* toxin negative, empiric treatment for CDI led to resolution of leukocytosis^[36]. CDI results in a protein losing enteropathy with resultant hypoalbuminemia^[37]. Serum albumin of < 2.5 or a fall in albumin of > 1.1 have been associated with a poor prognosis^[38]. Bartlett has noted that hypoalbuminemia in persons with antibiotic associated diarrhea may be a clinical clue suggesting CDI^[37,39]. Fecal leukocytes have been found in 28%-40% of cases^[40]. Detection of fecal lactoferrin (typically used as an indicator of inflammatory bowel disease activity) has been shown to be almost twice as sensitive (75%) as fecal leukocyte detection by methylene blue stain^[41]; however, both tests lack sensitivity and specificity and add little to the diagnostic evaluation.

Radiologic diagnosis of CDI

Radiologic studies such as acute abdominal series have been of little value with non-specific findings. Plain films of the abdomen may reveal colonic dilation, (especially cecal), and non-obstructive related small bowel air fluid levels indicative of ileus pattern. Abdominal computed tomography (CT) has been reported to be normal in 39% of cases, but often reveals a thickened colonic wall, which may be focal or diffuse^[42]. With fulminant colitis, there may be mucosal thumbprinting and an “accordion” appearance with oral contrast trapped in the thickened mucosal folds.

Table 2 Comparison of American College of Gastroenterology 2013 and Society for Healthcare Epidemiology of America/Infectious Diseases Society of America 2010 Guidelines for Treatment of *Clostridium difficile* infection (Differences between the guidelines are in bold)

Severity	SHEA/IDSA 2010 ¹		ACG 2013 ²	
	Definition	Treatment	Definition	Treatment
Mild-to-Moderate	WBC < 15000 cells/ μ L or lower and serum Cr < 1.5 times the premorbid level	Metronidazole 500 mg 3 times/d by mouth for 10-14 d	Diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria	Metronidazole 500 mg orally 3 times/d for 10 d. If no improvement in 5-7 d, consider change to vancomycin at standard dose
Severe	WBC > 15000 cells/ μ L or higher or a serum Cr > or equal to 1.5 times the premorbid level	Vancomycin 125 mg 4 times/d by mouth for 10-14 d	Serum albumin < 3 g/dL plus one of the following: WBC \geq 15000 or abdominal tenderness	Vancomycin 125 mg orally 4 times/d by mouth for 10 d
Severe, complicated	Hypotension or shock, ileus, megacolon	Vancomycin 500 mg four times/d by mouth or by nasogastric tube, plus metronidazole 500 mg every 8 h intravenously. If complete ileus, consider adding rectal installation of vancomycin	Any of the following attributable to CDI: ICU admission, hypotension with or without the need for vasopressors, fever \geq 38.5 °C, ileus or significant abdominal distension, mental status changes, WBC > 35000 cells/mm ³ or < 2000 cells/mm ³ , serum lactate > 2.2 mmol/L, end organ failure	Vancomycin 500 mg orally four times/d and metronidazole 500 mg IV every 8 h and vancomycin per rectum (500 mg in 500 mL saline as enema) four times a day

¹Society for Healthcare Epidemiology of America (SHEA)/Infectious Diseases Society of America (IDSA)^[31]; ²American College of Gastroenterology (ACG)^[71]. WBC: white blood cell; CDI: *Clostridium difficile* infection; ICU: Intensive care unit.

Endoscopic diagnosis of CDI

Endoscopy is usually reserved for special situations. The American College of Gastroenterology (ACG) guidelines recommend endoscopy when a rapid diagnosis is needed, when there is a delay in results of toxin assay or an initial negative toxin assay when CDI is strongly suspected, when there is an ileus and stool is not available and when other colonic diseases are in the differential^[43] (Table 2). Endoscopy is frequently normal with mild disease, but often reveals multiple typical yellowish-white plaques (pseudomembranes) elevated above the surrounding mucosa^[44]. The plaques vary from a few millimeters to 20 mm and may become confluent with advanced disease and may slough off leaving a denuded underlying mucosa. The intervening mucosa between the plaques may be normal or erythematous and edematous. Overall, pseudomembranes have been detected in 41% of cases of CDAD^[45]. Distal involvement of the colon is most common, making flexible sigmoidoscopy a reasonable initial test although in one series, false negative rate due to proximal involvement with rectal sparing was reported in 10% of cases^[46]. Histologically, the pseudomembranes, composed of fibrin, mucus, epithelial and inflammatory cells appear as “clouds” rising from points of superficial ulcerations. The lesions have been termed “volcano” lesions appearing like an eruption above underlying glandular lesions^[47]. In 22% of cases, pseudomembranes were visualized on endoscopy, but not present histologically^[48].

Laboratory diagnosis of CDI

The state of the art for best practice is controversial and confusing. Curry noted that “diagnosis of CDI remains one of the most vexing difficulties for hospital microbiology laboratories”, because there is no single accepted

gold standard^[49]. For many years, cell culture cytotoxicity neutralization assay (CCNA) was the accepted gold standard. By this method, stool filtrates are inoculated onto a monolayer of a cell culture in wells with and without *C. difficile* antitoxin. Rounding of the cells in the antitoxin-free well demonstrates a cytopathic effect and the presence of toxin. If there is no change in the antitoxin containing well, then the presence of *C. difficile* toxin in the stool is confirmed. CCNA is quite specific for CDI and can detect toxin in the stool as low as 10 picograms. However, the assay is expensive, has a slow turnaround time (2 d minimum), lacks standardization among laboratories and is generally unavailable outside the research setting. More recently, many investigators have considered toxigenic culture (TC) as the method of choice for diagnosis of CDI. With the toxicogenic culture method, stool is cultured for *C. difficile* on a selective differential medium (cycloserine, cefoxitin, fructose agar or CCFA). In the next step, the organism is tested for ability to produce toxin. Compared to TC, CCNA has only 67%-79% sensitivity^[49]. The Society for Healthcare Epidemiology of America (SHEA)/Infectious Diseases Society of America (IDSA) 2010 guidelines note that “the sensitivity and specificity of stool culture followed by identification of a toxigenic isolate (TC) as performed by an experienced laboratory provides the standard against which other clinical tests should be compared”^[31]. Despite the assertions of the superiority of TC as a gold standard, there are significant issues with using TC as a gold standard. The TC identifies the ability to produce toxin, but not actual toxin in stool. This can lead to false positives due to the fact that up to 7% of asymptomatic hospitalized patients may be colonized on admission with toxigenic *C. difficile*^[50]. Rates of asymptomatic colonization with toxin producing *C. difficile* can be even

higher among elderly patients in skilled nursing facilities, approaching 20%^[51]. Concern about using TC as the gold standard was raised by a recent study conducted by the National Health Service (NHS) Laboratories in the United Kingdom, which evaluated 12441 diarrheal fecal samples^[52]. The study showed that the presence of toxin in the fecal specimens was associated with poor clinical outcomes; however, culture of toxin producing *C. difficile* without detection of toxin in the diarrheal stool specimens was not associated with worse clinical outcomes than stools that were negative for toxigenic *C. difficile*. At best, which test should be the gold standard for diagnosis of CDI, TC or CCCNA, is currently undecided. One thing is clear if the gold standard being used is TC, then all the comparators, whether enzyme immunoassay (EIA), glutamate dehydrogenase (GDH) or polymerase chain reaction (PCR), will be less sensitive. If CCCNA is used as the gold standard, the comparators will appear more sensitive.

There is consensus that the EIA for toxin A/B, currently the primary test used in up to 90% of clinical laboratories^[53] is too insensitive and non-specific and no longer recommended as a stand-alone test^[54]. The EIA for toxin A/B has been adopted by most clinical laboratories because it is fast, convenient and inexpensive. Recent studies have shown however, that the sensitivity can be as low as 38%^[55]. The EIA requires 100-1000 picograms of toxin as compared to the ability of the CCCNA to detect less than 10 picograms of toxin^[53]. In addition to poor sensitivity, the EIA also has a positive predictive value (PPV) as low as 50% due to the low prevalence of *C. difficile* among all specimens submitted for testing from symptomatic patients^[54]. Historically, 15%-25% of antibiotic associated diarrhea has been felt to be due to *C. difficile*. However, most recent studies suggest a decreasing rate of positivity with only 5%-10% of samples testing positive^[54]. In 2001, 22% of samples tested were positive for toxin by EIA *vs* only 11% in 2007^[56]. If the prevalence of positive stools is 10%, then the PPV of a positive toxin EIA varies from less than 50%-90%. Falsely diagnosing a patient with CDI can lead to isolation of patients who are not infected. Isolation has been shown to have negative consequences, with a doubling of adverse events and days without a physician note and an increase in formal complaints by 8-fold^[57]. A false diagnosis of CDI can also lead to cohorting of uninfected patients with patients who have active CDI, particularly in skilled nursing facilities, as well as delay in finding the true etiology of the diarrhea and the unnecessary use of antibiotics. A systematic review of toxin detection kits concluded that the sensitivity and specificity of the different test kits were sufficiently heterogeneous between studies of the same test, such that meta-analytic methods could not be used to pool studies on a particular toxin EIA assay^[56]. They concluded that differences in test characteristics were most likely related to the threshold cutoff chosen for each test. Choosing a low threshold increased the sensitivity, but at the same time decreased specificity and vice versa. Overall, the au-

thors concluded that none of the EIA toxin assays had an acceptable predictive value and that a two-step testing strategy should be used.

The lack of sensitivity and specificity of the toxin A/B EIA assay has led to a search for more accurate test methods. The detection of GDH in stool has shown significant promise. The test is fast (15-45 min), convenient, inexpensive, and sensitive. The GDH is a common antigen expressed at high levels by all *C. difficile* strains. However, the test only documents the presence of *C. difficile*, but not the presence of a toxigenic strain (20% of *C. difficile* strains do not produce toxin) or the presence of toxin in stool^[58]. Therefore, GDH (+) stool requires confirmation of toxin production with a second test. Early studies reported sensitivities as high as 100% for detection of *C. difficile*^[59]. However, more recent studies have raised concern about the sensitivity of the GDH assay for non-epidemic B1 strains. For non-epidemic B1 strains, the sensitivity may be as low as 69%^[60].

The use of PCR to detect the gene for toxin production (*tdfB* gene) is promising as a stand-alone test for CDI. The PCR for the toxin gene is fast (2 h) and sensitive with a minimum detection limit of 105 per gram of stool^[61]. However, the cost can be 5-10 times greater than EIA for toxin A/B. Sensitivity has been 91% as compared to enzyme immunoassay at 67%^[62]. Overall, sensitivity has been 84%-94% in comparison to TC, similar to the CCCN^[60]. Many hospital laboratories will be able to offer ready availability of PCR testing with rapid turnaround.

There are currently four Food and Drug Administration (FDA) approved PCR assays, Gene Ohm (Becton Dickinson, San Diego), Gene Xpert (Cepheid, Sunnyvale, Ca.), which not only can identify the *toxin* gene but also the epidemic B1 strain, Progestro (Prodesse, Waukesha, Wi) and Simplexa (Quest Diagnostics, Madison NJ). In a meta-analysis of PCR *vs* TC, a pooled sensitivity of 92% and specificity of 94% was reported^[63]. However, as with TC mentioned earlier, the PCR detects the *toxin* gene, but does not detect toxin in stool raising concerns about over diagnosis by detecting asymptomatic carriers. In addition, the use of the PCR may increase CDI incidence rates by greater than 50%^[64]. This raises concern with mandatory reporting programs and inter-hospital comparisons. Some authors have noted an increase from 6.5% positive samples before the use of PCR to 15% after their laboratory changed to PCR for *C. difficile* detection^[65]. In addition, the PCR cannot be used for suspected relapse as up to 56% of patients will be positive by PCR at 1-4 wk after completion of therapy^[66]. However, despite its high sensitivity and specificity, at the recently noted prevalence of 10% of CDI among tested specimens, the positive predictive value may be only 63%^[65]. Despite these issues, some laboratories have now adopted PCR as a stand-alone diagnostic test for *C. difficile*^[67].

Another promising method for CDI diagnosis is detection of the *toxin* gene by loop-mediated isothermal amplification (LAMP), which does not require a large capital outlay for PCR^[63]. This non-PCR based gene amplification method detects the pathogenicity locus of

toxigenic *C. difficile*. The test is simple, rapid (1 h) and significantly less expensive than PCR based methods. The Illumigene (Meridian Bioscience, Europe) assay was found to have a 92% sensitivity, 98% specificity, 99% negative predictive value and 84% positive predictive value, respectfully¹⁶⁸. However, the same issues that raise concerns about TC and PCR, *i.e.*, detection of toxigenic *C. difficile*, but not toxin in stool, are true for LAMP.

The concerns with EIA for toxin A/B, PCR and GDH as stand-alone tests has led to the study of an algorithmic approach to the diagnosis of CDI, similar to HIV and syphilis testing. Larson *et al*⁶⁹ studied a 3 step algorithm with the initial test being a glutamate dehydrogenase. If the GDH is positive, this was followed by confirmation of toxin in stool with an EIA for toxin A/B. If both are positive, the test is reported as positive for CDI. If the EIA toxin A/B is negative, the final result is determined with a PCR. Using this algorithm, they found a sensitivity of 84% and specificity of 99.7% with very high PPV of 97.5% and NPV of 99.7% compared to a modified gold standard using CCCNA and PCR. In the previously mentioned United Kingdom NHS study using 12441 diarrheal fecal specimens, Wilcox concluded that a two-step protocol with an EIA for GDH or a nucleic acid amplification test (NAAT), such as a PCR for *toxin* gene, followed by confirmation of stool toxin by a EIA for toxin A/B was the most effective testing algorithm in distinguishing patients with *C. difficile* infection from those who did not have CDI⁵³. This two-step algorithm has now become the standard in NHS laboratories in England as of April 2012⁷⁰. The combination optimizes specificity and positive predictive value (90%)⁵². This same study found that using an algorithm that optimized for sensitivity such as a GDH followed by a PCR resulted in a 95% sensitivity, but a PPV that was only 60%. In other words, 4 of 10 positive tests did not really have CDI. This would be an optimal method for excluding CDI, but not a very good method for determining if CDI was really present. The American Society of Microbiology (ASM) recommends that if the toxin A/B EIA or CCCNA is used and is negative, specimens should be further tested by PCR or TC⁵⁴. The ASM noted that utilizing toxin A/B EIA for *C. difficile* diagnosis is insensitive and no longer recommended as a stand-alone test. The ASM also noted that laboratories can also use a PCR to detect *C. difficile toxin* genes as a stand-alone diagnostic test. The SHEA/IDSA guidelines suggested that an initial GDH test followed by confirmation with either TC or CCCN was an option³¹. However, as previously noted, the last two tests are rarely available in clinical laboratories and results would not be available in time for clinical use. The 2013 ACG guidelines recommend a NAAT such as PCR as a standard diagnostic test for CDI. The guidelines also suggest that a GDH EIA can be used an initial screening test in a two-or three-step algorithm with subsequent confirmation of positive results with an EIA for toxin A/B. If the EIA for toxin A/B is negative, then a NAAT test should follow. How-

ever, the ACG guideline notes that the sensitivity is lower than a strategy based on an initial PCR⁷¹.

Repeat testing for CDI

One aspect of testing about which there is broad agreement is that there are limited indications for repeat testing. Yassin *et al*⁷² have suggested that performing the EIA for toxin on two or three samples can increase sensitivity to about 90%. However, Renshaw *et al*⁷³ suggested that repeated assays accounted for 36% of all toxin assays ordered, but provided clinically useful information in only 1% of the cases and significantly increased cost. Aichinger *et al*⁷⁴ found that repeat testing within 7 d by EIA for toxin A/B or by PCR for *C. difficile* toxin resulted in < 2% positive tests. In another study, repeat testing accounted for 17% of all tests ordered, but only 1% were positive⁷⁵. Peterson *et al*⁵⁹ noted that with a sensitivity of 73% and a specificity of 97.6% that if the *C. difficile* EIA was negative on the first two tests, a positive result on the third test was three times more likely to be a false positive than a true positive due to decreasing pretest probability with consecutive negative tests. In fact, even on the second test after an initial negative, the positive predictive value is less than 50%, about as good as flipping a coin. The 2013 ACG guidelines make a strong recommendation that repeat testing not be performed.

There is clearly no indication for serial monitoring of stools or an end of treatment “test of cure” as 1/3 of patients will still have a positive assay at the end of successful treatment⁴⁰. Stool carriage has been noted to persist for 3-6 wk after successful treatment and has not been found to predict who will relapse⁷⁶. Requiring a negative test to come out of isolation or before transfer to a long term care facility is inappropriate. Again, the 2013 ACG guidelines make a strong recommendation that testing for cure should not be done.

Given the limitations of the available laboratory tests for CDI, a reasonable approach is: (1) if CDI is suspected on clinical grounds, perform *C. difficile* testing according to your hospital laboratory protocol. Be aware of the test or algorithm they are using. Many clinical laboratories are in the process of changing testing protocols; (2) if the test is positive, continue or initiate treatment, if not started empirically; and (3) if the test is negative, make a clinical decision on whether to treat based on the likelihood of CDI (recent exposure to antibiotics or prior CDI, elevated white blood count or elevated creatinine or decreased albumin, age or other risk factors). If CDI is still suspected after a negative test, empiric treatment is reasonable. Repeat testing yields minimal additional true positives and increases cost. The ACG Guidelines make a strong recommendation that “Repeat testing should be discouraged”⁷¹.

In summary, testing for CDI is in flux, confusing and controversial. As noted by Fang, “the clinical laboratory can place the perpetrator (*C. difficile*) at the scene of the crime, but only the clinician can establish whether a crime (CDI) has taken place”⁷⁷.

THE CONTROVERSY OVER BASIC TREATMENT CHOICES

Despite numerous treatment trials for *C. difficile* infection, dating back to 1978, the drug of choice for CDI remains controversial. In fact, Pepin noted that “there are few common infectious diseases in developed countries for which the treatments used in 2006 are essentially the same as those recommended one-quarter of a century ago”^[78]. The same can be said for 2013 and the foreseeable future. The recent Cochrane Collaboration review of antibiotic treatment for CDI vividly illustrates the ongoing problems related to treatment decisions^[79,80]. The authors reviewed randomized, controlled trials of antibiotic therapy for CDI. There were 15 studies considered evaluable with 1152 patients involved. There was only one placebo controlled trial, which was considered to be of small size with poor methodological quality. The authors concluded that even the most basic question of whether any antibiotic is effective, much less which one, has not yet been answered. The authors stated, “this review cannot establish the efficacy of antibiotic therapy for CDI as the only placebo controlled trial is inadequate”. In fact, they noted that there is “uncertainty whether mild CDI needs to be treated”. Further, they noted that “this review cannot definitively make a specific antibiotic recommendation for the treatment of CDI”. When looking at particular antibiotics, they concluded that “no single antibiotic is clearly superior to others”. Although, they did note that teicoplanin was superior to vancomycin. Unfortunately, teicoplanin is not available in the United States.

Part of the reason that there have been so few changes in our treatment of CDI over the last 30 years may be due to the lack of development of significant resistance. Fortunately, a number of recent studies have not revealed resistance to the main standbys for treatment of CDI: metronidazole and vancomycin. Aspevall *et al.*^[81] studied 238 isolates of *C. difficile* collected from 2000 to 2001 and found no evidence of resistance to metronidazole or vancomycin. Hecht *et al.*^[82] studied 110 strains collected between 1983 and 2004. All strains were sensitive to metronidazole at less than or equal to 0.5 µg/mL. Bourgault *et al.*^[83] looked at 251 isolates collected during the outbreak in Quebec, Canada, which started in 2003. Of these, 69% were the B1 epidemic strain, while 11% were the NAP2 strain by PFGE. All isolates were sensitive to metronidazole and vancomycin. There was no increase in MIC's compared to historical isolates.

Unfortunately, the same cannot be said for other antibiotics. Recently, the complete genome of *C. difficile* has been sequenced revealing a significant potential for development of antibiotic resistance^[84]. Significant portion of the genome (11%) consists of mobile genetic elements, mainly conjugative transposons, which can be used to transfer genetic material between bacteria. These mobile genetic elements are often involved in the transfer of antimicrobial resistance and virulence factors.

Bourgault *et al.*^[83] found that for the B1 epidemic strain the quinolones, macrolides and other commonly used antibiotics have succumbed to the antibiotic resistance mechanisms of *C. difficile*. All strains were resistant to bacitracin, ciprofloxacin, levofloxacin and clarithromycin, while 80% were resistant to gatifloxacin, moxifloxacin and ceftriaxone. All historical NAP1 isolates were resistant to quinolones, suggesting that the epidemic may be more associated with the increased use of fluoroquinolones, as opposed to the recent development of quinolone resistance by the epidemic strain. Of note, 69% of the B1 epidemic strains were sensitive to clindamycin, while only 11% of the non-epidemic strain strains were sensitive to clindamycin.

METRONIDAZOLE OR VANCOMYCIN

Having summarized the murky state of the evidence based treatment of CDI, it would be reasonable to look at the pros and cons of metronidazole and vancomycin. The oft-quoted reasons for metronidazole assuming the status of preferred agent for treating CDI has been the potential for development of vancomycin-resistant enterococci (VRE) and the higher cost of oral vancomycin. In contrast to this notion, a small study looking specifically at the issue of developing VRE found no patients developed VRE while being treated with oral vancomycin^[85]. Unfortunately, vancomycin capsules (Vancocin HCl Pulvules) are extraordinarily expensive, with an average wholesale price of \$31.83 per capsule or \$127.32 per day for a dose of 125 mg *qid* vs \$2.19 per day for generic metronidazole 500 mg *tid*^[86]. Further, retail costs are much higher. Most hospitals avoid the extraordinary cost of vancomycin capsules by using the generic intravenous formulation and compounding it in water as a liquid vancomycin solution. One pharmacy, close to our clinic, sells vancomycin intravenous formulation for \$5.85 per 500 mg *vial*. If this 500 mg of vancomycin powder is reconstituted in 20 cc of water (often with flavoring to hide the bitter taste of vancomycin), the cost of vancomycin approaches \$1.50 per dose. Stability of the vancomycin solution in the refrigerator (4 degrees C) is at least 75 d and at least 26 d at room temperature (25 degrees C)^[87].

Despite issues related to fostering VRE and cost, prior comparative studies of metronidazole and vancomycin have not revealed a statistically significant difference between the two antibiotics^[88,89]. In one study, 95% were cured with metronidazole vs 100% with vancomycin^[88]. In the second study, the cure rates were identical at 94% in each group^[89]. However, the number of patients was small and neither study was stratified by severity of disease.

Despite similar response rates, there are significant pharmacologic concerns related to metronidazole, which tilt the balance in favor of vancomycin. Metronidazole is rapidly absorbed from the gastrointestinal tract and excreted through the biliary system, with only about 14% of the drug excreted in the stool^[90]. Fecal metronidazole

levels have been noted to increase with colonic inflammation, probably from transudation into the lumen, but these levels decrease as inflammation subsides and are undetectable upon recovery^[37,91]. More recently, Musher noted a failure rate of 22% with standard doses of metronidazole^[92]. This was not due to resistance, as those strains tested, were all sensitive to metronidazole. Interestingly, in this study there was no difference in outcomes between those who were continued on metronidazole despite clinical failure compared to those who were changed to vancomycin. Musher *et al*^[92] suggested that patients with severe disease could have decreased blood flow to the colon, which would result in less transudation of metronidazole into the lumen and either a slower response or clinical failure^[93]. Despite this potential for low metronidazole levels, *in vitro* the drug has been shown to be very rapidly bactericidal at 8-times the minimum inhibitory concentration (MIC), a level which is usually reached in the colon. This rapid bactericidal effect can be compared to vancomycin, which has been shown to be only inhibitory of bacterial growth^[40]. As opposed to the poor pharmacokinetics of metronidazole, vancomycin has near perfect characteristics for a drug used to treat an infection limited to the lumen of the colon. Vancomycin achieves levels in the colon of about 1000 µg/mL in stool due to the fact that there is limited or no absorption from the colon. Al-Nassir *et al*^[94] have shown that vancomycin is much more effective than metronidazole in removing *C. difficile* from the stool as measured by *C. difficile* density cultures^[94]. By day 5 of treatment, patients treated with vancomycin were 3.3 times more likely to have undetectable *C. difficile* than metronidazole ($P = 0.015$). In this study, 10 of 34 patients were switched from metronidazole to oral vancomycin between days 2 and 10 due to suboptimal clinical response, of whom 8 of the 10 had less than a one log decrease in *C. difficile*. Once they were switched to oral vancomycin, 7 of these 8 patients had undetectable *C. difficile* by culture. Freeman *et al*^[95,96] confirmed the favorable characteristics of oral vancomycin in a human gut model composed of three vessels operating in a weir cascade system in an oxygen free nitrogen atmosphere. They found that cytotoxin titers were unaffected by metronidazole, while vancomycin resulted in a marked decrease in toxin and the *C. difficile* vegetative form, leaving only spores which do not produce toxin. Another issue which may decrease the effectiveness of metronidazole is inactivation by *Enterococcus faecalis*, which has been shown to allow protection of organisms which would normally be killed by metronidazole^[97]. There also appears to be a higher failure rate with metronidazole when the physician is forced to continue the offending antibiotics, which is often the case. In one series, all patients who could have the offending antibiotic discontinued had resolution of diarrhea by 14 d when treated with metronidazole^[98]. However, 41% of the patients who had antibiotics continued failed to have symptomatic resolution of diarrhea by day 14 ($P = 0.02$).

Because rifampin has been shown to have markedly

superior *in vitro* activity in comparison with other antimicrobials against *C. difficile*^[99] combination therapy has been studied as a means to improve outcomes with metronidazole therapy. Lagrotteria *et al*^[100] conducted a prospective, randomized, single-blind study of metronidazole alone *vs* metronidazole plus rifampin^[100]. There was a similar time to improvement, similar proportion of relapses, but significantly more deaths in the combination group as compared to metronidazole alone (32% *vs* 5%, $P = 0.04$). The authors concluded: “there is no role for rifampin as an adjunct to treatment with metronidazole.”

TREATMENT DECISIONS BASED UPON STRATIFICATION BY DISEASE SEVERITY

A concern with all of the preceding comparative studies of vancomycin with metronidazole has been that there was no stratification by disease severity. One of the most important recent advances in the treatment of CDI has been the development of scoring systems, which allow the physician to determine which patients are at highest risk for severe CDI. The development of scoring systems was started by Pepin *et al*^[78] who developed local recommendations, because of the overwhelming epidemic in Quebec caused by the new epidemic B1 strain. In January of 2004, they developed local recommendations for the use of oral vancomycin: a WBC greater than 20000 cells/mm³ and a serum creatinine greater than or equal to 200 µmol/L. This recommendation was based upon a reduction of complicated CDI by 79% if vancomycin was the initial treatment compared to metronidazole^[101].

Zar *et al*^[102] conducted the first randomized, double-blind, placebo controlled trial comparing metronidazole and vancomycin in the treatment of CDI that stratified patients at study entry based upon severity of disease. The authors developed a scoring system giving 1 point each for the presence of age greater than 60 years, temperature greater than 38.3 degrees centigrade, albumin less than 2.5 mg per deciliter, or a WBC count greater than 15000 cells per mm³. They also gave 2 points for endoscopic evidence of pseudomembranous colitis or treatment in an intensive care unit setting. Mild disease was defined as 0 or 1 points and severe CDI was defined as greater than or equal to 2 points. Clinical cure was noted in 90% of those with mild CDI randomized to metronidazole and 90% of those randomized to vancomycin. For those with severe CDI, clinical cure was noted in 76% who received metronidazole *vs* 97% who received vancomycin ($P = 0.02$). Recurrences were similar for both groups at 15% and 14% for the metronidazole and vancomycin groups, respectively. The authors concluded that metronidazole and vancomycin are equally effective for the treatment of mild CDI; however, vancomycin is superior for treating patients with severe CDI. Critiques of the Zar *et al*^[102] article were that one of the criteria for failure was persistent toxin positivity at day 6 and 10 of therapy. In addition, there was exclusion of 8 patients

Table 3 Comparative average wholesale price for antibiotics used in the treatment of *Clostridium difficile* infection

Antibiotic	Cost per dose ¹	Usual regimen	Cost per treatment ¹
Metronidazole	\$0.73		
Vancomycin capsules (Vancocin HCL pulvules)	\$31.83	125 mg 4 times/d × 10 d	\$1273.20
Vancomycin intravenous formulation (generic)	\$5.00/g (\$0.62 per 125 mg dose)	125 mg 4 times/d × 10 d	\$25.00
Fidaxomicin (Dificid)	\$168.00	200 mg 2 times/d × 10 d	\$3360.00
Rifaximin (Xifaxan)	\$19.02 400 mg	400 mg 3 times/d × 20 d ²	\$1141.20

¹Average Wholesale Price (AWP); Anon, edition. Red Book online. ²Dose as a “chaser” after a course of oral vancomycin for recurrent CDI^[151]. Via Drugdex System (internet database) Greenwood Village, CO: Thompson Healthcare, 2011^[86]. CDI: *Clostridium difficile* infection.

with early death^[103]. When the 2 patients who were judged as having failed therapy solely on the basis of persistent toxin positivity and the 8 early deaths were included, vancomycin was still superior to metronidazole for those with severe disease with a 90% cure rate for vancomycin *vs* 71% for metronidazole ($P = 0.04$).

The 2010 IDSA guidelines recommend oral metronidazole for CDI with a WBC 15000 cells/mm³ and < 50% increase in serum Cr from baseline. The guidelines define severe disease as CDI with a WBC 15000 cells/mm³ or a 50% increase of serum Cr from baseline. For severe CDI, they recommend starting therapy with oral vancomycin 125 mg *qid*^[50]. Most recently, the ACG has updated its practice guidelines to include summary recommendations based on CDI severity^[71]. Mild-to-moderate disease is defined as diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria. Notably, the ACG classification for severe disease has been redefined from the IDSA guidelines to use only three criteria: a serum albumin < 3 g/dL plus one of either WBC 15000 cells/mm³ or abdominal tenderness. The choice to limit the guidelines to these three criteria was based on a prospective observational study by Fujitani *et al*^[104] which found that the only independent risk factors for severe CDI were abdominal distention, fever, WBC > 20000 cells/mm³, and serum albumin < 3 mg/dL. The ACG guidelines recommend the same initial treatments of metronidazole 500 mg orally three times daily for 10 d for mild-to-moderate disease and vancomycin 125 mg orally four times daily for severe disease.

NEWER ANTIBIOTICS FOR CDI

Rifaximin

Rifaximin (Xifaxan, Salix Pharmaceuticals, Inc. Raleigh, NC) is a broad spectrum, non-absorbable antibiotic used for the treatment and prevention of traveler’s diarrhea. The drug is not inactivated by gastric fluids and is also poorly absorbed, thereby largely excreted unchanged in the feces reaching concentrations up to 8000 g/gm of stool after 3 d of therapy^[105]. Rifaximin treatment has demonstrated survival rates in animal models equivalent to vancomycin. Rubin *et al*^[106] conducted an open label pilot study to assess the efficacy of rifaximin as an initial treatment option in patients without recurrent CDI. Of the 8 patients who completed the 10-d course of rifaximin 400 mg three times daily, 7 (88%) had symptom

resolution with 10 d of rifaximin treatment with no relapse within 2 wk. Additionally, Boero *et al*^[107] compared the efficacy of rifaximin 200 mg *tid* and vancomycin in a study of 20 patients. Response rates were 90% and 100% for rifaximin and vancomycin, respectively. One concern about rifaximin is the potential for resistance, especially given the lack of sensitivity testing outside of a research laboratory. A study of rifaximin susceptibility of 80 different *C. difficile* isolates found resistance among 14 isolates, of which 64% were the epidemic B1 strain^[108]. At this point, it is difficult to ascertain the clinical impact of these findings, especially given the extremely high fecal concentrations achieved with rifaximin. While these small studies suggest a potential application for rifaximin for the initial treatment of CDI, more attention has been placed on a rifaximin “chaser” in the treatment or prevention of recurrent CDI (see section on Recurrent CDI) (Table 3).

Nitazoxanide

Nitazoxanide (Alinia, Romark Laboratories, Tampa, Florida) is a broad-spectrum antiparasitic agent currently approved for the treatment of giardiasis and cryptosporidiosis^[109]. Nitazoxanide is highly active *in vitro* against *C. difficile*. Studies have shown that two-thirds of the drug is excreted in the stool as an active metabolite with activity against *C. difficile* comparable to the parent compound^[110]. Nitazoxanide has been shown to prevent colitis in the hamster model^[111]. Further, nitazoxanide has been shown to very active against a panel of 127 *C. difficile* isolates from the United Kingdom’s *C. difficile* Ribotyping Network at an MIC range of 0.03-0.5 mg/L^[112]. A recent prospective, randomized, double blind study by Musher *et al*^[113] compared metronidazole 250 mg *qid* for 10 d to nitazoxanide 500 mg *bid* for 7 or 10 d^[113]. After 7 d of treatment, the metronidazole response was 82% compared to 90% for nitazoxanide. At 31 d after starting treatment, a sustained response was noted for 58% of patients treated with metronidazole *vs* 66% for the 7-d course of nitazoxanide and 74% for the 10-d course ($P = 0.34$). Musher *et al*^[114] also reported the use of nitazoxanide in 35 patients that failed to respond to metronidazole after 14 d of therapy or who had prompt recurrence on at least two occasions after an initial response. They noted that 74% of patients responded, however, 7 of the 26 recurred, leaving an overall cure rate of 54%.

Most recently, Musher *et al*^[115] completed a randomized, double-blind study of nitazoxanide *vs* vancomycin. After 10 d of treatment, resolution of CDI occurred in 20 of 27 vancomycin patients (74%) and 17 of 22 nitazoxanide patients (77%). For those completing therapy, both treatments had similar times to resolution with response rates of 87% for vancomycin and 94% for nitazoxanide. Subsequently, 2 vancomycin patients and 1 nitazoxanide patients relapsed, leaving a sustained response rate of 78% for vancomycin and 89% for nitazoxanide. The authors noted that, while the small sample size may not have the power to prove noninferiority *vs* vancomycin, as the first randomized control trial their results suggest nitazoxanide may be equally effective.

Fidaxomicin

Fidaxomicin (Dificid®, Optimer Pharmaceuticals, San Diego, CA) is a macrocyclic antibiotic with a narrow spectrum of activity against gram-positive cocci. Fidaxomicin has been 100% protective in a hamster model of CDI^[116]. Importantly, fidaxomicin has been shown to have a comparable safety profile to vancomycin^[117], have undetectable serum levels while achieving high fecal concentrations, averaging greater than 10000 times the MIC for *C. difficile*^[118], a bactericidal mechanism of action^[119], preserve the intestinal microbiome (by sparing of *Bacteroides* sp.), reduce both toxin reexpression and CDI recurrence^[120], and reducing the acquisition of VRE and *Candida* species during CDI treatment^[121,122].

Much of the attention centered on fidaxomicin has been based on findings from two prospective, multicenter, double-blind, randomized Phase III trials demonstrating non-inferiority to vancomycin. The first trial (003 in the United States and Canada) of 629 patients randomized to receive either fidaxomicin 200 mg twice daily (with intervening placebo) ($n = 302$) or vancomycin 125 mg four times daily ($n = 327$), revealed no significant difference in the clinical cure rates: 88.2% for fidaxomicin and 85.8% for vancomycin^[123]. Another interesting observation that arose from the 003 trial was that overall recurrence rates, as defined by the reappearance of more than three diarrheal stools per 24-h period within 4 wk after cessation of therapy, were lower in the fidaxomicin group at 15.4% compared to 25.3% in the vancomycin group ($P = 0.005$). However, recurrence rates with the epidemic B1 strain were similar between fidaxomicin and vancomycin with 24.4% and 23.6% recurrences, respectively. The second Phase III trial (004 conducted at 45 sites in Europe and 41 sites in the United States and Canada) also found fidaxomicin to be non-inferior with cure rates of 91.7% *vs* 90.6% for vancomycin (one sided 95%CI: -4.3)^[124].

Most recently, a post-hoc intent to treat meta-analysis was performed on the results of the combined 003/004 Phase III trials. Of the 1164 patients included, fidaxomicin when compared to vancomycin was associated with a 40% reduction in persistent diarrhea, recurrence, or death through day 40 (95%CI: 26%-51%; $P < 0.0001$)^[125]. Subgroup analysis limited to the epidemic B1 strain, revealed

a 22% non-significant reduction in persistent/recurrent diarrhea (95%CI: 44% reduction to 8% increase, $P = 0.14$). The authors point out that with only 292 of 814 strains testing positive for B1, the results from this analysis are too underpowered to conclude fidaxomicin lacks beneficial effect for the B1 strain.

One important aspect of fidaxomicin remains, cost. At \$168 per 200 mg tablet, a twice-daily 10-d treatment course costs \$3360 for a 10 d course^[86]. The pharmaceutical company selling this medication has recently developed several strategies to help reduce the patient cost if the medication is needed.

COMPLICATED CDI

Complicated CDI is defined in the 2010 IDSA guidelines as severe CDI plus intensive care unit (ICU) admission, need for colectomy, ileus, toxic megacolon, hypotension or colonic perforation^[31]. The 2013 ACG guidelines define severe complicated CDI as any of the following: admission to the ICU, hypotension with or without the need for pressors, fevers > 38.5 °C, ileus or significant abdominal distension, mental status changes, WBC ≥ 35000 cells/mm³ or < 2000 cells/mm³, serum lactate > 2.2 mmol/L, or end organ failure^[71].

For severe complicated CDI, the IDSA guidelines recommend high dose oral vancomycin 500 mg *qid* (by nasogastric tube, if necessary) and/or metronidazole 500-750 mg q8h intravenously. Metronidazole and vancomycin combination has been shown to be synergistic *in vitro* for 68% of *C. difficile* isolates^[99]. Apparently, the increased dose of vancomycin for complicated CDI is related to a delay in attaining adequate fecal levels with 125 mg *vs* a higher dose when given orally^[126]. For complete ileus, metronidazole intravenously plus vancomycin administered by retention enema is recommended. The critical point is that the vancomycin, needs to be retained and distributed in the colon to be effective. Specific orders should detail the administration lest the vancomycin be administered as a plain enema, providing no benefit for the patient and creating a hazard for nursing staff. The vancomycin should be administered using a # 18 French Foley catheter with a 30 mL balloon. The Foley catheter should be inserted into the rectum, the balloon inflated and the vancomycin instilled. The catheter is then clamped; some authors recommend turning the patient on their right side to assist distribution of the vancomycin solution throughout the colon. After 60 min, the balloon is deflated and the catheter is removed^[127]. Because there have been no controlled trials of vancomycin by intracolonic installation, the optimal dose and interval are unclear. Apisarnthanarak *et al*^[128] reported a descriptive case series of nine consecutive patients treated with intracolonic vancomycin as adjunctive therapy for severe CDI. Eight of nine patients had failed five to 7 d of standard therapy for CDI and had evidence of a severe ileus with resultant cessation of diarrhea. Further evidence of the severity of the colitis was suggested by the fact that six of the nine patients were hypotensive at the time CDI

was diagnosed. They administered intracolonic vancomycin 0.5-1.0 g in one to two liters of normal saline as a retention enema. Because this was a retrospective collection of cases, the dosing interval and duration of therapy were variable. Two patients received intra-colonic vancomycin at 4 h intervals, two at 6 h, two at 8 h and three at 12 h. The authors noted complete resolution of colitis in eight of nine patients with no relapses and no surgical interventions. As a note of caution, four patients were colonized with VRE prior to the intracolonic vancomycin and two of these 4 developed VRE bacteremia. However, none of five patients who were not colonized with VRE before therapy developed subsequent colonization.

The 2013 ACG guidelines offer slight changes in recommend therapy for severe and complicated CDI^[71]. Initial therapy for severe and complicated CDI without any significant abdominal distention is vancomycin orally 125 mg *qid* plus intravenous metronidazole 500 mg *tid*. For severe and complicated CDI with ileus, toxic colitis, or significant abdominal distention, the recommended therapy is vancomycin delivered both orally 500 mg *tid* and per rectum 500 mg in volume of 500 mL *qid* plus intravenous metronidazole 500 mg *tid*. Of note, the author's discuss the potential for development of electrolyte imbalances with the use of saline for delivery of the vancomycin enema, in particular hyperchloremia. In such a situation, the authors propose the use of Ringer's Lactate, which contains a lower concentration of chloride^[71].

Tigecycline is a broad-spectrum glycylicycline antibiotic with reportedly low MIC values against *C. difficile*, along with evidence that it does not promote growth or toxin production in both a mouse and human model^[82,129,130]. To date no clinical trials have been performed on the use of tigecycline; however, several case reports have reported the successful use of IV tigecycline in severe or severe complicated CDI in which patients failed prior treatment with metronidazole and vancomycin^[131]. There has also been a case report noting the successful treatment of severe refractory CDI using a combination of tigecycline (50 mg IV every 12 h for 10 d) and rifaximin (400 mg twice daily for 17 d)^[132].

SURGICAL INTERVENTION

Failure to respond to maximal medical management, including unrelenting sepsis, cecal dilatation greater than 10 cm and bowel perforation have been considered indications for surgical intervention. In large series, 0.4%-3.6% of patients have required surgery, with an overall mortality of 30%-80%^[133-135]. Series of severe CDI repeatedly emphasize how difficult the diagnosis may be. In a report of 14 patients requiring surgical intervention, only 50% had a preoperative diagnosis of CDI, because they required laparotomy before results of *C. difficile* testing became available^[136]. Of note, the survival was better (86% *vs* 33%) in those with a preoperative diagnosis of CDI, which may have been due to the surgeon being more aware of the need for a total colectomy. Longo *et al*^[137] noted some of the difficulties

in the diagnosis of severe CDI in a series of 67 patients who required colectomy; 37% of the patients had no history of diarrhea, 45% presented in shock and 64% presented as an acute surgical abdomen^[137]. Dallal *et al*^[17] in a review of 64 patients who required a colectomy or died directly from CDI noted that 20% of the patients presented without diarrhea due to ileus. Of note, in this study 35% of diagnoses of severe CDI were found only at autopsy and the author suggested that a significant number of ICU deaths from "sepsis" may actually be CDI. Overall, 13% were *C. difficile* EIA toxin assay negative. Longo *et al*^[137] found false-negative *C. difficile* cytotoxin assay in 18% of CDI severe enough to require colectomy^[17]. Better diagnostic accuracy for severe CDI has been reported for the abdominal CT (89%-100% positive) and colonoscopy (100% positive)^[17,136,137]. Of note, intravenous and oral contrast were not required for a correct diagnosis with CT of the abdomen. Flexible sigmoidoscopy was falsely negative in 25% (2 of 8), one due to poor prep and one due to right sided colitis^[17].

Lamontagne conducted a retrospective review of 165 cases of CDI which required ICU admission during the epidemic in Quebec between January 2003 to June 2005^[138]. Of note, 24% of these ICU admissions resulted from relapse of previously diagnosed CDI, confirming how serious relapses can be. Predictors of 30 d mortality included a WBC of greater than 50000, age greater than 75-year-old, requirement for vasopressors and immunosuppression. Thirty eight patients underwent colectomies, 15 because of shock despite vasopressors, 11 with toxic megacolon, 10 with a lack of response to medical therapy and 2 because of perforation. The authors noted a significant decrease in mortality in those who had a colectomy *vs* those who were treated medically, with an adjusted odds ratio of 0.22, suggesting a 78% reduction in mortality. The major surgical benefit was found in those patients greater than 65 years of age who were immunocompetent with a WBC greater than 20000 and a lactate between 2.2 and 4.9 mm per liter. No surgical benefit was found in those with a white blood cell count less than 20000, less than 65 years of age and those with a normal lactate.

Recent surgical series have revealed conflicting data on which surgical procedure is preferred. Koss *et al*^[136] presented a retrospective review of 14 patients who required surgery. The indications were systemic toxicity (*n* = 10), progressive toxic colonic dilatation (*n* = 4), and one with both colonic dilation and bowel perforation. Overall, mortality was 36%. Of those who underwent a total colectomy, mortality was 11% compared to 100% mortality in those whose surgical procedure was limited to a left hemicolectomy. Of note, at the time of surgery the exterior surface of the colon frequently was noted to be unremarkable, but all were distended and edematous. Longo *et al*^[137] conducted a population based study from all 159 Department of Veterans Affairs Hospitals of patients who required colectomy for fulminant CDI between 1997 and 2001. For the 67 patients, the postoperative 30 d mortality was 48%. Of those who underwent

segmental colectomy, the mortality was 14%, while the mortality was 57% for those who underwent total colectomy (80% of the cases). At surgery, 58% of the patients were noted to have perforation or colonic infarction. As opposed to the Koss study, 12 of 14 patients who underwent hemicolectomy survived, probably because the colitis was restricted to the involved segment. A study by Dallal *et al*^[17] confirms the possibility of segmental colectomy. This study was a retrospective review of 64 patients who died or underwent colectomy for pathologically proven CDI drawn from 2334 hospitalized patients with CDI, who were hospitalized between January 1989 and December 2000. There were 44 patients who required surgical intervention. Of those undergoing a right hemicolectomy, 100% survived. This was a select subgroup of 4 patients, all of whom had intraoperative colonoscopy confirming the fact that the colitis was restricted to the right hemicolon. Overall, in this study 89% of patients underwent a total colectomy, with a mortality of 63%. Most predictive of perioperative mortality was vasopressor requirement preoperatively, which increased postoperative mortality by four-fold. The authors suggested that hypotension requiring vasopressors may be too late a point for successful intervention. They noted that a white blood cell count greater than 30000 with a left shift almost always preceded the onset of shock and may be used as an early indicator of fulminant CDI, which may require surgical intervention.

Most recently, Neal *et al*^[39] have studied an alternative to total colectomy advocating a diverting loop ileostomy with colonic lavage. They studied 42 patients with severe, complicated CDI. Their surgical approach was creation of a laparoscopic loop ileostomy followed by intraoperative colonic lavage with a warmed polyethylene glycol/electrolyte solution thru the ileostomy. They also performed postoperative antegrade instillation of vancomycin solution through the ileostomy. Compared to well matched historical controls mortality was reduced from 50% to 19%. Delayed reversal of the ileostomy, after recovery from the acute episode, resulted in preservation of the colon in 93% of cases. Based on these improved outcomes, they suggested that all patients with severe CDI should be considered for surgical management.

The 2013 ACG guidelines also defined signs and symptoms in complicated CDI which warrant surgical consultation, including: hypotension requiring vasopressor therapy, clinical signs of sepsis and organ dysfunction, mental status changes, WBC 50000 cells/mm³, lactates 5 mmol/L, or complicated CDI with failure to improve on medical therapy after 5 d^[71]. The suggested operative management is subtotal colectomy and end-ileostomy, which has been associated with reduced mortality in fulminant CDI^[40].

RECURRENT CDI

Recurrence of CDI after initial successful treatment has been a significant problem. On average, recurrence can be expected in 20%-30% of cases. Once there has been

an initial recurrence, relapse may occur in up to 65% of patients^[141]. Risk factors associated with recurrence include older age (greater than 65), longer hospital stays (greater than 16 d), the presence of comorbidities and another course of antibiotics^[142,143]. The new epidemic strain has been associated with an even higher rate of recurrence; rates may be as high as 47%^[143]. Some authors have postulated that recurrence may be related to inability to mount an adequate antibody response as manifested by low IgG directed against toxin A^[50].

The severity of recurrent episodes of CDI should not be underestimated. Pépin *et al*^[144] reviewed the outcomes of a first recurrence of CDI with the epidemic strain during the Quebec outbreak^[144]. They noted that 11% of patients with a first recurrence had at least one severe complication of CDI, including shock, colectomy, megacolon, perforation or death within 30 d. Complicated recurrent CDI was strongly associated with three factors: older age, elevated white blood cell count and renal failure. For those patients greater than 65 years of age, 13% developed recurrent CDI that was severe *vs* 7.5% for those 18-64 years of age. Subgroup analysis revealed recurrent CDI with a white blood cell count > 20000 was associated with a 38.9% incidence of complicated CDI *vs* only 10.6% when the white blood cell count was 10000-19000^[141]. The long term negative impact of CDI was also explored by Musher *et al*^[145], who reviewed outcomes for 103 patients who were considered to be cured without recurrence at 90 d after completion of therapy. They found that 22% of these patients developed recurrent diarrheal disease more than 90 d after the initial episode, 83% of whom were toxin positive.

Clinical approach to recurrent CDI

Most authors have recommended, repeating a course of the antibiotic used in the initial treatment, usually metronidazole, as the first step in the treatment of a recurrence. This sentiment is backed by the 2013 ACG guidelines^[71]. For additional recurrences, a combination of a prolonged taper of the antibiotic with oral vancomycin, followed by pulsed dosing is often used. The original reports of tapered dosing utilized oral vancomycin as the preferred drug, since levels in stool are high, over 1000-fold higher than the level needed to inhibit *C. difficile* and do not decrease as diarrhea resolves^[146]. Early suggested courses were vancomycin 125 mg *qid* for 7 d, tapering to 125 mg *bid* for 7 d, then daily for 7 d^[147]. After the taper has been completed, pulsed dosing can begin. The pulsed dosing of vancomycin is thought to allow time for germination of residual spores during the days off antibiotics, with killing of the vegetative form when the antibiotic is given again. Although there is no standard well studied pulsing regimen, one suggestion has been to give vancomycin 250 mg every 2 or 3 d for 3 wk^[148]. Bartlett has noted that he always utilizes a 6 wk course as this is the approximate time for return of normal flora^[149]. More recently, some authors have recommended continued lengthening of the pulsing interval until the vancomycin is given only once every

10 d^[150]. Rare patients may require chronic pulsed dosing every 3-4 d, relapsing each time they try to lengthen the interval or discontinue the vancomycin. The 2013 ACG guidelines recommend a simplified pulsed dosing only regimen with vancomycin 125 mg orally every 3 d for 10 doses without tapering of the vancomycin (Conditional recommendation, low-quality evidence). For patients with more than 3 recurrences, the ACG guidelines now suggest considering fecal microbiota transplant (FMT)^[71].

A new approach to relapsing CDI using a rifaximin “chaser” has been described. Johnson *et al*^[151] conducted an empirical trial of a 14-d course of rifaximin following a variety of different treatments, mainly using vancomycin, for the treatment of recurrent CDI. The authors studied eight women from their clinical practices, who had suffered from 4-8 episodes of CDI. The patients ranged in age from 43-88 years of age, with six of the eight being greater than 65 years old. The onset of recurrences varied from 1-59 d (mean of 10.5 d) after completion of treatment for CDI. For five of the patients, recurrences were as early as 1 d after treatment ended. The patients had been treated with 79-372 d with a variety of different treatments including metronidazole, vancomycin tapered and/or pulsed, probiotics and vancomycin plus rifaximin. Rifaximin was used as a “chaser” when the patients were asymptomatic, immediately at the end of the vancomycin treatment. Six of the patients received 400 mg *bid* for 14 d. Rifaximin was well tolerated without side effects. Seven of the eight patients had no further recurrence, with follow up that varied from 51-431 d. The one patient who was noted to have a recurrence was immediately retreated while symptomatic (the only deviation from their basic protocol) for 14 d. This patient was noted to develop resistance to rifaximin. More recently, a randomized, double-blind, placebo controlled trial was conducted on the efficacy of the rifaximin “chaser”. Patients completing a standard antibiotic regimen for CDI were assigned to receive either placebo or 400 rifaximin mg 3 times daily for 20 d. Recurrent diarrhea occurred in 49% of placebo patients and 21% of rifaximin patients ($P = 0.018$). Actual CDI recurrence rates, as assessed by positive toxin assay, were 31% (11 of 35) in the placebo group and 15% (5 of 33) in the rifaximin group ($P = 0.11$)^[152]. Although the difference between rifaximin and placebo was not significant, the study was underpowered to exclude a statistically significant difference.

Rifaximin as a stand-alone treatment for recurrent CDI has also been a focus of interest. A retrospective study examining 32 patients with recurrent CDI who had undergone an average of 4.4 antimicrobial treatment courses for CDI, found treatment with 400 mg twice-daily rifaximin for 14 d was successful in preventing relapse in 53% (17 of 32) of cases^[153]. Interestingly, the authors empirically noted the success of rifaximin treatment appeared to be related to the MIC of the particular isolate, and that B1 isolates (30% in the study) had the highest MICs among those tested. There was, however, no statistically significant difference ($P = 0.11$) in relapse rates among those with the B1 strain, 42% (8 of 19) compared

to 53% overall. Among the proposed mechanisms for this increased efficacy in treatment and prevention of recurrent CDI are rifaximin’s anti-inflammatory properties; rifaximin has been shown to induce epithelial cell changes that alter bacterial attachment and internalization, while also reducing the release of inflammatory cytokines^[154]. Lastly, with the increasing prevalence of the B1 strain, clinicians should be aware of the potential for rifaximin resistance given the lack of commercial testing availability. At this point, however, it is difficult to ascertain the clinical impact of these findings, in particular when rifaximin has been noted to achieve such high fecal concentrations. The most recent consensus from the ACG notes that there is no convincing evidence at this point in time for the use of rifampin or rifaximin in the treatment of recurrent CDI^[71].

Fecal Transplantation

Rapidly emerging onto the scene, FMT represents the most promising candidate among non-antibiotic treatment options for patients suffering from multiple relapses or recurrences. Borody *et al*^[155] in an article subtitled “Toying with Human Motions”, reviewed the use of the ultimate natural probiotic, transplanted human stool. Although noted to be “aesthetically unpleasing”, the use of stool transplant from one individual, usually a close relative, to the patient with relapsing CDI has had a high success rate. They reviewed the published literature of the use of fecal transplantation in 84 patients, noting a rapid response without recurrence in 86%. The authors also reviewed the use of stool transplantation for inflammatory bowel disease and irritable bowel syndrome and provide a detailed method for donor screening, preparation and administration.

Since then, the potential impact of FMT in the treatment of recurrent CDI has been more clearly elucidated and now represents a focal point of ongoing research. A systematic review of published studies between 2000-2011 identified 124 patients in seven studies with recurrent or refractory CDI who underwent FMT^[156]. Among these patients, 83% reported immediate improvement following the procedure and further remained diarrhea free for months to years. The results from early studies all varied in protocol for pre-transplant antibiotic use, methods of delivery, amount of material delivered, long-term follow up, and none were controlled trials. Nonetheless, this systematic review of the early studies highlights the potential impact of fecal transplant for the treatment of recurrent or refractory CDI.

Brandt *et al*^[157] in a multicenter long-term follow up study of 77 patients undergoing colonic FMT for recurrent CDI monitored both primary and secondary cure rates for individuals undergoing the procedure. A primary cure was defined as resolution of symptoms without recurrence within 90 d of treatment, while a secondary cure was resolution of symptoms with one further course of vancomycin. Follow up revealed a primary cure rate of 91% and a secondary cure rate of 98%. Of interest, the study addressed, through patient surveys, one of the

major drawbacks to FMT: the fact that the procedure is inherently aesthetically unpleasing. The survey results of these 77 patients revealed that 97% of patients would undergo another FMT for a CDI recurrence and that 53% of the patients would choose FMT as their first treatment option. This represents a promising finding that the unappealing nature of FMT may eventually be overcome by the predictable efficacy of FMT for patients facing the debilitating consequences of multiple CDI recurrences.

One of the most pressing question that has not been fully elucidated about FMT remains, how does it compare with other treatment options? While no double-blind randomized controlled trials have been completed to this date, new evidence has emerged from an interim analysis of an open-label, randomized, controlled trial in the Netherlands^[158]. This study of recurrent CDI infection assigned patients to receive one of three treatments: initial vancomycin regimen (500 mg four times daily for 4 d) followed by bowel lavage and subsequent nasoduodenal infusion of donor feces, standard vancomycin regimen (500 mg four times daily for 14 d) with bowel lavage, or standard vancomycin regimen alone. With a primary endpoint measured as cure without relapse within 10 wk, the overall cure rate with FMT was 94% (15 of 16). Of these 16, 13 achieved cure on their initial treatment, with 2 more achieving cure after treatment with a different donor stool. This was compared to 31% (4 of 13) in the vancomycin alone group and 23% (3 of 13) in the vancomycin and lavage group. Lastly, post-FMT analysis of patient feces showed increased bacterial diversity, similar to that of the healthy donors.

Overall, the current literature suggests a promising future for the application of FMT in the treatment of recurrent CDI, however, some issue still remain, namely, the lack of a consensus protocol and viable sources of the donor feces. The majority of early FMT procedures utilized donor feces from spouses, intimate partners, or close family members, while potentially safer, also possesses many practical challenges in gathering the sample and administration. New evidence suggests that there may be equally efficacious alternatives to these close family donors. Between 2004-2010, a group of 32 patients with relapsing CDI at the Stockholm South General Hospital underwent FMT by either enema or colonoscopy using a fecal transplant suspension reconstituted from a single donor specimen obtained in 1994^[159]. Among the patients, 69% (22 of 32) had a durable cure. These findings suggest that, in the future, it may be possible to establish a donor bank of prescreened individuals or specimens, thereby improving the ease, efficiency and safety of the process. Perhaps even more promising are results from a proof-of-principle study demonstrating that a stool substitute was capable of curing an antibiotic-resistant hypervirulent strain of *C. difficile*, ribotype 078. Researchers in this “RePOOPulating” study extensively cultured a stool sample from a 41-year-old healthy female donor to make a synthetic sample consisting of 33 different purified isolates, which was then used to treat 2 patients who had failed traditional therapy^[160]. Both patients

returned to normal bowel patterns within 2-3 d and remained symptom free at 6 mo. The authors of the study highlight numerous potential benefits of synthetic stool over donor stool including, the ability to control and alter the exact bacterial composition, the ability to replicate the procedure with an identical specimen, increased stability of donor stool sample, improved safety from knowledge of the exact sample composition, and the ability to adjust the sample for antimicrobial sensitivity.

A systematic review and meta-analysis by Kassam *et al*^[161] provides new insight into variation between methods of FMT delivery and from donor type. This review of 11 studies including 273 CDI patients treated with FMT, performed a subgroup analysis comparing lower gastrointestinal delivery with upper gastrointestinal delivery. Lower gastrointestinal delivery (colonoscopy or enema) had clinical resolution rates of 91.4% (203/222) compared to upper gastrointestinal delivery (nasogastric/nasojejunal tube and gastroscopy) resolution rates of 82.3% (42/51). Further comparison between anonymous *vs* patient selected donors did not reveal a significant difference in clinical outcomes regardless of the follow-up time.

In April 2013, the United States FDA determined that FMT is a biologic product and drug that is regulated by the FDA. The FDA ruled that an investigational new drug (IND) application, a cumbersome and time consuming process, was needed for the use of FMT for any indication. In response to vocal and unified opposition by the gastrointestinal specialty societies, the FDA rapidly reversed this requirement and provided that the “treating physician obtains adequate informed consent from the patient or his or her legally authorized representative for the use of FMT products. Informed consent should include at a minimum, a statement that the use of FMT products to treat *C. difficile* is investigational and a discussion of its potential risks”^[162].

In conclusion, as more evidence continues to become available, fecal transplantation is becoming an increasingly viable option for the treatment of recurrent or relapsing CDI, in particular given the recent recommendation for FMT to treat 3 CDI recurrences in the 2013 ACG guidelines^[71]. While there remains no optimal protocol for administration or consensus on the ideal source of the transplant sample, future studies, including an NIH-funded blinded RCT and the pending FDA IND process, may provide valuable insight for these questions.

ADJUNCTIVE THERAPY: INTRAVENOUS IMMUNOGLOBULIN AND ANIONIC BINDING RESINS

There has been significant interest in the use of intravenous immunoglobulin (IVIG) to treat severe refractory and recurrent CDI. This interest is based upon the fact that development of *C. difficile* antitoxin antibody has been associated with protection from the development of CDI after colonization with *C. difficile*^[50,163]. Small se-

ries and case reports have suggested a possible response to IVIG^[164,165]. Of note, all immunoglobulin lots tested contained IgG against toxins A and B and were capable of neutralizing cytotoxicity in one series^[166]. However, with the cost of IVIG approaching \$10000 for an individual treatment course, proof of efficacy is important. McPherson conducted a retrospective review of 14 patients with either severe, refractory or recurrent CDI^[167]. They used an IVIG dose of 150-400 mg/kg. Nine of these 14 patients responded in a median of 10 d, a relatively slow response, and 3 of these 9 patients had recurrent CDI after initial resolution. The most instructive study on the use of IVIG for severe CDI was conducted by Juang *et al*^[168] at the University of Pittsburgh Medical Center. Because of the severity of CDI at their institution, a committee developed eligibility criteria for IVIG which was then used in a prospective manner to choose patients eligible for IVIG. Eighteen patients received IVIG at a dose of 200-300 mg/kg and these patients were pair matched by propensity scoring with other patients with severe CDI. There was no difference in mortality (3 patients in each group), colectomy (3 patients in each group) or length of stay. Although this study is not definitive, the results do not support the use of IVIG for severe CDI. The 2013 ACG guidelines addressed the use of IVIG in the treatment of recurrent CDI, and concluded that it does not have a role as sole therapy; however, they noted that it may be helpful in patients with hypogammaglobulinemia. This recommendation is based on the predisposition for CDI in patients following solid organ transplantation.

Anion binding resins, like cholestyramine and colestipol, have been used to treat CDI. The non-absorbable resin binds to *C. difficile* toxin removing 99% of the cytotoxic activity^[169]. However, concerns have been raised about the use of these toxin-binding agents, because they also bind to vancomycin^[170]. Thus, combination therapy should be used carefully, if at all, with separation of the anion binding resin and vancomycin by at least 2-3 h. Other sources have recommended giving the vancomycin either 1 h before or 4-6 h after the cholestyramine dose^[171].

PREVENTATIVE THERAPY

One of the most important issues related to CDI from the perspective of the practicing clinician is the approach to the patient with a known history of *C. difficile*, who requires a subsequent course of antibiotics for an infection such as urinary tract infection or pneumonia or who cannot stop the antibiotics which induced the original episode of CDI. The use of metronidazole or vancomycin in this setting can be referred to as preventative therapy. Unfortunately, there is no data from systematic studies of the use of preventative therapy. However, Miller noted that “on the basis of no prospective evidence but, often, a large body of clinical experience, some clinicians now start a parallel course of oral metronidazole or vancomycin along with treatment with the

potentially CDI-inducing antimicrobial, to prevent the appearance of symptomatic CDI”^[172]. He goes on further to note “that despite absence of guidelines for this approach, there is remarkable homogeneity in the approaches used by most clinicians, in that clinicians who practice this prophylactic strategy use oral metronidazole or vancomycin during the entire course of antimicrobial therapy and for an additional 7 d after the end of the administration period”. This preventative approach to *C. difficile* seems an intuitively reasonable approach, which can be utilized pending results of future clinical trials that would validate its effectiveness.

Probiotics

Probiotics, defined by the World Health Organization as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host,” have seen a recent surge in interest and use^[173]. Current estimates for sales of probiotics, as both supplements and foods, was estimated to be \$770 million in the US alone for 2012, with worldwide sales at \$2.25 billion, a 79% increase since 2010^[174,175]. Further, recent estimates have projected worldwide spending on probiotics to reach \$4 billion annually by 2016^[176]. Despite all the interest and sales of probiotics, their utilization for the prevention or treatment of CDI remains controversial and unproven.

Heavy marketing campaigns and choice labeling of products have helped fuel the dramatic growth of the probiotics markets. Further helping to shape the consumer image, clinical evidence for the use of probiotics in the prevention of antibiotic-associated diarrhea (AAD) appears promising. Systematic reviews and meta-analyses were completed for the use of probiotics in any AAD. In one analysis, the pooled results from 63 randomized control trials revealed a RR of 0.58 (95%CI: 0.50-0.68, $P < 0.001$) with an number needed to treat (NNT) of 13^[177]. Among those studies, a subset of 14 were randomized controlled trials for the prevention of CDI and pooled analysis revealed a RR of 0.29 (95%CI: 0.17-0.48, $P < 0.001$) with an NNT of 25. However, it was noted that poor adherence and limited reporting of the number of samples tested may have skewed the results. Another meta-analysis of 34 studies including 4138 patients showed a 0.53 relative risk for the development of antibiotic-associated diarrhea in the probiotics *vs* the placebo group (95%CI: 0.44-0.63), with an NNT of 8^[178]. Importantly, the authors of this study chose to omit any trials involving the use of probiotics for the prevention or treatment of CDI.

Although some may wonder why a variant of baker's yeast, which is not a part of the normal microflora of the gut, would be effective in preventing or treating CDI, there is some theoretical support for the use of *Saccharomyces boulardii*, which has been shown to prevent toxin A binding and also to inactivate toxins A and B by proteolytic digestion^[179,180]. Further, in the hamster model, *S. boulardii* has been shown to be effective in preventing deaths from acute disease^[181]. Other mechanisms

by which *Saccharomyces* may prevent CDI include inhibition of *C. difficile* adhesion, cellular protection from histologic damage and inhibition of pro-inflammatory cytokine gene expression^[182-184]. In fact, Czerucka used the term “immunobiotic” to describe *S. boulardii*^[182].

The clinical efficacy of *S. boulardii* has shown mixed results in a number of reviews and meta-analyses of randomized, controlled trials of CDI. Dendukuri *et al*^[185] concluded that the “studies conducted to date provide insufficient evidence for the routine clinical use of probiotics to prevent or treat CAD”. Szajewska *et al*^[186] found a reduction in antibiotic associated diarrhea of 57%, but no reduction in CDI. Katz in reviewing the use of probiotics for the prevention of CDI developed a proposed guideline which noted no evidence to support efficacy in the primary prevention of *C. difficile*, but suggested that “*S. boulardii* can be used to decrease recurrences of *C. difficile*”^[187]. McFarland *et al*^[188] found that *S. boulardii* was not effective in preventing recurrence after an initial episode of CDI. The authors did find, however, a 50% reduction among patients who had had a previous recurrence. A second study of the use of *S. boulardii* in patients with recurrent CDI confirmed a decrease in recurrences, but only when combined with a high dose of oral Vancomycin (500 mg *qid*). There was no reduction in recurrent CDI with lower doses of vancomycin or metronidazole^[189]. McFarland later conducted a meta-analysis and noted that “from six randomized trials, probiotics had significant efficacy for CDD”^[190]. Unfortunately, he combined the 2 studies using *S. boulardii* with studies using a variety of *Lactobacillus* preparations, which could lead to significant misinterpretation of the data. As noted by Gerding; “A recent meta-analysis suggested that probiotics are effective; nevertheless, because of the heterogeneity of study methods and patient populations, it is not scientifically possible to conduct a meta-analysis of findings in the probiotic literature”^[191].

Another aspect of *S. boulardii* in the treatment or prevention of CDI is the risk for adverse events. While generally considered safe, there have been increasing reports of fungemia due to *S. boulardii*, especially in those with intravascular catheters and antibiotic therapy. Of the 37 patients with *S. boulardii* fungemia in one report, use of *S. boulardii* as a probiotic was considered to be the source of infection in 64%^[192]. Of note, an additional five cases were reported in patients who were not receiving a probiotic. In these cases, there was evidence of healthcare associated acquisition from other patients who were being treated with *S. boulardii*. The authors suggested that special caution should be taken with probiotics in critically ill and immunocompromised patients. Segarra-Newnham in a review of the use of probiotics for CDI concluded that “there were numerous unanswered questions”^[193]. She also noted that “given the potential for complications in debilitated immunosuppressed patients, the risk may outweigh the benefits”. Czerucka went even further suggesting “the presence of indwelling catheters is a contraindication for the administration of *S. boulardii*”^[182]. Further evidence that the safety of probiotics cannot

be assumed comes from a recent double blind, placebo controlled trial of a multispecies probiotic (mostly *Lactobacillus* sp. and *Bifidobacterium* sp.) in the treatment of severe acute pancreatitis^[194]. In the probiotic group, 16% of patients died *vs* 6% in the placebo group. Nine patients (8 with fatal outcomes) developed bowel ischemia. Eight involved the small bowel. The authors concluded “probiotics can no longer be considered to be harmless adjuncts to enteral alimentation, especially in critically ill patients”.

While the early literature focused primarily on the application of *S. boulardii* for prevention and treatment of CDI, more recently there has been a shift towards the use of *Lactobacillus* sp. preparations, such as Lactinex (Becton Dickinson, San Diego, Ca) or *Lactobacillus* GG (Culturelle, Bloomfield, Ct.). Early support for *Lactobacillus* came from a randomized, double-blind, placebo controlled trial, published by Hickson *et al*^[195] reporting the use of Actimel (Danone, France) in the prevention of CDI. In the United States, a similar product would be DanActive by Dannon. No patients in the probiotic group developed CDI, while 17% (9 of 53) in the placebo group developed CDI ($P = 0.001$). The authors concluded that “this has the potential to decrease morbidity, health care cost, and mortality if used routinely in patients aged over 50”. Unfortunately, the article by Hickson *et al*^[195] adds little substantive new data to the argument, because of its very poor generalizability. The extraordinarily high exclusion rate resulted in only 6.4% of screened patients being evaluable in the efficacy analysis. Of the 1760 patients assessed for eligibility, 1625 (92%) were excluded and a further 148 refused to participate, leaving only 135 patients to be entered in the study. Of these, 16% were lost to follow up, leaving only 6.4% of the patients eligible for analysis. As noted in a Letter to the Editor “I was astounded to read in the study method that Hickson *et al*^[195] had excluded high risk antibiotics (as well as some misclassified low risk antibiotics). To do so is akin to performing a trial of an agent that claims to prevent type 2 diabetes, but excluding obese patients”^[196].

Since that time a significant number of trials have been conducted with varying levels of support for probiotics. A 2008 Cochrane Review of the use of probiotics in the treatment of CDI in adults identified 4 randomized control trials meeting inclusion criteria, all of which were noted to be small in size and have methodological problems^[197]. Of these studies, only one was found to have a statistically significant benefit for probiotics, the previously mentioned study by MacFarland *et al* on *S. boulardii*. The most promising evidence to date for probiotics comes from a systematic review and meta-analysis involving pooled data from 20 studies and 3818 patients, which revealed a pooled RR of 0.34 (95%CI: 0.24-0.49), in other words a reduction in the incidence of CDI of 66%^[198]. Calculating the optimal information size, which is the number of patients required for an adequately powered study, using the worst-plausible-assumption and applying a 5% population incidence of antibiotic-associated CDI, the authors suggest this moderate-quality

evidence predicts probiotics prophylaxis would prevent 33 episodes of CDI per 1000 persons. Additionally, while their study indicated a larger risk reduction in the use of multiple species preparation over single species, this was likely accounted for by heterogeneity between studies.

The newest evidence surrounding the use of probiotics for prevention of CDI comes from the PLACIDE trial, a multi-center, randomized, double-blind, placebo controlled trial for the use of lactobacilli and bifidobacteria in the prevention of AAD and CDD, for which inpatients over the age of 65 were randomized to either a microbial preparation or placebo. Relative risks between the groups were RR 1.04 for AAD (95%CI: 0.84-1.28) and RR 0.71 for CDD (95%CI: 0.34-1.57)^[199]. The authors concluded no evidence that multi strain preparation of lactobacilli and bifidobacteria was effective in the prevention of AAD or CDD.

Overall, interpretation of results from probiotic studies present many challenges. Lawrence, who conducted a study of a *Lactobacillus* preparation to prevent recurrent CDI noted that a number of problems were faced in attempting to determine the efficacy of probiotics^[200]. He noted the high percentage of patients receiving systematic antibiotics (66.7%) and a high number of patients receiving gastric acid suppression, both of which might interfere with the efficacy of a probiotic. Other problems with studies of probiotics for the prevention of recurrences of CDI, include the lack of randomization of the type or dose of the antibiotic used with the probiotics, which may have altered the outcomes. Doses of probiotics were not standardized and may have been too small or the preparations may have become nonviable after manufacture or may have a different strain than advertised. A number of authors have found that the number of colony forming units can be much lower than what is advertised on the label^[201]. The 2013 ACG guidelines concluded that there is insufficient evidence that probiotics prevent CDI (Strong recommendation, low quality evidence)^[71]. In summary, there is much more enthusiasm than data for the use of probiotics in the prevention or treatment of CDI.

The newest approach in the prevention of CDI focuses on targeting the infective spore to prevent germination into the vegetative toxin producing form. Since only the vegetative form produces toxin, theoretically prevention of spore germination would prevent symptomatic infection. Howerton *et al*^[202] demonstrated that a cholate meta-benzene sulfonic derivative (CamSA) is a strong competitive inhibitor of taurocholate-mediated *C. difficile* spore germination. Subsequently, they administered a single 50 mg/kg dose of CamSA to mice infected with *C. difficile* spores and were able to prevent any signs of CDI^[203]. The authors also noted that CamSA gave complete protection against an “unnaturally massive” *C. difficile* spore infection, equivalent to human ingestion of hundreds of grams of infective spores. While still early in the investigative process, CamSA represents an entirely new approach to preventing CDI.

FUTURE TREATMENT OPTIONS FOR CDI

With the increased virulence and decreased response to standard treatment, combined with an increase in recurrences, both due to relapse and acquisition of epidemic strains in hospitals, the need for newer approaches to the treatment of CDI becomes even more important. There are a number of exciting new antibiotics being studied for treatment of CDI, including rifalazil, ramoplanin and non-antibiotic based approaches, such as tolevamer, monoclonal antibodies against toxin A, and a vaccine.

Rifalazil

Rifalazil is an experimental, absorbable antibiotic in the rifamycin class, related to rifampin with a broad spectrum of activity against a wide range of organisms, including *Mycobacterium tuberculosis*, *Chlamydia*, *Helicobacter pylori* and *C. difficile*^[204]. Testing of rifalazil against 110 isolates of *C. difficile* collected from 1983-2004 revealed excellent activity with an MIC 90 of 0.03 µg/mL, with only one isolate from the United States found to be resistant^[82]. In a study of *C. difficile* in the hamster model, all animals treated with rifalazil or vancomycin were protected from disease. Histologically, the rifalazil treated animals had less edema and neutrophil infiltration than the vancomycin treated animals. When vancomycin was discontinued, 65% of the animals developed disease, while none of the rifalazil treated animals had positive toxin assays or disease^[202]. Future trials of rifalazil in humans with CDI are eagerly anticipated, especially given the low relapse rate in animal models.

Ramoplanin

Ramoplanin is an experimental broad spectrum, non-absorbable glycolipodepsipeptide. In the same study mentioned above, all isolates of *C. difficile* were sensitive to ramoplanin with an MIC 90 of 0.5 µg/mL^[204]. In another study, which included *C. difficile* isolates with reduced susceptibility to vancomycin and resistance to metronidazole, no resistance was found to ramoplanin^[205]. In a hamster model of CDI, both ramoplanin and vancomycin were uniformly effective in resolution of symptoms^[96]. In the vancomycin group, 100% of animals had spores detected *vs* only 30% treated with ramoplanin after 2 d of treatment. Ramoplanin was noted to have a profound effect on both the vegetative and spore forms of *C. difficile* with complete eradication of both forms of the organism by 24 h. Vancomycin, on the other hand, had no effect on spores. The authors hypothesized that the efficacy against spores may be related to the binding of lipid II. A related antibiotic, nisin, which has been used as a food preservative for decades, had been noted to inhibit transformation from spore to the vegetative form in *Bacillus* and other *Clostridial* species^[206].

REP3123

REP3123 is a novel inhibitor of methionyl tRNA synthetase, which is required for bacterial growth. REP3123 inhibits toxin formation, is active in animal models,

prevents death of human cells exposed to *C. difficile* toxin and decreases spore formation. REP3123 has shown activity against 108 different *C. difficile* isolates, including the B1 strain, with an affinity for bacterial MetRS over 1000 times that of human mitochondrial or cytoplasmic MetRS^[207]. In addition, REP3123 is highly selective for gram positive bacteria which may spare much of the normal colonic flora^[208]. Clinical trials are eagerly awaited.

Tolevamer

Tolevamer (Genzyme Corp. Cambridge, MA) is a high molecular mass, non-absorbable polymer that has been shown to be a potent neutralizer of *C. difficile* toxins A and B, with each polymer molecule irreversibly binding 3-4 toxin molecules^[209]. A proposed advantage of non-antibiotic approaches for the treatment of CDI is the fact that there is no disturbance of the normal intestinal flora, potentially decreasing the risk of recurrent disease. Louie, et al. reported a randomized, double blind trial of tolevamer in patients with mild to moderate CDI^[210]. The patients were randomized to 3 or 6 g of tolevamer for 14 d or vancomycin 125 mg *qid*. If the 6 patients who had recurrence of diarrhea while still on treatment with tolevamer (4 in the 3 g/d group and 2 in the 6 g/d group) are included in the efficacy analysis, resolution of diarrhea was found in 60% of the tolevamer 3 g group, 79% in the 6 g group and 91% with vancomycin. Recurrence rates were 10% in the tolevamer 6 g group *vs* 19% in the vancomycin group. The major side effect of tolevamer was noted be hypokalemia, found in 23% of those in the 6 g group *vs* 7% of those treated with vancomycin. Because tolevamer is an anionic polymer capable of binding cations in colonic fluid, the hypokalemia is not surprising. Addressing this issue, the next study on tolevamer utilized a modified product, which is liquid with potassium added, to allow net-neutral potassium balance. This randomized Phase I trial tested tolevamer at 6, 9, 12 and 15 g/d, normal potassium was maintained with the new product and researchers reported that tolevamer was generally safe and well tolerated in patients at does up to 15 g/d^[211].

Despite its demonstrated safety with the reformulated drug, two subsequent studies challenged the efficacy of tolevamer for the treatment of CDI. The first was a Phase III randomized trial of 544 patients on either tolevamer (3 g, 3 times a day for 14 d), vancomycin (125 mg, 4 times a day for 10 d), or metronidazole (375 mg, 4 times a day for 10 d)^[212]. Of the 278 patients on tolevamer only 42% achieved clinical success, thereby failing to demonstrate noninferiority to the 73% success rate of vancomycin. One interesting finding, however, was the patients on Tolevamer had a decreased rate of recurrence (6%) when compared to the vancomycin group (18%; $P = 0.009$) and metronidazole group (19%; $P = 0.006$). The authors attributed the decreased rate of recurrence to the flora-sparing activity of tolevamer. As a follow-up to the findings of this Phase III trial, researchers in the UK studied the neutralizing effects of tolevamer on the

C. difficile cytotoxins in an *in vitro* human gut model^[213]. In contrast to previous studies, these researchers found that tolevamer was not associated with loss of the *C. difficile* cytotoxic effect. These results support and may explain the poor results for the primary endpoint in the previously described Phase III trial.

Monoclonal antibodies

The proposed mechanism behind the use of monoclonal antibodies (MAbs) in CDI is the potential ability to directly modulate the effects of *C. difficile* cytotoxins A and B. In animal models, MAbs have been shown to reduce the severity and duration of diarrhea, death rate, and rate of recurrence^[214]. Literature concerning the administration of a single MAb against either toxin A or toxin B seems to be conflicting; one early study reports that a MAb against toxin A was sufficient to protect form death, while a MAb against toxin B had no effect^[215]. In contrast, it was more recently suggested that the MAb against toxin B was protective against CDI^[216]. Given these conflicting reports, clinical application of MAb therapy appears to be adopting a dual administration of MAbs for both toxin A and toxin B. A randomized, double-blind Phase II placebo controlled trial of MAbs against toxin A (CDA1) and toxin B (CDB1) was able to demonstrate a lower recurrence rate with the administration of a single infusion of 10 mg/kg of MAb compared to placebo in patients also receiving either metronidazole or vancomycin^[217]. Overall, recurrence rates were 7% for the MAb group *vs* 25% for the placebo group (95%CI: 7-29, $P < 0.001$), while for patients with more than one previous episode of CDI the recurrence rates were 7% for the MAb group compared to 38% for the placebo group ($P = 0.006$).

Many questions remain about the application of MAb therapy in the treatment of CDI. Concern has been raised that MAb therapy does not decrease the severity of diarrhea, duration of hospitalization, or time to resolution^[218]. Additionally, the clinical applications of the current studies may not be appropriate given differences in course of illness between different patient populations, in particular the elderly^[219]. Some of these questions may be answered by two Phase III trials currently underway^[220,221]. Also likely to emerge in the future is the application of new MAbs that specifically bind to epitopes in the neutralizing regions of toxins A and B. These MAbs, known as PA-50 and PA-41, were shown to confer a dramatically increased survival rate in a hamster model, where the administration of a dual PA-50/PA-41 MAb revealed long term survival rate of 95% *vs* 0% for placebo^[222].

Vaccine

Interest in a vaccine is based upon the fact that development of *C. difficile* antitoxin antibody has been associated with protection from the development of CDI after colonization with *C. difficile*. A vaccine against toxins A and B, that has been efficacious in animal models as

well as humans, and demonstrated successful prevention of recurrence in 3 case reports^[223]. More recently, six Phase I trials on 200 individuals have been completed by Sanofi Pasteur with a bivalent formalin-inactivated vaccines against toxins A and B showing seroconversion of 75% of participants by day 70^[224]. A Phase II trial of this vaccine, currently underway in the US, is being conducted to assess primary CDI prevention in 650 at risk adults^[225]. Also in development is a chimeric antitoxin vaccine using an endotoxin free expression system from *Bacillus metaerium*, which was capable of producing neutralizing antitoxins and preventing spore-induced relapse in CDI^[226].

CONCLUSION

The impact of CDI infection is significant. This infection places a tremendously onerous burden on the health care system worldwide and has major adverse clinical and economic impact. This topic will be a continued high priority for national guidelines and clinicians will need to pay close attention to any forthcoming revisions for diagnosis and management. Presently, best practice recommendations would be as follows: (1) only patients with diarrhea (a stool that takes the shape of the container) should be tested for CDI; (2) initial testing should be done with glutamate dehydrogenase or nucleic acid amplification test for CDI, without repeat testing unless high suspicion for infection and initial GDH testing is done; (3) patients with resolution of diarrhea should not be rested to document cure of CDI; (4) initial antibiotic treatment for patients with mild/moderate CDI infection should be metronidazole 500 mg *tid* orally (provided no drug allergy contraindication); (5) initial treatment for severe CDI or failure to respond to 5-7 d of metronidazole should be vancomycin 125 mg *qid* orally. If severe or complicated CDI, intravenous metronidazole 500 mg *tid* should be added; (6) in patients with severe ileus or complicated CDI, best antibiotic plan is intravenous metronidazole 500 mg *tid* plus vancomycin 500 mg *qid* (oral) plus vancomycin 500 mg in 500 cc fluid *qid* (rectal by retention enema); (7) use of intravenous formulation compounded by pharmacy into oral solution offers significant cost advantage; (8) the first recurrence of CDI can be treated with the initial regimen if it induced appropriate clinical response; (9) the second recurrence of CDI should be treated with pulsed vancomycin; (10) the third recurrence or unresponsive severe CDI, fecal microbiota transplant should be considered; (11) current data suggests limited if any value, of probiotics for CDI treatment or prevention of relapse. The use of these agents in patients with central venous catheters should be avoided given possible infectious complications; and (12) high level disinfection of environmental surfaces for bathroom and if inpatient, contact surfaces is recommended. We routinely have patients discard toothbrush and change any device or implement that may allow oral contact ingestion of aerosolized spores in patients with CDI.

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