

Diagnosis and Management of *Clostridium difficile* Infection

Tony M. Korman, MBBS, FRACP, FRCPA¹

¹ Monash Infectious Diseases, Monash Health, Monash University, Clayton, Victoria, Australia

Address for correspondence Tony M. Korman, MBBS, FRACP, FRCPA, Monash Infectious Diseases, Monash Health, Monash University, Clayton, Victoria, Australia (e-mail: tony.korman@monash.edu).

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Abstract

There have been dramatic changes in the epidemiology of *Clostridium difficile* infection (CDI), with increases in incidence and severity of disease, attributed to the emergence of a fluoroquinolone-resistant “hypervirulent” strain, ribotype 027. *C. difficile* is now the most common pathogen causing hospital-acquired infection in U.S. hospitals, and community-acquired infections are increasing. The diagnosis of CDI is based on a combination of signs and symptoms, confirmed by laboratory tests. Clinical manifestations of CDI can range from asymptomatic colonization to severe pseudomembranous colitis and death. Many aspects of laboratory diagnosis of CDI remain contentious. Toxin enzyme immunoassays are too insensitive to be used alone, while nucleic acid amplification tests have emerged as an option, either as a stand-alone test or as part of a multitest algorithm. Oral vancomycin and metronidazole have been the recommended antimicrobial therapy options, and fidaxomicin is an effective new alternative. There is ongoing concern regarding the potential inferiority of metronidazole, in particular for severe CDI. Management of severe CDI and recurrent CDI continue to represent major treatment challenges. Biological therapies for the restoration of the intestinal microbiota (e.g., fecal microbiota transplantation) and monoclonal antibody therapy are promising approaches for CDI management, in particular troublesome recurrent CDI. This review will concentrate on the diagnosis and management of CDI in adults.

Keywords

- ▶ *Clostridium difficile*
- ▶ antibiotic-associated colitis
- ▶ pseudomembranous colitis
- ▶ fecal microbiota transplantation
- ▶ fidaxomicin
- ▶ toxic megacolon

Clostridium difficile is a spore-forming anaerobic gram-positive bacillus. The *C. difficile* toxin pathogenicity locus (PaLoc) contains the toxin genes *tcdA* (encoding toxin A) and *tcdB* (toxin B) along with three accessory genes: *tcdC*, *tcdR*, and *tcdE*. The roles of the individual toxins in pathogenesis continue to be elucidated,¹ with evidence demonstrating the virulence of toxin B,^{2–4} toxin A,^{3,4} and possibly binary toxin (CDT).⁴

There have been dramatic changes in the epidemiology of CDI, which is now recognized as a global public health problem.⁵ In the early 2000s, reports emerged from North America and Europe of increases in severe CDI with high mortality rate, particularly in the elderly, associated with increased use of fluoroquinolone antibiotics.^{6,7} The epidemic

“hypervirulent” strain was identified as a previously uncommon strain of *C. difficile* (known by various typing methods as toxinotype III, restriction endonuclease analysis type B1, North American pulsed-field type 1, or polymerase chain reaction [PCR] ribotype 027), which was fluoroquinolone resistant, and contained binary toxin and an 18-base pair deletion in *tcdC*, a putative negative regulator of toxin genes,^{8–10} with possible hyperproduction of toxins A and B.⁹

C. difficile is now the most common pathogen causing hospital-acquired infection in U.S. hospitals.^{11,12} The CDC estimates that 250,000 people require hospital care and at least 14,000 people die from CDI each year in the United States.¹³ Almost half of infections occur in people younger than 65, but more than 90% of deaths occur in people 65 and

older. Deaths related to CDI increased 400% between 2000 and 2007. CDI is associated with at least \$1 billion in excess medical costs per year.¹⁴ In addition, community-acquired CDI is increasing, and these patients may not have the traditional risk factors for CDI.^{5,15–19}

Antibiotics are the major risk factor for the development of healthcare-associated²⁰ and community-associated CDI,^{21,22} due to disruption of endogenous intestinal microbiota which promotes *C. difficile* spore germination, vegetative growth, and toxin production.²³ Meta-analyses identify clindamycin, cephalosporins, and fluoroquinolones^{20–22} as the highest risk antibiotics. The highest risk for CDI is during treatment and in the first month after antibiotic use, but risk continues for up to 90 days.²⁴ Proton pump inhibitors also increase the incidence of CDI.²⁵

Strategies to prevent CDI in acute care hospitals include early detection and isolation with contact precautions, hand hygiene, environmental cleaning, and antibiotic stewardship.^{26,27} Patients with suspected or proven CDI should be placed under contact precautions, including assignment to a single room with dedicated toileting facilities or cohorting with other infected patients. Gloves and gowns should be worn by all health care personnel upon room entry. Hand washing with soap and water is more effective for physical removal of *C. difficile* spores than alcohol-based hand hygiene products.²⁸ Hand washing with soap and water has been recommended when caring for CDI patients, in particular in the setting of a *C. difficile* outbreak. However, contamination of hands is less common when gloves are worn for the patient encounter,²⁹ and several studies have not found an increase in CDI with alcohol-based hand hygiene products.²⁷

The contaminated hospital surface environment plays a key role in the transmission of *C. difficile*.³⁰ *C. difficile* spores can survive on dry surfaces for up to several months and resist killing by standard disinfectants. A *C. difficile* sporicidal disinfectant³¹ should be used for disinfection of patient rooms and bathrooms. Dedicated medical equipment should be used for patients with CDI whenever possible. Equipment that must be shared between patients should be cleaned and disinfected with a sporicidal agent between uses. Encouraging appropriate use of antimicrobials is recommended, and a meta-analysis concluded that restrictive antibiotic stewardship programs effectively decrease the incidence of CDI.³²

In England, the incidence of CDI and related mortality has been reduced markedly (>60%) coincident with a decrease in ribotype O27 (55–21% cases) following the introduction of a *C. difficile* control program including strain typing, antimicrobial stewardship (with reduced use of cephalosporins and fluoroquinolones), mandatory reporting, and reduction targets.³³

Diagnosis of *C. difficile* Infection

The diagnosis of CDI is based on (1) a combination of signs and symptoms, confirmed by microbiological evidence of *C. difficile* toxin and toxin-producing *C. difficile* in stools, in the absence of another cause, or (2) colonoscopic or histopathological findings demonstrating pseudomembranous colitis (PMC).³⁴

Clinical Manifestations

The clinical manifestations of infection with toxin-producing strains of *C. difficile* range from asymptomatic carriage, to mild or moderate diarrhea, to fulminant and sometimes fatal PMC.^{35,36} The type of disease and severity of disease are related to organism factors^{37,38} and patient risk factors, including the presence of antitoxin antibodies.³⁹ Over 20% of hospitalized adults^{29,40} and up to 50% of residents of long-term care facilities⁴¹ may have asymptomatic *C. difficile* carriage and can serve as a reservoir for environmental contamination and sources for *C. difficile* transmission.^{29,41,42} Watery, nonbloody diarrhea, defined as three or more stools per 24-hour period, is the hallmark of symptomatic illness. Mild disease is characterized by diarrhea in the absence of signs and symptoms of colitis. Patients with moderate disease have diarrhea with evidence of colitis characterized by fever and abdominal cramps and discomfort, usually in the lower quadrants. Severe disease is discussed later.

Laboratory Diagnosis

Many aspects of laboratory diagnosis of CDI remain contentious, including selection of samples, selection of test method(s) and testing algorithms, and clinical validation.⁴³ It is important to perform testing for *C. difficile* only on unformed stools, because asymptomatic colonization with *C. difficile* is not uncommon.^{44,45} In patients with ileus and a strong suspicion for CDI, stool of any consistency, including rectal swabs, can be tested.⁴⁵ All patients with diarrhea who have been hospitalized more than 72 hours should be tested for *C. difficile*, irrespective of the physicians' request,⁴⁴ but testing can also be performed on samples submitted within the first 72 hours of hospitalization. Patients with diarrhea who have been admitted in a health care facility within a period of 3 months before the development of diarrhea should also be tested for *C. difficile*.⁴⁴ *C. difficile* testing should be performed on unformed stool samples of all patients with potential infective diarrhea and negative tests for common enteropathogens, irrespective of age, prior antibiotic use, comorbidity, co-medication, and onset of diarrhea (community or nosocomial).⁴⁴ Repeat testing following a negative test within 7 days is rarely useful, using any test method.^{46–48} Repeat testing during the same episode of diarrhea is not recommended, no diagnostic test should be used as a "test of cure,"⁴⁹ and *C. difficile* tests may remain positive for many weeks.⁴⁶

Cell Culture Cytotoxicity Neutralization Assay

The cell culture cytotoxicity neutralization assay (CCCNA) has historically been considered to be the gold standard for diagnosis of CDI.⁵⁰ However, CCCNA has 75 to 85% lower sensitivity than toxigenic culture,⁵¹ a long turnaround time (24–48 hours), requires cell culture expertise, and is now seldom used as a routine diagnostic test.⁴³

Toxigenic Culture

Toxigenic culture comprises isolation of *C. difficile* from feces followed by confirmation of toxin production by the isolate. Toxigenic culture is labor intensive, has a long turnaround time (>48 hours), and is not practical for routine diagnostic

use. In addition, toxigenic culture detects the ability of a *C. difficile* strain to produce toxin in vitro and does not necessarily indicate in vivo production of toxin in the host. Toxigenic culture is now considered by many to be the gold standard for *C. difficile* detection.⁵⁰ The 2010 Society of Healthcare Epidemiology of America (SHEA)/Infectious Disease Society of America (IDSA) guidelines support the use of toxigenic culture as the gold standard in method comparison studies.⁴⁹ However, although toxigenic culture may result in more positive specimens, it is not superior to CCCNA for the diagnosis of clinical disease.^{43,50} *C. difficile* culture is also important to allow for strain typing in the setting of an outbreak or other epidemiological studies, for antimicrobial susceptibility testing for surveillance of antimicrobial resistance, for new test method evaluation, and to evaluate new therapies.⁵²

Toxin Enzyme Immunoassays

Enzyme immunoassays (EIAs) for detection of *C. difficile* toxins A and B are rapid, inexpensive tests which are simple to perform, and were widely adopted by many laboratories. Toxin EIAs have high specificity (>95%), but have unacceptably low sensitivity compared with CCCNA (67–83%)^{44,53} and toxigenic culture (45–66%),^{44,53} and are no longer recommended for use as stand-alone tests for CDI diagnosis.^{43,49}

Glutamate Dehydrogenase

Glutamate dehydrogenase (GDH) (“common antigen”) is produced by all *C. difficile* isolates (including both toxigenic and nontoxigenic strains).^{54,55} A meta-analysis confirms that GDH has high sensitivity (>90% vs. CCCNA assay, >80% vs. toxigenic culture), low false-positive rate (<2%), and high negative predictive value (NPV).⁵⁶ In a recent UK study of >12,000 specimens, GDH sensitivity was very high (96% vs. CCCNA, 95% vs. toxigenic culture).⁵³ GDH is a convenient, rapid, inexpensive test with a rapid turnaround time which can be used as a screening test.^{46,51,57} Positive GDH tests must be followed up with a confirmatory test, such as a toxin EIA or a molecular test for detection of toxin genes.⁴³

Nucleic Acid Amplification Tests

Nucleic acid amplification tests (e.g., PCR) for *C. difficile* usually detect toxin genes (e.g., *tcdB* which encodes toxin B). Some assays also detect other targets (e.g., binary toxin genes, *tcdC* deletion) which act as surrogate markers for presumptive identification of ribotype 027 strains, but can also detect other ribotypes.^{58,59} NAATs are rapid tests with high specificity (>95%), high sensitivity for *C. difficile* detection (>90% vs. CCCNA, >85% vs. toxigenic culture), and high NPVs.^{44,60} The use of NAAT as a stand-alone test for *C. difficile* has been advocated,^{61,62} while others suggest NAAT should be used as part of a multistep algorithm.^{43,61}

Key questions regarding the clinical utility of NAATs remain, particularly regarding specificity and positive predictive value (PPV).⁴³ NAATs can identify *C. difficile* isolates that harbor toxin genes which are not expressed. A positive *C. difficile* NAAT assay does not differentiate between active infection and asymptomatic carriage. To reduce possible false

positives, it is important to only test unformed feces from patients who have suspected CDI. Introduction of NAAT assays to replace toxin EIA or CCCNA testing is associated with a >50% increase in CDI incidence,⁶³ and CDI surveillance requires adjustment for testing methods.^{64,65} Rapid NAAT assays may enable earlier diagnosis of CDI and avoiding the costs of repeat testing.⁶⁶ NAAT are more expensive than toxin EIAs; however, the cost of some rapid NAATs may be favorable compared with other options (e.g., a GDH/CCCNA algorithm) when labor costs are considered.⁶⁷ Further studies are required to assess the overall cost-effectiveness of NAATs for *C. difficile* detection.⁴³

Testing Algorithm Approaches

Testing algorithm approaches have been used for *C. difficile* laboratory diagnosis. In one two-step algorithm, the GDH assay is used as a screening test, and if negative, the specimen can be rapidly reported as negative without additional testing. If the specimen is GDH positive, confirmatory testing (e.g., NAAT) can be performed, and reported as positive or negative.^{43,44} In a three-step algorithm, samples are tested for GDH, and if positive, they are tested by using a toxin EIA. Toxin-positive samples can be reported as positive, but GDH-positive/toxin-negative samples are then tested by using NAAT. Alternatively, the samples can be screened by using a GDH/toxin combination EIA, but as the toxin EIA component has low sensitivity, samples with discrepant results (i.e., GDH positive/toxin negative) should be tested using NAAT.⁴³

The 2010 SHEA/IDSA guidelines included an interim recommendation to use GDH as an initial screening test and then CCCNA or toxigenic culture as the confirmatory test for GDH-positive stool specimens only.⁴⁹ The 2009 European guidelines recommended two- or three-stage algorithms. One option was to use GDH or NAAT as an initial screen, and samples with a negative test result can be reported as negative. Samples with a positive test should then be tested for toxin, and if positive, CDI is confirmed. If the toxin test is negative, but GDH or NAAT is positive, CDI cannot be differentiated from asymptomatic colonization.⁴⁴

Multistage algorithms performed better than standalone assays in a large UK study. GDH/EIA had high specificity (>99%) and PPV (>90%), while GDH/PCR had high sensitivity (>90%) and specificity (>95%) but a lower PPV (81% vs. toxigenic culture, 60% vs. CCCNA).⁵³ The UK algorithm recommends NAAT or a GDH assay to screen for the presence of *C. difficile*, followed by a toxin test (e.g., EIA) to confirm the diagnosis. The initial screening test using NAAT and GDH have high NPVs and can rapidly exclude CDI.⁵³ However, this approach can rely on a “confirmatory” toxin EIA second step which may have unacceptably low sensitivity, and has not been accepted in the United States.⁴³

Clinical Validation of Laboratory Diagnosis

A large prospective multicenter UK study provides important clinical validation of the laboratory diagnosis of *C. difficile* infection. Multiple tests for *C. difficile* were performed on

more than 12,000 unformed fecal samples, and corresponding outcome data for more than 6,500 inpatient episodes was analyzed. Mortality was significantly higher in patients with positive CCCNA test and toxigenic culture (16.6%), compared with those with positive toxigenic culture alone (9.7%), whose mortality was similar with negative toxigenic culture (8.6%). These findings confirm that CCCNA may be the best indicator of disease, but as it lacks sensitivity compared with toxigenic culture, it is no longer recommended as a stand-alone test.⁶¹ A new diagnostic category of “potential *C. difficile* excretor” was proposed for patients who were toxigenic culture positive, but CCCNA negative, to characterize patients with diarrhea which may not be due to CDI, but who can cause crossinfection.⁵³

Endoscopy

PMC is diagnosed by direct visualization of pseudomembranes on lower gastrointestinal endoscopy (either sigmoidoscopy or colonoscopy) or by histopathologic examination. PMC may spare the rectum in approximately 10% of patients, so colonoscopy is preferred.^{68–71} Pseudomembranes appear as tightly adherent, raised yellow or off-white plaques up to 2 cm in diameter, which may be covered with mucus, often with intervening normal looking colonic mucosa.⁷² Histopathological findings include the typical “summit” or “volcano” lesion with an erupting “pseudomembrane” of inflammatory cell infiltrate and debris with focal mucosal necrosis.^{72,73} PMC is highly specific for CDI,^{35,72} but pseudomembranes are detected only in half of CDI cases with positive CCCNA.^{49,74} Endoscopy rarely identifies pseudomembranes in CDI in patients with underlying inflammatory bowel disease.^{75,76} Other endoscopic findings may include bowel wall edema, erythema, friability, and inflammation. There is a risk of perforation with endoscopy in cases of fulminant colitis.⁷⁷ Endoscopy techniques may also be utilized for delivery of donor feces infusion (see later).

Diagnostic Imaging

Plain abdominal radiography can demonstrate polypoid mucosal thickening, “thumbprinting” (wide transverse bands associated with haustral fold thickening), or gaseous distention of the colon. Toxic megacolon is suggested by acute dilatation of transverse colon to a diameter >6 cm associated with systemic toxicity and the absence of mechanical obstruction.⁷⁸ Abdominal CT scan findings include wall thickening, low-attenuation colonic mural thickening corresponding to mucosal and submucosal edema (“target” or “double halo” sign), trapping of oral contrast material with high attenuation in the colonic lumen alternating with thickened inflamed mucosa with low attenuation (“accordion” sign), wall thickening involving the entire colon (pancolitis), pericolonic fat stranding, and ascites. Abdominal CT scan findings (colonic wall thickness > 15 mm, pleural effusion) may be independent predictors of complicated CDI in addition to clinical and laboratory parameters.⁷⁹ However, radiographic findings are neither sensitive nor specific for CDI.^{77,79–83}

Management of *C. difficile* Infection

General Principles

Stopping the inducing antibiotic(s) as soon as possible is strongly recommended for CDI.⁴⁹ Patients who continue concomitant antimicrobial therapy are more difficult to treat successfully and have a higher CDI relapse rate.^{84,85} Up to 20% of CDI cases may resolve without antimicrobial therapy.⁸⁶ Supportive care should include careful fluid and electrolyte management. The use of antimotility agents for CDI treatment has traditionally been discouraged,^{34,49} but evidence that they worsen outcome is lacking.⁸⁷

Antimicrobial Therapy

Metronidazole and Vancomycin

The recommended treatment options for initial non-severe CDI are oral metronidazole 500 mg three times daily or oral vancomycin 125 mg four times daily for 10 days.^{34,49} Fidaxomicin 200 mg twice daily (see later) is another potential option.⁸⁸ In prospective trials, the mean time for diarrhea resolution is 3 to 4 days.⁸⁹ Failure to respond to metronidazole therapy within 5 to 7 days could prompt consideration of a change in therapy to vancomycin at standard dosing.⁶²

Systematic reviews have concluded that no antimicrobial agent studied has proven to be clearly superior for the initial cure of CDI.^{90,91} Metronidazole has been recommended as first-line treatment for mild/moderate disease due to low cost and possibly reduced risk of vancomycin-resistant enterococci (VRE).^{34,49} However, due to ongoing concern regarding potential inferiority compared with vancomycin,^{89,92} recommendations now often relegate metronidazole to treatment of mild disease only.^{93,94}

Following the emergence of ribotype 027, increased risk of treatment failure and recurrences after metronidazole therapy were recognized,^{36,95–97} in particular in patients with severe disease,⁹⁸ and with continuation of antibiotics.⁹⁹ Other evidence for inferiority of metronidazole compared with vancomycin include more rapid resolution of symptoms,^{97,100} and reduction of *C. difficile* stool levels.⁹⁷ Analysis of data from trials of the ineffective toxin-binder tolevamer demonstrated that the efficacy of metronidazole was inferior to vancomycin (73 vs. 81% overall, 66 vs. 79% for severe CDI).¹⁰¹

The reason for the poor performance of metronidazole is not well understood,^{34,89} but comparing the pharmacokinetic properties to vancomycin may provide some explanation.^{34,92,93} Oral metronidazole is almost completely absorbed in the upper gastrointestinal tract, but bactericidal fecal concentrations (>9 µg/g) are attained in acute CDI. Fecal concentrations decrease during recovery, with significantly lower concentrations in formed compared with semiformal or watery stools.¹⁰² In contrast, oral vancomycin maintains high stool concentrations (>3,000 µg/g) throughout the course of CDI therapy.^{102,103} Metronidazole resistance has not been recognized as a cause of treatment failure.^{34,92} However, *C. difficile* strains with reduced susceptibility to

metronidazole have been identified,¹⁰⁴ while vancomycin resistance is extremely rare.^{92,105}

Fidaxomicin

Fidaxomicin is a promising alternative therapy for CDI. Oral fidaxomicin attains high fecal concentrations with minimal plasma concentrations.¹⁰⁶ The safety profile of fidaxomicin is comparable to oral vancomycin.¹⁰⁷ Two large double-blind randomized controlled trials (RCTs) (total $n = 572$ received fidaxomicin) confirmed noninferiority to vancomycin for clinical cure rates.^{108–110} In patients taking concomitant antibiotics, fidaxomicin was significantly more effective than vancomycin in achieving clinical cure.^{85,109}

Treatment with fidaxomicin was associated with a significantly lower rate of recurrence than vancomycin in the 4 weeks after completion of treatment in both studies (15 vs. 25%²⁸ and 13 vs. 27%²⁹). Recurrence rates were higher among ribotype 027 than among non-ribotype 027 cases, and were not significantly different following treatment with vancomycin or fidaxomicin.¹¹¹ There may be a potential role for fidaxomicin in first-line CDI treatment for patients “with risk factors known to portend relapse and severe infection.”⁸⁸ Possible explanations for reduced recurrence rates include superior inhibition of *C. difficile* toxin production¹¹² and spore production,¹¹³ and better preservation of the intestinal microbiome during and after treatment of CDI.^{114–116} Whole-genome sequencing demonstrated that fidaxomicin was superior to vancomycin for preventing both *C. difficile* reinfection and relapse.¹¹⁷ Fidaxomicin was also significantly less likely than vancomycin to promote acquisition of VRE (7 vs. 31%) and *Candida* species (19 vs. 29%) colonization.¹¹⁸ A key issue to be resolved is the comparative cost-effectiveness of fidaxomicin.^{119,120}

Other Oral Antimicrobial Agents

Nitazoxanide may be as effective as vancomycin for CDI, although a small RCT (only $n = 23$ received nitazoxanide) was unable to confirm noninferiority.¹²¹ Many new drugs are in preclinical and clinical development, including Phase III clinical trials with cadazolid and surotomycin.^{94,122}

Severe Disease

Patients with severe disease may develop a colonic ileus or toxic dilatation and present with abdominal pain and distension but with minimal or no diarrhea. Complications of severe *C. difficile* colitis include dehydration, electrolyte disturbances, hypoalbuminemia, toxic megacolon, bowel perforation, hypotension, renal failure, systemic inflammatory response syndrome, sepsis, and death.⁴⁹

Management guidelines and treatment studies have proposed various definitions of severe/and or complicated CDI and use different severity criteria, which has important implications for comparison of treatment outcomes.⁹² The SHEA/IDSA CDI treatment guidelines use the criteria leukocyte count $>15 \times 10^9/L$, rise in serum creatinine (>1.5 times the premorbid level), hypotension, shock, ileus, or toxic megacolon to define severe/complicated CDI.⁴⁹

Other U.S. guidelines classify severe CDI with serum albumin <30 g/L plus one of leukocytosis or abdominal tenderness, and complicated CDI with one of a list of other clinical and laboratory criteria.⁶² The European guidelines define severe CDI as an episode of CDI with (one or more specific signs and symptoms of) severe colitis and/or one or more unfavourable prognostic factors (leucocytosis, albumin <30 g/L or rise in serum creatinine) or a complicated course of disease, with significant systemic toxin effects and shock, resulting in need for ICU admission, colectomy or death. A systematic review found that the most common risk factors for complicated CDI were older age, leucocytosis, renal failure, and comorbidities.¹²³ Leukocyte count $>20 \times 10^9/L$ and serum creatinine level >133 $\mu\text{mol/L}$ (>1.5 mg/dL) measured on the day of diagnosis were predictors of a complicated course of CDI in the fidaxomicin RCTs.¹²⁴

Several clinical prediction rules (CPRs) for adverse outcomes of CDI have been developed. A scoring system devised retrospectively to identify patients with severe infection in one RCT used the presence of PMC, treatment in an ICU, or two out of four parameters (age >60 years, $T > 38.3^\circ\text{C}$, albumin <2.5 dL, WBC $>15 \times 10^9/L$).⁹⁸ A combination of five clinical and laboratory variables measured at the time of CDI diagnosis (the ATLAS score: age, treatment with systemic antibiotics, leukocyte count, albumin, and serum creatinine) predicted treatment response to CDI therapy in the fidaxomicin RCTs and may be useful in stratifying patients.¹²⁵ A systematic review which evaluated the available CPRs demonstrated serious methodological limitations which led to suboptimal quality and debatable utility and recommended the development of evidence-based tools through appropriate prospective cohorts.¹²⁶

Strain Type

Several studies demonstrated the importance of strain type (e.g., ribotypes 027 and 078) on CDI outcome,^{127–129} while some studies did not.¹³⁰ Recent large studies confirm that *C. difficile* genotype (including ribotypes 027 and 078) predicts severe disease and mortality,^{37,38,131} and new virulent strains associated with severe disease continue to emerge.^{59,127,132}

Oral Antibiotic Therapy

There are no RCTs available to guide recommendations for the choice and dosing of antibiotic therapy for the treatment of patients with severe CDI. Oral vancomycin 125 mg four times daily is recommended as the preferred therapy for severe or refractory CDI.^{34,49} Although levels achieved may be equivalent to standard dose,¹³³ increased vancomycin dose (500 mg four times daily) has been suggested based on expert opinion only.^{34,49} The use of metronidazole alone for severe CDI is now discouraged.³⁴ The cure rate was significantly higher with vancomycin than with metronidazole (97 vs. 76%) in patients with severe CDI ($n = 69$) in a RCT post hoc subgroup analysis^{91,98} but not on an intention-to-treat analysis.^{90,91} There are no data available on the efficacy of fidaxomicin in severe CDI.³⁴

Intravenous Antibiotics

Patients with severe CDI and ileus may have delayed passage of oral antibiotics into the colon and may benefit from the addition of intravenous metronidazole (500 mg three times daily). Fecal concentrations in the therapeutic range can be achieved due to biliary and intestinal excretion.^{102,134} However, in one small retrospective report, there was no difference in treatment outcomes between oral vancomycin monotherapy and combination therapy with oral vancomycin and metronidazole (mostly intravenous) for severe CDI.¹³⁵ In a nonrandomized study, intravenous metronidazole was inferior to oral metronidazole for CDI,¹³⁶ so oral therapy should be administered whenever feasible.¹³⁶ Intravenous vancomycin is not useful for CDI therapy.¹³⁷

Tigecycline has potent in vitro activity¹⁰⁵ and suppresses both *C. difficile* toxin production and sporulation.¹³⁸ Tigecycline achieves high stool concentrations with relative sparing of indigenous anaerobic microflora.^{139,140} There are case reports and small case series using intravenous tigecycline as adjunctive or alternative therapy for severe and/or refractory CDI,^{141–143} but prospective clinical trials are lacking.

Intracolonic Vancomycin

Intracolonic vancomycin (vancomycin enema) may be an effective adjunctive therapy for severe CDI for patients unable to tolerate the oral preparation, or with toxic megacolon or ileus which would prevent oral vancomycin from reaching the colon, but optimal dosing is uncertain.^{144–146}

Surgery

In a systematic review of 31 studies with 1,433 patients, the 30-day mortality was 41% (range, 19–71%), and predictors of postoperative death included preoperative intubation, acute renal failure, multiple organ failure, and shock requiring vasopressors.¹⁴⁷ Notwithstanding selection bias in retrospective studies, emergency colectomy for patients with fulminant CDI may provide a survival advantage compared with ongoing medical therapy.^{148,149} However, the criteria for surgical intervention, optimal timing, and preferred surgical procedure remain uncertain.^{150–152}

Indications for surgical intervention in CDI include colonic perforation, toxic megacolon, and rapidly progressive and/or refractory disease with systemic inflammatory response syndrome leading to multiorgan system failure.¹⁵³ In a retrospective review, colectomy was most beneficial for immunocompetent patients aged ≥ 65 years with a leucocyte count $\geq 20 \times 10^9/L$ and/or a plasma lactate 2.2 to 4.9 mmol/L.¹⁴⁸ Early surgery is usually recommended, before the development of shock and the need for vasopressors, usually 3 to 5 days after diagnosis in patients who are worsening or not clinically improving.¹⁵² Further investigation is required to evaluate CPRs that can predict deterioration to better inform decision making with regard to surgery timing.¹⁵²

Subtotal colectomy (removal of the colon, with the rectum remaining in situ) with end ileostomy is the currently accepted surgical procedure of choice for fulminant CDI based on low-quality evidence.^{148,150,152} Diverting loop ileostomy and

colonic lavage (with polyethylene glycol and vancomycin) may be an alternative to subtotal colectomy. A study of 42 patients reported reduced morbidity compared with historic controls who had colectomy (19 vs. 50%) and preservation of the colon in 93% of patients.¹⁵⁴ This surgical approach is promising, but further data, ideally from RCTs, are required before this is accepted as standard practice.^{147,152,155}

Recurrent *C. difficile* Infection

Management of recurrent CDI continues to provide major challenges. In a systematic review of 26 studies, at least one recurrence occurred in 22% of cases of CDI treated mainly with metronidazole or vancomycin.¹²³ After a first recurrence, the risk of further recurrences may be up to 40%, and $>60\%$ after two or more recurrences.^{156,157} A systematic review found that the most frequent risk factors for recurrence were older age, use of antibiotics after diagnosis, use of proton pump inhibitors, and strain type.¹²³ Recurrence usually occurs 3 to 21 days (average 6 days)³⁵ after completion of a treatment course.

Recommendations for the first recurrence of CDI are ceasing any causative antibiotic therapy, and treatment with the same options as for the initial episode.⁴⁹ Oral vancomycin is recommended,³⁴ and oral metronidazole may be an option for non-severe recurrent CDI.⁴⁹ The incidence of a second recurrence after treatment of a first recurrence with oral metronidazole or vancomycin is similar.^{34,101} In patients with a first recurrence of CDI, fidaxomicin was similar to vancomycin in achieving an initial clinical response, but the recurrence rate was lower (19 vs. 35%).¹⁵⁸

For treatment after multiple recurrences of CDI, a repeat vancomycin course followed by tapering and/or pulse strategy over weeks^{157,159} is recommended^{34,49} without proven efficacy, “in the hope that *C. difficile* vegetative forms will be kept in check while allowing restoration of the normal flora.”³⁴ Fidaxomicin has been suggested as a “chaser” regimen following vancomycin for patients with recurrent CDI.¹⁶⁰ Following multiple recurrent CDI, metronidazole is not recommended because of the potential for neurotoxicity.⁴⁹

Rifaximin, an orally nonabsorbed rifamycin,¹⁶¹ is a promising option for recurrent CDI, with reports of small uncontrolled case series.^{162–165} In a pilot RCT, patients receiving a rifaximin “chaser” ($n = 33$) following standard anti-CDI antibiotics had significantly decreased incidence of recurrent diarrhea compared with placebo.¹⁶⁶ However, there is concern regarding emerging rifaximin resistance in *C. difficile*.^{167–170} Biological therapies for recurrent CDI are discussed below.

Biological Therapies

There is increasing evidence that CDI is a microbiome-related disease.²³ Using microbiome data, patients with CDI and non-CDI diarrhea can be distinguished from healthy controls.¹⁷¹ Patients with recurrent CDI have decreased diversity of their fecal microbiome.¹⁷² Restoration of the intestinal microbiota using biological therapies is an attractive approach for CDI management.¹⁷³

Nontoxigenic *C. difficile* Colonization

Colonization with nontoxigenic *C. difficile* strains is associated with a decreased risk of developing subsequent CDI.¹⁷⁴ In 1987, two patients with relapsing CDI responded to administration of a nontoxigenic *C. difficile* strain.¹⁷⁵ A nontoxigenic *C. difficile* strain (VP20621) was well tolerated and able to colonize the gastrointestinal tract in a Phase I study,¹⁷⁶ and reduced the incidence of CDI recurrence by at least 50% compared with placebo in a recent Phase II dose-finding study.¹⁷⁷

Probiotics

The role of probiotics for prevention and treatment of CDI remains contentious.^{173,178} Possible mechanisms of action of probiotics such as *Saccharomyces boulardii* include direct activity against *C. difficile* (inhibition of adherence,¹⁷⁹ toxin proteolytic digestion¹⁸⁰) and modulation of the host response (inhibition of proinflammatory signaling pathways¹⁸¹ and stimulation of specific IgA antitoxin production¹⁸²).

The clinical application of probiotics for prevention and/or treatment of CDI has been limited by a lack of data from large well-designed RCTs.¹⁷³ A Cochrane review concluded that there was insufficient evidence to recommend probiotic therapy as an adjunct to antibiotic therapy, and no evidence to support the use of probiotics alone in the treatment of CDI.¹⁸³ Systematic reviews and meta-analyses concluded that there was moderate quality evidence to suggest that probiotics may be both safe and effective for preventing CDI.^{184,185} However, a large double-blind RCT (PLACIDE study)¹⁸⁶ found no evidence that a multistrain probiotic of lactobacilli and bifidobacteria was effective in prevention of CDI or antibiotic-associated diarrhea.¹⁸⁷

Fecal Microbiota Transplantation (Donor Fecal Infusion)

Restoration of intestinal dysbiosis by reintroduction of normal flora is the rationale for donor fecal infusion or fecal microbiota transplantation (FMT) for CDI.^{188–190} Numerous small case series^{191,192} and meta-analyses have reported clinical “cure” rates over 90% following FMT for recurrent CDI.^{193–196} A small ($n = 16$ in treatment group) open-label RCT of duodenal infusion of donor feces for recurrent CDI was terminated early after interim analysis demonstrated benefit (resolution of diarrhea after one infusion 81% vs. 23–31% treated with vancomycin).¹⁹⁷ After receiving donor feces infusion, patients had microbiota diversity resembling the healthy donors.¹⁹⁷ There are reports of FMT used successfully in patients with severe CDI,^{198,199} and cases series demonstrating efficacy and safety for treatment of CDI in immunocompromised patients.²⁰⁰ FMT may represent a cost-effective strategy for treatment of recurrent CDI.²⁰¹

Although FMT shows great promise, many practical, procedural, technical, ethical, safety, and regulatory issues are yet to be fully addressed. Although initially designated as an investigational new drug, the U.S. FDA later provided interim guidance to exercise discretion regarding these requirements, provided that the treating physician obtains adequate informed consent that the use of FMT for CDI is investigational and discusses potential risks.^{202,203}

Rigorous donor screening protocols to minimize the risk of transmitted infection have been proposed.^{192,204} A systematic review concluded that FMT using stool from a related donor had a slightly higher cure rate.¹⁹³ Fresh stool (within 8 hours of passage) is usually recommended, but this has not been studied rigorously.¹⁸⁸ However, excellent results (>90% overall success rate) were reported using a standardized frozen preparation of stool from “universal” donors.^{188,205,206} The optimal route of administration has not been determined. In a systematic review, instillation by gastroscopy, nasogastric tube, or nasojejunal tube was marginally less effective than other methods (e.g., rectal tube/enema, colonoscopy).¹⁹³ Ideally, large RCTs should be undertaken to confirm the efficacy and define best practices for FMT.^{193,203}

Defined Bacteriotherapy

Defined bacteriotherapy shows promise for the treatment of intestinal dysbiosis associated with recurrent CDI.^{207,208} In 1989, rectal instillation of a mixture of 10 bacterial strains cured five patients with re-establishment of fecal anaerobic bacteria.²⁰⁹ Recently, targeted restoration of the intestinal microbiota with defined bacteriotherapy resolved relapsing CDI in a mouse model.²¹⁰ Colonoscopic delivery of a synthetic “stool substitute” of purified cultures of 33 bacterial species derived from a single healthy donor cured two patients correlated with more diverse posttreatment fecal microbiota.²¹¹ Further developments of targeted bacteriotherapy (“synthetic microbial communities” or “microbial ecosystem therapeutics”) may provide effective new approaches for CDI therapy.^{190,208,212}

Immunotherapy

Low serum antibody levels against *C. difficile* toxins predispose patients to symptomatic³⁹ and recurrent^{213,214} CDI. Intravenous immunoglobulin has been used for treatment of CDI, but the benefit is questionable.²¹⁵ Monoclonal antibodies are a promising approach for treatment of CDI.²¹⁶ In a Phase II double-blind RCT, the addition (to standard anti-CDI antibiotic therapy) of a single intravenous infusion of monoclonal antibodies against *C. difficile* toxins had no effect on initial cure rate but significantly reduced CDI recurrence (7 vs. 25%).²¹⁷ Low serum antitoxin levels are associated with recurrence after therapy.^{217,218} The results of Phase III clinical trials near completion (ClinicalTrials.gov identifiers NCT01241552 and NCT01512239) are awaited with interest. Active immunization strategies aiming to provide long-term protection against CDI are also being developed. There are multiple candidate *C. difficile* vaccines in preclinical and clinical development.^{219,220}

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