INVITED REVIEW

Clostridium difficile and Antibiotic-associated Diarrhea

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Clostridioides (formerly Clostridium) difficile is a spore-forming, gram-positive bacilli responsible for *Clostridium difficile* infection (CDI), which is currently considered as a healthcare-associated infection of utmost importance. The prevalence rates of *C. difficile*-related infection in India vary from 7.1 to 26.6% in several studies.[1]

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Important Terminologies

Terminology	*Features*
C. difficile colonization	• Detection of the organism • No symptoms • Shedding of spores
C. difficile infection (CDI)	• Identification of *C. difficile* toxin or a toxigenic strain in stool • Symptoms present • Shedding of spores
C. difficile-associated diarrhea (CDAD)	• Diarrhea with *C. difficile* toxin found in stool sample or stool culture becomes positive for *C. difficile*
C. difficile colitis	• Glutamate dehydrogenase (GDH) and *C. difficile* toxin or nucleic acid amplification test (NAAT) and toxin are positive in stool • Mucosal inflammation proven by endoscopy • Pseudomembranous colitis—whitish raised multiple lesions, interspaced by normal mucosa, which later coalesce to form pseudomembranes, found by endoscopy

Roles of the Microbiota

Bile Acid Metabolism

Primary bile acids, cholic acid and chenodeoxycholic acid, after being produced in the liver, help in digestion of fat. They stimulate germination of *C. difficile* spores[2]. Primary bile acids are released into the small intestine and reabsorbed through the enterohepatic circulation. However, a small amount of the primary bile acids escapes enterohepatic circulation and enters the colon, where in presence of gut bacteria, they are converted to secondary bile acids, deoxycholic acid and lithocholic acid. Secondary bile acids are known to inhibit *C. difficile* growth[2]. Amino acids and short-chain fatty acids (SCFAs) may play a role in *C. difficile* colonization. Gross alteration of intestinal microbiota may diminish production of SCFAs and thus may enhance growth of *C. difficile*.[3]

Communicability

From Human

Shedding of *C. difficile* may occur during the phase of diarrhea as well as after completion of therapy. In a study done by Lawley et al., mean time taken to become stool culture negative was 4.2 days in patients receiving treatment for CDI. By the time diarrhea got resolved, but skin contamination persisted in 60% and environmental contamination was 37%. *C. difficile* remained positive in stool up to 4 weeks posttreatment in 56%, so also skin contamination in 58%, and sustained environmental shedding in 50%, in patients recovering from CDI. Hence, a patient with CDI can potentially be a source of CDI to other people even after they are declared cured clinically. Patient requiring continued antibiotic treatment may land up in a "supershedder" state, in which *C. difficile* spores get excreted in high concentrations in feces.[4] In this kind of patients, the organism may continue to germinate in the intestine and may persist as low-grade infection with intermittent flare.

An increasing number of literature suggests that asymptomatic carriers can also be a source of infection to the population, although the rate of transmission from one individual asymptomatic carrier may be low.[5] However, it is hypothesized that the asymptomatic carrier may outnumber symptomatic CDI patients; they could

contribute significantly to the disease transmission in society and long-term health care facility. More recently, Lanzas et al. concluded that patients colonized on admission may play a significant role in transmission to ward patients.[6]

From Food

C. difficile can be recovered from retail meat, including ground beef, and other meat products. The prevalence of it may vary from 20 to 63%, as it is revealed in different studies from the United States and Canada.[7,8]

From Environment

Toxigenic strains of *C. difficile* could be isolated from different environmental surfaces, especially which are prone to fecal contamination like floors, mops, toilet seats, bedpans, door handles of toilets, and so on, and sewage of a known positive subject. The spores are resistant to heat, desiccation, and many of the disinfectants. Hence, they can survive in the environment for months or years. The frequency of environmental contamination depends on whether the patient is carrying *C. difficile* or not. In areas where there is no known carrier, it may be found in less than 8% of rooms. However, 8–30% of rooms may be positive for *C. difficile*, which were shared by asymptomatic subjects, and 9–50% of rooms of patients with diarrhea.[9,10]

Virulence of *C. difficile*

C. difficile colonization occurs in the large intestine as the first step of infection when the gut microbiota gets disrupted and at that point of time *C. difficile* becomes the dominant organism. The pathogen being noninvasive in nature, the source of infection remained confined in the gut; however, the bacteria secretes enzymes, such as collagenase, hyaluronidase, chondroitin sulfatase, as well as toxins (A, B, and *C. difficile* transferase), which cause severe damage to the gut epithelium. The junctions between cells get disrupted, fluid accumulates in-between layers, and neutrophils get adhered leading to local inflammation. The enzymatic virulence is mostly responsible for loss of integrity of the intestinal barrier mechanism.[11] The toxin produced by *C. difficile* is both enterotoxic and cytotoxic.

The new variant of *C. difficile* (BI/NAP1/027) strain is a hypervirulent strain.[12,13] This strain changes the severity dramatically with more incidence of septic shock toxic megacolon, gut perforation, and death. This type of strains can secrete much higher amounts of toxins A and B and are more resistant to standard antibiotic therapy.

Clinical Manifestation

Symptomatology of CDI follows a spectrum, which ranges from the asymptomatic carrier state, mild or moderate diarrhea, to life-threatening fulminant colitis. The incubation period is variable in different reports. Ulcerations can occur in any part of the large intestine, but the sigmoid colon gets usually affected first and most commonly. Recovery from diarrhea occurs mostly within 5–10 days of antibiotic therapy in uncomplicated cases. Relation of antibiotics usage and the onset of diarrhea may vary and diarrhea can start during or directly after antimicrobial therapy, or may be delayed for couple of weeks after stopping of antibiotics. Symptoms could be gut-specific like watery diarrhea, abdominal pain, nausea and vomiting, abdominal distension, and loss of appetite only or could be associated with systemic symptoms like fever, weakness, generalized edema, dehydration, circulatory shock, and multiorgan failure. The fecal occult blood test is often positive, although frank bleeding is rarely present.[14] Significant hypoalbuminemia and raised creatinine with peripheral edema indicate severe disease and significant morbidity and mortality. Toxic megacolon and colon perforation may warrant emergent management including surgical intervention to save the life. The disease may cause intestinal paralysis with absence of diarrhea and delaying diagnosis. Small intestine infiltration, reactive arthritis, and bacteremia are some of the rare extracolonic manifestation of the disease.[15]

The antibiotics most commonly implicated in *C. difficile* colitis are clindamycin, fluoroquinolones, penicillin, and cephalosporin.

Though there is no clear-cut demarcation between mild, moderate, and severe CDI, following features may help to identify patients with severe disease. Presence of two of the following markers indicates that the patient is suffering from severe disease: hypoalbuminemia (serum albumin < 3 g/dL), white blood cell count $\geq$ 15,000 cells/mm^3, creatinine $>$ 1.5 $\times$ baseline (or glomerular infiltration rate reduced by 25% from baseline), or temperature $>$ 38.5°C. Fulminant (severe complicated CDI) is defined as CDI that presents with or develops at least one of the following signs or symptoms: admission to an intensive care unit, hypotension with or without use of vasopressors, ileus, toxic megacolon, mental status changes, serum lactate levels $>$ 2.2 mmol/L, or any evidence of end organ failure.

The CDI relapse is considered if diarrhea recurs within the first week of stoppage of antibiotic after the initial episode. Mostly the patient is found to have impaired immune response to *C. difficile* toxins, though a new exposure to spores may be a significant probability.

Symptomatic vs Asymptomatic

Differentiation of CDI and colonization is needed to avoid overtreatment. Mortality in patients harboring a toxigenic *C. difficile* strain did not increase if the toxin was not detectable in stool (Planche et al.).[16]

Most of the diagnostic tests are unable to differentiate CDI from colonization. The diagnostic test for toxigenic *C. difficile* should only be considered in patients with risk factors, having diarrhea, or other features suggestive of inflammatory colitis. For day-to-day patient care, rapid assays are more suitable as they are less time-consuming. From the epidemiologic point of view, identifying toxigenic *C. difficile* colonization in asymptomatic patients may be helped by culture. An algorithmic approach may be followed to optimize diagnostic ability of the tests. The algorithm should start from pretest probability and presence of symptoms and signs. The set of tests should include a Tox A/B EIA to test for free toxins in stool as well as rapid assays (NAATs). The asymptomatic colonization state can be confirmed by the absence of clinical symptoms or the presence of an alternative diagnosis along with toxigenic culture or PCR confirmation.

Diagnosis

Useful diagnostic tests (the best way to optimize diagnosis of CDI is to combine two tests in algorithm)

Tests	*Turnaround time*	*Sensitivity/ specificity (%)*	*Limitations*
C. difficile toxins assay (EIA)	1–2 hours	(75–85)/ (95–100)	The test lacks sensitivity but is rapid and available for all laboratories
GDH	15–45 minutes	58–68/100	Do not distinguish whether the strain is toxigenic
NAAT	2 hours	(80–100)/ (87–99)	High cost Confirms the presence of *C. difficile* toxin-producing strain, but it does not necessarily mean that the strain produces any toxins at the moment.

Diagnostic Algorithm of *Clostridium difficile* Infection (Source Up-to-date)

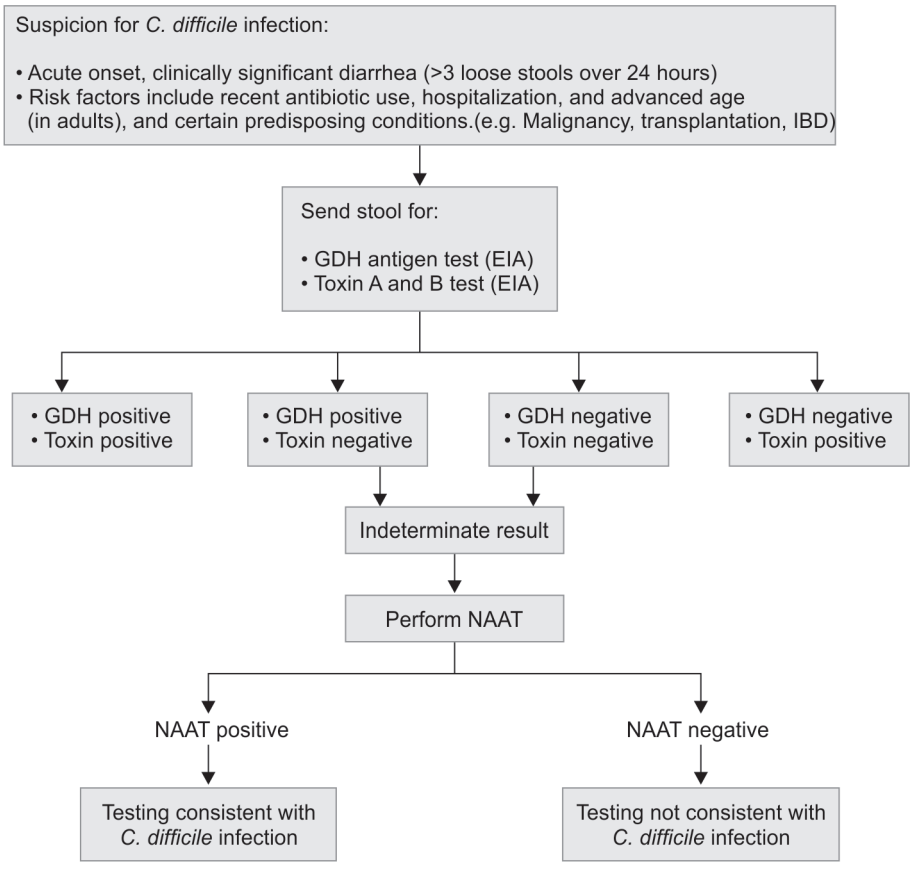
Diagnostic algorithm remains the same for initial infection and recurrent or relapsed infection.

Sample Management

The toxin present in a stool sample is heat labile and gets destroyed at normal room temperature. It may not be detected in stool after approximately 2 hours if the sample is kept at room temperature. Once the stool sample is obtained, testing it at the earliest is the best. Stool should be stored at 4°C if the test is planned within next 24 hours.[17] The best sample is diarrheal stool sample, or test can be performed with rectal swab if ileus is suspected. Repeat testing for *C. difficile* toxin after successful treatment is not warranted as they may remain positive; however, treatment is not needed for only positive stool in a person without symptoms.[18,19]

Endoscopic evaluation is indicated if diarrhea does not respond to the standard course of anti *C. difficile* antibiotic or associated disease, and a tissue diagnosis is needed. A quick look at flexible sigmoidoscopy is a preferred method as it requires minimal or no air insufflation, which avoid procedural complication like perforation of the gut. Pseudomembranous colitis, a very typical finding of *C. difficile* colitis, can also be found in different other diseases, like Behcet's disease, inflammatory bowel disease, ischemic colitis, and infections, like CMV or enterohemorrhagic *Escherichia coli* O157:H7.[20]

Abdominal imagings like X-ray, ultrasound, and CT scan help diagnosing complications of CDI. Important findings are distended bowel loops, often with wall thickening, toxic megacolon, and bowel perforation.[21] Ultrasound imaging is emerging as a good and easily available bedside point-of-care method for monitoring the width of the colon and free fluid in the abdomen, which may indicate some ominous complications.



Box 1: Risk factors for an initial episode of *Clostridium difficile* infection

- Antibacterial therapy
- Treatment during the previous 3 months
- Multiple antibacterial agents
- Older age (>65 years)
- Severe underlying illness
- Immunocompromised patients
- Immunosuppressive drugs
- HIV infection
- Antineoplastic agents
- Tube feeding and gastrointestinal surgery
- Gastrointestinal medications, including gastric acid reduction therapy
- ICU stay
- Prolonged hospitalization (median periods of 20 days)

Box 2: Factors associated with increased incidence of nosocomial *Clostridium difficile* infection

Increasing numbers of patients who:

- Are aged >65 years
- Are immunocompromised
- Suffer from other comorbidities
- Use of high-risk antibacterials such as 8-methoxy fluoroquinolones
- Use of enemas, gastrointestinal stimulants, and stool softeners
- Overburdened healthcare workers and reduced compliance with hand hygiene
- Cost constraints leading to inadequate environmental disinfection
- Rapid turnover in hospital beds
- Overcrowding in hospitals
- Prolonged periods of hospitalization
- Shared toilet facilities between patients
- Inadequate isolation facilities for infected patients
- Emergence of epidemic strains

Risk Factors for CDI

Different risk factors have been identified for CDI and are being summarized above (Boxes 1 and 2).

Differential Diagnosis

C. difficile colitis should be differentiated from other infectious and noninfectious causes of acute diarrhea in the intensive care unit. These might be increasingly difficult if the patient is colonized with toxin-bearing *C. difficile* spores. Presence of fever, leukocytosis, abdominal pain, and pseudomembranous colitis favors *C. difficile* colitis than other diagnoses.

- Food-induced diarrhea: This is common in patients with delayed initiation of enteral feed in ICU with secondary lactose intolerance and food-induced diarrhea. Specially, hyperosmolar feed, high albumin content food, and large volume of food can induce it. Stoppage of diarrhea after stopping feed usually clinches the diagnosis.
- Drug-induced diarrhea: Many drugs including antibiotics, laxatives, and syrups can induce diarrhea in susceptible ICU patients. The mechanism could be osmotic or allergic in nature.
- Inflammatory bowel disease (IBD): Undiagnosed IBD may present initially as ICU-associated diarrhea. The IBD flare-up could be result of antibiotic exposure in ICU. These may be particularly difficult to diagnose as they are associated with inflammatory features and can have pseudomembranous colitis on endoscopy.
- Postinfectious irritable bowel syndrome (IBS): An IBS may be precipitated after any episode of infectious diarrhea including *C. difficile*. These are not inflammatory in nature and subside with symptomatic treatment.
- Other infectious diarrhea: Other infectious agents like *Salmonella*, Amebiasis, *Staphylococcus*, and *Clostridium perfringens* may also cause inflammatory diarrhea. The diagnosis can be established after identifying specific pathogen.

Clostridium difficile Infection Treatment

Treatment of CDI represents a challenge to the clinicians. Patients with symptoms of colitis with positive *C. difficile* toxin assay in stool and patients with high suspicion of CDI having a negative ELISA test should receive treatment. For an asymptomatic carrier of *C. difficile* or with mild symptoms, the indication for treatment is more doubtful. A key step to control CDI is discontinuation of current antibiotic therapy. This allows the intestinal flora to regrow. Simultaneously, anti-CDI treatment with appropriate antibiotics with adequate dose and duration must accompany to eradicate the infection along with reduce the recurrence.

According to ESCMID guidelines, two drugs metronidazole and vancomycin were considered as the cornerstone of CDI treatment. Metronidazole was first-line drug in nonsevere CDI, while vancomycin was the drug of choice for severe cases.[22] A statistically nonsignificant superiority has been shown by RCTs relative to metronidazole.[23] Fidaxomicin, a bactericidal antibiotic with a spectrum of activity against Gram-positive pathogens, has been available since 2011. It is poorly absorbed, achieves high fecal concentrations, and has minimal effect on fecal flora. It inhibits RNA synthesis in *C. difficile* and reduces toxin level and reduces spore formation. Efficacy wise, fidaxomicin is comparable to vancomycin as far as cure is concerned and may be more effective in reducing CDI recurrence.[24] In the 2017 IDSA and SHEA guideline, fidaxomicin is declared to be the cornerstone of CDI treatment. Two antibiotics are now being evaluated for CDI treatment—tigecycline is a glycylcycline that acts as a protein synthesis inhibitor and teicoplanin is a glycopeptide antibiotic that inhibits cell wall synthesis. Both showed variable results in different studies.

Currently for nonsevere CDI, oral vancomycin or fidaxomicin is advocated. Oral metronidazole is a second choice because of lower efficacy of this drug. The therapy is for 10 days. The test of cure by repeat stool testing is not indicated. The treatment plan remain similar in pregnant and breastfeeding women with oral vancomycin. Concurrent antibiotics may increase risk of prolonged diarrhea and recurrence of colitis. Antibiotics associated with lower risk of CDI, such as macrolides, aminoglycosides, sulfonamides, vancomycin, or tetracyclines, may be considered or current antibiotics may be converted to antibiotics that are used for CDI whenever indicated,

if such therapy is indispensable. The role of probiotics frequently used in diarrheal diseases is still not defined.

Naturally developing anti-toxin antibodies play a great role in development of immunity against CDI with some protective effects.[25] It has been found that monoclonal antibodies targeted against toxins A and B (actoxumab and bezlotoxumab, respectively) markedly reduce CDI recurrence. Bezlotoxumab was approved by the FDA in 2016 for prevention of recurrent CDI in patients with high risk of CDI recurrence.

Fecal Microbiota Transplantation

Fecal microbiota from stool of normal volunteers has the potential to reconstitute the colonic bacteria in a patient receiving multiple antibiotics due to other infection or a *C. difficile*-infected person and expected to be of promising therapeutic benefit.

Fecal microbiota transplantation (FMT) may be considered in the following situations to reduce the dysbiosis of gut:[26]

- Recurrent or relapsing CDI: Three or more episodes of mild-to-moderate CDI and failure of a 6–8-week taper with vancomycin with or without an alternative antibiotic (e.g., rifaximin, nitazoxanide, or fidaxomicin).
- Two or more episodes of severe CDI requiring hospitalization.
- Moderate CDI with no clinical response to standard therapy (vancomycin or fidaxomicin) for at least a week.
- Severe or fulminant CDI with no clinical response to standard therapy after 48 hours.

The FMT procedure has not yet been standardized. Donor stool is delivered to the institution within a few hours of passage. It then undergoes the following processes: (1) dilution, generally with normal saline; (2) homogenization with a blender to achieve a liquid slurry; and then (3) filtration to remove particulate matter to facilitate administration. Fecal transplant can be administrated via oral capsules, colonoscopy, retention enema, or the nasojejunal/nasoduodenal tube. Colonic perforation during colonoscopy and aspiration pneumonia with upper GI administration are two potential but grave complications in this sick group of patients. Lower GI tract FMT may result in better effectiveness and retention enema may be a low-cost safe and easy procedure, though patients may require repeated enema.[27] Fecal microbiota transplantation may be done using fresh feces or human microbiota mixed with a cryoprotectant, which is frozen for storage at −80°C.

Recurrent Infection

Recurrent CDI is defined as new occurrence of symptoms within 8 weeks of cure of the initial episode with successful treatment. The risk for first recurrence is about 10–20%, while the risk is going up to 40–65% after second or third recurrence.[28] Various risk factors may be responsible for the disease recurrence. This specially include older age, other antibiotic, immunosuppression, and proton pump inhibitors, inadequate cure, and severe disease.

Clostridium difficile Infection Prevention

Prevention of CDI requires implementation of various strategies. Correctable risk factors need to be reduced, which finally decrease susceptibility of a patient to CDI. Second important intervention should target to reduce the horizontal transmission of *C. difficile* spores from patients, asymptomatic carriers, and environmental surfaces. One episode of outbreak may involve many residents at a time enlarging the chain of transmission. The first instrument acts at the patient level and the second one acts at the institution level. The prevention tools are identified as bundles of CDI prevention strategies, often termed "standard prevention measures." Emphasis is given on timely detection of disease, hand hygiene, and environmental disinfection. Alcohol or chlorhexidine handwash do not eradicate *C. difficile* spores. Handwashing with soap water is mandatory after contact with *C. difficile* patient or environment. The choice of agent for environmental cleaning is also defined and should be with hypochlorite or bleach.

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