Clinical spectrum & pathogenesis of *Clostridium difficile* associated diseases

Chetana Vaishnavi

Department of Gastroenterology, Postgraduate Institute of Medical Education & Research, Chandigarh, India

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*Clostridium difficile* is the major aetiological agent of antibiotic associated diarrhoea and colitis. The majority of hospitalized patients infected by *C. difficile* are asymptomatic carriers who serve as silent reservoirs for continued *C. difficile* contamination of the hospital environment. *C. difficile* associated disease (CDAD) is a serious condition with mortality up to 25 per cent in frail elderly people. *C. difficile* infection may present itself in several forms with both colonic and extracolonic manifestations. Several factors are involved in determining whether or not a patient develops *C. difficile* infection. These include factors related to the pathogen as well as the host. Transmission of *C. difficile* can be endogenous or exogenous. Colonization of the pathogen occurs when the gut flora gets disrupted due to various factors. The main virulence factors for CDAD are the two potent toxins: toxin A and toxin B which share 63 per cent of amino acid sequence homology and act on small guanosine triphosphate binding proteins. The emergence of the global hypervirulent *C. difficile* strain has been a cause of concern. Diagnosis of CDAD infection can be done by detection of *C. difficile* toxin in the stool specimen. Vancomycin is the drug of choice for severely ill patient, whereas metronidazole can be used for mild to moderately ill patients. Clinical spectrum, the factors precipitating CDAD, pathogenesis, diagnostic assay and treatment of the disease are reviewed.

**Key words** Clinical spectrum - *Clostridium difficile* - diagnosis - pathogenesis - predisposing factors - treatment

**Introduction**

*Clostridium difficile*, a Gram-positive spore bearing anaerobic bacteria is the major aetiological agent of diarrhoea and colitis associated with antibiotics. Hall and O’Toole\(^1\) originally identified the organism as a component of normal colonic flora of newborn infants. *C. difficile* is commonly present in the stools of 5 per cent of healthy adults usually in low numbers\(^2\) and in about 15-70 per cent of infants\(^3-5\). The majority of hospitalized patients infected by *C. difficile* are asymptomatic carriers who serve as silent reservoirs for continued *C. difficile* contamination of the hospital environment\(^6\). However *C. difficile*-associated disease (CDAD) is a serious condition with mortality up to 25 per cent in frail elderly people\(^7\). *C. difficile* is now recognized as the primary cause of hospital acquired colitis in patients who receive antibiotics, chemotherapeutics or other drugs that alter their normal flora.

When *C. difficile* was first discovered, infection by the organism was regarded primarily as an outcome of
antibiotic intake and not as a life threatening disease. During the recent outbreaks of CDAD in at least 12 hospitals in the entire Estrie region in Quebec, a three-fold rise in the incidence of CDAD, and a higher number of cases involving toxic megacolon, colectomy or death have been reported. The mutant hypervirulent strain was typed as NAP1/BI/027 (North American PFGE type I/restriction endonuclease analysis BI/ribotype 027) and was found to produce greater than 16 times toxin A and 23 times toxin B in addition to the binary toxin. McDonald et al. found NAP1/BI/027 strain in eight institutions, in six different States in United States and it represented more than 50 per cent of the isolates from five institutions. This global epidemic strain has also been reported to cause outbreaks in parts of continental Europe, Great Britain, The Netherlands and Belgium with increased morbidity and mortality. The risk for CDAD has increased not only by usual factors, as pseudomembranous colitis (PMC), toxic megacolon and perforations in C. difficile was rare before 2002, but their incidence increased dramatically after that, particularly due to emergence of fluoroquinolone resistant strains.

C. difficile is also being reported more frequently even from non hospital-based settings, such as from the community. Domestic as well as wild animals are probably transmitting this as the same ribotypes found in them were found to be associated with human infection. In this review the clinical spectrum, the factors precipitating CDAD, pathogenesis, diagnostic assay and treatment of the disease are discussed.

Clinical spectrum

C. difficile, like virtually all bacterial enteric pathogens, causes a spectrum of clinical conditions with both colonic and extracolonic manifestations. The different manifestations are detailed below:

(a) Colonic manifestation

(i) Asymptomatic carriage: Colonization with C. difficile is the presence of the organism in a person with no clinical symptoms like diarrhea. Asymptomatic carriage of C. difficile is quite common in hospitalized patients. Epidemiologic studies have reported that 10-16 per cent of hospital inpatients in high-risk units become carriers after receiving antibiotics. Symptomatic disease is less often seen in carriers, despite the fact that most of the C. difficile isolates are toxin producing. Riggs et al. suggested that asymptomatic carriers of epidemic and non epidemic C. difficile isolates have the potential to contribute significantly to disease transmission in long-term care facilities. Asymptomatic carriage can be predicted by taking into account certain clinical factors such as recent antibiotic exposure or previous occurrence of CDAD. Patients with C. difficile colonization and a serum IgG response to C. difficile enterotoxin usually become asymptomatic carriers.

(ii) C. difficile diarrhoea: Usually mild to moderate diarrhoea, sometimes accompanied by lower abdominal cramps is seen with C. difficile infection. Symptoms usually begin during or shortly after antibiotic therapy. Occasionally these may be delayed for several weeks. C. difficile toxins can be usually detected from faecal specimens, even though endoscopic and histologic features may be normal in patients with mild disease. The diarrhoea resolves with the stoppage of antibiotics.

(iii) C. difficile colitis: The most common clinical manifestation of C. difficile infection is colitis without pseudomembrane formation. This is a more serious illness than benign or simple antibiotic-associated diarrhoea and presents as malaise, abdominal pain, nausea, anorexia, and watery diarrhoea. Abdominal pain and cramps in some patients are relieved by passage of stool. Sometimes dehydration and a low-grade fever with a systemic polymorphonuclear leukocytosis may occur. Levels of lactoferrin released from the secondary granules of intestinal leukocytes, as well as other inflammatory markers rise significantly in patients having advanced CDAD compared to patients with a milder form of the disease. Faecal lactoferrin assay performed simultaneously with the C. difficile toxin assay can help rule out asymptomatic carriage of C. difficile. A nonspecific diffuse or patchy erythematous colitis without pseudomembrane may be seen under sigmoidoscopy.

(iv) Pseudomembranous colitis (PMC): Finney was the first to describe PMC as a post-operative complication of gastroenterostomy. PMC is the classic manifestation of full-blown C. difficile colitis and is accompanied by similar, but often more severe symptoms than those observed in colitis. The classic pseudomembranes, which are raised yellow plaques ranging from 2-10 mm in diameter scattered over the colorectal mucosa are best revealed by sigmoidoscopic examination. White blood cell counts of 20,000 or greater and hypoalbuminaemia of 3.0 g/dl or lower may be observed in severely ill patients. Most patients with PMC have involvement of the rectosigmoid area. However, colonoscopy is required because as many as one third of patients...
have pseudomembranes limited to the more proximal colon\textsuperscript{24}. In patients with hypoalbuminaemia or acquired immunodeficiency syndrome a neutrocytic ascites with low serum to ascites albumin gradient may occur\textsuperscript{25, 26} with ascites being the only presenting manifestation of PMC.

(v) Fulminant colitis: \textit{C. difficile} infections may present as fulminant colitis in approximately 3 per cent of patients and account for most of the serious complications including perforation, prolonged ileus, megacolon and death\textsuperscript{35}. Patients with fulminant colitis complain of severe lower quadrant or even diffuse abdominal pain, diarrhoea, and distension and some of them may exhibit high fever, chills and marked leukocytosis. Diarrhoea may occur as a usual symptom, but may be minimal in patients with ileus as a consequence of which secretions accumulate in the dilated, atonic colon. Severe protein-losing enteropathy may result in hypoalbuminaemia. A patient with toxic megacolon has a dilated colon with signs and symptoms of severe toxicity that include fever, chills, dehydration and high white blood count. On plain abdominal radiograph the patient may also have dilated small intestine with air-fluid levels mimicking an intestinal obstruction or ischaemia or pseudo-obstruction\textsuperscript{28}. The risk of perforation and precipitation of megacolon\textsuperscript{29} deters a barium enema examination. However, computed tomographic scan of the abdomen is most valuable in severe cases and those localized to the proximal colon may reveal colonic distension, thickening, pericolonic inflammation, or free air\textsuperscript{29, 30}.

Signs and symptoms of bowel perforation may be present in some patients with fulminant \textit{C. difficile} infection. Further morbidity and mortality can be prevented in patients with fulminant \textit{C. difficile} colitis by aggressive diagnostic and therapeutic interventions\textsuperscript{25}. Though the risks of perforation are generally uncommon, limited flexible sigmoidoscopy or colonoscopy may be performed at the bedside\textsuperscript{31}. \textit{C. difficile} infection has also been reported to be involved in the exacerbation of ulcerative colitis\textsuperscript{32}. Hookman & Barkin\textsuperscript{33} observed that fulminant colitis is reported more frequently during outbreaks of \textit{C. difficile} in patients with inflammatory bowel disease (IBD) and carries higher mortality than those without underlying IBD.

(vi) Recurrent CDAD: Recurrent CDAD is a difficult clinical problem due to repeated recurrences of the manifestation. The pathophysiology is not quite clear and may be due to persistently altered faecal flora. Repeat antibiotics may subsequently be unable to suppress \textit{C. difficile} overgrowth. Alternatively, impaired immune response may also be responsible. It has been estimated that approximately 15-20 per cent of patients treated for CDAD, relapse following successful therapy\textsuperscript{34}. This condition is manifested by the sudden re-appearance of diarrhoea and other symptoms usually within a week of stopping treatment with vancomycin or metronidazole. Patients who relapse once are at greater risk of further relapses. McFarland \textit{et al}\textsuperscript{35} reported a relapse rate of as high as 65 per cent in patients who had suffered two or more previous relapses. Relapse is generally not related to antibiotic resistance because in some patients re-infection can occur with the same or different strain. The small bowel and the appendix may also act as reservoirs of \textit{C. difficile} spores that enter the colon and result in relapse\textsuperscript{36}.

The basis for variability in response to \textit{C. difficile} infection is not entirely clear, but host factors appear to be more important than bacterial virulence factors\textsuperscript{37}. It was suggested that there might be an association between the \textit{C. difficile} strains, production of toxins, and clinical manifestation of the infection\textsuperscript{38}. However, a few studies where \textit{C. difficile} was not an epidemic strain have shown that there was no difference between strains causing symptomatic cases and asymptomatic carriage\textsuperscript{39, 40}.

(b) Extracolonic features

Recent literature mentions that CDAD is no longer limited to the colon. \textit{C. difficile} may infrequently cause disease in a variety of other organ systems and except for bowel involvement and reactive arthritis most of the cases do not appear to be strongly related to previous antibiotic exposure though they are preceded by specific or nonspecific gastrointestinal (GI) disease. Some of the features of extracolonic diseases can be summed up as follows:

(i) Small bowel: Jacobs \textit{et al}\textsuperscript{41} reviewed literature on extracolonic manifestation of \textit{C. difficile} and revealed that small intestinal \textit{C. difficile} infections seem to be increasing in incidence. Small bowel CDAD with formation of pseudomembranes on ileal mucosa may occur when previous surgery on it has been carried out and is associated with a high mortality rate. Testore \textit{et al}\textsuperscript{42} examined jejunal specimens from 100 patients who died without any immediate history of GI symptoms and mucosal cultures in 3 cases treated with antibiotics were positive for \textit{C. difficile}. Boland and Thomson\textsuperscript{43} presented a case of \textit{C. difficile} enteritis in a 42 yr old
patient with ileal pouch-anal anastomosis and flexible endoscopy revealed copious amounts of mucus with adherent pseudomembranes throughout pouch and distal small bowel.

Navaneethan & Giannella\(^\text{44}\) reported that small bowel involvement is more frequently reported in IBD patients who have undergone total colectomy or in patients with ileal-anal anastomosis. The increase in the number of these patients may actually reflect an increase in the rising incidence of \textit{C. difficile} infection in general or increasing virulence of the infecting organism.

\textbf{(ii) Bacteraemia:} Like with other colonic bacteria, \textit{C. difficile} is also known to cause bacteraemia with about 20 per cent mortality\(^\text{45}\). Bacteraemia due to \textit{C. difficile} has previously been described in 14 patients with underlying GI processes\(^\text{46}\). These authors also reported a unique case of monomicrobial \textit{C. difficile} bacteraemia in a young woman with an underlying haematologic malignancy but without any GI symptoms.

\textbf{(iii) Reactive arthritis:} \textit{C. difficile}-related polyarticular kind of reactive arthritis may involve joints of the knee and wrist in about a 50 per cent of the cases\(^\text{47}\). Reactive arthritis begins an average of 11.3 days after the onset of diarrhoea and is a prolonged illness, taking an average of 68 days to resolve\(^\text{41}\). Ducroix-Roubertou \textit{et al}\(^\text{48}\) reported a case of a monoarticular arthritis of the left knee following PMC in a 45 yr old man, 8 days after the onset of a \textit{C. difficile} enterocolitis.

\textbf{(iv) Miscellaneous entities:} Other extracolonic manifestations due to \textit{C. difficile} include cellulitis, necrotizing fascitis, osteomyelitis, prosthetic device infections, intra-abdominal abscess, empyema, localized skin infections, \textit{etc}.

\textbf{Factors precipitating CDAD}

The following factors determine whether or not a patient develops a \textit{C. difficile} infection:

\textbf{(a) General factors:} An indepth review on established and potential risk factors for CDAD has recently been published\(^\text{49}\). Briefly these include (i) long duration or multiple antibiotic intake; (ii) the nature of the faecal flora; (iii) the size of the \textit{C. difficile} population; (iv) production of the requisite cytotoxins; (v) the presence of other organisms that affect toxin expression or activity, and (vi) the presence of host risk factors, including advanced age, presence of a nasogastric tube\(^\text{50}\), receiving anti-ulcer medication\(^\text{50}\), severe underlying illness, prolonged hospital stay, use of enemas, GI stimulants and stool softeners. Johnson & Gerding\(^\text{51}\) derived a model of pathogenesis for infection with \textit{C. difficile}. They hypothesized that a patient admitted to hospital was at negligible risk for infection until an antimicrobial agent was administered. If during or after treatment such a patient gets subsequently exposed to \textit{C. difficile}, he/she either develops CDAD or becomes colonized without diarrhoea or potentially does not get infected at all. But once established as an asymptomatic carrier, a patient is at decreased risk for CDAD. Patients are at continuous risk of exposure to \textit{C. difficile} during the period of hospitalization and become vulnerable to infection after they have been exposed to antimicrobials. The two most important components essential for CDAD is exposure to antimicrobials followed by exposure to \textit{C. difficile} and majority of the patients do not get ill with these till the third additional factor related to host immunity, virulence of infecting \textit{C. difficile} strain or to type and timing of exposure come into play.

\textbf{(b) Specific factors:} Immunosuppressive drugs have also been reported to be associated with the development of CDAD\(^\text{52,53}\). Faulty immune response to \textit{C. difficile} toxins has been quoted as one of the major host factors predisposing patients to the development of symptomatic CDAD\(^\text{54,55}\). Patients receiving immunosuppressive drugs are debilitated and therefore are unable to mount an effective IgG antibody response against \textit{C. difficile} toxin A thereby increasing the risk for CDAD\(^\text{15}\).

\textit{C. difficile} colonization is more frequent in intensive care and oncology units, where broad spectrum antibiotics and immunosuppression are wide spread\(^\text{56}\). Administration of tacrolimus, an immunosuppressive agent resulted in the development of CDAD\(^\text{57}\). Ulcerative colitis patients unresponsive to corticosteroids\(^\text{58}\) may require long time immunosuppressive treatment, which may result in multiple infections, inclusive of \textit{C. difficile}\(^\text{59}\). Five leukemic patients treated with immunosuppressives, died from secondary complications of PMC\(^\text{60}\). As the use of immunosuppressives increase, the incidence of CDAD will also rise and may account for as many as 20 per cent of patients of CDAD without prior use of antibiotics\(^\text{61}\).

Gastric acid suppressive use due to raised pH of stomach results in increased risk of CDAD\(^\text{62,63}\). Proton pump inhibitors (PPI) may thus contribute to the pathogenesis of CDAD, due to increased survival of spores. PPI use was a significant risk
factor for development of CDAD in a retrospective case control study. However, Pepin et al. reported that elevated risk of CDAD with PPI occurred only in univariate analysis but not so after adjustment for co-morbidities on multivariate analysis. Kaur et al. found that BALB/c mice treated with PPI had a higher experimental colonization with C. difficile, enhanced myeloperoxidase activity as well as greater level of epithelial damage, oedema and neutrophil infiltrates in the colon as compared to control untreated animals. Jayatilaka et al. in a five year study period found that PPI usage correlated exactly with the overall annual increased CDAD incidence and believed that the widespread prescription of PPI could be responsible. PPI therapy was reported as an independent and the only risk factor associated with reported as an increased length of hospital stay in CDAD patients. Thus the risk of CDAD in hospitalized patients receiving antibiotics may be compounded by exposure to PPI therapy.

Administration of cancer chemotherapeutic agents possessing antibacterial properties may also result in sufficient disturbance of the intestinal microflora to allow colonization with C. difficile. Emoto et al. reported severe CDAD in 6.1 per cent of patients receiving cisplatin based combination chemotherapy for ovarian malignancies. Resnik & Lefevre described development of fulminant C. difficile colitis in a 66 yr old patient with ovarian cancer who received paclitaxel and carboplatin chemotherapy. Kumar et al. reported that 32.7 per cent patients treated with methotrexate or mesalamine for psoriasis were positive for C. difficile toxins. Exposure to corticosteroids was also significantly associated with an increased risk of CDAD relapse.

Thus the combination of the environmental presence of C. difficile in health care settings and the number of people receiving antibiotics, immunosuppressives, PPI or cancer therapeutics in these settings can result in frequent outbreaks.

(c) Factors responsible for recent increase in CDAD in the West: Zerey et al. demonstrated that the incidence of C. difficile infection was increasing in surgical patients in United States and was most prevalent after emergency operations particularly among patients having intestinal tract resections.

The increased incidence of nosocomial CDAD in the West with marked increase in severity of cases requiring colectomy or ending in death was attributed to liberal use of fluoroquinolones and cephalosporins. The NAP1/BI/027 strain poses a great risk as it is also found to be resistant to fluoroquinolone. In 2007, severe cases of CDAD with this epidemic strain was detected in Germany for the first time and was strongly associated with receipt of cephalosporins and fluoroquinolones in the 3 month before onset of symptoms.

Pathogenetic mechanisms

Transmission of C. difficile occurs both endogenously as in the carrier state and exogenously through nosocomial source. When an individual is exposed to C. difficile or its spores, an initial disruption of the normal colonic bacterial flora occurs resulting in colonization of the organism through surface proteins. Damage to enterocytes due to C. difficile toxins occur as soon as C. difficile colonizes the intestine resulting in cytoskeletal changes and the release of fluids and inflammatory products. Pathogenic C. difficile produces two high molecular weight potent toxins - A and B - which bind to specific receptors on the luminal aspect of the colonic epithelium. The role of surface proteins and toxins of C. difficile in the pathogenesis of CDAD are detailed below:

(a) Surface proteins of C. difficile: Colonization process is thought to be a necessary preliminary step in the course of C. difficile infection. Calabi et al. investigated tissue binding of C. difficile surface layer proteins (SLPs) which are the predominant outer surface components encoded by sIpa gene. They demonstrated that SLPs play a role both in the initial colonization of the gut by C. difficile and in the subsequent inflammatory reaction. Different adhesins implicated in the colonization process of C. difficile are (i) flagella, composed of the flagellin Fli C and the flagellar cap protein Fli D, involved in cell and mucus attachment (ii) a cell-surface protein with adhesive properties, Cwp 66 (iii) a fibronectin-binding protein, Fbp68 and (iv) s-layer protein.

These adhesins are able to induce an immune response, which could play a role in the defense mechanism of the host. Janoir et al. observed that cwp84, a surface protein exhibited proteolytic activity which could contribute to the degradation of the host tissue integrity and to dissemination of the infection.

(b) Role of C. difficile toxins: The main virulence factors for CDAD are the two potent toxins - toxin A and toxin B that share 63 per cent of amino acid sequence homology. Both the toxins induce mucosal injury and colitis as seen by neutrophil infiltration, which is a prominent feature of CDAD.
Toxin A is an enterotoxin that causes haemorrhage and fluid secretion in the intestines of rodents whereas toxin B is a cytotoxin detectable by its cytopathic effects in tissue culture. However, both toxins affect the cytoskeletal features, even though their activity differs in potency. Toxins A and B have been shown by nucleotide sequencing to be located in close proximity to each other within a 19.6 kbp pathogenicity locus (PaLoc) encoded by two separate genes (tcdA and tcdB) on the same chromosome. C. difficile can be divided into 24 toxinotypes based on the changes in both toxin genes. Some toxinotypes possess a third kind of toxin known as the binary toxin described elsewhere in this review.

The toxins get transported into the cytoplasm where these act on small guanosine triphosphate binding proteins known as the Rho proteins. The Rho proteins are associated with actin polymerization, maintenance of the cytoskeletal architecture, and the cell movement. A severe inflammatory reaction in the lamina propria with the formation of micro-ulcerations of the colonic mucosa that is covered by a pseudomembrane occurs due to the activity of the toxins.

(i) Toxin A - Toxin A is a 308-kDa lethal enterotoxin and minute quantities can stimulate fluid secretion in animal intestinal loops similar to cholera toxin though the mechanism of action is quite different. Toxin A causes extensive damage to the epithelial lining of the intestine and accounts for nearly all of the GI symptoms. The villus tips of the epithelium are initially disrupted followed by damage to the brush border membrane. Katyal et al. reported a disturbance in the intestinal brush border membrane enzymes in CDAD patients. Denuding of the mucosa eventually is accompanied by extensive neutrophil infiltration resulting in massive inflammation. The fluid response is partly an outcome of the damage to the intestinal epithelium. Toxin A also acts as a cytotoxin resulting in disruption of the tight junctions of the intestinal epithelium and might be an important mechanism of toxin A enterotoxicity.

Toxin A also elicits the production of various cytokines and neurokinins, the biological reactants, which are believed to be playing an important role in pathogenesis. Purified toxin A has potent effect on human colonic epithelial cells as seen in vitro. Toxin A initially induces cell rounding which results in detachment of the cell from the basement membrane, followed by apoptosis. Toxin A also brings about a rapid loss of resident cells such as macrophages, T cells and eosinophils and induces changes in the shape of adherent polymorphonuclear leukocytes. At least two pathophysiologic pathways are involved in changes in the epithelial cell barrier via glycolysation of the Rho proteins. These are (i) disaggregation of actin microfilaments leading to epithelial cell destruction and opening of tight junctions, and (ii) early release of proinflammatory cytokines from intestinal epithelial cells probably via activation of mitogen-activated protein kinase. The spherical cells become thin and rope like with rearrangement of F-actin cytoskeleton into aggregates. Thus the toxins alter the actin cytoskeleton, cause epithelial cell damage and result in increased permeability of the tight junctions. A severe acute necro-inflammatory reaction is produced by toxin A in the intestine resulting in activation of mast cells, vascular endothelium, and immune cells.

(ii) Toxin B - Toxin B is also a very large and potent cytotoxin with 279 kDa molecular weight. It causes a number of non specific in vitro responses in mammalian cells. This includes disorganization of the actin filaments, loss of intracellular potassium, decrease in the level of protein and nucleic acid synthesis. Under normal physiological conditions toxin B by itself cannot cause damage or a fluid response in intestinal loops probably due to its inability to bind to the specific carbohydrate receptors on the intestinal brush border membrane. After toxin A has bound to the receptor initiating the damage, toxin B joins in and gains access to the underlying tissue as supported by animal experimentations.

The cytotoxic activity of toxin B is similar to that of toxin A, but is 1000-fold more potent than the former. There is formation of neurite-like retraction fibers resulting in partial detachment of cells. Next the cell-spanning stress fibers disappear and the remainder of the actin filaments accumulates in the perinuclear space. Both toxins disrupt the function of the Rho family of protein. Decreased transepithelial resistance and increased flux of paracellular marker such as mannitol and raffinose indicate the disruption of the tight junction.

(iii) Binary toxin - Another toxin, which is an iota-like toxin, was described from the C. difficile strain CD196 and has been named binary toxin CDT. Binary toxin contains both toxins A and B and is a product of both toxin genes (cdtB for the binding component and cdtA for the enzymic component). It is located on the chromosome outside the PaLoc and is actin-specific
ADP-ribosyltransferase toxin that could be acting synergistically. It has not been found to be essential for eliciting *C. difficile* associated colitis. Binary toxin CDT is produced by most of *C. difficile* isolates with mutations in the *tcdA* and *tcdB* genes\(^\text{100}\) and up to 2 per cent A B strains of *C. difficile* are estimated to produce only binary toxin CDT\(^\text{101}\).

*Diagnosis by toxin assay: C. difficile* toxins can be detected in the faecal samples by several methods as mentioned below:

(i) **Tissue culture:** Tissue culture can detect as little as 1.0 pg of toxin B making it the most sensitive test available and has therefore been regarded as the gold standard in laboratory diagnosis of *C. difficile* toxin. Toxin identification can be confirmed with *C. difficile* antitoxin or antitoxin against *C. sordellii*, which produces the cross-reacting toxins\(^\text{102}\). Commercial tests recommend a final dilution of 1:40 to 1:50 of the stool sample\(^\text{103}\). Cell lines that may be used include Vero, Hep 2, Chinese hamster ovary, HeLa cells and MRC-5 lung fibroblasts.

Many disadvantages accompany tissue culture technique because it is the least controlled test. Specimens may at times cause nonspecific cell rounding that is neutralized not only by the specific antitoxin but also by neutral serum. The addition of too much faecal material to the tissue culture well can cause false positive reactions. The maintenance of cell cultures is also very difficult. The procedure is cumbersome, expensive and time consuming and requires a well-developed laboratory. Sometimes a non specific cytopathic effect can also occur in a few of the cases due to a viral agent or another bacterial enterotoxin such as that of *C. perfringens*, rendering any interpretation difficult. False negatives can occur in stored samples due to several reasons such as (i) toxin degrading enzymes, (ii) delay in transportation of sample, (iii) medication of the patient, and because (iv) some cell lines are less sensitive than others to the cytopathic effect of the toxin. Infact, a negative cytotoxicity assay does not completely rule out *C. difficile* as the cause of diarrhoea.

(ii) **Counter immunoelectrophoresis:** This method for detection of toxin is expensive, cumbersome, and lacks the required levels of sensitivity and specificity for satisfactory diagnostic test.

(iii) **Latex agglutination test:** Commercially available latex agglutination test (LAT) is rapid, but is

unaffordable for routine use because of the high cost per test. Moreover commercially available LAT is known to detect a non toxic marker antigen for *C. difficile* and therefore frequently results in false positive reactions. Now a days commercial LAT is less frequently available and has been replaced by enzyme-immunoassays.

(iv) **Enzyme immunoassays:** Enzyme-linked immunoassays (ELISA) are commercially available to detect either toxin A alone or both toxins A and B in stool specimens. ELISA has sensitivity and specificity ranges of 50 to 90 per cent and 70 to 95 per cent respectively. About 100 to 1000 pg of toxins must be present for the test to be positive. The advantage of using ELISA is because of the lesser time required for the test. But the high cost per single test may necessitate batching of samples. A high percentage of indeterminate readings may also occur.

Most ELISA have a sensitivity of more than 80 per cent compared to that of tissue culture assay. However, ELISA that detect only toxin A may miss out on toxins from A B isolates resulting in wrong interpretation. Thus, ELISA that detect both toxin A and B are recommended to detect these atypical isolates. Such tests will also take care of specimens containing low levels of toxin A and B.

(v) **Dot immunobinding assay:** The test is carried out on the surface of individual membrane cassette employing the principle of enzyme immunoassay (EIA). Stool supernatant is made to pass through a filter onto the membrane and then made to react to mouse monoclonal antibody to *C. difficile* toxin. Appropriate enzyme conjugate and substrate are added to visualize the blue coloured dot. Sometimes the presence of excessive amount of debris can cause difficulty in interpretation of the results.

(vi) **Rapid membrane tests:** These are lateral flow devices with coloured conjugates or flow through formats that require multistep processing. Such tests utilize peroxidase tagged antibodies and a wash step followed by the addition of a substrate. The sample preparation for these tests requires centrifugation or filtration. These tests have sensitivity in the range of 60 to 89 per cent. These tests are toxin A specific and therefore do not detect A B isolates.

(vii) **Polymerase chain reaction:** The polymerase chain reaction (PCR) technique is used to detect enterotoxin or toxin B gene in isolates or faeces and has sensitivity similar to cytotoxin testing. The advantage of PCR
is the rapidity of the test. But PCR needs appropriate infrastructure and technical expertise, and is time-consuming. Most assays target only one of the two genes, potentially missing isolates carrying only one of them. Feces may contain PCR inhibitory components, which can cause difficulties in the assay. Moreover, PCR detects even minute number of C. difficile genome copies present even in healthy individuals thereby overemphasizing the aetiology.

(viii) Immunochromatography assay: This technique is a single test EIA for detection of toxins A and B in faecal samples. It can be done within 20 min and without any requirement of pre-treatment.

(ix) Loop mediated isothermal amplification: Loop mediated isothermal amplification (LAMP) is a rapid and simple method for detecting toxin B gene in stool samples as well as in isolates. Detection of tcdB by LAMP from overnight cultures in cooked meat medium could be an alternative method of diagnostic testing at clinical laboratories without special apparatus. Even though the technique is easier to perform it is not as sensitive as the PCR.

Therapy and management

Withdrawal of the antibiotic therapy that precipitated the disease or at least changing antibiotic regimens results in early resolution of the diarrhoeal symptoms even in some cases of established PMC. Fluid replacement and electrolyte balance maintenance is important. About 25 per cent of patients respond within a few days to these simple measures. In case of non response, they should be treated with specific antimicrobial therapy, which is crucial to prevent the progression of C. difficile pathogenesis. The drug of choice for seriously ill patients is oral vancomycin because it has no side effect and is not absorbed by the intestine. Diarrhoea generally resolves over an average of 5 days even though up to 50 per cent relapse rate may occur after vancomycin treatment. Vancomycin administered in the form of capsules, help to camouflage its bitter taste. However, in seriously ill patients, oral suspensions help to achieve high concentrations in the colon more quickly. Vancomycin can also be administered by nasogastric tube, a long intestinal tube, by enema, or by direct instillation through a colostomy or ileostomy in patients too ill for oral therapy. Intravenous administration of vancomycin to patients unable to take oral medication can be done with the hope that some of the drug will reach the colonic lumen through the inflamed mucosal surface. Other desperate measures in patients who continue to do poorly are cecostomy or colectomy. Some patients develop a series of relapses, thereby extending the illness. Use of vancomycin in routine is discouraged because it is expensive and there is a risk of development of vancomycin-resistant enterococci.

Oral metronidazole can be used in place of vancomycin and is favoured because it is less expensive. Randomized trials show excellent initial responses in approximately 95 per cent of patients treated with metronidazole. But, the disadvantage of the drug is the near complete absorption such that the levels achieved in the colon are virtually nil. Resistance to metronidazole has been found in some isolates of C. difficile. Metronidazole therefore is used for patients with mild or moderate illness but should not be used for critically ill patients.

Antibiotics that are active against C. difficile include ampicillin, bacitracin, fusidic acid and teicoplanin, which have been tried with little success. C. difficile also shows good in vitro susceptibility to various antimicrobials, such as rifaximin, ramoplanin and nitazoxanide and these could be used in future trials. Antimicrobial treatment also kills the normal bacterial flora thereby causing the disease to recur. Once the colon has been injured, it seems to be more susceptible to reinfection. Nelson suggested that in order to improve the patient’s clinical condition and prevent the spread of C. difficile infection to other patients if one does decide to treat, one should choose the antibiotic that brings both symptomatic and bacteriologic cure and that teicoplanin appears to be the best choice.

Limited success can be achieved by administration of intravenous gamma globulin. Probiotics like Lactobacilli species or Saccharomyces boulardii can also be used to replace the pathogenic C. difficile flora. Even rectal instillation of fresh stool in saline from living related donors and rectal instillation of mixed broth cultures of stool flora have been tried to replenish the normal flora. Binding agents like cholestyramine, Isabgol husk and tlevamix may also bind to administered drugs and delay elimination of C. difficile toxins.

Parenteral administration of C. difficile toxoid vaccine might protect high-risk individuals against CDAD by development of antibody response.

Indian experience with CDAD

The prevalence of C. difficile-associated colitis is global and the incidence varies considerably from place
to place. In India, the studies on C. difficile-associated diarrhoea have been limited, probably due to the lack of technology and the difficulty in culturing the pathogen. Available reports from India estimate a prevalence of 15-30 per cent of paediatric and adult patients taking antibiotics. Gupta & Jadav reported 25.3 per cent isolation of C. difficile from diarrheal patients of all age groups. Niyogi et al. reported C. difficile in 8.4 per cent and cytotoxin in 7 per cent of faecal samples in children between 0-14 yr of age. C. difficile was the only pathogen in 7.3 per cent of patients with acute diarrhoea whereas 3.1 per cent control children without diarrhoea harboured the organism. Niyogi et al. isolated C. difficile in 11 per cent hospitalized patients with diarrhoea and 2.9 per cent non diarrhoeic controls; 87 per cent isolates produced cytotoxin even though the diarrheic patients had no history of antibiotic use. Bhattacharya et al. investigated 233 patients with acute diarrhoea and isolated C. difficile as a sole pathogen from 7.3 per cent, of which, 82.4 per cent produced cytotoxin. In another study Niyogi reported that of the 43 C. difficile isolates, 100 per cent were inhibited by low concentrations of metronidazole, penicillin G, tetracycline and ampicillin, but were highly resistant to gentamycin, trimethoprim, sulphamethoxazole, nalidixic acid, cycloserine and cefotaxime.

Kochhar et al. demonstrated that infectious agents like C. difficile were responsible for some of the exacerbations in ulcerative colitis patients without any history of recent exposure to antimicrobial drugs or hospitalization. Vaishnavi et al. reported 30 per cent positivity for C. difficile toxin in hospitalized patients of all age group receiving single to multiple antibiotics for various ailments, but only in 7 per cent of samples from patients not receiving antibiotics. When only adult population were investigated, the positivity for C. difficile toxin was 19.4 per cent in the antibiotic receiving hospitalized patients. Kang et al. reported that C. difficile-associated diarrhoea was more common in the post transplantation period in India than in developed countries.

Gogate et al. observed that C. difficile was an important pathogen for antibiotic associated diarrhea in children of age group 5-12 yr. Vaishnavi et al. reported the association of C. perfringens with antibiotic associated diarrhea either by itself or in synergy with C. difficile infection. Balamurugan et al. reported overgrowth of C. difficile in the stool of Indian patients with ulcerative colitis compared to healthy controls using real time PCR. A decrease in the number of C. difficile positive cases has been reported during a 5 year study period and attributed the reduction to stringent surveillance and an improved antibiotic policy adopted in the hospital.

Conclusions

C. difficile associated disease is a growing nosocomial and public health problem. Hospitalized patients receiving antibiotics for their ailments are at great risk of acquiring CDAD. Clinical suspicion is more important than ever before because stool assays for diagnosing CDAD are not widely available. Wherever available it is fraught with inherent problems and therefore diagnosis may be missed or delayed. Several measures significantly reduce the incidence of CDAD. Infection control procedures that should be followed to prevent spread of the disease include environmental hygiene, washing hands with ordinary soap and water or using 0.03 per cent Triclosan and isolating patients with CDAD. Environmental cleaning should be done with phenolic disinfectant. Isolated patients should have equipment dedicated to them to reduce cross-contamination. Infection control plays a key role in controlling CDAD outbreaks. The spores of C. difficile can survive in the hospital environment for months, providing a reservoir for new infections. Active and aggressive surveillance activity is the key to reduce incidence. Monitoring should enable the development and implementation of policies and procedures that minimize the risk of this nosocomial pathogen. Preventing C. difficile infection offers a potentially significant improvement in patient’s outcomes, as well as a reduction in hospital costs and resource expenditures.

References


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Reprint requests: Dr C. Vaishnavi, Additional Professor (GE Microbiology), Department of Gastroenterology, Postgraduate Institute of Medical Education & Research, Chandigarh 160 012, India
e-mail: cvaishnavi@rediffmail.com, cvaishnavi@sify.com