REVIEW

Pathophysiology, Diagnosis and Treatment of Clostridium difficile Infection

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Abstract. Clostridium difficile infection has become in recent years an important nosocomial threat. Prevention of the spread of C. difficile infection among long term hospitalized patients is a major challenge since C. difficile spores can persist indefinitely in the hospital environment. Following antibiotic therapy that disrupts the normal bacterial flora of the colon, C. difficile can colonize the large intestine. The bacteria releases two large protein toxins that bind to colonocytes and mediate an acute inflammatory diarrhea characterized by an abundant exudate rich in neutrophils and proteins that in some cases can form the typical "pseudomembrane". C. difficile infection shows a spectrum of severity from asymptomatic carrier to fulminant acute pseudomembranous colitis. The gold standard for the laboratory diagnosis of C. difficile infection is the stool-cytotoxin test, however recently developed immunoassays represent a good alternative. The treatment of C. difficile infection is based on the severity of the clinical picture. In patients with mild diarrhea discontinuation of the causing antibiotic can be an adequate therapeutic approach, whereas patients with more severe symptoms require antibiotic therapy or, in the most severe infections, even colectomy. (Keio J Med 48 (4): 169-174, December 1999)

Key words: Clostridium difficile, colitis, enteric infection, antibiotics, toxins

Clostridium difficile is the cause of antibiotic-associated diarrhea in humans, now recognized as the commonest enteric infection in US hospitals, with a 10% symptomatic infection rate for hospitalized patients. The organism colonizes the human colon after the normal colonic flora has been altered by antibiotic therapy and then releases two protein exotoxins, toxins A and B. C. difficile toxins bind to specific receptor(s) on colonocytes to stimulate a massive fluid secretion (diarrhea) and necrosis of the surface mucosa associated with an acute inflammatory infiltrate. Infection with C. difficile produces a spectrum of clinical responses, varying from the asymptomatic carrier state to fulminant pseudomembranous colitis.

Clostridium difficile Bacteriology

C. difficile was first described in 1935 by Hall and O'Toole who were investigating the acquisition of normal bacterial flora in healthy newborns. They decided to name this gram-positive bacillus of the genus Clostridia as the "difficult clostridia" because it was resistant to isolation and culture on conventional media. Although the bacteria was toxigenic and caused rapid death of animals injected with culture filtrate of C. difficile, initially it was considered a harmless intestinal commensal since infants colonized by this organism had no signs of illness. Not until 1978 was it discovered that C. difficile was the source of the cytotoxin found in the stools of patients with antibiotic-associated pseudomembranous colitis. This organism is now recognized as a major nosocomial pathogen with substantial morbidity in elderly hospitalized patients. However, not all strains of C. difficile are toxigenic. During outbreaks of C. difficile infection in hospitals, some patients may be colonized by non-toxigenic strains. C. difficile strains can be categorized as low, medium and high toxin producers, but disease severity does not appear to correlate with the production and concentration of toxins in the stools. C. difficile strains have been also classified upon serotype, bacteriophage, electrophoretic profiles of bacterial proteins, however these classifications have

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little clinical utility except to track hospital outbreaks.\textsuperscript{5}

**Pathophysiology of Clostridium difficile Colitis**

A critical chain of events is necessary to induce \textit{C. difficile} colitis. Following antibiotic therapy the normal intestinal flora is disrupted allowing \textit{C. difficile} to colonize the intestinal lumen. The fact that infection occurs only after the normal microbial intestinal flora is altered by administration of antibiotics suggests that certain organisms in the normal flora prevent colonization by \textit{C. difficile}.\textsuperscript{6} For example \textit{Bacteroides} accounts for over 90\% of the total fecal flora, and some of these species disappear in patients with \textit{C. difficile} diarrhea and colitis, whereas recovery from the infection is accompanied by repopulation with \textit{Bacteroides}.\textsuperscript{7} Almost all antibiotics including vancomycin and metronidazole which are generally used to treat patients with active colitis\textsuperscript{8} and even some cancer chemotherapeutic agents can predispose to colonization by \textit{C. difficile}.\textsuperscript{9} However some antibiotics are more likely to be associated with symptomatic \textit{C. difficile} infection, probably due to differential effects on the normal colonic flora.\textsuperscript{10} Clindamycin is the most common antibiotic associated with infection and diarrhea, however broad spectrum penicillins and cephalosporins are also commonly associated with \textit{C. difficile} colitis.

When pathogenic strains of \textit{C. difficile} colonize the colon they release two unique protein exotoxins, toxin A (308 kDa) and toxin B (275 kDa) (Table 1).\textsuperscript{11} These toxins have no structural or functional similarities with the classic bacterial enterotoxins, such as cholera toxin, \textit{E. coli} enterotoxin or shiga toxin.\textsuperscript{12} \textit{C. difficile} toxins possess potent cytotoxic activity against cultured fibroblast and other cells.\textsuperscript{13} The cytotoxic effect results in cell rounding which is due to disaggregation of filamentous actin and dysfunction of tight junctions caused by inactivation of the small GTP-binding protein Rho, which is required to maintain actin integrity.\textsuperscript{14} Both toxins bind to glycoprotein receptors on the human colonocyte brush border and cause necrosis and shedding of epithelial cells into the lumen.\textsuperscript{15} Toxin A, but not toxin B, mediates inflammatory diarrhea in experimental animals.\textsuperscript{11} \textit{In vivo} studies in rodents showed that injection of purified toxin A into closed intestinal loops elicits an acute inflammatory diarrhea with massive fluid secretion, mucosal damage and a prominent neutrophils infiltration which is evident 2–4 hours after injection of the toxin.\textsuperscript{8,11}

The pathogenesis of \textit{C. difficile} toxin A inflammation involves interaction between epithelial cells, sensory nerves and inflammatory cells of the intestinal lamina propria. Sensory nerves appear to be involved in the early stages of the inflammatory cascade induced by \textit{C. difficile} toxin A.\textsuperscript{16} Several studies showed that the release of the peptide substance P from intestinal sensory nerves is a critical early step in the inflammatory cascade.\textsuperscript{17,18} This peptide appears to activate macrophages and mast cells of the lamina propria, which in turn release inflammatory mediators that up-regulate the expression of adhesion molecules on endothelial cells and neutrophils, mediating neutrophil recruitment and migration into the intestinal mucosa. Neutrophil-derived inflammatory mediators are major elements in the pathogenesis of \textit{C. difficile} colitis causing acute destruction and necrosis of enterocytes. The importance of neutrophils is supported by experiments showing that preventing neutrophil recruitment during toxin A-mediated colitis markedly reduced intestinal inflammation, epithelial damage and diarrhea.\textsuperscript{19}

**Table 1** Clostridium difficile Toxins A and B

<table>
<thead>
<tr>
<th></th>
<th>Toxin A</th>
<th>Toxin B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>308 kDa</td>
<td>275 kDa</td>
</tr>
<tr>
<td><strong>Receptor</strong></td>
<td>Glycoprotein</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Cytotoxicity</strong></td>
<td>+</td>
<td>100-fold more potent than Toxin A</td>
</tr>
<tr>
<td><strong>Molecular Mechanisms</strong></td>
<td>Rho-glucosylation</td>
<td>Rho-glucosylation</td>
</tr>
<tr>
<td><strong>Neurotoxic</strong></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Enterotoxic</strong></td>
<td>experimental animals</td>
<td>no</td>
</tr>
<tr>
<td><strong>human colon</strong></td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

**Pathology of Clostridium difficile Infection**

On sigmoidoscopic inspection of the colonic or rectal mucosa of patients with \textit{C. difficile} colitis, pseudomembranes appear as yellow or off-white raised plaques of 0.2 to 2 cm in diameter scattered over a fairly normal or hyperemic intervening mucosa. The exposure of human colon to \textit{C. difficile} toxins is followed by shedding of cells from the basement membrane into the lumen leaving a shallow ulcer on the mucosal surface. Then serum protein, mucus and inflammatory cells flow outward from the ulcer, creating the typical colonic pseudomembrane. The spewing forth of the inflammatory exudate from the mucosal ulcers produces the typical "volcano" or "summit" lesions of \textit{C. difficile} colitis. The patchy distribution of the pseudomembranes is probably related to a toxin-dose response effect. Exposure \textit{in vitro} of colonic mucosa to low toxin B concentrations causes patchy cellular damage, whereas at higher toxin concentrations the damaged area becomes nearly confluent.\textsuperscript{20}
Epidemiology and Transmission of *Clostridium difficile* Infection

*C. difficile* is an important cause of morbidity in hospital and nursing home patients since they are more likely to take antibiotics in an environment that is highly contaminated with *C. difficile* spores (Fig. 1). An estimated three to four million new cases of *C. difficile* diarrhea and colitis occur in United States hospitals each year, affecting as many as 10% of patients hospitalized for more than two days.1 *C. difficile* typically infects elderly, debilitated inpatients.2 In contrast, the infection is quite rare in the outpatients setting with only 20,000 estimated cases of infection yearly.2 Risk factors for infection include exposure to multiple antibiotics, gastrointestinal tract surgery, enteral tube feeding and exposure to an infected roommate.2,4 The presence or absence of serum antibodies to *C. difficile* toxins may also play an important role in determining the susceptibility to and severity of colitis.25

Once antibiotic therapy has rendered the bowel susceptible to infection, fecal-oral colonization by *C. difficile* occurs by ingestion of *C. difficile* heat-resistant spores that persist in the environment for months. Contamination in the environment is especially common in hospitals and facilities providing long term care.2,6,7 *C. difficile* can be cultured from swabs taken from toilets, bedpans, floors, mops, scales and furniture in hospitals and nursing homes. Fecal carriage of the organism by health care workers is unusual, but carriage of *C. difficile* on the hands, clothing and stethoscopes of hospital staff probably allows passive spread of the infection. The use of vinyl examining gloves and careful hand washing after examining patients can drastically reduce the spread of infection.28

Newborns also acquire *C. difficile* from the hospital environment where the organism is commonly cultured from environmental surfaces in newborn nurseries and neonatal intensive care units.2 Newborns as young as 1 day of age can harbor *C. difficile* spores for several weeks or months, and the organism can be cultured from stools for 1 to 2 months in 10% of asymptomatic newborns.29 The asymptomatic carriage rate of *C. difficile* is as high as 60% or 70% in several longitudinal studies of healthy infants less than one year of age. Many infants carry toxigenic strains with high titers of toxin in the stools, but they are completely asymptomatic.29 This suggests that one or more host factors required for pathogenesis are lacking in the first year of life. One possibility is that infant intestine lacks specific receptors for the toxins, which then develop later in life. Indeed, specific toxin A binding sites are absent in newborn rabbit intestine and are then expressed after weaning, gradually reaching adult levels.30

### Clinical Presentation

The clinical presentation of *C. difficile* infection is quite variable and includes asymptomatic carriage, antibiotic-associated colitis without pseudomembrane formation, pseudomembranous colitis, and fulminating colitis.

#### The carrier state

Whereas over 50% of healthy neonates are asymptomatic carriers, less than 1% of adults carry *C. difficile* spores in their intestine. The number of adult carriers increases in hospitalized patients recently treated with antibiotics. The host and bacterial factors that determine asymptomatic carriage are not clear. Asymptomatic carriers are an important reservoir of *C. difficile*, since they can contaminate the environment and perpetuate the chain of infection.31 However, treatment of asymptomatic carriers with antibiotics is not recommended, since it does not permanently reduce the rate of carriage.32

#### Diarrhea without colitis

The most common clinical presentation of *C. difficile* infection is mild to moderate diarrhea sometimes accompanied with lower abdominal cramping whereas systemic symptoms are generally absent. Typically symptoms begin either during or shortly after antibiotic therapy, but can be delayed for several weeks. In these patients with mild disease physical examination and sigmoidoscopy are frequently normal whereas *C. difficile* toxins are present in the stools. The diarrhea often subsides when the initiating antibiotic is stopped, thus making specific treatment unnecessary.

#### Colitis

*C. difficile* infection can cause severe colitis without formation of pseudomembranes and is generally associated with profuse diarrhea, abdominal distension and pain. Systemic manifestations include fever, anorexia,
nausea and leukocytosis. Sigmoidoscopy reveals diffuse or patchy, non-specific colitis. Pseudomembranous colitis has a clinical picture similar to C. difficile colitis except that local and systemic manifestations are more severe. In addition sigmoidoscopy reveals characteristic adherent yellow plaques or pseudomembranes which may coalesce to form a confluent membrane. In about 10% of cases only the proximal colon is affected; therefore the pseudomembranes can be missed by sigmoidoscopy alone.

Patients with C. difficile colitis occasionally present with acute abdomen and fulminant colitis. As a consequence of greatly diminished colonic muscular tone toxic megacolon can result. Plain abdominal radiographs may reveal a dilated colon and ileum or air in the abdominal cavity if perforation has occurred. Massive mucosal edema of the colon may be evident as "thumbprinting" on an abdominal film or as marked thickening of the colonic wall on a computed tomographic scan. Sigmoidoscopy should be avoided to prevent perforation, but a proctoscopy with minimal air insufflation is safe and may be useful for the diagnosis.

**Laboratory Diagnosis**

The gold standard for the laboratory diagnosis of C. difficile infection is the stool-cytotoxin test. This is a tissue culture assay based on the induction of cell rounding by C. difficile toxins in stool filtrate. This test is extremely sensitive (94-100%) and specific (99%), since only a few picograms of toxin B are sufficient to induce cell rounding. Neutralization of cell rounding by using specific anti-toxin B sera confers specificity to the assay. The test is generally reported as positive or negative, as there is no direct correlation between toxin B levels in the stool and severity of the disease. Stool culture for C. difficile is a less specific method for establishing a diagnosis, since non-toxigenic strains of C. difficile can also be isolated with this approach.

More recently rapid immunoassays for the detection of C. difficile antigens or toxins have been developed and are now commercially available. The latex-agglutination test has been widely used but has poor sensitivity and specificity, since it recognizes a protein present in non-toxigenic strains of C. difficile can also be isolated with this approach. Patients with confirmed or suspected C. difficile infection is to discontinue antibiotic therapy, if possible (Table 2). In patients with only mild symptoms this measure alone may be sufficient to allow recovery. Specific therapy to eradicate C. difficile is required if symptoms are more severe and persistent or the antibiotic therapy that induced the disease cannot be discontinued. Metronidazole (250 mg po tid a day for 7-10 days) and vancomycin (125-500 mg tid for 10 days) are equally effective in treating C. difficile induced colitis. Because of the high cost of vancomycin, metronidazole is the drug of first choice. Symptomatic improvement can be expected within 72 hours, and diarrhea and colitis resolve completely in more than 95% of patients after 10 days of treatment. Patients who cannot tolerate oral medication because of ileus can be treated with intravenous metronidazole. Excretion of the drug into the bile and exudation from the inflamed colonic mucosa will result in bactericidal concentrations in the intestinal lumen. The absence of significant improvement after 48 to 72 hours of antibiotic therapy may indicate a more serious infection that requires surgery.

**Table 2 Treatment of Antibiotic-associated Colitis**

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<thead>
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<th>Step</th>
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<tbody>
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</tr>
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<td>3.</td>
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**Treatment of Recurrent C. difficile Colitis**

About 10 to 20% of patients with an acute episode of C. difficile colitis will experience a relapse of diarrhea, usually within one week after the antibiotic therapy is discontinued. A possible reason for relapsing disease may be the failure to completely eradicate the organism from the colon during treatment or re-infection of a receptive host from the contaminated environment. The diagnosis of recurrent diarrhea from C. difficile infection should be confirmed by a stool-toxin assay.

Relapses characterized by mild diarrhea may be treated conservatively without a new cycle of antibiotics, since they may resolve spontaneously (Table 3). This approach will reduce the risk of new relapses since the normal flora is allowed to reconstitute itself. Patients with more severe symptoms or several relapses may benefit of a new course of metronidazole. There is no clear rationale for using vancomycin as a treatment for relapses after metronidazole therapy, since the development of antibiotic resistance is not usually the

**Treatment of Acute C. difficile Colitis**

The first step in managing diarrhea and colitis in patients with confirmed or suspected C. difficile infection is to discontinue antibiotic therapy, if possible (Table 2). In patients with only mild symptoms this measure alone may be sufficient to allow recovery. Specific therapy to eradicate C. difficile is required if symptoms are more severe and persistent or the antibiotic therapy that induced the disease cannot be discontinued. Metronidazole (250 mg po tid a day) and vancomycin (125 mg four times a day) are equally effective in treating C. difficile induced colitis. Because of the high cost of vancomycin, metronidazole is the drug of first choice. Symptomatic improvement can be expected within 72 hours, and diarrhea and colitis resolve completely in more than 95% of patients after 10 days of treatment. Patients who cannot tolerate oral medication because of ileus can be treated with intravenous metronidazole. Excretion of the drug into the bile and exudation from the inflamed colonic mucosa will result in bactericidal concentrations in the intestinal lumen. The absence of significant improvement after 48 to 72 hours of antibiotic therapy may indicate a more serious infection that requires surgery.

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Table 3  Treatment of Recurrent Clostridium difficile Diarrhea

| 1. Consider conservative therapy if symptoms are mild |
| 2. Second course of therapy with metronidazole or vancomycin |
| 3. Tapered antibiotic course |
| 4. Other options: |
| Combined therapy with antibiotic and cholestyramine |
| Therapy with microorganisms |
| Bacteriotherapy with enteric flora |
| Saccharomyces boulardii |
| Lactobacillus GG |
| Intravenous immunoglobulin treatment |

cause of the relapse.

Although symptoms of the relapse respond promptly to the antibiotic therapy, new episodes of diarrhea are common after antibiotics are discontinued and these patients may experience numerous episodes of diarrhea. Various approaches have been suggested for the management of repeated relapses such as slow tapering of antibiotic therapy,42 use of cholestyramine,43 bacteriotherapy with fecal enemas and oral administration of nontoxicigenic C. difficile.7 In early clinical trials the administration of Saccharomyces boulardii, a non-pathogenic yeast, in combination with metronidazole was shown to reduce the rate of recurrence in patients with multiple recurrences.44 The rationale of this treatment is that the yeast competes with C. difficile for a metabolically favored niche in the colonic microflora and possibly releases factor(s) that reduce the enterotoxic effects of C. difficile toxins.45 The yeast itself does not colonize the bowel chronically and is eliminated spontaneously after treatment is discontinued.

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