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CLOSTRIDIUM DIFFICILE INFECTION- A PANDEMIC IN EVOLUTION

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Abstract

Clostridium difficile infection (CDI) is the leading cause of antibiotic associated diarrhea and recently there has been an alarming trend of increasing rates and severity of CDI in the United States and Europe. C. difficile produces a wide variety of manifestations ranging from asymptomatic carrier state, mild diarrhea to pseudomembranous colitis, sepsis, multiorgan failure and death. Pathogenic strains of C. difficile produce two potent toxins, toxins A and B, which mediate the inflammatory diarrhea seen with CDI.

The pathogenesis of CDI includes the initial alteration of the colonic bacterial flora by antibiotics or cancer chemotherapy; ingestion of C. difficile or its spores with colonization; and finally toxin mediated intestinal injury and inflammation. The onset of diarrhea is typically during or shortly after completion of a course of antibiotic therapy. However, up to a third of patients develop diarrhea up to 12 weeks after antibiotics have been discontinued. Diagnosis is based on the demonstration of toxins A or B or both in stool samples. Cell cytotoxicity assay for toxin demonstration is the gold standard test for diagnosis of CDI. The first step in treatment involves stopping the antibiotic, if possible, and empirical therapy should be initiated for C. difficile immediately after stool procurement for patients with severe symptoms consistent with CDI. Metronidazole in a dose of 250-500 mg four times a day for 10-14 days or oral vancomycin at 125-500 mg four times a day for 10-14 days is the treatment of choice in patients with mild to moderate CDI.

Vancomycin is the first line treatment for severe CDI and also in patients with mild to moderate CDI who do not improve within 72 hours of initiation of treatment with metronidazole. Up to 20% of patients who develop CDI will have a recurrence following discontinuation of antibiotic treatment and recurrences may be multiple. More appropriate and more limited use of antibiotics and preventive measures are the key if we are control this infection in the near future.

Key Words

Clostridium difficile, Diarrhea, Pseudomembranous colitis, Antibiotics, Morbidity

Abbreviations

CDI-Clostridium difficile infection
IBD- Inflammatory bowel disease
CDC-Center for Disease Control and Prevention
EIA-Enzyme immunoassay
PCR-Polymerase Chain Reaction
GDH-Glutamate dehydrogenase
PMC-Pseudomembranous colitis

Introduction

C.difficile infection (CDI) is the leading cause of antibiotic associated diarrhea and is associated with substantial morbidity and mortality. C. difficile is a gram-positive bacillus and found in the human intestine in 1% to 3% of healthy adults and about 20% in patients receiving antibiotics [1]. Since the initial report of this bacterium as a cause of antibiotic associated pseudomembranous colitis in 1978, [2] it has increased in frequency over the years correlating with the increased use of broad spectrum antibiotics. C. difficile causes a wide gamut of manifestations ranging from mild diarrhea to pseudomembranous colitis, sepsis, multiorgan failure and death. [3]Knowledge of the epidemiology, pathogenesis, risk factors and management of disease caused by C. difficile has increased dramatically during the past three decades but has not yet led to any decline in the frequency of C. difficile diarrhea and colitis. Recent papers highlight an alarming trend of increasing rates and severity of CDI. This trend is observed not just in the US, but also in Europe [4-10].

The emergence of a hypervirulent C.difficile strain, BI/NAP1/027 is associated with a more severe and complicated disease with a higher mortality has worsened the situation further. This epidemic strain has been reported from the United States and several European countries [9, 10]. In addition to its effect on morbidity and mortality, CDI is also associated

with increasing duration of hospitalization and hence increasing health care costs. The expected health care cost due to CDI alone is estimated up to 3.2 billion dollars per year in the US. [11] Clearly the impact of CDI on the health care system is significant and continues to become more complicated with emergence of community-acquired CDI. [12, 13] The emergence of CDI in Inflammatory bowel disease (IBD) patients associated with longer duration of hospitalization and higher mortality is also a major emerging concern[14].

This review article addresses the present understanding of CDI and its rising incidence, treatment and the future management strategies to control this infection.

Review criteria

In September 2008, we searched MEDLINE from 1966 to the present using the Medical Subject Headings terms Clostridium difficile diarrhea, Clostridium difficile diarrhea and pathogenesis, Clostridium difficile diarrhea and treatment and the key word “Clostridium difficile diarrhea”. Full papers and abstracts without language restrictions were considered.

Microbiology

C. difficile is a gram positive spore forming rod that is an obligate anaerobe. It was first described in 1935 as part of the normal flora of healthy neonates. [15] It is a relatively large bacillus (2–17 μm in length) and CCFA medium (consisting of cycloserine, cefoxitin, and fructose agar in an egg-yolk agar base) provides a highly selective microenvironment for its growth in the laboratory.

Pathogenic strains of C. difficile produce two potent toxins, toxin A, an enterotoxin, and toxin B, a cytotoxin. These toxins mediate the inflammatory diarrhea as seen with CDI. The genes encoding toxin A and B are encoded in the C. difficile pathogenicity locus (tcdA and tcdB) which also encode two additional regulatory genes (tcdC and tcdD).[16] The tcdD gene product up-regulates toxin transcription, while tcdC probably encodes a toxin gene repressor. [16] The fifth gene of the pathogenicity locus, tcdE is postulated to release both toxins A and B into the colonic lumen by lysing the cell walls. [17] Both toxins A and B have a 49% amino acid homology and possess a N-terminal domain that possess cytotoxic activity, a transmembrane domain that facilitates toxin entry into the cytoplasm and a C-terminal domain that favors toxin binding to the epithelial cells. [16] Both toxins A and B are UDP-glucose hydrolases and glucosyltransferases and contribute to the inflammatory diarrhea; however toxin B may be the major inflammatory toxin[18].

Epidemiology of Clostridium difficile infection

CDI occurs predominantly in hospitalized patients and the incidence is increasing across the United States. Three million new cases of CDI occur in the United States each year; as many as 10% of patients are affected within 2 days of hospitalization. [19] Intestinal carriage in healthy adults varies from 0-3% which increases to 20% in hospitalized patients. Interestingly, only one third of all infected patients developed diarrhea, while the remaining two thirds remain as asymptomatic carriers. Antibiotic-resistant C. difficile spores survive in the hospital environment and can be demonstrated on toilets, bedrails, floors, telephones, call buttons, stethoscopes, and the hands of healthcare workers. [19-22] Also sharing a room with an infected patient increases the risk of infection[19].

Antibiotic therapy is the major risk factor for CDI and animal studies have shown that as little as two organisms can cause infection in antibiotic treated animals. [23] With as many as 109 organisms per gram of feces from every infected human patient and the common contamination of hospitals with C. difficile spores, the prospect of eradication of this infection in the near future is remote.

The CDC reports of community acquired - C. difficile colitis in the US has made the picture more concerning. The incidence of community-acquired infection was 6.9 per 100,000 population in 2006 in Connecticut and was 7.6 per 100,000 population in Philadelphia. [12, 13] The traditional risk factors like recent hospitalization, being elderly or having an underlying health condition were frequently absent; close to 25% of patients who developed community acquired - C. difficile colitis were young, healthy patients with no recent hospitalization in the past 1-year. This was truly surprising given that CDI is seldom thought off in young healthy patients with diarrhea seen in the clinic.

A recent study from Spain also reported a rising incidence of CDI and postulated that increased clinical awareness and suspicion, widespread antibiotic use, and increasing age were responsible for this [24].

Immunity and host factors

The normal bacterial flora in the bowel is an important defense and inhibits the growth of C. difficile. [25] The gastric acid barrier is another important defense mechanism to protect against ingested microorganisms. The use of proton pump inhibitors and the risk of CDI is a subject of controversy and the results of studies have been conflicting. [26] Similarly, the question whether gastric bypass or any gastrectomy procedure increases the risk is not known, but is of potential research interest.

The immune response, particularly humoral arm appears to be important in determining the duration and seve-

rity of C. difficile colitis. [27] Animal studies show that antibodies to toxin A protects against C. difficile colitis. [28] However the protective effect of circulating anti-toxin A and B antibodies in humans is not known. This is because antibodies against C. difficile toxins are present in the majority of the adult population. [29] Secretory immunoglobulin A antitoxin inhibits the binding of the toxin and provides protection against infection. 30In fact, severe or recurrent C. difficile colitis is postulated to be due to ineffective immune protection[31].

Pathogenesis

The various stages in the pathogenesis of CDI include the initial alteration of the normal colonic bacterial flora by antibiotics or other antimicrobial agents or cancer chemotherapy; ingestion of C. difficile or its spores with colonization; and finally toxin mediated intestinal injury, inflammation, and intestinal secretion.

The bacterial flora constitutes an important defense mechanism and the protection afforded by the bacterial flora has been termed as colonization resistance. [32] The importance of colonization resistance is demonstrated in studies where the growth of C.difficile was inhibited in vitro by human fecal extracts but not by sterile extracts. [33] Also infants and children below 24 months of age who have poorly developed microflora, colonization resistance is less and hence get colonized by C.difficile in close to 80%. [34] However the development of clinically significant infection is rare because of the lack of receptors for toxins in the immature colonic epithelium[35].

Antibiotic Use

Almost any antibiotic has been associated with the development of CDI. (Table 1) The risk of CDI varies depending on the type of antibiotic, the frequency of their use, the use of combination therapy, duration of use and the route of administration. [36-38] However, even short-term use of prophylactic antibiotics can cause CDI. [39] The most common implicated antibiotics associated with CDI till recently were ampicillin, amoxicillin, cephalosporins, and clindamycin. [36-38] However with the widespread use of fluoroquinolones, they are now one of the common predisposing factors for CDI. [39-42] Cancer chemotherapy, particularly methotrexate [43] and bowel preparation regimens used before colon surgeries disrupt the bacterial colonic flora and predispose to CDI. [37] Restoration of the normal colonic flora allows elimination of C. difficile and clinical recovery[44].

Other Predisposing factors

In addition to the use of drugs disrupting the bacterial flora, intensive care or prolonged hospital stay, and, possibly, proximity to an infected individual are also risk factors for CDI. [19, 37] Elderly patients are particularly at high risk of CDI and their risk is 20-fold higher than younger patients. [41, 42, 44, 46] Other predisposing factors include the severity of underlying disease in hospitalized patients, a defective immune system [47] and, possibly, the use of proton pump inhibitors. The potential role of altered gastric function has been mentioned above. The various predisposing factors are summarized in table 1.

These risk factors should be considered when investigating new onset diarrhea in the hospitalized patient.

Mechanism of CDI

C. difficile produce two potent toxins, toxin A, an enterotoxin, and toxin B, a cytotoxin. The toxins initially attach to non-proteinaceous disaccharide Gal beta 1-4GlcNac residues in the colon. Both toxins play a role in the initial binding to the colonic epithelial cells. After adhesion, the toxin enters the cell through receptor-mediated endocytosis and catalyzes the transfer of a glucose residue from UDP-glucose to guanosine triphosphate-binding rho proteins, [16, 48] the intracellular signaling molecules regulating cytoskeletal organization and gene expression. Glucosylation of rho proteins in turn leads to disruption of protein synthesis, and cell death. [49] NF-κB and MAP kinases are also activated leading to the release of interleukin (IL)-1β, tumor necrosis factor-α, and IL-8, [49] contributing

Table I. Predisposing factors to Clostridium difficile infection (CDI)

1. Antibiotic use

   Frequent:

   - Fluoroquinolones
   - Cephalosporins
   - Ampicillin and Amoxicillin
   - Clindamycin

   Less Frequent or Rare:

   - Tetracyclines
   - Sulfonamides
   - Macrolides
   - Aminoglycosides
   - Metronidazole

   Cancer chemotherapy

2. Recent hospitalization
3. Intensive care unit stay
4. Hemodialysis
5. Advanced age (Age > 65)
6. Co-morbid diseases
7. Proximity to CDI positive patient
8. ? Proton pump inhibitor use

to the marked intestinal inflammatory response and secretion that is seen. The colonic injury also results from alteration of the enterocyte cytoskeleton with disruption of tight junction function[18].

Clinical presentation

CDI produces a wide variety of clinical manifestations varying from an asymptomatic carrier state to fulminant colitis with megacolon. The various clinical syndromes caused by CDI are listed in table 2.

The most common clinical presentation of CDI is diarrhea associated with antibiotic use. The onset of diarrhea is typically during or shortly after completion of a course of antibiotic therapy. However, up to a third of patients develop diarrhea after antibiotics have been discontinued. Studies show that diarrhea occurs as long as 8 - 12 weeks after the termination of therapy[50].

Antibiotic-Associated Diarrhea

Although diarrhea may occur as a complication during treatment with antibiotics, only 20% of these cases are due to CDI. The osmotic effect of unabsorbed carbohydrate is probably responsible for the diarrhea. [51] Patients have watery diarrhea, although mucoid or soft stools also occur, with a characteristic foul odor. Gross bloody diarrhea is rare. Systemic symptoms are rare and sigmoidoscopic examination usually reveals normal colonic mucosa or mild hyperemia of the rectum. Diarrhea stops when antibiotics are discontinued in a majority of patients.

Asymptomatic Carrier

The colonization rate of C. difficile increases with hospitalization and these patients act as reservoirs of infection and the perpetuation of infection. [19, 20] However treatment of these carriers is not recommended as the carrier state may be prolonged by treatment[52].

Table II. Clinical syndromes of Clostridium difficile infection (CDI)

Clinical syndrome	Features
Asymptomatic Carrier	• Colonization of Clostridium difficile in hospitalized patients. Asymptomatic. • Source of infection • No treatment
Antibiotic-Associated Diarrhea	• Watery diarrhea • Gross bloody diarrhea is rare. • Systemic symptoms are rare • Diarrhea stops when antibiotics are discontinued
C. difficile Colitis without pseudomembrane Formation	• Malaise, abdominal pain, and watery diarrhea • Systemic symptoms and low-grade fever with a polymorphonuclear leukocytosis • Sigmoidoscopy reveals colitis without pseudomembranes.
Pseudomembranous Colitis	• Malaise, abdominal pain, and watery diarrhea. (more severe) • Systemic symptoms are more severe. • Sigmoidoscopy reveals colitis with pseudomembranes.
Fulminant Colitis	• Severe disease with paralytic ileus. • May evolve to toxic megacolon. • Increasing abdominal pain, bloody diarrhea, severe hypoalbuminemia • Leukemoid reaction • Bowel perforation may occur • Requires colectomy

C. difficile Colitis without Pseudomembrane Formation

Patients usually present with malaise, abdominal pain, and watery diarrhea. They also have systemic symptoms and low-grade fever with a polymorphonuclear leukocytosis. Sigmoidoscopic examination may reveal colitis without pseudomembranes.

Pseudomembranous Colitis

Pseudomembranous colitis (PMC) is characterized by malaise, abdominal pain, and watery diarrhea, but often more severe, than those of patients with colitis who do not have pseudomembranes. Sigmoidoscopic examination reveals pseudomembranes, raised yellow plaques scattered over the colorectal mucosa. Rectosigmoid area is usually affected in patients with pseudomembranous colitis, but some patients may have disease isolated to the more proximal colon[53] in which an abdominal computed tomography scan reveals thickening of the colonic wall.

Fulminant Colitis

CDI can present with or evolve into a fulminant colitis. Severe CDI with pseudomembranes occurs in 3-5% of patients who develop C.difficile infection, particularly in patients who have underlying co morbid illness and associated with a high mortality rate of 65%. [54] Severe disease may cause paralytic ileus, which may evolve into toxic megacolon characterized by a dilated colon (>7 cm in its greatest diameter), and signs and symptoms of severe toxicity (fever, chills, dehydration, high white count). There is associated dilatation of the small intestine in patients with megacolon mimicking an intestinal obstruction. Bowel perforation may also occur. [55, 56] Diarrhea may be absent because of paralytic ileus, particularly in postoperative patients who receive narcotics for pain control. Other indicators of severe disease include increased abdominal pain, the development of bloody bowel movements, severe hypoalbuminemia resulting in anasarca. [57] Patients may present without diarrhea but only with abdominal pain, fever and leukocytosis (a leukemoid reaction with a white blood cell count up to 100,000 cells /cu.mm.) [57] A high degree of suspicion is required to diagnose CDI in these settings. The complications of CDI are listed in table 3.

Extracolonic manifestations

CDI in addition to affecting the colon also affects the small intestine. Reports thus far are in patients with surgically altered small bowel, which may be a predisposing factor in patients. [58] In normal patients, peristaltic activity of the small intestine and the mechanical action of the ileocaecal valve prevents C.difficile from colonizing the small intestine. [59] However this protective mechanism is lost in patients with surgically altered small bowel and also in IBD patients with surgical resection of the colon. Reactive arthritis may also occur in patients with CDI, particularly in patients with HLA-B27 antigen[58].

Diagnosis of CDI

A variety of laboratory and imaging techniques are used for the diagnosis of CDI. Laboratory diagnosis of stools has superseded imaging techniques because of the ease of use, cost and specificity. Laboratory testing is recommended in patients with a history of current or recent antibiotic use with evidence of diarrhea/colitis. Diagnosis is based on the demonstration of toxins A or B or both in stool samples. In contrast to other infectious diarrheas, stool culture is not used clinically.

Laboratory Investigations

The diagnosis of CDI should be suspected in any patient who develops diarrhea after hospital admission or diarrhea at admission with any recent antibiotic use in the previous 8-12 weeks. In addition, in view of the new hypervirulent stain and its ability to infect healthy, non-hospitalized patients, it should also be considered in young, healthy outpatients with diarrhea.

Table III. Complications of Clostridium difficile infection (CDI)

1. Relapse and/or recurrent CDI
2. Paralytic ileus
3. Toxic megacolon
4. Intestinal perforation
5. Hypoalbuminemia
6. Ascites
7. Leukemoid reaction
8. Hypovolemic or septic shock
9. Multi-organ failure
10. Colectomy
11. Extra-intestinal manifestations:
    - Small bowel enteritis
    - Arthritis
12. Increased severity of inflammatory bowel disease (IBD)
13. Pouchitis (following ileal pouch-anal anastomosis)

Although a variety of laboratory tests are used for the diagnosis of CDI, enzyme immunoassay (EIA) are the most commonly used tests to detect the toxin.

Enzyme linked Immunoassay

These assays are based on the detection of toxins A and/or B using either a monoclonal antibody or a polyclonal antiserum that recognizes the specific toxin. EIA are inexpensive and the results are available within 2-6 hours. The most widely used EIA's for detection of both toxins A and B in stool are somewhat less sensitive (70-90%) than the cell cytotoxicity assay (see below). Up to 30% of tests may be falsely negative in comparison to the cell cytotoxicity assay or culture [60, 61]. They do demonstrate excellent specificity (99%). [61] The lower sensitivity of these tests can be improved by performing EIAs on 2 or 3 specimens rather than on 1 specimen, which increases the diagnostic yield by 5%–10%. [62] The lower sensitivity of these tests is because of the higher detection limits up to 10000 pg of toxin [63] as compared to 10 pg for cell cytotoxicity assay. Thus, a negative EIA does not reliably exclude a diagnosis of CDI.

Latex Agglutination Assay

Latex agglutination assay is based on the glutamate dehydrogenase (GDH) enzyme produced by C.difficile. The sensitivity of these tests approached almost 96%–100% in a recent study. [64] However certain other organisms can also produce GDH and also the positivity indicates only the presence of the organism, rather than in vivo production of C. difficile toxin. It is not recommended for routine clinical use.

Cell Cytotoxicity Assay

Cell cytotoxicity assay is the gold standard test for diagnosis of CDI. It detects as little as 10 picograms of toxin and it is the most sensitive available test for detection of toxin B. [65-66] It is based on the principle that the toxins in the stool exert a cytopathic effect characterized by cell rounding which can be demonstrated in tissue culture. A centrifuged and filtered sample of stool diluted in phosphate buffered saline is added to a layer of cultured monolayer of cells, usually fibroblast or Chinese hamster ovary cells and examined after 24-48 hours later for cell rounding. A positive result is defined as a stool sample which causes cell rounding which is blocked by preincubation of the sample with specific C. diffcile antitoxin.

The high sensitivity (94-100%) and specificity (99%) of the cytotoxicity assay is its major advantage. Disadvantages are its relatively high technical expertise and the 24-48 hours needed to complete the assay[67].

Clostridium difficile culture

Stool culture is seldom used for routine diagnosis because test turnaround takes 24–48 h and because it is not specific for in vivo production of toxins and can be positive among hospitalized patients who are asymptomatic carriers. However, because culture permits molecular typing of the organisms, it is essential for monitoring molecular epidemiology and antibiotic susceptibility. [65] We do not recommend its routine use in the diagnosis of CDI in clinical practice.

Polymerase Chain Reaction for toxin gene detection

Polymerase Chain Reaction (PCR) based primers for the detection of genes for toxins A and is highly sensitive and specific for the diagnosis of CDI. [68, 69] Culture of the organisms may be required for PCR, which makes the process more technically demanding and challenging. A recent study based on the nested PCR assay reported a 99% concordance with the cytotoxicity assay and a sensitivity of 96.3% and a specificity of 100%[69].

Imaging

Imaging techniques are not routinely used for the diagnosis of CDI. Colonoscopy or sigmoidoscopy are useful when the diagnosis is doubtful or to assess the disease severity. Colonoscopy is the preferred diagnostic technique because PMC in up to a third of patients will involve the right colon only and will be missed by sigmoidoscopy[53].

Plain radiography is usually normal in patients with CDI, unless they have complications like ileus or toxic megacolon or perforation. CT imaging is useful in the diagnosis of PMC or fulminant CDI and the characteristic features include colonic-wall thickening, pericolonic stranding, the "accordion sign," and the "double-halo sign". [70] The accordion sign is seen with oral contrast and shows the high attenuation in the colonic lumen alternating with a low attenuation inflamed mucosa, while the double-halo sign is seen with intravenous contrast. [70] The presence of these signs in the right clinical setting justifies a probable diagnosis of CDI.

Treatment of CDI

The first step in treatment involves discontinuing the antibiotic, if possible. The Society for Healthcare Epidemiology of America recommends initiating empirical therapy for C. difficile immediately after stool procurement for patients with severe symptoms consistent with CDI. [36] Early initiation of treatment is critical in improving the outcome. If the inciting antibiotic cannot be stopped because of other sites of infection, then the antibiotics with the least likely risk of CDI should be used like aminoglycosides, trimethoprim or erythromycin/azithromycin. Agents that decrease the intestinal motility like narcotics, loperamide should be avoided because of the risk of decreasing toxin clearance and increasing the risk of ileus and/or megacolon[71].

Specific antibiotic therapy should be initiated as soon as possible. Metronidazole in a dose of 250-500 mg four times a day for 10-14 days or oral vancomycin at 125-500 mg four times a day for 10-14 days is the treatment of choice in patients with CDI. Bacitracin, teicoplanin and fusidic acid have been used in the treatment of CDI, but their efficacy has not

Table IV. Characteristics of infection with hypervirulent BI/NAP1/027 Clostridium difficile infection (CDI)

1. Epidemiology

- Atypical, hypervirulent
- Isolated in 1984
- Increasing in incidence and severity
- Increased duration of hospitalization and health care costs
- Associated with fluoroquinolone use
- Isolated from 38 states in the US
- Also isolated from Canada, UK, Netherlands, Belgium, France, Austria, Ireland, Spain, Poland, Germany, Scotland, Sweden, Norway, Hungary, Luxembourg, Finland and Japan.

2. Virulence characteristics

- Mutation in TcdC at nucleotide position 117, a negative regulator of toxin production
- Increased toxin production (20-fold)
- Increased sporulation
- Additional gene encoding the binary toxin
- Increased resistance to fluoroquinolones

3. Treatment characteristics

- Associated with recurrent and severe disease
- Decrease in treatment response and increase in treatment failure to metronidazole.
- Oral vancomycin is the drug of choice

been proved superior to vancomycin/metronidazole in large systematic meta-analysis. [72, 73] A recent large meta-analysis of 1157 patients from 12 randomized trials assessed the efficacy of eight antibiotics for the treatment of CDI. None of the antibiotics are superior for symptomatic cure and/or reduction in complications. [74] Thus metronidazole is the initial drug of choice because of similar efficacy, lower cost and lesser risk of selecting vancomycin resistant enterococci in mild to moderate disease. However in patients with severe disease, multiple studies have shown a failure rate of 22-38% with metronidazole. [75] Studies have shown similar cure rates in patients with mild disease with either use of metronidazole or vancomycin, while in severe disease the cure rate with metronidazole is 76%, as compared to vancomycin, which gives a cure rate of 97%. [76] These data support the use of vancomycin as the first line treatment for severe CDI, also in patients with mild to moderate CDI who do not improve within 72 hours of initiation of treatment with metronidazole should be switched to vancomycin. Severe CDI requires aggressive treatment and doses up to 2 g/d of vancomycin may be required in patients with severe disease.

Patients with fulminant colitis require stoppage of the inciting antibiotic, initiating treatment with oral vancomycin at a high dose of 500 mg every 6 hours which may be administered with a nasogastric tube because of paralytic ileus.

Emergent surgery is required for patients who do not respond to the above medical management and in patients with impending perforation and toxic megacolon. Patients usually undergo a sub total colectomy and a temporary ileostomy and are associated with a high perioperative mortality rate approaching close to 40%[77].

Decreasing Responsiveness of Clostridium difficile

Recent population based studies demonstrate that over the past 11 years the nature of C difficile colitis is changing with increased rate of C difficile diagnoses, increasing fatality, an increased total mortality rate, and an increased colectomy rate. [78-81] The increasing severity is thought to be due to increasing resistance of C difficile to standard first-line therapy. [78] Previously, the response rates to metronidazole was high (90%) and there was a lower recurrence rates with the use of first line metronidazole, but unfortunately recent reports [41, 82] show that the overall effectiveness of metronidazole is decreasing, particularly in patients with severe CDI and in intensive care unit patients[83].

Recurrent Disease

Recurrent CDI, including relapse or reinfection is a major problem in CDI and as many as 20% of patients who develop CDI will have 1 more recurrence following discontinuation of the antibiotic treatment. Diarrhea can recur within a week up to a month. Some patients have 5 or more recurrences. The probiotic Saccharomyces boulardii 500 mg twice daily is used in addition to antibiotics in studies and has reduced the likelihood of recurrence in two controlled trials. [84] However S. boulardii should not be used in immunocompromised patients and those with central intravenous lines because of the risk of fungemia.

A new antibiotic exposure, advanced age more than 65 years, underlying disease severity, a low serum albumin concentration (<2•5 g/dL) and stay in an intensive care unit are all important risk factors for recurrence. [85, 86] Studies using serotyping, PCR ribotyping, or chromosomal restriction endonuclease analysis prove that new strains of C difficile are responsible for 10–50% of recurrent infections favoring re-acquisition of hospital-based strains. [87] Also a higher concentration of anti-toxin antibody is associated with a decreased risk of recurrence[85].

Patients with first relapse, respond well to a repeat 14-day course of metronidazole or vancomycin. Long tapering courses of vancomycin or pulsed treatment reduce recurrence and are suggested for treating second relapse. [88] Because of the risk of often-irreversible neuropathy with long-term use of metronidazole, it is not used for treatment of second relapse. Recently, several small series reported the efficacy of rifaximin in treating recurrent CDI. [89] Similarly reconstitution of the fecal flora by administration of stool is effective in small series

[90] and other treatments including the use of active and passive immunization by administration of immunoglobulins or oral administration of antibodies from colostrum of cows immunized against toxins are under investigation for future use[86].

Increasing severity of CDI

In a recent study on the C.difficile related deaths in the US, the mortality rate has increased from 5.7 per million populations in 1999 to 23.7 per million in 2004. [91] In fact the paper reported a 35% per year increase in the mortality rate associated with C. difficile infection. During the same time, there was a 23% increase in the hospitalizations attributed to CDI from 2000 to 2005. Also there was an absolute increase in CDI hospitalizations, more than double in all age groups with increase in adjusted case-fatality rate for all CDI hospitalizations[92].

Recent papers have highlighted a hypervirulent form of C. difficile strain, BI/NAP1/027 that is associated with a more severe and complicated disease with a higher mortality. The characteristics of the hypervirulent strain are listed in table 4. This strain appears to spread in the US and in a recent CDC report on the number of states with the BI/NAP1/027 strain of C. difficile, 38 states are identified to carry the hypervirulent strain of the bacterium. [12, 13] This particular strain of C difficile: toxinotype III, North American PFGE type 1, and PCR ribotype 027 (NAP1/027) carries the binary toxin gene cdtB (cytolethal distending toxin B gene) and an 18–base pair deletion in tcdC; it produces 16-23 times more toxin A and B than the routine strain. [8, 9, 93] In addition, this hypervirulent strain is also associated with increased disease severity [6, 41] and possibly transmissibility and has caused outbreaks in Europe and the United States [9, 10, 42].

The rising use of fluoroquinolones may be one of the reasons for selecting the hypervirulent BI/NAP1/027 C difficile strain since it is resistant to this class of antibiotics and possibly less responsive to other antibiotics. This is occurring not only in the United States but also in UK and across Europe. Fluoroquinolones were most strongly associated with the development of C difficile (attributable hazard ratio, 3.4) when compared to other antibiotics. [41] The increasing severity of C difficile colitis may also be because of the increasing acuity of illness of hospitalized patients in the United States. Recent papers have reported a 23% increase in the hospitalizations and 35% per year increase in the mortality rate associated with C. difficile infection[91, 92].

Inflammatory bowel disease and CDI

Almost three decades before, LaMont et al postulated that C difficile toxin complicates chronic inflammatory bowel disease (IBD) and contribute to relapse in some patients. [94] Since then, isolated case series of C difficile toxin contributing to symptomatic relapse in patients with IBD were reported. 95More recently, studies from single tertiary care institutions as well as large nationwide inpatient databases have demonstrated an increase in incidence and severity of CDI in patients with IBD. [14, 96-97] C difficile was the most common infection occurring in 5% of hospitalized IBD patients and 20% of those patients requiring colectomy. [96] In addition to the increased susceptibility of acquiring CDI among patients with IBD, studies show that IBD may also be a risk factor for worse outcome when associated with CDI as compared to those without underlying IBD. [14] In all the studies published thus far, patients with Ulcerative colitis (UC) are at a greater risk of CDI as compared to Crohn's disease (CD) patients and non-IBD patients. The high frequency of immunomodulator use is proposed to be responsible for increased infection rate in the IBD population. [96] Also colonic disease was an independent predictor of CDI. [96] In addition to the increased mortality, CDI was associated with close to 46% increase in the length and cost of hospitalization among UC patients and 63 % increase in the length and cost of hospitalization among CD patients[97].

Economic impact of CDI

CDI is associated with rising health care costs. Kyne et al reported that each patient with CDI incurred a 54% higher hospitalization cost and stayed 3.6 days longer as compared to patients without CDI with a direct impact of $1.1 billion per year. [98] This did not include patients in nursing homes or long-term facilities with CDI and thus the health care costs may be much higher than what has been studied. In fact the health care expenditure is expected to be close to $ 3.2 billion dollars. [99] The effects of C. difficile infection certainly go beyond costs. A Retrospective analysis of its morbidity in surgical patients has shown that C. difficile was an independent predictor of length of stay, which increased by 16 days, cost of hospitalization increased by $77,483 and the mortality rate increased by 3.4-fold compared with patients who did not acquire C. difficile[100].

Conclusions

CDI has continuously evolved over the years rising from a benign diarrhea with antibiotic use to a significant public health problem with epidemic proportions. CDI poses substantial challenge to epidemiologists, infection control practitioners, gastroenterologists, surgeons and hospital administration. The rising incidence, with increasing hospitalization rates and duration, and increasing morbidity and mortality is of great concern. Heightened public awareness of the increasing disease burden of CDI is an important first step in controlling this epidemic. In addition, preventive measures are the key and require concerted effort from all quarters from epidemiologists to hospital administration and clinicians if we are to control this infection in the near future.

REFERENCES

1. Weir E, Flegel K. Protecting against Clostridium difficile illness. CMAJ. 2005; 172: 1178.
2. Bartlett JG, Chang TW, Gurwith M, et al: Antibiotic-associated pseudo membranous colitis due to toxin-producing clostridia. N Engl J Med 1978; 298:531-534
3. Bartlett JG. Historical perspectives on studies of Clostridium difficile and Clostridium difficile infection. Clin Infec Dis. 2008; 46 (Suppl 1). S4-11.
4. Morris AM, Jobe BA, Stoney M, et al. Clostridium difficile colitis: an increasingly aggressive iatrogenic disease? Arch Surg. 2002; 137:1096-1100.
5. Dallal RM, Harbrecht BG, Boujoukas AJ, et al. Fulminant Clostridium difficile: an underappreciated and increasing cause of death and complications. Ann Surg. 2002; 235:363-372.
6. Pepin J, Valiquette L, Alary ME, et al. Clostridium difficile–associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ. 2004; 171:466-472.
7. Wysowski DK. Increase in deaths related to enterocolitis due to Clostridium difficile in the United States, 1999–2002. Public Health Rep. 2006; 121:361–2.
8. Warny M, Pepin J, Fang A, et al. Toxin production by an emerging strain of Clostridium difficile associated with outbreaks of severe disease in North America and Europe. Lancet. 2005;366(9491):1079-1084
9. McDonald LC, Killgore GE, Thompson A, et al. Emergence of an epidemic, toxin gene variant strain of Clostridium difficile responsible for outbreaks in the United States between 2000 and 2004. N Engl J Med. 2005; 353:2433–41.
10. Kazakova SV, Ware K, Baughman B, et al. A hospital outbreak of diarrhea due to an emerging epidemic strain of Clostridium difficile. Arch Intern Med. 2006; 166: 2518-24.
11. O'Brien JA, Lahue BJ, Caro JJ, Davidson DM. The emerging infectious challenge of clostridium difficile-associated disease in Massachusetts hospitals: clinical and economic consequences. Infect Control Hosp Epidemiol. 2007; 28: 1219-27. Epub 2007 Oct 3.
12. Centers for Disease Control and Prevention (CDC). Surveillance for community-associated Clostridium difficile--Connecticut, 2006. MMWR Morb Mortal Wkly Rep. 2008; 57(13): 340-3.
13. CDC. Severe Clostridium difficile--associated disease in populations previously at low risk---four states, 2005. MMWR 2005; 54:1201-5.
14. Ananthakrishnan AN, McGinley EL, Binion DG. Excess hospitalization burden associated with Clostridium difficile in patients with inflammatory bowel disease. Gut 2008;57;205-210
15. Hall IC, O'Toole E. Intestinal flora in newborn infants with description of a new pathogenic anaerobe. Am. J. Dis. Child 1935; 49:390-402
16. Warny M and Kelly CP. Pathogenicity of Clostridium difficile toxins. In: Microbial Pathogenesis and the Intestinal Epithelial Cell, 2003; 503-523. ed. Hecht G. Washington, DC: ASM Press.
17. Tan KS, Wee BY, Song KP: Evidence for holin function of tcdE gene in the pathogenicity of Clostridium difficile. J Med Microbiol 2001; 50:613-619.
18. Riegler M et al. Clostridium difficile toxin B is more potent than toxin A in damaging human colonic epithelium in vitro. J Clin Invest 1995; 95:2004-2011.
19. McFarland LV, Mulligan ME, Kwok RY, et al. Nosocomial acquisition of Clostridium difficile infection. N Engl J Med. 1989; 320:204-210.
20. Johnson S, Clabots CR, Linn FV, et al. Nosocomial Clostridium difficile colonization and disease. Lancet 1990; 336: 97-100
21. Fekety R, Kim KH, Brown D, et al. Epidemiology of antibiotic-associated colitis; isolation of Clostridium difficile from the hospital environment. Am. J. Med. 1981; 70:906-908
22. Kim KH, Fekety R, Batts DH, et al. Isolation of Clostridium difficile from the environment and contacts of patients with antibiotic-associated colitis. J. Infect. Dis. 1981; 143:42-50
23. Larson HE, Price AB, Honour P, et al: Clostridium difficile and the etiology of pseudomembranous colitis. Lancet 1978; 1:1063-1066.
24. Asensio A, Vaque-Rafart J, Calbo-Torrecillas F, et al. Increased rates in Clostridium difficile infection among hospitalized patients, Spain 1999-2007. Euro Surveill. 2008; 13. 18943
25. Borriello SP. The influence of the normal flora on Clostridium difficile colonization of the gut. Ann. Med. 1990; 22:61.67
26. Choudhry MN, Soran H, Ziglam HM. Overuse and inappropriate prescribing of proton pump inhibitors in patients with Clostridium difficile-associated disease. QJM. 2008; 101:445-8. Epub 2008 Apr 14.
27. Kelly CP. Immune response to Clostridium difficile infection. Eur. J. Gastroenterol. Hepatol. 1996; 8:1048-53
28. Kim PH, Iaconis JP, Rolfe RD. Immunization of adult hamsters against Clostridium difficile-associated ileocecitis and transfer of protection to infant hamsters. Infect. Immun. 1987; 55:2984-92
29. Viscidi R, Laughon BE, Yolken R, et al. Serum antibody response to toxins A and B of Clostridium difficile. J. Infect. Dis. 1983; 148:93-100
30. Kelly CP, Pothoulakis C, Orellana J, et al. Human colonic aspirates containing immunoglobulin A antibody to Clostridium difficile toxin A inhibit toxin A-receptor binding. Gastroenterology 1992; 102:35-40
31. Warny M, Vaerman JP, Avesani V, et al M. Human antibody response to Clostridium difficile toxin A in relation to clinical course of infection. Infect. Immun. 1994; 62:384-89
32. Wilson KH, Freter R: Interaction of Clostridium difficile and Escherichia coli with microfloras in continuous-flow cultures and gnotobiotic mice. Infect Immun 1986; 54:354-358.
33. Borriello SP, Barclay FE: An in vitro model of colonization resistance to Clostridium difficile infection. J Med Microbiol 1986; 21:299-309.
34. Kelly CP, LaMont JT: Clostridium difficile infection. Annu Rev Med 1998; 49:375-390
35. Eglow R, Pothoulakis C, Itzkowitz S, et al: Diminished Clostridium difficile toxin A sensitivity in newborn rabbit ileum is associated with decreased toxin A receptor. J Clin Invest 1992; 90:822-829.
36. Gerding DN, Johnson S, Peterson LR, eet al. Clostridium difficile–associated diarrhea and colitis. Infect Control Hosp Epidemiol 1995; 16:459–77.
37. Bignardi GE. Risk factors for Clostridium difficile infection. J Hosp Infect 1998; 40:1–15.
38. Bartlett JG. Clinical practice: antibiotic-associated diarrhea. N Engl J Med 2002; 346:334–9.
39. Sunenshine RH, McDonald LC. Clostridium difficile–associated disease: new challenges from an established pathogen. Cleve Clin J Med 2006; 73:187–97.

40. Gaynes R, Rimland D, Killum E, et al. Outbreak of Clostridium difficile infection in a long-term care facility: association with gatifloxacin use. Clin Infect Dis 2004; 38:640–5.
41. Pepin J, Saheb N, Coulombe MA, et al. Emergence of fluoroquinolones as the predominant risk factor for Clostridium difficile–associated diarrhea: a cohort study during an epidemic in Quebec. Clin Infect Dis 2005; 41:1254–60.
42. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multiinstitutional outbreak of Clostridium difficile–associated diarrhea with high morbidity and mortality. N Engl J Med 2005; 353:2442–9.
43. Anand A, Glatt AE. Clostridium difficile infection associated with antineoplastic chemotherapy: a review. Clin Infect Dis 1993; 17:109–13
44. Viscidi R, Willey V, Bartlett JG. Isolation rates and toxigenic potential of Clostridium difficile isolates from various patient populations. Gastroenterology 1981; 81:5-9
45. Aronsson B, Mollby R, Nord CE. Diagnosis and epidemiology of Clostridium difficile enterocolitis in Sweden. J Antimicrob Chemother 1984; 14(Suppl D): 85–95.
46. Karlstrom O, Fryklund B, Tullus K, et al. A prospective nationwide study of Clostridium difficile–associated diarrhea in Sweden. The Swedish C. difficile Study Group. Clin Infect Dis 1998; 26:141–5.
47. Yolken RH, Bishop CA, Townsend TR, et al. Infectious gastroenteritis in bone-marrow-transplant recipients. N Engl J Med 1982; 306:1010–2.
48. Pothoulakis C: Pathogenesis of Clostridium difficile-associated diarrhea. Eur J Gastroenterol Hepatol 1996; 8:10411047.
49. Warny M, Keates AC, Keates S, et al: p38 MAP kinase activation by Clostridium difficile toxin A mediates monocyte necrosis, IL-8 production, and enteritis. J Clin Invest 2000; 105:1147-1156.
50. Mogg GA, Keighley MR, Burdon DW, et al. Antibiotic-associated colitis—a review of 66 cases. Br J Surg 1979; 66:738–42.
51. Young VB, Schmidt TM. Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. J Clin Microbiol 2004; 42:1203–6.
52. Johnson S, Homann SR, Bettin KM, et al. Treatment of asymptomatic Clostridium difficile carriers (fecal excretors) with vancomycin or metronidazole. A randomized, placebo-controlled trial. Ann. Intern. Med. 1992; 117:297-302
53. Tedesco FJ, Corless JK, Brownstein RE. Rectal sparing in antibiotic associated pseudomembranous colitis: a prospective study. Gastroenterology 1982; 83: 1259-60
54. Rubin MS, Bodenstein LE, Kent KC: Severe Clostridium difficile colitis. Dis Colon Rectum 1995; 38:350-354.
55. Morris JB, Zollinger RM Jr, Stellato TA. Role of surgery in antibiotic induced pseudomembranous enterocolitis. Am. J. Surg. 1990; 160:535-39
56. Morris LL, Villalba MR, Glover JL. Management of pseudomembranous colitis. Am. Surg. 1994; 60:548-52
57. Tedesco FJ, Barton RW, Alpers DH. Clindamycin-associated colitis: a prospective study. Ann Intern Med 1974; 81:429–33
58. Jacobs, A, Bernard K, Fishel R, et al. Extracolonic manifestations of Clostridium difficile infections. Presentation of 2 cases and review of literature. Medicine (Baltimore) 2001 Mar; 80 (2): 88-101.
59. Kralovich KA, Sacksner J, Karmy-Jones RA et al. Pseudomembranous colitis with associated fulminant ileitis in the defunctionalized limb of a jejunal-ileal bypass. Report of a case. Dis Colon Rectum 1997; 40: 622-624.
60. Delmee M, Van Broeck J, Simon A, et al. Laboratory diagnosis of Clostridium difficile–associated diarrhea: a plea for culture. J Med Microbiol 2005; 54:187–91
61. O'Connor D, Hynes P, Cormican M, et asl. Evaluation of methods for detection of toxins in specimens of feces submitted for diagnosis of Clostridium difficile–associated diarrhea. J Clin Microbiol 2001; 39:2846–9.
62. Manabe YC, Vinetz JM, Moore RD, et al. Clostridium difficile colitis: an efficient clinical approach to diagnosis. Ann Intern Med 1995; 123:835–40.
63. Viscidi R, Laughon BE, Hanvanich M, et al. Improved enzyme immunoassays for the detection of antigens in fecal specimens. Investigation and correction of interfering factors. J Immunol Methods 1984; 67:129–43.
64. Ticehurst JR, Aird DZ, Dam LM, et al. Effective detection of toxigenic Clostridium difficile by a two-step algorithm including tests for antigen and cytotoxin. J Clin Microbiol 2006; 44:1145–9.
65. Wilkins TD, Lyerly DM. Clostridium difficile testing: after 20 years, still challenging. J Clin Microbiol 2003; 41:531–4.
66. Merz CS, Kramer C, Forman M, et al: Comparison of four commercially available rapid enzyme immunoassays with cytotoxin assay for detection of Clostridium difficile toxin(s) from stool specimens. J Clin Microbiol 1994; 32:1142-1147.
67. National Clostridium difficile Standards Group: report to the Department of Health. J Hosp Infect 2004; 56(Suppl 1):1–38.
68. Morelli MS, Rouster SD, Giannella RA et al: Clinical application of polymerase chain reaction to diagnose C. difficle in hospitalized patients with diarrhea. Clin Gastroenterol Hepatol 2004; 2: 669-674.
69. Alonso R, Munoz C, Gros S, et al: Rapid detection of toxigenic Clostridium difficile from stool samples by a nested PCR of toxin B gene. J Hosp Infect 1999; 41:145-149.
70. Kawamoto S, Horton KM, Fishman EK. Pseudomembranous colitis: spectrum of imaging findings with clinical and pathologic correlation. Radiographics 1999; 19:887–97.
71. Walley T, Milson D: Loperamide-related toxic megacolon in Clostridium difficile colitis. Postgrad Med J 1990; 66:582
72. Kelly CP, LaMont JT: Treatment of Clostridium difficile diarrhea and colitis. In: Wolfe MM, ed. Therapy of Digestive Disorders, 2nd ed. Philadelphia: WB Saunders; 2006: 734-744.
73. Zimmerman MJ, Bak A, Sutherland LR: Review article: Treatment of Clostridium difficile infection. Aliment Pharmacol Ther 1997; 11:1003-1012.
74. Nelson R. Antibiotic treatment for Clostridium difficile associated diarrhea in adults. Cochrane Database Syst Rev 2007; 3: CD004610.
75. Miller MA. Clinical management of Clostridium difficile associated disease. Clin Infect Dis 2007; 45 (Suppl 2): S122-28.
76. Zar FA, Bakkanagari SR, Moorthi KM, et al. A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile associated diarrhea, stratified by disease severity. Clin Infect Dis 2007; 45: 302-7.
77. Synnott K, Mealy K, Merry C, et al: Timing of surgery for fulminating pseudomembranous colitis. Br J Surg 1998; 85:229-231.
78. Layton BA, McDonald LC, Gerding DN, et al. Changing patterns of Clostridium difficile disease: a report from infectious diseases physicians. In: Program and abstracts of the Infectious Diseases Society of America 2004 Annual Meeting; September 30-October 4, 2004; Boston, MA. Abstract 563

79. Ricciardi R, Rothenberger DA, Madoff RD, et al. Increasing Prevalence and Severity of Clostridium difficile Colitis in Hospitalized Patients in the United States. Arch Surg. 2007; 142:624-631

80. DuPont HL, Garey K, Caeiro JP, et al. New advances in Clostridium difficile infection: changing epidemiology, diagnosis, treatment and control. Curr Opin Infect Dis. 2008; 21:500-7.

81. Dubberke ER, Butler AM, Reske KA, et al. Attributable outcomes of endemic Clostridium difficile-associated disease in nonsurgical patients. Emerg Infect Dis. 2008 ;14:1031-8.

82. Musher DM, Aslam S, Logan N, et al. Relatively poor outcome after treatment of Clostridium difficile colitis with metronidazole. Clin Infect Dis. 2005; 40: 1586-90.

83. Fernandez A, Anand G, Friedenberg F. Factors associated with failure of metronidazole in Clostridium difficile–associated disease. J Clin Gastroenterol. 2004; 38:414-18.

84. McFarland LV, Surawicz CM, Greenberg RN, et al. A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease. JAMA 1994; 271: 1913–18

85. Kyne L, Warny M, Qamar A, et al. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhea. Lancet 2001; 357: 189–93.

86. Nair S, Yadav D, Corpuz M, et al. Clostridium difficile colitis: factors influencing treatment failure and relapse—a prospective evaluation. Am J Gastroenterol 1998; 93: 1873–76

87. Noren T, Akerlund T, Back E, et al. Molecular epidemiology of hospital-associated and community-acquired Clostridium difficile infection in a Swedish county. J Clin Microbiol 2004; 42: 3635–43

88. McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. Am J Gastroenterol 2002; 97:1769–75

89. Johnson S. Schriever C, Galang M et al: Interruption of recurrent C. difficile-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. Clin Infect Dis 2007; 846-848.

90. Tvede M, Rask-Madsen J. Bacteriotherapy for chronic relapsing Clostridium difficile diarrhea in six patients. Lancet 1989; 1:1156–60.

91. Redelings MD, Sorvillo F, Mascola L. Increase in Clostridium difficile–related mortality rates, United States, 1999–2004. Emerg Infect Dis [serial on the Internet]. 2007 Sep [date cited]. Available from http://www.cdc.gov/EID/content/13/9/1417.htm

92. Zilderberg MD, Shorr AF, Kollef MH. Increase in adult Clostridium difficile-related hospitalizations and case fatality rate, United States, 2000-2005. Emerg Infect Dis 2008; 14: 929-31.

93. Curry SR, Marsh JW, Muto CA, et al. tcd C genotypes associated with severe TcdC truncation in an epidemic clone and other strains of Clostridium difficile. J Clin Microbiol 2007; 45:215–21

94. LaMont JT, Trnka YM. Therapeutic implications of Clostridium difficile toxin during relapse of chronic inflammatory bowel disease. Lancet 1980; 1: 381-83

95. Trnka YM, LaMont JT. Association of Clostridium difficile toxin with symptomatic relapse of chronic inflammatory bowel disease. Gastroenterology 1981; 80: 693-96

96. Issa M, Vijayapal A, Graham MB, et al. Impact of Clostridium difficile on inflammatory bowel disease. Clin Gastroenterol Hepatol 2007; 5: 345-51

97. Nguyen GC, Kaplan GG, Harris ML, et al. A national survey of the prevalence and impact of Clostridium difficile infection among hospitalized inflammatory bowel disease patients. Am J Gastroenterol. 2008 ;103:1443-50. Epub 2008 May 29.

98. Kyne L, Hamel MB, Polavaram R,et al. Health care costs and mortality associated with nosocomial diarrhea due to Clostridium difficile. Clin Infect Dis. 2002 1; 34:346-53. Epub 2001 Dec 17.

99. O'Brien JA, Lahue BJ, Caro JJ, et al. The emerging infectious challenge of clostridium difficile-associated disease in Massachusetts's hospitals: clinical and economic consequences. Infect Control Hosp Epidemiol. 2007; 28: 1219-27. Epub 2007 Oct 3.

100. Zerey M, Paton BL, Lincourt AE, et al. The burden of Clostridium difficile in surgical patients in the United States. Surg Infect (Larchmt). 2007; 8: 557-66