

CLOSTRIDIUM DIFFICILE INFECTION: RISK FACTORS, DIAGNOSIS AND CONTROL

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Abstract

The epidemiology of *Clostridium difficile* infections (CDI) has changed over the past decade. In addition to dramatic worldwide increases in incidence, new CDI populations are emerging. These populations include patients with community acquired infections with no previous antibiotic exposure, children, pregnant women and patients with IBD. Diagnosis of CDIs requires the identification of *C. difficile* toxin A or B in diarrheal stool. Current diagnostic tests, however, remains inadequate and an optimal diagnostic testing algorithm has not yet been defined. Metronidazole and vancomycin are currently first-line agents for CDI treatment. Vancomycin, however, has demonstrated superior efficacy and therefore is the preferred agent in patients with severe infections. As with many antibiotics, the incidence of treatment failure with metronidazole is increasing, thereby emphasizing the need to find alternative treatments. Disease recurrence continues to occur in 20-40% of patients and its treatment remains challenging. In patients who develop fulminant colitis from a CDI, early surgical consultation is essential. Intravenous immunoglobulin and tigecycline have been used in patients with severe refractory disease, however delaying surgery may be associated with worse outcomes. Due to the risk of horizontal transmission of *C. difficile* infection control measures are necessary. Animals may serve as reservoirs for humans. Ongoing research by human and veterinary scientist into, epidemiology, diagnosis, effective treatment protocols and prevention are essential.

1. Introduction

Clostridium difficile is a member of the genus *Clostridium* which is comprised of over 100 species. Of these, only about 20 are of pathogenic importance for animals and human [57]. *Clostridium difficile* is one of the most common causes of nosocomial diarrhea in humans. It is responsible for ~15-25% of human antibiotic-associated diarrhea and for more than 95% of pseudomembranous colitis in humans [2; 15]. Recently, morbidity and mortality rates for *C. difficile* infection (CDI) have increased markedly in many developed countries where CDI surveillance has been well established [32]. *C. difficile* is responsible for an estimated 3 million cases of diarrhea and colitis each year. Its prevalence has increased two fold in recent years and the case fatality rate associated with CDIs is reportedly as high as 17% [33]. Recent increases in the incidence and severity of CDIs in North America and Europe has been linked to the emergence of a highly virulent, fluoroquinolones-resistant strain, which is characterized as toxinotype III, restriction

endonuclease type BI, PCR ribotype 027, and North American pulsed-field gel electrophoresis (PFGE) type 1 (NAP1) [32]. Clinical presentations of CDIs range from asymptomatic carriers, patients with mild diarrhea and in extreme cases death. Although infections are generally localized in the intestines, there is increasing evidence of extra-intestinal CDI cases [11]. Non-intestinal CDIs includes bacteremia with or without focal infection, intra-abdominal infections as well as extra-abdominal abscesses [11]. In addition, there are reports of more than 40 cases of reactive arthritis [14], in which one or more peripheral joints have become inflamed post CDI [5].

While *C. difficile* is of primary importance for public health; veterinarians have also reported an increasing prevalence of CDI in domestic animals. Currently many efforts are focused in determined if *C. difficile* is a significant zoonotic pathogen. *C. difficile* has been isolated from horses, pigs, calves, dog, cats, and rats [55, 58, 61, 62, 63, 64]. It has been implicated in fatal cases involving two captive Asian elephants [17]. Household pets have also been reported to be common carriers of *C. difficile* [18].

Some animal and human isolates of *C. difficile* share identical genotypic and phenotypic features, however, the epidemiological relationship between these isolates remains unclear and requires further investigation. *Clostridium difficile* PCR ribotype 078 is the most frequent ribotype in calves and swine and is often found in meat products [51]. In the Netherlands ribotype 078 is the third most frequent type in humans after ribotype 027 and 014 [51, 52].

A CDI in most cases is considered a conditional disease that depends on various factors including host-pathogen interaction, antibiotic treatment and medical management of the host. Patients who are asymptomatic or improve clinically after treatment may serve as carriers, thus becoming important sources of CDIs. Infection are most commonly seen in hospitalized elderly while rare cases are found among children and infants. The epidemiology of CDIs has changed in recent years to include the following; (1) low risk populations now being affected; (2) the virulence and mortality rates being higher than in the early “era” of CDI; and (3) the emergence of CDIs in patients that are not under intensive treatment or have not undergone previous antibiotic therapy [8, 12]. The aim of this paper is to present current published information relating to the epidemiology, diagnosis, treatment, and prevention of CDI.

2. Virulence factors of *C. difficile*

C. difficile is an encapsulated anaerobic gram-positive, rod bacterium that is spore-forming and motile. Known virulence factors include (a) toxins, (b) adhesins that facilitate the attachment *C. difficile* on specific receptors of colon epithelial cells [18] (c) cell walls containing paracrystalline arrays (S-layer) [58], (d) carbohydrate capsules that protects the bacterium from phagocytosis [18, 58] (e) and outer membrane proteins, such as collagen-binding and fibronectin-binding proteins [7, 30, 58].

The ability of *C. difficile* to produce spores is of considerable significance. The vegetative form cannot survive in aerobic conditions and either dies quickly or transforms into a spore when exposed to oxygen or nutritional stresses. These spores contaminate the environment, and are especially problematic in hospitals. The spores are resistant to heat, radiation, antibiotics, and common disinfectants. Contaminated surfaces are a major source of infection, and only a limited number of disinfectants have proven to

effective in reducing number of *C. difficile* spores. While chlorhexidine is useful for removing the organism from gloves, hand washing with soap or chlorhexidine gluconate will effectively remove *C. difficile* from one's hands [13]. One study investigating the survival of spores and vegetative bacteria in contact copper-based compounds and stainless steel showed usefulness of copper alloys in reducing survival rate of *C. difficile* cells and spores in hospital environments [68]. *C. difficile* spores are resistant to thermal decontamination procedures commonly used at hospitals to eliminate spores from bedpans [3]. The increasingly common use of alcohol-based hand rubs to reduce transmission of methicillin resistant *Staphylococcus aureus* strains, provides no protection from spread of *C. difficile* [19].

The most potent virulence factors of *C. difficile* are its following toxins; toxin A (TcdA), toxin B (TcdB) and binary toxin. The effect of TcdA includes a cytotoxic effect on enterocytes, stimulation of the enteric nervous system resulting in an influx of neutrophils, induction of the release of substance P and mast cell deregulation, as well as activation of various inositol-signaling pathways within affected host cells, which typically are intestinal epithelial cells. This results in secretion of chloride ions and water into the intestinal lumen [1, 2, 30, 32]. TcdB is a cytotoxin, glycosyltransferase. While both TcdA and TcdB are cytopathic toxins, toxin B is ~1000-fold more potent in non-intestinal cell lines [10]. Most pathogenic *C. difficile* strains produce both TcdA and TcdB, however, clinically relevant TcdA- TcdB+ (A-B+) strains of *C. difficile* that cause diarrhea and colitis in humans have been isolated from time to time [47].

Binary toxin is similar to iota toxin of *C. perfringens*. It is composed of a binding portion (CdtB) that binds to target intestinal cells, and an enzymatically active portion, CdtA. The binary toxin is unique to *Clostridium* species. In addition to *C. difficile*, the toxin is also present in *C. botulinum*, *C. perfringens*, and *C. spiroforme* [7, 57, 58]. As previously mentioned, an increase in the incidence and severity of CDIs in Canada, the United States, and other countries has been associated with the hypervirulent strain BI/NAP1/027. This strain possesses binary toxins, *tcdC* deletion and is able to release 16 and 23 times more TcdA and TcdB respectively, compared with other common strains [28, 56].

3. Pathogenesis of *C. Difficile*

C. difficile spores pass through the gastric acid pH in the stomach and then begin to germinate in the small intestine, particularly the terminal part of ileum. Upon reaching the colonic lumen, the bacteria begin to multiply. Adhesion of *C. difficile* to epithelial cells in the large intestine is facilitated by cell surface proteins [18]. Pathogenic strains of *C. difficile* produce toxins that act upon and cause the death of epithelial cells, through the disruption of the actin cytoskeleton and tight junctions. The intense inflammatory response to these toxins results in fluid and electrolyte secretion from host cells into the intestinal lumen [30]. Epithelial cell apoptosis, local necrosis and pseudomembranous colitis may follow [18, 30]. CDI may be accompanied by toxic megacolon, electrolyte imbalance and occasional bowel perforation. The onset of symptoms is frequently abrupt, characterized by explosive, watery, foul-smelling diarrhea that accompanied by abdominal pain. Other symptoms include the absence of frank blood in the stool in most cases, an elevated white blood cell count and in some cases fever [15]. CDIs may also occur in extra-intestinal tissues [11].

4. Risk factors

4.1 Age

There is a significant difference in risk of CDI between age groups. The elderly people, particularly those over 60 years of age, are considered to be at high risk for CDIs. The increasing frequency of vascular, heart, kidney and lung disease in older patients has been identified as a risk factor for acquiring a CDI [1]. Recently, CDIs have been reported in healthy persons living in the community and in peripartum young women [12; 28; 59]. Children are thought to be less susceptible than elderly people because specific receptors on colonic epithelium are not fully developed [67]. Breast milk contains inhibitor factors to many pathogens including *C. difficile* [60], therefore it is not common to find CDI in healthy children. It has been reported that children in Canada and India are susceptible to virulent strains of *C. difficile*. During the years 2000-2003, more than 200 children in Canada with an average of 5.4 year old and 250 children aged 5-12 years required hospitalization with relapsing CDI [30].

4.2 Immunoglobulin level

Sougioultzis *et al* [65] reported that asymptomatic carriers of *C. difficile* showed ~3-fold higher serum anti-TcdA antibody levels compared to patients with CDI. Patients with a high level of anti-TcdA antibody during the first episode of CDI are 48-fold less likely to develop recurrent diarrhea compared to patients with the lower anti-TcdA antibody [65]. It is worthy to note that immunocompromised patients, such as those whom are HIV-positive are at high risk for CDIs [41, 42].

4.3 Antibiotic treatment

Antibiotic treatment can lead to development of CDIs through disruption of normal intestinal flora, particularly those of colon. This disruption favors the overgrowth of *C. difficile* [22]. *Staphylococcus aureus* was originally implicated in antibiotic-associated diarrhea and colitis, yet in 1978 *Clostridium difficile* was found to be the main infective agent [9]. Certain antibiotics including clindamycin, ampicillin, amoxicillin, penicillin, cephalosporins, carbapenems, tetracyclines, fluoroquinolones, sulfamethoxazole, metronidazole, vancomycin, amino glycosides, antifungals, and fluconazole appear to increase the risk of acquiring CDI [10, 67]. In recent years, CDI cases have been more frequent, more severe, more refractory to standard therapy, and more likely to relapse [10]. Fluoroquinolone use seems to significantly increase the risk of developing resistance, particularly in the BI/027/NAP-1 strain of *C. difficile* [8]. There is a positive correlation between the numbers of antibiotics, the duration of a given course, the number of courses, and morbidity/mortality of CDI [67].

4.4 Surgical manipulation

Surgical procedures can serve to increase the risk of CDI [5, 15, 67] due stress, antibiotic treatment and anesthetic drugs that reduce the motility of gastrointestinal tract and induce changes in normal gut flora [22, 34, 39]. Patients who have undergone organ transplantation are at high risk of CDIs. The growth of toxin producing *C. difficile* is marked in patients who undergo postoperative X-ray therapy [35]. Other diagnostic or treatment interventions such as nasogastrofibroscopy or antibiotics administration prior to and during tube feeding also increase the risk of CDIs [53].

4.5 Antacid and other treatment regimes

Patients who are administered proton pump inhibitors, particularly those that are hospitalized, often suffer from multiple co-infections [40]. The use of multiple antibiotics in combination with histamine blockers greatly elevates the risk for CDIs because a reduction in the acidity in the stomach allows spores to survive and pass into the intestine where bile salts promote spore germination [34, 43]. It has been reported that acid-suppressive therapy has increased the risk of a CDI from 1 case per 100,000 in 1994 to 22 per 100,000 in 2004 [39, 40].

5. Modes of Transmission

C. difficile is found widely in the environment, in soil and in marine sediments and sand [44]. It has been isolated from several species including human, horses, donkeys, dogs, cats, domestic birds, cattle, ducks, geese, seals, snakes and camels [18, 61, 62]. It has been reported that 3% of the human population carries *C. difficile* asymptotically. However, the prevalence of carriers in a hospital setting may be as high as 20%. Commonly touched surfaces in hospitals including bedrails, telephones, call buttons, door knobs, toilet seats, and bedside tables in rooms of patients with CDIs are sources of infection [48]. It is not clear if domestic animals can serve as reservoirs for human infection. Skin contamination often persists on a patients' chest and abdomen after a CDI [16]. The common reservoir and source of the organism is the intestinal tract [44], and the main route of transmission is oral-fecal. Mothers can serve as sources of *C. difficile* for their children [25], and vaginal transmission from carriers to their babies has been reported [66].

6. Diagnosis

The range of diagnostic methods varies from classical bacteria isolation to modern molecular techniques. The routine diagnostic test for determining a CDI is the cytotoxin assay [15, 30]. The enzyme immunoassay has replaced the conventional cytotoxin assay because of speed of results and technical ease of performance [15, 33, 46]. Despite costs and technical challenges, stool cultures are crucial for understanding the epidemiology and pathogenesis of CDAD. However, improved rapid identification methods are needed, and additional, in-depth studies on the genetic

mechanisms of resistance and spore formation among NAP1 and NAP2 strains are required for better understanding the epidemiology of CDAD [54].

A relatively new diagnostic method, flow cytometry allows for quantitative evaluation of adherence of *C. difficile*, as well as positive and negative toxins from human colon and small intestine epithelial cells. Additionally this method plays an important role in the explanation of pathogenesis [46]. Algorithm diagnostic methods are very sensitive (92%) and results can be achieved within 4 hours [50].

A wide range of molecular techniques for diagnosing and research purpose are available today [50, 51, 55, 57, 68].

7. Treatment and control measures

Metronidazole and vancomycin are the first choice antibiotics for treatment of CDIs [24, 31]. However, due to the emergence of resistant *C. difficile* strains, other antibiotics such as ramoplanin are being used [31]. Avoidance of unnecessary proton pump inhibitor administration may help reduce the incidence of this infection [29]. To help prevent infection outbreaks, it is essential to put in place a surveillance and control program that includes, staff education, increased vigilance, strict hygiene precautions; cohort nursing in a designated ward, rigorous cleaning procedures, restriction of staff and patient movement, and restriction of antibiotic use [24, 33]. Ideally, patients with a CDI should be in private rooms until their diarrhea has resolved. It is important for medical personnel to avoid contact with contaminated areas and medical equipment. Healthcare workers must use gloves, especially when they deal with infected patients and their environments. Appropriate substances such as cellulose acetate, which is able to kill a range of nosocomial microbes including *C. difficile* should be used to decontaminate surfaces in hospitals [38]. Disinfection with 10% bleach has also been shown to significantly reduce CDIs [48]. Germicide exposure experiments revealed different results for *C. difficile* vegetative cells, compared with those for spores. *C. difficile* spores are resistant to disinfectants at recommended working concentrations, and chlorine-based compounds are able to inactivate *C. difficile* spores at high concentration only. In case of outbreak of epidemic strains of *C. difficile* showed a greater sporulation capacity than non-epidemic strains, so it

is recommended to use as environment disinfectant chlorine compounds in a high concentration [49].

Preservation and restoration of the microbial flora is a useful goal of adjunct therapy [25, 26, 27]. Probiotics such as *Lactobacillus* spp. or *Saccharomyces boulardii* have been shown to be useful in the prevention and treatment of diarrhea, including CDIs [23, 45]. *S. boulardii* protease inhibits the intestinal effects of *C. difficile* TcdA by proteolysis of the toxin and inhibition of TcdA binding to brush border receptors [26]. Oral administration of *S. boulardii* reduces morbidity and mortality of CDI [20, 21].

Inhibition of spore germination of *C. difficile* may be a useful approach [4, 36, 37]. Polysaccharide fucoidin reduces tissue injury inflammation responses significantly in mouse intestines infected with *C. difficile* toxin-A. It reduces mucosal disruption and myeloperoxidase and adenosine deaminase activities [6]. Vaccination with both toxoids A and B not only showed a good antibody response of IgA to TcdA and IgG to TcdB, but it also resolved diarrhea in recurrent CDI patients [65].

8. Conclusions

CDI is an important emerging diseases that is now becoming a very costly public health issue. The ability to develop spores makes *C. difficile* extremely difficult to control. Spores can survive in feces for up to 4 years and in the hospital environment for up to 5 months. Standard cleaning and disinfection procedures are not sufficient to decontaminate the environment, and only a few very potent disinfectants are effective at killing spores. Reinforcement of hand-washing, selection of appropriate disinfectants and daily environmental disinfection has been shown to decrease spread of infection. Establishing a surveillance system is an important step towards reducing the incidence of CDIs. The medical community must consider the risk of developing a CDI before prescribing a combination of antibiotics, antihistamines, and antacids. In addition, the use of antibiotics must be monitored carefully in order to reduce the possibility of a CDI. The zoonotic capacity of *C. difficile* is an important issue for both veterinary public health and human health research institutions.

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