**Clostridium difficile** infection: a review

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*Clostridium difficile*, as one of the main bacterial causes of diarrhea, is an important healthcare-associated pathogen. It is also the main causative agent of antibiotic-associated diarrhea and pseudomembranous colitis. The *C. difficile* infection is a life threatening disease, and there is an urgent need to control its spread in healthcare centers. This review summarizes the most recent work on epidemiology and interactions between host and *C. difficile*.

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**Introduction**

*Clostridium difficile* is a Gram-positive, anaerobic, spore-forming rod and is currently one of the most important healthcare-associated pathogens in both developed and developing countries [1,2]. *C. difficile* was first discovered in the stool of neonates in 1935 by Halland O’Toole. It has been shown that *C. difficile* strains are colonized in 60–70% of infants. Furthermore, some people carry the bacterium in their intestines as carriers, and they can spread the infection to others [2]. Despite the commensal nature of *C. difficile*, it is also an important cause of morbidity and mortality – in spite of reasonably good diagnostic methods – because of a high probability of recurrence after treatment [3]. *C. difficile* is one of the most prominent causes of antibiotic associated diarrhea and most important causes of pseudomembranous colitis (PMC). Patients presenting with diarrhea should be tested for *C. difficile* infection (CDI) after a hospitalization for 3 or more days [1]. An accurate diagnosis is essential for the management and prevention of CDI and for assessing its epidemiology. CDI incidences are increasing in severity and are often life threatening [1,4]. There is an urgent need to control the spread of this infection in healthcare centers. After gut colonization, the bacterium produces two types of toxins including A and B toxin. These toxins are responsible for diseases like mild diarrhea, colitis, PMC and toxic mega colon that can be lethal [1,5]. In addition to toxin A and toxin B, it may produce a number of other putative virulence factors including fimbriae, SlpA S-layer, fibronectin-binding protein (Fbp)A, Cwp84 cysteine protease, a *Clostridium difficile* binary toxin (CDT) binary toxin (third toxin), putative Cwp66 and CwpV adhesions, and para-cresol [6]. In the early-to-mid-2000s, with the emergence of hyper-virulent strains (ribotypes NAP1/BI/O27 and NAP1/BI/O78), the incidence and severity of CDI increased significantly [4]. Hospitalization, old age and comorbidities increase the risk of CDI [2]. Recently, in some healthcare centers, CDI has surpassed methicillin-resistance *Staphylococcus aureus* as the most common healthcare-associated infection [6,7]. The diagnosis and management of CDI have been improved by the introduction of more advanced tests such as glutamate
Biology and pathogenesis of *Clostridium difficile*

*Clostridium difficile* is a spore-forming bacillus [8]. Spores are ubiquitous, persist in aerobic environments, are resistant to stomach acidity and commonly used decontaminants and are transmitted via the fecal–oral route, particularly within the healthcare setting. Spores are highly relevant and vital in *C. difficile* pathological process [2,9]. Because of these features, the key to *C. difficile*’s success as a nosocomial pathogen is its dormant spore form, which leads to the problem of recurring infection [8]. *C. difficile* disease is caused in multiple steps. The gut microbiota, as a colonization barrier, plays a key role against pathogenic bacteria. Antibiotic treatment can cause a disruption of the gut microbiota, which allows *C. difficile* spores to germinate sufficiently to establish an infection. Normally, in the absence of antibiotics, the gut microbiota prevents the overgrowth of ingested *C. difficile* spores. In the next step, putative virulence factors are produced, followed by intestinal epithelial attachment [2,8].

Colonization and adherence

Colonization is an important characteristic and a crucial step for the pathogenesis of CDI. Following the colonization on intestinal epithelial cells, *C. difficile* is able to produce toxins [10–13]. In addition, accessory virulence factors that could play a role in adherence and intestinal colonization of *C. difficile* include cysteine protease Cwp84 and S-layer (a para crystalline array of protein external to the cell wall), a 66-kDa cell-wall protein Cwp66, GroEL heat-shock protein, a 68-kDa Fbp, the flagella components FlIC (Flagellin) and FlID (Flagellar cap protein) [9,13–15]. The main *C. difficile* virulence factors are TcdA enterotoxin and TcdB cytotoxin, both located within a 19.6-kb region of the chromosome known as the pathogenicity locus that also contains three further genes: tcdR encoding an RNA polymerase sigma factor, which is considered as a negative regulator of toxin expression (tcdC), tcdE, which is a holin-like protein, and the binary toxin CDT with an enzymatical activity (tcdA), which causes ADP-ribosylation of G-actin [8,14]. Although the biological significance of CDT during infection remains unclear, in-vitro studies have shown that purified CDT is toxic to Vero cells and may increase the adherence of *C. difficile* to intestinal epithelial cells by the formation of netlike microtubule protrusions. Some strains produce toxin B only and can cause PMC [15,16]. *C. difficile* can release toxins A and B, which access the intestinal epithelial cells and glycosylate proteins in various intracellular signaling pathways which finally leads to inflammation, cell death and clinical manifestations of CDI. Toxins A and B are cytotoxic and cause the disruption of actin cytoskeleton and tight junctions that result in decreased Tran’s epithelial resistance, fluid accumulation and the destruction of the intestinal epithelium leading to clinical symptoms and signs of CDI-like watery diarrhea and the inflammation of the colonic mucosa (Fig. 1) [9,14].

Interactions between host and *Clostridium difficile*

**Effect of metabolic products of Clostridium difficile on gut microbiota**

Human gut contains a normal microbiota consisting of diverse bacterial species that maintain a metabolic equilibrium in the gut, which include promoting intestinal homeostasis through diverse mechanisms, such as degradation of xenobiotic substances, synthesis of vitamins and other beneficial metabolites, immune system regulation and the prevention of the colonization of invading pathogenic microorganisms (antagonism microbial). The gut microbiota helps to maintain the colonization resistance, which allows the resident microbiota to out-compete pathogens for niches and nutrients. Specifically for *C. difficile*, the gut microbiota regulates the production of sodium taurocholate, a bile salt analog needed for the germination of *C. difficile* spores to its disease-causing vegetative form, thereby controlling the life cycle of *C. difficile*. The composition of the microbe may be altered by the use of antibiotic agents. These antibiotics can directly inhibit the growth of susceptible bacteria, induce changes in a gut mucosa with altered epithelial function, cause the loss of protective Toll-like receptor signaling and the accumulation of proinflammatory T helper 17 T cells which leads to increased tissue damage and increased epithelial permeability [5,17,18].

**Function of immune system response in Clostridium difficile infections**

**Innate immune responses:** The host immune responses play a role in the clinical manifestation of CDI. Innate immune responses by inducible intestinal inflammation can play an early process role in pathogenesis of CDI. Previous studies have shown that the toxin A acts rapidly on intestinal epithelial cells causing cellular rounding, detachment,
apoptosis and the secretion of proinflammatory cytokines. Following the loss of epithelial cells, the exposure of lamina propria to TcdA would lead to apoptosis of macrophages, eosinophils and T cells. This in turn will trigger the dissemination of inflammatory cascade via further release of proinflammatory cytokines and chemokines [e.g. IL-12, IL-18, IFN-γ, IL-1β, TNF-α, macrophage inflammatory protein (MIP) 1 and MIP-2, IL-8] which may be responsible for tissue damage. Neutrophil activation, aided by mast cell degranulation, can lead to extensive host-cell damage. However, a hallmark of CDI activation of innate immune sensors, followed by the release of cytokines and chemokines, are followed by local neutrophil infiltration. Intestinal dendritic cells also respond to C. difficile antigens, including surface layer proteins (SLPs) and C. difficile toxins, by promoting the release of regulatory and anti-inflammatory cytokines such as IL-10, IL-23 and IL-4. These cytokines initiate cellular repair processes, dampen the inflammatory response and activate regulatory T and B lymphocytes to promote the protective adaptive antibody response. Actually, it is still unknown whether innate immune responses are, as a whole, beneficial or harmful to the human host [2,18].

Adaptive immune responses: Reports from multiple animal models and human studies clearly indicate that humoral immune responses to TcdA and TcdB influence the outcomes of CDI. Several studies have suggested an inverse association between levels of anti-TcdB antibodies and disease. The importance of the adaptive immune response in modulating CDI outcome is perhaps best highlighted by the number of experimental vaccines currently under development [2,17,19,20]. In a majority of adults and older children, antibodies against C. difficile are present through transient exposure with C. difficile. The role of antitoxin antibodies in the prevention of primary disease is well established. Patients with serum IgG antibodies directed against TcdA, in comparison with control group at the time of spore colonization generally remain asymptomatic. Furthermore, previous studies have suggested that serum IgG antibodies, directed against TcdA, are protective against the recurrence of disease. High mucosal antitoxin antibody (IgA) antibody concentrations as well as the presence of antitoxin antibodies in stool have been associated with the protection against severe or recurrent CDI. In addition, adaptive immune responses to nontoxin virulence factors such as the S-layers proteins, cell-wall proteins and flagella have been reported in CDI patients [2,18–23].

Changing epidemiology of Clostridium difficile infection

Changes in the virulence of causative strains coupled with changes in antibiotic usage patterns are probably responsible for increased morbidity and mortality of CDI [8,24]. In the past 20 decades, the total number of high-severity causes of CDI has increased. For example, in the USA, the number of cases of CDI was previously at 30–40 per 100,000 population. It increased to 50/100,000 cases in 2001 and 84/100,000 cases in 2005 (nearly three times more than the baseline rate of 31/100,000 in 1996) [25,26]. With improved infection control measures, antibiotic stewardship and advanced diagnostic techniques, we may begin to witness an actual drop in prevalence in the near future [27].

Hyper-virulent strains of Clostridium difficile

In the early 2000s, with the emergence of the North American pulsed-field gel electrophoresis type 1 (NAP1), strain changes occurred in the epidemiology of CDIs [28]. The NAP1 strain, with a mutation in the regulatory gene of tcdC, causes an increased production of toxins A and B and mutations in the SLPs, which increases its adherence to intestinal epithelium and the production of more spores in comparison with historical strains. Furthermore, this strain, which was associated with the use of fluoroquinolones, produces the binary toxin. In the years between 2002 and 2006, NAP1/BI/O27 strain was responsible for a deadly new epidemic, and in 2010, it spread in the USA, Canada, England, Europe and Asia [8,29]. The hypervirulent strains, belonging to the BI/NAP1/027 group, were responsible for an increased incidence of CDI and greater disease severity [30,31]. Another hypervirulent strain of C. difficile, designated as NAP8/078, was foodborne and associated with community-acquired CDI in young patients and was predominately found in pigs and calves. Similar to the BI/NAP1/027, ribotype 078 is a hypervirulent strain of C. difficile associated with severe disease and mortality. The most important identification factor for the NAP8/078 strain was a 39-bp deletion in the tcdC portion of the pathogenicity location that produces binary toxin, and variable resistance to clindamycin, erythromycin and fluoroquinolones [29,32–36].

Prevalent Clostridium difficile ribotypes in Asia

The true prevalence of CDI remains unknown in Asia, but limited studies have indicated that CDI is a significant nosocomial pathogen with a comparatively high prevalence rate. In South East Asian countries like China, Japan, Singapore, Hong Kong, Taiwan and Korea, relatively high prevalence rates were noted. The predominant ribotypes in Asia appeared to be 017, 018, 014, 002 and 001 [37–39]. Unlike the other major epidemic strains 027 and 078, these ribotypes do not produce binary toxin, and ribotype 018 does not appear to possess variant toxin genes. Ribotypes 017 and 018 have caused widespread disease in Asia and across the world. Ribotype 018 is the fourth most prevalent ribotype in Europe and is resistant to clindamycin and fluoroquinolones. In Japan, smz/018 appears to have persisted as the most common ribotype for over a decade.
Meanwhile, ribotype 017, A B⁺ and toxinotype VIII strains are widespread in China, Korea, Japan, Taiwan and Hong Kong causing epidemics worldwide. The exposure to antineoplastic agents, use of nasal feeding tubes and care in a particular hospital ward were associated with ribotype 017 infections. Despite these revelations, CDI was likely to occur at similar rates in Asia as in other continents where CDI is more commonly researched [37,40,41].

Community-associated infections
Up to 41% of all cases of CDI are classified as community-acquired CDIs (CA-CDI). The disease was seen in populations that were previously thought to be at low risk, such as young individuals and pregnant women. So, it appears as though people in the community are also at risk for CDI as are hospitalized patients. Community sources for CA-CDI include soil, water, pets, animal products, meats and vegetables [42–45].

Risk factors
Hospitalization, changing patterns of antibiotics use and age more than 65 years are among the risk factors attributed to increased rates of CDI. The most prominent risk factor for the development of CDI lies in the history of antibiotic usage (particularly clindamycin, ampicillin or amoxicillin, cephalosporins and fluoroquinolones). Hospitals are considered not only as reservoirs, but also as a transmission vector. Other important risk factors are the manipulation of the gastrointestinal tract (e.g. surgery) and an immunosuppressed state (e.g. chemotherapy, HIV-positive patients and medical comorbidities leading to immunosuppression). Patients are at risk for the acquisition of C. difficile through ingestion of spores, which are usually transmitted from other patients, hands of healthcare personnel or the environment [25–27,46].

Recurrence of Clostridium difficile infection
Recurrence of CDI is defined as the presence of diarrhea and a positive C. difficile stool assay within at least 10 days after the first episode. There are little data about the relative frequency of this condition. The endogenous persistence of C. difficile spores and the acquisition of a new strain from an exogenous source may explain the physiopathology of recurrences. The rates of clinical recurrences could therefore be reduced by implementing strict isolation precautions [47,48].

Laboratory diagnosis of Clostridium difficile infection
There are a variety of diagnostic methods available to laboratories for the diagnosis of CDI, but all of these methods have limitations [49]. The ideal laboratory test for CDI would be sensitive, specific, rapid, simple to perform and inexpensive. Culture is the most sensitive method, but it is not very specific due to the possibility of isolating nontoxigenic isolates and is also time-consuming. Selective agar plate called cycloserine cefoxitin fructose agar has often been used for the isolation of the bacterium from stool specimens. The selective agents are cycloserine at a concentration of 500 mg/l and cefoxitin at 16 mg/l [8]. Fecal diagnostic tests, anaerobic toxigenic culture and the cell culture cytotoxicity neutralization assay (CCCNA) are the most sensitive techniques for the diagnosis of CDI, but all are time-consuming, expensive, lack standardization among laboratories and are generally unavailable outside the research settings [5]. For many years, the CCCNA was the accepted gold standard. By this method, stool filtrates are inoculated onto a monolayer of a cell culture in wells with and without C. difficile antitoxin [7]. The enzyme immunoassay (EIA) became broadly used because of its rapidity in performance, but EIA used for toxin detection suffers from sensitivity problems and is considered suboptimal for diagnosing CDIs [7]. A laboratory assay measuring the clostridial enzyme, GDH, represents a rapid, convenient, inexpensive and sensitive test for the bacterium but does not identify C. difficile toxin(s) [5,7]. Nucleic acid amplification methods, which detect the toxin genes, are the newest methods to be implemented for the diagnosis of CDI and are promising to become a stand-alone test for CDI [7,49]. PCR is superior to EIA in terms of sensitivity and specificity, but is much more costly, and false positives and false negatives (if stool specimen collection is delayed and the patient treated empirically for suspected CDI) may result in an inappropriate treatment [25]. In most laboratories, real-time PCR has become the preferred laboratory test for diagnosing CDI based on high test sensitivity. However, the accuracy for using real-time PCR for diagnosis depends on the prevalence of CDI and the rate of asymptomatic C. difficile carriage in the population studied [5]. There is some concern about molecular testing as testing cannot distinguish between C. difficile-associated diarrhea and asymptomatic carriage and may result in overdiagnosis and overtreatment [50]. The loop-mediated isothermal amplification, a non-PCR-based gene amplification method, was developed for detecting the pathogenicity locus of toxigenic C. difficile [7,51]. A comparison of the available assays for C. difficile detection is shown in Table 1. Other indirect tests include direct gas liquid chromatography (GLC) on stool specimens, latex agglutination or computed tomography scan. In a typical GLC profile, C. difficile is displayed with large amounts of butyric and isocaproic acids. Furthermore, a rapid and accurate test for the identification of C. difficile production of proline amino peptidase by a disc test was developed and is used in conjunction with the typical morphology of the colonies [25,43]. Endoscopy is required to diagnose the presence of PMC found in up to half of the CDI patients. An
abdominal computed tomography examination is useful in diagnosing CDI in a patient suspected with the disease in whom mucosal thickening of the colon is seen [5,25]. The recommended route for testing is the use of a two-step algorithm, a GDH assay (C. difficile antigen), followed by EIA (toxin testing). If the antigen test is positive and the toxin is negative, a final PCR confirmatory testing may be necessary. The American Society of Microbiology recommends that if the toxin A/B EIA or CCCNA is negative, PCR or toxigenic culture should be further tested [7,52].

Prevention of Clostridium difficile infection

There is a need of infection control measures for the prevention of CDI in hospitals and for preventing C. difficile spores from reaching patients [42]. Appropriate cleaning of environment and medical equipment is useful for the prevention of CDI. A challenge to hospital control is how to effectively eradicate C. difficile due to the persistent nature of its spores [8]. The C. difficile spores are transmitted by patients in the room, and by the hands of healthcare workers. The transmission rate in the rooms of patients with CDI and active diarrhea, that of the rooms of patients who are colonized with C. difficile but do not have diarrhea, and that of the rooms of patients who do not harbor C. difficile were reported to be more than 50%, ~25% and less than 10%, respectively [53]. Hand hygiene is essential for controlling CDI infection. Traditional hand washing especially with chlorhexidine-based soaps is preferred due to the fact that C. difficile spores are resistant to the commonly used alcohol hand rubs or gels [8,42]. Antimicrobial stewardship programs, which promote rational and reduced use of antibiotics, are essential to CDI control. Control programs and monitoring the use of antibiotics should be implemented. Physicians should also restrict the use of broad-spectrum antibiotics, particularly cephalosporins and clindamycin, with careful consideration of current efforts for standardizing regimens for surgical prophylaxis [8,27,29].

Treatment of Clostridium difficile infection

There are two most difficult challenges for CDI treatment which include the management of multiple recurrences and the fulminant or severe complicated CDI. Patients with multiple recurrences of CDI typically respond to vancomycin or metronidazole. Newer agents are desperately needed for treatment of multiple relapsing and fulminant CDI. Several approaches are under investigation including the use of flora-sparing antibiotics, vaccines, toxin-binding agents and monoclonal antibodies [42]. A novel therapy for CDI is fidaxomicin (an oral minimally absorbed macrocyclic antibiotic) that became the second drug approved by the Food and Drug Administration for CDI (vancomycin was the first). Fecal transplant and probiotics used for the reestablishment of a normal colonic flora constitute an intuitive therapeutic approach [54]. However, the data are too retrospective and insufficient to recommend their use for that purpose [55]. Although there is no vaccine against C. difficile, it is a promising field of investigation. Substantial progress in the development of a preventive vaccine seems to be in program [10,36]. If there are clinical manifestations like perforation, peritonitis with systemic toxicity and toxic colon dilatation, surgery is necessary. Serum lactate may serve as a marker for severity [21,50,51].

Conclusion

Awareness and surveillance of CDI in Asia is relatively poor. The incidence of CDI in most Asian countries remains largely unknown, partly tends to be underdiagnosed. This has resulted in a lack of recognition of CDI as a health problem.

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Conflicts of interest

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