Update on *Clostridium difficile*

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Introduction

Clostridium difficile represents one of the main causes of infectious diarrhea due to a bacterial strain in the hospital setting. *C. difficile* is a common nosocomial pathogen, particularly among intensive care unit (ICU) patients, whose clinical characteristics often include important risk factors for *C. difficile* infection, such as severe underlying disease and treatment with antimicrobials. Prolonged ICU stay has been identified among the risk factors for *C. difficile* infection [1]. Furthermore, *C. difficile*-associated disease may cause fulminant colitis requiring admission to the ICU [2]. Rates of *C. difficile* infection have risen rapidly over the past decade, along with a trend to increased rates of complications, nosocomial outbreaks, difficult-to-treat recurrent infection, and all-cause mortality within 30 days of *C. difficile* infection [2, 3]. The severity of *C. difficile*-associated disease reflects the emergence of isolates with increased pathogenicity, replicative capacity, and antibiotic resistance. Furthermore, the appearance of *C. difficile* as a community-acquired disease, and the increasing use of immunosuppressive therapies in elderly and debilitated patients has contributed to the spread of *C. difficile*-associated disease. Associated complications include toxic megacolon, bowel perforation, and septic shock. Patients with complicated *C. difficile*-associated disease

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J.-L. Vincent (Ed.), *Annual Update in Intensive Care and Emergency Medicine 2013*,
DOI 10.1007/978-3-642-35109-9_4,

display mortality rates of up to 38 %, correlated with significantly prolonged hospitalization especially in the ICU [4].

An increase in treatment failure with metronidazole and challenges related to *C. difficile*-associated disease relapses are other new features of *C. difficile* infection [5]. Although controversial, some authors also report an increased incidence in populations previously considered at low risk [6, 7].

Reduction in antibiotic use, development of infection control committees, and prevention of infection transmission through prompt isolation of infected patients, hand hygiene, and cleaning procedures remain key factors in reducing the incidence of *C. difficile*-associated disease in the critical care setting [8].

The Changing Epidemiology of *C. difficile*

In European hospitals, the number of cases of *C. difficile*-associated disease has increased each year since 2000, and in North America a greater than 3-fold increase in *C. difficile* infection rates during the 5-year period from 2000–2004 has been registered, especially in the elderly [3, 9, 10]. Recent published data in the US report 336,600 hospitalizations related to *C. difficile*-associated disease in 2009, corresponding to 1 in 100 of all hospital stays. In the critical care setting, Lawrence et al. reported an incidence of 0.4–100 cases of infection per 1,000 patient-days per 1,000 admissions, but rates may be higher in outbreak settings and have regional variation [11]. Although elderly hospitalized patients are the main group at risk for developing *C. difficile*-associated disease, recent evidence showed an increased incidence of *C. difficile* infection in populations with no previous antibiotic therapy and low risk groups, such as children [6].

In 2005, molecular analysis identified a new strain of *C. difficile* defined as BI/NAP1/027 (by restriction endonuclease analysis, pulse-field gel electrophoresis, and PCR ribotyping, respectively) responsible for large outbreaks in North America and Europe capable of *in vitro* production of higher levels of toxins A and B [12, 13]. The epidemic, toxin-gene variant ribotype 027 strain is associated with accelerated kinetics *in vitro* and toxin synthesis during stationary growth phases, and mutation in the negative regulator gene (*tcdC*) for production of the binary toxin CDT involved in actin-specific ADP ribosyl transferase activity leading to cytoskeleton disorganization [14, 15]. Furthermore, *C. difficile* ribotype 027 is capable of *in vitro* replication in the presence of non-chloride cleaning agents and displays resistance to fluoroquinolones (MIC > 32 mg/l) [16, 17].

Although only sub-inhibitory concentrations of metronidazole, vancomycin, and linezolid induced toxin production, fluoroquinolones and cephalosporins have been shown to promote ribotype 027 spore germination, cell growth and toxins [18, 19]. Of note, the same *in vitro* model showed that neither piperacillin-tazobactam nor tigecycline induced *C. difficile* toxin production [19]. Finally, ribotype 027 strains with reduced susceptibility to metronidazole have also been found to be transmitted between patients, but their clinical significance in

terms of response to antibiotic treatment remains unclear and is still under investigation [20].

Ribotype 027 infections are mostly described in hospitalized patients, but there is recent evidence of community-acquired cases, especially in the community surrounding a hospital in which other cases were diagnosed. A recent study by Wilcox et al. [13] showed an increase in incidence, severity, recurrence, complications and mortality related to *C. difficile*-associated disease with a correlation to ribotype 027 in patients above 65 years. Control of the epidemic *C. difficile* ribotype 027 correlated with a 61 % reduction in cases of *C. difficile* infection between 2007–2010 [21].

Pathogenesis of and Risk Factors for *C. difficile* Infection

C. difficile, a Gram-positive, spore-forming, anaerobic rod can colonize the gut if the normal intestinal flora is altered or absent. Often, asymptomatic colonization is seen in the fecal flora of new-born infants and elderly patients [22]. *C. difficile*-associated disease is a toxin-mediated intestinal disease with highly variable clinical manifestations, ranging from mild diarrhea to severe syndromes, including toxic megacolon, bowel perforation, sepsis, septic shock, and death [23]. Abdominal pain, fever, leukocytosis, and presence of mucus in the stool are the commonest clinical manifestations associated with symptomatic *C. difficile* infection, although they are reported in less than half of patients [24]. Melena or extraintestinal manifestations, such as bacteremia, abscesses, or osteomyelitis are rare [25, 26].

C. difficile is implicated as the causative organism in up to 25 % and 50–75 % of patients who develop antibiotic-associated diarrhea and antibiotic-associated colitis, respectively [4, 27]. Other risk factors associated with *C. difficile*-associated disease are summarized in Table 1 [8, 10, 28–36]. Even though some cases are not associated with previous antibiotic exposure, this remains the principal risk factor for the development of *C. difficile*-associated disease, occurring typically 2 to 3 months before infection [37]. Although all antibiotics can potentially be associated with the development of *C. difficile*-associated disease, some carry a higher risk than others, including clindamycin, cephalosporins and, more recently, fluoroquinolones [38].

Antibiotics play an important role in the development of *C. difficile*-associated disease by disrupting the normal microbiota in the gut and favoring the multiplication and colonization of *C. difficile*. Susceptibility to *C. difficile*-associated disease in patients treated with antibiotics persists for a variable period after the administration of the last dose depending on the molecule administered, i. e., longer time for clindamycin compared to cephalosporins [39, 40]. Hospitalization may expose the patient to a highly-resistant spore contaminated environment, along with the risk of health care workers' sub-optimal hand hygiene. Older patients show greater mortality associated with *C. difficile*-associated disease and more recurrent disease because of their inability to mount a specific serum IgG immune response when exposed to the toxins [41]. Patient exposure to the spores of the microorganism

Table 1 Risk factors associated with *C. difficile*-associated disease in the hospital setting

Risk Factor	Reference
Age ≥ 65 years	[28, 29]
Immunocompromised state (e. g., immunosuppressive drugs, HIV infection, antineoplastic agents)	[29, 30, 31]
Multiple antimicrobials during the previous 3 months	[10, 32, 33]
Severe underlying illness	[28, 29]
Gastrointestinal surgery	[28]
ICU stay	[28]
Multiple antibacterial exposure within 3 months	[28]
Gastrointestinal stimulants and stool softeners	[28, 29]
Reduced health-care worker hand hygiene	[34]
Inadequate environmental disinfection	[8, 34]
Overcrowding and rapid turnover in hospital beds	[34]
Prolonged hospitalization (> 20 days)	[35]
Shared toilet facilities among patients	[34]
Inadequate isolation measures for infected patients	[8, 34]
Emergence of epidemic strains	[36]

HIV: human immunodeficiency virus

occurs mainly through contact with the hospital environment or health care workers. Nevertheless, Best et al. demonstrated the possibility of airborne spread of *C. difficile* spores from patients with symptomatic *C. difficile*-associated disease, recovering *C. difficile* from air sampled at heights up to 25 cm above the toilet seat following flushing a toilet [42].

Following spore germination, the replicating vegetative cells can adhere and penetrate the enterocytes via flagella and proteolytic enzymes, and adhere to the cells through adhesins to colonize the gut. Then, cytotoxic enzymes A and B, the main *C. difficile* virulence factors, cause colonic mucosa cytoskeleton disorganization with inflammatory cytokine production, fluid accumulation and destruction of the intestinal epithelium. As mentioned, the binary toxin produced by *C. difficile* BI/NAP1/027 can increase toxin A and B toxicity and lead to more severe disease [43].

Severe Forms of *C. difficile*-associated Disease

Symptoms of *C. difficile*-associated disease range from a mild self-limited diarrhea to life-threatening colitis. About 30 % of patients with *C. difficile*-associated

disease are febrile, and 50 % have leukocytosis. A white blood cell (WBC) count > 20,000/µl may herald a patient at risk for rapid progression to fulminant colitis with systemic inflammatory response syndrome (SIRS) and shock. It is important to recognize that presentation of fulminant *C. difficile*-associated disease colitis may be atypical, especially if the patient is immunosuppressed or elderly, and may not necessarily be associated with antibiotic usage [44].

Pseudomembranous colitis and toxic megacolon are pathognomonic of severe *C. difficile*-associated disease. However, pseudomembranes are present in only 50 % of patients with *C. difficile* colitis. Fulminant disease is a potential complication of *C. difficile*-associated disease and colectomy in this group can be life-saving [45]. Unfortunately, hospital mortality in this group of patients ranges from 35 % to 57 % [46]. Although diarrhea is the hallmark of symptomatic *C. difficile*-associated disease, severe abdominal pain and lack of diarrhea could indicate that the patient has ileus with toxic megacolon. High mortality in fulminant colitis is largely the result of lack of timely recognition, for this reason the intensivist should evaluate and manage patients with *C. difficile*-associated disease in order to identify fulminant disease in a timely manner so that colectomy and its timing can be optimized.

There are no validated methods to identify patients at risk for poor outcomes due to *C. difficile* infection, but some factors include advanced age, acute renal insufficiency, WBC count > 20,000/µl, immunosuppression, hypoalbuminemia, and at least one organ system failure [46].

Recurrences of *C. difficile*-associated Disease: A Challenging Issue

High rates of *C. difficile*-associated disease recurrence probably represents one of the most challenging aspects of *C. difficile* management. Up to 30 % of patients may experience a second event within 60 days (usually in the first two weeks) from discontinuation of successful treatment with standard therapies, i. e., metronidazole or vancomycin. Recurrence appears to be related to a combination of factors: Failure to re-establish the colonic microflora, persistence of *C. difficile* spores in the intestine, and sub-optimal host immune response to the infecting organism and its toxins. Risk factors for recurrent episodes include: Immunocompromise, exposure to antibacterial agents that disrupt the normal colonic microflora, previous episode of *C. difficile* infection, renal impairment, older age (≥65 years), severe underlying disease, prolonged hospitalization, and ICU stay. Factors that are common in patients hospitalized in ICU, such as the lack of restoration of enteric microbiota, the persistence of *C. difficile* spores within the gut, and deficient host immune response all appear to be related to the chance of recurrence. Furthermore, hospitalized patients who are colonized by the bacteria or experience acute or recurrent infection may represent a reservoir of infection for other patients who share the same environment. Usually, clinical severity does not

change significantly between primary events and recurrences; a second cycle of treatment with metronidazole or vancomycin can be efficacious in this scenario, but the therapy remains suboptimal and 40 to 60 % of patients will have one or more relapses [47].

Diagnosis and Therapy of *C. difficile*-associated Disease

The diagnosis of *C. difficile* infection consists of clinical history (i. e., antimicrobial use or/and other risk factors) and presence of diarrhea in combination with laboratory tests. Diagnostic laboratory protocols measure *in vivo C. difficile* toxin production, which is responsible for *C. difficile*-associated disease. Since a rapid and accurate microbiological diagnosis is key, diagnostic algorithms that can provide high sensitivity, rapid turnaround time, and ease of performance are mandatory [48]. Although availability of a rapid diagnostic algorithm for *C. difficile*-associated disease would reduce unnecessary antibiotic treatment and speed implementation of infection control precautions, pre-emptive antibiotic therapy is often started empirically by clinicians.

The detection of toxin A/B from fecal samples by immunoenzymatic methods has been the cornerstone of laboratory *C. difficile* infection diagnosis for over two decades. However, its sensitivity and specificity are suboptimal when used as a standalone assay and it relies on the prevalence of *C. difficile* toxins in stool [49]. Thus, a two-step diagnostic algorithm using a rapid test for both toxin A and B by immunoassay methods followed, in selected cases, by stool culture including isolate toxin testing is performed. Troublesome specimens should always be sent to reference laboratories for culture cytotoxicity neutralization assay (CCNA) confirmatory testing. Other tests include rapid antigen detection of a cell wall-associated enzyme, glutamate dehydrogenase (GDH), as a screening test to rule out negative specimens and test the positive ones for toxin production [50]. Recently, last generation polymerase chain reaction (PCR)-based commercial kits and ribotyping have become available for *C. difficile*-associated disease outbreak monitoring and epidemiological surveys [51, 52].

In addition to microbiological tests, computed tomography (CT) scanning can be useful for recognizing more severe forms of disease detecting colonic mural thickening, intramural gas, and pleural effusion. Laboratory tests showing high WBC counts, low albumin level, and immunosuppression have also been correlated with severe *C. difficile*-associated disease [53].

Treatment of *C. difficile* infection can be challenging. When possible, any antibiotic treatment should be discontinued to allow restoration of the intestinal flora [54]. Often this option is not possible in critically ill patients: In this case, therapy goals are to eradicate the infection despite continuation of concomitant therapy, and to minimize the incidence of recurrence. Metronidazole and vancomycin represent the mainstay for *C. difficile*-associated disease treatment. In a prospective, randomized, double-blind, placebo-controlled trial comparing vancomycin and metronida-

zole for the treatment of mild and severe *C. difficile*-associated disease [55], metronidazole or vancomycin resulted in clinical cure in 90 % and 98 % of mild forms, respectively; in severe *C. difficile*-associated disease, clinical cure was reached for 76 % and 97 % patients treated with metronidazole or vancomycin, respectively ($p = 0.02$). Thus, a superior efficacy of vancomycin was demonstrated for severe cases and the authors recommended it as first-line treatment for severe *C. difficile*-associated disease. Study of *C. difficile*-associated disease recurrences showed no inferiority for metronidazole compared to vancomycin [56]. Thus, vancomycin should be considered as treatment for a first *C. difficile* infection recurrence only in the presence of markers of severe disease (i. e., pseudomembranous colitis, hypotension, rising serum creatinine level) [54]. Conversely, further recurrences should be treated with tapered and/or pulsed vancomycin therapy [54]. Although different tapering schemes have been proposed, this approach has not been validated in comparative studies. Treatment indication and doses are shown in Table 2.

Tigecycline, a glycylcycline derivative of minocycline, also achieves fecal concentrations above the minimum inhibitory concentration (MIC) for *C. difficile*. Tigecycline is not licensed for treatment of *C. difficile*-associated disease and there are no randomized trials but only case reports showing its potential efficacy; thus, it is currently only recommended in cases in which other standard options have failed.

Other antimicrobial treatments include rifaximin, proposed as a rescue option in the treatment of second and later recurrences although high levels of resistance have emerged; ramoplanin, a new lipoglycodepsipeptide, that showed similar results when compared to vancomycin in terms of *C. difficile*-associated disease cure and relapse rates with no emergence of resistance; nitazoxanide, a nitrothiazolide compound with good antimicrobial activity against helminthic and protozoal parasites is still being studied [57, 58]. Fidaxomicin, a macrocyclic antibiotic with good *in vitro* activity against clinical isolates of *C. difficile* (including NAP1/BI/027 strains), has also shown promising results in clinical trials and superiority in recurrence cure rates compared to vancomycin [59]. Furthermore, in individuals taking concomitant antibiotics for other concurrent infections, fidaxomicin was superior to vancomycin in achieving clinical cure (90 % vs. 79.4 %, respectively; $p = 0.04$) [60].

Non-antibiotic treatments include toxin-binding agents such as tolevamer, which also neutralizes toxins produced by the NAP1/BI/027 strain, has shown good results on *C. difficile*-associated disease recurrences but lower cure rates when compared with vancomycin and metronidazole, and has a potential place in the treatment of recurrent conditions as supplemental therapy [61]. Treatment with intravenous immunoglobulin (IVIG) to neutralize toxin A by IgG anti-toxin A antibodies has been utilized off-label to treat both refractory and fulminant *C. difficile* infection despite the lack of large randomized controlled trials and few reports of successful treatment in recurrent or severe *C. difficile*-associated disease [62]. Because alterations in the intestinal flora play a critical role in *C. difficile*-associated disease pathogenesis, the use of probiotics (especially *Saccharomyces boulardii* and *Lactobacilli*) is also being studied for treatment of *C. difficile* infection, although the evidence in the literature is not yet sufficient to recommend their

Table 2 Treatment options in *Clostridium difficile* infection in ICU patients

First episode	
Non-severe disease	Stop causative antibiotics if possible Metronidazole 500 mg tid orally for 10–14 days (if oral therapy is possible) Metronidazole 500 mg tid intravenously for 10–14 days (if oral therapy is impossible)
Severe disease	Vancomycin 125 mg qid orally for 10–14 days (if oral therapy is possible) Metronidazole 500 mg tid intravenously for 10 days + intracolonic vancomycin 500 mg in 100 ml of normal saline every 4–12 h and/or vancomycin 500 mg qid by nasogastric tube (if oral therapy is impossible) Fidaxomicin 200 mg bid orally
First recurrence	Treat as the first episode, according to severity of the disease. Fidaxomicin 200 mg bid orally
Second recurrence	
If oral therapy is possible	Fidaxomicin 200 mg bid orally for 10–14 days Vancomycin 125 mg qid orally for 14 days Consider tapering after initial 14 days therapy: 125 mg bid for 7 days, then 125 mg qid for 7 days, then 125 mg once every 2 days for 8 days (4 doses) and lastly 125 mg once every 3 days for 15 days (5 doses) Consider rifaximin 200–400 mg bid for 10–14 days after vancomycin Consider Saccharomyces boulardii 500 mg bid for 21–28 days
If oral therapy is impossible	Metronidazole 500 mg tid intravenously for 10–14 days + retention enema of vancomycin 500 mg in 100 ml of normal saline every 4–12 and/or vancomycin 500 mg qid by nasogastric tube
Third and later recurrences	Eliminate risk factors Consider tigecycline Consider intravenous immunoglobulin (IVIG) and monoclonal antibodies (ongoing trials) Consider "fecal transplantation"

routine use [63]. Finally, vaccination against *C. difficile* with a toxoid vaccine has proved protective against recurrent *C. difficile*-associated disease [64], and intestinal microbiota transplantation (fecal bacteriotherapy) seems very promising [65].

Conclusion

C. difficile-associated disease diagnosis and treatment is challenging and recurrences are frequent, contributing to its difficult management. A key measure for treating *C. difficile* infection includes discontinuation of antibiotic therapy to allow

restoration of the intestinal flora, although this approach is not often applicable in critically ill patients. New therapies aim to eradicate the infection even in the presence of antimicrobial therapy, and to reduce the incidence of recurrence. Metronidazole has shown a poorer response when compared to vancomycin in severe forms of *C. difficile*-associated disease. Oral metronidazole is usually recommended for initial treatment of non-severe *C. difficile*-associated disease. Fidaxomicin may be promising in those patients who cannot tolerate vancomycin, although additional data are needed. New compounds are also under investigation. Nevertheless, infection control measures, awareness of the multiple risk factors along with consideration of possible nosocomial transmission within the ICU, and correct antimicrobial management to limit antibiotic use are key factors to reduce the incidence of *C. difficile*-associated disease in the ICU.

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