

***Clostridioides difficile* infection: an update**

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SUMMARY

Clostridioides difficile (*C. difficile*) is a Gram-positive, spore-forming anaerobic bacterium emerged as a leading cause of diarrhea globally. CDI's (*Clostridioides difficile* infection) impact on healthcare systems is concerning due to high treatment cost and increased hospitalisation time. The incidence of CDI has been influenced by hypervirulent strains such as the 027 ribotype, responsible for significant outbreaks in North America and Europe. CDI's epidemiology has evolved, showing increased community-acquired cases alongside traditional hospital-acquired infections. Mortality rates remain high, with recurrent infections further elevating the risk. Transmission of *C. difficile* primarily occurs via spores, which survive in healthcare settings and play a pivotal role in transmission. Not only health workers, but also the food chain could have a significant impact on the transmission of infection, although no confirmed foodborne cases have been documented. Pathogenicity of *C. difficile* involves spore germination and toxin production. Toxins A and B can cause cellular damage and inflammatory responses in the host, leading to colitis. Clinical picture can range from mild diarrhea to fulminant colitis with toxic megacolon, and bowel perforation.

Risk factors for CDI include antibiotic exposure, advanced age, hospitalization, and use of proton pump inhibitors. Patients who experience abdominal surgery or patients with inflammatory bowel disease (IBD) are particularly susceptible due to their compromised gut microbiota. Management of CDI has evolved, with fidaxomicin emerging as a superior treatment option over vancomycin for initial and recurrent infections due to its reduction of recurrence rate. Faecal microbiota transplantation (FMT) is effective for recurrent CDI, restoring gut eubiosis. Bezlotoxumab, a monoclonal antibody against *C. difficile* toxin B, has shown promise in reducing recurrence rates. Severe cases of CDI may require surgical intervention, particularly in instances of toxic megacolon or bowel perforation. In conclusion, CDI remains a significant clinical entity. Further research are needed to improve patients' outcome and reduce the burden on healthcare systems.

Keywords: *Clostridioides difficile*, bezlotoxumab, fidaxomicin.

INTRODUCTION

Clostridioides difficile is a Gram-positive, spore-forming anaerobic bacterium that has emerged as a leading cause of nosocomial diarrhoea, with a concerning increase in community-onset cases in recent years. According to the ECDC, the European incidence of CDI exceeds

120,000 cases per year, making it one of the major economic burdens on healthcare systems, due to the high treatment costs and prolonged hospital stay [1]. The European report by COMBACTE-CDI survey documented a median CDI incidence rate of 4.1 cases per 10,000 bed-days [2]. Interestingly, community acquired CDI (CA-CDI) have increased in the last decades, reflecting important changes in epidemiology and risk factors. However, recent Italian data reported a relevant missed CA-CDI diagnosis rate, probably resulting from younger patients with no previous antibiotic exposure or history of hospitalisation [3]. An Italian

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surveillance study showed a lower-than-expected incidence of CDI (0.96 cases/10000 patient days-bed), reflecting disease under-estimation [4].

Concurrently, recent 20 years have seen the spread of epidemic strains, such as the hypervirulent 027 ribotype, which was responsible for an outbreak in the US and UK [5].

Latest guidelines have highlighted some changes in the therapeutic approach of CDI [6, 7]. *Clostridioides difficile* illness can be considered a subject of primary interest in terms of epidemiological changes, burden of disease and therapeutic options, remaining both a central topic in scientific debate and a concern in clinical practice.

Given this background knowledge, our narrative review aims to give an overview of *C. difficile* epidemiological and pathogenetic features, together with current updates on therapeutical approach.

■ METHODS

We performed a narrative review on *Clostridioides difficile* using PubMed electronic databases. Keywords were: “*Clostridioides difficile*”, “CDI”, “colitis”, “epidemiology”, “strains”, “risk factors”, “pathogenesis”, “toxins”, “dysbiosis”, and “therapy” including “FMT”. The search string used was “(*Clostridioides difficile*) AND (colitis) OR (epidemiology) OR (risk factors) OR (pathogenesis) OR (toxins) OR (dysbiosis) OR (therapy) OR (FMT)”. According to this criterion, all original and review articles were considered. Further studies were collected by screening the reference lists of selected articles for any relevant sources not retrieved in the initial search. Finally, 96 articles were considered pertinent and were thus selected for inclusion.

■ EPIDEMIOLOGY AND BURDEN OF DISEASE

By the first years of 2000s, an increased incidence of CDI in Canada, US and Europe was noticed [8]. Since then, several studies attempted to investigate the mechanisms underlying these epidemiological changes. Innovative molecular biology techniques allowed us to amplify the 16S-23S intergenic spacer regions of *C. difficile*, in order to identify a new epidemic strain of *C. difficile* defined as *BI/NAP/PCR ribotype 027*, an hypervirulent strain responsible for several outbreaks in the US [9]. This strain is characterised by fluoroquinolones resistance and a higher MIC₉₀ to met-

ronidazole when compared with non-027 isolates [10]. Between 2004-2006 an outbreak of *C. difficile 027/BI/NAP1* presented in the UK with a documented rising incidence from 25.9% to 41.3% [11]. Moreover, an Italian study reported a significant increase in RT027 incidence up to 64% between 2009 and 2012 [12]. Outbreaks of 027-like ribotypes have been described all over Europe. Between 2016 and 2019, RT181 was identified as the most frequent and virulent ribotype in Greece, found in approximately 70% of hospitals. Despite the similarities, RT181 appears to have a lower virulence than RT027. Furthermore, while RT027 is associated with cases of both CA-CDI and HA-CDI, RT181 has only been observed in nosocomial outbreaks [13, 14].

An Italian study conducted from 2015 to 2018 also showed that strains *ST-378*, *ST-379* (belonging to clade 1) and *ST-37*, *ST-11* (belonging to clades 4 and 5) were mostly isolated in patients with CDI, while *ST-5* and *ST-380* were isolated only in outpatients [15].

Recent data reported a marked high raise in CDI incidence in American hospitals, with an incidence in US up to 8.3 CDI cases per 10000 patient-days [16].

COVID-19 pandemic significantly impacted on CDI epidemiology, although the available data are quite heterogeneous. According to an US analysis conducted nationwide in 2020, 4920 cases of CDI were found among a total of 1,045,125 patients with COVID-19. Furthermore, CDI was independently associated with higher mortality-rates [17]. Other data from the National Healthcare Safety Network, later confirmed by Italian and Mexican studies, showed an overall reduction in hospital acquired-CDI (HA-CDI) in 2020 compared to 2019 [18-21]. Improved infection prevention and control practices, increased emphasis on hand hygiene and the use of personal protective equipment might have had a positive impact on HAIs rates during the COVID-19 pandemic.

On the other hand, further studies conducted in America and Europe reported a rise of CDI cases, especially in the early months of the pandemic. This may be explained by the limited resources available at the beginning of the COVID-19 pandemic, when small ward spaces, small staff and lack of personal protective equipment could have limited infection prevention and control practices [22].

Mortality rates related to CDI are still high, ranging from 11% to 18%, with substantial risk of complications even after 12 months from hospital admission [23, 24]. The estimated death per year due to CDI amounts to 29,000 and 1,800 deaths in the USA and UK, respectively [25, 26]. This adds up to the large economic impact related to hospital management of CDI. A recent study conducted in Germany revealed that healthcare resource usage and associated costs were consistently higher in CDI patients compared to non-CDI patients [23]. The incremental cost per patient was € 4,101 in CDI patients, increasing to € 13,291 in case of ≥ 3 recurrences [23]. An American study published in 2022 revealed how CDI-associated deaths were almost 10 times higher for patients with recurrent CDI than primary CDI (25.4% versus 2.7%), with higher hospitalisation rates and higher healthcare costs [27].

Other changes in epidemiology concern the recent emergence of clusters of CA-CDI. CA-CDI is defined when the onset of symptoms is in the community setting, or within 48 hours after admission to a health-care facility [28, 29]. A population-based study exploring risk factors for CA-CDI revealed that community-acquired patients were more likely to be younger, females and with a lower comorbidity score than hospital-acquired patients [29]. Moreover, patients with CA-CDI were more likely to be antibiotic *naïve*, less likely to be on gastric acid suppressing therapy, and less likely to have cancer [29].

■ TRANSMISSION

C. difficile requires the spore formation for its survival. Spore is a metabolically inert bacterial form, which provides resistance to oxygen, UV, desiccation and heat.

Sporulation is ruled by master regulator *Spo0A* and the subsequent sigma factor cascade, which allows an asymmetric separation of the cell, bringing to the formation of the spore [9]. Once ingested, spores survive the acid gastric environment and reach the duodenum, where the interaction with bile acids allows germination into toxin-producing bacteria and colonisation of the large intestine [25]. Hospital-outbreaks are the most common sources of transmission via the oral-faecal route, even though the relationship between antibiotic exposure, spore exposure and symptom onset often re-

main unknown. Health-care workers play a pivotal role in *C. difficile* transmission, stressing how hand hygiene programmes need to be pursued to limit CDI outbreaks [30]. In parallel, it is also crucial to enforce infection control measures in case of hospital outbreaks. Besides hand hygiene with water and soap, isolation procedures for infected patients in single rooms or with cohort isolation, should be applied. Correct hygiene of high-touch surfaces, and all shared equipment prior to use with another patient, including toilets, wheelchairs and gurneys, should be guaranteed to prevent spore's contamination and CDI intra-hospital diffusion [31].

Besides HA-CDI, several studies focussed on *C. difficile* diffusion through the food chain. Although there are no confirmed cases of any foodborne disease caused by *C. difficile*, its occurrence in livestock and foods suggests that contaminated food products with spores could be a vehicle to spread CDI [32-36]. Studies confirmed that detection of similar *C. difficile* strains and spores in food is present in meat retails, vegetables and seafood [32]. PCR ribotype analysis confirmed that RT 017, 027, 078, were concurrently identified in both CA- and HA-outbreaks but also in foods and livestock [33-36].

Interestingly, an analysis of *C. difficile*-ribotype distribution and a culture investigation among retail potatoes crops were performed in 2018, showing a *C. difficile* positivity rate above 10% in 9/12 countries. The most frequently identified ribotypes (014/020, 078/126, 010 and 023) were the same as those found in human, animal and environmental samples [37].

■ PATHOGENICITY

As aforementioned, sporulation and germination can be included amongst the pathogenic factors of *C. difficile* [38]. Besides from spores' germination, different molecules are involved in the pathogenic pathways of CDI. The *slpA* gene encodes for a particular group of adhesines, which are surface layer proteins (SLPs) of *C. difficile* that differs from SLPs of several other bacteria. Interestingly, it was proven that chemical removal of SPLs results in a missing adherence of *C. difficile* to human Henrietta Lacks cells (HeLa) [39].

The group of SLPs also includes cell wall proteins (CWPs), a cluster of highly immunogenic proteins

implicated in CDI pathogenesis. Amongst them, *Cwp22* seems to be involved in toxin production, sporulation, bacteria motility and cell viability. Studies revealed that *Cwp22* inactivation resulted in an increased cell permeability and autolysis, with an impaired cellular adherence in vitro and a decreased cytotoxicity [25, 40]. CD283 is another relevant *C. difficile* surface protein, whose over-expression increase biofilm formation on abiotic surface [25, 41].

Toxin production is the major virulence factor in CDI pathogenesis. Toxin A and toxin B are encoded by a group of genes implicated in toxin expression and secretion (*TcdR*, *TcdE*, *TcdL*, *TcdC*). Toxin A/B mechanism concerns glycosylation of host GTP-ases, whose inactivation leads to changes in the actin cytoskeletal structure, cytokines secretion, cell cycle arrest and cell death [42]. All these factors can lead to CDI pathogenesis under favourable conditions. Patient's risk factors can lead

to a gut microbiota alteration, defined as dysbiosis, which favours the proliferation of toxigenic strains of *C. difficile* and sporulation and subsequent toxin production. Toxins trigger an inflammatory response with fluid secretion and tissue damage, then causing diarrhoea [43] (Figure 1).

Moreover, *C. difficile* strains exhibit intrinsic resistance to different classes of antibiotics. RT001 ribotype is intrinsically resistant to fluoroquinolones, ceftriaxone, and erythromycin, while RT00 express resistance to fluoroquinolones and clindamycin. Notably, RT027 is known to be intrinsically resistant to fluoroquinolones [42].

Therefore, broad-spectrum antibiotics contribute to gut microbiota, promoting intestinal colonisation by *C. difficile*, reducing its clearance and finally increasing the risk of infection and recurrence [25]. Moreover, since conventional therapies with oral vancomycin and metronidazole contribute to alteration of the gut microbiota, the use of these

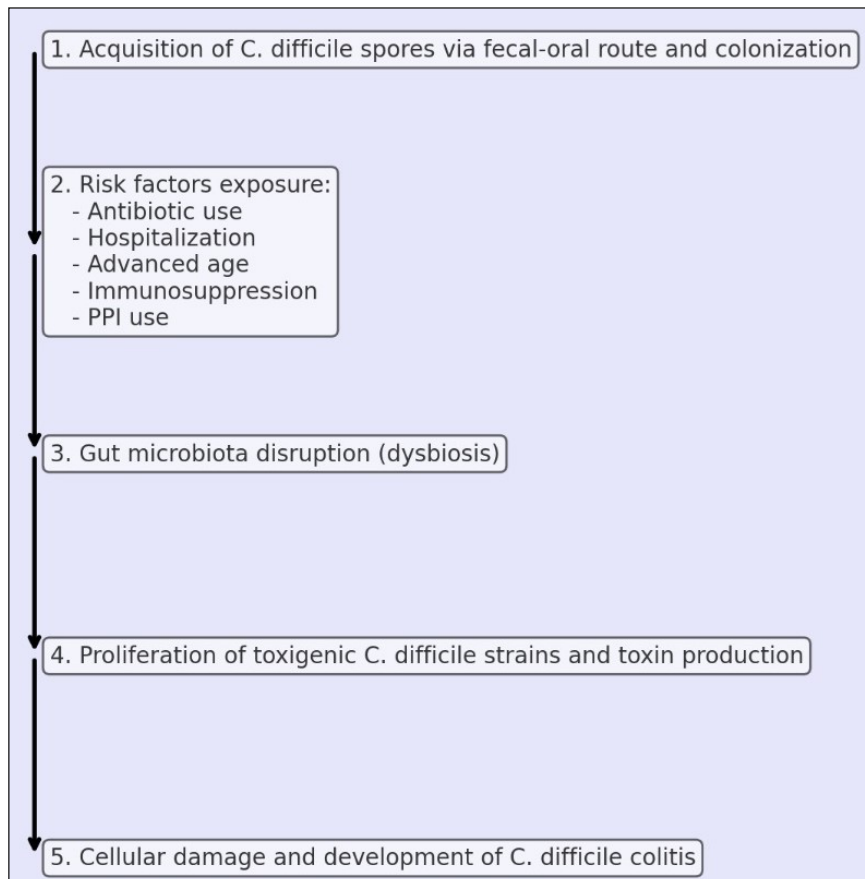


Figure 1
Pathogenesis
of *C. difficile* colitis.

drugs may increase the risk of reinfection. The introduction of fidaxomicin, a non-absorbable antibiotic that is less toxic to intestinal commensal bacteria, appears to play a role in reduction of CDI recurrence [44].

■ RISK FACTORS

CDI features multiple risk factors that contribute to its development. Antibiotic exposure is primarily linked to CDI, due to the disruption of gut microbiota, then creating a favourable environment for *C. difficile* proliferation and toxin production [45].

Cephalosporins are typically associated with CDI, especially third- and fourth-generation molecules [46]. Beta-lactams/beta-lactamase inhibitor combinations are also linked to an increased risk for CDI, due to a greater dysbiosis caused by beta-lactamase inhibitors [47]. Duration of antibiotic exposure has also a significant impact, as reported in a work by Brown et al. where 10- and 14-day courses of antibiotic therapy were significantly associated with increased risk of CDI when compared to 7-day courses (respectively 12% and 27%) [48]. Hospitalization represents another critical risk factor due to the increased likelihood of exposure to *C. difficile* in healthcare settings, paired with higher prevalence of susceptible populations in this environment [49]. Advanced age is strongly associated with CDI. Elderly patients often have weakened immune systems, co-morbidities, experience frequent hospitalizations with high antibiotics exposure, which all relate to an increased risk for CDI [50]. Furthermore, older age is typically linked to an increased risk of recurrent diseases [51], also due to changes in gut's inter-species diversity, with less protection against pathogenic bacteria [52]. Moreover, when considering the gender distribution, women typically have an increased risk of CDI [53]. Abdominal surgery is a relevant risk factor for CDI. Recent data report a considerable CDI incidence after abdominal surgery, of which laparoscopic colorectal surgery appears to be the procedure with the highest risk of CDI occurrence [54]. Historically, proton pump inhibitors (PPIs) have been linked to a higher risk of CDI. PPIs reduce gastric acidity, potentially leading to an alteration of the gut microbiome and so creating an environment more conducive to *C. difficile* colonization and toxin production. A meta-analysis by Trifan et al. found that PPIs use was associated with a 65%

increased risk of CDI [55]. More recently, Meta et al. highlighted the linkage between PPIs and recurrent CDI, showing that patients treated with PPIs were 64% more likely to develop recurrent CDI [56]. This association underscores the importance of cautious PPI use, particularly in patients with other risk factors, and suggests that healthcare providers should strongly evaluate the actual indication of PPI therapy in this category of patients for de-prescribing. Chronic kidney disease (CKD) has been also identified as a significant risk factor for CDI, as reported in a study by Thonprayoon et al. [57]. Patients with CKD often have a compromised immune system and frequently require antibiotic treatments, further disrupting their gut microbiota and enhancing the risk of CDI. Moreover, a systematic review focusing on CDI risk factors in American patients reported how CKD was a relevant risk factor for primary CDI and recurrent CDI [58]. This data emphasizes the need for vigilant infection control and appropriate antibiotic stewardship in this vulnerable population. Obese patients are predisposed to CDI as well due to less diversity of gut microbiota. Obesity is also associated with chronic inflammation and impaired immune responses, which can lead to increased susceptibility to infections. There are few data in literature showing how obesity is linked to a significantly increased risk of CDI compared to patients with normal body mass index [59]. On the contrary, a study by Malick et al. did not find association between obesity and death among patients with CDI [60]. Patients with chronic inflammatory bowel disease (IBD) present anatomic-functional alterations that promote the onset of CDI. In addition, biological drugs used for IBD treatment favours the occurrence of CDI [61]. Lastly, a relevant risk factor for CDI development are previous episodes of CDI, with recurrence rates progressively more frequent after subsequent infections, highlighting the difficulty to achieve a complete eradication of the infection [62]. Intervening on risk factors appears to be crucial in combating CDI. STOPP/START criteria (Screening Tool of Older Person's Prescriptions/Screening Tool to Alert doctors to Right Treatment) collect a number of indications to identify relevant prescribing problems related to potentially inappropriate drugs and potential prescription omissions [63]. When applied to CDI, START criteria promote the use of probiotics in combination with

antibiotics to prevent *C. difficile* diarrhoea, especially in non-immunocompromised or severely debilitated patients.

■ CLINICAL PICTURE

CDI occurs with a broad spectrum of clinical manifestations, ranging from mild diarrhoea to severe, life-threatening conditions. The severity of CDI is typically classified into mild, moderate, severe, and fulminant/severe-complicated colitis. Mild CDI is characterized by watery diarrhoea without relevant systemic symptom, while moderate CDI is often associated with moderate abdominal pain, fever and leucocytosis [64]. Patients with severe colitis exhibit more relevant symptoms such as profuse diarrhoea, severe abdominal pain, significant leucocytosis, hypoalbuminemia and increased serum creatinine levels [65]. The most severe form of colitis is fulminant CDI, with patients experiencing severe abdominal distension, ileus and profound systemic and life-threatening toxicity with signs of shock [66]. Fulminant CDI is often characterised by the onset of toxic megacolon and gut perforation. Prevalence of toxic megacolon ranges between 0.4% and 3% among cases of CDI, with higher rates occurring in patients older than 80 years. It is a serious complication which prompts rapid surgical evaluation, defined by the radiological finding of the diameter of the transverse colon >6 cm, with loss of haustration [67]. Recent guidelines by European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Infectious Diseases Society of America (IDSA) have proposed a classification of CDI into non-severe, severe and severely complicated (*i.e.*, fulminant) colitis [6, 7]. Although they differ slightly in some clinical and laboratory criteria, the cornerstones in the diagnosis of severe colitis are a white blood cell count >15000 cells/ml and an increased creatinine level (> 1.5 mg/dl for IDSA and >50% baseline according to ESCMID's guidelines). Notably, ESCMID also includes additional radiological supporting factors such as imaging evidence of distention of the large intestine, pericolic fat stranding or colonic wall thickening. Fulminant or severe-complicated colitis is characterized in both cases by the presence of signs of haemodynamic impairment (hypotension, septic shock), ileus or radiological evidence of megacolon. Again, ESCMID also includes signs of severity such as in-

creased serum lactates, intestinal perforation or any fulminant course of disease (*i.e.* rapid deterioration of the patient). Besides a clinical severity classification, a distinction is made between first episode and recurrent CDI. Recurrent CDI is defined as a return of diarrhoea and a positive stool test for *C. difficile* toxins or nucleic acid amplification test within two to eight weeks after completing treatment for the initial infection [45]. Approximately 15%-25% of patients with an initial CDI episode will develop a first recurrence, with a growing risk of recurrence up to 60% after the second recurrences as the number of CDI episodes increases [67]. Given the pivotal role of CDI recurrences in the clinical management of patients, early intervention with optimal therapy is crucial.

■ DIAGNOSIS

C. difficile diagnostics should be performed in patients with diarrhoea, defined as the occurrence of ≥ 3 unformed stool evacuations within 24 hours, while formed stool samples should not be tested for CDI [45]. There are various tests available, each with a different target for detecting the presence of *C. difficile*. Glutamate dehydrogenase (GDH) test is typically used as an initial screening test. GDH is an enzyme expressed by all strains of *C. difficile*, therefore it does not distinguish between the presence of toxigenic and non-toxigenic strains [68]. The sensitivity of the glutamate dehydrogenase test is 96%, with a high negative predictive value ranging from 98.4% to 100% [68]. Detection of *C. difficile* toxins A and B is a key diagnostic test for CDI diagnosis, given the toxins' central role in the pathogenesis of the disease. Although cytotoxicity neutralization assay and toxigenic culture represent the reference diagnostic methods, they are no longer used in clinical practice due to their lack of resource efficiency [69]. Enzyme immunoassays (EIAs) for toxins A and B are currently the main toxins detection test due to their rapid turnaround and simplicity. A stool sample is collected from the patient, then incubated with specific antibodies that bind to toxin A and B. If toxins are present, a change in colour made by a specific substrate indicates a positive result, which is later measured using a spectrophotometer providing quantitative or qualitative results. The entire process is typically completed in a few hours, yielding results rapidly [69]. However, the sensitivity of toxin EIAs can

range from 60% to 80%, mainly due to the lability of the toxin in the faecal sample, and potentially leading to false-negative results. Interestingly, a better sensitivity is described for the new ultrasensitive toxin assays, with overall higher accuracy than multistep algorithms, but they are not yet commercially available [70]. Moreover, repeating the toxin test after treatment is not recommended, as toxins can be detected on stools even 6 weeks after treatment and resolution of diarrhoea. Nucleic acid amplification tests (NAATs) consist of a variety of molecular tests which use polymerase chain reaction, loop-mediated isothermal amplification, helicase dependent amplification assay, and microarray technologies for detecting a variety of toxigenic *C. difficile* gene targets [68]. NAATs tests show high sensitivity (up to 95%) and high negative predictive value for diagnosis of *C. difficile* infection. On the other hand, these tests also detect asymptomatic carriers of toxigenic *C. difficile* strains, so the correct stool sample collection is crucial. To enhance CDI diagnostics, the recent European and American guidelines recommend a multi-step diagnostic algorithm. ESCMID guidelines propose a two-step algorithm consisting of a sensitive screening method (NAATs or GDH test) followed, in case of a positive result, by an EIA for toxin A/B [6]. An alternative is the concomitant screening of both GDH and toxin A/B EIA, considering samples with a GDH-positive test but negative toxin A/B test as samples with CDI or *C. difficile* carriage. In these cases, NAATs can be performed. IDSA guidelines propose the same approach, with the exception that NAATs alone can be considered diagnostic in setting of appropriate stool sample selection [7]. Further supporting this approach, a systematic review by Kraft et al. sustains the use of diagnostic algorithms that use NAAT alone or in combination with GDH or GDH plus toxin EIA [71].

The use of radiology and endoscopy plays a complementary role in the diagnosis of CDI, particularly in assessing complications and severe cases. Abdominal X-rays are often used first to assess the extent of colonic distention and to check for perforations. CT scans provide detailed images of the colon, revealing the extent of inflammation and complications such as abscesses, perforation or megacolon [72]. CT findings of CDI typically include colonic wall thickening, "accordion" or "target" signs, and pericolonic stranding. Endoscopy,

particularly flexible sigmoidoscopy or colonoscopy is not routinely performed, due to the high risk of perforation and bleeding, especially in cases of fulminant CDI [72]. On the other hand, it could be a helpful tool when the suspicion is high and stool tests are negative, or when other diseases must be ruled out, especially in patient with persistent symptoms after an adequate treatment. Endoscopy reveals the typical colic manifestation of CDI, which is pseudomembranous colitis (PMC). PMC is characterized by the presence of pseudomembranes, which are 2 cm mucosal yellow/white lesions irregularly distributed in the colon. However, pseudomembranes are present in about 40-60% of CDI cases and could be found also in other bacterial infections and non-infectious diseases [73, 74].

■ THERAPY

Treatment strategies of CDI have evolved significantly. In the past, metronidazole has been the main therapy choice for a first mild CDI episode. Oral vancomycin gradually replaced metronidazole, due to multiple evidence supporting its superiority in the treatment of both severe and mild/moderate CDI [75,76]. Vancomycin is poorly absorbed from the gastrointestinal tract when administered orally, ensuring high colon concentrations while minimizing systemic absorption and thus reducing the risk of systemic side effects [45]. Both ESCMID and IDSA guidelines had recommended vancomycin as the first line in the treatment of mild and severe CDI [45]. However, recent guidelines suggested fidaxomicin over vancomycin for both the first episode and up to the second recurrence of CDI, with vancomycin being acceptable for a first episode only if other agents are unavailable [6, 7]. Fidaxomicin was introduced into clinical practice following its FDA approval in 2011. It is a macrocyclic antibiotic with high stool concentration and a narrow spectrum of activity, primarily targeting *C. difficile*, therefore probably causing less gut microbiome dysbiosis when compared to vancomycin [77]. Two randomized controlled trials showed the non-inferiority of fidaxomicin over vancomycin for clinical cure of mild and severe CDI, with a significantly lower recurrence rate [78,79]. Moreover, a subgroup analysis population from both studies highlighted how recurrence within 28 days occurred in 35.5% of patients from the vancomycin group and 19.7% in

the fidaxomicin group [80]. Also, patients with CDI treated with fidaxomicin experienced less colonisation and overgrowth of VRE and *Candida* species [81].

For both vancomycin and fidaxomicin, the standard duration of therapy for mild/severe CDI is 10 days, at a dosage of 125 mg vancomycin every 6 hours and 200 mg fidaxomicin every 12 hours, respectively. Interestingly, the updated guidelines introduce the extended-pulsed fidaxomicin regimen consisting of 200 mg 12 hourly for 5 days followed by 200 mg every other day for 20 days. Evidence comes from the EXTEND trial, in which extended-pulsed fidaxomicin was superior to standard-dose vancomycin for sustained cure of CDI, reporting also the lowest recurrence rates observed in a randomized clinical trial of antibiotic treatment for CDI [82]. Considering different durations of therapy, an insight can be found in this recent observational study including patients with CDI treated with a short course of vancomycin (5-7 days) and fidaxomicin (5 days), compared to standard 10-days regimen. Interestingly, shortened treatment regimens were shown to be effective, with lower recurrence rate [83].

There is still no standardised therapeutic strategy in the treatment of fulminant severe-complicated CDI. While IDSA recommends higher-dose vancomycin (500 mg 6 hourly) in association with IV metronidazole, ESCMID consider the use of fidaxomicin and suggest the addition of IV tigecycline, avoiding IV metronidazole [6, 7]. Interestingly, evidence supporting the combination of high-dose vancomycin, and IV metronidazole are scarce.

Early data about the effectiveness of dual therapy came from an American study conducted in 2015 reporting a lower mortality in patients treated with vancomycin and IV metronidazole combination [84]. More recently, a retrospective study by Wang et al. showed no association between dual therapy and improved outcomes compared with vancomycin alone in patients with fulminant and non-fulminant CDI [85]. Interestingly, a recent Italian meta-analysis examining data from three retrospective studies revealed a mild, not statistically significant difference in mortality between the dual therapy group and the monotherapy group [86]. Considering the lack of solid evidence, RCT comparing the efficacy of the combination regimen versus vancomycin alone in patients with fulminant or severe-complicated CDI are current-

ly needed. Interestingly, despite the ESCMID indication, there is no current data on the efficacy of fidaxomicin in severe life-threatening disease.

Faecal microbiota transplantation (FMT) has emerged in the past years as a pivotal therapeutic option for the treatment of recurrent CDI [87]. Were it not for organizational issues, FMT could possibly become a first line treatment due its efficacy and tolerability. FMT procedure consists of a stool infusion from a healthy donor into the gastrointestinal tract of the patient with CDI, in order to restore gut eubiosis and suppress the overgrowth of *C. difficile*. Studies have shown the FMT effectiveness for treating recurrent and refractory *C. difficile* infection while some evidence is emerging on the potential benefits of FMT for treating initial episodes of CDI [88-90]. A study by Popa et al. showed how in 25 patients with primary CDI (19 with a severe and 6 with a non-severe form) the FMT success rate was 94.7% [91]. Stronger evidence is provided by a Danish randomized trial comparing the efficacy of FMT with placebo after a 10-days course of oral vancomycin at standard dosing in patients with first or second CDI episode. Interestingly, FMT was highly effective and superior to the standard of care including vancomycin alone in achieving sustained resolution from *C. difficile* [92].

Notably, FMT could play a relevant role in treating fulminant disease. In 2021 a systematic review on FMT efficacy in refractory severe or fulminant CDI was performed [93]. 209 patients from 10 studies where analysed, revealing that FMT could be effective in treating fulminant CDI but requires multiple treatments and additional anti-CDI antibiotics to achieve resolution. However, considering the limits of the procedure, stronger evidence with randomized trials is needed to better assess the therapeutic role of FMT in these patients.

Other molecules besides antibiotics also have their place in the therapeutic management of CDI. Bezlotoxumab is a new drug approved by the FDA in 2016 and included in the latest guidelines for the treatment of CDI recurrences alongside standard antibiotic treatment [6, 7]. Bezlotoxumab is a monoclonal antibody specifically targeting *C. difficile* toxin B. MODIFY I and MODIFY II trials demonstrated that bezlotoxumab significantly reduced the rate of CDI recurrence within 12 weeks of treatment compared to placebo [94].

According to IDSA recommendations, patients with a primary CDI and risk factors for CDI recur-

rences could be treated with bezlotoxumab in addition to standard-of-care therapy in settings where logistics are not an issue while bezlotoxumab is only considered in the management of patients with CDI starting from the second recurrence onwards according to the ESCMID indications. Interestingly, given the data from post-hoc analyses of the MODIFY studies, bezlotoxumab was effective in reducing the rate of relapse even in patients with primary CDI infection [6, 7, 95]. Therefore, considering the limitations of high costs and the need for intravenous therapy, further studies would be necessary to promote bezlotoxumab as a possible therapeutic choice in patients at high risk of relapse.

Going beyond medical therapy, patients with severe-complicated or fulminant forms of CDI may require surgical treatment. Undoubtedly, an urgent surgical assessment could be necessary for patients with signs of megacolon, colonic perforation, acute abdomen, septic shock or organ failure. When comparing IDSA and ESCMID guidelines, IDSA suggest surgical treatment for patient with fulminant CDI with high WBC count, while ESCMID consider surgical evaluation for clinically deteriorating patients not responding to CDI therapy [6, 7]. Timing for surgical evaluation is crucial. Data from a Canadian study reported a survival benefit in patients treated with emergency colectomy with lactate levels ≥ 5 mmol/L and WBC lower than $20 \times 10^9/L$, suggesting the importance of early surgical evaluation in at-risk patients prior to severe clinical deterioration [96].

CONCLUSIONS

C. difficile infection remains a major clinical entity of growing scientific importance. The incremental risk of recurrence characteristic of this pathogen is the cornerstone on which treatment choices must be strengthened to improve patient outcomes and reduce healthcare costs. Furthermore, intervening and addressing the risk factors for CDI in both hospital and out-of-hospital settings may be the key to reducing the spread of the disease and the exposure of patients at high risk of CDI recurrence. Overall, the management of CDI has evolved significantly, with advances in both pharmacological treatments and innovative therapies. Further studies will be needed to investigate the therapeutic landscape to best optimise the management of CDI.

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