Clostridioides difficile: diagnosis and treatments

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ABSTRACT

Clostridioides difficile (formerly *Clostridium*) is a major cause of healthcare associated diarrhea, and is increasingly present in the community. Historically, *C difficile* infection was considered easy to diagnose and treat. Over the past two decades, however, diagnostic techniques have changed in line with a greater understanding of the physiopathology of *C difficile* infection and the use of new therapeutic molecules. The evolution of diagnosis showed there was an important under- and misdiagnosis of *C difficile* infection, emphasizing the importance of algorithms recommended by European and North American infectious diseases societies to obtain a reliable diagnosis. Previously, metronidazole was considered the reference drug to treat *C difficile* infection, but more recently vancomycin and other newer drugs are shown to have higher cure rates. Recurrence of infection represents a key parameter in the evaluation of new drugs, and the challenge is to target the right population with the adapted therapeutic molecule. In multiple recurrences, fecal microbiota transplantation is recommended. New approaches, including antibodies, vaccines, and new molecules are already available or in the pipeline, but more data are needed to support the inclusion of these in practice guidelines. This review aims to provide a baseline for clinicians to understand and stratify their choice in the diagnosis and treatment of *C difficile* infection based on the most recent data available.

Introduction

Clostridioides difficile (formerly *Clostridium*) is responsible for virtually all cases of pseudomembranous colitis and is implicated in 10-25% of antibiotic associated diarrhea.[1 2] It has been recognized as a major cause of healthcare associated diarrhea in adult patients,[3 4] and is responsible for large outbreaks in hospital settings.[5 6] Physicians face two major challenges. The first is management of fulminant life threatening colitis (defined by hypotension or shock, ileus, or megacolon)—this complication is rare (≤5%) but associated with a high mortality (35-50%). The second challenge is preventing recurrence, which occurs in 15-25% of cases in the two months following the initial episode.[7] A patient presenting with a first recurrence has a higher risk of subsequent recurrence and may enter a cycle of multiple episodes, leading to exhaustion and long courses of antimicrobial therapy. Besides *C difficile* infection, asymptomatic colonization (defined by the presence of the microorganism in the absence of *C difficile* infection symptoms) ranges from 4% to 15% of healthy adults.[8] A recent study showed that asymptomatic colonization by a toxigenic strain on admission to hospital increases the risk of developing subsequent *C difficile* infection.[9] In addition, asymptomatic carriers of *C difficile* shed the microorganism in the environment, and a growing body of evidence shows that asymptomatic carriers play an important role in introducing and maintaining transmission in the ward.[10]

C difficile infection is mediated by two toxins, TcdA and TcdB, which disrupt tight junctions and destroy the actin cytoskeleton of enterocytes. The toxins induce an inflammatory response by recruiting neutrophils and mastocytes, which release cytokines, leading to the formation of pseudomembranes.[11] The toxins are encoded by two genes *tcdA* and *tcdB* which form, with three accessory genes, a 19.6 kB pathogenicity locus. Not all patients colonized with *C difficile* develop *C difficile* infection. This suggests that other factors (immune response and intestinal microbiota balance), in addition to *C difficile*, are important in disease pathogenesis.

The metabolism of bile acids has been shown to play a major role in the mechanism of *C difficile* infection.[12] In 1983, Wilson et al showed that primary bile acid cholic acid and its taurine conjugated derivative taurocholic acid could stimulate germination of *C difficile*.[13] Other bile acids, including chenodeoxycholate, inhibit taurocholate induced germination.[14] Chenodeoxycholate competitively inhibits germination at a concentration 10-fold lower than cholate, and the resulting effect at homeostasis

is a suppression of *C difficile* invasion in vivo.[15] Response to biliary acids also varies across strains and ribotypes.[16] Moreover, it has also been shown that antibiotic treatments, recognized as a risk factor for *C difficile* infection, induce a shift in fecal bile acid composition. An increase in primary bile acids favours germination and a decrease in secondary bile acids inhibits germination, thereby promoting *C difficile* infection.[17]

Sources and selection criteria

The references used in this review were identified through PubMed and Medline searches of articles published between 1958 and 2018. Search terms included "bacteriophage", "bezlotoxumab", "cadazolid", "*Clostridium difficile*", "*Clostridioides difficile*", "*Clostridium* infection", "diarrhea", "fecal microbiota transplantation", "fidaxomicin", "ileus", "intensive care unit", "metronidazole", "non-toxigenic strains", "pseudomembranous colitis", "RBX2660", "ridinilazole", "rifaximin", "surotomycin", "teicoplanin", "tigecycline", "tolevamer", "toxic megacolon", "toxoid vaccine", "vaccine", and "vancomycin." We prioritized recent (after 2000) high quality reviews and randomized controlled trials in which multiple references would be relevant. When randomized controlled trials were not available, we considered observational studies, case reports, and case series. For diagnostic and therapeutic algorithms, we chose to present only scientific societies' guidelines. In the therapeutic area, we favoured randomized controlled trials powered in size to show a statistical difference on the primary parameters such as global clinical cure and recurrence. When not available, we selected other designs and underlined the potential limitations to the conclusions observed.

C difficile epidemiology

The incidence of *C difficile* infection has increased markedly worldwide over the past two decades.[4 18-20] This change is assumed to be owing in part to the emergence and rapid dissemination of the clone PCR ribotype (RT) 027 but also to increased awareness among physicians of *C difficile* infection, and the use of more sensitive methods (ie, nucleic acid amplification test) for diagnosis. Other clones have also emerged at a regional or national level, such as RT 176[21] in eastern Europe, RT 244[22] in Australia and New Zealand, RT 018 in Italy, and RT 017 in Asian countries (South Korea, China, and Japan).[23] *C difficile* is frequently encountered in animals and in meat such as pork, veal, and horse.[24] Knetsch et al reported that asymptomatic farmers and their pigs can be colonized with clonal isolates of *C difficile* RT 078, indicating that spread between animals and humans might occur.[25] *C difficile* has also been found in vegetables and seafood, suggesting that *C difficile* infection might be a foodborne pathogen.[24] Given widespread colonization of livestock and contamination of outdoor environments, and the demonstration of clonal groups of *C difficile* shared between humans and food animals, management and control of *C difficile* infection should be holistic, taking into account these factors.

In the US, the estimated incidence of *C difficile* infection in 2011 was 453 000 on the basis of data from active population and laboratory based surveillance across diverse geographic locations.[4] The estimated annual mortality within 30 days of diagnosis was 29 500. In 2013, the Centers for Disease Control and Prevention categorized *C difficile* infection in the highest priority category of antimicrobial resistance threats.

In Europe, the estimated number of cases is 124 000 per year[19] and *C difficile* was the sixth most frequent microorganism responsible for healthcare associated infections during the 2016-17 European point prevalence study.[26]

In many countries, *C difficile* infection presents a substantial burden to healthcare facilities in terms of morbidity and mortality,[27 28] resulting in increased length of hospital stay and extra cost.

C difficile infection is no longer restricted to hospital settings, and is increasingly prevalent in the community. Currently, more than a quarter of all cases of *C difficile* infection are estimated to be community acquired,[29-31] although community acquired infection is still under-recognized because of a lack of screening by community physicians.[32 33] Epidemiological studies have shown that community associated *C difficile* infection affects groups not previously at risk (younger patients and those with no exposure to antibiotics in the 12 weeks before infection).[29] In a prospective study of 2541 patients visiting their general practitioners (GPs) for gastrointestinal disorders, the incidence of patients with a positive toxigenic culture and a positive cell cytotoxicity assay was 3.27% (95% confidence interval 2.61 to 4.03) and 1.81% (95% confidence interval 1.33 to 2.41), respectively. GPs requested *C difficile* testing in only 12.9% of stool samples, and therefore detected only 52.3% of patients who had tested positive with toxigenic culture. *C difficile* infection may occur out of hospital in patients without traditional risk factors.

C difficile diagnosis

A rapid and accurate diagnosis of *C difficile* infection is essential to guide treatment and to prevent nosocomial transmission. Prompt diagnosis will shorten the time to treatment initiation for patients with a positive diagnosis and the time to discontinuation of empirical treatment in patients with a negative result. It is also crucial to obtain reliable data with which to monitor incidence over time and to compare the incidence across different healthcare facilities. Recent innovations and progress in the field of *C difficile* diagnosis led the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) to update the guidelines for *C difficile* infection diagnosis in 2016.

Underdiagnosis

There is considerable underdiagnosis and mis-diagnosis of *C difficile* infection in Europe, as

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suggested by the prospective point prevalence EUCLID study.[34] In this study, 7297 stool samples from 482 healthcare facilities were collected in a single day and tested routinely at a central laboratory using a reference and standardized method (GDH+Toxins A/B). Results of local and national *C difficile* infection testing were compared. Overall, 148 of 641 samples (23%) positive for *C difficile* were not diagnosed by participating laboratories owing to a lack of clinical suspicion. There were also 68 (1.5%) false negative results, resulting in misdiagnosis *of C difficile* infection. Thus, a substantial burden of undetected cases remains, which is deleterious for patients, and hampers control measures.

Indications of *C difficile* testing—implementation of stool rejection criteria

Systematic *C difficile* testing is recommended where diarrhea occurs in a healthcare setting, or where tests for common enteropathogens are negative and other causes of diarrhea (eg, inflammatory colitis, enteral nutrition) have been ruled out. To improve laboratory test accuracy, *C difficile* testing is not advised in patients who have received laxatives in the past 48 hours or in those without clinical diarrhea (defined as three unformed stools in 24 hours). European guidelines do not currently recommend routine surveillance of *C difficile* colonization; however, one quasi-experimental controlled study shows that identification of colonized patients on admission and their isolation can effectively reduce the transmission of the disease and the incidence of *C difficile* infection.[35] The results of this study must be reproduced in other settings before being considered for widespread implementation. In addition, screening strategy (universal versus targeted screening on high risk patients) should be addressed in the future.

At the laboratory level, only diarrheic stools (defined as stools taking the shape of the container or stools corresponding to Bristol stool chart types 5 to 7) should be accepted to lessen the chance of obtaining positive culture results from patients merely colonized.

The stool sample should be sent to the laboratory in a leakproof container and processed within two hours of collection. Daily testing (including weekends) with restitution of the results within the same day is highly recommended.[36] If stool testing is delayed, stools should be stored at 4°C for maximum 72 hours, or frozen at −80°C. Freezing at −20°C is not recommended because it alters the toxins.[37 38] Appropriate storage conditions and management of stool samples are essential to avoid toxin degradation, which might result in false negative results by enzyme immunoassays or stool cell cytotoxicity neutralization assay. Rectal or perirectal swabs are inadequate for toxin detection but can be used for culture or nucleic acid amplification tests, more particularly in the case of epidemiological studies or ileus.

C difficile testing should not be routinely performed in infants ≤1 year because asymptomatic colonization with toxigenic strains of *C difficile* is frequent.[39 40] Testing of these infants should be limited to those with Hirschsprung disease or other severe motility disorders or in an outbreak situation.

Repeat testing used to be common practice when the first enzyme immunoassays for toxins with poor sensitivity came on the market in the 1980s. This practice should now be strongly discouraged because the diagnostic gain (defined by the frequency of tests converted from negative to positive) is very low. Of note, repeat testing using a test with suboptimal specificity may generate false positive results. When enzyme immunoassays for toxins are used, the diagnostic gain of a repeat sample within seven days is 1.9%.[41] For nucleic acid amplification testing, the percentage of repeat tests that turned out to be positive within seven days of a first negative sample is between 1% and 3.2%.[41-44] However, in an epidemic situation, the diagnostic gain (8.2%) is higher and might be of value.[45]

Stool samples should be also taken before initiating a specific treatment for *C difficile* to avoid false negative results. Sunkesula et al[46] showed that the cumulative number of patients converting from positive to negative polymerase chain reaction was 7/51 (14%), 18/51 (35%), and 23/51 (45%) after days 1, 2, and 3 treatment, respectively.

Sometimes physicians order *C difficile* testing after treatment for *C difficile* infection as a test of cure. This practice is not recommended as spores and/or toxins remain detectable in 7% (2/28) of patients at the end of treatment for *C difficile* infection and as many as 56% (15/27) of patients *with C difficile* infection have positive stool cultures 1-4 weeks after therapy,[47] despite resolution of diarrhea.

Nevertheless, despite these recommendations for stool selection, inappropriate testing is still frequent: Dubberke et al reported that 36% of patients tested for *C difficile* did not have diarrhea (defined as ≥3 diarrheal bowel movements (type 6 or 7 stool on the Bristol Stool Chart) in the 24 hours preceding stool collection), and 19% had received a laxative.[48] Ongoing education of physicians and nurses can reduce inappropriate testing.[49]

Reference methos

Despite recent advances, diagnosis of *C difficile* infection remains challenging as there is no single assay combining high sensitivity and specificity, rapid turnaround time, and low cost. Historically, reference methods have been the stool cell cytotoxicity neutralization assay and toxigenic culture. These methods detect different targets (free toxins in the cytotoxicity neutralization assay, and presence of a strain with potential to produce toxins in toxigenic culture) and, therefore, results of these tests are not directly comparable.

The stool cell cytotoxicity neutralization assay involves observing a cytopathic effect (rounding off of the cells) after inoculation of a stool filtrate on cell culture. The specificity of the effect is confirmed by its neutralization assay using a toxin B antitoxin.

Different cell lines can be used (MCR-5, Vero, HeLa, Hep-2).[50] This method can detect picograms of toxins. However, drawbacks include a lack of standardization and a slow turnaround time (>48 hours). In addition, cell cytotoxicity neutralization assay is cumbersome, laborious, and requires trained personnel. Laboratories have progressively abandoned this method for routine testing, although it is still used as a comparator for other diagnostic methods that detect free toxins.

The toxigenic culture is a two step method, which starts with the isolation of *C difficile* on a selective medium followed by demonstration that the isolate can produce toxins in vitro. Several selective media derive from the historical cycloserine cefoxitin fructose agar medium from George et al.[51] Subsequently, taurocholate or lysozyme was added to stimulate spore germination.[1 52-54] To make isolation of *C difficile* easier, a spore selection step, based on heat or alcohol shock, can be applied to the stool before media inoculation. Plates are usually incubated for 48 hours (or up to seven days, depending on the methods used) in an anaerobic atmosphere at 35-37°C. Colonies of *C difficile* are yellowish to white, circular to irregular, and flat, with a ground glass appearance. The colonies have a distinctive odor similar to para-cresol (or horse manure). In addition, *C difficile* colonies on cycloserine cefoxitin fructose agar fluoresce a chartreuse (yellow-green) color under ultraviolet light. Chromogenic agars have been developed to facilitate the identification of *C difficile* colonies.[55] However, some specific polymerase chain reaction ribotypes (ie, RT 023) fail to produce black colonies because they lack the ability to hydrolyse esculine.[56] In practice, definitive identification relies on biochemical characterization of isolates, or by matrix assisted laser desorption ionization-time-of-flight mass spectrometry. Culture is essential to determine antimicrobial susceptibility and subsequent typing. Routine antimicrobial susceptibility testing is not mandatory to guide treatment but can occasionally be performed where treatment has failed clinically, or for epidemiological purposes. Typing of *C difficile* is increasingly important to improve our understanding or *C difficile* infection epidemiology, to investigate outbreaks, and to detect early the emergence of new hypervirulent strains. Polymerase chain reaction ribotyping has become the reference method in Europe. However, whole genome sequencing has a higher discriminatory power, and the availability of next generation sequencing platforms allows laboratories to use whole genome data routinely in epidemiologic investigations.

Detection of the toxigenic status of the isolate can be achieved directly from colonies using nucleic acid amplification testing, cell cytotoxicity neutralization assay, or enzyme immunoassays for toxins.

Other methods

Enzyme immunoassays for toxins

The first micro well enzyme linked immunosorbent assays (ELISA) for toxin A became available in the late 1980s. These now detect toxins A and B using chromatographic/lateral flow membrane devices. Some evidence (in older studies) shows that newer enzyme immunoassays have improved sensitivity compared with those detecting toxin A only; however, the overall sensitivity remains relatively poor compared with cell cytotoxicity neutralization assay (from 29% to 86%) and preclude their use as standalone tests for diagnosis of *C difficile* infection.[57] According to a systematic review of the literature by Crobach et al, lateral flow membrane devices for toxins seem to a have a lower sensitivity compared with well type enzyme immunoassays for toxins (0.79, 95% confidence interval 0.66 to 0.88 versus 0.85, 95% confidence interval 0.77 to 0.91, respectively).[57]

Glutamate dehydrogenase assay

Glutamate dehydrogenase is a metabolic enzyme expressed at high levels by all strains of *C difficile*, both toxigenic and non-toxigenic. A positive result merely indicates the presence of *C difficile*, although some other *Clostridium* species may occasionally cross react. Glutamate dehydrogenase assays are easy to perform and cheap; they exist as a solid phase microtiter plate format or as a lateral flow immunochromatographic membrane in a single test or a combined test with a toxin A and B.[1 52 53] The overall sensitivity of glutamate dehydrogenase assay is 96% (86-99% compared with toxigenic culture). Because of the high negative predictive value of glutamate dehydrogenase assays (ranging from 98.4% to 100%), they are now often used as an initial step of a two step algorithm. Any negative result rules out the presence of *C difficile*. However, interpretation should be cautious and depends on the prevalence of *C difficile* infection: for a prevalence of 10% and a glutamate dehydrogenase assay with a negative predictive value of 99%, one positive stool sample out of 10 will be missed if glutamate dehydrogenase is used as a screening method. Any positive glutamate dehydrogenase test result must be confirmed by a more specific method detecting toxins.

Nucleic acid amplification tests

Nucleic acid amplification assays became commercially available in 2009, and a variety of tests are now available. These tests use polymerase chain reaction, loop-mediated isothermal amplification, helicase-dependent amplification assay, and microarray technologies.[53 58] Some platforms are designed for on-demand testing, whereas others are more amenable to high-throughput testing. These assays detect a variety of gene targets, including conserved regions of *tcdA*, *tcdB*, *cdt* and the Δ117 deletion in *tcdC*, the latter two as surrogate markers for RT 027. Like glutamate dehydrogenase assays, nucleic acid amplification assay tests have been shown to be very sensitive in detecting toxigenic strains (pooled sensitivity of 95%) and to display a high negative predictive value for diagnosis of *C difficile* infection.[57] Concerns regarding their specificity and positive predictive values emerged rapidly because,

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in addition to cases of *C difficile* infection, these tests also detect asymptomatic carriers of toxigenic *C difficile*. Another theoretical concern is the potential variation in *tcdA and tcdB* regions targeted by nucleic acid amplification assay test primers, which could result in a false negative result.

Several multiplex gastrointestinal panels used for syndromic diagnostics (xTAG Gastrointestinal Pathogen Panel, Luminex; FilmArray Gastrointestinal Panel, Biomérieux; Seeplex Diarrhea ACE Detection, Seegene) also target the *C difficile* toxin B gene. However, the targets of these assays are seldom evaluated and compared with the gold standards for diagnosis of each pathogen. During a multicenter evaluation of the FilmArray Gastrointestinal Panel, Buss et al[59] found a sensitivity and specificity for *C difficile* detection of 98.8% (95% confidence interval, 95.7 to 99.9) and 97.1% (95% confidence interval, 96.0 to 97.9), respectively, compared with toxigenic culture.

Value of free toxin versus presence of toxigenic culture

Over the past decade, the merits of the different tests and targets (free toxin versus presence of a toxigenic strain) for diagnosis of *C difficile* infection have been intensively discussed. The potential for asymptomatic carriage of a toxigenic strain (and toxins, to a lesser extent, as suggested by Pollock et al[60]) added confusion to the debate. A growing body of evidence shows that detection of free toxin in stools best correlates with clinical symptoms and clinical outcome.

A prospective one year study showed that patients with *C difficile* infection detected by nucleic acid amplification assay test alone were less likely to develop a complication of the infection (ie, 30 day mortality, colectomy, admission to intensive care, or readmission for recurrence) compared with *C difficile* infection detected by both nucleic acid amplification assay test and enzyme immunoassay/cell culture cytotoxicity assay (3% versus 39%, respectively; P<0.001). This suggested that the decrease in complication rate could be due to earlier detection and treatment of *C difficile* infection or to the detection of *C difficile* carriers who develop diarrhea for another, unrelated reason.[61] In a very large prospective multicenter study from the UK that included 12 420 stool samples, the authors found that the presence of free toxins was statistically significantly associated with poor clinical outcomes (higher all-cause 30 day mortality rate and higher white blood cell count), whereas the presence of toxigenic C *difficile* in feces in the absence of a positive toxin assay was associated with a clinical outcome that was no worse than for samples that tested negative for C *difficile*.[62] The authors concluded that the use of nucleic acid amplification assay testing leads to overdiagnosis of *C difficile* infection. They suggested that nucleic acid amplification assays could be used as first stage tests to exclude the presence of *C difficile*, followed by a more specific toxin test to identify patients most likely to have *C difficile* infection. These results were subsequently confirmed by an independent study from a single academic medical center in the US, which showed that, in patients positive for toxins, the number of stools per day, the rate of complications, the 30 day mortality, and the level of digestive inflammation assessed by fecal lactoferrin were substantially higher compared with patients testing positive by nucleic acid amplification assay but negative for toxins.[63] The authors concluded that the use of molecular tests alone is likely to lead to overdiagnosis and overtreatment.

Nevertheless, despite the higher specificity of enzyme immunoassays for toxins, detection of free toxins may lack sensitivity, and enzyme immunoassays may be negative in patients with complicated *C difficile* infection[64] or in those with endoscopically proven pseudomembranous colitis.[65] Although *C difficile* infection is the cause of most cases of pseudomembranous colitis, clinicians should consider less common causes (ie, infectious colitis with *E coli* 0157:H7, CMV, *Entamoeba histolytica*, or treatments such as cisplatin or cyclosporin), especially if pseudomembranes are seen on endoscopy but testing remains negative for *C difficile*.[66] In a small retrospective study of 143 true *C difficile* infection patients from a single center, Humphries et al did not find any difference in toxin enzyme immunoassay positivity between patients with mild versus severe disease (49% *v* 58%; P=0.31) and concluded that the presence of stool toxin measured by enzyme immunoassay does not correlate with disease severity.[67]

Recommended algorithm

Different algorithms have been proposed for diagnosis of *C difficile* infection. ESCMID recommends a two step algorithm based on a sensitive screening method (nucleic acid amplification assay or glutamate dehydrogenase assay) followed, in the case of a positive result, by a more specific technique to detect free toxins in stools (enzyme immunoassay test for toxins or cytotoxicity neutralization assay)[68] (fig 1). An optional step is to perform nucleic acid amplification assay for confirmation of glutamate dehydrogenase assay-positive, toxin enzyme immunoassay-negative samples. Another recommended option is to perform a combined test detecting glutamate dehydrogenase and toxins with an optional reflex nucleic acid amplification assay test in the case of glutamate dehydrogenase array-positive, toxin-negative results. Indeed, such a result can correspond to either the presence of a non-toxigenic strain or the presence of toxigenic strains with toxins below the detection threshold of enzyme immunoassay.

The Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) have published similar guidelines, with the exception that nucleic acid amplification assay tests alone can be considered if appropriate stool selection is guaranteed (fig 1).[69] This recommendation is supported by several studies showing that nucleic acid amplification assay outperforms other diagnostic test methods when applied to patients

who meet clinical criteria for *C difficile* disease (at least three loose or unformed stools in ≤24 hours with history of antibiotic exposure).[70 71]

These algorithms were proposed to combine sensitivity and specificity. In a setting with a *C difficile* infection prevalence of 5% among stool samples, Crobach et al[57] calculated the positive and negative predictive values of diagnosis algorithms based on nucleic acid amplification assay followed by enzyme immunoassay tests for toxins. The values were 98.5% (positive predictive value) and 98.9% (negative predictive value), whereas a standalone nucleic acid amplification assay would result in positive and negative predictive values of 45.7% and 99.8%, respectively.

These multistep algorithms also present some drawbacks, however. They are more time consuming, especially when using a unitary test for screening and cell cytotoxicity neutralization assay as confirmation. Patients with a negative screening assay can be rapidly ruled out for *C difficile* infection, but those with a positive result must be confirmed by a second test, which can dramatically increase time to delivery of the final result to the physician, more particularly when cell cytotoxicity neutralization assay is used. These delays may negatively affect patient outcome.[36] The use of a single test combining glutamate dehydrogenase assay and toxins detection enables laboratories to complete the diagnosis on the same day.

Other studies have shown a correlation between cycle threshold (Ct) of polymerase chain reaction, presence of free toxins in stools, and patient outcome.[72-77] Senchyna et al[75] estimated that an Xpert Ct cut-off of 26.4 had a negative predictive value of 97.1% for excluding the presence of toxin in stool. Crobach et al showed that the accuracy of Ct values of their home-made polymerase chain reaction to predict toxin A/B enzyme immunoassay results varies between 78.9% and 80.5%.[76] Dionne et al[72] have shown that Ct of polymerase chain reaction is lower in patients with a positive enzyme immunoassay for toxins (mean Ct=28.4) compared with patients negative for toxins (mean Ct=39.1). In another study where patients with *C difficile* infection were stratified according to the severity defined by the IDSA/SHEA criteria, Jazmati et al[73] found a lower Ct in patients with severe *C difficile* infection compared with patients with mild to moderate disease. Reigadas et al[74] showed that low toxin B Ct values from samples collected at the initial moment of diagnosis appear to be a strong marker for poor outcome.

The second limitation of these diagnostic algorithms is that a sensitive and rapid assay for detection of toxin does not exist, and therefore among patients suspected to have *C difficile* infection and harboring a toxigenic strain (nucleic acid amplification-positive), up to one third do not have any detectable toxin. In this case, it seems difficult to discriminate between true *C difficile* infection or carriage of a toxigenic strain.

New methods—biomarkers

Ultrasensitive assays that use single molecule array technology[60 78 79] can detect and quantify *C difficile* toxins A and B over a five log range of concentrations, starting from an analytical cut-off of approximately 1 pg/mL. Preliminary results indicate that single molecule array assays can detect toxins in 24% more samples with laboratory defined *C difficile* infection than the high performing toxin enzyme immunoassay, and therefore have the potential to improve diagnosis of *C difficile* infection.[80] During a clinical evaluation that included frozen stool samples from 311 patients with suspected *C difficile* infection, Sandlund et al[79] showed that the sensitivity and specificity of the Singulex Clarity *C difficile* toxins A/B assay were 97.7% (95% confidence interval, 93.0% to 99.4%) and 100% (95% confidence interval, 95.4% to 100%), respectively, compared with a multistep polymerase chain reaction and toxin testing procedure (nucleic acid amplification test+enzyme immunoassay for toxins+cell cytotoxicity neutralization assay). In another study, Pollock et al[60] compared the toxin levels of diarrheal nucleic acid amplification test-positive patients with those of non-diarrheal patients who tested positive in nucleic acid amplification. Surprisingly, they showed that toxin concentration did not differentiate *C difficile* infection from asymptomatic carriage. Nevertheless, when *C difficile* infection /carrier cohorts were restricted to those with detectable toxin, respective medians were notably different (toxin A, 874.0 *v* 129.7 pg/mL, P=0.021; toxin B, 1317.0 *v* 81.7 pg/mL, P=0.003, toxins A+B, 4180.7 *v* 349.6 pg/mL, P=0.004; Ct, 25.8 *v* 27.7, P=0.015). No cut-off adequately distinguished between patients with *C difficile* infection and those who were carriers of *C difficile*. In conclusion, single molecule array technology for detection of *C difficile* toxins is ultrasensitive and has the potential to be a standalone test to replace the multistep testing algorithms currently recommended for *C difficile* diagnosis.

Lactoferrin (an iron binding glycoprotein) and calprotectin (a calcium binding protein) are two proteins derived from polymorphonuclear neutrophils, which are released by the gastrointestinal tract in response to infection and mucosal inflammation.[81] These markers are used routinely to monitor levels of inflammation in patients with inflammatory bowel disease. As *C difficile* infection is histologically characterized by an infiltration of neutrophils, fecal lactoferrin or calprotectin may represent potential biomarkers for disease activity.

Many studies have shown that patients with *C difficile* infection have higher lactoferrin and calprotectin levels[82-86] compared with diarrheal patients testing negative for *C difficile* and with non-diarrheal controls. These two markers were found to be highly correlated with each other, which is not surprising considering their common cellular origin. However, these studies also reported a great variability of fecal lactoferrin and calprotectin levels, with an overlap between patients with *C difficile* infection and

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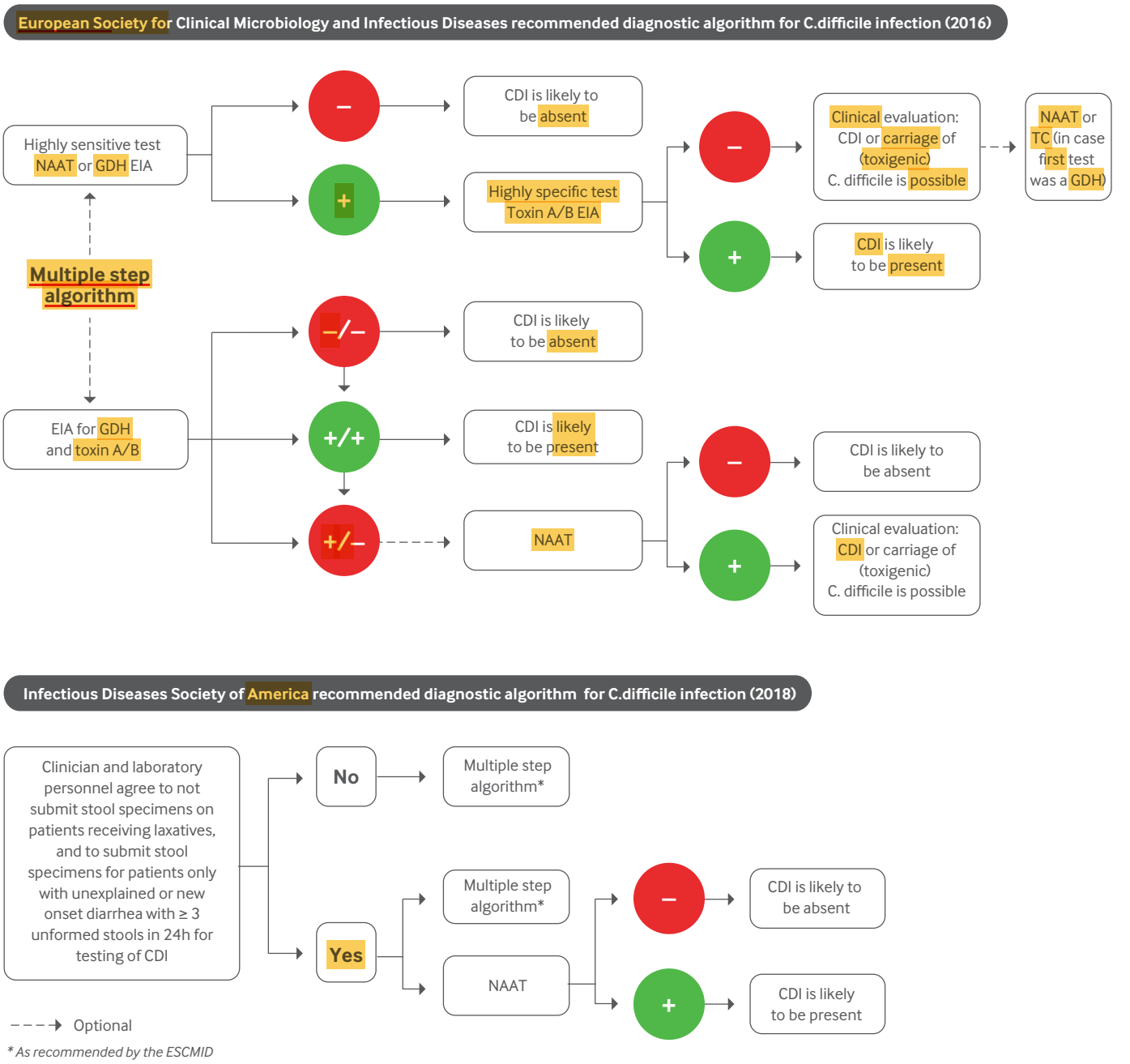

Fig 1 | Algorithms for the diagnosis of *C difficile* infection recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the Infectious Diseases Society of America (IDSA). CDI, *C difficile* infection; NAAT, nucleic acid amplification test; GDH EIA, glutamate dehydrogenase enzyme immunoassay; TC, toxigenic culture.

controls.[87 88] This observation reduces the predictive accuracy of both markers for *C difficile* infection and makes it difficult to determine an optimal cut-off value.[82] In summary, the sensitivity and specificity of lactoferrin and calprotectin are too low to recommend their routine use for screening of patients.

Treatment

Antibiotics

Metronidazole and vancomycin

When *C difficile* infection was described in 1893, no specific treatment was available.[89] In 1979, an experimental work performed on hamsters showed the efficacy of metronidazole and vancomycin.[90] Thirteen patients with antibiotic induced colitis were given vancomycin (500 mg four times daily) and none experienced a recurrence during a follow-up ranging from one to six months.[91] The choice of vancomycin over metronidazole in this initial work was based on its minimal absorption after oral administration, it reaching high colonic concentrations (measured at a mean level of 3.100 mg/g of stool), and the optimal results in the hamster given vancomycin over metronidazole. Eleven of 13 patients presented

with a severe infection according to definitions from IDSA/SHEA, with a leucocytosis between 17 000 and 45 000 cells/mm^3. An editorial suggested that, although vancomycin was highly efficient, it may be advisable to lower the dose to limit damage to the gut microbiota.[92]

Measurement of fecal metronidazole and hydroxymetronidazole was performed in nine patients with *C difficile* infection.[93] The authors showed that concentration of these two molecules decreased substantially with recovery. The concentrations obtained were, proportional to the minimum inhibitory concentration, lower than vancomycin (usually below 20 µg/g of stool for an minimum inhibitory concentration ranging between 0.25 and 1 mg/L).

The first prospective randomized trial comparing metronidazole with vancomycin analyzed 52 patients in the vancomycin group and 42 in the metronidazole group.[94] The main result of this study was that these drugs had equivalent efficacy and recurrence rate. However, this trial was not blinded, recurrence was evaluated over a three week period, and most importantly, according to the number of patients analyzed, this study was not powered to show a difference in efficacy. The second trial, published 10 years later, presents exactly the same limitations comparing vancomycin, teicoplanin, metronidazole, and fucidic acid.[95] This trial was also unblinded, included a limited number of patients (31 with vancomycin and 31 with metronidazole), and the follow-up period was limited to 30 days after discontinuation of therapy. From these two studies, metronidazole was nevertheless considered to be the drug of choice in *C difficile* infection because it was less expensive and was not associated with the potential increase in vancomycin resistant organisms.[96] Two later studies changed these conclusions. A randomized, prospective, double blind, placebo controlled trial with 172 patients compared vancomycin with metronidazole.[97] The patients were stratified according to disease severity and were followed for 21 days. The overall cure rate was 84% in the metronidazole group and 97% in the vancomycin group (P=0.006). No difference was observed for patients with mild disease, but in severe forms, treatment success was seen in 76% and 97% of the cases for metronidazole and vancomycin, respectively (P=0.02). However, the definition of severity was based on a score not previously validated and the follow-up for a recurrence was limited to 21 days. Nevertheless, this study showed the superiority of vancomycin over metronidazole for patients with severe *C difficile* infection. A further study comparing metronidazole and vancomycin was designed to evaluate the efficacy of tolevamer, a non-antibiotic, toxin binding polymer.[98] The study authors pooled and analyzed the results of two multinational randomized controlled trials, where 563 patients were treated with tolevamer, 289 with metronidazole, and 266 with vancomycin. Tolevamer was inferior to metronidazole and vancomycin, but more importantly, clinical success of metronidazole was also inferior to vancomycin (72.7% *v* 81.1%, P=0.02). A retrospective propensity matched cohort study evaluated the risk of recurrence and all cause 30 day mortality among patients receiving metronidazole or vancomycin.[99] One drawback of this study, besides the retrospective design, is the definition of recurrence which is restricted to another positive laboratory test result for *C difficile* more than 14 days, but fewer than 56 days after the initial diagnosis date. No clinical parameter was included, making the results on this parameter not relevant. Nevertheless, the risk of 30 day mortality was statistically significantly reduced among patients receiving vancomycin. Globally, these data confirm that metronidazole has a lower efficacy compared with vancomycin and support the use of vancomycin over metronidazole in *C difficile* infection. To optimize vancomycin treatment evaluated in recurrent *C difficile* infection, it might be possible to use a pulsed or tapered regimen of vancomycin. Evidence for this approach includes observational studies showing recurrence rates ranging from 31% to 6%[100-102] and one randomized trial that included 12 patients, where the recurrence rate reached 41.7%.[103]

Fidaxomicin

Fidaxomicin was discovered in the late 1970s and was developed by pharmaceutical companies under different names between the late 1980s and mid-2000s.[104 105] Fidaxomicin is a narrow spectrum antibiotic with activity against Gram positive aerobes and anaerobes. The drug is not active against Gram negative pathogens, thus preserves the normal gastrointestinal microbiota. Fidaxomicin inhibits bacterial protein synthesis via transcription inhibition.[106] Two phase 3 trials with the same design—one in patients in the US and Canada (629 patients),[107] and one in Europe (535 patients)[108]—provided consistent and reproducible results comparing fidaxomicin with vancomycin in *C difficile* infection. Rates of clinical cure with fidaxomicin were non-inferior to those after treatment with vancomycin, but in both studies, fidaxomicin was associated with a significantly lower rate of recurrence (15.4% *v* 25.3%,[108] 12.7% *v* 26.9%[107]). As expected from the earlier studies, the efficacy of fidaxomicin on recurrence (compared with vancomycin) is linked to preservation of gut microbiota. In fact, analysis of a subset of 89 patients from the phase 3 trial[108] showed that major components of the microbiome persisted after fidaxomicin administration, whereas vancomycin was associated with a further 2-4 $\log_{10}$ reduction in colony forming units of Bacteroidetes/Prevotella group organisms.[109] Among the other potential mechanisms, a reduction in toxin A and B production could also be involved.[110] Finally, fidaxomicin was also shown to improve control of environmental contamination with *C difficile*. After analyzing surfaces in the rooms of 134 patients treated with fidaxomicin, metronidazole, or vancomycin,[111] the authors showed that fidaxomicin was associated

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with reduced environmental contamination with *C difficile* (57.6% *v* 36.8%, P=0.02).

One method proposed to improve the efficacy of fidaxomicin was suggested by using an in vitro human gut model.[112] A randomized, controlled, open label, superiority study enrolled 364 patients to test the hypothesis that a pulsed regimen could improve the rate of recurrences.[113] Patients received fidaxomicin 200 mg twice daily on days 1-5, then once daily on alternate days on days 7-25 (extended pulsed fidaxomicin), or commercially available oral vancomycin 125 mg capsules four times daily on days 1-10. The primary endpoint was sustained clinical cure 30 days after the end of treatment. The results showed that 70% of patients with extended pulsed fidaxomicin achieved sustained clinical cure, compared with 59% of patients receiving vancomycin. This study provided the lowest recurrence rate observed in a randomized clinical trial for *C difficile* infection. The greatest criticism of the study relates to the choice of comparators: extended fidaxomicin versus vancomycin. Comparing extended pulsed fidaxomicin with a routine 10 day regimen of fidaxomicin may have given the results more relevance. From these studies, fidaxomicin seems to represent a drug of choice in *C difficile* infection, although one report describes the first strain of *C difficile* to have markedly reduced susceptibility to the treatment.[114]

Teicoplanin

Teicoplanin is a glycopeptide antibiotic that is active against *C difficile*. Few studies on its efficacy have been performed. A prospective randomized study comparing several drugs showed that clinical cure was obtained in 96% of cases, which is similar to vancomycin.[95] The number of recurrences was, however, lower (7% *v* 16%) but the difference was not statistically significant; however, the study was not powered to answer this question. A prospective observational study compared vancomycin with teicoplanin in patients with severe infection.[115] Treatment with teicoplanin resulted in higher clinical cure rate (90.7% versus 79.4%) and fewer recurrences (9.3% *v* 34.3%), both percentages reaching statistical significance.

Tigecycline

Tigecycline is a glycylcycline class antibiotic which has been proposed for treating severe cases of *C difficile* infection.[116] A retrospective observational cohort study compared patients receiving tigecycline monotherapy with patients given standard treatment.[117] Patients treated with tigecycline had a statistically significantly better clinical cure (34/45, 75.6% *v* 24/45, 53.3%; P=0.02), less complicated disease course, and less *C difficile* infection sepsis compared with patients receiving standard therapy. These data support the use of tigecycline in complicated and severe *C difficile* infection. Two retrospective studies confirm the safety of the drug in severely infected patients[118] but failed to show an improvement compared with standard therapy in clinical cure or recurrence rate.[119] If tigecycline is used to treat severe infections, it should be considered a substitute to metronidazole as adjunctive therapy to vancomycin.

Combination of molecules

Use of combined molecules is recommended in complicated forms of *C difficile* infection,[69 120] although evidence for this is limited. A single center retrospective observational comparative study evaluated 88 patients in the intensive care unit with *C difficile* infection.[121] The results showed a statistical improvement of survival: mortality was 36.4% and 15.9% in the monotherapy and combination groups, respectively. Oral vancomycin may play a key role in the association: in a mouse model, the addition of metronidazole to vancomycin improved clinical outcome.[122] In a retrospective analysis on the efficacy of intracolonic vancomycin, patients treated with intracolonic vancomycin and standard treatment compared with standard treatment alone had comparable mortality rates, but severity score, transfer to the intensive care unit, and percentage of toxic megacolon were higher in the group receiving intracolonic vancomycin.[123] A randomized study in this subset of patients is needed.

Fecal microbiota transplantation

Efficacy

Fecal microbiota transplantation is proposed to treat recurrent *C difficile* infection. It was initially described in 1958 in the treatment of pseudomembranous enterocolitis.[124] The first randomized study was published in 2013 and studied the effect of duodenal infusion, through a nasoduodenal tube, of donor feces in patients with recurrent *C difficile* infection.[125] The study was stopped after an interim analysis. Of the 16 patients in the infusion group, 13/16 had resolution of diarrhea and three patients received a second infusion, after which symptoms resolved in two, to reach an overall cure rate of 93.8% The control groups with vancomycin alone or vancomycin with bowel lavage were cured in 31% and 23% of cases, respectively. This study concluded that infusion of donor feces was substantially more effective for the treatment of recurrent *C difficile* infection than using vancomycin. A potential limitation of this study concerning the efficacy of vancomycin treated patients would be that only one trial of vancomycin was allowed, whereas it might have been reasonable to propose in these recurrent forms either pulsed or tapered vancomycin regimens. A comparable study was performed via colonoscopy, also stopped after a one-year interim analysis, with 20 patients.[126] Eighteen of the 20 patients treated with fecal microbiota transplantation exhibited resolution of *C difficile* infection compared with 5/19 of the patients treated with vancomycin. This was confirmed in several other randomized trials.

Since these initial trials, results have been published from seven randomized clinical trials, including open label randomized trials with no controlled group or placebo, using fecal suspension.[103 127-132] Overall

efficacy rates range between 80% and 94% after one or multiple fecal microbiota transplants in all clinical trials but one.[103] In this single center, open label, randomized controlled trial, the authors compared the effectiveness of 14 days of oral vancomycin followed by a single fecal microbiota transplantation by enema with a 6 week taper of oral vancomycin in patients experiencing acute episodes of recurrent *C difficile* infection. Seven out of 16 patients were cured with fecal microbiota transplantation compared with 7/12 with tapered vancomycin. In this study, which was stopped at the interim analysis, fecal microbiota transplantation was performed by single enema, whereas studies by Cammarota and Lee[126 130] showed that the efficacy increased with multiple infusions. Moreover, 6/16 patients did not retain at least 80% of the enema. In other words, these patients did not receive an optimal treatment with fecal microbiota transplantation.[133]

Two recent meta analyses on randomized controlled trials found consistent conclusions that enema was less efficient than oral or colonoscopy administration of fecal microbiota transplantation, and had efficacy equivalent to capsules or colonoscopy. No difference was seen between fresh and frozen stool.[134 135] In a systematic review published by Tariq et al, the authors concluded that fecal microbiota transplantation was associated with a lower cure rate in randomized controlled trials compared with open label and observational studies,[135] and attributed this to the heterogeneity of the recurrence definition, but also to the inclusion of microbiota based drugs (SER-109), or administration by enema. A systematic review including 18 observational studies with 611 patients showed a primary cure rate of 91.2% (95% confidence interval 87.6% to 94.8%).[136]

Data show that frozen stools are as efficient as fresh stools,[130 137 138] and lyophilized products have a lower efficacy.[131] An unblinded randomized trial comparing fecal microbiota transplantation by capsule and by colonoscopy[129] found that prevention of recurrent *C difficile* infection after a single treatment was achieved in 96.2% in both capsule and colonoscopy groups. Fecal microbiota transplantation via oral capsules was not inferior to delivery by colonoscopy over a 12 week period.

Fecal microbiota transplantation is therefore a highly effective treatment in recurrent *C difficile* infection, although the methods to deliver it are varied.

New indications in C difficile infection

Severe and complicated C difficile infection—No consensus exists on the definition of severity, which varies among scientific societies[69 120] and clinical trials.[97 107 108] Ianiro et al compared single and multiple infusions in severe refractory *C difficile infection*.[139] This randomized clinical trial included 56 patients and showed that multiple fecal infusions were more effective than a single transplantation (respectively 100% *v* 75% cure rate). Another study reported four patients treated with fecal microbiota transplantation for severe *C difficile* infection refractory to antibiotic treatment.[140] All patients had a clinical response to the procedure. Fecal microbiota transplantation is not yet recommended in severe *C difficile* infection and randomized clinical trials are needed to evaluate its use in this specific indication. Fecal microbiota transplantation has been proposed in complicated forms of *C difficile* infection as an alternative to surgery.[141-143] Evidence for use of fecal microbiota transplantation remains limited, and this option should be considered as part of a multidisciplinary approach.

First episode of C difficile infection—Using fecal microbiota transplantation in a first episode of *C difficile* infection has also been evaluated. In a randomized clinical trial that compared oral vancomycin with first line fecal microbiota transplantation, symptoms resolved in 8/9 patients treated with vancomycin versus 4/7 in the fecal microbiota transplantation arm.[144] A proof of concept trial randomly assigned 21 patients to oral metronidazole or fecal microbiota transplantation by enema.[145] Evaluation was performed at days 4, 35, and 70. Seven patients in the transplantation group responded to treatment (78%; 95% confidence interval 40 to 97), as compared with five in the metronidazole group (45%; 95% confidence interval 17 to 77) (P=0.20), suggesting that fecal microbiota transplantation could be an option in a first episode. Transplantation in this setting is challenging, however, because the long term effects have yet to be explored.

Immunocompromised patients—A multicenter retrospective study included 75 adults and 5 children[146] with immunosuppression related to solid organ transplant, oncologic conditions, HIV/AIDS, and immunosuppressive therapy. The overall cure rate reached 89%. None of these high risk patients developed related infectious complications. Similarly, several reports show a good efficacy of fecal microbiota transplantation in severe and/or complicated forms of *C difficile* infection in the intensive care unit.[141 147-151] The largest cohort is retrospective and described the evolution of 111 patients: 66 in the fecal microbiota transplantation group and 45 in the non-fecal microbiota transplantation group.[152] The authors showed that fecal microbiota transplantation improves survival in severe cases, concluding that early fecal microbiota transplantation reduces mortality and should be proposed as a first line treatment for severe *C difficile* infection; however, no formal society guidelines recommend use of fecal microbiota transplantation in severe *C difficile* infection. More data are required to confirm a clinical benefit.

Mechanism of action

The mechanism explaining the high efficacy of fecal microbiota transplantation is multifactorial and not yet completely understood. Restoration of gut microbiota diversity is probably an important factor, and an association between clinical cure and increased diversity was shown in a pivotal trial.[125] Staley et al showed that complete microbiota

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engraftment was not essential for recovery following fecal microbiota transplantation.[153] The authors underline the key role of bacteria associated with secondary bile acid metabolism, which were associated with an increased resistance to infection. Consistent with these data, analysis of patients' feces before and after fecal microbiota transplantation showed that feces before fecal microbiota transplantation induced germination, and after fecal microbiota transplantation inhibited vegetative growth.[154] A study evaluating 10 patients after fecal microbiota transplantation showed consistent results, with a restoration of secondary bile acid levels in all patients receiving transplants.[155] Finally, if the structure of the microbiota is important, function probably counts also.[156] The efficacy of sterile fecal filtrate also suggests that bacterial components, bacteriophages, or active metabolites play a major role in efficacy and should be further evaluated.[157 158]

Donor selection

One challenge to implementing fecal microbiota transplantation is how to obtain a validated screened donor.[159] Screening practices vary between countries, and several guidelines are now published.[160 161] The US Food and Drug Administration issued a safety alert in June 2019 following the death of a patient who had received transmission of multi-drug resistant organisms via fecal microbiota transplantation.[162] However, analysis of this event was complicated because screening of donors for multi-drug resistant organisms is normally always performed, and a link between death and fecal microbiota transplantation, as well as the cause of death, was not reported. It is therefore difficult to perform a comprehensive analysis of this serious adverse event.

To conclude, fecal microbiota transplantation is an efficient treatment for recurrent *C difficile* infection, and potentially for other forms of the disease, such as severe or complicated *C difficile* infection. The mechanisms associated with its success are not yet completely understood and questions are pending regarding alternative methods (microbiota based preparations, sterile filtrate) and more importantly, long term safety.

Emerging treatments

Ridinilazole

Ridinilazole is a novel antibiotic with a targeted activity on *C difficile*. A phase 1 study showed a positive safety profile, supporting its clinical development.[163] A phase 2 trial compared ridinilazole with vancomycin in 100 patients, the primary endpoint being a sustained clinical response.[164] Ridinilazole was found superior to vancomycin, the sustained clinical response reaching 66.7% in the treatment group compared with 42.4% in patients treated with vancomycin. Two phase 3 studies are due to start in 2019 (NCT03595553 and NCT03595566).

Ursodeoxycholic acid

Bile acids play a key role in *C difficile* infection. Some compounds are potent inhibitors of germination.[14] A stable inhibitor could represent a useful prophylaxis with a direct effect on *C difficile* germination, for example before antibiotic treatment. A proof of concept study was performed with a patient with recurrent *C difficile* infection ileal pouchitis treated with ursodeoxycholic acid.[165] The patient remained free of infection for more than 10 months after initiation of treatment. A phase 4 clinical trial under way to measure the efficacy of ursodeoxycholic acid supplementation following *C difficile* infection (NCT02748616). The primary outcome is the return to a normal pattern of fecal bile acids.

Other drugs

Several molecules are being tested in *C difficile* infection: CRS3123 inhibits bacterial methionyl-tRNA synthetase,[166] and LFF571 blocks protein synthesis.[167] Two toxin binders, calcium aluminosilicate antidiarrheal (NCT01570634) and GT160-246 (NCT00466635), need more clinical data, and further research is needed. Finally, three drugs have been evaluated in *C difficile* infection: cadazolid,[168 169] tolevamer,[98] and surotomycin.[170] but the clinical results did not show any difference against the comparator and development was stopped.

Bacteriophage

Phage tail-like particles have been assessed in vitro and have shown capability to eradicate *C difficile*.[171] To date, no clinical trials are recorded.

Non-toxigenic strains

Spores of non-toxigenic *C difficile* are protective against toxigenic strains.[172] A case report showed the potential for non-toxigenic *C difficile* in preventing recurrence of *C difficile* infection.[173] An oral suspension was evaluated for tolerance in healthy subjects showing the expected gastrointestinal colonization.[174] A phase 2 randomized placebo controlled clinical trial with 173 patients treated with metronidazole or vancomycin showed that non-toxigenic *C difficile* colonized the gastrointestinal tract and substantially reduced recurrence of *C difficile* infection from 30% in the placebo group to 5% in the patients receiving 10^7 spores/day for seven days.[175]

To summarise, five drugs are currently available to treat *C difficile* infection: metronidazole, vancomycin, fidaxomicin, tigecycline, and teicoplanin. A systematic review and network meta-analysis screened 23 004 studies and selected 24 trials including a total of 5361 patients and compared treatments for non-multiply recurrent infections with *C difficile*.[176] For sustained clinical cure, fidaxomicin and teicoplanin were better than vancomycin. Vancomycin, fidaxomicin, teicoplanin, ridinilazole, and surotomycin were all better than metronidazole. A Cochrane analysis was consistent with those conclusions: in mild infections, vancomycin is superior to metronidazole and fidaxomicin to vancomycin.[177]

Prevention

Drugs

Rifaximin

Rifaximin is a non-absorbable rifamycin antibiotic that has been tested in a randomized double blind

study to prevent recurrence after completion of standard antibiotic therapy (generally with metronidazole or vancomycin).[178] In the study, recurrence of *C difficile* infection decreased from 31% in the placebo group to 15% after rifaximin. A placebo controlled trial confirmed these results, with a decrease of recurrence at 12 weeks from 29.5% to 15.9%.[179] In another trial, where the drug was used to prevent encephalopathy in patients with cirrhosis, rifaximin was associated with an outbreak of *C difficile* infection with rifaximin resistant strains of *C difficile*.[180] To date, no trials have been registered to evaluate rifaximin in *C difficile* infection.

Probiotics

Probiotics are live microorganisms administered to restore a dysbiotic environment and potentially prevent *C difficile* infection. Bio-K is a probiotic associated with three species of *Lactobacillus*. A phase 3 trial, randomized and double blinded, showed that Bio-K prophylaxis during antibiotic treatment was associated with a lower incidence of antibiotic or *C difficile* associated diarrhea.[181] Two other probiotics with different associations were clinically tested: VSL#3 and Howaru Restore. VSL#3 was tested in a multicenter randomized double blind study in patients exposed to an antibiotic course.[182] The results showed a decrease in cases of antibiotic associated diarrhea, but no cases of *C difficile* infection were reported. Howaru Restore was also evaluated in a randomized trial at different dosages.[183] The results showed a decrease in *C difficile* associated diarrhea in the probiotic group.

Microbiota based drugs

Microbiota based treatments, which include RBX2660 and SER-109, are suspensions of microbiota prepared from human stool that have a mechanism similar to fecal microbiota transplantation. A randomized placebo controlled trial of RBX2660 showed superiority, with an overall efficacy of 88.8%.[184] SER-109 is an encapsulated mixture of purified Firmicutes spores. A phase 2 trial of the drug that included 30 patients showed that SER-109 successfully prevented *C difficile* infection.[185] Two phase 3 trials (ECOSPOR III and IV) in the treatment of recurrent *C difficile* infection are recruiting (NCT03183141, NCT03183128). Another drug from the same company, SER-262, is being tested in a phase 1 study (NCT02830542).

Antibodies and vaccines

Antibodies

Fully human monoclonal antibodies targeting *C difficile* toxins A and B have been developed and showed a statistically significant efficacy in a hamster model.[186] A randomized, double blind, placebo controlled study evaluated these two monoclonal antibodies (actoxumab for *C difficile* toxin A, and bezlotoxumab for toxin B) in 200 patients who had initially been treated with metronidazole or vancomycin.[187] The rate of recurrence was substantially lower among patients treated with the antibodies (7% *v* 25%).

Two phase 3 clinical trials (MODIFY I and MODIFY II) evaluated the two antibodies' ability to reduce recurrence in 2655 patients. In the trials, patients received standard oral antibiotics for primary or recurrent *C difficile* infection, plus an infusion of either bezlotoxumab, actoxumab plus bezlotoxumab, or placebo.[188] Actoxumab alone was administered in MODIFY I but discontinued after interim analysis. Addition of actoxumab did not improve efficacy; however, bezlotoxumab alone was associated with a substantial reduction in recurrent infection compared with placebo (17% *v* 28%). No difference was seen in the rates of clinical cure between bezlotoxumab and placebo (80%), and sustained clinical cure was 64% for bezlotoxumab and 54% for placebo. Rates of adverse event were similar among the treatment groups. A phase 3 trial in children is currently recruiting to evaluate safety, tolerability, and efficacy of bezlotoxumab (NTC03182907).

Vaccines

Two vaccines are under evaluation.

A formalin inactivated toxoid based vaccine is currently the most advanced. Formulation is by inactivation of toxins A and B to produce toxoids A and B, which elicit a protective immune response. A phase 1 study of *C difficile* toxoid vaccine was performed in healthy volunteers.[189] Vaccination was well tolerated and more than 90% of the volunteers developed a strong serum antibody response to both toxins. The phase 2 study found that a high dose (100 µg) with adjuvant treatment administered at 0, 7, and 30 days elicited the best immune response through day 180.[190] A phase 3 study was started (NCT00772343), but was interrupted after a planned interim analysis. The Independent Data Monitoring Committee for the phase 3 Cdiffense clinical trial program concluded that the probability that the trial would meet its primary objective was too low.

Another phase 1 study evaluated VLA84, a recombinant fusion protein with relevant epitopes of toxins A and B, as a vaccine candidate in a healthy population and in older adults.[191] VLA84 was well tolerated and induced high antibody titer against toxins A and B in both populations. Comparable results were found with another formulation.[192] No information is available on a potential phase 3, planned or ongoing.

Emerging treatments

Beta-lactamase—Preserving the gut microbiota during systemic β-lactam treatment can be achieved using non-absorbable β-lactamases. SYN-004 is a recombinant β-lactamase designed to reduce the effect of systemic β-lactam. Two phase 2 trials confirmed that the molecule fully degraded ceftriaxone that had entered the gut after systemic administration.[193]

DAV132—is an activated charcoal based product that irreversibly captures antibiotics. As with β-lactamase, the goal is to inactivate the gut dysbiosis induced by systemic antibiotics.[194]

Lactoferrin—has been proposed prophylactically in long term care for patients who require broad spectrum antibiotics (NCT00377078).

Probiotics—Several probiotics are currently being tested, specifically *Lactobacillus reuteri*. Two phase 3 trials have recently completed (NCT02127814, NCT01295918) and a randomized trial is proposed but is not yet recruiting (NCT03647995).

Polyclonal oral antibodies—are being evaluated in phase 2/3 trials, and include hyperimmune bovine colostrum (NTC00747071) and whey protein concentrate 40% (NTC001177775). Results are not yet available.

Vaccines

- A DNA vaccine is being developed with encouraging results from animal models[195 196]
- Genetically modified toxins A and B were recently tested in a phase 1 trial[197] and a phase 2 trial (NCT02561195). A phase 3 trial is recruiting (NCT03090191)
- CDVAX is an oral vaccine that uses spores from a genetically modified bacterium. A phase 1 trial was terminated and results are not yet available (NCT02991417).

International guidelines—a critical view

A turning point in the treatment of *C difficile* occurred in 2014 with the publication of the European treatment guidance,[120] which included new definitions for severity and the identification of subgroups with an increased risk of complications and an increased risk of recurrence. Before this recommendation, IDSA/SHEA based the definition of severity on leucocytosis and the serum creatinine level.[87] ESCMID defines severity with signs and symptoms from clinical evaluation, laboratory investigations, colonoscopy, and imaging (table 1), and defines prognostic factors to identify patients with an increased risk of developing severe *C difficile* infection. Four factors were classified with a strong recommendation: age ≥65, marked leucocytosis (>15×10^9/L), decreased blood albumin (<30 g/L), and rise in serum creatinine level (≥133 µmol or ≥1.5 times the pre-morbid level). A comparable approach was proposed for recurrence with four additional factors with a high level of recommendation: age ≥65 years, continued use of antibiotics (not for *C difficile* infection), comorbidity, and a previous history of *C difficile* infection. In this recommendation, metronidazole is still proposed for non-severe *C difficile* infection. For severe infection, or in patients at risk of developing severe *C difficile* infection, vancomycin is the first choice. For a first recurrence, or for patients at risk of recurrence, both vancomycin and fidaxomicin are proposed as first line therapy. Finally, for multiple recurrent *C difficile* infection, fecal microbiota transplantation is recommended.

In 2018 IDSA/SHEA updated recommendations on treatment of adults.[69] In non-severe *C difficile* infection, both vancomycin and fidaxomicin were proposed, and metronidazole was downgraded to an alternative treatment if the two other agents are unavailable. Both vancomycin and fidaxomicin are recommended in severe forms of *C difficile* infection, and fecal microbiota transplantation remains the first choice in multiple recurrences of *C difficile* infection where appropriate antibiotic treatments have failed (for at least two previous recurrences).

Bezlotoxumab is not included in any algorithm for the treatment of *C difficile* infection (the study results pertaining to treatment with bezlotoxumab were released after the most recent IDSA/SHEA guidelines). With the data currently available, it would be difficult to include the antibody in any guideline, most importantly because the population for whom bezlotoxumab would be most appropriate is patients with a high risk of recurrence. Such patients are likely to be immunosuppressed (hematology, biotherapy, cancer) and exposed to broad spectrum antibiotics. We need clinical studies to evaluate the efficacy of bezlotoxumab in this subset of patients, and obtaining an adequate population to prescribe monoclonal antibodies needs development.

Table 1 | Severity criteria for *C difficile* infection, defined by ESCMID

Category	Signs/symptoms
Physical examination	Fever (core body temperature >38.5°C) Rigors (uncontrollable shaking and a feeling of cold followed by a rise in body temperature) Haemodynamic instability including signs of distributive shock Respiratory failure requiring mechanical ventilation Signs and symptoms of peritonitis Signs and symptoms of colonic ileus. Mixture of blood with stools is rare in *C. difficile* infection and the correlation with severity of disease is uncertain
Laboratory investigations	Marked leucocytosis (leucocyte count >15 9 10^9/L) Marked left shift (band neutrophils >20% of leucocytes) Rise in serum creatinine (>50% above baseline) Elevated serum lactate (≥5 mM) Markedly reduced serum albumin (<30 g/L)
Colonoscopy or sigmoidoscopy	Pseudomembranous colitis Insufficient data is available on the correlation of endoscopic findings compatible with *C. difficile* infection (eg, edema, erythema, friability, and ulceration) and the severity of disease
Imaging	Distension of large intestine (>6 cm in transverse width of colon) Colonic wall thickening, including low-attenuation mural thickening Pericolonic fat stranding Ascites not explained by other causes. The correlation of haustral or mucosal thickening, including thumbprinting, pseudopolyps and plaques, with severity of disease is unclear

Conclusion

The diagnosis of *C difficile* infection is still challenging. Testing should be performed routinely in healthcare associated diarrhea or any unexplained diarrhea in the community. European and North American scientific societies recommend a strategy based on a two step algorithm that includes a sensitive screening method followed by a more specific method to detect free toxins. Treatment of *C difficile* infection currently relies on two molecules: vancomycin and fidaxomicin in mild and severe forms of the infection. Metronidazole is no longer recommended, being inferior to vancomycin and fidaxomicin. Fecal microbiota transplantation is the treatment of choice in recurrent disease. We still need clinical trials to have a better idea of the target population for bezlotoxumab. *C difficile* infection used to be a simple pathology with a simple answer. Now, a better understanding of the pathophysiology has led to improvement of diagnostic techniques and refinement of the definitions unravelling the complexity of the pathology. If metronidazole and vancomycin were the backbone of treatment in previous years, we now have new molecules: fidaxomicin and tigecycline, and new approaches such as fecal microbiota transplantation to treat and prevent *C difficile* infection.

RESEARCH QUESTIONS

1. Determine the prevalence of nosocomial *C difficile* infections in European hospitals
2. Test the feasibility of adopting a standardized polymerase chain reaction ribotyping method
3. Compare the types of *C difficile* prevalent in European hospitals
4. Survey the antimicrobial susceptibility of European strains of *C difficile*
5. Draw up European guidelines on the prevention, diagnosis, treatment, and surveillance of *C difficile* infections
6. Determine the burden of *C difficile* infection in hospitals and in the community
7. Establish the role of *C difficile* in animals and its associations with humans
8. Determine the development and spread of plasmid mediated metronidazole resistance in toxigenic and non-toxigenic *C difficile* isolates
9. Determine the significance of asymptomatic carrier status for the individual patient and their environment (community, food, contact with animals) on short term and long term follow-up
10. Evaluate the potential role of screening for colonization in selected settings and patients
11. Evaluate the potential methods to manipulate human and animal gut microbiota to prevent *C difficile* colonization and infection
12. Evaluate the effect of *C difficile* infection in long term follow-up
13. Determine which group to treat with monoclonal antibodies for prevention of *C difficile* infection
14. Determine the best treatment for complicated forms of C difficile infection
15. Identify the factors that determine stool efficacy in fecal microbiota transplantation to design targeted approaches
16. Evaluate the potential role of fecal microbiota transplantation in treating first recurrence of *C difficile*

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