New concepts in *C. difficile* management

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Abstract

Background: *C. difficile* infection is transmitted via spores and the disease is mediated via secreted toxins. It represents a significant healthcare problem and clinical presentation can range from asymptomatic carriage to life-threatening pseudomembranous colitis.

Sources of data: publications in the field, with a focus on recent developments and concepts

Areas of agreement: infection control measures, antibiotic stewardship, current management of the initial episode of *C. difficile* infection

Areas of controversy: selection and sequence of interventions for the management of recurrent *C. difficile* infection; management of persistent carriers of toxigenic *C. difficile* in patients at high risk of subsequent *C. difficile* infection

Growing points: use of faecal microbiota transplantation for recurrent *C. difficile* infection

Areas timely for developing research: role of specific microbiota-mediated interventions and vaccination in the treatment and prevention of *C. difficile* infection

Introduction

Description of *Clostridium difficile* was reported in 1935, following its isolation from healthy infant faeces and it was originally named *Bacillus difficile* (1). *C. difficile* is a spore-forming, Gram-positive anaerobic bacterium, which was identified in the 1970s as the aetiological agent of antibiotic-associated pseudomembranous colitis (2). Recently, based on phenotypic, chemotaxonomic and phylogenetic analyses, reclassification of *Clostridium difficile* as *Clostridioides difficile* has been proposed (3).

In 2011, the estimated number of incident *C. difficile* infections in the United States was 453,000 and an estimated 29,300 associated deaths (4). There was a marked increase in the number of reported cases of *C. difficile* infection around mid-2000 (in 2007/08, >55,000 cases were reported by NHS Trusts in England), which included outbreaks associated with a more virulent strain (ribotype 027) of *C. difficile*. The emergence of ribotype 027 is believed to have been driven by the use of fluoroquinolone antibiotics and a recent study has proposed a role for the increase (since 2000) in the human diet of the disaccharide trehalose, which this strain is able to metabolise at low concentrations (5). Trehalose is a stable sugar, which may be found in foods such as pasta, minced beef and ice cream. There is also increasing recognition of the occurrence of *C. difficile* infection in the community, often in patients who have previously had in-patient and/or out-patient exposure to the hospital setting.

Since 2007/08 there has been a progressive decline in the number of reported cases of *C. difficile* infection in UK and the numbers have been fairly stable since 2013/14, (when 13,362 cases were reported). Over 12 months to 31st March 2018, 13,286 cases of *C. difficile* infection were reported by NHS Trusts in England to Public Health England. As in the past, rates of infection are highest among those over the age of 65 years, especially those over the age of 85 years (6). The decrease in incidence rates of *C. difficile* infection since 2007/08 appears to be due largely to infection prevention and control measures and antimicrobial stewardship interventions.

Although there have been large reductions in incidence rates, *C. difficile* infection continues to represent a significant healthcare problem. This was illustrated in a recent population-based study that showed an almost three-fold increase in 30-day all-cause mortality and more than 20% mean increase in additional length of stay beyond the infection. The greatest impact of *C. difficile* infection was seen in the elderly. Over the 6-year period of the study (2010 – 2016),

there appeared to be no improvement on the impact of *C. difficile* infection on mortality or additional length of stay (7), implying the need to ensure timely and optimal clinical management of patients with this infection.

Pathogenesis

The normal resident colonic bacteria are widely recognised to provide protection against colonisation by pathogenic bacteria such as *C. difficile* and this defence is designated colonisation resistance (8, 9). There is significant current interest in the characterisation of the protective resident bacteria and the mechanisms by which they resist colonisation by *C. difficile*. Recognition of normal protection by resident microbiota provides the rationale for the use of faecal microbiota transplantation in patients with recurrent *C. difficile* infection (see below), which aims to repopulate the colon with the bacteria that mediate colonisation resistance. Such protection is disrupted by broad-spectrum antibiotics, which represent a major risk factor for *C. difficile* infection.

High risk broad-spectrum antibiotics that predispose to *C. difficile* infection include clindamycin, fluoroquinolones and second/third generation cephalosporins. Recent studies using metagenomic, metatranscriptomic and metaproteomic analyses and metabolomics (study of microbe-derived small molecules) have shown that these antibiotics lead to major depletion and disruption of the resident colonic bacteria present in the lumen and mucosal surface, with associated changes in the functions of the microbial communities (10, 11). The functional impact includes alterations in bacterial metabolism of carbohydrates, amino acids and bile acids.

The association between *C. difficile* infection and the use of chemotherapy for cancer treatment (without antibiotics) has been recognised for >20 years (12). The risk may be increased in patients with prolonged hospitalisation and those who receive both chemotherapy and antibiotics.

In view of the reported association with this class of drugs, the diagnosis of *C. difficile* infection usually leads to the review of the indication(s) for the use of proton pump inhibitors. Further studies are required to determine the impact of stopping proton pump inhibitor treatment on the subsequent risk of *C. difficile* infection.

Only toxigenic strains of *C. difficile* are responsible for the infection leading to inflammation in the colon that is mediated by secreted toxins A and B (2, 13). Non-toxigenic strains of *C. difficile* are non-pathogenic and one strain has been studied as a treatment option in patients with recurrent disease (see below).

Secreted toxins A and B are major determinants of the disease induced by *C. difficile*. These large (>200 kDa) toxins are potent inducers of the secretion of pro-inflammatory mediators and cell death. There has also been significant interest in host cell receptors, cellular uptake and intracellular mechanisms of actions of the toxins (13).
Studies have demonstrated the importance of the host immune response to the toxins in determining the susceptibility to disease and risk of recurrence (2). Some of the therapeutic approaches discussed below have targeted *C. difficile* toxins.

Clinical features and assessment of disease severity

Following colonisation with toxigenic *C. difficile,* the wide spectrum of clinical presentation ranges from asymptomatic carriage to mild diarrhoea to life-threatening pseudomembranous colitis (14).
A number of criteria have been used to define severe disease and have included clinical features (bowel frequency, abdominal pain/tenderness, pyrexia) results of blood tests (peripheral white blood cell count, serum creatinine level, albumin level), the presence of pseudomembranous colitis (during endoscopic examination) and extent / severity of colitis upon imaging via CT scan.
Patients with mild *C. difficile* infection may be considered to be those with bowel frequency less than 4 times over 24 hours and with normal white blood count and creatinine level. In recent guidelines (15), patients with non-severe disease are defined as those with peripheral white blood count <15,000 cells per ml and a serum creatinine level <1.5 mg/dl (<132.6 µmol/L). Those with severe disease are deemed to have white blood count and creatinine level above these values. Severe complicated or fulminant colitis is considered to be present in those with hypotension or shock, ileus, megacolon and some of these patients may require admission to intensive care unit.

Recurrence of *C. difficile* infection

Recurrence of *C. difficile* infection occurs in 10 – 35% of patients, usually within 8 weeks of completion of treatment and may be due to the original strain or new strain of *C. difficile* (14,

15). Risk factors for recurrence include previous episodes of *C. difficile* infection, host immune response to *C. difficile* toxins, additional antibiotics, old age, severe underlying disease(s) and the use of proton pump inhibitors. Treatment options for recurrent *C. difficile* infection are discussed below.

Inflammatory bowel disease (IBD) and *C. difficile* infection

A number of studies have reported an increase in the risk of *C. difficile* infection in patients with inflammatory bowel disease (2, 15). The risk may be greater in patients with ulcerative colitis but a recent study, which reported a 4.8-fold increase in *C. difficile* infection in patients with IBD, found no difference between those with ulcerative colitis and Crohn's disease (16). It should be noted that pseudomembranes (or characteristic histological changes) may not be seen in IBD patients with *C. difficile* infection (2). Worse clinical outcomes have been reported in IBD patients with *C. difficile* infection, including longer duration of residence in hospital, increased colectomy rates and higher mortality rates (17). The potential mechanisms by which *C. difficile* infection may enhance mucosal inflammatory responses in IBD have been reviewed (2).

Diagnosis and investigations

The diagnosis of *C. difficile* infection is usually considered in those presenting with diarrhoea following exposure to antibiotics and requires collection of stool samples. Before the establishment of the relevant enzyme immunoassays (EIAs) and molecular tests for toxin genes, the diagnosis of *C. difficile* infection was based predominantly on culture of toxigenic *C. difficile* (followed by confirmation of its capacity to produce toxins) or a positive cell culture cytotoxicity neutralization assay. These tests are time consuming and now represent reference methods. Additionally, *C. difficile* culture and molecular typing is used for the detection of outbreaks and epidemiologic studies. Because of their convenience, enzyme immunoassays for toxin A or both toxins (A and B) were introduced in clinical laboratories in the late 1980s (15). The performance of these commercial EIAs was variable, with reasonable specificity but low sensitivity. Newer EIAs have tended to perform better but in order to improve sensitivity, other tests were introduced. They include immunoassays for glutamate dehydrogenase (GDH, which is expressed by all isolates of toxigenic and non-toxigenic *C. difficile*) and molecular [nucleic acid amplification test (NAAT) and polymerase chain reaction (PCR)] tests for the detection of toxin gene. These assays are highly sensitive (>90%) for the presence of *C. difficile* in a stool sample and therefore have a high negative predictive value (>95%) for *C. difficile*

infection. However, GDH immunoassays and molecular tests for toxin gene have a low (<50%) positive predictive value for the infection.

From 2012, a two-test protocol has been established in UK hospitals for the diagnosis of *C. difficile* infection. An initial screening test is undertaken to look for the presence of *C. difficile* via GDH immunoassay or molecular test for toxin gene. If positive, a second test is undertaken to look for the presence of *C. difficile* toxins using a sensitive enzyme immunoassay (18).

If the molecular test for *C. difficile* toxin gene is positive but the stool sample is negative for toxin EIA, the patient is deemed to be a carrier (excretor) of toxigenic *C. difficile* and has potential for transmission to others (see below).

Since the stool samples are usually only tested from patients with diarrhoea and since sensitivities of the toxin EIAs are usually <90%, some patients with *C. difficile* infection may be misdiagnosed as carriers (and the diarrhoea attributed to another cause). The demonstration of characteristic pseudomembranous colitis at flexible sigmoidoscopy (which can be undertaken without bowel preparation) may enable the diagnosis of *C. difficile* infection when the stool tests are equivocal or negative despite strong clinical suspicion. Together with biopsies (which on histological examination may show "summit lesions" characteristic of pseudomembranous colitis), such endoscopic examinations may also be helpful in the rapid diagnosis and assessment of those with severe symptoms, and may also identify other causes of diarrhoea such as such as inflammatory bowel disease, ischaemic colitis and microscopic/collagenous colitis (19). Additionally, stool samples can be collected during the endoscopic procedure.

Assessment of disease severity

Predictors of 30-day mortality include a high leukocyte count and elevated creatinine and lactate levels (14, 20). In those with significant clinical features, CT abdomen enables assessment of the extent and severity of colonic inflammation. Abdominal x-ray is often helpful for the detection of complications such as toxic megacolon or perforation. For those with features of colitis on CT imaging but equivocal stool test result, flexible sigmoidoscopy & biopsy may be required to confirm the diagnosis of *C. difficile*-associated pseudomembranous colitis.

Infection control

Patients with suspected infectious diarrhoea should be accommodated in a single room with a self-contained toilet and its own hand basin. If such facilities are not available, patients with

confirmed *C. difficile* infection may be nursed in a dedicated ward or multibed room, with strict infection control measures.

In addition to isolation, contact precautions should be undertaken and include wearing gowns and gloves when caring for patients with *C. difficile* infection. Hand washing with soap and water is recommended to remove *C. difficile* spores. Following discharge, the room that had been occupied by a patient with *C. difficile* infection should be disinfection.

With increasing appreciation of antibiotic resistance in clinical practice, antibiotic stewardship programs are widely adopted with the aim to use narrow-spectrum antibiotics for documented infection, for the shortest duration. For the control of *C. difficile* infection, this usually involves restriction in the use of fluoroquinolones, third-generation cephalopsorins, clindamycin and amoxicillin. Such measures are deemed particularly important for those at greatest risk, including those with previous episodes of *C. difficile* infection and carriers of toxigenic *C. difficile*.

Carriage of toxigenic *C. difficile*

In a systematic review and meta-analysis, asymptomatic patients colonised with toxigenic *C. difficile* had a 5.9 times higher risk of subsequent *C. difficile* infection compared to those who were not colonised (21). There is also an increased risk of *C. difficile* infection in hospitalised patients exposed in the ward to asymptomatic carriers of toxigenic *C difficile* (22). Moreover, detection and isolation of asymptomatic carriers of toxigenic *C. difficile* has been reported to lead to a decrease in the incidence of health care-associated *C. difficile* infection (23). The risk of transmission is likely to be greater in carriers of toxigenic *C. difficile* with diarrhoea due to another cause. With the use of the two-test protocol for the diagnosis of *C. difficile* infection, there is increasing recognition of this group of patients, in which infection control measures should be undertaken.

Since asymptomatic carriers of toxigenic *C. difficile* are at an increased risk of developing (and transmitting) *C. difficile* infection, selected patients could be considered for intervention. Such an approach is being investigated for high risk patients such as those undergoing bone marrow transplantation (24, 25).

Treatment of *C. difficile* infection.

Oral vancomycin and metronidazole have been used for the treatment of *C. difficile* infection since the 1970s. Although initial small studies reported no significant difference in responses

to these two antibiotics, more recent studies have demonstrated the superiority of vancomycin for not only severe *C. difficile* infection, but also mild-to-moderate disease (reviewed in (15, 20). Together with reduction in its cost (especially the use of intravenous formulation for oral administration), vancomycin has increasingly been the antibiotic of choice for *C. difficile* infection of any severity.

In 2011 fidaxomicin was approved by the European Medicines Agency and the Food and Drug Administration for the treatment of *C. difficile* infection. It is a macrocyclic antibiotic with a narrow spectrum of antibacterial activity against *C. difficile*, with moderate activity against some other Gram-positive bacteria. It is very poorly absorbed systemically and achieves high faecal concentrations after oral administration. In two randomised controlled trials, fidaxomicin (dose 200 mg twice daily for 10 days) was non-inferior to vancomycin (125 mg four times daily for 10 days) in rates of clinical cure (defined as resolution of diarrhoea and no further need for treatment) in patients with mild to severe *C. difficile* infection. Recurrence rates were significantly lower in those who received fidaxomicin, except in the subgroup of patients infected with the ribotype 027 strain of *C. difficile* (26, 27). Adverse events did not differ significantly between vancomycin and fidaxomicin. Thus, vancomycin or fidaxomicin have recently been recommended for the treatment of *C. difficile* infection that is mild to severe (15). In view of its cost, vancomycin is often used for an initial episode of *C. difficile* infection and fidaxomicin reserved for those with recurrence. Because of higher cure rates (compared to vancomycin) in patients receiving concomitant antibiotics for other infections, fidaxomicin is currently the antibiotic of choice in this group of patients.

Patients with severe complicated or fulminant *C. difficile* infection were not included in the above studies and is currently usually treated with high dose (500 mg q.d.s) oral vancomycin and intravenous metronidazole. In the presence of ileus, adequate amounts of oral vancomycin may not reach the colon but intravenous metronidazole is secreted in the lumen of the inflamed colon. Rectal vancomycin may also be used in such patients.
Surgery may be required for some with severe complicated or fulminant disease. This usually involves a subtotal colectomy but a recent report suggests a role for loop ileostomy (which could be undertaken laparoscopically in the majority of the patients) and instillation of vancomycin into the preserved colon via the ileostomy (28).

Treatment for recurrent *C. difficile* infection

A number of approaches have been used for the treatment of first recurrence: course of vancomycin (if metronidazole was used to treat the initial episode), fidaxomicin (if vancomycin was used to treat the first episode). Subsequent recurrence is often treated with vancomycin in a tapered and pulsed regimen.
Recently, there has been increasing use of faecal microbiota transplantation for those patients who have had 2 or more recurrences of *C. difficile* infection (see below).

Recently-investigated non-antibiotic-based treatment strategies

Since the *C. difficile* infection is often preceded by the use of broad-spectrum antibiotics, there is interest in the development of non-antibiotics-based approaches to treatment and prevention of this infection. Such approaches are based on the knowledge of disease pathogenesis such as the permissive environment created by broad spectrum antibiotics that allows colonisation by toxigenic *C. difficile* due to the loss of protective resident bacteria. Following colonisation, there is an essential requirement for secreted toxins to mediate the intestinal inflammation.
For treatment of established infection, the secreted *C. difficile* toxins have been targeted with aim of inhibiting their interactions with the host mucosal cells. Tolevamer is an anionic polymer that noncovalently binds *C. difficile* toxins A and B and following a promising phase 2 study, was investigated for the treatment of mild to moderately severe *C. difficile* infection in two randomised controlled trials (29). Less than 50% of patients responded to tolevamer as monotherapy, which was significantly less effective than either metronidazole or vancomycin. Compared to metronidazole and vancomycin, recurrence of disease was significantly lower in those who responded tolevamer, which could be due persistence of protective components of the microbiota. It is of interest that those who responded to tolevamer retained high counts of *C. difficile*, which gradually declined and by day 42 levels were similar to those in the antibiotic treated groups (30).

Bezlotoxumab is a human monoclonal antibody that is capable of neutralizing toxin B by blocking its binding to host cells. In a report of two phase 3 trials, bezlotoxumab as single infusion during standard antibiotic treatment for *C. difficile* infection was associated with significantly lower rate of recurrent infection, when compared with placebo (in pooled analysis, 27% of those who received placebo had recurrence of *C. difficile* infection at 12 weeks, compared to 17% of those who had bezlotoxumab) (31). Bezlotoxumab has been approved for use in many countries for the prevention of recurrence of *C. difficile* infection. It is anticipated

that further studies will enable assessment of the role of this treatment, which is given in addition to standard antibiotics.

Non-toxigenic strains of *C. difficile* do not secrete toxins as they lack the relevant genes and therefore do not cause disease. In studies undertaken in 1980s, hamsters colonised by non-toxigenic strains of *C. difficile* were shown to be protected from infection by a toxigenic strain (32). In a more recent phase 2 study involving patients that had recently completed a course of antibiotics for *C. difficile* infection, oral administration of spores of a non-toxigenic strain of *C. difficile* significantly reduced the recurrence of *C. difficile* infection (33). It is postulated that colonisation by non-toxigenic *C. difficile* would provide protection by competing against toxigenic strains of *C. difficile* for the relevant niche in the colon.

Faecal microbiota transplantation (FMT)

FMT has been of interest for many years, with many anecdotal reports of success in the treatment of recurrent *C. difficile* infection. Over the last few years, there has been resurgence of interest following the report in 2013 of the first randomized trial in which duodenal infusion (via nasoduodenal tube) of donor faeces was significantly more effective than vancomycin for the treatment of recurrent *C. difficile* (34). Subsequent randomized trials have demonstrated efficacy of FMT, administered via different routes (oral capsules, nasogastric tube, colonoscopy, enema), in the treatment of recurrent *C. difficile* infection (15, 35). Whilst studies suggest that instillation at colonoscopy may be lead to highest rates of success, procedure-related risks of adverse events are likely to be lower following administration via oral capsules or enema and these routes also offers the scope for more convenient repeat administration. Studies to date have reported short-term efficacy and safety of FMT with predominantly mild to moderate self-limited adverse events that are largely related to the gastrointestinal tract (15, 35). However, there is a need for the demonstration of long-term safety of this treatment. Currently, FMT treatment is usually considered in a patient with two or more recurrences of *C. difficile* infection (20).

The mechanism(s) by which FMT mediates therapeutic benefit in patients with recurrent *C. difficile* infection is unknown but is of significant current interest. It is postulated to be via the restoration of the characteristics of the resident microbiota that mediate colonisation resistance to *C. difficile*. Patients with recurrent *C. difficile* infection have been shown to express a decrease in diversity of the gut microbiota (36). Structural and functional features of the

resident microbiota that may be re-establish by FMT include the metabolism of carbohydrates, amino acids, lipids (including fermentation into short chain fatty acids) and bile acids (10, 36). A number of studies suggest that colonization resistance to *C. difficile* may be restored via bacteria-mediated re-establishment of bile acid metabolism that leads to the generation of secondary bile acids following 7 alpha-hydroxylation of primary bile acids that reach the colon (2). Competition for metabolites and nutrients may represent other mechanisms by which FMT restores colonization resistance against *C. difficile.*

Antibiotic-induced loss of colonization resistance has been reported to be re-established in mice using a mixture of 6 bacterial species (37), implying future prospect for the development of similar defined bacteriotherapy for patients with recurrent *C. difficile* infection. However, challenges for the development of such therapy are illustrated by the recent demonstration that some probiotic bacterial species may delay the re-establishment of the microbiome to the state that existed prior to disruption by antibiotics (11).

In an open-label pilot study, donor stool suspensions that were sterilized by filtration have been reported to lead to the resolution of recurrent *C. difficile* infection in 5 patients (38). This study suggests that the beneficial effects of FMT may not require bacteria to mediated therapeutic benefits which could be derived from the bacterial products, components and/or bacteriophages. It is anticipated that future controlled studies will determine the role of sterile faecal filtrates in the management of patients with recurrent *C. difficile* infection.

Vaccination

Active immunization aims to generate a protective systemic and / or mucosal immune responses in those at greatest risk of developing *C. difficile* infection. Although no vaccine is currently approved for clinical use, there are ongoing clinical trials that have been undertaken following studies in animals (39).

Majority of the studies have targeted toxins A and B because they represent the main virulence determinants of *C. difficile* infection, and anti-toxin antibodies have been associated with protection against *C. difficile* infection and its recurrence (2). Inactivated whole toxins and their recombinant fragments have been used as vaccines. More recently, DNA vaccines have also been studied. Since vaccines against the toxins may not provide protection against colonisation, bacterial surface antigens involved in adhesion to intestinal epithelial cells represent additional targets.

Clinical trials

Following phase II trials for protection against recurrence and for prophylactic use, a phase III clinical trial was initiated to assess the efficacy of a highly purified formalin inactivated full length toxins A and B toxoid vaccine in preventing symptomatic primary *C. difficile* infection in adults aged 50 yrs or older (39, 40). This trial was initiated in 2013, but after recruitment of >9,000 participants, the trial was terminated because the Independent Data Monitoring Committee concluded that the probability that the study will meet its primary objective is low (40). A genetically modified toxins A and B toxoid vaccine is currently recruiting to a phase III trial, aiming for >17,000 participants (41). The trial is evaluating the ability of the vaccine to provide protection against *C. difficile* infection in at risk adults aged 50 years or older. Immunogenicity and safety of this vaccine was reported in phase I and II studies (39). A recombinant fusion protein consisting of truncated *C. difficile* toxins A and B completed a phase II study in 2015 in healthy adults (42) and a phase III trial is expected to start in the near future.

Conclusion

C. difficile infection continues to represent a significant healthcare problem in which the majority of those affected are in the older age group and have recently been on antibiotics. Vancomycin is increasingly used for an initial episode of *C. difficile* infection of any severity. However, persistent disruption of the protective resident colonic bacteria is believed to be responsible for recurrence of *C. difficile* infection that occurs in a significant proportion of patients. There is therefore significant interest in non-antibiotics based treatments and of those recently investigated, faecal microbiota transplantation (FMT) is increasingly used in clinical practice for the management for those who have had multiple recurrences of *C. difficile* infection. It is anticipated that greater understanding of mechanisms by which FMT mediates therapeutic benefit will lead to the identification of new forms of treatment. Infection prevention and control measures and antimicrobial stewardship interventions remain important aspects of management, which are especially relevant for those with previous episodes of *C. difficile* infection and asymptomatic carriers (excretors) of toxigenic *C. difficile*. Protection via active immunization is currently under investigation in at risk adults aged 50 years or older.

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