

# Clostridium difficile infection and antibiotic-associated diarrhoea

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## ABSTRACT

Antibiotic-associated diarrhoea is among the most common adverse events related to antibiotic use. Most cases are mild, but *Clostridium difficile* infection causes a spectrum of disease, ranging from occasional diarrhoea to colitis, toxic megacolon, and potentially death. Recent developments in our understanding of the biology of the gut microbiota have provided new insights into the pathogenesis of these conditions, and have revealed a role for manipulation of the gut microbiota as a novel therapeutic approach. This review will give an overview of the assessment of these conditions, before focusing on the rapidly developing area of their treatment.

## Introduction

In addition to being a potential reservoir for pathogenic bacteria, the gut also has an extensive ecosystem of microorganisms (~10<sup>11</sup> bacteria per gram of intestinal content) with no overt pathogenicity. It is now appreciated that this ecosystem (often called the 'gut microbiota') performs roles that are essential for the maintenance of host health, including short chain fatty acid and bile acid metabolism.<sup>1</sup> Antibiotic-associated diarrhoea (AAD) is now understood to represent an imbalance of the gut microbiota resulting from antibiotic use, with several mechanisms appearing to contribute to the disease process.

Most cases of AAD are mild and self-limiting, and are associated with negative stool culture results. Given the widespread use of antibiotics, it is not surprising that this condition is so common, affecting 5–39% of people treated with antibiotics.<sup>2</sup> One key mechanism of AAD appears to be changes in the gut microbiota that cause decreased short-chain fatty acid absorption, resulting in osmotic diarrhoea.<sup>3</sup>

One particular form of AAD – *Clostridium difficile* infection (CDI) – can cause more severe gastrointestinal disease. *Clostridium difficile* (now also referred to as *Clostridioides difficile*) is a Gram-positive, spore-forming anaerobic bacillus. Spores of *C difficile* can survive for long periods on inanimate objects (resisting heat, acid and antibiotics), a major reason why this bacterium can cause such problems within healthcare settings. *Clostridium difficile* is spread

via the faeco-oral route, and causes disease in humans through the production of two protein exotoxins (toxin A and toxin B), which are cytotoxic to colonic epithelial cells.<sup>4</sup> The host's adaptive immune response following exposure to *C difficile* also appears to be important in determining the severity of the disease, with high IgG antitoxin level production being protective.<sup>4</sup> Antibiotic use is the major risk factor for CDI, causing antibiotic-related loss of gut microbial communities that protect against gut infection, thereby facilitating the germination and vegetative growth of the organism when it enters the gut of vulnerable people. Risk factors for CDI are summarised in Table 1. The range of clinical disease that can occur in CDI is wide: diarrhoea and fever occur in almost all cases, but the most severe cases are characterised by colitis, toxic megacolon (dilatation of the colon, with the risk of perforation), multi-organ failure, or even death.

## Key points

Antibiotic use may result in diarrhoea through different mechanisms, including: osmotic diarrhoea (through the loss of gut bacteria that absorb short-chain fatty acids); and colonisation and overgrowth of toxin-secreting *Clostridium difficile*

Typical laboratory tests for the presence of *C difficile* do not have high sensitivity and specificity in isolation, so a combination of tests should be used when making the diagnosis of CDI

CDI has recently become harder to treat with conventional antibiotics for several reasons, including increased treatment failure with metronidazole, rising rates of CDI recurrence, and the emergence of hypervirulent strains of *C difficile* (eg NAP1/027)

Fidaxomicin is a new antibiotic that has considerable efficacy in treating recurrent CDI, though there is uncertainty as to its use in particular situations, including treatment of the NAP1/027 strain

Randomised clinical trial evidence demonstrates that faecal microbiota transplant (FMT) is more effective than vancomycin for the treatment of recurrent or refractory CDI, and this treatment is now approved in guidelines

KEYWORDS: *Clostridium difficile*, diarrhoea, antibiotics, faecal microbiota transplant, microbiota ■

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**Table 1. Risk factors for *Clostridium difficile* infection**

Risk factor	Details
Antibiotics	Almost all antibiotics can increase vulnerability to <i>Clostridium difficile</i> infection (CDI), but cephalosporins, fluoroquinolones, clindamycin and certain penicillins (eg co-amoxiclav) increase risk to the greatest extent
Acid-suppressant medications	Both proton-pump inhibitors (PPI) and H <sub>2</sub> -receptor antagonists appear to increase risk (risk is increased with PPI especially)
Age	Rates are ten-fold higher in those of >65 years than in the younger population
Hospitalisation	<ul style="list-style-type: none"> <li>&gt; Recent hospitalisation, prolonged hospitalisation (&gt;7 days), and/or prolonged antibiotic courses all increase risk of CDI</li> <li>&gt; Being admitted to a room where the previous patient had CDI is itself a risk factor for infection</li> </ul>
Immunosuppression	

Rates of CDI (and associated morbidity/mortality) peaked in the UK approximately 10 years ago (with about 70,000 cases in the UK, around 4000 of which were fatal, attributed to CDI in 2007).<sup>5</sup> Rates have since decreased rapidly. There are currently around 12,000 cases per year in the UK,<sup>5</sup> with the sharp recent decline in incidence being attributed to infection prevention and control interventions, including antibiotic stewardship.<sup>6</sup>

## Diagnosis

Typical AAD is a clinical diagnosis, based on the relationship between recent antibiotic use and the development of diarrhoea, and on the exclusion of potential alternative causes of diarrhoea.

Diagnosis of CDI may be difficult for several reasons. First, the currently available tests lack ideal sensitivity and specificity. Second, *C difficile* may either cause asymptomatic colonisation or active gastrointestinal infection, and conventional tests do not clearly differentiate between these two states. Therefore, guidelines recommend testing for CDI only when a patient has diarrhoea and grounds for suspecting an infective aetiology.<sup>7</sup> Laboratory tests for CDI are summarised in Table 2.

Several other investigations may also be useful in the assessment of people with CDI. Leucocytosis (particularly neutrophilia) is common, and may be very marked. Abdominal radiograph is the preferred test for toxic megacolon, whereas abdominal CT is sensitive for the presence of CDI-related colitis. Lower gastrointestinal endoscopy may show only oedema or erythema, or may show the classical finding of pseudomembranes, ie raised yellowish plaques that may be intermittently scattered throughout affected colonic mucosa (Fig 1).

Where clinicians have a strong clinical suspicion of CDI, empirical therapy should be considered regardless of the laboratory result;

**Table 2. Tests for *Clostridium difficile* infection**

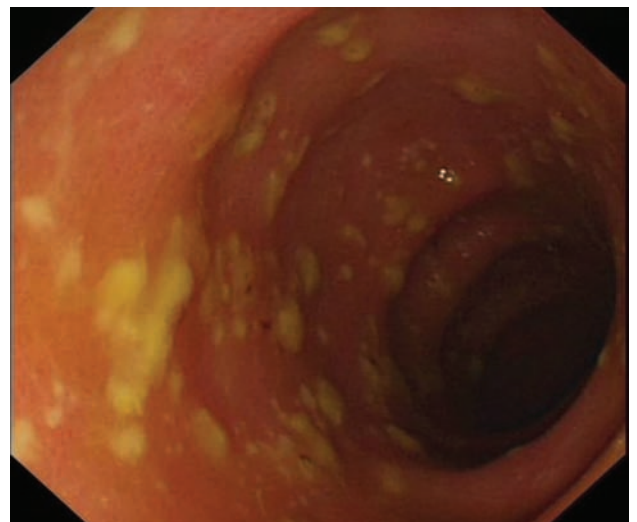
Test	Details
GDH EIA	GDH is a protein that is present in all isolates of <i>C difficile</i> . This test is sensitive, quick and relatively cheap, but lacks high specificity for toxigenic forms of <i>C difficile</i> . It is therefore often used as an initial screening test
Toxin A and B EIA	This test has high specificity but variable degrees of sensitivity
Toxin A and B PCR	This PCR test is specific for genes encoding toxins that are produced by <i>C difficile</i> , but a positive result does not help to distinguish active toxin production from asymptomatic carriage of toxigenic <i>C difficile</i>
Cell culture cytotoxicity assay	This is still widely recognised as the reference standard test, although it is rarely performed in most clinical laboratories

Given the variability of the sensitivity and specificity of these tests, a two-stage testing algorithm is widely applied. In the UK, toxin A/B enzyme immunoassay (EIA) testing is often used after an initial glutamate dehydrogenase (GDH) or polymerase chain reaction (PCR) screen<sup>7</sup>

this is because with a high pre-test probability, the negative predictive value of the tests that are currently available is insufficient to exclude disease.

## Treatment

In all cases of AAD, initial care includes the removal of precipitating factors (ie cessation of antibiotics). This will resolve the majority of cases of typical AAD (and some cases of CDI), but most cases of CDI will require more specific intervention. In treating CDI, a severity assessment is important to establish the most appropriate treatment. The assessment described in



**Fig 1. Pseudomembranous colitis.**

the current Public Health England (PHE) guidelines<sup>8</sup> is the most commonly used version:

- > white cell count >  $15 \times 10^9/L$
- > acutely rising creatinine (eg >50% increase above baseline)
- > temperature > 38.5°C
- > severe colitis (either from clinical abdominal signs or from radiological investigations).

### Conventional treatment of CDI

Until recently, the antibiotics metronidazole and vancomycin were the only pharmacological options for the treatment of CDI. Metronidazole may be administered orally or intravenously, whereas vancomycin may be given orally or per rectum for this indication. Vancomycin is not used intravenously to treat CDI because it has very limited penetration into the gut mucosa. Intravenous immunoglobulin (IVIg) also has a role in treating severe disease. Until recently, these treatments were effective for most cases of CDI, with surgical intervention (colectomy) reserved for the small number of severe cases that were refractory to medical therapy.<sup>8</sup>

However, over the past decade, CDI has become more difficult to treat with conventional therapies for several reasons. First, the rate of CDI treatment failure with metronidazole is increasing, with rate of non-response now quoted at >20%;<sup>9</sup> this is one of the reasons why patients with CDI and markers of severity should be treated with vancomycin in preference to metronidazole.<sup>8</sup> Second, rates of recurrent CDI (ie return of disease within a short period after stopping antibiotic therapy) are also increasing.<sup>9</sup> Third, hypervirulent strains of *C difficile* have emerged, particularly the NAP1/027 strain, which is associated with the secretion of large quantities of toxin and poor response to conventional antibiotics.<sup>10</sup> Novel approaches for therapy for CDI are urgently sought.

### New antibiotics: fidaxomicin

Fidaxomicin is a novel macrocyclic antibiotic, administered orally, with a narrow spectrum of activity. Although fidaxomicin has been shown in clinical trials to have efficacy in treating recurrent CDI<sup>11</sup> (and has consequently been added into clinical guidelines<sup>8</sup>), there are still a number of concerns regarding its use. These include its apparently limited efficacy against the NAP1/027 strain, the lack of evidence regarding its use in CDI with severe colitis, and its considerable expense (since it is 600 times more expensive than metronidazole.)

### Manipulation of the gut microbiota: probiotics and faecal microbiota transplantation

Given that all forms of AAD represent a perturbation of the gut microbiota, restoration of the usual commensal microbiota is an appealing means of treating the condition.

One approach to achieve this is through the use of probiotics, ie the delivery of live microorganisms into the host (sometimes through a suspension or drink). There are limited data regarding the use of probiotics to treat AAD/CDI, with relevant studies suggesting a possible modest benefit in treating non-severe CDI. However, significant uncertainties still exist in determining the specific organisms that should be included in the formulation, the dose to be used and so on, and probiotics are therefore not recommended at present.

Another approach has been the use of faecal microbiota transplantation (FMT), that is, taking a stool sample from a healthy screened donor (with presumed 'normal' gut microbiota), processing this in a laboratory into a liquidised bacterial suspension, and delivering it into the gut of affected people. A number of case reports and small case series have described this treatment over the past 60 years, but the first major randomised trial investigating its efficacy was carried out in 2013.<sup>12</sup> In this study, patients with recurrent CDI were randomised to receive either bowel lavage followed by FMT (via a nasoduodenal tube), vancomycin alone, or vancomycin with bowel lavage. The study was stopped early on ethical grounds after a planned interim analysis because 81% of patients ( $n=13/16$ ) had recovered in the FMT arm, compared to 31% ( $n=4/13$ ) receiving vancomycin alone, and 23% ( $n=3/13$ ) receiving vancomycin and bowel lavage. Two of the three patients who did not respond to an initial FMT were successfully treated with a second. There were no major adverse effects. A subsequent randomised trial demonstrated that FMT was superior to vancomycin in treating recurrent CDI when administered colonoscopically.<sup>13</sup>

These and other studies have helped to establish optimal practical aspects related to FMT administration (Table 3). FMT appears generally safe with mild, self-limiting gastrointestinal and constitutional symptoms being the most common complications described. However, there have been a small number of potential transmissions of infection, perforations associated with treatment administration, and even deaths related to its use (eg through aspiration of transplant material).<sup>14</sup> As a result of these clinical studies (coupled with other studies demonstrating its cost effectiveness), FMT has now been accepted as an approved treatment for recurrent or refractory CDI by NICE<sup>15</sup> and in PHE guidelines.<sup>8</sup> FMT is now available as treatment for CDI within a growing number of UK centres, but the expense of establishing and maintaining a service, and ongoing discussions about the regulation and governance of FMT within the UK, have impacted upon more rapid adoption.<sup>16</sup>

Given the obvious potential drawbacks of FMT (including its unpalatable nature and the invasive means of administration), there is great interest in refining FMT from its current state into a more acceptable pill or drink. 'Capsulised' FMT (in which faecal slurry or freeze-dried stool is placed into capsules) is now starting to become available and has recently been demonstrated to be of similar efficacy to colonoscopic FMT in a large randomised trial.<sup>17</sup>

### Other novel approaches

A number of novel approaches for the treatment of CDI are at various stages of development; these are summarised in Table 4.

### Conclusions

AAID and CDI remain a common problems. Many cases are mild, but a significant number – especially of CDI – can be difficult to treat and potentially life-threatening. Developments in our understanding of the importance of the gut microbiota, and the marked efficacy of FMT in clinical trials, have raised the profile of CDI research and the search for more refined, targeted therapies. ■

**Table 3. Practical aspects of faecal microbiota transplantation (FMT). (Adapted from Mullish *et al*<sup>18</sup> and Cammarota *et al*<sup>19</sup>)**

Criteria	Details	
Donor selection	Screening questionnaire	People >60 years of age, those with recent antibiotic use (within six weeks), significant risk factors for blood-borne viruses, recent constitutional illness, and/or recent travel to countries with high rates of infectious diarrhoea are typically excluded from further assessment
	Medical questionnaire	People with a personal or family history of disorders associated with perturbation of the gut microbiota (including inflammatory bowel disease, irritable bowel syndrome, metabolic syndrome, autoimmune disorders and/or gastrointestinal malignancy) are typically excluded from further assessment
	Laboratory testing, repeated at regular intervals, typically includes:	<p>Blood screening</p> <ul style="list-style-type: none"> <li>&gt; Hepatitis A serology</li> <li>&gt; Hepatitis B serology and hepatitis B DNA</li> <li>&gt; Hepatitis C serology and hepatitis C RNA</li> <li>&gt; HIV-1 and -2 and HTLV-1 and -2 serology</li> <li>&gt; Syphilis serology</li> <li>&gt; Cytomegalovirus and Epstein-Barr virus serology</li> <li>&gt; <i>Entamoeba histolytica</i> serology</li> <li>&gt; <i>Strongyloides stercoralis</i> serology</li> </ul> <p>Stool screening</p> <ul style="list-style-type: none"> <li>&gt; Microscopy, culture and sensitivity for enteric bacteria (at least three samples over three separate days)</li> <li>&gt; Analysis for ova, cysts and parasites (at least three samples over three separate days)</li> <li>&gt; Acid-fast staining for <i>Cyclospora</i>, <i>Isospora</i> and <i>Cryptosporidium</i></li> <li>&gt; Screening for carriage of antibiotic-resistant organisms, carbapenemase-producing <i>Enterobacteriaceae</i></li> <li>&gt; <i>Clostridium difficile</i> toxin and polymerase chain reaction (PCR)</li> <li>&gt; Analysis for rotavirus and adenovirus</li> <li>&gt; <i>Helicobacter pylori</i> faecal antigen</li> </ul>
Choice of recipients	Indications	<ul style="list-style-type: none"> <li>&gt; Recurrent or relapsing CDI</li> <li>&gt; Limited data regarding efficacy in severe or fulminant CDI colitis</li> </ul>
	Contraindications	<ul style="list-style-type: none"> <li>&gt; There are only a small number of absolute contraindications, eg a life-threatening food allergy</li> <li>&gt; FMT seems to have similar efficacy (and no additional infective complications) when given to immunocompromised individuals, including solid organ transplant recipients, recipients of recent chemotherapy, people with HIV infection and people with inflammatory bowel disease (IBD) taking B/T cell immunomodulatory therapy<sup>20</sup></li> </ul>
Transplant preparation	<ul style="list-style-type: none"> <li>&gt; Typically &gt;50–60 g of stool is mixed with 250–300 mL of diluent (most often saline), before filtering of the slurry. The exact protocols used vary between different centres</li> <li>&gt; Transplants are either prepared from fresh stool on the day of delivery, or prepared in advance and frozen (using glycerol as a bacterial cryopreservative) before thawing on the day<sup>21</sup></li> </ul>	
Transplant delivery	<ul style="list-style-type: none"> <li>&gt; The transplant may be delivered either via the upper gastrointestinal route (gastroscopy, or nasogastric or nasoenteric tube) or by the lower gastrointestinal route (enema or colonoscopy). FMT capsules are an emerging delivery modality</li> <li>&gt; Anti-CDI antibiotics are typically stopped 12–72 hours prior to transplant; a polyethylene glycol preparation may be given on the day prior to administration</li> <li>&gt; A proton-pump inhibitor (PPI) and/or a prokinetic (ie metoclopramide) are often administered prior to upper gastrointestinal administration. Loperamide is often administered after lower gastrointestinal administration to facilitate retention within colon</li> </ul>	

**Table 4. Novel approaches for the treatment of *Clostridium difficile* infection (CDI)**

Approach	Details	
Antimicrobials	Antibiotics	A number of novel antibiotics are in clinical trials
	Ribaxamase	This is a beta-lactamase that may be co-administered with systemic broad-spectrum antibiotics, with the aim of degrading the antibiotics in the gut before they can affect the gut microbiota and create a propensity to CDI
Immunisation	Passive immunisation	Co-administration of bezlotoxumab (an anti-toxin B antibody) together with conventional antibiotic therapy has been shown to reduce the rate of recurrence of CDI compared to antibiotic therapy alone <sup>22</sup>
	Active immunisation	Anti-toxin vaccines are now in clinical trials. However, early data suggest reduced seroconversion in older people, those most at risk of CDI
Microbiome manipulation	Non-toxicogenic <i>C difficile</i> <sup>23</sup>	In a clinical trial, non-toxicogenic <i>C difficile</i> was administered to those with CDI, with the aim of outcompeting toxigenic <i>C difficile</i> from its reservoir within the gut. CDI recurrence rates were 30% in those receiving placebo in comparison with 11% in those receiving non-toxicogenic <i>C difficile</i>

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