The management of *Clostridium difficile* infection

O. Martin Williams and Robert C. Spencer*

Health Protection Agency Regional Laboratory South West, Level 8, Queens Building, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW, UK

**Introduction/background:** *Clostridium difficile* is the commonest cause of nosocomial diarrhoea. The epidemiology and clinical phenotype of the disease has dramatically changed with the global emergence of a virulent strain of *C. difficile*.

**Source:** This review was compiled using data from individual studies and review articles identified from PubMed. The retrieved articles were also examined for additional references.

**Areas of agreement:** Appropriate and timely infection control measures are required to control *C. difficile* infection (CDI) in the hospital environment, and either oral metronidazole or vancomycin remains the mainstay of treatment depending on the severity of infection.

**Areas of controversy:** The optimal method for diagnosing CDI remains unclear, as does the best therapeutic strategy for the management of multiple relapses.

**Growing points/areas timely for developing research:** Studies of new antimicrobial agents with activity against *C. difficile* are required to improve the management of multiply relapsing disease. The use of novel therapeutic approaches that do not require antimicrobials requires urgent research, including the use of immunological or vaccine-based regimen, bacteriotherapy or *C. difficile*-specific bacteriophages.

**Keywords:** *Clostridium difficile*/risk factors/treatment

**Introduction**

*Clostridium difficile* is a Gram-positive anaerobic bacillus that has become the most commonly identified cause of nosocomial gastroenteritis. First described in 1935, it remained a laboratory curiosity until the 1970s when the role of *C. difficile* in pseudomembranous colitis (PMC) was realized. It can cause a spectrum of disease from asymptomatic colonization to severe diarrhoea, PMC, toxic megacolon, colonic perforation and death. *Clostridium difficile* associated extra-intestinal disease, including reactive arthritis, has been described, but is rare. The organism is widely distributed in soil and the intestinal tracts of animals, and is capable of forming spores, which are resistant
to heat, desiccation and chemical agents including gastric acid and disinfectant.

Pathogenesis

Toxins A and B are the main virulence factors of *C. difficile* and non-toxigenic strains are non-pathogenic. The two toxins are transcribed from a five gene pathogenicity locus: two toxin genes *tcdA* (Toxin A) and *tcdB* (Toxin B) together with three regulatory genes one of which *tcdC* encodes a probable negative regulator of toxin transcription. The two toxins bind to the surface of intestinal epithelial cells, are then internalized whereupon they catalyse the glucosylation of cytoplasmic Rho proteins, which ultimately lead to cell death.8

Laboratory diagnosis

The ‘gold standard’ for the laboratory diagnosis of *C. difficile* infection (CDI) is the detection of faecal toxins using tissue culture cell lines such as African green monkey kidney (Vero) cells. Cytopathic effects are observed, which can be neutralized with specific antiserum.9 However, because many laboratories lack tissue culture facilities, commercial enzyme immunoassays are now commonly used. The majority detect toxin A10 but now kits that can detect both toxins A and B are recommended because not only do they have apparent increased sensitivity over toxin A alone kits, but because of their ability to detect disease caused by strains of *C. difficile*, which only produce toxin B.11 The sensitivity (range 0.75–0.99) and specificity (range 0.92–1.0) of the commercially available assays are reasonable,12 but they offer the advantage of allowing laboratories to give a same day diagnosis.

Glutamate dehydrogenase (GDH), also called the common antigen, is produced constitutively by all *C. difficile* isolates. Commercial assays for the detection of GDH are available, although early GDH-based tests cross-reacted with other bacterial species including *Clostridium sporogenes*, proteolytic *Clostridium botulinum* and *Peptostreptococcus anaerobius*.13 As tests that utilize GDH are unable to differentiate between toxin-producing and non-toxigenic *C. difficile* isolates, a two-stage testing protocol has been proposed.14 Stool samples would be initially screened using GDH-based assays. As these tests have a high negative predictive value (>99%),14–16 a negative result would reliably exclude a diagnosis of CDI. A positive GDH test would require further confirmation using culture and/or toxin-based detection methods. The introduction of a two-stage testing protocol would incur additional
time and cost pressures for a laboratory, although these could be offset by reducing unnecessary antibiotic and infection control measures, and streamlining the use of isolation facilities in a hospital.

Lactoferrin, a glycopeptide that is expressed by activated neutrophils, has been shown to be significantly elevated in the faeces of patient with inflammatory bowel conditions, including severe CDI.\(^{17,18}\) Faecal lactoferrin measurements, in conjunction with other tests for \textit{C. difficile}, may be useful to discriminate between patients with advanced disease and mild CDI or \textit{C. difficile}-colonized individuals.\(^{16}\)

Isolation of \textit{C. difficile} by culture is unnecessary for laboratory diagnosis but essential for epidemiological typing. Enhanced detection can be achieved by use of media with the selective agents cycloserine and cefoxitin together with selection of \textit{C. difficile} spores by alcohol treatment.\(^{19}\)

**Changing epidemiology**

Recently, there has been a significant change in the incidence and severity of disease associated with \textit{C. difficile}. This was first highlighted following a review of 1721 cases of \textit{C. difficile}-associated diarrhoea (CDAD) from Sherbrooke, Quebec.\(^{20}\) The authors reported a 4-fold increase in the annual incidence of CDI over a 13 year period, from 35.6 per 100 000 population to in 1991 to 156.3 per 100 000 population in 2003.\(^{20}\) The greatest increase in incidence was observed in patients \(>65\) years of age (from 102.0 to 886.5 per 100 000 population), associated with an increase in disease severity and mortality.\(^{20}\)

A recent health statistics report from the UK reported that the number of deaths associated with \textit{C. difficile} had increased from 975 in 1999 to 2246 in 2004,\(^{21}\) due, in part, to improved laboratory testing and reporting. This also coincided with high profile outbreaks of CDI at Stoke Mandeville Hospital (Buckinghamshire Hospitals NHS Trust) between October 2003 and June 2004 involving 174 cases and 19 (11\%) deaths that were definitely or probably due to \textit{C. difficile}, and a second outbreak occurring between October 2004 and June 2005, involving 160 new cases and 19 (12\%) further deaths.\(^{22,23}\)

The major shift in the epidemiology of hospital-acquired CDI can be attributed, in part, to the emergence of a virulent new strain of fluoroquinolone-resistant \textit{C. difficile}, variably designated PCR ribotype 027, North American pulsed field type 1 (NAP1), restriction enzyme analysis type ‘B1’ and ‘toxinotype III’ depending on the typing methodology used.\(^{24}\) The NAP1/B1/027 strain has been associated with the recent outbreaks reported in Canada, USA, UK, France, Belgium and the Netherlands.\(^{25–27}\) In addition to the toxin A and B (\textit{tdcA} and \textit{tdcB})
genes recognized as the main virulence factors of \textit{C. difficile}, the NAP1/B1/027 strain has an 18-bp deletion within the \textit{tcdC} gene, and has been shown \textit{in vitro} to produce between 16 and 23 times more toxin A and B than other strains of \textit{C. difficile}, respectively.\textsuperscript{28} NAP1/B1/027 also possesses another potential virulence determinant designated the binary toxin (\textit{cdtA} and \textit{cdtB}).\textsuperscript{24} This binary toxin is unrelated to the pathogenicity locus, which encodes for toxins A and B. It is homologous to the \textit{iota} toxin of \textit{Clostridium perfringens} and composed of a 48-kD enzymatic component and a 99-kD binding structure. The binary toxin has enterotoxigenic activity in its own right, but its exact role in the pathogenesis of CDI remains unclear, as strains which produce binary toxins in the absence of classical A and B toxin appear to be non-toxigenic. However, the observation that NAP1/B1/027 strains have increased pathogenicity raises the spectre that binary toxins acts in synergy with toxins A and B in causing a more severe form of colitis with a corresponding increase in morbidity and mortality.\textsuperscript{28}

\section*{Risk factors for CDI}

The combination of nosocomial exposure to \textit{C. difficile} spores and the disruption of the normal gastrointestinal microflora, which forms an essential protective barrier preventing colonization and subsequent infection by opportunistic pathogens can result in CDI.\textsuperscript{29} The major contributors to the disruption of the colonic microflora are antibiotics, but other medications or procedures such as intestinal surgery also increase the risk of CDI. A history of treatment with antimicrobial or antineoplastic drugs within the previous 3 months is present in virtually all patients who develop CDI.\textsuperscript{30–32} Virtually every class of antibiotics, apart from the aminoglycosides, have been implicated as a risk factor for CDI with the greatest risk being attributed to second and third generation cephalosporins.\textsuperscript{33–35} Clindamycin has also been implicated as a significant risk factor for many years, compounded by a multi-state outbreak of clindamycin resistant \textit{C. difficile} in the USA.\textsuperscript{36} Fluoroquinolones were, until recently, perceived to be low risk for CDAD.\textsuperscript{37} This belief has recently been challenged. The earliest report of a significant association between ciprofloxacin usage and CDI came as part of a quality assurance project to reduce the rates of CDI in Ontario.\textsuperscript{38} This association has subsequently been confirmed by at least four other studies,\textsuperscript{39–42} with the risk increasing with longer courses of therapy (adjusted hazard ratio for 1–3 days of therapy was 2.42 [95\% confidence interval (CI): 1.62–3.62] increasing to 4.33 (95\% CI: 3.21–5.84) if the duration of therapy was \geq 7 days).\textsuperscript{42}
The duration of treatment also appears to be a significant risk factor for other antibiotic agents, although the estimated risk of developing CDI even after a single dose of peri-operative prophylaxis was 14.9 per 1000 surgical procedures. Recently, intravenous vancomycin has also been implicated as a risk for CDI. Contrary to popular belief, intravenous vancomycin given for ≥5 days can produce detectable levels in bile [mean 7.6 µg/ml (range 3.0–20.8 µg/ml)], and has been shown to alter gastrointestinal flora in mice.

The current increase in the incidence of CDI also appears to coincide with a widespread increased use of proton-pump inhibitors (PPI). A recent systematic review found a positive association between PPI use and CDI [pooled odds ratio (OR) of 1.94 (95% CI: 1.28–3.00)]. There was also a similar trend with the use of the less potent H2 receptor antagonists [OR: 1.40 (95% CI: 0.85–2.29)]. Others have failed to demonstrate an association between antacid therapy and CDI and this issue remains controversial. Although C. difficile spores are resistant to gastric acid, vegetative C. difficile are not but have been shown to survive in hypochlorhydric gastric juice from a patient with pernicious anaemia and in the gastric contents of patients taking PPI with a pH >5. In a hamster model, approximately 80% of ingested spores germinated within 1 h, although it is unclear where or how quickly germination occurs in humans. Similarly, vegetative C. difficile has been shown to remain viable for up to 6 h on moist surfaces, making the observed association between PPI use and susceptibility to CDI biologically plausible.

**Treatment/prophylaxis of asymptomatic carriers**

Asymptomatic colonization is very common in healthy newborns and infants ranging from 15 to 80%, although it is only rarely associated with overt disease. It has been postulated, based on a rabbit model, that the apparent lack of pathogenicity in neonates is due to the absence of C. difficile toxin receptors in the infant colon. This, however, remains unproven in humans, and does not appear to be the case in an infant hamster model. Colonization rates in children drop to levels comparable with healthy adults (<4%) by approximately 2 years of age. In contrast, asymptomatic carriage rates of 20–50% have been reported in patients in long-term health care facilities.

Because of the concerns that asymptomatic carriers of C. difficile can contribute significantly to nosocomial transmission of C. difficile in long-term facilities, some investigators have adopted a policy of treating asymptomatic carriers. On a leukaemia unit with high rates of infection, the frequency of CDI fell from 16.3 to 3.9% after all...
symptomatic and asymptomatic *C. difficile* carriers were treated with vancomycin. Others, however, have failed to demonstrate significant benefit of giving metronidazole to asymptomatic carriers on the frequency of CDI. Johnson *et al.* treated 30 asymptomatic carriers in a randomized control trial with either vancomycin (125 mg qds), oral metronidazole (500 mg bd) or placebo for 10 days. Immediately after completion of therapy, the rates of *C. difficile* carriage were 10% in the vancomycin group, 70% in the metronidazole group and 80% in the placebo group (*P* = 0.02). After a 2-month follow-up period significantly more patients in the vancomycin treated group had re-acquired *C. difficile* (67%) compared with those who had received placebo (11%) (*P* < 0.05). In addition, one asymptomatic patient who was originally colonized with a non-toxigenic strain of *C. difficile* became re-infected and symptomatic with a toxigenic strain after treatment with vancomycin.

Some clinicians have also adopted a policy of giving prophylactic metronidazole or oral vancomycin to asymptomatic patients when an antibiotic treatment is administered. Although this might seem logical, exposure to any antimicrobial increases the risk of CDI, and for patients with established CDI, antimicrobial exposure increases the likelihood of relapse. Prophylaxis also increases the cost of treatment and may promote resistance to metronidazole and vancomycin in other bacteria.

**Management of first episode of CDI (Fig. 1)**

The onset of unexplained diarrhoea, defined on the Bristol Stool Chart as types 5–7, especially in the elderly and vulnerable populations who have received broad-spectrum antibiotics, should alert the clinician to the possibility of CDI. Similarly, a sudden and unexplained increase in the white cell count (WCC), or deterioration in renal function in this cohort of patients, despite an absence of diarrhoea, should also prompt urgent investigations into the possibility of ‘silent’ CDI. A stool sample should be sent immediately for *C. difficile* toxin testing, and empiric therapy for CDI should be considered in the light of clinical presentation pending the results of this test. More than one test per patient, repeated after 24 h, may be required if the first test is negative, especially where the clinical suspicion of CDI is high. Other diagnostic procedures, including sigmoidoscopy or abdominal computerized tomography may be required in patients with ‘silent’ CDI.

Once CDI has been suspected, the inciting antibiotic(s) should be discontinued if possible. In the past, this strategy combined with supportive care, resulted in resolution of symptoms in up to 25% of
Fig. 1 Therapy for patients with diarrhoea with either (a) a positive *C. difficile* toxin (CDT) test or (b) pending CDT test results deemed at risk of CDI (for first or second episode of *C. difficile* infection). The severity of CDI can be graded as non-severe (mild or moderate), severe or life-threatening disease. Mild CDI is characterized by a well patient with normal inflammatory markers and <3 type 5–7 stools/day. Moderate disease is characterized with 3–5 type 5–7 stools/day, with a WCC \(< 15 \times 10^9/\text{l}\) and a CRP \(< 150 \text{ mg/l}\). Severe disease is characterized by the presence of any of: a WCC \(> 15 \times 10^9/\text{l}\); a temperature \(> 38.5 \text{ C}\); an acute rise in serum creatinine (\(> 50\%\) above base line); a C-reactive protein \(> 150 \text{ mg/l}\); evidence of severe colitis on clinical or radiological investigations. Life-threatening disease includes features of severe disease with hypotension, or partial or complete ileus or toxic megacolon, a serum lactate \(\geq 5 \text{ mmol/l}\), colitis in neutropaenia, perforations of the colon or sepsis. Footnote 1: Intra-colonic vancomycin 500 mg in 100–500-ml saline given as a retention enema (18 gauge Foley catheter with 30-ml balloon inserted per rectum). The vancomycin is instilled and the catheter clamped for 60 min. After this the catheter is unclamped, the balloon deflated and the catheter removed. Repeat 4–12 hourly depending on severity of CDI. Footnote 2: Colectomy is best performed before the serum lactate \(\geq 5 \text{ mmol/l}\). Total or subtotal colectomy rather than hemi-colectomy or caecostomy is preferred.
patients without the need for further antibiotics. However, withholding specific therapy must be done with caution because of the concerns about the rapid deterioration of patients with CDI associated with the epidemic strain. If the inciting antibiotic cannot be stopped, then other agents that are less likely to drive the CDI should be given for the shortest possible duration.

Supportive care should be given with meticulous management of fluid and electrolyte losses, and early consideration for nutritional support. An assessment of severity should be made, although there are no validated scoring systems of severity in CDI. Markers that are commonly used to define severe CDI are age (>65 years), a significantly elevated leukocyte count at diagnosis, and deteriorating renal function. Louie et al. defined severe CDI as the presence of either more than 12 stools in 24 h, severe, persistent abdominal pain or distension (>2 h in duration), two or more episodes of vomiting, or a temperature >38.9°C. However, a definition of severity based on the number of diarrhoeal stools may suffer from difficulties in the recording of such episodes, particularly in the elderly. Also, severe CDI may be complicated by an ileus with no diarrhoea. In contrast, Zar et al. defined severe CDI as the presence of two or more of age >60 years, temperature >38.3°C, albumin level <2.5 mg/dl and peripheral WBC count >15,000 cells/mm. Patients with endoscopic evidence of PMC or treatment in the intensive care unit for CDI were also defined as severe. However, the inclusion of age may be too non-specific as a predictor of severity.

Recent guidance from the Department of Health has suggested that the presence of either a WCC >15 x 10⁹/l, an acutely rising blood creatinine (>50% increase above baseline), a temperature >38.5°C or evidence of severe colitis either by abdominal signs or radiology should be considered as severe CDI, and treated accordingly.

A recent meta-analysis of antibiotic treatments for CDI was unable to make specific recommendations about the best form of therapy because of the quality of the published studies. The choice of initial antibiotic therapy depends on the severity of disease. Traditional treatments for CDI have relied on the use of either oral vancomycin or metronidazole, two agents known to be bactericidal for C. difficile. A variety of other antibiotics have been used, including teicoplanin, rifampicin, fusidic acid and bacitracin, with clinical and bacteriological outcome measures that were more or less equivalent to either metronidazole or vancomycin.

Oral vancomycin has been used for the treatment of PMC since the 1950s, when the suspected pathogen was Staphylococcus aureus. Vancomycin is poorly absorbed when administered orally, and results in faecal concentrations that are generally in excess of 1000 µg/g
dose of 125 mg qds), which are maintained throughout the course of treatment. Although 3.1% of isolates in a Spanish study appeared to have reduced susceptibility to vancomycin (minimum inhibitory concentration (MIC) 4–16 μg/ml), true resistance to vancomycin (MIC >32 μg/ml) has not been reported, and the colonic levels achieved after oral administration are usually >100-fold higher than the highest MIC recorded.

Metronidazole is rapidly and completely absorbed from the small bowel when administered orally, and up to 15% of its metabolites are excreted in faeces. Faecal concentrations of metronidazole are undetectable in healthy volunteers, but achieve concentrations of 9.3 μg/g in watery stool, subsequently dropping to 1.2 μg/g in formed stool. When administered intravenously the concentrations of metronidazole in faeces are comparable with levels achievable following oral administration. Generally, most strains of *C. difficile* are sensitive to metronidazole with median MICs ≤1.0 μg/ml, although rates of resistance (MIC >32 μg/ml) of 6.3% have been reported in one study.

As early studies found that metronidazole compared favourably with vancomycin and because of the significant cost saving associated compared with vancomycin, metronidazole has become the usual first choice for treatment of CDI. Similarly, there have been concerns about the over-use of vancomycin promoting the spread of vancomycin-resistant organisms. Interestingly, recent studies have suggested that the rates of colonization with vancomycin-resistant enterococci (VRE) were no different between patients treated with either oral vancomycin or metronidazole.

For the reasons described, and because it appears to be as effective as vancomycin for non-severe CDI, metronidazole should remain the first-line treatment for mild-to-moderate disease (400–500 mg tds for 10–14 days). Patients should be reviewed daily, paying particular attention to the fluid and electrolyte balance. If a patient has failed to improve, or shows signs of deterioration by day 5, then the metronidazole should be switched to oral vancomycin of 125 mg qds.

Recent publications have suggested that the use of metronidazole is associated with increased rates of treatment failure and relapse. In a small, randomized control trial comparing metronidazole (250 mg qds) with vancomycin (125 mg qds), the two agents had similar response rates in mild infection (90 versus 98%, respectively; \( P = 0.36 \)). In contrast, vancomycin was significantly more effective than metronidazole in patients with severe CDI (97 versus 76%; \( P = 0.02 \)), although the relapse rates were not significantly different between the two agents regardless of initial disease severity. Similarly, a prospective randomized control trial, published in abstract form, of
tlevam and versus vancomycin versus metronidazole showed similar results. Of note, the study by Zar et al. enrolled patients between 1994 and 2002, before the emergence of the BI/NAP1/027 epidemic strain and stratification of clinical response according to *C. difficile* genotype in the tlevam and study is awaited.

A retrospective review was conducted in order to examine the effects of the introduction of epidemic BI/NAP1/027 strain on treatment outcomes. For the period between 1991 and 2002, before the emergence of the epidemic BI/NAP1/027 strain, treatment with vancomycin was associated with a lower probability of developing severe/complicated CDI compared with metronidazole [adjusted OR: 0.21 (95% CI: 0.05–0.99)]. However, for the period 2003–2006, when BI/NAP1/027 was the predominant strain, the perceived benefit of vancomycin over metronidazole was lost [adjusted OR: 0.90 (95% CI: 0.53–1.55)].

Oral vancomycin (125 mg qds) should be given as the first-line agent for patients with severe CDI. In an effort to reduce costs, some hospitals have opted to administer the intravenous form of vancomycin orally, and apart from an unpleasant taste it is likely to be equally effective as the vancomycin Matrigel capsules. Patients with fulminant infection with ileus or toxic mega-colon should also receive intravenous metronidazole 500 mg tds. Intra-colonic vancomycin has been advocated by some for the treatment of life-threatening disease. Higher doses of vancomycin (≥2 g/day) have been recommended for severe CDI, although the vancomycin concentrations achieved after administration of 125 mg qds are >100-fold higher than the highest MIC recorded. Also, there appears to be no added benefit from the increased dose during the first episode or recurrent CDI.

Some clinicians use combination of antibiotics, including giving metronidazole with vancomycin, although there are no clinical trials to support this strategy. Although the addition of rifampicin (600 mg bd) to vancomycin (125 mg qds) for 7 days resulted in a good response in seven patients with multiple relapses of CDI, the addition of rifampicin to metronidazole failed to improve the time to symptom resolution, or reduce the rate of relapse, compared with metronidazole alone.

Fusidic acid is sometimes used alone, or in combination with either vancomycin or metronidazole for the treatment of CDI. A prospective randomized, double-blind trial comparing metronidazole 400 mg tds (*n* = 55) with fusidic acid 250 mg tds (*n* = 59) for 7 days found that there was no significant difference in respect of clinical cure and recurrence between the two groups, although the rates of fusidic acid resistance in patients who remained culture-positive was 55%. The high rates of post-treatment resistance to fusidic acid is likely to limit its usefulness.
A clinical dictum, perpetuated by expert opinion, has emerged that anti-motility agents (e.g. loperamide) should not be used in patients with CDI. A recent review of the literature found 55 patients with CDI who were given anti-motility agents. Seventeen patients who experienced complications or died after receiving anti-motility agents did not receive appropriate initial antibiotic therapy for CDI. However, 23 patients who received either metronidazole or vancomycin co-administered with the anti-motility agent experienced no complications. This data should be interpreted cautiously, pending the results of further study. Anti-motility agents could be considered in patients with prolonged symptoms despite treatment (>20 days), who have no markers of severity (normal WCC and CRP, afebrile, with no abdominal pain or distension), although the patients should be monitored closely for early signs of complications.

Patients who develop signs of toxic megacolon (dilation >10 cm), perforation, or shock should be considered for colectomy. Although the mortality in this group remains high despite surgery (up to 48%), a subgroup of individuals may benefit from emergency colectomy (age >65 years, immunocompetent, with a leukocytosis ≥20 × 10⁹/l). Surgery should be performed before the blood lactate rises above 5 mmol/l. Conversely, patients who were either >75 years, immunosuppressed, had a WCC >50 × 10⁹/l, had mental status changes, longer trials of medical management before surgery, or significant vasopressor requirements, had higher mortality rates after surgery. Total or subtotal colectomy should be performed, rather than a hemicolectomy or caecostomy because of the risk of disease recurrence. If disease recurs in a rectal stump that has been preserved for subsequent ileo-rectal anastomosis, then topical vancomycin could be administered.

**Treatment of relapses (Fig. 2)**

Between 20 and 25% of patients experience a recurrence of symptoms after the initial episode of CDI has resolved, although it is unclear whether this is because of re-activation of disease or re-infection. Up to 45% of those who have had one recurrent CDI episode will go on to suffer further recurrences, and up to 65% will relapse after two or more recurrences. A recent meta-analysis has demonstrated that the most important risk factors for recurrent disease are the continued use of non-C. difficile antibiotics after diagnosis of CDI [OR: 4.23 (95% CI: 2.10–8.55; \( P < 0.001 \)], older age (>65 years) [OR: 1.62 (95% CI: 1.11–2.36; \( P = 0.0012 \)] and concomitant receipt of antacid medications [OR: 2.15 (95% CI: 1.13–4.08; \( P = 0.019 \)]. Patients...
receiving therapy for CDI whilst taking a PPI have been shown to be more than four times more likely to relapse compared with patients not taking a PPI.99

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**Fig. 2** Therapy for patients with diarrhoea with 3 or more episodes of CDI. Footnote 3: Tapering/pulsed therapy: vancomycin 125 mg po qds for 1 week, vancomycin 125 mg po tds for 1 week, vancomycin 125 mg po bd for 1 week, vancomycin 125 mg po od for 1 week, vancomycin 125 mg po od every other day for 1 week, vancomycin 125 mg po od every third day for 1 week, then stop.
It is important to remember that not all patients who develop diarrhoea after the discontinuation of metronidazole or vancomycin have recurrent CDI. Other conditions, including post-infectious irritable bowel syndrome, microscopic colitis or inflammatory bowel diseases should be considered. Similarly, patients who fail to respond to prolonged treatment with metronidazole or vancomycin should have other causes of diarrhoea excluded. A repeat toxin test may not be helpful, as a positive result may reflect colonization only. If diagnostic doubt exists then sigmoidoscopy or colonoscopy is helpful.

Once a recurrence of CDI has been diagnosed, it is essential to stop any precipitating antibiotic. Similarly, the role of antacid medication should be reviewed, and discontinued if not deemed essential. An assessment of severity should be made, but in general, as resistance to metronidazole and vancomycin is uncommon the first episode of recurrence should be treated with the same agent that was successful for the treatment of the first episode100 (Fig. 1). Subsequent recurrences should be treated with oral vancomycin (Fig. 2). There are no proven or standard treatment regimens for multiple relapses of CDI, although tapering or pulsed dosing of vancomycin has gained popularity. Administering extended treatment courses with a reducing dose (tapering regime) or intermittent dosing (pulsed regime) is believed to eradicate C. difficile carriage by allowing spores to germinate, and may aid restoration of the normal colonic microflora. Tedesco et al.101 reported a case series of 22 patients with recurrent CDI successfully treated with a tapering regimen of vancomycin (500 mg/day for 1 week, 250 mg/day for 1 week, then 125 mg/day for 1 week) followed by a pulsed dose regimen of vancomycin (125 mg every third day for 21 days). Similarly, a tapering dose of vancomycin (over a mean of 21 days) or a pulsed dosing of vancomycin (125–500 mg every third day over a mean of 27 days) resulted in significantly fewer relapses compared with a standard vancomycin regimen (1 g/day for 10 days) (31, 14.3 and 54%, respectively).85 Although the results of these observational studies are encouraging there is a concern, particularly for pulsed dosing, that such treatment may promote the emergence of resistant strains of C. difficile.

It has been proposed that patients who develop recurrent CDI do so because they fail to mount a protective immune response to C. difficile and its toxins.102–104 Commercial preparations of pooled human immunoglobulin have been shown to contain IgG that can neutralize toxins A and B, although there are batch-to-batch variations in activity. Accordingly, passive immunization against C. difficile toxins has been attempted in patients with multiple relapses of CDI. Although a variety of dosing strategies have been given with favourable outcome, no data from randomized control trials are available. A single dose of 400 mg/kg
of pooled human immunoglobulin can be given for severe or refractory CDI, and repeated after 21 days.

In an effort to repopulate the colonic microflora, mono- or mixed-cultures of live microorganisms have been given either as prophylaxis, or as treatment of CDI. *Lactobacillus* species (vancomycin resistant Gram-positive rods) or *Saccharomyces boulardii* (yeast) have been the focus of most research. Although a recent randomized, double-blind, placebo-controlled trial showed a beneficial effect of a proprietary probiotic *Lactobacillus* preparation as prophylaxis against antibiotic associated diarrhoea,\textsuperscript{105} the methodological flaws in the study prevents the generalizability of the results. Two recent meta-analyses failed to find sufficient evidence to recommend the use of probiotics as prophylaxis\textsuperscript{106} or as an adjunct to antibiotic therapy for CDI.\textsuperscript{106,107} In addition there are concerns about the administration of live microorganisms to compromised patients. A randomized, double-blind, placebo-controlled trial of a proprietary probiotic *Lactobacillus* preparation failed to show a reduction in infectious complications when given as prophylaxis to patients with severe pancreatitis and was associated with an increased risk of mortality.\textsuperscript{108} Similarly, the use of *S. boulardii* has been associated with invasive disease in compromised patients,\textsuperscript{109,110} and their widespread use is not recommended.\textsuperscript{111}

An alternative strategy to restore resistance to recolonization has been through the administration of non-toxigenic strains of *C. difficile*. This approach was effective in animal models,\textsuperscript{112–114} and in two patients who had multiple relapses of CDI.\textsuperscript{115} Another bacteriotherapy approach has been to administer faeces from healthy donors to patients with relapsing CDI.\textsuperscript{116} Fresh stool from a healthy donor is homogenized with normal saline, and can be administered as either an enema or via a nasogastric tube. The preferred stool donors are individuals who have intimate physical contact with the recipient (spouse or significant partner), or family household members, although if none is available then stool from any other healthy volunteer could be used. Family members are preferred donors as they are likely to share the same environment as the recipient, which would theoretically reduce the risk of introducing potentially new bacterial pathogens into an already compromised host.\textsuperscript{117} A number of case reports suggest that the success rate is high (up to 90%),\textsuperscript{117} although there are no comparative studies to verify its effectiveness.

**Potential future therapies**

With the recent increase in the incidence and severity of CDI other novel therapies have been examined. Itazoxanide, (2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide, is a nitrothiazole benzamide compound,
that is approved for the treatment of cryptosporidiosis and giardiasis.\textsuperscript{118} This drug is active against a variety of parasitic and bacterial pathogens, and has been shown to have \textit{in vitro} and \textit{in vivo} antimicrobial activity against \textit{C. difficile}.\textsuperscript{119}

A randomized double-blinded, controlled study comparing metronidazole (250 mg qds for 10 days) with nitazoxanide (500 mg bd for 7 days or 10 days) demonstrated the non-inferiority of nitazoxanide for the treatment of moderately severe to severe \textit{C. difficile} colitis.\textsuperscript{120} Also, 66\% of patients who had failed to respond to treatment with metronidazole had a sustained responded with nitazoxanide (500 mg bd for 10 days).\textsuperscript{121} In a recent small double-blinded study, patients were randomized to receive either oral vancomycin (125 mg qds) or nitazoxanide (500 mg bd) for 10 days for the treatment of moderate-to-severe CDI. Nitazoxanide was shown to be non-inferior to vancomycin with sustained response rates of 89\% for nitazoxanide-treated patients compared with 78\% for vancomycin-treated patients.\textsuperscript{122}

Overall, nitazoxanide may be useful for the treatment of CDI, although economic considerations preclude its use as first-line treatment compared with metronidazole. It may be considered for the treatment of multiple relapses, and although it could be used instead of vancomycin to avoid the risk of selecting vancomycin-resistant organisms in hospitalized patients, further research is required.

Rifaximin is a poorly absorbed broad-spectrum oral antibiotic belonging to the rifamycin family of antibiotics. It has antimicrobial activity against a number of aerobic and anaerobic Gram-positive and Gram-negative bacteria, including \textit{C. difficile}.\textsuperscript{123} It is currently licensed by FDA for the treatment of \textit{E. coli}-induced traveller’s diarrhoea at a dose of 200 mg tds for 3 days.

Rifaximin has been used successfully as a prophylactic and therapeutic agent in animal studies,\textsuperscript{124} and in small studies compared with vancomycin,\textsuperscript{125} or as follow-up therapy to prevent relapse of CDI.\textsuperscript{126} Although initial studies demonstrated a very low incidence of \textit{in vitro} rifaximin resistance in \textit{C. difficile} (<1 \times 10^{-9}),\textsuperscript{123} three resistant strains (MIC >256 \mu g/ml) were found among 110 toxigenic clinical isolates (two from Argentina in 1998 and one from Chicago in 1995).\textsuperscript{127} A rifaximin-resistant isolate (MIC >256 \mu g/l) was also identified in a patient following a course of rifaximin for relapsed CDI\textsuperscript{126} O’Connor \textit{et al.}\textsuperscript{128} reported that 14 of 80 different clinical isolates of \textit{C. difficile} were found to be resistant to rifaximin (MIC >32 \mu g/l). Point mutations in the \textit{rpoB} gene were detected in all the resistant isolates, which also conferred cross-resistance to rifampicin. Interestingly, 9 of the 14 isolates belonged to the BI/NAP1/027 epidemic strain of \textit{C. difficile}. Similarly, Curry \textit{et al.}\textsuperscript{129} reported rates of rifampicin-resistance (MIC > 32 \mu g/l) in \textit{C. difficile} in a single
institution of 36.8%, of which 96.5% belonged to the BI/NAP1/027 epidemic strain. The mutations found in the \textit{rpoB} gene were predicted to confer cross-resistance to rifaximin despite the very high luminal concentrations of rifaximin after oral administration. Although this phenomenon was likely to have represented a clonal outbreak in a single centre, in conjunction with the other data, it raises concerns about the wider use of rifaximin for the treatment of CDI, especially in institutions with high rates of the BI/NAP1/027 epidemic strain.

Rifaxalazil, also known as KRM-1648 or benzoaxazinorifamycin, is a rifamycin derivative related to rifampin and rifabutin that has been shown to be active against \textit{C. difficile in vitro}, and had good prophylactic and therapeutic effects in an animal model. \cite{130}

Ramoplanin, a glycolipodepsipeptide, has been shown to be active \textit{in vitro} against \textit{C. difficile}, \cite{131,132} and in both hamster and \textit{in vitro} gut models of clindamycin-induced CDI. \cite{133} Similarly, oritavancin, a lipoglycopeptide, has been shown to be more active than vancomycin \textit{in vitro} \cite{134} and in an \textit{in vitro} gut model, \cite{135} and is efficacious for treatment and prophylaxis in an animal model. \cite{136}

OPT-80, also known as tiacumicin B, lipiarmycin or PAR-101, is a macrocyclic antimicrobial secreted from the actinomycete \textit{Dactylosporangium aurantiacu}. It appears to have little or no systemic absorption after oral administration and narrow activity spectrum against Gram-positive aerobic and anaerobic bacteria, including \textit{C. difficile}. \cite{127,137} It appears to be well tolerated in phase I and II studies, \cite{138,139} and was associated with a cure rate of 94% when given at a dose of 200 mg twice daily for 10 days. The use of OPT-80 did not significantly reduce the \textit{Bacteroides fragilis} counts, when compared with vancomycin, suggesting it may be beneficial in preventing recurrent disease. \cite{140}

As CDI is a toxin-mediated disease, agents with the potential of binding these targets have been tested. A variety of agents have been tried including cholestyramine, and Synsorb 90, although tolevamer has shown most promise in clinical trials. Tolevamer, also known as GT160-246, is a high-molecular weight inert polymer that was shown to bind efficiently toxins A and B of \textit{C. difficile} both \textit{in vitro} and in animal studies. \cite{141,142} In a phase II study tolevamer, given at a dose of 6 g/day, for mild-to-moderate CDI was shown to be non-inferior to oral vancomycin (500 mg/day), and was well tolerated apart from hypokalaemia. \cite{68} In two subsequent phase III trials, tolevamer was inferior to vancomycin and metronidazole for the resolution of CDI, although the rates of recurrent infection in those who responded to tolevamer (3%) were significantly lower compared with vancomycin and metronidazole (23 and 27% respectively, \(P < 0.001\) for both comparisons). \cite{82}
A variety of novel strategies are at an early stage of development, but may be useful future developments in the treatment of CDI. These including bovine colostrum\textsuperscript{143} and specific human monoclonal antibodies aimed at neutralizing toxin A.\textsuperscript{144} A variety of proteins, including toxins A and B, have been targeted as potential candidates for a \textit{C. difficile} vaccine\textsuperscript{145} and a novel temperate bacteriophage has been identified that possesses an endolysin that is biologically active and capable of lysing cells of \textit{C. difficile},\textsuperscript{146} offering possible alternatives for the prevention and treatment of CDI.

\section*{Infection control}

Much attention has been paid to the prevention of CDI and reports/recommendations concentrate on three main elements of prevention.\textsuperscript{147,148}

(i) Restricted use of antibiotics.

(ii) Strict enteric precautions when caring for any patient with diarrhoea.

(iii) Meticulous cleaning of clinical areas and emphasis on effective handwashing with soap and water \textit{not} the alcohol-hand rubs in widespread use in healthcare facilities.

\section*{Conclusion}

The global landscape of nosocomial CDI has altered radically with the emergence of a virulent strain that is associated with greater morbidity and mortality. Although the traditional treatments remain useful, and newer antibiotics will become available in due course, novel therapeutic strategies that are not reliant on antimicrobial agents will be required to deal with the increasingly complex problems associated with multiply relapsing CDI.

\section*{References}


Management of CDI


