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Recent advances in *Clostridium difficile*-associated disease

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ABSTRACT

The main purpose of this article is to review recent developments in the management of acute and recurrent *C. difficile*-associated disease, with consideration of existing and new antibiotic and non-antibiotic agents for treatment. Details of the current developmental stage of new agents are provided and the role of surgery in the management of severe disease is discussed. Infection control measures considered comprise prudent use of antimicrobials, prevention of cross-infection and surveillance. Other topics that are covered include the recent emergence of an epidemic hypervirulent strain, pathogenesis, clinical presentation and approaches to rapid diagnosis and assessment of the colonic disease.

C. difficile is a Gram-positive, anaerobic spore-forming bacillus that was identified as an aetiological agent of antibiotic-associated pseudomembranous colitis in the late 1970s^{1 2}. It is believed to be responsible for 15-20% of antibiotic-related cases of diarrhoea and nearly all cases of pseudomembranous colitis³. Over the last decade, the incidence of *C. difficile*-associated disease has progressively increased and is now a significant clinical problem in North America and Europe.

EPIDEMIOLOGY

In the United Kingdom, the Health Protection Agency's data for voluntary reporting of positive stool samples show that *C. difficile* infection has been an increasing clinical problem since the early 1990s^{4 5}. Whilst some of the increase may be due to improved reporting, it is widely accepted that there has also been an increase in the number of cases. Mandatory reporting of *Clostridium difficile*-associated disease (CDAD) in people aged 65 years and over has been included in the healthcare-associated infection surveillance system for acute hospital trusts in England since January 2004. There were 51,681 reports of CDAD in people aged 65 years and over in 2006, a 26.2% increase on 2004⁶.

There have also been reports of more cases of CDAD in North America, which have been associated with the use of fluoroquinolones and more severe disease than that seen previously, especially in those over the age of 65 years⁷⁻⁹. Molecular studies in *C. difficile* isolates from a number of US and Canadian healthcare facilities suggest that a more virulent strain of *C. difficile* is responsible for outbreaks of CDAD and also more severe disease^{10 11}. Characteristics of the epidemic strain, designated NAP1/027 are discussed below. In one report, NAP1/027 strain was isolated from 67% with healthcare-associated disease and 37% associated with community-acquired disease¹². The same genotype as NAP1/027 (also known as type BI) has been found in historic isolates obtained before 2001 but was not previously reported to cause either severe disease or outbreaks¹².

C. difficile infection may induce a relapse in patients with inflammatory bowel disease^{13 14}. Two retrospective studies have recently reported a 2- to 3-fold increase in the frequency of *C. difficile* infection in patients with inflammatory bowel disease over a 5- to 6- year period^{15 16}.

Another recent trend has been the reporting of more severe disease in non-traditional hosts such as young seemingly healthy adults and children in the community and some without antimicrobial exposure^{17 18}. Potential sources of *C. difficile* in the community include domestic (dogs, cats) and farm (horses, pigs, cows) animals¹⁹⁻²¹. There have also been recent reports of a marked overlap between isolates from calves

and humans, including the predominant type 027^{22 23}. In a recent Canadian study, *C. difficile* spores were identified in 20% of retail ground meat (beef and veal) samples²⁴. Moreover, a toxigenic strain of *C. difficile* has been isolated from raw turkey-based food for dogs and cats²⁵. Further studies are required to determine the clinical relevance of these findings but they raise the possibility that *C. difficile* could also be transmitted via food contaminated with spores.

RISK FACTORS

In addition to microbial virulence determinants, it is likely that host factors contribute to the increase in the incidence of CDAD. Those over the age of 65 years are particularly susceptible and numbers in this age group have progressively increased over the last century and are predicted to continue to do so²⁶. Disruption of the protective colonic flora (designated colonization resistance) by broad-spectrum antibiotics is the commonest predisposing factor to CDAD and different antibiotics may have distinct effects on not only the gut microbiota but also *C. difficile*²⁷. Immunosuppression has also been shown to be an independent risk factor for developing *Clostridium difficile* infection. Particularly susceptible patients include those exposed to chemotherapeutic or immunosuppressant agents on nephrology, haematology and oncology wards²⁸⁻³⁰.

An association between acid suppression and increased risk of *C. difficile* infection has been reported in a number of studies. In a recent systematic review³¹, there was an increased risk of taking antisecretory therapy in those infected with *C. difficile* (pooled OR 1.94, 95% CI 1.37–2.75). The association was greater for proton pump inhibitor use (OR 1.96, 95% CI 1.28–3.00) compared with the use of H₂ receptor antagonists (OR 1.40, 95% CI 0.85–2.29). Whilst further studies are required to determine whether the association is causal, a potential mechanism could be survival of vegetative forms of *C. difficile* in gastric contents with proton pump inhibitor-induced raised pH³².

PATHOGENESIS

Diarrhoea and colonic inflammation following infection with toxigenic strains of *C. difficile* are believed to be due to two large molecular weight secreted toxins, designated toxin A and B. Some strains of toxigenic *C. difficile* (such as the epidemic NAP1/027 strain) also secrete binary toxin, but its role in disease pathogenesis remains to be determined. The NAP1/027 strain has been reported to produce more toxin A and B than other strains of *C. difficile*¹², which may be due to deletion in the *tcdC* gene that negatively regulates toxin expression. Both of the two large molecular weight toxins (A and B) are cytotoxic to mammalian cells and the role of toxin A in disease pathogenesis has been better characterized than that of toxin B³³. Initial effects of toxin A on intestinal epithelial cells include induction of the expression of cytokines such as interleukin(IL)-8 (potent chemoattractant for neutrophils) and loss of barrier function, with subsequent cell death by apoptosis³⁴⁻³⁷ (Fig 1). Epithelial cells and monocytes are more sensitive to *C. difficile* toxin A-induced cell death than lymphocytes^{35 38 39}, implying differential effects of the toxin on cells of the innate and adaptive immune system.

Histologically, the disease is characterised by focal epithelial ulceration associated with an inflammatory exudate⁴⁰ that appears as a characteristic pseudomembrane on endoscopic examination. Reasons for the focal nature of the colonic inflammation remain unknown but could be due to high concentrations of toxins secreted by bacteria in close vicinity to the affected epithelium, resulting in high expression of IL-

8 and rapid epithelial cell death. The ensuing focal epithelial ulceration would enable the toxins access to a blood vessel in the superficial lamina propria, leading to migration of neutrophils through the breach in the epithelial barrier. By contrast, epithelial cells distant to the bacteria would be exposed to lower concentrations of the toxins and the subsequent induction of transforming growth factor-beta, which has been shown to not only protect against loss of barrier function, but also facilitates epithelial restitution³⁶.

CLINICAL PRESENTATION

Following colonisation with toxigenic *C. difficile*, individuals may become asymptomatic carriers⁴¹⁻⁴³ or develop colonic disease. In those that develop *C. difficile*-associated disease, clinical features can range from mild diarrhoea to life-threatening pseudomembranous colitis. Severe disease is characterised by abdominal pain, profuse diarrhoea (which is often non-bloody) and systemic symptoms such as fever, anorexia, nausea and malaise.

In severe CDAD, leukocytosis, raised C-reactive protein and low albumin levels are frequently seen. In one recent report, of those patients with severe CDAD that required admission to the intensive care unit, independent predictors of 30-day mortality were leukocytosis $\geq 50 \times 10^9/L$, lactate level ≥ 5 mmol/L, age ≥ 75 years, immunosuppression and shock requiring vasopressor treatment⁴⁴. Some patients with severe pseudomembranous colitis may have little or no diarrhoea as a result of toxic megacolon and paralytic ileus. In these patients, abdominal distension, marked leukocytosis and dilated and inflamed colon on abdominal x-ray and CT scan may provide important clues regarding the diagnosis.

Despite adequate initial treatment, recurrence of disease may occur in 15 – 30% of patients with CDAD⁴⁵⁻⁴⁷. This may occur due to either relapse or re-infection. It is possible that those with pseudomembranous colitis are at an increased risk of relapse⁴⁸, but further studies are required to confirm this. Studies also suggest that those with relapsing disease have an impaired immune response to *C. difficile* toxins⁴⁷, including reduction in the number of mucosal IgA-producing cells⁴⁹.

DIAGNOSIS

C. difficile-associated disease is usually diagnosed following the demonstration of toxins A and/or B in stool samples. Demonstration of the presence of *C. difficile* toxins by the characteristic cytopathic effect on a monolayer of cells is considered by many to be the 'gold standard'^{50,51}. The main disadvantage of this assay is that it takes 24 – 48 hours to obtain a result. Many laboratories now use enzyme-linked immunosorbent assays (ELISAs) for toxin A or toxins A and B. Since toxin A-negative, toxin B-positive strains have been reported to cause disease⁵², assays that test for the presence of both toxins are preferable. A number of studies have shown that false negative rates can be high for both assays^{16,48,51,53}. In some patients with pseudomembranous colitis, repeat stool test may also be negative⁴⁸. Reasons for false negative rates are not fully understood, but may include inappropriate handling or storage of stool samples prior to testing.

In view of the characteristic appearance of pseudomembranous colitis at endoscopy and also on histological examination of biopsies, flexible sigmoidoscopy (with biopsies) has been proposed in those patients suspected to have *C. difficile*-associated diarrhoea but who have a negative stool test for *C. difficile* toxins⁴⁸. The identification of pseudomembranous colitis at the endoscopic examination enables a rapid diagnosis to be made and appropriate clinical management to be initiated. In those patients with

non-specific changes in the colonic mucosa, a second stool sample can be collected during the endoscopic examination and further management could be guided by the results of the repeat stool test and histological examination of biopsies. For patients suspected to have CDAD, flexible sigmoidoscopy by the bed-side, without any bowel preparation, has been shown to be safe and well tolerated⁴⁸. Avoidance of moving the patient to the endoscopy unit minimises the risk of contamination of other sites with *C. difficile* spores. Flexible sigmoidoscopy may also facilitate the identification of other causes of diarrhoea, such as ischaemic colitis and inflammatory bowel disease. It should be noted that in patients with relapse of ulcerative colitis due to *C. difficile* infection pseudomembranes may not be seen at endoscopy, nor the characteristic histological changes (of pseudomembranous colitis) on biopsy¹⁶. Colonoscopy may be required to diagnose the predominantly right-sided antibiotic-associated haemorrhagic colitis, which has recently been shown to be due to *Klebsiella oxytoca*⁵⁴.

Stool samples may also be cultured on selective medium for *C. difficile*. However, further tests would be required on the cultured *C. difficile* to confirm that it is a toxigenic strain by demonstration of its ability to express toxin A and/or B. Thus, it would take 3 – 4 days to get a result and this test is not routinely undertaken in most hospitals for diagnostic purposes. However, culture is useful for investigating outbreaks of *C. difficile* infection as isolates can be genotyped, and for measuring the antimicrobial susceptibility of *C. difficile* strains.

Stool enzyme immunoassays for glutamate dehydrogenase antigen are available for the diagnosis of *C. difficile*-associated disease but are not widely used⁵⁵ and have not been as extensively evaluated as cytotoxicity assays and ELISAs for *C. difficile* toxins.

INFECTION PREVENTION AND CONTROL

Whilst infection with *C. difficile* may occur in both the hospital and community setting, the majority of these infections now occur in relation to healthcare. *C. difficile* is fast becoming one of the most important healthcare-associated infections and the prevention and control of this infection is posing a significant challenge. Control measures are based on three main strategies: (i) Prudent use of antimicrobials (ii) Prevention of cross-infection and (iii) Active surveillance of cases

Prudent use of antimicrobials

The principle risk factor for *C. difficile*-associated disease is prior antimicrobial therapy, especially with broad-spectrum antibiotics. Some antibiotics appear to have a much higher propensity to cause disease than others (see Table 1), although this may change over time. For instance the fluoroquinolone antibiotics have previously been shown to have a low risk for causing *C. difficile*-associated disease compared to cephalosporins^{56 57}. However high-level fluoroquinolone resistance has now emerged in some strains of *C. difficile*, including ribotypes 027 and 106, and fluoroquinolones have recently been shown to be a major risk factor for infection with the hypervirulent strain NAP1/027⁵⁸.

Table 1
Risk of *C.difficile* for different antibiotics

Low risk	Medium Risk	High Risk
Aminoglycosides	Co-amoxiclav	2 nd /3 rd generation cephalosporins
Vancomycin	Macrolides	Clindamycin
Trimethoprim	Amoxicillin/ampicillin	Fluoroquinolones
Tetracyclines		
Piptazobactam		
Benzylpenicillin		

It is now accepted that the prudent use of antibiotics is an essential component of *C. difficile* control measures, particularly within high risk areas of healthcare. Components of a good antimicrobial control programme include: prescribing short durations of antibiotics including one dose prophylaxis, avoiding broad-spectrum antibiotics where possible, restricting intravenous antibiotics, using automatic stop dates, monitoring antibiotic use by specialty and employing antibiotic pharmacists⁵⁹. Most of the published interventions on antibiotic prescribing as a method of *C. difficile* control have looked at the benefits of reducing broad-spectrum antibiotics in favour of restrictive or narrow-spectrum antibiotic policies. There are now a number of reported studies demonstrating that a significant reduction in *C. difficile* infections can be achieved by introducing new antibiotic policies (see Table 2).

Table 2
Reports of successful control of *C.difficile* by restriction of high-risk broad-spectrum antibiotics.

Setting	Antibiotics reduced	Antibiotics increased	Reference
Elderly care	Cephalosporins	Benzylpenicillin, trimethoprim +/- gentamicin	138
Elderly care	Co-amoxiclav	Benzylpenicillin, amoxicillin, trimethoprim	139
Elderly care	Cefotaxime	Ciprofloxacin	56
Medicine, surgery	Ceftriaxone, oral cephalosporins	Levofloxacin	57
Elderly care	Cefotaxime	Piptazobactam	140
Medicine, elderly care	Ceftriaxone, cefuroxime	Moxifloxacin, piptazobactam	141
Hospital-wide	Clindamycin	Various	142

Prevention of cross-infection

The basic infection prevention and control measures for patients with *C. difficile* are well established. The mode of spread is via the faecal-oral route. The principal factor that makes the control of *C. difficile* particularly difficult within the healthcare setting is that the organism is capable of producing highly resistant spores. These survive readily within the hospital environment for long periods and there can be widespread contamination of the environment, including mattresses, bed frames, commodes, toilets, radiators and medical equipment in the vicinity of symptomatic patients⁶⁰.

Patients with suspected CDAD should be isolated as soon as possible, preferably in a room with an en-suite toilet or else with a dedicated commode. During outbreaks, patients may be cohort nursed together if there are insufficient single rooms, and consideration should be given to a dedicated *C. difficile* isolation ward for institutions with hyperendemic *C. difficile*. Failure to isolate symptomatic patients promptly was one of the principal contributory factors in the outbreaks at Stoke Mandeville Hospital⁶¹.

Enhanced environmental and equipment cleaning is necessary for symptomatic patients. *C. difficile* spores are relatively resistant to a wide range of disinfectants, with perhaps chlorine containing agents retaining at least some sporicidal activity^{8 62}. There is now interest in the use of vaporised hydrogen peroxide as a reliable sporicidal method for environmental disinfection of *C. difficile* spores⁶³, although this type of technology cannot be used in the same room as patients or staff.

Staff looking after symptomatic patients, or who are in direct contact with their immediate environment or equipment should wear the appropriate protective clothing (gloves, aprons). Strict hand hygiene should be observed with soap and water. Alcohol rinses or gels are much less effective against *C. difficile* spores compared with other organisms causing healthcare associated infections e.g. MRSA⁶⁴. Each healthcare organization should have policies in place to support the control of *C. difficile* infection, re-inforced through adequate programmes of teaching, training and audit.

Surveillance

Active surveillance for cases of *C. difficile* infection should be undertaken to monitor local, regional and national rates of infection, to provide timely feedback to clinicians and other healthcare professionals, to detect outbreaks, and to monitor the effectiveness of particular interventions. Mandatory reporting of all cases of *C. difficile* infection in the over 65s was introduced for all NHS Trusts in 2004. This has been supplemented by a planned national programme of culturing for *C. difficile* isolates, and submitting isolates to the Anaerobic Reference Laboratory of the National Public Health Service, Wales for molecular typing and antibiotic susceptibility testing.

Local surveillance programmes should aim to provide timely feedback on *C. difficile* rates, especially to high-risk areas of secondary care including elderly care, general and renal medicine and clinical haematology. It is also recommended that NHS Trusts regard outbreaks of *C. difficile* as serious untoward incidents associated with infection, and that investigation of these outbreaks should include a root-cause analysis.

MEDICAL TREATMENT

Prophylaxis

In susceptible patients, prophylaxis against the development of CDAD, administered at the same time as antibiotics (e.g. for pneumonia) is an attractive concept. Probiotics are live micro-organisms which confer a health benefit on the host and have been of interest for a number of years. They may provide protection against colonisation by *C. difficile*, and may also act via other mechanisms that remain to be fully characterized. Most of the studies to date have investigated the effect of probiotics on antibiotic associated diarrhoea, of which (as outlined above) 15 – 20 % will be due to *C. difficile* infection.

A recent meta-analysis concluded that *Saccharomyces boulardii*, *Lactobacillus rhamnosus* GG, and probiotic mixtures significantly reduced the development of antibiotic-associated diarrhoea⁶⁵. *S. boulardii* is a non-pathogenic yeast, which has also been shown to be effective in preventing the recurrence of CDAD (see below). Recently, a randomised double blind, placebo controlled trial involving older patients in hospital who were receiving antibiotics, has reported that a commercially available probiotic yoghurt preparation led to a significant reduction in both the incidence of antibiotic-associated diarrhoea and *C. difficile*-associated diarrhoea⁶⁶. The probiotic yoghurt drink contained *Lactobacillus casei* DN-114 001 (*L casei* imunitass) (1.0×10^8 colony forming units/ml), *S thermophilus* (1.0×10^8 cfu/ml), and *L bulgaricus* (1.0×10^7 cfu/ml). Further studies are expected to determine the role of probiotic treatment in prophylaxis against CDAD in routine clinical practice.

Prophylactic intragastric administration of bovine IgG concentrated from colostrum has been reported to protect hamsters against CDAD⁶⁷. However, diarrhoea developed when the IgG was stopped, implying the additional requirement for reconstitution of the normal colonic flora for continuing protection. Orally administered anti-toxin A avian antibody has also been shown to protect hamsters against CDAD⁶⁸ and appropriate clinical studies are awaited.

Treatment of acute episode

Stopping offending antibiotics

Early studies suggested that in those with mild disease, discontinuation of the offending antibiotics may lead to resolution of CDAD. In the prospective randomised trial of metronidazole vs vancomycin, in 22.8 % of patients with CDAD, the diarrhoea resolved during 48 – 72 hour observation period before recruitment into the study⁶⁹. In another early randomised controlled trial⁷⁰, diarrhoea resolved or improved in 2 patients with pseudomembranous colitis on placebo. In our more recent prospective study, diarrhoea resolved in 30% of patients over median 4.0 days, after discontinuation of offending antibiotics⁴⁸. Out of those that had had flexible sigmoidoscopy, diarrhoea in four patients without pseudomembranous colitis resolved within 24 h of discontinuation of the offending antibiotics⁴⁸. Thus in mild CDAD, specific antimicrobial treatment may not be required if the offending antibiotics can be discontinued and flexible sigmoidoscopy may facilitate the identification of such patients. For those requiring treatment, established and new agents for management of acute CDAD are listed in Table 3.

Table 3
Established and New Agents for Treatment of Acute CDAD

Treatment	Type	Evidence Base/Developmental Stage
Antimicrobials		
Metronidazole	Imidazole derivative	RCT ⁷¹
Vancomycin	Glycopeptide	RCT ⁷¹
Teicoplanin	Glycopeptide	RCT ⁷¹
Bacitracin	Cyclic polypeptides derived from <i>B. subtilis</i>	RCT ⁷¹
Fusidic Acid	Steroid antibiotic derived from <i>Fusidium coccineum</i>	RCT ⁷¹
Nitazoxanide	Nitrothiazole benzamide	RCT ⁹³
PAR-101	Macrocyclic antibiotic	Phase III studies ¹⁰³
Rifaximin	Rifamycin derivative	Phase III studies ¹⁴³
Ramoplanin	Lipoglycopeptide	Phase II studies ⁸⁵
Non-antimicrobials		
Tolvamer	Anionic polymer	Phase III studies ¹¹⁰
CDA1 (MDX-066)	Human monoclonal antibody to Toxin A	Phase II studies ¹¹⁴
MDX-1388	Human monoclonal antibody to Toxin B	Phase II studies ¹¹⁴

RCT – randomised controlled trial

Antimicrobial treatment of CDAD

Previous controlled clinical trials have demonstrated efficacy of vancomycin, metronidazole, bacitracin and fusidic acid in the treatment of CDAD⁷¹. Metronidazole and vancomycin are widely used in clinical practice. Many guidelines recommend the use of metronidazole in those deemed to require treatment^{3 50 72}. This guidance is based on (i) reported equivalent efficacy of metronidazole and vancomycin in controlled clinical trials (ii) risk of colonization with vancomycin-resistant enterococci⁷³ and (iii) cost of vancomycin. However, in a retrospective study, the mean duration of symptoms was significantly shorter with vancomycin than metronidazole⁷⁴. Moreover, recent studies have reported high failure rates of metronidazole^{75 76}. Although resistance to metronidazole has been reported^{77 78}, clinical CDAD treatment failures with this antibiotic may not be attributable to decreased susceptibility of the causative *C. difficile* isolate to metronidazole⁷⁹.

Metronidazole is not detected in faeces of healthy subjects^{80 81} and in asymptomatic carriers of *C. difficile*⁸². By contrast, metronidazole has been detected in watery and semiformal stool samples of patients with *C. difficile*-associated colitis⁸³ and in Crohn's disease patients with colonic inflammation⁸¹. Metronidazole has also been detected in stool samples of patients with *C. difficile*-associated disease that have received the antibiotic intravenously⁸³. Thus, it is likely that metronidazole is secreted only by the inflamed colonic mucosa.

In a small retrospective case series, the use of intracolonic vancomycin therapy has been reported to be an effective adjunctive treatment in patients with severe *Clostridium difficile*-associated colitis⁸⁴.

Teicoplanin

A recent Cochrane review⁷¹ concluded that, in terms of symptomatic cure (defined as initial symptomatic resolution without symptomatic recurrence at any time during the

follow-up period that ranged from two to six weeks), teicoplanin may be slightly more effective than vancomycin with a relative risk of 1.21 [95% CI 1.00 – 1.46; p=0.06]. For initial symptomatic resolution (described as cessation of diarrhoea as defined by each individual study during the treatment period ranging from 7 to 10 days), teicoplanin was as effective as vancomycin⁷¹. Teicoplanin may not currently be available in all countries.

New antimicrobial agents for CDAD

The search for new antimicrobial agents against *C. difficile* (either newly developed antibiotics or established antimicrobial drugs investigated for their use in patients with CDAD) is driven by the desire to find an alternative to metronidazole and vancomycin. Thus, the new agents may be active against both *C. difficile* and vancomycin-resistant enterococci (e.g. ramoplanin⁸⁵), and/or be predicted to reduce the risk of recurrence because of a narrow spectrum of activity (e.g. PAR-101/OPT-80⁸⁶), in anticipation of the maintenance of colonization resistance. These new agents are initially tested for their *in vitro* activity against clinical isolates of *C. difficile*⁸⁷ and include nitazoxanide, rifaximin, tinidazole, PAR-101 (also known as OPT-80, tiacumicin B) and ramoplanin. Some of these agents have also been tested in the hamster model of *C. difficile*-associated colitis^{88 89}, prior to clinical trials.

Nitazoxanide

Nitazoxanide is a nitrothiazole benzamide compound that is active against many intestinal parasites, including *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum*, *Encephalitozoon intestinalis*, *Isospora belli*, *Blastocystis hominis*, *Balantidium coli*, *Enterocytozoon bieneusi*, *Ascaris lumbricoides*, *Trichuris trichura*⁹⁰. After oral administration, a significant proportion (approximately 66%) of the drug is excreted in faeces in its deacetylated form designated tizoxanide⁹¹, which is active against *C. difficile*⁹². In a recent randomised double-blind study, nitazoxanide (500 mg every 12 h) was shown to be as effective as metronidazole (250 mg every 6 h) in the treatment of CDAD⁹³. In an open-label study, nitazoxanide was given to 35 patients who failed treatment with metronidazole for *C. difficile* colitis, and 26 (74%) responded, of whom seven later had recurrent disease⁹⁴.

Rifaximin

Rifaximin is a semi-synthetic derivative of rifamycin, which is very poorly absorbed from the gastrointestinal tract. It has been available in Italy for many years and more recently has been approved by the US Food and Drug Administration for use in non-dysenteric traveller's diarrhoea⁹⁵. Rifaximin has been used in combination with ciprofloxacin in patients with treatment-resistant pouchitis⁹⁶ and has also been studied in patients with diverticular disease⁹⁷ and hepatic encephalopathy⁹⁸. These studies have shown that this antibiotic is generally well tolerated. Rifaximin has good *in vitro* activity against *C. difficile*^{87 99} and has been reported to be as effective as vancomycin in initial resolution of symptoms in CDAD⁷¹.

PAR-101

PAR-101 (formerly OPT-80), also known as tiacumicin b, is an 18-membered macrocyclic antibiotic that was originally isolated from fermentation broth of *Dactylosporangium aurantiacum* subsp. *Hamdenensis*^{100 101}. It has high *in vitro* activity against *C. difficile*, but poor activity against eubacteria and anaerobic Gram-negative rods^{86 102}, suggesting that it may disrupt the colonic flora to a lesser degree

than current antibiotic treatment of CDAD. Orally administered tiacumicin b was effective in the treatment of *C. difficile*-induced colitis in hamsters⁸⁸. Poor absorption of this antibiotic was illustrated by lack of detectable levels in the serum of treated hamsters but it was present in their caeca. In an open-label phase IIa clinical trial¹⁰³, PAR-101 demonstrated therapeutic efficacy against CDAD. Phase III studies consisting of randomized double-blind comparisons of PAR-101 and vancomycin for the treatment of CDAD were commenced in 2006¹⁰³.

Ramoplanin

Ramoplanin is a novel lipoglycopeptide antibiotic with bactericidal activity against aerobic and anaerobic Gram-positive bacteria (including *C. difficile*, vancomycin-resistant enterococci and methicillin resistant *S. aureus*) but has no activity against Gram-negative organisms⁸⁵. Ramoplanin acts by inhibiting the assembly of bacterial peptidoglycan cell walls and its activity against *C. difficile* did not change when the level of susceptibility to either vancomycin or metronidazole was reduced¹⁰⁴. In the hamster model of *C. difficile*-induced colitis, ramoplanin demonstrated comparable therapeutic efficacy to vancomycin⁸⁹. Following oral administration, ramoplanin does not appear to be absorbed, with significant concentrations of the drug detected in faeces⁸⁵. It has been reported to be safe and effective in temporarily suppressing asymptomatic gastrointestinal carriage of vancomycin-resistant enterococci¹⁰⁵. In a Phase II study, similarity in response rates of ramoplanin compared with vancomycin for treatment of CDAD has been reported at a scientific meeting⁸⁵.

Non-antimicrobial treatment of CDAD

Toxin-binding compounds

These treatments are based on the concept that interference with *C. difficile* toxin-host cell interactions lead to therapeutic efficacy. Colestipol and cholestyramine are anion-exchange resins that have been shown to bind *C. difficile* toxins but have not been deemed clinically efficacious⁷². Synthetic oligosaccharide sequences attached to an inert support (SYNSORB) have been shown to bind and neutralise the activities of *C. difficile* toxin A^{106 107} but clinical studies have not been reported.

Tolvamer is the salt of a soluble, high molecular weight anionic polymer which non-covalently binds *C. difficile* toxins A and B, but has no significant antimicrobial activity¹⁰⁸. It has been shown to have therapeutic efficacy in the hamster model of CDAD¹⁰⁹. The results of a multicentre, randomised, double-blind phase II clinical trial were recently reported¹¹⁰. A total 289 patients with a first ever or recurrent episode of CDAD were randomized to tolevamer monotherapy (3g or 6g/day) or vancomycin (125mg/qds). Tolvamer at a dose of 6g/day demonstrated non-inferiority to vancomycin with a trend towards reduced recurrence (p=0.05). There was an increased risk of hypokalaemia in patients treated with tolevamer, thought to be due to the binding of potassium in the intestinal lumen. Current phase III trial is investigating the therapeutic effect of 9 g/day of tolevamer, the formulation of which has been changed to minimise the risk of hypokalaemia¹⁰⁸.

Antibody-based treatment

In case reports, intravenous human immune globulin has been reported to be beneficial in patients with severe CDAD^{111 112}, but a recent retrospective analysis does not support the use of this treatment outside of a controlled clinical trial¹¹³.

Intraperitoneal administration of a human monoclonal antibody to toxin A led to significant reduction in mortality in the hamster model of CDAD but combination treatment with the addition of anti-toxin B antibody provided greater protection¹¹⁴. Phase II clinical trials using these human monoclonal antibodies are currently being undertaken¹¹⁴.

Oral administration of avian antibodies to toxin A and B has also been reported to provide full protection against CDAD in hamsters, when administration was initiated 8 h after challenge with *C. difficile* (and continued for 4 days)⁶⁸. Thus clinical studies in which anti-toxin antibodies are administered orally, for treatment of CDAD, will also be of interest.

Treatment of recurrent CDAD

Recurrent disease occurs in 15 – 35% of patients with CDAD^{45-47 115}, which may reflect relapse of infection due to the original strain or by reinfection with a new strain of *C. difficile*. The risk of recurrent disease is higher in those that have previously had more than one episode of CDAD¹¹⁶ and has been shown to be related to the host immune response^{49 117}. Whilst persistence of spores of the original strain may germinate and lead to relapse after treatment, in many patients disease recurrence may be due to reinfection by a different strain of *C. difficile*^{118 119}. Treatment for recurrent CDAD is summarised in Table 4.

Table 4

Treatment of Recurrent CDAD

Treatment	Type	Evidence Base/Developmental Stage
Antimicrobials		
Vancomycin (with <i>S. boulardii</i>)	Glycopeptide	RCT
Vancomycin, tapered-pulsed	Glycopeptide	Uncontrolled trial, clinical experience
Rifaximin	Rifamycin derivative	Small, uncontrolled trial
Non-antimicrobials		
Faecal enema	Faecal bacteriotherapy	Case reports, retrospective review
Oligofructose	Prebiotic	RCT
<i>Saccharomyces boulardii</i>	Probiotic	RCT
<i>Lactobacillus plantarum</i>	Probiotic	Small RCT
Human gamma globulin	Pooled Ig	Case reports
Anti- <i>C. difficile</i> whey protein concentrate	Immunotherapy	Open-label pilot study
<i>C. difficile</i> vaccine	Toxoid vaccine	Phase I studies

RCT – randomised controlled trial

Antibiotic treatment

Tapered and pulsed regimens of vancomycin have been reported to be beneficial in the treatment of recurrent CDAD^{115 120}. The rationale for such treatment is that antibiotic-resistant spores may convert to antibiotic-sensitive vegetative forms during gradual withdrawal of antibiotics (tapered) and given on alternate days (pulsed)⁴⁷.

In an uncontrolled study, rifaximin administered for 2 weeks and given immediately after vancomycin (before recurrence of symptoms) was effective in preventing predictable recurrences in 7 out of 8 patients¹²¹. The one patient with further

recurrence responded to a second course of rifaximin but resistant *C. difficile* was recovered after treatment. A controlled trial is awaited.

Faecal bacteriotherapy

In an effort to restore the disrupted colonic microflora in patients with recurrent CDAD, several case reports describe the use of faecal enemas prepared from healthy donor stools^{122 123}. Most of these patients reported no further *C. difficile* recurrence. In a study in 5 patients in whom a mixture of different facultative aerobic and anaerobic bacteria were administered¹²³ *Bacteroides* species in the normal bowel flora appeared to provide protection against colonization by *C. difficile*.

In a more recent retrospective review, donor stool (provided by healthy family members) was administered via nasogastric infusion in 18 patients with recurrent CDAD, over a 9 year period¹²⁴. Ninety days after receipt of the 'stool transplant', 15 of the 18 patients remained relapse free.

Probiotics and prebiotics

In a double-blind, randomised, placebo-controlled trial, the addition of the probiotic yeast *S. boulardii* to standard antibiotics significantly reduced the rate of recurrent CDAD¹¹⁶. When combined with *S. boulardii*, recurrent CDAD may respond better to a short course of high-dose vancomycin, compared to high-dose vancomycin alone¹²⁵. It should be noted that although *S. boulardii* is generally well tolerated, fatal fungaemia has been reported during treatment¹²⁶.

Prebiotics are non-digestible oligosaccharides which selectively stimulate the growth, activity, or both, of probiotic-like bacteria normally present in the gut. A randomized trial of 142 CDAD cases treated with standard antibiotics, with or without an adjunctive prebiotic (oligofructose), showed that significantly fewer patients in the prebiotic group experienced a recurrence within 60 days compared with those on placebo¹²⁷. However, there was no difference between the two groups in the stool culture rate for *C. difficile* at days 30 and 60. Thus the mechanism for the reduction in recurrence rate with oligofructose remains to be determined.

A small double-blind, placebo-controlled multicentre trial comparing metronidazole and *L. plantarum* vs metronidazole and placebo in patients with recurrent *C. difficile*-associated diarrhoea reported no statistically significant differences between the groups¹²⁸. Since persistent disruption of the colonic flora appears to be an important determinant of recurrent CDAD, further studies investigating the role of probiotics, prebiotics and synbiotics (combination of probiotics and prebiotics) in the management of patients with relapsing CDAD are likely.

Antibody-based treatment

Rationale for intravenous gamma globulin treatment in recurrent CDAD is that these patients may have an impaired antibody response to *C. difficile* toxins and that anti-toxin specific antibodies may be present in the gamma globulin preparations administered^{129 130}. Controlled clinical trials investigating the role of intravenous gamma globulin in recurrent CDAD are awaited.

In a small open label pilot study, 9 adults with relapsing CDAD were treated orally with anti-*C. difficile* whey protein concentrate daily for 2 weeks, following completion of a course of metronidazole and/or vancomycin¹³¹. During a median follow-up period of 333 days (range 35 days to 12 months), there were no further episodes of CDAD. Anti-*C. difficile* whey protein concentrate was prepared from mature milk (not colostrum) of cows immunized with *C. difficile*-inactivated toxins

and killed whole-cell *C. difficile* to generate mainly secretory IgA against *C. difficile*, toxin A and toxin B¹³².

Toxoid vaccine

In an open-label study, three patients with multiple episodes of recurrent CDAD received *C. difficile* vaccine containing toxoid A and toxoid B¹³³. After vaccination, none of the subjects had any further episodes of CDAD over the subsequent 6 months. Two of the three subjects showed an increase in levels of anti-toxin A and anti-toxin B IgG, compared to baseline values.

SURGERY

The role of surgery for treatment of severe CDAD has been recognised for many years¹³⁴. Indications for surgery include toxic dilatation of the colon, bowel perforation, systemic toxicity and failure to respond to medical treatment. However, postoperative mortality is often high^{135 136}. Compared to partial resection, total colectomy is reported to be associated with lower mortality rate¹³⁷. In a recent retrospective observational cohort study of 165 cases of CDAD that required admission to intensive care unit, emergency colectomy seemed more beneficial in patients aged 65 years or more, those who were immunocompetent, those with leukocytosis $\geq 20 \times 10^9/L$ or lactate level between 2.2 and 4.9 mmol/L⁴⁴. A combined medical and surgical cooperative approach in the early management of patients with severe CDAD is likely to improve outcome^{134 135}.

CONCLUSIONS

Recent changes in the epidemiology of *C. difficile* infection and the emergence of an epidemic hypervirulent strain serve to emphasise the need for greater attention to infection control, early diagnosis of CDAD, and more effective treatments for those with severe and recurrent disease.

A significant proportion of patients with mild disease may not require any further treatment, if the offending antibiotics can be discontinued. Currently, a number of therapeutic avenues are being pursued for treatment of acute and recurrent CDAD. It is anticipated that the results of a number of recent and currently ongoing clinical trials will provide a robust evidence base for future recommendations for the management of patients with *C. difficile* infection. Surgical resection of the inflamed colon is a therapeutic option in those with severe CDAD and a combined medical and surgical approach is recommended for such patients.

Key points Table 1

C. difficile-associated disease (CDAD) is an important healthcare-associated infection

CDAD may recur in 15 – 30% of patients

A virulent strain of *C. difficile* has been responsible for recent outbreaks

Control measures are based on prudent use of antimicrobials, prevention of cross-infection and active surveillance of cases

Key points Table 2

Rapid diagnosis of CDAD will facilitate infection control measures and early treatment

Significant false negative rates for stool *C. difficile* toxin assays have been reported

Flexible sigmoidoscopy enables rapid diagnosis of *C. difficile*-associated pseudomembranous colitis

Patients with mild CDAD may respond to discontinuation of offending antibiotics, without the need for specific treatment

Those with severe CDAD may require admission to the intensive care unit and a combined medical and surgical approach to management

Vancomycin and metronidazole are widely used for the treatment of CDAD

A number of new antimicrobial and non-antimicrobial agents are currently under investigation for the treatment of acute and recurrent CDAD

Legend to Figure 1.

Transmission electron micrographs (TEMs) of control and toxin A-exposed human colonic biopsy specimens in organ culture. The control (A) shows preservation of epithelial integrity although some cells have processes (arrow) in their lateral aspects, with some separation from neighbouring cells. In TEMs of toxin A-exposed specimens (B & C) epithelial cells are round and many have become detached from the basement membrane (large arrow). Cells undergoing apoptosis are also present (small arrows). Reproduced from reference 31.

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Figure 1

