Review

Clostridium difficile-associated disease (CDAD) causes substantial morbidity and mortality. The pathogenesis is multifactorial, involving altered bowel flora, production of toxins, and impaired host immunity, often in a nosocomial setting. Current guidelines recommend treatment with metronidazole; vancomycin is a second-line agent because of its potential effect on the hospital environment. We present the data that led to these recommendations and explore other therapeutic options, including antimicrobials, antibody to toxin A, probiotics, and vaccines. Treatment of CDAD has increasingly been associated with failure and recurrence. Recurrent disease may reflect relapse of infection due to the original infecting organism or infection by a new strain. Poor antibody responses to C difficile toxins have a permissive role in recurrent infection. Hospital infection control and pertinent use of antibiotics can limit the spread of CDAD. A vaccine directed against C difficile toxin may eventually offer a solution to the CDAD problem.

Introduction

Clostridium difficile is the leading identified cause of nosocomial diarrhoea associated with antibiotic therapy (figure). Occurrence of diarrhoea in hospitalised inpatients who receive antibiotics ranges from 3% to 29%.1 C difficile has been implicated as the causative organism in 10–25% of patients with antibiotic-associated diarrhoea, 50–75% of those with antibiotic-associated colitis, and 90–100% of those with antibiotic-associated pseudomembranous colitis.2–4 Mortality of C difficile-associated disease (CDAD) ranges from 6% to 30% when pseudomembranous colitis is shown to be present,4–6 and is substantial even in the absence of colitis. The incidence of CDAD has increased in the past decade, with a 10-fold increase reported in Quebec,7 as has the proportion of patients who have severe, refractory, or recurrent disease.7,8 Hospital costs attributable to this condition in the USA8 and UK9 exceed US$4000 per case.

History of C difficile colitis

First described in the 1950s, pseudomembranous enterocolitis was attributed either to Staphylococcus aureus, an organism that had become prevalent in hospital inpatients who had received antibiotics,10–12 or to Candida albicans.13 In 1974, a prospective study of 200 patients treated with clindamycin detected diarrhoea in 21% and pseudomembranous colitis in 10%.14 A toxin produced by a Clostridium species was proposed as the cause of clindamycin-induced ileocaecitis in hamsters in 1977;15 later this toxin was isolated from patients’ stool samples, with evidence and counter-evidence presented for C difficile and Clostridium sordelli as causative organisms.16–18 However, by 1978, C difficile had been clearly identified as the causal agent of antibiotic-associated colitis.19

The initial treatment for CDAD was oral vancomycin. In the early 1980s, metronidazole was also shown to be effective, perhaps equally so, and with a strong preference to avoid the use of vancomycin in hospital inpatients, reinforced by several sets of therapeutic recommendations,20–23 has led to increasing reliance on metronidazole. In 1997, the American Gastroenterology Association published recommendations for treating CDAD that included discontinuation of antibiotics, supportive non-specific therapy, and addition of metronidazole for those who failed to respond within 2–3 days.24 Oral vancomycin was recommended for the following categories of patients: those who were critically ill, unable to tolerate metronidazole, pregnant, or under the age of 10 years; those who failed initial therapy with metronidazole; or those whose infecting organism proved to be metronidazole resistant. The past few years have witnessed an increase in the failure rate of antimicrobial therapy.8,9,25 Some patients simply fail to respond to conventional therapy, and others promptly relapse after discontinuation of treatment. The
Cochrane database reports only nine well-designed randomised trials that have assessed treatments for CDAD. The purpose of this review is to examine current recommendations for therapy, to assess other possible modes of treatment, and to cite areas for future investigation.

Microbiology and epidemiology

Hall and O’Toole first described *C. difficile* in 1935 as part of the normal flora of neonates. The organism is a Gram-positive, spore-forming rod that is an obligate anaerobe. It is relatively large (2–17 μm in length) and fast growing; CCFA medium (consisting of cycloserine, cefoxitin, and fructose agar in an egg-yolk agar base) is highly selective for its growth. Toxin A, toxin B, and the binary toxin of *C. difficile* may contribute to CDAD. Toxin A causes fluid secretion and intestinal inflammation when injected into rodent intestine and is a chemoattractant for neutrophils in vitro. Toxins A and B both activate the release of cytokines from monocytes. It is unclear whether the binary toxin is pathogenic; however, a recent study has shown a trend toward more severe disease in patients who carry the strain of *C. difficile* that produces binary toxin. *C. difficile* can be cultured from the stool of 3% of healthy adults. Stool carriage of *C. difficile* reaches 16–35% in hospital inpatients, with the percentage proportional to the duration of hospital stay and increasing with exposure to antibiotics. Newly admitted patients who are already colonised seem to be an important source of contagion in hospitals. CDAD may also occur in outpatients, although incidence is low.

Pathogenesis and immunity

The pathogenesis of CDAD is complex and incompletely understood. The congruence of debilitating diseases and antibiotic therapy (sometimes chemotherapy) in hospital inpatients is thought to alter the bacterial flora of the colon, thus creating conditions that favour new acquisition and proliferation of *C. difficile*. Individuals who acquire *C. difficile* may be colonised or develop disease, and the immune status of the host is an important determinant of the outcome. Patients with more severe underlying illnesses are more likely to develop CDAD. People who carry *C. difficile* without developing colitis have higher concentrations of serum antibody to toxin A than do symptomatic patients, and are less likely to develop clinical disease. For patients who develop CDAD, higher concentrations of anti-toxin A antibody are associated with a shorter duration of illness and a decreased risk of recurrence.

Stopping the offending antibiotic

In three early studies, 15–23% of patients with CDAD had spontaneous resolution of symptoms within 48–72 h of stopping the antibiotic that was associated with the disease episode and without specific antimicrobial therapy. Continuation of systemic antibiotics has been associated with refractoriness to treatment. However, one cannot predict which patients will clear the infection spontaneously, and to discontinue antibiotics is often not feasible. In addition, the time between the onset of symptoms and the confirmation of CDAD may be a few days in the usual clinical scenario, and further delay in starting treatment in symptomatic patients may lead to clinical deterioration. Thus, in theory, although discontinuing the antibiotics and observing the patient might be effective in a small proportion of patients, it is difficult to apply this approach in practice.

Specific therapy

Vancomycin

Oral vancomycin was used to treat so-called staphylococcal enterocolitis and clindamycin-associated diarrhoea before the discovery that *C. difficile* was responsible for the disease. Recognition of this organism’s role was followed by additional studies of vancomycin for treatment. Between 1977 and 1980, most physicians prescribed oral vancomycin for 7–14 days to treat confirmed cases of CDAD, with clinical resolution observed in almost 90% of treated patients. Doses of vancomycin ranging from 125 mg to 500 mg four times daily were found to be equally effective. Subsequent studies showed the percentage of patients cured by oral vancomycin to be 86–100%. The table summarises treatment failures and recurrences in published series of cases.

In vitro, *C. difficile* is susceptible to vancomycin; the reported minimum inhibitory concentration (MIC) required to inhibit 90% of strains (MIC90) is 0.75–2 µg/mL. A recent study from Madrid found that 3% of *C. difficile* isolates had intermediate resistance to vancomycin (MIC 4–16 µg/mL), but clinical correlation was not provided. Orally administered vancomycin has limited absorption and has a stool concentration of up to 3100 µg/g, suggesting that the resistance reported to date is not clinically important.

Metronidazole

In 1982, Cherry and colleagues described 13 patients with CDAD who were treated with 1.5–2 g oral metronidazole daily for 7–10 days. All 13 patients responded, although two (15%) had recurrent disease. Soon thereafter, a randomised trial in 92 patients compared 250 mg oral metronidazole four times daily with 500 mg oral vancomycin four times daily; treatment was given for 10 days. Responses to treatment (88% for vancomycin, 90% for metronidazole) and recurrence within a 21-day follow-up period (12% for vancomycin, 5% for metronidazole) were similar.

The apparent equivalence of these two drugs and continuing concern over the selection of vancomycin-
resistant bacteria, especially in hospitals, led the US Centers for Disease Control and Prevention to recommend that metronidazole be used as first-line therapy for CDAD. One study reported a 98% response with 7% recurrences in 632 CDAD patients treated with metronidazole. A response of 90–98% with the use of metronidazole has also been noted in other studies. However, Nair and co-workers described a small series of patients treated either with vancomycin or metronidazole in which 25% failed to respond to a 2-week course of treatment, and 26% had a recurrence within 3 months. Our experience in a large observational study was similar. Infectious disease physicians in North America have also noted a recent increase in the number of refractory and recurrent disease episodes.

In vitro, the MIC of metronidazole for C difficile ranges from 0·20 µg/mL to 2·0 µg/mL (median 0·05). A retrospective review of 61 studies of antibiotic treatment in CDAD: treatment failure and recurrences.

<table>
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<tr>
<th>Studies</th>
<th>Treatment failures [n/total (%)]</th>
<th>Recurrences [n/total (%)]</th>
<th>Duration of follow up (days)</th>
<th>Percentage failure plus recurrence</th>
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<td>Table: Studies of antibiotic treatment in CDAD: treatment failure and recurrences</td>
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Bacitracin

Bacitracin was successfully used to treat isolated cases of CDAD in the 1980s, and was subsequently compared with vancomycin in two randomised clinical trials. There was no difference between the drugs in the clinical response, which ranged from 76% to 100%. On completion of therapy, 53% of patients receiving bacitracin and 14% of those receiving vancomycin still had C difficile toxin in the stool (p<0·05), but this result did not affect the number of clinical recurrences.

Teicoplanin and fusidic acid

Teicoplanin and fusidic acid, neither of which is available in the USA, have been shown to have similar efficacy to oral vancomycin and metronidazole. A European study prospectively compared oral vancomycin, metronidazole, teicoplanin, and fusidic acid in 119 patients with CDAD and found that 93–96% were clinically cured for all regimens. However, 10 patients with CDAD who were initially treated with intravenous metronidazole for a mean of 4 days showed clinical improvement in nine patients. We have used this approach successfully in patients who have toxic megacolon (SA, RJH, DMM; unpublished data). As a note of caution, there is a case report that described failure of intravenous metronidazole in the treatment of pseudomembranous colitis. To our knowledge, no trial has compared oral with intravenous metronidazole for the treatment of CDAD.
treatment with fusidic acid was associated with a significantly higher recurrence (28%, p=0.04) and a higher proportion of adverse events (31% had gastrointestinal discomfort, p=0.001).

Nitazoxanide
Approved for treating protozoan and helminthic infections in the USA in December, 2003, nitazoxanide has already been used to treat 5 million people around the world for these diseases. This drug blocks anaerobic metabolic pathways of microorganisms and is effective against C difficile in vitro (MIC₉₀ 0·06–0·5 µg/mL). In vivo, nitazoxanide prevents colitis after challenge of hamsters with C difficile,89 although it has not been shown to treat established disease. In human beings, approximately two-thirds of the oral dose is excreted in faeces as an active metabolite called tizoxanide, which has an MIC₉₀ of 0·06 µg/mL for C difficile.90 This metabolite has been found at a concentration of 200 µg/mL in human bile after a 1000 mg oral dose (information on file with the US Food and Drug Administration [FDA]) and thus high intraluminal concentrations can be achieved. An open-label, prospective, compassionate-use study in our hospital has shown that treatment with nitazoxanide cured 75% of patients who had failed metronidazole therapy, although one-third of these later relapsed (SA, RJH, DMM; unpublished data); and a double-blind, controlled trial comparing these two drugs is currently underway.

Non-antimicrobial treatments
Antiperistaltic agents—eg, loperamide and diphenoxylate—should be avoided in CDAD. Several case reports have linked the use of antiperistaltic agents in patients with CDAD with the development of toxic megacolon,91,92 probably because they delay excretion of toxin. Pooled human immunoglobulin (200–500 mg/kg) has been used with variable success to treat refractory CDAD in individual patients.93,94 Anti-C difficile bovine immunoglobulin neutralises the effects of toxin B in the cell cytotoxicity assay, and has been used to treat and prevent C difficile colitis in rodents.95,96 Use of a monoclonal antibody to toxin A has shown promising results in animals, and phase II studies in human beings are currently in progress.97,98 The anion exchange resins colestipol and cholestyramine bind the toxin produced by C difficile, but lack clinical efficacy,99,100 and their potential is further compromised by the possibility that they also bind drugs that are used to treat the disease, such as vancomycin.101 Short courses of intravenous methylprednisolone have also been used to effectively treat CDAD in a few patients,102 although because steroids have only been used on a case report basis, formal recommendations cannot be made.

The panel summarises treatment recommendations.

Panel: Treatment recommendations
- Stop treatment with the offending antibiotic, if possible.
- Replete fluid and electrolytes.
- Do not use antimotility agents.
- If specific treatment is required, use metronidazole 500 mg orally every 6–8 hours for 7–10 days. Vancomycin at a dose of 125 mg orally every 6 hours is a second-line alternate agent. If the patient cannot tolerate the drug orally, use intravenous metronidazole, but this should be switched to oral therapy once able to tolerate it. In the case of ileus or toxic megacolon, use intravenous metronidazole, perhaps adding vancomycin retention enemas in a dose of 500 mg mixed in 100 mL normal saline.
- Avoid using vancomycin unless metronidazole seems ineffective, the patient is pregnant or allergic to metronidazole, or true resistance is shown.
- In case of recurrence, re-treat with the agent that had been used to treat the initial episode of CDAD, usually metronidazole.
- In case of multiple recurrences or refractory disease, consider the use of probiotics, immunoglobulin, or steroids.
- In all cases, strict contact isolation of the patient is essential in controlling the spread of the disease to other patients.
- Do not treat symptom-free carriers.

CDAD in patients with HIV infection
Patients with HIV/AIDS are as likely to respond to therapy as other patients,103,104 a finding that might be thought of as surprising in light of the putative role for humoral immunity (see below). Our own observations suggest that patients who have AIDS and develop CDAD, many of whom are younger adults, tend to respond better to treatment than do debilitated, elderly individuals with CDAD.

Recurrences
CDAD recurs after treatment in 8–50% of cases,4,5,7–9,105–107 with recent reports of increases in both recurrent and refractory disease.4,7 A single recurrence tends to be followed by repeated episodes, perhaps in as many as 65% of patients.108,109 New exposure to antibiotics, especially multiple antibiotics, is an important risk factor for recurrence,108,110 as is age older than 65 years,111 severity of underlying illness,112 a low serum albumin concentration (<2·5 g/dL),113 stay in an intensive care unit,114 and hospital stay of 16–30 days.7 It was initially assumed that infection recurred because C difficile sporulated during treatment and then germinated once treatment was completed. However, by use of serotyping, PCR ribotyping, or chromosomal restriction endonuclease analysis, several reports have implicated new strains of C difficile in 10–50% of
occurs. A higher concentration of anti-toxin antibody detected in most patients by 3 days after colonisation decreased risk of recurrence. Our experience has been that repeated courses of metronidazole or vancomycin seem to have similar responses of 70–78% with further recurrences in an additional 25%. The issue of recurrent CDAD remains a vexing problem with no satisfactory resolution at present.

With a better understanding of the pathogenesis of CDAD, treatment has been directed either to restoring a normal colonic ecosystem (which is presumably inimical to the growth of C difficile), or to bolstering the immune response. Stool infusions, in an effort to repopulate the colon with normal colonic flora, have been reported effective in refractory cases. Such therapy lacks aesthetic appeal, not to mention the risk of transferring communicable diseases.

There has also been growing interest in probiotics—the use of non-pathogenic organisms to repopulate the colonic microflora, and thus, presumably, restrict the growth of toxigenic C difficile. Agents that have been studied include a non-toxigenic strain of C difficile, Saccharomyces boulardii, and Lactobacillus spp. McFarland and colleagues studied the addition of S boulardii to vancomycin or metronidazole in a prospective, double-blind study; in 60 patients with recurrent CDAD, there were fewer recurrences in those patients who received S boulardii (35% vs 65%, p=0.04). However, treatment with S boulardii did not decrease recurrences in patients treated for their first episode of CDAD. A placebo-controlled pilot study noted a trend towards a decreased incidence of CDAD in hospital inpatients who were given Lactobacillus and Bifidobacterium spp at the time antibiotic therapy was started, although the results did not reach significance. Although a meta-analysis found that odds ratios from available randomised studies favoured a role for probiotics over placebo, the US FDA was not convinced that the data supported efficacy of S boulardii.

Recurrent CDAD has been treated with some success with intravenous immunoglobulin. No prospective clinical trial has been reported, and it is not known whether the commercial globulin preparations contain antibody to C difficile toxin, or whether antibody to some other antigen is responsible for the putative beneficial effect. On the basis that recurrence is more likely in individuals who lack anti-toxin A antibody, infusion of such antibody or vaccination with a toxoid might be beneficial.

### Treatment of symptom-free individuals

Symptom-free carriers of C difficile are at a relatively low risk of developing CDAD, and treatment is not recommended. However, symptom-free, colonised patients may be a source for spread in hospitals, and there have been attempts to interrupt epidemics of CDAD by treating such individuals. Treatment with oral vancomycin successfully suppresses the organism but may be followed by extended carriage; metronidazole is ineffective. For these reasons, and because symptom-free people are, in general, less likely to be sources of infection than those who have diarrhoea, we do not regard treating them as a viable option.

### Preventive strategies

Implementation of a comprehensive infection control programme that included strict application of universal precautions, periodic educational programmes, phenolic disinfection for environmental cleaning, and strict handwashing was associated with a decrease in the incidence of CDAD from 155 per year to 67 per year in an acute care facility. Use of hypochlorite solution as a disinfectant and disposable rectal thermometers also decrease the risk of spread. Because of the central role of antibiotics in predisposing to CDAD, restrictive antibiotic policies (eg, restricting clindamycin, cephalosporins, and gatifloxacin) have been effective in reducing disease.

The apparent role of immunity in controlling CDAD has prompted research into the development of a vaccine. Various vaccines have been tested with some success in animals, including a formalin-inactivated C difficile toxoid vaccine, live vaccines with Vibrio cholerae and Salmonella typhimurium acting as vector strains and expressing an attenuated toxin A, and conjugate vaccines combining the non-toxic peptide of toxin A covalently with polysaccharides from pneumococcus, Shigella flexneri, and Escherichia coli. A parenteral C difficile toxoid vaccine has been shown to be highly immunogenic in healthy human volunteers, and a trial is underway to test its efficacy in elderly patients as well as in those with recurrent or relapsing CDAD.
Conclusion

*Clostridium difficile* is an important cause of nosocomial morbidity and mortality, and has been implicated in recent epidemics. Current data support the treatment of CDAD with oral metronidazole in a dose of 1·0–1·5 g daily, with oral vancomycin as a second-line agent, although a search for alternate antimicrobial drugs is underway. Treatment of symptom-free patients is not recommended. Current treatment strategies seem to be increasingly ineffective, especially for patients who have multiple recurrences. Biotherapy and vaccination are currently being explored as treatment options for patients with recurrent disease. Greater attention should be paid to hospital infection control policies and restriction of broad-spectrum antibiotics.

Conflicts of interest

DMM is currently involved in a clinical trial assessing the use of nitazoxanide in the treatment of *C difficile*-associated disease with a grant from Romarck Laboratories. SA and RJH have no conflicts of interest.

References

Clostridium difficile


