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Clostridium difficile-Associated Disease
Changing Epidemiology and Implications for Management

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Abstract

Clostridium difficile-associated disease (CDAD) is increasingly being reported in many regions throughout the world. The reasons for this are unknown, are likely to be multifactorial, and are the subject of several current investigations. In addition to the upsurge in frequency of CDAD, an increased rate of relapse/recurrence, disease severity and refractoriness to traditional treatment have also been noted. Moreover, severe disease has been reported in non-traditional hosts (e.g. younger age, seemingly healthy, non-institutionalised individuals residing in the community, and some without apparent antimicrobial exposure). A previously uncommon and more virulent strain of *C. difficile* has been reported at the centre of multiple transcontinental outbreaks. The appearance of this more virulent strain, in association with certain environmental and antimicrobial exposure factors, may be combining to create the 'perfect storm'. It is human nature to be reactive; however, the successful control of *C. difficile* will require healthcare systems (including administrators, and leadership within several departments such as environmental services, infection control, infectious diseases, gastroenterology, surgery, microbiology and nursing), clinicians, long-term care and rehabilitation facilities, and patients themselves to be proactive in a collaborative effort. Guidelines for the management of CDAD were last published over a decade ago, with the next iteration due in the fall (autumn) of 2007. Several newer therapies are under investigation but it is unclear whether they will be superior to current treatment options.

Bacillus difficilis was first described in the laboratory in the mid-1930s. *Clostridium difficile*, as it is now known, was later linked to clinical disease in 1978. Since that time, *C. difficile*-associated disease (CDAD) has largely been thought of as a clinical nuisance (except when causing periodic outbreaks), with the infection itself responding to the discontinuation of the offending antimicrobial(s) in many patients, while some cases progressed to more severe forms of the disease. *C. difficile* has been associated with a full spectrum of disease, ranging from self-limiting diarrhoea to fulminant life-threatening disease including sepsis, colonic perforation and toxic megacolon. *C. difficile*, though responsible for up to 33% of antibacterial-associated diarrhoea (AAD) cases, is not the only cause.[1] *C. perfringens* and *Staphylococcus aureus* are also among other common causes of AAD,[1] and should not be forgotten with all of the attention paid to *C. difficile* of late.

For reasons not fully understood, CDAD appears to be increasing worldwide in both incidence[2-4] and severity of disease.[3,5] Since its discovery as the cause of antibacterial-associated pseudomembranous colitis, outbreaks due to specific strains of *C. difficile* have occurred. In the late 1980s, *C. difficile* restriction endonuclease analysis (REA) type J9 was implicated in outbreaks and was strongly associated with clindamycin exposure.[6] More than a decade later, yet a different and previously uncommon strain, REA type BI (also known as North American Pulse field type 1 [NAP1]/polymerase chain reaction [PCR] ribotype 027) has been associated with recent outbreaks in North America, the UK and other parts of Europe.[7] It is concerning that CDAD is also being reported in a subset of the population previously thought to be at minimal risk for the disease, e.g. younger patients, peripartum, community-dwelling outpatients, some without admitted exposure to antimicrobials.[8] Recently published observations suggest that metronidazole is not as effective in the management of CDAD; but is this really true? Because of the serious consequences of CDAD, a variety of poorly studied or ineffective treatment strategies have crept their way into clinical practice: are they helpful? In addition, several new treatment options are being studied. This paper focuses on the changing epidemiology of *C. difficile*, reviews recent findings related to virulence characteristics, and provides an update on newer agents being studied for the management of CDAD.

1. Clinical, Pathological and Laboratory Diagnosis

In brief, CDAD typically presents as watery diarrhoea without the presence of visible blood in stool. Fever, abdominal pain and leukocytosis typically accompany diarrhoea. In some patients, diarrhoea is not a presenting feature, particularly when ileus or toxic megacolon is evident. Radiographic studies of the abdomen can reveal a thickened colonic wall, ascites or colonic dilatation (typically indicating toxic megacolon). The use of CT scans may be helpful in patients with severe CDAD to provide additional data for surgical decisions. Colonoscopy usually reveals inflammation with or without pseudomembranes. Histopathological studies of biopsy specimens obtained at colonoscopy tend to show classic 'volcanic' lesions.

A laboratory diagnosis is typically made by performing tests to determine the presence of toxins A and/or B, with the most common test in use in the US being the enzyme immunoassay (EIA) kit for toxins A and B. The more resource-intensive cell culture cytotoxin assay is also used in some countries and is more sensitive than EIA testing. Using culture (alone) to diagnose CDAD is useless because non-toxigenic strains of *C. difficile* are prevalent and are not causes of disease. A renewed interest in culturing *C. difficile*, however, has emerged in an effort to learn more about toxigenic strains and antimicrobial susceptibility. Central laboratories in the US, Canada and the UK, and perhaps other geographical areas, have increased their capabilities to conduct more widespread testing of clinical isolates of *C. difficile*. Tests may include REA typing, PCR ribotyping, pulsed-field gel electrophoresis (PFGE), toxinotyping and susceptibility testing.

2. The Epidemic Strain (NAP1/027)

2.1 Toxin Production

NAP1/027, similar to other strains of *C. difficile*, produces the two traditional toxins, toxin A and toxin B.[9] Both toxins are typically expressed in patients with clinical disease; however, toxin A-negative and toxin B-positive strains have been identified in patients with severe CDAD.[10] Both toxins are large (270–308 kDa) and are encoded for on a chromosome within the pathogenicity locus (PaLoc) of the organism. Also located within the PaLoc are regulatory genes such as *tcdC*, which is a downstream negative regulatory gene that modulates the expression of toxins A and B. Akerlund and colleagues[11] reported that when a large proportion of the *C. difficile* population is in vegetative form, toxin production is at its greatest level, and conversely when the majority of cells are in spore form, toxin production is greatly diminished. Therefore, the organism's ability to produce toxin and to sporu-

late may reflect opposing and alternative survival mechanisms during nutrient shortages (stationary growth phase).[11] The same investigators were unable to correlate disease severity with faecal toxin levels and PCR ribotype (the NAP1/027 strain was not studied). Thus far, all NAP1/027 strains contain an 18-base pair *tcdC* gene deletion that is thought to be responsible for the accelerated kinetics of toxin production.[7,12] *In vitro* studies demonstrated that NAP1/027 strains produce 16- and 23-fold more toxin A and B, respectively, compared with toxinotype 0 strains.[13] Freeman et al.,[14] however, point out that characterising *in vitro* toxin production is subject to several limitations and *in vivo* toxin production may not be readily inferred from these experiments.

2.2 Binary Toxin

NAP1/027 strains also possess a previously uncommon binary toxin gene (noted to be present in 6% of an historical sample of clinical isolates).[7] The structure and function of this toxin is similar to that of other binary toxins, such as iota toxin found in *C. perfringens.* Although patients infected with binary toxin-positive strains of *C. difficile* trended towards having greater disease severity,[15,16] toxin A- and B-negative but binary toxin-positive strains of *C. difficile* have been shown to be non-pathogenic in classic nonclinical models of infection.[17]

2.3 Toxinotyping

In addition to *C. difficile* identification methods that include PFGE, PCR and REA typing, strains can also be distinguished by toxinotyping studies. In short, these studies examine subtle sequence variations in the PaLoc of the *C. difficile* strain. To date, at least 22 different toxinotypes have been reported.[18] Toxinotype III, to which NAP1/027 belongs, was previously rare, accounting for only 2–3% of clinical isolates. Other toxinotypes have been identified (e.g. XIV/XV and IX) in patients with community-associated and peripartum disease that are 64% and 85%, respectively, related to the epidemic toxinotype III (NAP1/027) strain.[8] These strains also contain the 18-base pair gene deletion and harbour the binary toxin.[8] Whether toxinotyping can be used to distinguish virulence potential among strains has yet to be demonstrated.

2.4 Sporulation Capacity

C. difficile, like *Bacillus anthracis*, possesses an uncommon virulence factor in that it is able to form spores in response to a hostile environment or a nutrient-deprived milieu. Therefore, *C. difficile* is capable of surviving for long periods in the environment as well as in sanctuaries within the gastrointestinal tract. Some genotypically distinct strains of *C. difficile* demonstrate a greater propensity to hypersporulate and are often associated with outbreaks.[19] NAP1/027, like other outbreak strains, has demonstrated the capacity to hypersporulate compared with other non-outbreak strains (figure 1).[20] As with the other recently identified characteristics of this organism, future studies are required to elucidate the exact role of hypersporulation in the transmission or pathology of *C. difficile*.

3. Linking NAP1/027 to Severe Disease

In 2000, reports from the University of Pittsburgh noted increased CDAD incidence and severity as measured by a doubling of disease rates, increased number of colectomies performed, and deaths.[5,15,21] A CDAD outbreak attributed to NAP1/027 in Quebec resulted in an attributable mortality of 17%[22] and more than 1400 deaths,[23] as well as an increased number of colectomies and additional

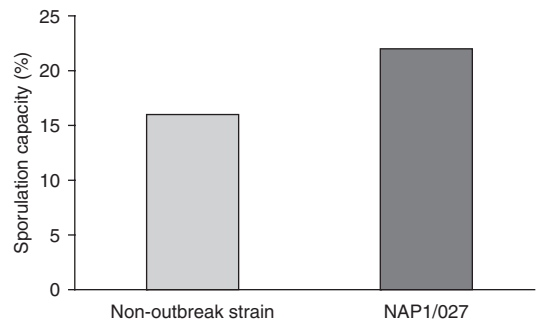

Fig. 1. Sporulation capacity of *Clostridium difficile* NAP1/027 compared with non-outbreak strains. BI/NAP1 vs non-outbreak: $p < 0.05$.

length of hospitalisation for those who survived. Unusually high recurrence rates were also reported (58%) in patients over the age of 65 years.[24] While circumstantial clinical evidence can be used to link the appearance of NAP1/027 strains of *C. difficile* with more severe disease, clinical studies better able to answer this question are under way.

4. Risk Factors for *Clostridium difficile*-Associated Disease (CDAD)

Risk factors for acquiring CDAD are complex and consist of exposure to toxigenic strains of the organism,[25] prior use of any antimicrobial agent,[26] duration of antimicrobial therapy,[27] the degree *in vitro* activity against *C. difficile* of an antimicrobial,[28] exposure to gastric acid suppressants,[29] poor host serum immunoglobulin levels,[30] poor colonic IgA production,[31] advanced age,[32] and severity of underlying illness of the host.[32] Risk factors can be broken down into exposure risks (environmental) and host risks.

4.1 Environmental Risks

Since *C. difficile* is a spore-forming organism, it is not surprising that it is able to survive on inanimate surfaces for long periods.[33] Admission to inpatient acute care facilities, long-term care facilities and rehabilitation centres have long been risk factors for acquiring *C. difficile*. Length of stay at these high-risk facilities, as well as proximity to symptomatic patients with CDAD at these facilities, also increases the risk of acquiring toxigenic strains of *C. difficile*. It has been shown that as levels of environmental contamination rise, so does the prevalence of *C. difficile* found on the hands of healthcare workers.[34] The risk of acquiring CDAD during hospitalisation is exponentially increased at dilapidated healthcare facilities, facilities with communal toilets and showers, those institutions where cutbacks involving environmental services budgets/personnel have negatively affected both the frequency and the extent of surfaces in the patient's room being properly cleaned, and where hand hygiene compliance among healthcare workers is poor. Feeding tubes have been identified as risk factors for CDAD,[35] as they are both surrogate markers for increased contact with healthcare personnel and a means of delivering the organism directly into the gastrointestinal tract.

4.1.1 Handwashing Versus Alcohol Hand Rubs

The use of alcohol-based hand rubs is a convenient, efficient and effective method for improving hand hygiene in inpatient facilities. While effective in its action against most pathogenic bacteria found in hospital settings, alcohol is ineffective against spore-forming organisms. In a recent study, 18–60% of the initial inoculation of *C. difficile* spores on a contaminated hand could be readily transferred by a handshake after using commercially available alcohol gels.[36] In contrast, the mechanical action of handwashing in a sink with soap and water has proven effective in removing *C. difficile* from the hands of healthcare workers.[36] While some have attempted to blame reliance on alcohol hand rubs for escalating *C. difficile* rates, a retrospective study did not support this notion.[37] The US Center for Disease Control and Prevention (CDC) does recommend substitution of alcohol-based hand rubs with handwashing using soap and water during outbreak situations, and also recommends the use of contact precautions in all CDAD patients.[38] When caring for patients with CDAD, handwashing with soap and water is a vital part of decreasing the risk of healthcare worker-to-patient (and vice versa) transmission of *C. difficile*, and alcohol products should be avoided; for all other patients, alcohol hand rubs should continue to be encouraged.

4.1.2 Cleaning Agents

Traditional quaternary ammonium-based cleaning agents typically used in healthcare settings are not sporicidal, and may actually encourage vegetative forms of *C. difficile* to sporulate.[19,20] Thus, similar to widespread use of alcohol with its relative ineffectiveness in killing *C. difficile* spores, standard hospital cleaning agents are also ineffective in removing *C. difficile* from the environment. Chlorine-based solutions (hypochlorite and isocyanuric acid-based compounds) are sporicidal. Several studies have demonstrated the effectiveness of replacing standard hospital cleaning products with 10% sodi-

um hypochlorite solutions during outbreaks, resulting in significant reductions in the number of CDAD cases as well as reducing the environmental spore burden.[39-41] It is important not only to use a sporicidal cleaning agent, but also to ensure that all 'high contact' surfaces that surround the patient and healthcare worker are actually cleaned. Taken a step further, patients who are discharged home or who are being managed as outpatients may be advised to clean their bathrooms (toilet seats, sinks and other surfaces) with 10% bleach solutions that are prepared fresh each day. Chlorine-based cleaning agents have demonstrated efficacy in killing vegetative and spore forms of NAP1/027 as well as other hypersporulating outbreak strains.[20]

4.2 Host-Related Risk Factors

4.2.1 Host Immunity

Host-related risk factors play an important role in who becomes infected as well as who remains susceptible to multiple recurrences. In one study, those who were not able to develop serum anti-toxin A IgG titres in response to colonisation with *C. difficile* were 48 times more likely to develop diarrhoea compared with those who mounted an adequate immune response.[42] In addition, it has been noted that a significant reduction in colonic mucosal IgA-producing cells and macrophages has been linked to recurrent CDAD.[31] The senescence of immunity is probably why the majority of patients who develop CDAD are older, but this can be an oversimplification of the issue. Older adults are also more likely to be hospitalised or institutionalised, to receive antimicrobials, have multiple co-morbidities and to remain hospitalised for longer periods.[27]

4.2.2 Antimicrobials

Another important host-related risk factor is antimicrobial use. In fact, it has always been a basic tenant of CDAD that antimicrobial use precedes infection due to *C. difficile*. Recent observations and population-based studies have challenged the paradigm that clinical exposure to antimicrobials is required in patients in order for *C. difficile* to cause disease.[29] For example, 61% of patients who developed community-acquired CDAD did not admit to having antimicrobial exposure within the 90 days prior to developing disease.[29] Another recent study also demonstrated that 59% of patients who developed community-acquired CDAD did not have documented antimicrobial exposure.[43] These findings may be unique to patients with community-acquired CDAD, where other risk factors remain to be determined, such as exposure to gastric acid suppressants or the innate virulence of the organism.

All antimicrobials (including certain chemotherapeutic agents) have the potential to disrupt colonisation resistance and increase the risk of CDAD; however, some agents may pose greater risks than others.[26] All antimicrobials disrupt the normal microbiota enough to allow toxigenic strains of *C. difficile* to initiate disease; this includes exposure to the treatments themselves (oral vancomycin, metronidazole).[44,45] Variables that augment risk include prolonged exposure to antimicrobials[27] and exposure to antimicrobials lacking *in vitro* activity against the infecting strain of *C. difficile*.[28] It is a common, but unproven, perception that antimicrobials associated with anti-anaerobic activity pose greater risk than drugs lacking activity against anaerobes,[39] but this has not been supported by quality evidence.[46-48] What seems to be more clear is that antimicrobials formerly thought to pose low-to-moderate risk, but over time become used more frequently, may become more commonly associated with CDAD.[21,27,47,49] This appears to be the case with the fluoroquinolones. Also exacerbating their role in CDAD is the fact that NAP1/027 is pan-resistant to all agents within the fluoroquinolone class. Traditional higher-risk agents remain at relatively high risk (e.g. cephalosporins), while clindamycin has shown variable risk. The variable risk of clindamycin may stem from the varying degree of susceptibility to NAP1/027 strains (in contrast to the uniform resistance observed in the J-type outbreak strains) as well as its infrequent use in adults (at least in the US).

As a result of numerous and complex relationships between variables and the failure to control for them, the literature prior to 2001 is replete with

dubious studies. In fact, most studies have failed to adequately address important confounding variables, resulting in serious threats to the validity of their findings.[50] Since the published critique of past CDAD risk factor ascertainment studies, several studies have been presented or published that are better designed to assess risk.[12,21,27,51] However, one of the remaining chief difficulties in risk factor ascertainment studies for CDAD is the fact that many patients have received multiple antimicrobials in the 6–8 weeks before developing CDAD, further complicating the ability to assign blame to a particular agent. In addition, as a result of the retrospective nature of the study designs, it is difficult to obtain a completely accurate outpatient antimicrobial history.

4.2.3 Gastric Acid Suppression

The suppression of gastric acid can increase host susceptibility to a variety of infections. Dial and colleagues[52] used cohort and case-control study designs to determine whether exposure to proton pump inhibitors (PPIs) was an independent risk factor for CDAD. Multivariable analyses revealed statistically significant adjusted odds ratios (95% CI) of 2.1 (1.2, 3.5) and 2.7 (1.4, 5.2) in the two studies identifying PPI use as a risk factor for CDAD. The use of gastric acid suppressants has also been associated with the development of community-acquired CDAD.[29] Others have also implicated the use of PPIs as an independent risk factor for CDAD,[21,53,54] while some investigators have not.[27,55] PPIs have also been shown to cause diarrhoea with or without specific histological findings from biopsy specimens obtained during colonoscopy (forms of microscopic colitis such as lymphocytic and collagenous colitis).[56,57] These patterns are different to the 'volcanic eruption' type histological findings from biopsy specimens taken from patients with CDAD. Clinicians should be mindful of this, as colitis caused by PPIs may interfere with or delay the diagnosis of CDAD. Future prospective studies of this association are warranted; however, given the current evidence, it seems logical to increase our vigilance regarding the stewardship of PPI use.

5. Management Strategies

Treatment algorithms used at my hospital and developed in collaboration with experts from North America and the UK are presented in figure 2 and figure 3.[47] The first step to managing a patient with CDAD is to re-evaluate the need for the offending antimicrobial agent (if the patient is still on therapy). If the patient is receiving an antimicrobial, its continued use must be justified or otherwise discontinued. Because studies continue to report that antimicrobials are misused (e.g. given for sinusitis without regard to duration of symptoms, or prescribers admitting to issuing antibiotics to satisfy a patient's request rather than based on objective evaluation for actual infection),[58,59] it is sensible to require clinicians to formally justify the need for the antimicrobial. One message was clear from the recent Cochrane review on interventions to improve antimicrobial prescribing practices among hospital inpatients,[60] programmatic strategies to improve antimicrobial use have been shown to reduce rates of CDAD.

It is also worthwhile mentioning that for patients without CDAD but who are receiving antimicrobials for the treatment of an underlying infection, 'prophylaxis' to prevent CDAD is unwarranted. This is because the very antimicrobials that treat CDAD are also capable of causing CDAD.[26,44,45] In addition, they have no effect on *C. difficile* spores and can further disrupt the intestinal microbiota, and may select for other resistant organisms.

Metronidazole and oral vancomycin are discussed primarily in this section because of their extensive use worldwide. Teicoplanin,[61] fusidic acid[62] and bacitracin[63,64] have demonstrated efficacy similar to metronidazole or vancomycin. Investigational agents are also discussed in section 8 (table I).

5.1 Therapy for First or Second Episodes

For patients with first or second episodes of non-severe CDAD and who have a functioning gastrointestinal tract, metronidazole is still recommended as first-line therapy (figure 2). Data supporting this are derived from randomised comparative clinical trials with vancomycin,[65] and a recent retrospective ob-

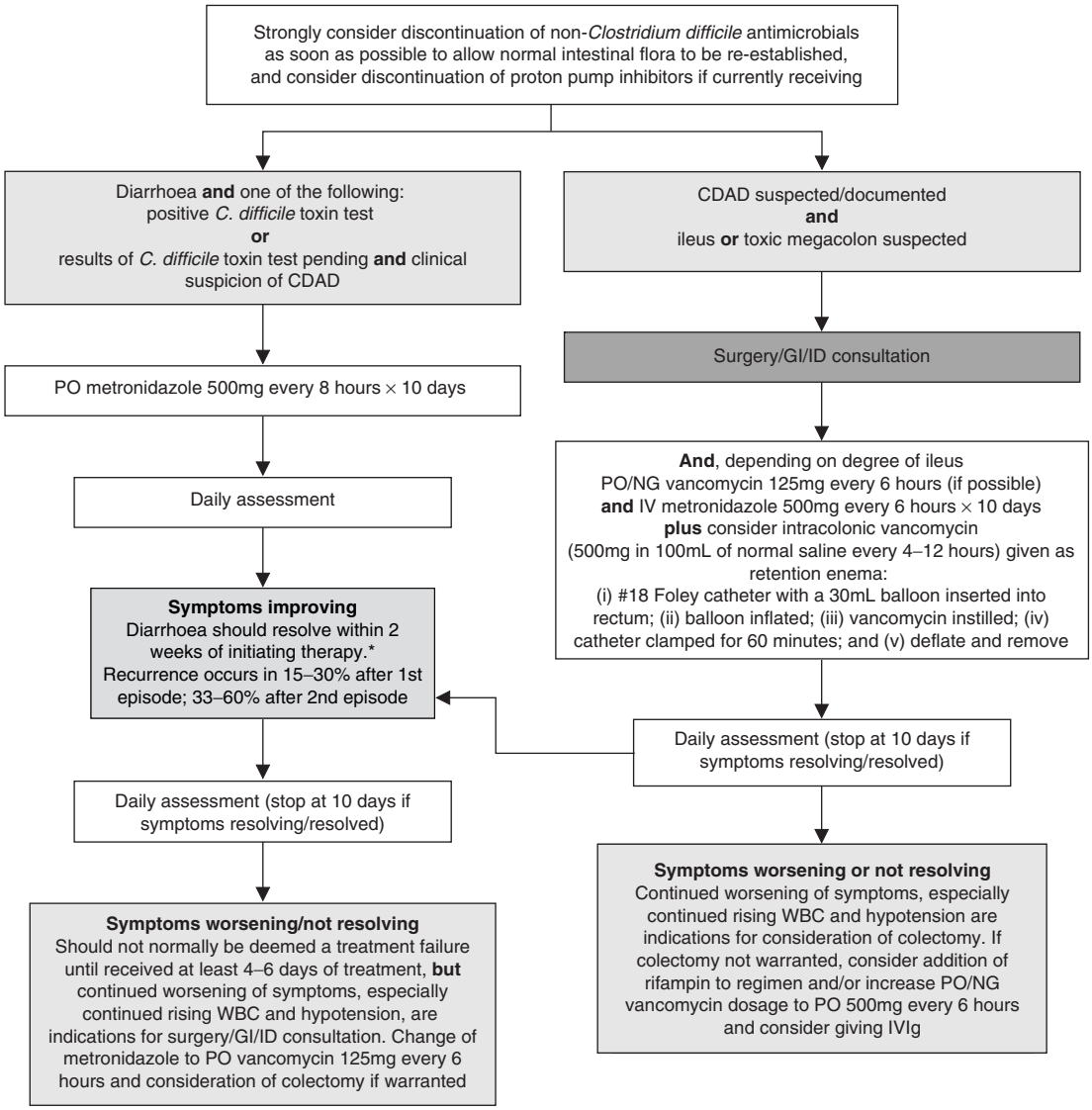

Fig. 2. Treatment algorithm for first and second episodes of *Clostridium difficile*-associated diarrhoea (CDAD) within 6 months. * Clinicians should be cognisant of the number and description of bowel movements per day to gauge clinical response. It is important to avoid unnecessarily long treatment durations that further disrupt the commensal flora. **GI** = gastrointestinal; **ID** = infectious disease; **IV** = intravenous; **IVIg** = IV immunoglobulin; **NG** = nasogastric; **PO** = oral; **WBC** = white blood cell count.

servational study that included infection with NAP1/027 strains.[66] In fact, regardless of whether metronidazole or vancomycin was used for second-episode cases, the complication rates were higher for both options compared with historical controls.[66] A slightly delayed response has historically been observed with metronidazole, with the mean length of time with symptoms being 4.6 days compared with vancomycin (3.0 days), but patients ultimately responded equally and shared similar relapse rates.[67] In most circumstances, metronidazole remains the drug of choice. Vancomycin is preferable when multiple episodes of CDAD have been documented (see section 6.2 and figure 3), for severe

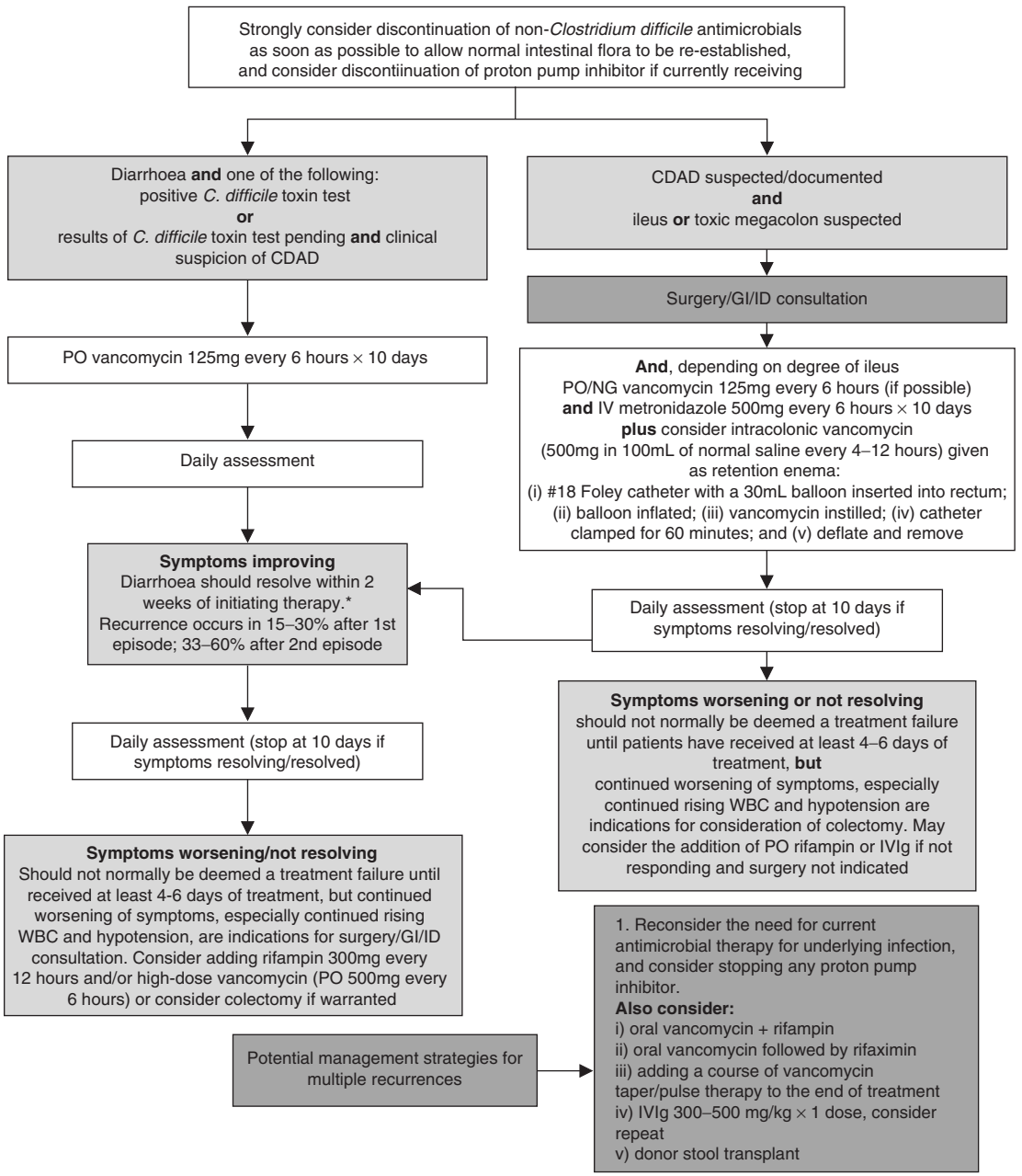

Fig. 3. Treatment algorithm for third or further episodes of *Clostridium difficile*-associated diarrhoea (CDAD) within 6 months. * Clinicians should be cognisant of the number and description of bowel movements per day to gauge clinical response. It is important to avoid unnecessarily long treatment durations that further disrupt the commensal flora. **GI** = gastrointestinal; **ID** = infectious disease; **IV** = intravenous; **IVIg** = IV immunoglobulin; **NG** = nasogastric; **PO** = oral; **WBC** = white blood cell count.

CDAD and if intolerance to metronidazole exists. When oral vancomycin is used, evidence dictates that 125mg administered every 6 hours is equivalent to 500mg every 6 hours in terms of efficacy,[68] but

Table I. Treatment options and biologicals in development for *Clostridium difficile*-associated disease

Product (trade name if available)[a]	Type	Stage of development	Manufacturer
C. difficile vaccine	Vaccine	Phase I	Acambis
Monoclonal antibodies (anti-toxin A: MDX-066; anti-toxin B: MDX-1388)	Antibodies	Phase II	Medarex
Nitazoxanide (Alinia®)	Antimicrobial	Phase III	Romark Laboratories
Ramoplanin	Antimicrobial	Phase III	Oscient Pharmaceutical
Rifaximin (Xifaxan®)	Antimicrobial	Phase III	Salix Pharmaceutical
PAR-101 (OPT-80)	Antimicrobial	Phase III	Optimer Pharmaceutical
Tolevemar	Polymer	Phase III	Genzyme Corporation

a The use of trade names is for product identification purposes only and does not imply endorsement.

costs significantly less. There also appears to be no value to adding rifampicin (rifampin) to metronidazole for patients with first-episode CDAD.[69] After the first episode of CDAD, 12–20% of patients can be expected to relapse, while if a second episode of CDAD occurs, 33–60% of patients can be expected to have a relapse.[70] The second episode should be treated in the same manner as the first, unless mitigating circumstances (e.g. ileus, severe disease markers) are present.

In most parts of the world, *in vitro* resistance among clinical isolates of *C. difficile* to either metronidazole or vancomycin has not been reported;[71] therefore, the fear of *in vitro* resistance to these primary therapies should not guide the selection of treatment in most geographical areas. A small number of strains with elevated minimum inhibitory concentrations (MICs) to metronidazole and vancomycin have been reported,[72] but validation by an outside laboratory has not occurred and the clinical meaning is unclear (as concentrations in stool, particularly for vancomycin, far exceed the MICs reported).

5.2 Therapy for Third or Further Episodes (Recurrent Disease)

In the clinic it is difficult to determine whether a multiple episode of infection is due to re-infection with a new strain or a relapse involving the original infecting strain of *C. difficile*. Vancomycin appears to be the drug of choice for multiply recurring cases of CDAD (figure 3).[73] One approach is to use vancomycin 125mg four times daily for 10 days, and follow this up with either pulse-dosed or tapered vancomycin regimens.[73,74] For endogenously recurring CDAD, spores may be the source of the problem.[73,75] A regimen we favour is vancomycin administered in a pulsed fashion, 125–500mg given as a single dose every 3 days for 2–3 weeks as described by McFarland et al.[73] It is theorised that persistent spores may be encouraged to recrudesce in the absence of antimicrobials; hence, the newly transformed vegetative cells become susceptible to pulsed-vancomycin. Although higher dose vancomycin (500mg four times daily) is effective when given for 10 days to patients with recurrent CDAD, it is associated with higher recurrence rates than standard therapy followed by a pulsed or tapered vancomycin regimen.[73] Higher dose metronidazole was not effective at reducing future recurrences.[73] The addition of rifampicin to vancomycin has been reported to be effective in a small study of patients with recurrent CDAD.[76] We tend to use lower doses of rifampicin (300mg twice daily) than that studied (600mg twice daily), for tolerability reasons.[47] Donor stool transplantation has been shown to be effective in a small number of patients and in a small number of our own patients.[77-80] However, for obvious reasons that include the potential spread of other infections, this should be considered near the end of the list of treatment strategies.

Administration of non-toxigenic strains of *C. difficile* has demonstrated efficacy in nonclinical models of infection as well as in a limited number of patients.[81,82] Toxigenic strains of *C. difficile* maintain their populations with a large fitness cost compared with non-toxigenic strains of *C. difficile*. The hypothesis has demonstrated efficacy; non-toxigenic strains introduced to antimicrobial-treated ani-

mals outcompete subsequently introduced toxigenic strains into the same animal, resulting in no mortality post-infection.[81]

A variety of management options – including probiotics, cholestyramine and antiperistaltic agents – with theoretical benefits have gained in popularity with some clinicians in light of recurrent and severe CDAD. Unfortunately, quality data cannot be found to recommend their use, and there is good evidence that significant harm is associated with their use. For instance, studies evaluating the use of probiotics for recurrent CDAD have been plagued by small sample sizes or heterogeneous samples (cases of CDAD mixed with other causes of AAD, where in the latter, probiotics have demonstrated efficacy). In brief, the current literature does not support the use of probiotics in adult patients with CDAD, as summarised in a recent systematic review.[83] Some have even made the mistake of conducting meta-analyses to determine the efficacy of probiotics;[84,85] however, because the study populations and designs/methods are so heterogeneous (patients, definitions of disease, lack of control for confounding, single organism versus multi-organism formulations), a meta-analysis cannot be reliably performed or interpreted. One study evaluating *Saccharomyces boulardii* for the prevention of recurrent CDAD demonstrated a slight benefit;[86] however, it was not sufficiently convincing when evaluated by the US FDA.[44] Moreover, an increasing body of literature demonstrates the potential harm of probiotics when used in patients with CDAD, chiefly in the form of bacteraemias due to *Lactobacillus* spp. and fungaemias due to *S. boulardii* in both immunocompetent and immunocompromised hosts.[87-90] Similarly, anion binding resins or adsorbants (cholestyramine, colestipol) have crept their way into review articles as viable treatment options. These agents theoretically bind *C. difficile* toxins; however, a placebo-controlled trial demonstrated that colestipol was no more effective than placebo in reducing faecal excretion of *C. difficile* toxins.[91] Like probiotics, there is potential for harm, as cholestyramine has been shown to bind known effective therapies (e.g. vancomycin), rendering them potentially less effective at the site of infection.[92] In addition, antiperistaltic agents such as loperamide should not be used, as some investigators have associated their use with developing toxic megacolon.[93]

5.3 Special Situations

5.3.1 CDAD with a Nonfunctioning or Partially Functioning Gastrointestinal Tract

Not many treatment choices are currently available for patients with a non-functional gastrointestinal tract. Intravenous metronidazole 500mg four times daily has been used, with or without oral vancomycin (if possible) and intracolonic vancomycin (if possible),[94] while realising that the treatment of CDAD with intravenous metronidazole has not been rigorously studied and failures have been noted.[95] Intracolonic vancomycin should be used with caution because of the friable state of the colonic mucosa and surgical consultation is strongly advised in these patients.

5.4 Therapy for Non-Responders

For patients with refractory disease (not responding at the day 4–6 evaluation point or who are worsening during treatment upon daily assessments), a few decisions need to be made. If markers for severe disease are present at any time during therapy (high white blood cell count, ascites, obstruction, colonic perforation, toxic megacolon), surgical consultation is obligatory. Because of the high mortality associated with severe CDAD, colectomy needs to be considered; however, in one series of 67 patients undergoing colectomy for severe CDAD, surgical morbidity was 81% and overall mortality was 48%.[96] A recent study examined the impact of emergency colectomy versus medical therapy alone for fulminant CDAD.[97] A reduction in 30-day mortality was noted in the surgical intervention group after adjusting for independent risk factors that included leukocytosis $\geq 50 \times 10$/L, lactate ≥ 5 mmol/L, age >75 years, immunosuppressors and shock requiring vasosuppressors.

If a patient was started on oral metronidazole, therapy should be changed to oral vancomycin. If vancomycin was chosen initially, a higher dose of

vancomycin (500mg every 6 hours) can be chosen or rifampicin can be added to vancomycin (although there are no data to support the latter option, we have found it helpful). Intravenous immunoglobulin (IVIg) has also been studied in a limited number of patients with refractory disease, with mixed results. The premise for therapy with IVIg is based on the fact that patients who develop severe disease and/or relapsing disease mount a poor antitoxin antibody response. Pooled IVIg may contain antitoxin A IgG. The largest study to date of IVIg was a retrospective, observational evaluation of 14 patients.[98] Six of 14 patients were cured (clinically responded and no relapse occurred within the timeframe reported). The doses used ranged from 150 to 400 mg/kg administered as a single dose, while one patient received a second dose. For those who responded to IVIg, the median response time was 10 days. In another study, five patients were treated with varying doses of IVIg (300–500 mg/kg), with the 400 mg/kg dose being most commonly chosen.[99] Three of the five patients were deemed successfully treated, with resolution occurring within 11 days. While it may provide a means for response for patients with severe/relapsing disease who are otherwise without alternative therapy, the disadvantages include marginal efficacy, unknown optimal dose, high cost (≈US$1500/dose for a 70kg patient),[100] and that it is often in short supply.[100]

6. Currently Marketed Drugs with Activity against CDAD, but Limited Supporting Data Available

6.1 Rifaximin

Rifaximin, a non-absorbed rifamycin derivative, is approved for use in traveller's diarrhoea and has good *in vitro* activity against *C. difficile*. However, *in vitro* high-level resistance (MIC >256 μg/mL) already exists to this compound (3% of strains tested in one series).[101] Because of documented resistance coupled with the absence of clinical efficacy data, this drug should not be used for the treatment of CDAD until its efficacy can be confirmed in adequate trials and we better understand the clinical meaning of the resistance reported to rifaximin.

6.2 Nitazoxanide

Nitazoxanide is currently marketed for the treatment of a variety of parasitic diarrhoeal illnesses. Nitazoxanide has *in vitro* activity against *C. difficile* and has recently been studied as a 500mg twice daily regimen given for either 7 or 10 days versus metronidazole 250mg four times daily for 10 days.[102] This was a randomised, double-blind study in adult hospitalised patients, enrolling between 36–40 patients in each of the three treatment arms. Response rates after 7 days of treatment and 31 days after beginning treatment for metronidazole, nitazoxanide for 7 days and nitazoxanide for 10 days were 82.4/57.6%, 90/65.8% and 88.9/74%, respectively.[102] Nitazoxanide demonstrated non-inferiority to metronidazole in this relatively small study.

6.3 Tinidazole

Tinidazole, like metronidazole, is a nitroimidazole. Tinidazole is currently approved by the FDA for the treatment of trichomoniasis, giardiasis and amoebiasis. Tinidazole is active *in vitro* against clinical isolates of *C. difficile*.[103] Clinical studies evaluating the efficacy of tinidazole for the management of CDAD are lacking and as such this compound also cannot be recommended for CDAD at present.

7. Investigational Treatment/Prevention Options for CDAD

7.1 PAR-101

PAR-101 (or tiacumicin b complex, formerly OPT-80) is an 18-membered macrocyclic compound with limited activity against intestinal flora, and is highly active against *C. difficile*. Phase III studies of PAR-101 are ongoing.[104] Similar to other novel treatments for *C. difficile*, PAR-101 is minimally absorbed, demonstrates low MIC values against *C. difficile*, and has been shown to be effective in nonclinical models of CDAD.[105]

7.2 Ramoplanin

Ramoplanin seems to be stalled in phase III development at present. Ramoplanin demonstrated similar efficacy compared with vancomycin in the clindamycin-induced *C. difficile* infection model in hamsters.[106] Interestingly, results from the *in vitro* gut model showed that ramoplanin appeared to have a beneficial effect on *C. difficile* spores and spore recrudescence compared with vancomycin ($p < 0.05$).[106]

7.3 Tolevamer

Tolevamer is a liquid polystyrene preparation that binds to *C. difficile* toxins A and B. The results of a randomised, double-blind, active-controlled phase II study in patients with mild-to-moderate CDAD were recently reported.[107] Two doses of tolevamer (3 g/day, 6 g/day) were evaluated against vancomycin (125mg every 6 hours). Tolevamer 6 g/day, but not 3 g/day, demonstrated non-inferiority to vancomycin, with a trend towards reduced recurrence in the high-dose tolevamer arm ($p = 0.05$).[107] Because tolevamer is not an antimicrobial *per se*, commensal microbiota are not adversely affected and its therapeutic effect is purely due to toxin neutralisation. Overall, tolevamer was well tolerated except for the finding of hypokalaemia, which occurred in 23% of the tolevamer-treated patients versus 7% of vancomycin recipients ($p < 0.05$).[107] As a result of this study, a new liquid formulation of tolevamer that allows higher doses to be administered and that contains potassium as a counter ion to minimise hypokalaemia has been studied in a phase I trial.[107]

7.4 Toxoid Vaccine

A *C. difficile* toxoid vaccine is currently in phase I trials. A very small study of three patients with recurrent CDAD (patients requiring 7–22 months of continuous vancomycin therapy) evaluated this parenterally administered *C. difficile* vaccine containing toxoid A and toxoid B.[108] Two of the three patients demonstrated increased IgG antitoxin A antibodies (3- and 4-fold increases), while an increased IgG antitoxin B antibody response was observed (20- and 50-fold).[108] All three patients discontinued use of vancomycin without further recurrence following vaccination.

7.5 IgG, Monoclonal Antibody

Human monoclonal antibodies against toxins A (MDX-066) and B (MDX-1388) have been evaluated in cell neutralisation assays, the hamster model of CDAD, and have begun studies in humans. In the hamster model, a combination of both anti-toxins A and B reduced mortality from 100% to 45% ($p < 0.0001$).[109]

8. Conclusions

C. difficile has periodically diversified. In the late 1980s, the REA 'J-type' strains were linked to outbreaks in acute care facilities and demonstrated *in vitro* resistance to clindamycin. Currently, REA 'BI-type' strains (NAP1/027) are being implicated as causes of outbreaks, demonstrate variable resistance to clindamycin, but are uniformly resistant to all fluoroquinolones. Like other outbreak strains, NAP1/027 has the potential to hypersporulate, which, in concert with cutbacks in healthcare spending leading to reduced environmental services and nursing resources and infrastructure dilapidation, may explain its widespread dissemination. In contrast to other outbreak strains, certain virulence characteristics, specifically hypertoxin production, seem to provide a rationale for more severe disease resulting in delayed response to traditional therapies, increased morbidity and increased mortality. The attributable financial impact of increased hospital rates of CDAD has been quantified,[110] but few administrators have reacted to such studies. The cure for this attention deficit will come in the form of:

- lost revenue due to decreased bed-turnover as a result of excess length of stay;
- high census hospitals with unoccupied beds due to bed A being occupied by a symptomatic CDAD patient requiring isolation, with the failure to cohort and fill bed B for various reasons

(similar to our current observations with the impact of MRSA);

- the expected spike in the pharmacy drug budget as a result of clinician demand for the array of new and guaranteed expensive treatments/vaccines that will be trickling out of the pipeline; and/or
- the potential for public reporting of infection due to *C. difficile*.

While it is too simplistic to assume that a basic change in an antimicrobial formulary or antimicrobial restrictions as single intervention strategies will be successful in reducing CDAD rates where NAP1/027 strains are endemic, administrative support for establishing formal antimicrobial stewardship programmes in concert with infusing adequate resources for infection control and environmental services departments are likely to have the greatest impact on minimising the morbidity, mortality, and financial burden associated with CDAD.

From the clinician's perspective, using antimicrobials more judiciously (withholding antimicrobials with close observation for mild infections not likely to be caused by bacteria, stopping antimicrobials when infection is ruled out, using short-course therapy), initiating treatment for CDAD early and closely monitoring the patient's clinical progress, washing hands with soap and water rather than using alcohol hand rubs when caring specifically for CDAD patients, and educating patients regarding proper disinfection at home are likely to have a positive impact. Finally, a host of treatments and preventative strategies are being investigated and we can help by participating in clinical trials.

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