Clostridium difficile—a moving target
Glenn S. Tillotson¹* and Joni Tillotson²

Addresses: ¹Optimer Pharmaceuticals, Inc., 10110 Sorrento Valley Road, Suite C, San Diego, CA 92121, USA; ²Department of Biology, Immaculata University, Immaculata, PA 19345, USA

* Corresponding author: Glenn S. Tillotson (gtillotsonconsult@yahoo.com)

F1000 Medicine Reports 2011, 3:6 (doi:10.3410/M3-6)

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial License (http://creativecommons.org/licenses/by-nc/3.0/legalcode), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. You may not use this work for commercial purposes.

The electronic version of this article is the complete one and can be found at: http://f1000.com/reports/m/3/6

Abstract

Clostridium difficile has been recognized as a pathogen in humans for over 40 years, but in the past decade the incidence has increased and, more importantly, the clinical presentation and consequences have become more serious, with increased morbidity and mortality. The emergence of a new, more pathogenic strain, BI/NAP1/027, has driven these shifts. Treatment of this disease has been with two antibiotics, metronidazole and vancomycin, but increasing recurrence, not uncommon with C. difficile infections, has prompted research into several alternative therapies. These include a new class of antibiotic (fidaxomicin), a monoclonal antibody, a vaccine, and most recently a biotherapeutic (which, in this case, is a nontoxin-producing strain of C. difficile). The future management of C. difficile infection will probably require a combination of these approaches once we have the data from ongoing studies.

Introduction

It is clear that Clostridium difficile is gaining ground on us. New virulent strains are harder to remove by cleaning the environment and more deadly once they infect the patient. Data from 2006 by Henrich et al. [1] showed the crude mortality rate in the US to be 10.1%, which was even higher in those over 70 years of age, at 15.4%. No large studies of mortality have been published based on data from the past 3 years. Coupled with the reduced effectiveness of first-line therapy, it is clear that we need to adopt new approaches to treatment to keep it at bay. Thankfully, several new approaches have been developed and are in clinical trials.

C. difficile was first described as a human pathogen in 1978 as a cause of antibiotic-associated diarrhea or colitis. This condition was usually associated with courses of antibiotics, namely clindamycin or cephalosporins, which severely disrupt the normally protective bowel flora and thus enable C. difficile to adhere and produce toxins that lead to an inflammatory response and gastrointestinal disease. The condition also tended to be limited to the hospital setting. For approximately 30 years the disease was a significant cause of death in the elderly. However, in the past 5–10 years the organism has evolved to become more virulent (acquired from multiple sources), more antibiotic resistant, and infectious to new types of patients. A new hypervirulent epidemic strain has also emerged in the US and Europe, which presents major clinical problems for infection control as it leads to higher rates of disease recurrence and thus environmental contamination.

We will discuss the changing epidemiology in terms of which patients are now being infected, the possible sources they may have been colonized from, the emerging types of C. difficile and their virulence (or capacity to cause disease), and the mortality-related and financial impacts on the healthcare system. We will also examine the management options of C. difficile infection and the relative strengths and weaknesses of current and future potential agents. Future agents encompass new antibiotics, monoclonal antibodies, vaccines, and biotherapeutics.
Epidemiology

C. difficile accounts for 20–30% of cases of antibiotic-associated diarrhea and is the number one cause of hospital-associated diarrhea in healthcare settings including long-term nursing facilities. However, establishing an absolute incidence of the disease in the US is not possible as C. difficile infection is not a reportable disease (although there are individual states, such as Ohio, where it is).

The situations are clearer in Canada and Europe where surveillance mechanisms exist; indeed, the emergence of an epidemic of C. difficile was initially reported in Quebec, Canada by Pépin et al. in 2004 [2]. These authors were the first to report an almost fivefold increase in cases of C. difficile infection over a 13-year period, increasing from 35.6 cases per 100,000 population in 1991 to 156.3 cases per 100,000 population in 2003 [2]. Strikingly, the proportion of severe or complicated C. difficile infections also more than doubled in this period. A similar epidemiological picture emerged from Pittsburgh, USA, with more cases of C. difficile infection being reported from other US sites, and data from the National Hospital Discharge Survey encompassing 500 hospitals showed a doubling of discharges listing C. difficile infection as the cause of hospitalization.

It transpired that the main reason for this increase in cases of C. difficile infection was the emergence of a new strain, which has been termed BI/NAP1/027 (or just ribotype 027). The BI/NAP1/027 name is based on three different molecular or biochemical typing methods used to differentiate strains of bacteria [3]. Because of the confusion surrounding these three terms, the ribotyping system is the preferred method, thus in North America the epidemic strain is known as the 027 ribotype. This strain has now been reported in at least 40 US states so it is reasonable to assume it is distributed nationally [4].

In the UK, a sustained and high-profile outbreak of C. difficile infection leading to multiple deaths led to government intervention mandating total surveillance and reporting of all cases of C. difficile infection to the central health authority. Again, the 027 ribotype was the most common, but types 106 and 001 were also not uncommon in England. There was a quadrupling of death certificates where C. difficile infection was mentioned in England and Wales over the period 2004–2007 but this was followed by a 29% decrease in 2008 [5] after the institution of strict infection control policies and other mechanisms.

An excellent review by Freeman et al. [6] describes in detail the epidemiology of C. difficile infection in Europe, North America, and other parts of the world. Clearly the rapid spread of the 027 ribotype has caused significant alarm in the infection control community leading to Clements et al. [7] analyzing the risks of further worldwide spread of this strain. Until 2007, this strain was not reported outside Europe and North America, but a survey of the period 2008–2010 showed intercontinental spread had occurred. The question was, how?

The movement of people, animals, vectors, and inanimate objects across international borders has spread many infectious diseases, including influenza, acute respiratory distress syndrome, malaria, and others. The issues of identifying carriers of C. difficile strains, especially 027, are impossible as carriers are not screened. Clearly, an asymptomatic carrier can move between countries and healthcare systems and be the source of an outbreak. The contamination of foodstuffs and the carriage and infection of animals, including domestic pets and livestock (such as dogs, horses and pigs), has also been reported [8]. It would appear an almost impossible task to prevent 027 and other strains of C. difficile moving between countries, so reducing the risks of C. difficile infection in high-risk patients becomes a priority.

Prior to 2000, C. difficile infection was almost exclusively found in the hospital setting among older adults. However, as the new strain has emerged, C. difficile has been reported to be a cause of community-acquired infection among a wider age range of patients, including children. Indeed it was long thought that children under 1 year of age could not contract C. difficile, possibly due to the lack of toxin receptors in their colon compared with adults. Recently, Zilberberg et al. [9] examined two large national US databases in an effort to determine the incidence of C. difficile infection among children over the period 1997–2006. These large databases showed a marked increase in rate of pediatric C. difficile infection-related hospitalizations from 7.24 to 12.80 per 10,000, with most of the increase occurring between 2000 and 2006. Children aged 1–4 years were the most likely to have the disease, while newborns were the least likely. There were no differences in incidence between gender and races.

This new strain of C. difficile has incurred a significant financial impact as well as increasing the morbidity and mortality burden. Historically, the attributed mortality to C. difficile infection was less than 2% but this has increased to over 10% in recent years [1]. Additionally, the excess costs of C. difficile infection have been estimated to be more than 55,000 inpatient days and over $55 million annually in the state of Massachusetts. Extrapolating from this, the estimated annual excess
costs in the US over the period 2000–2002 would be $3.2 billion per year [10]. As the number of cases has increased since this estimate, it is clear that *C. difficile* infection is a major healthcare burden in need of urgent and appropriate management.

**Virulence**

So why has this disease altered over the past decade? The picture is complex and is only just coming into focus. There have also been some controversies along the way. *C. difficile* produces two toxins that have been shown to cause disease in humans, toxin A and toxin B, although there has been much recent controversy as to the relative role of the two proteins. Lytras et al. [11] reported that only toxin B, not toxin A, was essential for virulence and disease. However, Kuehne et al. [12] subsequently demonstrated that this was not the case by constructing gene-knockout mutant strains of *C. difficile*. Strains producing either toxin A or toxin B alone both caused disease, and those lacking the ability to produce both toxins completely lacked virulence, providing evidence that both toxins play a part in the disease and must be considered equally in the development of diagnostic tests for *C. difficile*.

Adding to the complexity of the evolving *C. difficile* story, Merrigan et al. [13] recently described phase-dependent hyperproduction of the two toxins and, perhaps more intriguingly, increased production of spores by the 027 hypervirulent strain. In the exponential growth phase, both hypervirulent and nonhypervirulent strains produced low levels of toxin A and toxin B, but in contrast to previous reports that tested toxin levels at a different growth phase, the levels of toxin were below the detectable thresholds. During the stationary phase of growth, however, hypervirulent isolates produced elevated levels of toxin. More significantly though, they produced more spores, and did so earlier, than all other isolates. The authors postulated that increased sporulation, potentially in synergy with elevated toxin production, may contribute to the widespread disease now associated with hypervirulent *C. difficile* strains such as the 027 ribotype. Another factor was identified by studies of the role of S-layer proteins in the cell wall when it was shown that the outer coat of *C. difficile* may also play a role in increasing the adhesion of the organism to various surfaces, including the gastrointestinal lining and perhaps inanimate surfaces, thus making spore removal more difficult (G. Vedantam, personal communication). Indeed, Dang et al. [14] have recently explored these S-layer proteins as possible novel drug targets.

**Current management of *C. difficile* infection**

Since the original description of *C. difficile* infection in 1978, two antibiotics have been the mainstay of treatment: metronidazole and oral vancomycin (only the latter is approved by the US Food and Drug Administration). Because of the relative impact of the disease, there was little concerted effort to develop new agents until the emergence of reduced susceptibility to metronidazole and the more recent occurrence of 027 and other hypervirulent strains, which led to a renewed effort to develop more effective drugs.

The recent Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) guidelines for the management of *C. difficile* infection make recommendations for the selection of drugs; notably, the most important action before initiating any specific antibiotic therapy is the cessation of the inciting antimicrobial as soon as possible [15]. The next step is to determine whether the infection is severe, characterized by a white blood cell count or a serum creatinine level that is 1.5 times greater than the premorbid level. If neither of these parameters is present, then the patient is deemed to have mild to moderate infection and warrants the first line of defense, metronidazole. However, if the patient is deemed to have severe *C. difficile* infection based on either of the above two parameters and their general clinical condition, then vancomycin, the second line of defense, is recommended.

Presently, most patients (75%) are treated initially with metronidazole, but the rate of recurrence tends to be 25–30% or higher depending on underlying disease and other factors. Indeed, resistance to this drug has been increasingly reported in various parts of the world, with Spain reporting rates of 6.3–7.7% resistance to metronidazole in the early part of this century; however, this could not be confirmed in other laboratories. In the UK, 24.4% of *C. difficile* strains were reported as having a reduced susceptibility to metronidazole, but were not resistant. The exact mechanism of this reduced susceptibility is not fully understood.

The relative merit of these two antibiotics has been the subject of some debate. Recent studies have compared metronidazole and vancomycin in terms of clinical cure and recurrence 30 days post-therapy. Zar et al. [16] stratified patients using a novel severity scoring system into mild or severe infection prior to randomly assigning patients to receive either metronidazole or vancomycin for 10 days. In the mildly infected cohort there was no difference between cure rates with the two regimens (90% metronidazole versus 98% vancomycin), while in the severely ill group the cure rates were 76% for metronidazole and 97% for vancomycin (p = 0.02). There was no statistically significant difference in the recurrence rates.
Louie et al. [17] reported one of two recent large randomized comparative studies of a toxin binder, tolevamer, and metronidazole and vancomycin, which showed vancomycin to be superior to metronidazole in severely ill patients, but with negligible differences in the mild or moderately sick C. difficile infection patients (Table 1). Confusingly, a second similar tolevamer trial in Europe failed to show any difference between metronidazole and vancomycin, even in the treatment of severe C. difficile infection. Incidentally, tolevamer was not shown to be effective in treating C. difficile infection either, and development was discontinued.

Oral vancomycin would probably be used more frequently in treating C. difficile if not for concerns that potentially difficult-to-treat pathogens such as methicillin-resistant Staphylococcus aureus (MRSA), Enterococcus faecalis, and E. faecium might develop vancomycin resistance. Recent data from Miller et al. [18] examined four different agents similar to tolevamer and metronidazole and vancomycin, which were compared in a randomized comparative study involving more than 1,100 patients. Recent presentations have shown fidaxomicin to be equal to vancomycin in terms of clinical cure rate but superior in terms of lower recurrence rates and global clinical cure rates (i.e., clinical cure rates combined with recurrence rates) (see Figure 1). The pooled data from the two studies (which were conducted in North America and Europe) were analyzed to elucidate the efficacy of the two drugs among the epidemic BI/NAP1/027 strains and the nonepidemic isolates [20].

Recurrence rates were similar for the two drugs in patients infected with the epidemic isolates, although the rate was lower for fidaxomicin (23.3% versus 31.2% for vancomycin), whereas analysis of recurrence in the non-epidemic-infected patients revealed that fidaxomicin was superior to vancomycin (8.4% versus 25.3%; p < 0.003). The cure rates in the epidemic- and nonepidemic-infected patients treated with vancomycin or fidaxomicin were not statistically different from each other, but cure rates for the 027 strain were significantly lower than for non-epidemic strains for both vancomycin (p < 0.022) and fidaxomicin (p < 0.009) [20]. Fidaxomicin is currently under review by the regulatory authorities in the US and Europe.

Future C. difficile therapies

With first-line therapy beginning to fail, it is clear that new approaches are needed. As one might expect, several of these new approaches are targeted at those most at risk. It is well known that some patients, especially the elderly and/or those who have been exposed to antibiotics, do not mount a robust immune response to C. difficile and are particularly vulnerable to recurrent or severe disease. Consequently, ways to boost the immune system, such as with a vaccine or antibody enhancement or modification of the host gut flora, are being investigated. Also under development is a novel antibiotic, fidaxomicin.

Fidaxomicin is a macrocyclic antibiotic with a narrow spectrum of antibacterial activity, in that it acts only against certain Gram-positive species and appears to have minimal activity against other members of the bowel flora. It has been shown to be safe in two large phase III clinical trials involving more than 1,100 patients. Recent presentations have shown fidaxomicin to be equal to vancomycin in terms of clinical cure rate but superior in terms of lower recurrence rates and global clinical cure rates (i.e., clinical cure rates combined with recurrence rates) (see Figure 1). The pooled data from the two studies (which were conducted in North America and Europe) were analyzed to elucidate the efficacy of the two drugs among the epidemic BI/NAP1/027 strains and the nonepidemic isolates [20].

Recurrence rates were similar for the two drugs in patients infected with the epidemic isolates, although the rate was lower for fidaxomicin (23.3% versus 31.2% for vancomycin), whereas analysis of recurrence in the non-epidemic-infected patients revealed that fidaxomicin was superior to vancomycin (8.4% versus 25.3%; p < 0.003). The cure rates in the epidemic- and nonepidemic-infected patients treated with vancomycin or fidaxomicin were not statistically different from each other, but cure rates for the 027 strain were significantly lower than for non-epidemic strains for both vancomycin (p < 0.022) and fidaxomicin (p < 0.009) [20]. Fidaxomicin is currently under review by the regulatory authorities in the US and Europe.

It will be interesting to see the reaction of the normally cautious infectious disease community to this new agent, which, according to the two controlled studies, displays similar clinical cure rates to vancomycin but a superior

Table 1. Clinical cure rates from a phase III randomized comparative trial of tolevamer, vancomycin, and metronidazole in Clostridium difficile infections (CDIs) in North America [17]

| CDI disease stratification | Tolevamer | Vancomycin | Metronidazole |
|----------------------------|-----------|------------|---------------|
| Mild                       | 45 (59.2%)| 23 (85.2%)| 26 (78.8%)    |
| Moderate                   | 44 (46.3%)| 58 (79.5%)| 40 (75.5%)    |
| Severe                     | 35 (36.8%)| 28 (84.8%)| 37 (64.9%)    |

*p = 0.04.
rate in terms of preventing recurrences (and global clinical cure rates), which occur in almost a quarter of all *C. difficile* infection cases and even more often in the sicker patients.

Immunological approaches are being developed by two other companies, one of which is Merck, who are developing the use of monoclonal antibodies directed against the two toxins A and B. Lowy et al. [21] reported a phase II double-blind placebo-controlled trial of the efficacy of CDA1 plus CDB1 involving 200 patients, 101 in the antibody group and 99 in the placebo cohort. Patients received either metronidazole or vancomycin for treatment of their *C. difficile* infection, chosen by the local physician. Patients were randomly assigned to receive either a single intravenous infusion of 10 mg each of CDA1 and CDB1 per kg body weight or a placebo. The patients were then followed for 84 days, during which they monitored stool frequency, consistency, and other relevant parameters. The primary endpoint was the recurrence of *C. difficile* infection; the secondary endpoints included number of days until the resolution of the initial episode, severity of the initial episode, and failure of antibiotic treatment. Levels of serum antibodies against toxins A and B were measured in all patients. The initial results look promising. The rate of recurrence of *C. difficile* infection was lower among those who received the monoclonal antibodies, 7% versus 25%, (95% confidence interval 7–29; \( p < 0.001 \)). The recurrence rates among those infected with the epidemic strain were 8% in the antibody cohort and 32% in the placebo group (\( p = 0.06 \)). In patients having more than one prior infection the recurrence rates were 7% and 38% for the antibody and placebo groups, respectively (\( p = 0.006 \)). There were also fewer adverse events in the antibody group: 18 versus 28 (\( p = 0.09 \)).

An alternative immunologic approach to managing *C. difficile* infection is the use of a vaccine; consequently, Sanofi-Aventis are currently developing the ACAM-CDIFF™ for this purpose. Data presented by Foglia [22] showed that the initial intramuscular-delivered vaccine directed towards both toxin A and B was well tolerated in 200 subjects in six phase I studies. The vaccine successfully induced IgG against toxin A and B in most subjects but the response rate was lower in those aged over 70 years compared with those aged 25 years. Preliminary data in three patients with recurrent *C. difficile* infection were promising but it is too early to determine the exact role of vaccine therapy in managing this aspect of the disease.

The most recent development in managing *C. difficile* infection has been a phase I trial on a biotherapeutic: nontoxigenic *C. difficile* (NTCD) [23]. NTCD is a strain of *C. difficile* that does not produce toxins but attaches itself to the usual bowel receptors thereby denying access to the pathogenic variety of *C. difficile* and thus preventing it from producing toxins. Phase I data showed that NTCD colonized the stools of normal subjects following vancomycin treatment. The administration of this novel agent was well tolerated and persistence of the clostridial
spores was seen for over 28 days. Phase II studies, initially testing NTCD as a recurrence preventative regimen, will commence in 2011. This approach raises a totally new approach to managing infections by manipulating the human microbiome, a new sphere of microbiology based on 16s RNA analysis of samples of the gastrointestinal tract, oral cavity, and other anatomical areas in which the disruption of the normal flora leads to ingress by other species, which then leads to disease.

Summary and speculation

*C. difficile* is an evolving organism and with these changes our ability to recognize the disease and manage it is also shifting. Previously, two drugs provided an adequate therapy, but emerging clinical failure of metronidazole, increasing recurrence rates, associated higher mortality, and emergent hypervirulent strains with a heightened ability to persevere in the environment necessitate new therapeutic approaches. Presently, the use of a new antibiotic, perhaps in combination with both an immunological and “biotherapeutic” modality, can help not only the treatment of *C. difficile* infection but also reduce the incidence of the disease and its impact on human lives and economics.

Abbreviation

NTCD, nontoxicogenic *C. difficile*.

Competing interests

GT has worked for ViroPharma Incorporated, makers of Vancocin and developers of NTCD, and is a full-time employee of Optimer Pharmaceuticals. JT has no competing interests.

References

1. Henrich TJ, Krakower D, Bitton A, Yokoe DS: Clinical risk factors for severe *Clostridium difficile*-associated disease. Emerg Infect Dis 2009, 15:415-22.
2. Pépin JL, Valiquette L, Abary ME, Villemure P, Pelletier A, Forget K, Pépin K, Chouinard D: *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. CMAJ 2004, 171:466-72.
3. McDonald LC, Killgore GE, Thompson A, Owens RC Jr, Kazakova SV, Sambol SP, Johnson S, Gerding DN: An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med 2005, 353:2433-41.
4. McDonald LC, Owings M, Jernigan DB: *Clostridium difficile* infection in patients discharged from US short-stay hospitals, 1996-2003. Emerg Infect Dis 2006, 12:409-15.
5. Hudson M: Statistical bulletin–Deaths involving *Clostridium difficile*: England & Wales, 2009. Newport, Wales: Office for National Statistics; 2010.
6. Freeman J, Bauer MP, Baines SD, Corver J, Fawley WN, Goorhuis B, Kuijper EJ, Wilcox MH: The changing epidemiology of *Clostridium difficile* infections. Clin Microbiol Rev 2010, 23:529-49.
7. Clements AC, Magalhaes RJ, Tatters AJ, Petason DL, Riley TV: *Clostridium difficile* PCR ribotype 027: assessing the risks of further worldwide spread. Lancet Inf Dis 2010, 10:395-404.
8. Songer JG: Clostridia as agents of zoonotic disease. Vet Microbiol 2010, 140:399-404.
9. Zilberberg MD, Tillotson GS, McDonald C: *Clostridium difficile* infections among hospitalized children, United Sates, 1997-2006. Emerg Infect Dis 2010, 16:604-9.
10. O’Brien JA, Lahue BJ, Caro JJ, Davidson DM: The emerging infectious challenge of *Clostridium difficile*-associated disease in Massachusetts hospitals: clinical and economic consequences. Infect Control Hosp Epidemiol 2007, 28:1219-27.
11. Lyras D, O’Connor JR, Howarth PM, Sambol SP, Carter GP, Phumoonna T, Poon R, Adams V, Vedantam G, Johnson S, Gerding DN, Rood JJ: Toxin B is essential for virulence of *Clostridium difficile*. Nature 2009, 458:1176-9.
controlled trials with 1105 patients [abstract]. Presented at 48th Annual Conference of the Infectious Disease Society of America, October 21-24, 2010, Vancouver, Canada.

21. Lowy I, Molrine DC, Leav BA, Blair BM, Baxter R, Gerding DN, Nichol G, Thomas WD Jr, Leney M, Sloan S, Hay CA, Ambrosino DM: Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *New Engl J Med* 2010, 362:197-205.

22. Foglia G: ACAM-CDIFF™: An active vaccine against *Clostridium difficile* infection [abstract]. Presented at 10th Anaerobe Society of the Americas, July 7-10, 2010, Philadelphia, PA.

23. Villano SA, Tatarowicz W, Seiberling M, Gerding DN, Gomez A, Monnot-Chase E: Phase I evaluation of an oral suspension of VP 20621, spores of an non-toxigenic *C. difficile* strain (NTCD) in healthy older subjects pretreated with oral vancomycin [abstract]. In Proceedings of the 50th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, September 12-15, 2010; Boston, MA. Washington, DC: ASM Press; 2010.