REVIEW

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Clostridium difficile infection

An overview of the disease and its pathogenesis, epidemiology and interventions

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Clostridium difficile infection (CDI) is the primary cause of antibiotic-associated diarrhea and is a significant nosocomial disease. In the past ten years, variant toxin-producing strains of *C. difficile* have emerged, that have been associated with severe disease as well as outbreaks worldwide. This review summarizes current information on *C. difficile* pathogenesis and disease, and highlights interventions used to combat single and recurrent episodes of CDI.

Background

Clostridium difficile is a fastidiously anaerobic, Gram-positive bacillus and the causative agent of the diarrheic disease *Clostridium difficile i*nfection (CDI). CDI is one of the most common healthcare-acquired infections in the western hemisphere. According to the United States (US) Centers for Disease Control and Prevention (CDC), US CDI rates doubled from 2000–2003.[1] CDI is the most common cause of infectious diarrhea in hospitals, and accounts for 15–39% of antibiotic-associated diarrheas.[2,3] In the US, an estimated 400,000 cases of CDI occur annually, with a corresponding burden on the healthcare system in excess of $3 billion.[4] While hospitalized patients, especially those receiving antibiotics prophylactically or therapeutically, are at increased risk for CDI, community-acquired CDI is also on the rise, with alarming increases being reported in some parts of North America[5] and in populations historically thought to be at low risk.[6] "Hypervirulent" *C. difficile* variant strains have been associated with CDI outbreaks and epidemic in the past eight years, and are only just beginning to be rigorously characterized at a molecular level.

The Disease and Risk Factors

CDI symptoms range from mild to moderate diarrhea, which can include, or progress to, pseudomembranous colitis and/or toxic megacolon.[7] Classic CDI is precipitated by antibiotic suppression of normal gut flora that facilitates the colonization of the gastrointestinal tract by environmentally-present *C. difficile* spores. Spores ingested following contact with contaminated biotic or abiotic surfaces, germinate in the gut to a vegetative cell-type that can colonize the host, and produce gut-damaging toxins during a late growth stage.[8] The toxins enter intestinal epithelial cells and glucosylate Rho GTPases, resulting in cytoskeletal rearrangements and ultimately, apoptosis.

Unusual disease manifestations associated with CDI include extra-intestinal infections,[9] ileal infections,[10] post-colectomy enteritis,[11] reactive arthritis[12] and bacteremia.[13] Clearly established risk factors include: age above 65 years, co-morbidities, immune-suppression, cancer, gastrointestinal disorders, previous antibiotic use, and previous hospitalization.[14] Use of proton pump inhibitors[15] and residence in extended-care facilities[16] are also postulated to predispose patients to CDI. Recovery is complicated by the potential for disease recurrence that occurs in approximately 15–35% of infections.[17] In some intransigent cases, multiple CDI recurrences occur over the course of months or years, severely impacting quality of life.[17]

Susceptibility to CDI increases with age, with a majority of human CDI cases occurring in patients 65 years or older. Strong retrospective data are available from multiple published reports showing a direct correlation between CDI rate/mortality and patient age.[18] High rates of infection in the elderly likely result from the failure to mount an effective immune response, as well as the inability of the commensal microbiota to fully and rapidly recover after suppression (sometimes long-term) by anti-CDI antibiotics.[19]

The potential for disease recurrence also complicates CDI treatment. Recurrent CDI is thought to be mainly due to persistent alterations in patient gut flora (as well as the inability to mount an effective anti-CDI immune response). Both age and co-morbidities appear to contribute to relapses. A large retrospective study performed in the US Department of Veterans Affairs (VA) Healthcare System revealed that that 11% of VA CDI patients were admitted to the hospital a second time, 2.5% a third time, and 0.8% a fourth time for recurrent CDI.[20] Other studies have detailed higher recurrence rates, reaching 33% following an initial CDI episode,[21] and 45% for infections occurring after the first recurrence.[22] Recurrent CDI usually occurs soon after cessation of anti-CDI antibiotic therapy; multiple

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reports have been published showing that patients with relapsing CDI had diarrheic symptoms re-appearing within 14–45 days.[23] In many patients, the offending *C. difficile* strain is molecularly indistinguishable from the one originally infecting the patient (relapse), and in the remaining cases, new strain(s) are the cause of disease (re-infection).[23] Studies documenting CDI recurrence reveal that anywhere from 33%–50% of re-infections are due to new strains of *C. difficile*.[23-25] These observations strongly suggest that recurrent infections are complicated in etiology, arguing for a role (or lack thereof) for host immunity. In general, patients with recurrent CDI have severely impacted quality of life.

Transmission

The bacterial spore is the etiologic agent of CDI. Spores are a unique cell-type formed as a result of bacterial exposure to stress, transforming viable bacteria to dormant entities that are resistant to common environmental insults such as temperature and pH. Spores are ubiquitous, especially in the nosocomial setting, where they adhere tenaciously to fomites, providing a continuous source of infectious particles.[26] In the hospital, healthcare worker-to-patient transmission and environment-to-patient transmission is common. Stringent infection control and eradication approaches have to be employed to control CDI outbreaks, since the organism is resistant to killing by most routine cleaning measures.[27] Studies performed to correlate CDI rates with hospital conditions reveal that increased use of particular antimicrobials, and poor infection control practices are strongly associated with increased CDI frequency.[28,29]

Pathogenesis

C. difficile strains are either toxigenic or non-toxigenic. Toxigenic strains harbor a 19.6 kb genomic island called the Pathogenecity Locus (PaLoc).[30] Most PaLoc's contain five genes; some rare isolates carry six.[31] Two PaLoc genes encode the glucosyltransferase toxins TcdA (309 kDa) and TcdB (267 kDa), respectively, which target host-cell Rho, Rac and Cdc42 proteins. Intoxication of host cells by TcdA/B also results in re-distribution of tight-junction proteins such as occludin, and consequent alteration of epithelial barrier function.[32] Prolonged exposure to the toxins leads to host-cell apoptosis.[33]

TcdC and TcdR are two other PaLoc-encoded proteins. TcdC is a negative regulator and modulates toxin gene expression.[34,35] TcdR is an activator (sigma factor) required for *tcdA/B* expression.[36] Binary toxin (orthologous to the *C. perfringens* iota toxin), is another putative virulence factor produced by a number of *C. difficile* isolates including hypervirulent strains, and is encoded outside the PaLoc by the *cdtA/B* genes.[5] In the most usual route of infection, *C. difficile* enters antibiotic-treated hosts as spores that germinate in the intestine to vegetative cells, and that produce toxin at the stationary phase of growth. Entry into stationary phase, when toxin gene expression increases, corresponds to the disease-causing period.[37]

Genetic manipulation of *C. difficile* has been historically difficult due to many factors including (a) the lack of convenient molecular tools, (b) the presence of apparently stringent restriction-modification system(s) that prevent acquisition and maintenance of exogenous DNA,[38] (c) the lack of genetic information associated with clinical isolates and (d) the relative paucity of selectable antibiotic resistance markers required for constructing multiple mutations. However, in the past three years, multiple reports have highlighted the use of different approaches to genetically manipulate *C. difficile* and construct isogenic mutants.[39-41] These technologies show promise, and will result in a tremendous boost to *C. difficile* genetics. To date, they have been limited to use in only a few strains of the organism, and will need to be applied with the same efficiency to diverse clinical isolates to realize their true potential.

Recently, chromosomal disruption mutants of the toxigenic *C. difficile* strain 630 were used to evaluate the contributions of toxins TcdA and TcdB, respectively, to virulence in the hamster model of CDI.[40] Hamster inoculations with wild-type and mutant strains revealed that TcdB was the primary virulence factor in this model, since a *tcdB* disruption (with functional TcdA) was avirulent, while a *tcdA* disruption (with functional TcdB) was fully virulent.

Other *C. difficile* Virulence Factors

Multiple non-TcdA/TcdB virulence factors have been proposed as being important for CDI as well as *C. difficile* dissemination.[5] CDT, the binary toxin encoded by the *cdtA* and *cdtB* genes has been associated with the newly-emerged epidemic strains of *C. difficile* at high frequency.[5] CDT ribosyl-transferase activity inactivates host-cell signaling pathways, leading to cytoskeletal re-organization and cell death.[42] The contribution of CDT to human *C. difficile* pathogenesis and disease has not been rigorously assessed.

Bacterial adherence is also thought to be an important virulence attribute of *C. difficile*, with surface-layer proteins (SLPs) playing a key role in the process.[43] We and others have shown that SlpA is a major contributor to *C. difficile* adherence, and that inhibition of adherence can be exploited as a strategy to prevent *C. difficile* binding to biotic surfaces.[44] SLPs have also been implicated in immune modulation associated with CDI;[45] thus, these proteins are critical non-toxin virulence factors.

Molecular Typing of *C. difficile* Isolates

Multiple tests are used to characterize *C. difficile* clinical isolates at the molecular level.[46] Of these, the most widely used involve electrophoretic analyses of variably-sized fragments amplified from 16S-23S ribosomal DNA gene spacer regions (ribotyping[47]), *tcdA* and *tcdB* gene polymorphisms (toxinotyping[48]), whole genome restriction (REA typing[49] and pulse-field typing[50]). Other typing methods involve phylogeny-based analyses [multi-locus sequence typing[51] (MLST) and multi-locus variable number of tandem repeats (MLVA) typing[51]], as well as proteomic approaches (surface-layer protein profiling[52]) and bacteriophage-based typing.[53] The common US and Canadian human epidemic strains are characterized as ribotype 027, North American pulse-field

Table 1. The changing face of *Clostridium difficile* infection?

CDI	Pre-2000	2000-present	Reference
The data presented below are representative; specific references are thus provided where appropriate.			
Rate (USA)—all adults	5.5 cases/10,000 population (2000)	11.2 cases/10,000 population (2005)	142
Rate (USA)—elderly (>65)	13.7 cases/10,000 population (1993)	38.8 cases/10,000 population (2004)	143
Mortality (USA)	5.7 per million (1999) 1.2% (2000)	23.7 per million (2004) 2.2% (2004)	142, 144
Mortality (Canada)	4.5% (1991)	22% (2004)—outbreak associated	145
Risk factors	Antibiotics, age, multiple co-morbidities, immune-compromising conditions, IL-8 polymorphism	Antibiotics, age, multiple co-morbidities, immune-compromising conditions, IL-8 polymorphism, PPIs (?)	5, 97
Recurrence	~20% after first episode	~33% (and up to 45% for multiple episodes)	23
Outbreaks	Infrequently associated with NAP1/027 strains	Frequently associated with NAP1/027 strains, especially in the USA, Canada, UK	5
Community-acquired	<1 case/100,000 population (UK) 1994 8–12 cases/100,000 population (USA)	22 cases/100,000 population (2004); U. K. 6.9 cases/100,000 population (2006); Connecticut 7.6 cases/100,000 population (2005); Philadelphia	82, 83, 146
CDI in children, young adults and peripartum women (USA)	Children: 7.24 cases/10,000 hospitalizations (1997) Peripartum women: 0.02% (1985–1995)	Children: 12.8 cases/10,000 hospitalizations (2006) Peripartum women: 24 cases reported (2003–2009)	14, 94

type 1 (NAP1), restriction endonuclease type BI and toxinotype III. Common veterinary epidemic strains (now also isolated from human patients) are NAP7 or NAP8, restriction endonuclease type BK and toxinotype V.

The Changing Face of CDI—Emergence of Hypervirulent Variants (Table 1)

Retrospective and prospective studies have been performed to monitor CDI, with some epidemiological correlations especially during outbreaks. In Canada, since 2002, there have been large CDI outbreaks in hospitals in the Southern Quebec province.[18,54] From 2003–2004, 14,000 nosocomial cases of CDI were reported.[55] Between 1991 and 2003 the incidence of CDI in all adults increased from 102 to 210 cases per 100,000 population, and in those patients 65 years and older, from 102 to 866 cases per 100,000 population.[54] It has been estimated that almost 2,000 deaths occurred during these outbreaks,[56] and that the epidemics caused significant healthcare costs to the Canadian Government. The cause of the Canadian epidemics is now known to be the NAP1/027/BI molecular strain type of *C. difficile*.[18]

In the US, there were CDI outbreaks in seven hospitals in six states between 2001 and 2004.[57,58] Six hospitals reported that a NAP1/027/BI strain caused the outbreaks, and that there was a corresponding increase in the number of colectomies and deaths.[59] In March 2006, the number of states with hospitals reporting outbreaks had increased to 19. At least five states reported outbreaks in VA hospitals (Arizona, Georgia, Illinois, Indiana and Texas). As of October 2008, the number of states in the USA with ≥1 hospital reporting the presence of NAP1/027/BI strains of *C. difficile* was 40.[5]

The United Kingdom (UK) has also had CDI outbreaks caused by NAP1/027/BI strains. In 2003, in one hospital in Stoke-Mandeville, Aylesbury, there were 300 infections and 12 deaths, and in a Devon hospital, there were 265 infections and 13 deaths in a 6-month time period[60] (January–June 2005). Overall, in the UK in 2004, there were 43,672 positive reports of CDI, a 23% increase from 2003.[60] The UK now has a nation-wide CDI surveillance system (www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/ClostridiumDifficile/), which has revealed that there were 50,465 infections in 2007.

Of concern, many epidemic-associated *C. difficile* strains are resistant to fluoroquinolone (FQ) antibiotics. FQs have been strongly associated with CDI, and are considered agents of CDI precipitation along with cephalosporins/clindamycins, representing a change in CDI epidemiology.[55,61] However, any molecular relationship between FQ resistance and hypervirulence is yet to be established.

Little is known about the virulence of *C. difficile* epidemic strains. MLST and micro-array analyses have revealed that epidemic strains clade into a discrete group, related to, but not identical with, other toxigenic *C. difficile* isolates.[51,62] In addition, most epidemic strains also harbor early stop codons as well as deletions in the negative modulator of toxin production, TcdC.[5]

It was previously proposed that epidemic-associated NAP1 *C. difficile* isolates produced toxins earlier (during the logarithmic phase of growth), and in greater amounts (16–23-fold) than non-NAP1 strains isolated during the same time-period from the same hospital.[63] However, it has since become clear that toxin production does not occur during logarithmic growth of epidemic *C. difficile* isolates.[64,65] Further, NAP1 *C. difficile* isolates produce only 3–5-fold higher toxin levels compared with outbreak-associated *C. difficile* isolates that are not considered hypervirulent, nor associated with severe disease sequelae[64,66,67] (and that clade close to NAP1 isolates on phylogenetic analyses). Increased toxin production is thus likely not the sole distinguishing predictor of NAPI strain hypervirulence because (a) the choice of comparator strains used for determining toxin variations can profoundly

influence the final fold-differences in toxin level,[65] and (b) multiple non-hypervirulent *C. difficile* isolates produce copious amounts of toxin, but are not associated with increased disease severity either in humans or in animal models of CDI.[68]

Toxin gene expression in *C. difficile* is modulated by the regulators TcdC, TcdR, CodY, as well as additional molecules.[34,37,69-71] In NAP1 isolates, *tcdC*, which encodes the negative regulator of *tcdA*/*B* expression harbors both a point mutation at base pair (bp) 117 as well as 12–39 bp deletions. These deletions are likely irrelevant, since the point mutation occurs proximal to them, and results in a truncated TcdC protein. The absence of functional TcdC may account for the net 3–5-fold increase in toxin production observed in NAP1 strains in the stationary phase of growth. The absence of detectable toxins in the logarithmic phase of growth, however, is related to *tcdR* expression. *tcdR*, which encodes a positive activator of toxin gene expression, is itself expressed at extremely low (basal) levels during the exponential phase of growth, but highly expressed only in stationary phase, correlating with a corresponding increase in toxin levels.[36,72] Thus, the absence of TcdC in NAP1 strains is not sufficient to permit toxin production during exponential growth, and underscores the critical requirement of TcdR for toxin gene expression.

What then, is a distinguishing feature of hypervirulent *C. difficile* isolates? Recent reports have highlighted *C. difficile* sporulation efficiency as a likely contributor to virulence. Work from several groups suggest that NAP1/027/BI strains of *C. difficile* have higher efficiencies of sporulation than comparable non-hypervirulent strains,[65,73] suggesting that increased numbers of spores could contribute not only to dissemination of infectious particles, but also serve as reservoirs for recurrent disease.

Antibiotic Resistance

Multiple studies highlight the existence and emergence of antibiotic resistance in *C. difficile* clinical isolates,[74] against agents such as metronidazole, vancomycin, the fluoroquinolones, tetracyclines and the macrolides. Resistance is still rare, but has been reported both for metronidazole[75] and vancomycin,[76] the two agents most commonly used to treat CDI. Strains with reduced susceptibility to metronidazole (breakpoint 16 μg/mL) were recently recovered from one hospital in the UK; these clinical isolates were of the ribotype 001, a common epidemic-associated molecular type in that country.[77] The mechanism of metronidazole resistance is yet to be described for *C. difficile*; orthologs of the classic resistance determinants (*nimA-F*) have not been described to date.[78] *C. difficile* strains with reduced susceptibility to vancomycin have also been recovered;[79] however, as with metronidazole, the mechanism of resistance is still unclear. Orthologs of the enterococcal *van* genes have yet to be identified in *C. difficile*. While the reduced susceptibility to both metronidazole and vancomycin has not yet been linked to clinical resistance, the very emergence of these strains is of concern.

Fluoroquinolone (FQ) resistance has increased in *C. difficile*[80] and NAP1/027/BI isolates are typically FQ resistant, though this is not always the case.[81] Resistance is primarily mediated by mutations in the DNA gyrase-encoding genes *gyrA*/*B*, leading to altered enzymes that are insensitive to the replication-inhibiting FQ drugs.[78] Resistance may also occur due to altered expression of bacterial pumps that extrude the antibiotics.[78]

Other antibiotics to which *C. difficile* isolates may be moderately or highly resistant are the tetracyclines and the macrolide-lincosamide-streptogramin (MLS) family of drugs[74] (especially erythromycin and clindamycin). Multiple mechanisms of resistance have been identified, and include ribosomal protection and pumps (tetracyclines) and ribosomal protection, efflux and inactivation (MLS family).

The rifamycins are another class of antibiotics that have been used to treat CDI—especially disease recurrences. These drugs inhibit the β-subunit of bacterial RNA polymerase (RpoB), and affect gene transcription. Rifamycin (rifaximin and rifampicin) resistance (*rpoB* point mutations) is relatively easy to acquire, and has been reported in multiple studies; interestingly, in vivo development of resistance has also been observed.[17]

CDI in the Community and CDI Tropism

Although CDI has been historically considered to be a healthcare-associated infection, recent reports have highlighted the prevalence[6] as well as increase in frequency, of the disease in the community.[6,82,83] A primary confounding factor in correctly identifying community-acquired CDI has been the lack of standardized criteria to define point-of-disease acquisition as well as prior antibiotic or risk factor exposure. Community-associated CDI (up to 16.2/100,000 cases[6]), has been observed in individuals with no previous recent antibiotic exposure (up to 90 days), no hospitalization and few or no co-morbidities.[6,84] Interestingly, one risk factor may be close association with infants less than 2 years of age, likely due to their asymptomatic carriage of *C. difficile*.[85,86] Another risk factor appears to be residence in a long-term care facility, where it has been demonstrated that anywhere from 9[87] to 50%[88] of the population may be asymptomatically colonized with *C. difficile*, thus acting as reservoirs of infection.

Infants and young children were thought at one time to be relatively resistant to CDI, presumably due to lack of a receptor(s) for toxin binding. *C. difficile* colonization rates can be very high in this cohort (>84%), with multiple studies reporting both bacteria and toxin in the GI tracts of neonates and children up to the age of 2 years.[89,90] Historically, symptomatic disease was presumed to have tropism skewed toward older adults. However, it has become clear in the past few years that CDI (particularly that associated with NAP1/027/BI strains), is not restricted to defined patient age or co-morbid conditions. CDI has now been observed in previously healthy children and young adults, post-partum women, and persons with little or no previous antimicrobial exposure or hospitalization.[91-95] Since comparative rates (post-partum women) and functional implications of increased toxin-positive tests (children) are not available, these observations are likely best interpreted with caution. Nonetheless, the recent revelations raise the possibility that new paradigms may need to be considered while assessing CDI risk factors.

Immunity

There are large gaps in our understanding of the role of the innate immune response to *C. difficile* infection. Central to the recognition of pathogen-associated molecular patterns is the Toll-like receptor (TLR) pathway, which signals via the adaptor molecule MyD88 to activate the innate immune response. In the mouse model of infection, MyD88-deficient mice were more susceptible to severe *C. difficile* infection, suggesting a protective role for the innate immune response against CDI.[96] In some instances, however, innate immune responses may actually exacerbate infection-related sequelae. A single nucleotide polymorphism within the interleukin-8 (IL-8) promoter that results in higher concentrations of the pro-inflammatory cytokine IL-8 in the lumen is associated with a greater propensity for developing symptomatic CDI.[97]

A number of studies have explored innate immune signaling in response to *C. difficile* toxins, but very little work has been done to directly examine the effects of bacterial colonization itself. *C. difficile* toxins activate the pro-inflammatory transcription factor NFκB in a number of different cell lines[98-101] and consequent neutrophil recruitment is known to contribute to intestinal injury.[102,103] On the other hand, toxin A-mediated rapid apoptosis of IKK-deficient mouse ileal loops suggest that NFκB activation may also have a protective role at some stages of the disease.[104] Antimicrobial peptides may also have a protective effect against *C. difficile* and its toxins. The sheep antimicrobial molecule SMAP-29 was shown to be effective against *C. difficile*.[105] Interestingly, human alpha-defensins have been shown to interact with high affinity to Toxin B, but not Toxin A and competitively inhibit its glucosyltransferase activity.[106]

Adaptive immunity does occur, but a wide spectrum of responses has been observed. Most human patients have anti-*C. difficile* IgA, likely from having encountered the bacterium in a non- or sub-clinical infection setting during their early years.[107] Patients that do recover from an initial episode of CDI have circulating IgA as well as IgG.[107] The IgG2 and IgG3 subtypes are specifically induced in response to CDI; patients with recurrent disease generally do not display an effective IgG response.[107,108]

CDI in the Veterinary Setting and the Potential for Zoonotic Transmission

C. difficile infects a variety of non-human mammals and the disease sequelae often mirrors human CDI.[109,110] Interestingly, CDI in the most susceptible non-humans is primarily limited to neonates; with disease outbreaks being reported in mammals of agricultural importance such as piglets, calves and foals.[109] CDI has also been reported in other mammals and birds, including, but not limited to, zoo animals such as elephants, ostriches and bears.

Infection of pigs with *C. difficile* was first noted >20 years ago, following accidental exposure of gnotobiotic pigs. Onset of porcine CDI occurs at 1–7 days of age, with diarrhea beginning soon after birth.[111] Morbidity varies from 10 to 90% in affected farrowing units, but the case fatality rate rarely exceeds 10%. However, survivors may be a source of economic loss, due to subnormal weaning weights and, ultimately, reduced slaughter weights. Outbreaks of severe CDI have occurred in piglets ~5 days of age, characterized by profuse diarrhea,[112] ascites and mesocolonic edema. *C. difficile* is also a common cause of neonatal porcine typhlocolitis,[112] and is thus likely the most important uncontrolled cause of neonatal diarrhea in pigs.[110]

In the past two years, multiple reports have raised the possibility of zoonotic transmission of *C. difficile*, particularly from retail foods.[113] The prevalence of *C. difficile*, including epidemic strains, has been documented in cooked and un-cooked meats,[109,114-116] as well as in produce.[117,118] Molecular typing of organisms isolated from food has revealed, in multiple cases, identical genotypes to *C. difficile* strains recovered from human CDI patients as well as food animals.[115,119]

Colonization Resistance

In most cases, normal gut flora prevent establishment by *C. difficile*, a phenomenon referred to as colonization resistance. Therefore, suppression of normal flora by broad spectrum antibiotics is considered to be the main predisposing factor in the development of CDI.[120] This is highlighted by the fact that introduction of normal cecal homogenates into antibiotic-treated hamsters curtails *C. difficile* colonization and concomitant inflammation.[121] The transplantation of colonic flora from closely-related normal individuals, has also been shown to be an effective treatment for human patients with recurrent *C. difficile* infection.[17,122] A number of different mechanisms have been proposed for colonization resistance. Co-culturing experiments on agar plates suggest that various bacterial genera, particularly Lactobacilli and group D enterococci, can directly inhibit the growth of *C. difficile*.[123] Similarly, in vitro experiments using a continuous flow culture model demonstrated direct inhibition of *C. difficile* growth by various bacterial species. The inhibition correlated with a decrease in pH and the depletion of specific amino acids; restoration of the pH and addition of the depleted amino acids relieved the growth inhibition on *C. difficile*, suggesting that colonization resistance may be mediated by a direct competition for nutrients.[124]

In the intestine, commensal bacteria may additionally compete for attachment sites favored by *C. difficile*, since a non-toxigenic strain of *C. difficile* can effectively interfere with colonization by toxigenic *C. difficile* strains.[125] While the mechanism for this interference has not been established, our in vitro experiments suggest that the non-toxigenic strain directly interferes with subsequent attachment by other *C. difficile* strains.[44] The native flora may also produce metabolites and/or toxins that are inhibitory to *C. difficile* growth. In a study exploring the age-restriction of *C. difficile* colonization of hamsters, Rolfe et al. demonstrated that the production of volatile fatty acids such as butyrate can directly inhibit *C. difficile* growth.[126] Based on their studies on the differential growth of *C. difficile* on various bile salts, Sorg & Sonnenshein propose yet another mechanism by which the native flora may impede CDI.[127,128] Their hypothesis is that native flora convert cholate to deoxycholate, a compound that is toxic to vegetative *C. difficile* and inhibits germination of *C. difficile* spores.

Depletion of the flora by antibiotics should result in an accumulation of cholate, a compound that supports germination as well as growth of *C. difficile*. Finally, the native flora may directly or indirectly activate the host innate immune system, resulting in the production of antimicrobial compounds that are inhibitory to *C. difficile* growth and colonization.[129]

Interventions and Future Studies

The only US Food and Drug Administration-approved antibiotic for treatment of CDI is oral vancomycin.[5] Oral metronidazole has historically also been used as first-line therapy for CDI, and other agents, tested in randomized trials, include teicoplanin, nitazoxanide, fusidic acid, bacitracin, the macrocycle narrow-spectrum agent fidaxomicin and toxin-binding anion-exchange resins.[7,17,130,131] Invariably, treatment depends on severity of disease and often involves discontinuation of the antimicrobial responsible for precipitating CDI.[132] Recurrent CDI has been very difficult to treat and it is estimated that 15–35% of patients with one previous episode of CDI will experience a recurrence, while 33–65% of patients with >2 previous CDI episodes will recur.[132] If disease symptoms reappear within two weeks of completion of therapy, there is significant likelihood that a relapse (with the same strain), rather than re-infection (with a new strain), has occurred. The source of the organisms may be environmental (due to poor infection-control practices) and/or a gut niche of *C. difficile* spores that germinate upon cessation of antimicrobial therapy. Common regimens for recurrent CDI are extended courses of vancomycin, which, in those patients with multiple recurrences, may need to be continued for months. Other approaches attempting to treat refractory CDI have included the use of intravenous immunoglobulin (IVIG) administration, pulsed and tapering doses of vancomycin, vancomycin plus rifampin, probiotics (lactobacilli and *Saccharomyces boulardii*) and a *C. difficile* toxoid vaccine.[17,23,91,130,132-136] Another successful approach appears to be the use of fecal transplants. This procedure involves the reconstitution of patient gut flora from a donor sample, usually administered via nasogastric tube.[137-139]

Competitive exclusion of toxin-producing *C. difficile* from gut niches has also been explored as a preventive measure. This approach was initially tested in the Syrian golden hamster, an established animal model for studying CDI. Antibiotic-treated hamsters challenged with toxigenic *C. difficile* strains typically die within 48 hours. However, *C. difficile* strains which lack toxin A and B (and may or may not lack binary toxin) efficiently and asymptomatically colonize the hamster gut. This colonization persists for weeks to months.[125,140] Further, hamsters first colonized with a non-toxigenic strain are protected from challenge by a toxigenic *C. difficile* strain; protection extends against both CDI and death. Different non-toxigenic strains have varying efficacies of colonization and thus, protection.[125]

The above data indicate that colonization with non-toxigenic *C. difficile* may be a creative strategy for preventing infection by toxigenic strains. This directed "probiotic" approach is currently being explored as an option to prevent nosocomial CDI (Gerding DN, personal communication); one study with 2 patients reported partial success with this approach.[141] Patients at highest risk for CDI would be given a non-toxigenic *C. difficile* strain after commencing antibiotic therapy; if they are efficiently colonized, they would be protected from CDI caused by toxigenic strains.

Conclusions

CDI remains a significant nosocomial problem, and the community-acquired/associated manifestation of the disease poses a serious threat to human and non-human patients, especially those with underlying morbidities. Epidemiological evidence accumulated over the past 10 years has revealed that global spread of hypervirulent *C. difficile* variants occurs easily and rapidly. Thus, new treatments, and more important, new preventive measures are urgently required to combat this old pathogen that appears to be exceptionally adept at acclimatizing to changing clinical and sociological practices.

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