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The Ecology and Pathobiology of Clostridium difficile Infections: An Interdisciplinary Challenge

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Summary

Clostridium difficile is a well recognized pathogen of humans and animals. Although C. difficile was first identified over 70 years ago, much remains unknown in regards to the primary source of human acquisition and its pathobiology. These deficits in our knowledge have been intensified by dramatic increases in both the frequency and severity of disease in humans over the last decade. The changes in C. difficile epidemiology might be due to the emergence of a hypervirulent strain of C. difficile, aging of the population, altered risk of developing infection with newer medications, and/or increased exposure to C. difficile outside of hospitals. In recent years there have been numerous reports documenting C. difficile contamination of various foods, and reports of similarities between strains that infect animals and strains that infect humans as well. The purposes of this review are to highlight the many challenges to diagnosing, treating, and preventing C. difficile infection in humans, and to stress that collaboration between human and veterinary researchers is needed to control this pathogen.

Keywords

Clostridium difficile; infection; human; veterinary

Introduction

Clostridium difficile is an anaerobic organism that was first described in the 1930's, when it was termed Bacillus difficilis because of difficulties in culturing this bacterium *in vitro*. B. difficilis was initially believed to be part of the normal intestinal flora of newborns (Hall & O'Toole, 1935). However, in 1978 Bartlett, *et al.* implicated this organism (by then known as C. difficile) in pseudomembranous colitis, a disorder often associated with antibiotic use (Bartlett *et al.*, 1978). Prior to that report, antibiotic-associated colitis was thought to be caused by Staphylococcus aureus (Oeding & Austarheim, 1954, Prohaska, 1959). Since that time, C. difficile infection has evolved from the role of a nuisance complication of

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antimicrobial therapy to being one of the most rapidly rising and feared nosocomial pathogens worldwide (Zilberberg *et al.*, 2008).

The incidence and severity of CDI are increasing in North America and Europe (Figure 1) (Barbut *et al.*, 2007, McDonald *et al.*, 2006, Redelings *et al.*, 2007, Gravel *et al.*, 2009). Recent outbreaks of CDI have been associated with increased severity, leading to more colectomies and attributable deaths (Figure 2) (Redelings *et al.*, 2007, Pepin *et al.*, 2005b, McEllistrem *et al.*, 2005, Pepin *et al.*, 2004, McDonald *et al.*, 2005, Dallal *et al.*, 2002, Loo *et al.*, 2005). These increases in CDI incidence and severity have been associated a new, hypervirulent strain of *C. difficile*, commonly referred to as the epidemic strain. Because of the numerous methods available for molecular typing of *C. difficile*, this strain has been given several different names based on the type of typing performed: NAP1, 027, and BI (McDonald *et al.*, 2005). This epidemic strain has a mutation in an important toxin production down regulatory gene, the *tcdC* gene that renders this gene non-functional. As a result, this strain is able to produce up to 16 times more toxin A and 23 times more toxin B in vitro than what have historically been the most common strains of *C. difficile* (Warny *et al.*, 2005). Other potential virulence factors of the epidemic strain include presence of the genes for binary toxin and high-grade fluoroquinolone resistance.

CDI also causes significant morbidity and mortality in endemic, and community settings, and might be responsible for as many as 20,000 deaths and costs as much as \$3.2 billion per year in US acute care facilities alone (Campbell *et al.*, 2009, Dubberke *et al.*, 2008a, O'Brien *et al.*, 2007). There are concerns that CDI incidence in the community is increasing, and an expanding number of studies report that *C. difficile* contaminates food (Weese, 2010, Wilcox *et al.*, 2008). This pathogen is clearly a target for pre-ingestion control and prevention. The purpose of this review is to provide an overview of the many clinical challenges posed by *C. difficile* and the illnesses it causes in humans, and to prompt collaboration between veterinary and human microbiologists and ecologists and human clinical investigators.

The Organism

C. difficile is a Gram-positive bacillus that forms oval, subterminal spores. It is a strict anaerobe that forms non-hemolytic, rhizoid colonies on blood agar plates and has a characteristic "horse barn" odor. *C. difficile* is motile and is catalase, indole and urease negative. It is also negative for lipase and lecithinase on egg-yolk agar. *C. difficile* can be cultured and isolated from clinical specimens using well established methodology (Clabots *et al.*, 1989), but culture is rarely used to diagnose CDI, as discussed below. When employed, alcohol shock increases the yield of recovering *C. difficile* from stool compared to direct plating onto selective media (Clabots *et al.*, 1989). In this technique, equivalent volumes of stool and 95% ethanol are incubated and gently mixed. After washing, the specimen is inoculated onto pre-reduced cycloserine-cefoxitin-fructose agar with taurocholate (TCCFA) agar and incubated at 37°C in an anaerobic environment for up to one week. To optimize *C. difficile* recovery from rectal swabs, rectal swab specimens can be first inoculated into TCCFA broth (Arroyo *et al.*, 2005). Identification of candidate *C. difficile* is made using commercial systems, such as the RapidID™ ANA II system or by traditional biochemical methodologies, such as a positive reaction with the Pro disk and a negative spot indole reaction.

C. difficile virulence factors

C. difficile toxins A and B

The principal C. difficile virulence factors are a pair of closely related large toxins known as C. difficile Toxins A (TcdA) and B (TcdB). The two large toxins are believed to account for most of the clinical manifestations of CDI. Toxin non-producing strains do not appear to cause disease in colonized individuals, regardless of recent antibiotic usage. Based on these observations, inhibition of Tcd toxin activity might be expected to prevent or resolve the clinical features of CDI.

The genes encoding TcdA and TcdB share 66% nucleotide sequence identity. TcdA and TcdB are large molecules (308 and 270 kD respectively) that also share extensive amino acid sequence homology (74%) within the enzymatic and substrate recognition domains (Reinert *et al.*, 2005). The *tcdA* and *tcdB* genes appear to have arisen from a gene duplication event, are transcribed as single open reading frames, and are found within a large pathogenicity island (Jank *et al.*, 2007). Recent *in vivo* studies demonstrated TcdB to be most crucial to virulence (Lyras *et al.*, 2009).

The Tcd toxins are believed to bind to host cell receptors (but such receptors have not been identified with certainty), and are then internalized and transported into the cytoplasm where they are enzymatically active. Both of these toxins are glucosyltransferases that target the Rho family of GTPases (Jank *et al.*, 2007). After they are glucosylated, these targets, which include RhoA, Rac1 and Cdc42, become inactive. This inactivation results in a variety of effects: disruptions in signaling cascades, arrest of cell cycle progression, and damage to cytoskeletal integrity (Jank *et al.*, 2007). An immediate effect of this injury is the increased secretion of fluid and electrolytes from enterocytes and a decreased permeability barrier of the intestinal mucosa. Clinical and experimental evidence indicate that inflammation is an early response to toxin exposure. Within a few hours of toxin exposure, enterocytes become rounded and an inflammatory cascade characterized by release of inflammatory cytokines is activated (Ishida *et al.*, 2004, Hippenstiel *et al.*, 2000).

Some C. difficile strains also produce an actin-ADP-ribosylating toxin termed the C. difficile transferase (CDT) toxin (also called binary toxin) (Martin *et al.*, 2008, Geric *et al.*, 2004, Stubbs *et al.*, 2000, Goncalves *et al.*, 2004). Though CDT is frequently found in C. difficile strains associated with severe CDI, establishing the role of this toxin in pathogenesis remains elusive (Barbut *et al.*, 2005, McDonald *et al.*, 2005). A recent report suggests that CDT elicits protrusions from epithelial cells, and augments bacterial adherence (Schwan *et al.*, 2009). It is plausible that this phenotype leads to increased host mucosal inflammation during symptomatic CDI in patients infected with CDT-expressing C. difficile.

Animal Models

The rabbit was used in the original characterization of *B. difficilis* by Hall and O'Toole (Hall & O'Toole, 1935). Culture supernatants injected intra-peritoneally caused seizures and death, with mouse lethal dose₅₀ testing near that for C. botulinum, but there was no description of intestinal pathology. In 1943, the guinea pig was used in studies putatively to show the value of penicillin for treatment of gas gangrene, but the animals developed large fluid-filled and hemorrhagic cecae (Hamre *et al.*, 1943). It was also noted that stool from these animals caused cytopathic changes in Vero cells, but this was attributed to a virus (Green, 1974). The association between C. difficile and pseudomembranous colitis in the 1970's brought clarity to the field, and prompted the need for new animal models to study this now recognized pathogen.

Bartlett, et al identified the etiology and pathology of clindamycin-associated enteritis as possibly due to *C. difficile* using the Syrian Golden hamster (Bartlett *et al.*, 1977). The pathology of the Syrian Golden hamster infection model is generally regarded as closest to human antibiotic-associated enterocolitis. Recently, the key role of toxin B in *C. difficile* virulence was clarified using $\Delta tcdA$ and $\Delta tcdB$ genetic constructs in the hamster model (Lyras *et al.*, 2009). In regard to treatment strategies, the hamster infection model has also been used to demonstrate protection from CDI using colonization with a nontoxigenic strain of *C. difficile* and response to antibiotics (Sambol *et al.*, 2002, Anton *et al.*, 2004). Unfortunately, hamster genome sequencing data, and hamster-specific serologic reagents to further investigate candidate cellular and immune targets that have been described in mouse pathogenicity models of CDI, are not available, so extensive use of this animal model is hindered.

Mouse studies are not limited by these constraints, and various strains of mice have been used to study CDI pathology and transmission, immunology and signal transduction, vaccine efficacy, and probiotics (Gardiner *et al.*, 2009, Ishida *et al.*, 2004, Anton *et al.*, 2004, Chen *et al.*, 2008, Adams *et al.*, 2007, Warny *et al.*, 2000, Ghose *et al.*, 2007). However, murine intestinal pathology differs from that observed in severe human CDI. Also, no model addresses the severe systemic response seen in humans with fulminant CDI. A recent notable exception is the gnotobiotic piglet model, which demonstrated systemic toxemia and circulating Interleukin-8 production with severe but not mild disease (Steele *et al.*, 2010). This suggests that complications of severe CDI such as multiple organ dysfunction syndrome (Dobson *et al.*, 2003), abdominal compartment syndrome (Shaikh *et al.*, 2008) and adult respiratory distress syndrome (Jacob *et al.*, 2004) might be due to toxemia and abnormal modulatory responses. The above hypothesis is supported by an embryonic zebrafish model that demonstrated cardiotoxicity of *C. difficile* toxin B (Hamm *et al.*, 2006).

Clinical disease in humans

CDI presents a broad spectrum of clinical manifestations. The spectrum includes an asymptomatic carrier state, colitis with or without pseudomembranes (and colitis might or might not be manifest as bloody diarrhea), and, in its most feared incarnation, fulminant colitis with megacolon or perforation. Recent antibiotic exposure is identified in >90% of hospitalized patients who develop CDI. However, most diarrheas associated with antibiotics are independent of CDI (Bartlett, 2002). It is not at all clear why there is such a spectrum of CDI, but it is possibly explained by host in addition to bacterial factors. Such factors might include variable cellular susceptibility to the toxins made by *C. difficile*, host response to *C. difficile* and/or its toxins, underlying status of the health of the host and the constituents of the ambient, non-*C. difficile*, intestinal microbiota, and of circulating antibodies against bacterial toxins (Jiang *et al.*, 2006, Janvilisri *et al.*, 2009, Louie *et al.*, 2009, Kyne *et al.*, 2000).

Asymptomatic colonization with *C. difficile* is common (Marciniak *et al.*, 2006, Riggs *et al.*, 2007). It is quite likely that this subset of *C. difficile*-infected individuals serves as this organism's reservoir. However, there are no data in support of treating asymptomatic (or post-symptomatic) carriers of *C. difficile* with antibiotics in attempts to clear this organism from the gut (Johnson *et al.*, 1992).

Symptoms of CDI most commonly start about a week after antibiotic therapy starts. However, diarrhea can develop even after antibiotic treatment has ceased. This interval can complicate the diagnosis of CDI, because a clinician might not be aware of this previous risk factor. *C. difficile* can rarely affect the small bowel of humans, typically in patients post colectomy (Malkan *et al.*, 2009, Causey *et al.*, 2009). Additional (and also uncommon) non-

colonic complications of *C. difficile* infection include extraintestinal dissemination (Libby & Bearman, 2009, Gregg & Alexander, 2009), and reactive arthritis (Birnbaum *et al.*, 2008).

Most patients symptomatically infected with *C. difficile* have nonbloody diarrhea. Additional symptoms include abdominal pain (usually crampy), malaise, nausea, vomiting, dehydration and fever. Laboratory tests commonly demonstrate elevated white blood cell counts and neutrophilic predominance. Indeed, the sudden occurrence of leukocytosis in hospitalized patients who might be at risk for CDI (most particularly those receiving antibiotics or having recently been administered these agents) should prompt consideration of testing for *C. difficile* infection (Bulusu *et al.*, 2000).

Pseudomembranous colitis is the entity most often associated with *C. difficile* infection. However, this disorder occurs in less than 50% of people demonstrated to have CDI (Olson *et al.*, 1994, Bouza *et al.*, 2005). Pseudomembranous colitis can present several weeks after antibiotics have been stopped (Pear *et al.*, 1994). In addition to the symptoms described above for *C. difficile* associated non-pseudomembranous colitis diarrhea, pseudomembranous colitis often is accompanied by fever, chills, and tenesmus. Physical findings are non-specific and include diffuse abdominal tenderness and distention. Hematochezia is unusual in pseudomembranous colitis, and fecal leukocytes are demonstrated in only about half of the patients. However, as with non-pseudomembranous colitis, peripheral leukocytosis is common. Three leukocytosis patterns are recognized: a sudden increase in the white blood count simultaneous with, or preceding symptoms of *C. difficile* colitis, or exacerbation of already existing leukocytosis (Bulusu *et al.*, 2000).

C. difficile can also cause fulminant colitis, manifest as transmural inflammation of the colon, and fulminant colitis has many serious associated complications. Fulminant colitis represents well under 10% of symptomatic CDI, but it might be more common in those infected with the recently described hypervirulent epidemic strain (McDonald *et al.*, 2005, Hubert *et al.*, 2007, Miller *et al.*, 2009, Loo *et al.*, 2005). Fulminant colitis can present *de novo*, or evolve from a milder infection. Severe leukocytosis (over 20,000 cells/ μ l) and hypoalbuminemia can accompany fulminant *C. difficile*-associated colitis (Lamontagne *et al.*, 2007). Paradoxically, diarrhea can decrease as severe colitis evolves because of colonic dilation and ileus. Colectomy might be necessary for impending or established colonic perforation (Gash *et al.*, 2010).

Relapsing CDI

Up to 30% of patients develop symptoms after apparently successful initial treatment of *C. difficile* infections regardless of which antibiotic was chosen as initial therapy (Bouza *et al.*, 2005). Infection could be from endogenous strain recurrence, or acquisition of a different strain (Barbut *et al.*, 2000).

CDI and its many Clinical Challenges

Challenges to Prevention of Human Disease

By the early 1990's, several observations led investigators in the field to conclude that CDI was largely hospital acquired, and that the chief source of transmission was patients with diarrhea. The mode of transmission was believed to be health care workers in these settings, more precisely, health care workers who did not use gloves when handling feces from patients infected with *C. difficile* (Clabots *et al.*, 1992, Samore *et al.*, 1996, McFarland *et al.*, 1989, Johnson *et al.*, 1990). Infection control recommendations logically included isolation of patients with diarrhea in the hospital, and use of gloves when handling feces in the health care setting (Gerding *et al.*, 1995, Fekety, 1997). However, only the wearing of gloves while handling stool earns an "A1" quality of evidence rating for prevention of CDI (Johnson *et*

al., 1990), and we are left with the disturbingly increased incidence of these infections: (Barbut *et al.*, 2007, Kuijper *et al.*, 2006, McDonald *et al.*, 2006, Paltansing *et al.*, 2007, Redelings *et al.*, 2007, Kyne *et al.*, 2002, Valiquette *et al.*, 2007, Muto *et al.*, 2007, Loo *et al.*, 2005). It is disheartening that few practical advances in CDI prevention have emerged in the three decades following its description.

It is possible that secular changes in CDI epidemiology might have rendered current infection control recommendations (i.e., those that focus on fomite avoidance in the hospital from symptomatic patients) less helpful. There is increasing recognition of outpatient CDI (Dubberke, 2009, Klein *et al.*, 2006) and asymptomatic *C. difficile* carriage in the community (Dubberke *et al.*, 2009a, Wilcox *et al.*, 2008, Pituch, 2009, Limbago *et al.*, 2009, Dubberke *et al.*, 2009b). These patients could enter the hospital without symptoms but with *C. difficile* in their intestinal tract, which through unknown mechanisms causes disease later in hospitalization or is transmitted to other patients (Clabots *et al.*, 1992). Disease control strategies that rely on limiting spread of this pathogen only from symptomatic patients would therefore not be effective if this was true.

Since the early 2000's, alcohol-based hand hygiene products have been recommended as the primary form of hand hygiene in healthcare settings (Boyce *et al.*, 2002). This is problematic, because *C. difficile* spores resist the bactericidal effects of alcohol, use of alcohol-based hand hygiene products is not associated with a reduction in *C. difficile* spores on hands, and contact with asymptomatic *C. difficile* carriers can contaminate the hands of unwitting healthcare workers (Riggs *et al.*, 2007, Oughton *et al.*, 2009). It is recommended to use soap and water after caring for a patient with CDI during a CDI outbreak (Dubberke *et al.*, 2008b). However, asymptomatic carriers are not identified. This is problematic because gloves are not worn when caring for, and soap and water for hand hygiene is not used after contact with, asymptomatic carriers, thus potentially increasing their contribution to *C. difficile* transmission. It is notable that the increase in CDI incidence started in the early 2000's, at the same time as the wide spread use of alcohol hand hygiene products (Figure 1). In addition, the increase in CDI onset outside of healthcare settings could indicate that there has been an increase in the number of asymptomatic carriers who develop symptomatic CDI, and also an increase in the number of asymptomatic carriers that are admitted to hospitals. The role that asymptomatic carriers play in the transmission of *C. difficile* in today's healthcare settings, the proportion that become symptomatic, and the factors that influence the likelihood of developing CDI after *C. difficile* acquisition must be established.

While antibiotics play an enduring role as a risk factor for CDI, a wider range of antibiotics seem to pose greater risk than had been previously considered (i.e., fluoroquinolones), and use of gastric acid suppression agents are also emerging as risk factors (Dial *et al.*, 2005, Dial *et al.*, 2006, Planche *et al.*, 2008). Clearly, we need to take a broader view of potential modes of acquisition, including the colonized host, the environment, or food, as methods through which hospitalized patients acquire symptomatic CDI.

CDI and challenges to its diagnosis in humans

We remain constrained in our ability to diagnose CDI (Bartlett, 2008b, Planche *et al.*, 2008, Kvach *et al.*, 2009). Stools are rarely cultured to find *C. difficile*, because culture is cumbersome with a protracted turn-around-time. In addition, after isolating *C. difficile*, further characterization needs to be performed because nonpathogenic as well as pathogenic *C. difficile* are equally recovered by culture, and >10% of hospitalized patients might be colonized with non-pathogenic *C. difficile* (Belmares *et al.*, 2009). For these reasons non-culture diagnostic tests are often employed. The oldest is the cytotoxicity assay, and this test

remains the clinical laboratory “gold standard.” This technique applies fecal filtrates to cultured fibroblasts, and seeks microscopic evidence of cell death or injury (which is also neutralized for purposes of specificity testing with antibodies to *Clostridium sordellii* toxin) (Bartlett, 2002). However, the minimum time from specimen processing to results for cytotoxicity assays is 24 hours, and time from original order for the test to results often takes several days. In addition, there can be variability between microscopist when interpreting the results. It should also be noted that *C. difficile* toxin is unstable and will degrade rapidly in clinical specimens left at room temperature following collection, so results are compromised if transport of the specimen is delayed.

Antigen tests (usually enzyme immunoassays for *C. difficile* toxins A and B or the *C. difficile* common antigen) fare variably well against cytotoxicity assays, but are typically less sensitive (Musher *et al.*, 2007b). In addition, molecular assays detecting *tcdB* have become commercially available. Recent publications suggest that detection is improved with multistep algorithms that encompass culture, toxin detection, and gene amplification (Shin *et al.*, 2009, Ticehurst *et al.*, 2006, Fenner *et al.*, 2008, Delmee *et al.*, 2005, Reller *et al.*, 2007). However, we cannot, with current data and technology, apply uniform diagnostic recommendations to all specimens in which we suspect CDI (Bartlett & Gerding, 2008). Ideally, future testing technology will be available at or near point of care, be economical, performed in a clinically relevant time frame (hours, at most) so that infection control and therapeutic action can be implemented rapidly, and be interpreted by personnel not requiring full microbiologic technical expertise.

Various typing methodologies have been described for characterizing and comparing *C. difficile* isolates, including PCR-ribotyping, pulse-field gel electrophoresis, multi-locus sequence typing, toxinotyping, each with different advantages and performance characteristics (Killgore *et al.*, 2008). Typing of *C. difficile* is typically reserved for research or epidemiological purposes, with few laboratories routinely typing *C. difficile* isolates. Use of non-culture based diagnostics for CDI limit the number of isolates available for typing as well.

In recent years, new challenges to CDI diagnosis have emerged with the identification of the putatively hypervirulent epidemic strain (Loo *et al.*, 2005, Warny *et al.*, 2005, McDonald *et al.*, 2005). This strain seems to have evolved into its current pathogenic genotype in the past two decades (Stabler *et al.*, 2009), and further study is needed to determine whether the future diagnosis of CDI will need to consider the sub-type of infecting *C. difficile* and the presence or absence of *C. difficile* Toxin A and/or B (or the toxin genes), toxin expression regulatory gene deletions (*tcdC*), and/or the *C. difficile* binary toxin (CDT) (Rupnik *et al.*, 2009).

On occasion the diagnosis of pseudomembranous colitis is made by sigmoidoscopy (though, if there is access to good clinical microbiology, it is rarely necessary to do so to make this diagnosis). Pseudomembranes are striking and their appearance is characteristic: there are yellow-white raised plaques that are up to 10 mm in diameter (Figure 4). These small plaques can also coalesce.

The tissue of pseudomembranous colitis contains inflammatory cells, fibrin, and mucus, and on histopathology, there is a “volcano” lesion “erupting” from the mucosa (Figure 5).

Although sigmoidoscopy can be helpful, it is important to remember that pseudomembranes can be restricted to the proximal colon (Tedesco *et al.*, 1982), or be completely missing.

These limitations of current diagnostic strategies for CDI oblige a coordinated approach to this infection with non-culture methodology, and a specimen collection that results in new and useful approaches.

Human CDI and challenges to its treatment

Since the late 1970s, metronidazole and vancomycin have been the pillars of CDI therapy. Metronidazole has been preferred as the first line therapy for mild CDI; it is well absorbed and tissue levels in the colon are easily achieved, no matter the route of administration (oral, intravenous, or rectal) (Bolton & Culshaw, 1986). Resistance is quite unusual (Barbut *et al.*, 1999, Johnson *et al.*, 2000, Hecht *et al.*, 2007), but treatment failures are common (15–30%) for unclear reasons (Hu *et al.*, 2008, Belmares *et al.*, 2007, Musher *et al.*, 2005). Metronidazole's use is sometimes limited by nausea and vomiting, and, less commonly but more ominously, by peripheral neuropathy.

Vancomycin is a glycopeptide bacterial cell wall synthesis inhibitor that is often used in severe CDI (Bartlett, 2008a, Zar *et al.*, 2007). Intravenously administered vancomycin does not achieve concentrations in the colon of such a magnitude to treat CDI. For this reason, orally administered vancomycin is preferred. Orally dosed absorbed vancomycin is minimally absorbed and is neither nephrotoxic nor ototoxic. However, oral administration of this antibiotic sometimes poses difficulties, because patients severely ill with CDI cannot take medications by mouth, or might have an ileus that precludes access of the oral vancomycin to the region of the gut with the highest *C. difficile* burden. There has also been understandable hesitancy to use vancomycin to treat CDI because of concern about selecting for vancomycin resistant enterococci (Al-Nassir *et al.*, 2008, Shin *et al.*, 2003), but in the setting of a severely ill patient, this concern should not deter use of vancomycin.

Rifaximin (an oral nonabsorbable form of rifampin) demonstrates some efficacy in CDI (Johnson *et al.*, 2007, Garey *et al.*, 2008a, Garey *et al.*, 2008b), but reports of rifampin resistant *C. difficile* suggest some limitation to rifaximin's utility for treating CDI (O'Connor *et al.*, 2008, Curry *et al.*, 2009). Nitazoxanide, a new anti-parasitic/anti-bacterial agent (Musher *et al.*, 2006, Musher *et al.*, 2007a), ramoplanin (Freeman *et al.*, 2005) and teicoplanin (Nelson, 2007) also show promise. Toxin binders (e.g., cholestyramine (Sinatra *et al.*, 1976, Kreutzer & Milligan, 1978)) have been used in uncontrolled studies. Telovamer, a toxin-binding resin, showed promise in in vitro (Hinkson *et al.*, 2008), animal models (Hinkson *et al.*, 2008) and early human studies (Louie *et al.*, 2006), but more recent studies in humans have been less promising (Weiss, 2009).

Intravenous immune globulin (Leung *et al.*, 1991, Wilcox, 2004) monoclonal antibody therapy (Lowy *et al.*, 2010) and therapeutic vaccines have (Sougioultzis *et al.*, 2005) also been considered, because circulating antibodies to *C. difficile* toxins are associated with protection from severe CDI in hospital (Kotloff *et al.*, 2001, Aboudola *et al.*, 2003, Kyne *et al.*, 2000, Kyne *et al.*, 2001). None of these agents are approved by the FDA to treat CDI, however, and cannot now be considered for first-line therapy (Balagopal & Sears, 2007, Abougergi *et al.*, 2010).

Recurrent CDI presents treatment challenges, and occurs after cessation of first line therapy in 20–30% of patients. It is important to remember that antibiotic resistance of *C. difficile* is rare, and that there is no justification to using higher doses of metronidazole or vancomycin to treat recurrences of symptomatic CDI. Recommendations regarding the treatment of recurrent CDI are informed by extensive experience (Leffler & Lamont, 2009), and limited data.

Microbial influences on CDI development in humans

Despite the use of antimicrobials to which *C. difficile* is rarely resistant, we still encounter a 5–10% mortality from symptomatic CDI in hospitalized patients. We need better knowledge of host and microbial ecology and pathobiology so as to develop new ways to treat, or ideally, prevent, CDI,

A large body of circumstantial evidence suggests that CDI occurs because of perturbations in enteric microbial ecology. First, and most compellingly, and as noted above, antibiotics remain strong and independent risk factors for CDI. A recent Canadian study vividly demonstrates this risk: one in every 67 adults who received a peri-operative (i.e., once or twice only) antibiotic developed symptomatic CDI (Carignan *et al.*, 2008). Antibiotics profoundly alter the microbial composition of the human gut (Antonopoulos *et al.*, 2009), but there is considerable inter-individual variation in the extent of this effect (Dethlefsen *et al.*, 2008). Though controversial (Miller, 2009), probiotics might prevent some cases of CDI (McFarland, 2006). Fecal “transplants,” in attempts to alter the human microbial biomass, have been used in severe refractory CDI and to prevent CDI relapses (Nieuwdorp *et al.*, 2008, Persky & Brandt, 2000, You *et al.*, 2008, Tvede & Rask-Madsen, 1989, Aas *et al.*, 2003).

CDI stools have less overall bacterial diversity than control stools, as assessed by 16S rRNA gene sequencing (Chang *et al.*, 2008). However, this study compared only four subjects with an initial episode and three subjects with a recurrent episode of CDI (all of whom had taken antibiotics), and three controls who had not taken antibiotics. Thus, the significance of the diminished bacterial diversity cannot be determined because it could relate simply to antibiotic use. A similar study of 11 patients who develop CDI after outpatient use of antibiotics demonstrated (by 16S rRNA gene sequencing and temporal temperature gradient gel electrophoresis), some potentially “permissive” antecedent (to the antibiotics) bacteria that might facilitate colonization with, or expansion of, asymptomatically carried *C. difficile* (De La Cochetiere *et al.*, 2008).

Veterinary and Foodborne Aspects of CDI

C. difficile is a demonstrated enteric pathogen to many different animal species, including horses, hares, pigs, nonhuman primates, dogs, cats, ostriches and laboratory animals such as Syrian hamsters, mice, rats, and guinea pigs (Keel & Songer, 2006). In horses, *C. difficile* causes acute colitis in mature horses treated with antibiotics and foals (Baverud, 2004), and has been described as a nosocomial pathogen in equine veterinary hospitals (e.g., (Baverud *et al.*, 1997, Madewell *et al.*, 1995)/ *C. difficile* has been documented as a major cause of neonatal enteritis in piglets since 2000 (Songer & Anderson, 2006). The case definition of porcine CDI includes piglets of 1–7 days of age with a history of scouring since shortly after birth (Songer & Anderson, 2006). More recently, *C. difficile* has been implicated as a causative agent of diarrhea in calves (Hammitt *et al.*, 2008).

C. difficile is also found in apparently healthy animals, including food animals such as poultry (Indra *et al.*, 2009, Zidaric *et al.*, 2008), swine (Indra *et al.*, 2009, Avbersek *et al.*, 2009, Norman *et al.*, 2009), and cattle (Indra *et al.*, 2009), and household pets (Weese *et al.*, 2009d). As in humans, young animals have greater colonization rates than older animals (Keel & Songer, 2006). A longitudinal study in one swine operation reported a 50% colonization of suckling piglets, but only 8.4% in weaned pigs and 3.9% in grower-finisher pigs (Norman *et al.*, 2009). Little is known of the epidemiology and genotype distribution of *C. difficile* in animal populations. Studies that have genotyped animal isolates suggest lower strain diversity in animal populations compare to human populations (Avbersek *et al.*, 2009, Keel *et al.*, 2007). Keel *et al.* (2007) ribotyped *C. difficile* from piglets, calves, horses and

dogs. Porcine isolates comprised four PCR ribotypes; one (ribotype 078), a toxinotype V strain, predominated (83% from a total of 144 isolates). This was also the most common ribotype (94%) among 33 calf isolates but was rarely identified in dogs and horses (Keel *et al.*, 2007). In humans, ribotype 078 has caused severe cases of CDI at the community level in young populations (Goorhuis *et al.*, 2008a). The *C. difficile* type 078 isolates from humans and pigs were highly genetically related, which suggested that pigs may be a potential reservoir for this strain (Goorhuis *et al.*, 2008a).

The increasing recognition of human *C. difficile* as a community-acquired infection has prompted examination of its potential sources at the community level, including potential foodborne origins. A well recognized risk factor for community-onset *C. difficile* infection is recent discharge from a hospital, a risk that extends to 12 weeks after discharge (McDonald *et al.*, 2007). The median incubation period for *C. difficile* is 2 to 3 days and stays in the hospital are becoming shorter (Clabots *et al.*, 1992). Therefore it is possible patients recently discharged from a hospital remain at increased risk for developing CDI, but are acquiring their pathogenic *C. difficile* in the community after discharge (Weese, 2010). In the mid-1990's, *C. difficile* was identified in spoiled vacuum packed meat products (Broda *et al.*, 1996). Since then, *C. difficile* has been found in both animal and vegetable food products. *C. difficile* spores have been isolated in raw meat for dogs (Weese *et al.*, 2005), in meats prepared for human consumption (Rodriguez-Palacios *et al.*, 2007), as well as in vegetables in South Wales (al Saif & Brazier, 1996) and ready to eat salads in Scotland (Bakri *et al.*, 2009). The strains more commonly found in animal products are toxinotype V (Songer *et al.*, 2009, Weese *et al.*). This is notable because toxinotype V strains of *C. difficile* cause proportionately more cases of community onset infections than hospital onset infections (Limbago *et al.*, 2009). However, in a Canadian study, meat *C. difficile* isolates contained genes encoding TcdA, TcdB and CDT belonged to toxinotype III (Rodriguez-Palacios *et al.*, 2007). Interestingly, these strains have the *tcdC* deletion associated with hyperexpression of TcdA and TcdB. Follow-on studies in the United States (Arizona) and Canada have expanded the list of foods containing *C. difficile* (ground beef, turkey, pork, and sausages and braunschweiger), and have suggested possible seasonality in the prevalence of contaminated product, with higher prevalence (20 % of retail meat) in winter (Rodriguez-Palacios *et al.*, 2009). The density of *C. difficile* contamination is low (<60 spores/g) (Weese *et al.*, 2009c).

Household pets might be another source of community-acquired CDI, and human-pet transmission might be common. A recent study documented 10% of dogs and 21% of cats were colonized with *C. difficile* (Weese *et al.*, 2009d). The most prevalent strain to colonize the pets was ribotype 001, the most common strain of *C. difficile* in the hospital setting prior to the emergence of the current epidemic strain. Having an owner identified as being immunocompromised was associated with almost an 8-fold increased association of the pet being colonized. This is notable because dogs that enter healthcare facilities (i.e. "pet therapy") are 2.7 times more likely to be colonized with *C. difficile* compared to dogs that participate in animal assisted interventions that do not enter healthcare facilities (Lefebvre *et al.*, 2009). Dogs that entered healthcare facilities that licked patients or accepted treats were more likely to be colonized with *C. difficile* compared to dogs who did not lick patients or accept treats as well (Lefebvre *et al.*, 2009).

Studies have identified strains isolated in food that are genetically identical to strains isolated from humans with CDI as well as isolates from humans with CDI genetically identical to strains isolated from animals, which has suggested that food animals may be a source of food contamination with *C. difficile* (Goorhuis *et al.*, 2008b, Jhung *et al.*, 2008). Nevertheless, the prevalence of some important human strains, such as the ribotype 027/NAP1, appears to be disproportionately high in food compared to food animals (Weese,

2010). Therefore, it has been postulated that other sources such as slaughterhouse environments, the processing facility, or the hands of personnel manipulating meat may also contribute to the contamination of food with *C. difficile* spores (Weese, 2010). *C. difficile* spores are also isolated in other environments including households, soil, pets, water (al Saif & Brazier, 1996, Weese et al., 2009d), and therefore, the relative importance of food as a source of *C. difficile* remains unclear.

Table 2 reviews isolation rates and organism characterization from recent world-wide surveys, and is derived from a recent and thorough review of this topic (Weese, 2010). It should be noted, however, that isolation techniques for *C. difficile* from food is far from standardized, and differences in procedures might account for recovery rate differences between studies.

The above data make a case for the foodborne origin of human *C. difficile* strains. Systematic food and animal surveys will enable us to confirm or refute this postulated linkage.

Conclusion

CDI poses clinical and infection control problems to degrees not anticipated a decade ago. CDI's implications compel us to include broad sets of disciplines to try to control this pathogen and the diseases it causes. Expertise from the fields of diagnostic microbiology, nursing, hospital management and infection control, pharmacology, microbial pathogenesis, genome biology, microbial ecology, microbial pathogenesis, and therapeutics development will be needed to help clinicians mitigate the effects of *C. difficile* once this organism gains access to human hosts. It appears likely that food plays a role in transmission and colonization of hosts by *C. difficile*, and, by implication, there will be an animal and/or environmental reservoir for this pathogen. We predict that the interface between veterinary microbiology and ecology, food technology and science, and human biology will be a fertile and worthwhile area for collaboration and research *C. difficile*.

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Three bullet points

- Clostridium difficile infection frequency and severity are increasing.
- Diagnosing, treating and preventing C. difficile infection remain a challenge.
- More research is needed to define the pathobiology of C. difficile infection and to identify the major determinants and sources of C. difficile transmission and acquisition in the hospital and in the community

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Figure 1.
 Increasing incidence of CDI, mid 1990s to mid 2000s, United States (from (McDonald *et al.*, 2006) with permission):

Figure 2.
CDI Mortality rates, United States, expressed as fatalities per million, 1999–2004 (from (Redelings *et al.*, 2007), with permission).

Figure 3.
Resected colon from a patient with CDI fulminant colitis (from (Tarr *et al.*, 2009, Tarr *et al.*, 2008) with permission).

Figure 4.
Pseudomembranes in CDI (from (Bartlett, 2002) with permission from Massachusetts Medical Society).

Figure 5.
“Volcano” lesion (from (Tarr et al., 2009, Tarr et al., 2008) with permission).

Table 1

Treatment of Recurrent CDI (modified from (Leffler & Lamont, 2009) with permission).

Treatment of Recurrent <i>C. difficile</i> Infection
Initial recurrence
14-day course of oral metronidazole or vancomycin
Second Recurrence
Tapered pulse dose oral vancomycin
_ 125 mg 4 times daily for 1 week
_ 125 mg twice daily for 1 week
_ 125 mg daily for 1 week
_ 125 mg every other day for 1 week
_ 125 mg every third day for 2 weeks
Consider 1-month course of probiotics starting in the final 2 weeks of antibiotic therapy
Third or subsequent recurrence
Tapered pulse dose oral vancomycin (see above)
Followed by
14-day course of rifaximin, nitazoxanide, or toxin-binding resins
Consider 1-month course of probiotics starting in the final 2 weeks of antibiotic therapy
Consider intravenous immunoglobulin or fecal bacteriotherapy
Consider chronic low-dose suppressive therapy with oral vancomycin for elderly patients and those with multiple comorbidities

Table 2

(from (Weese, 2010) with permission).

Prevalence of isolation and ribotype distribution of *Clostridium difficile* from food animals and retail meat

Country	Sample type	Prevalence (%)	Ribotype 027/ toxinotype III (%)	Ribotype 078/ toxinotype V (%)
Canada (Rodriguez-Palacios <i>et al.</i> , 2006)	Calves	15	12	26
USA (Hammit <i>et al.</i> , 2008)	Calves	25	0	94
Canada (Costa <i>et al.</i> , 2009)	Veal calves	49	0/1	65
Slovenia (Pirs <i>et al.</i> , 2008)	Calves	1.8	0	0
Austria (Indra <i>et al.</i> , 2009)	Cows	4.5	0	0
Slovenia (Zidaric <i>et al.</i> , 2008)	Chickens	62	0	0
Austria (Indra <i>et al.</i> , 2009)	Chickens	5	0	0
Zimbabwe (Simango & Mwakurudza, 2008)	Chickens	29	NT	NT
Slovenia (Pirs <i>et al.</i> , 2008)	Piglets	52	0	0/77
USA (Yaeger <i>et al.</i> , 2007)	Piglets	79	NT	NT
USA (Keel <i>et al.</i> , 2007)	Piglets	NA	0	83
Austria (Indra <i>et al.</i> , 2009)	Pigs	3.3	0	0/50
Canada (Weese <i>et al.</i> , 2009b)	Piglets	95	0	94
Canada (Rodriguez-Palacios <i>et al.</i> , 2007)	Beef, veal	20	0/67	0
USA (Songer <i>et al.</i> , 2009)	Various	42	27	73
Canada (Rodriguez-Palacios <i>et al.</i> , 2009)	Beef, veal	6.1	0/27	0
Slovenia (Pirs <i>et al.</i> , 2008)	Piglets	52	0	0/77
USA (Yaeger <i>et al.</i> , 2007)	Piglets	79	NT	NT
USA (Keel <i>et al.</i> , 2007)	Piglets	NA	0	83
Austria (Indra <i>et al.</i> , 2009)	Pigs	3.3	0	0/50
Canada (Weese <i>et al.</i> , 2009b)	Piglets	95	0	94
Canada (Rodriguez-Palacios <i>et al.</i> , 2007)	Beef, veal	20	0/67	0
USA (Songer <i>et al.</i> , 2009)	Various	42	27	73
Canada	Beef, veal	6.1	0/27	0

Country	Sample type	Prevalence (%)	Ribotype 027/ toxinotype III (%)	Ribotype 078/ toxinotype V (%)
(Rodriguez-Palacios <i>et al.</i> , 2009)				
Canada (Metcalf <i>et al.</i> , 2009)	Pork	1.8	43/57	0
Canada (Weese <i>et al.</i> , 2009a)	Chicken	15	0	96
Canada (Weese <i>et al.</i> , 2009b)	Pork Beef	12 12	7.1/14 7.1	71 86

NT, typing was not performed; NA, not applicable, as the study was an evaluation of previously collected isolates.