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Clostridium difficile

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SYNOPSIS

Clostridium difficile infections (CDI) have emerged as one of the principal threats to the health of hospitalized and immunocompromised patients. Nucleic acid testing for *C. difficile* toxin genes has eclipsed traditional clinical diagnostics for CDI in sensitivity and is now widespread in clinical use, but preliminary evidence suggests that this may have come at a cost of substantially reduced positive predictive value. The importance of *C. difficile* colonization is increasingly recognized not only as a source for false positive clinical testing but also as a source of new infections within hospitals and other healthcare environments. In the last five years, several new treatment strategies that capitalize on the increasing understanding of the altered microbiome and host defenses in CDI patients have completed clinical trials, including fecal microbiota transplantation (FMT). This article highlights the changing epidemiology, laboratory diagnostics, pathogenesis, and treatment of CDI.

Keywords

Clostridium difficile; CDI; diagnosis; epidemiology; fecal microbiota transplantation; FMT

Overview

Clostridium difficile was identified as the leading cause of antibiotic-associated diarrhea and colitis in 1978, but since 2001 *C. difficile* infections (CDIs) have evolved from sporadic complications of antimicrobial therapy to severe, sometimes fatal, events that have become an endemic threat to the health of hospitalized and immunosuppressed patients worldwide. This article discusses the changing epidemiology, clinical and laboratory diagnosis, pathogenesis and treatment of CDI.

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The Author has nothing to disclose.

Microbiology

Clostridium difficile is an obligate anaerobic, spore-forming, Gram-positive rod first described in 1935 as *Bacillus difficilis* in the fecal flora of healthy infants.[1] The organism remained unrecognized as a cause of human infection until 1977 when it was identified as the cause of what had previously been referred to as antibiotic-associated colitis.[2, 3] Hall and O'Toole's species name reflected difficulty of isolating *C. difficile* from other anaerobic and facultative stool flora, which was mostly attributable to its relatively slow growth (40–70 minutes doubling time).[1] *C. difficile* is exquisitely aero-intolerant during logarithmic growth phases when vegetative cells predominate,[4] making it difficult for laboratories not equipped with anaerobic chambers to passage the organism before sporulation occurs at approximately 48–72 hours. Laboratories equipped with anaerobic jar systems are delayed in the ability to isolate the organism because of the need for a minimum 48 hours between passages in order to avoid fatal oxygen intoxication of fresh cultures.

Culture of *C. difficile* has been unfairly perceived as difficult, however, as selective media have been refined to isolate the organism from fecal and environmental specimens (Fig. 1).[5–7] These media capitalize on the organism's intrinsic resistance to cefoxitin and cycloserine and the ability of *C. difficile* to utilize fructose and mannitol as carbohydrate sources; Stickland reactions play a central role in the organism's biosynthetic pathways and account for the organism's alkalization of undefined peptone-based media which are widely used with neutral red to indicate *C. difficile* grows in both liquid and agar media (Fig. 1), usually within 48 hours of incubation.[8] Sodium taurocholate, a bile salt, has been shown to be vital to the germination of *C. difficile* endospores and is an essential component in selective media.[9, 10]

Once isolated, *C. difficile* can be readily sub-cultured to non-selective media such as 5% sheep blood agar on which it assumes its characteristic irregular ground-glass colonial morphology without hemolysis (Fig. 2). While *C. difficile* colonies vary greatly in size (more motile strains have maximum colony widths of 12 to 15 mm), they fluoresce under UV illumination and exhibit a characteristic odor often referred to as "horse dung" or "barn" odor. This phenotypic feature results from the ability of the organism to ferment tyrosine into *p*-cresol, a phenolic compound for which the species has considerable tolerance and which can be used to distinguish the organism from other clostridia using high performance liquid chromatography.[11, 12] Smaller colonies of *C. difficile* can be difficult to distinguish morphologically from those of other non-hemolytic *Clostridium* spp, but rapid biochemical confirmation with L-proline aminopeptidase spot disk activity can provide confirmation of suspected *C. difficile*.[13]

Chromogenic commercial media have also been developed which allow for more rapid isolation of *C. difficile* (usually as colorless or grey-black colonies on a blue background within 24 hours), but these media often allow for growth of non-*difficile* clostridia.[14] Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF) platforms are readily able to distinguish *C. difficile* from other clostridia in these circumstances.[14–16]

Culture of *C. difficile* is primarily a research tool used in the evaluation of diagnostic tests as well as to recover isolates for outbreak investigation and molecular epidemiology. *C. difficile* isolates can be maintained nearly indefinitely at room temperature as spore stocks or as vegetative cells in chopped meat medium, although many laboratories are in the habit of storing the organism in frozen glycerol stocks.[17, 18] Because non-toxigenic strains of *C. difficile* are commonplace, culture of the organism for clinical purposes must be followed by confirmation of toxin production. This can be done either using molecular tests for the presence of toxin genes or by performing cytotoxicity assays or enzyme immunoassays on cell-free culture supernatants(see **Pathogenesis** and **Diagnosis** below). For phenotypic toxin testing, use of complex media without glucose, fructose, and mannitol (present in most of the primarily selective media for the organism) may be important to minimize the effect of rapidly metabolized carbon sources in repression of toxin synthesis.[19] The glucosyltransferase activities of toxins A and B in *C. difficile* have been used to devise selective media that can identify toxigenic strains in a single step using the presence of an insoluble blue product around colonies of toxigenic strains at 48 hours of incubation, but to date this methodology has not undergone a wide-scale validation and may miss some strains expressing toxin at low levels.[20]

Molecular epidemiology and strain typing

For the purposes of tracking global and local molecular epidemiology of *C. difficile*, several strain typing systems have been devised (Table 1). For tracking global linages, pulsed field gel electrophoresis (PFGE) is the method generally preferred by the US Centers for Disease Control and Prevention,[21, 22] while European centers and others have preferred PCR ribotyping.[23] Both methods are band-based and require sets of reference isolates and suffer from a degree of subjectivity, but both methods are able to separate *C. difficile* lineages that correspond to a large degree with the 380 lineages of *C. difficile* identified using a more objective and technically demanding method based on Sanger sequencing of *C. difficile* housekeeping genes, multilocus sequence typing (MLST).[24] A single locus typing scheme based on sequencing of the *tcdC* gene has been devised and can serve to distinguish the lineages of toxigenic strains, but this scheme is not suited for typing of non-toxigenic strains.[25, 26] MLST and *tcdC* typing are both entirely portable, with a public database of sequence types maintained by the University of Oxford (http://pubmlst.org/cdifficile). PFGE, ribotyping, MLST, and *tcdC* genotyping are insufficiently discriminatory for source attributions or tracking hospital outbreaks, for which restriction endonuclease analysis (REA), multilocus variable number of tandem repeats analysis (MLVA), and whole genome sequencing (WGS) are more suited, each representing progressively increased discriminatory capacity.[27–30] There has been considerable enthusiasm for adoption of WGS-based methods, but the high cost and lack of a standardized analysis scheme for WGS data are barriers to the wide-scale adoption of this method.

Ecology, host range, and distribution

C. difficile is ubiquitous and widely distributed in nature (Table 2).[31] It can be found easily in soil and sewage and has been found in the feces of most mammals.[31] *C. difficile* has occasionally been described as a veterinary pathogen, particularly in piglets, calves, and

some avian species,[32, 33] but its epidemiology in animals is not nearly as well-described as it is for *C. perfringens* in animal husbandry. The original animal model for CDI was the Syrian hamster, which suffers a lethal colitis after antibiotic pre-exposure. [34] More recently, mouse models of CDI have been developed which more closely parallel non-lethal CDI in humans, including the development of relapsed infection after recovery.[35] To date, however, there is scant evidence that CDI occurs in nature in the absence of antimicrobial use. Despite initial concern that *C. difficile* is widespread in the food supply; most prevalence studies suggest that the prevalence of *C. difficile* is low and occurs at very low colony counts (Table 3).[36, 37] *C. difficile* strain typing has suggested that some high initial prevalence estimates of *C. difficile* in meat products may have arisen from laboratory contamination events, and thus far there is no direct evidence that *C. difficile* is a foodborne illness.[38, 39] In contrast, environmental studies have consistently identified healthcare environments, including outpatient clinics, as heavily contaminated with *C. difficile*.[6, 40, 41]

Disinfection, Survival, and Laboratory Infection

The endospores of *C. difficile* are resistant to heat, 70% ethanol used in hand sanitizers, and the quaternary ammonium detergents used as hospital and laboratory disinfectants, but sodium hypochlorite-based solutions are capable of inactivating spores.[42] *C. difficile* spores are known to have nearly indefinite viability, demonstrating only $0.5\log_{10}$ reduction in viability after 14 months of storage on steel disks at room temperature.[18] The viability of C*. difficile* spores contaminating hospital environments also declines over time, but not at a rate that is below the infectious inoculum (ID_{50} =5 spores/cm^2) in the mouse model of CDI.[41, 43] Sodium hypochlorite (chlorine bleach) at a concentration of 5000 ppm (achieved with 10% aqueous solution of standard household bleach in distilled, deionized water) at 10 minutes of contact time results in a 6 $\log_{10}$ (≥ 99.9999%) reduction in the viability of *C. difficile* spores, although lesser concentrations of bleach take up to 30 minutes for the same level of activity.[44]

Occupancy of a room previously occupied by both known CDI patients and by patients previously receiving antimicrobials have both been identified as significant risks for development of CDI among subsequent hospital room occupants, highlighting the need to provide adequate terminal disinfection to prevent transmission to new patients.[45, 46] Previous studies have reported adoption of sodium hypocholorite cleaning of hospital environments as key elements in the control of CDI epidemics in hospitals,[47] but more recently less caustic alternative agents such as combinations of hydrogen peroxide and peracetic acid have been developed as terminal disinfectants.[48] The development of fully automated ultraviolet irradiation devices and hydrogen peroxide vapor systems for disinfection of hospital rooms holds promise as a means to enhance the effectiveness of hospital disinfection, but these devices do not eliminate the need to perform routine cleaning of the organic soil burden on hospital surfaces and add 30–60 minutes to the process of cleaning a hospital room, adding considerably to the cost of this approach.[49, 50] Nonetheless, centers that have adopted these systems have observed significant decreases in healthcare-associated CDI rates.[51–53]

Laboratory-acquired CDI has been anecdotally reported. Both reported cases involved young females who were working with *C. difficile* on open benches and using alcohol (which is

inactive against *C. difficile* spores) to disinfect laboratory work surfaces. [54] Only one of the two cases had known antibiotic exposures, although the case in which no antibiotic exposure was noted was self-limited and resolved without treatment. [54] Nonetheless, Bouza *et al.* recommended that all laboratory work with *C. difficile* take place in biological safety cabinets using appropriate barrier precautions and appropriate terminal disinfectants active against *C. difficile* spores.[54]

Asymptomatic carriage of *C. difficile*

Healthy, non-hospitalized ambulatory adults are commonly colonized with *C. difficile*, and the colonization of 5–15% of adults is often transient.[55–57] A prospective survey found that 26% of 428 hospitalized patients in a medical ward acquired *C. difficile*. In this study, only 38% developed symptoms consistent with CDI by 11 months, indicating that 62% of patients who acquired *C. difficile* were asymptomatically colonized. [58] In a later study by Clabots and colleagues,[59] 21% of all admissions to a single medical ward at a Veterans Affairs hospital were positive for *C. difficile* carriage during a 9-month period. Of these, 86% were asymptomatic carriers. The predominant mode of acquisition in this study was hospital-associated in 41% of admissions. Molecular typing of recovered isolates revealed 19 instances of within-hospital transmission among asymptomatic patients. Acquisition of the same strains was documented within the same hospital room separated in time by up to 24 weeks, suggesting that environmental contamination contributes to C*. difficile* transmission. The study also documented transmission between pairs of patients who were in rooms far separated from each other, suggesting transmission of C*. difficile* spores on the hands of health care workers to new ward admissions.[59] Subsequent mathematical models of *C. difficile* infection have suggested that transmission within hospitals solely from symptomatic *C. difficile* patients is inadequate to account for sustained endemic transmission within hospitals; the contribution of asymptomatic carriers was estimated to be particularly significant if transmission from these carriers was common.[60]

The contribution of asymptomatic carriers to incident *C. difficile* infections has been further estimated in recent studies. Eyre and colleagues performed a population-based study of 1250 CDI cases occurring 2007–2011 in Oxfordshire, UK. Using whole genome sequencing of *C. difficile* isolates, they showed that 45% of all incident CDI cases were genetically distinct from all previous cases diagnosed in the region, suggesting that sources beyond symptomatic patients were important in CDI transmission.[28] Using MLVA genotyping of incident CDI and a concurrent cohort of individuals colonized with *C. difficile* at a single medical center in Pittsburgh, Pennsylvania over a 119-day period, Curry and colleagues determined that 17/56 (30%) and 16/56 (29%) of incident CDI cases could be traced to other symptomatic CDI patients and *C. difficile* carriers, respectively.[61] In a 15-month prospective study in six Canadian hospitals, 117/4143 (2.8%) and 123/4143 (3.0%) had healthcare-associated *C. difficile* infection and colonization, respectively, although direct links between these cohorts was not established by strain typing.[62] Longtin and colleagues performed a controlled intervention study to examine the effect of screening incoming admissions to a single Canadian hospital for *C. difficile* colonization 2013–2015, demonstrating that simple contact isolation measures for the 4.8% of admissions identified as carriers of *C. difficile* resulted in an observed rate of hospital-acquired CDI of 3.0 infections/10,000 patient-days compared to

6.9 infections/10,000 patient-days in the pre-intervention period.[63] Vertical controls based on active surveillance for *C. difficile* within healthcare facilities hold promise as a means to control CDI.

Clinical presentation and prognosis

As above, asymptomatic carriers are by far the most prevalent group among those individuals who are culture-positive for toxin-producing *C. difficile*, representing 62% to 86% of hospitalized individuals with *C. difficile* -positive stools in previous prospective studies.[58, 59] For the minority who develop CDI, the infection exists on a continuum from mild diarrhea to fulminant colitis. Few descriptive series of CDI have been published, but in the pre-2001 era, development of semi-formed diarrhea more than 7 days after antimicrobial exposure was the most common presentation among a series of 43 inpatients.[64] Leukocytosis is a common feature of CDI, which has been found to be the most common cause for unexplained leukocytosis among inpatients and the fourth most common cause for leukocytosis overall. [65, 66] Fever is seen in only 50% of cases, and bloody diarrhea is seen in a distinct minority of cases.[64] Rapid increases in leukocytosis, abdominal distension or pain, and sudden cessation of diarrhea are poor prognostic signs that have been anecdotally reported in cases of fulminant colitis.[67]

Several severity score indices for CDI have been developed to best predict CDI patients at risk for death, colectomy, or intensive care unit admission attributable to CDI; fever, leukocytosis, hypoalbuminemia, and abdominal distension were independent predictors of severe CDI in one early study.[68] Subsequent multicenter cohort studies have suggested that a 3-point scale based on age≥65 years, peak serum creatinine ≥2 mg/dL, and peak peripheral blood leukocyte count≥20,000 cells/μL can be used to predict ~75% of patients at risk for severe CDI.[69] A subsequent prospective cohort has resulted in a bedside scoring system based on age, treatment with systemic antibiotics, leukocyte count, albumin, and serum creatinine (ATLAS score 0 to 10) to predict cure among CDI patients,[70] and similar prediction rules for recurrence have also been developed.[71] The clinical utility of these scores in management of CDI, an infection characterized by occasionally abrupt changes in clinical severity, is not yet established. The incubation period for CDI remains unknown, particularly since the incubation period can be defined either from the time of first exposure to the organism, from the first exposure to antibiotics, or from the first appearance of organism in stools until the onset of symptoms. In studies of healthy volunteers without diarrhea, oral administration of non-toxigenic *C. difficile* spores results in fecal shedding within 2–4 days,[72] but this may not reflect the dynamics of fecal shedding in hospitalized patients. Early studies of hospitalized patients suggested that CDI probably occurred within 7 days of the first appearance of *C. difficile* in stool,[73] and beyond this several inpatient cohort studies suggested that patients who did not manifest symptoms of CDI by 7 days of appearance in stool were at reduced risk of subsequent CDI compared to non-colonized patients in the same cohorts.[74] Observational evidence indicates that patients remain at risk for CDI for up to 12 weeks after exposure to antimicrobials, however,[75, 76] with the onset of CDI occurring up to 60 days (median 20.3 days) from the time of hospital discharge.[77] The discrepancies in these observations regarding the potential incubation period for CDI may reflect that follow-up in the initial hospital cohort studies was limited to hospital discharge,

and the cohorts were relatively small and may not have included large numbers of severely immunosuppressed patients at high risk for CDI.

Epidemiology of CDI

Age ≥ 65, exposure to antimicrobials, and length of stay in a health care facility have all been independently associated with risk for CDI.[78] In addition, age greater than 65 years has been repeatedly associated with an increased risk of symptomatic infection.[79, 80] CDI epidemiology changed dramatically after 2000, with an increase in disease incidence and severity, including fulminant colitis, colectomy, and death described at several large hospitals worldwide.[21, 81, 82] In the United States, *C. difficile* incidence doubled from 1996 to 2003.[79] A newly emergent epidemic strain of C. *difficile*, which was rarely encountered before 2000 which became the most prevalent strain causing 30% to 50% of CDI cases, was associated with this phenomenon.[21, 81] The epidemic strain has been named NAP1 by PFGE, 027 by PCR ribotyping, and BI by REA typing, and ST1 by MLST/*tcdC* genotyping and is usually referred to as NAP1/BI/027.[83]

The causal association of the emergence of this epidemic lineage of *C. difficile* and severe CDI is uncertain. In a large population-based surveillance study of 2057 CDI cases with available stain typing data, severe outcomes and death occurred in 4.9 and 2.7% of cases, respectively, and NAP1 strains (28.4% of cases) were associated with severe outcomes and death after adjusting for other confounding risks with adjusted odds ratios of 1.66 and 2.12, respectively.[84] A European study noted that ribotype 078 CDI had higher observed mortality than other lineages, including ribotype 027, however.[85] Several studies have failed to observe any relationship between epidemic lineage CDI and severe clinical outcome.[86–88] Enhanced toxin production originally observed for epidemic lineage stains actually varies widely across strains of ribotype 027, and the significance of lineage with respect to clinical outcome has been cast into considerable question.[88–90] An association between epidemic lineage CDI and recurrence, however, has been consistently observed in many study settings.[91, 92]

In 2005, severe CDI was reported in patients previously assumed to be at a low risk for CDI, including pregnant women and outpatients without known exposures to antimicrobials.[93] Population-based studies were subsequently undertaken which showed rates of community disease ranging between 6.9 and 23.4 cases per 100,000 person-years, but these were all based on laboratory surveillance.[94–97] These estimates of the incidence of community-acquired *C. difficile* are far exceeded by incidence rates of hospital-acquired *C. difficile*, which are approximately 5000 times higher. A subsequent study of the prevalence of CDI among community-dwellers testing positive for CDI in the evaluation of acute diarrheal syndromes showed that 43 of 1091 (3.9 %) such patients tested positive for *C. difficile*, but only 3 patients (0.3%) remained as possible CDI patients when more recognized causes of outpatient gastroenteritis such as norovirus were excluded.[98] CDI remains extremely unlikely as a cause of outpatient gastroenteritis based on available evidence.

Among patients with traditional risk factors for CDI (Table 4), several sub-groups who experience exceptional rates of CDI have emerged, including solid organ transplant patients,

bone marrow transplant patients, and patients with inflammatory bowel disease (Crohn's disease and ulcerative colitis) (Table 5). The rates of CDI observed in these populations can be readily expressed in denominators of 100, reflecting disease incidences 100 to 1000 times those observed in general inpatient populations and long-term care facilities. The unifying clinical feature of these groups is widespread use of broad-spectrum antimicrobials needed to manage conditions such as febrile neutropenia and opportunistic infections, although all of these groups also share significant immunosuppression and exposure to healthcare facilities. Unfortunately, most clinical trials for CDI have traditionally excluded patients with underlying diarrheal disorders such as inflammatory bowel disease, creating an evidence gap in the management of these patients. The impact of CDI on these populations can be significant. In one study of kidney recipients, the attributable mortality of CDI was 0.7%, higher than the observed loss of transplant grafts due to thrombosis and rejection.[99] In a study of lung transplant recipients, 54% of CDI occurred within the first 6 months of transplant; and these early CDI patients had a higher risk of death.[100] In this cohort, 70% of CDI occurred after discharge from the initial hospitalization, such as in the outpatient setting or during readmissions for complications, rather than immediately post-operatively.[100]

The public health impact of CDI is substantial. The burden of CDI has been estimated at $2,454 to $6,326 in excess healthcare costs per case, resulting in additional US healthcare expenditures of $4.8 billion per year for acute care facilities alone.[101–103] Beyond the economic impact, CD results in an estimated 453,000 cases and 29,000 deaths annually in the United States and has replaced methicillin-resistant *Staphylococcus aureus* as the most common cause of healthcare-associated in U.S. hospitals.[104–106] In 2013, CDI was classified by Centers for Disease Control and Prevention as one of three urgent antibiotic resistance threats in the U.S. that "require urgent public health attention to identify infections and to limit transmission."[107]

Pathogenesis

The secretory diarrhea and colonic inflammation seen in CDI is attributable to the effects of 2 large clostridial toxins, toxin A and toxin B, both encoded by *tcdA* and *tcdB* respectively as part of the 19.6-kb pathogenicity locus (PaLoc) on the *C. difficile* chromosome.[108–110] Non-toxigenic strains of *C. difficile* in which the entire PaLoc is replaced with a 115-bp sequence are commonly encountered both in colonized humans and the environment.[31, 111] TcdA and TcdB, similar to other large clostridial toxins, glycosylate small guanosine triphosphatases in the Rho and Ras families when endocytosed into epithelial cells, leading to actin filament disassembly, disruption of tight junctions, and ultimately cell death.[112] While toxin B is observed to intoxicate a wide variety of mammalian cell lines, candidate receptors responsible for cell surface binding and endocytosis in intestinal epithelial cells have only recently been characterized. Using an insertional mutagenesis technique in Caco-2 cells, LaFrance and colleagues demonstrated that poliovirus receptor-like 3 (PVRL3) is involved in binding of TcdB to its target, disruption of or pre-treatment by antibodies to which creates resistance to toxin B in this cell line.[113] Tao and colleagues, however, failed to confirm this finding in HeLa or Caco-2 cells.[114] Instead, their CRISPR– Cas9-mediated genome-wide screens identified members of the Wnt-binding frizzled family, particularly FZD1, 2 and 7, as the predominant receptors for TcdB. The finding that TcdB may compete

directly with Wnt for FZD binding suggests that colonic epithelial disruption may be a direct consequence of TcdB, as Wnt signaling is particularly important for maintaining colonic stem cells.[114]

Previous studies using pure TcdB and TcdA pointed to a synergistic role for the 2 toxins in CDI, but the recent ability to create gene knockouts in *C. difficile* has led to the demonstration that TcdB is necessary and sufficient to cause disease in both the hamster and mouse models.[115, 116] This finding is concordant with the clinical observation that TcdA −TcdB+ *C. difficile* strains are fully capable of causing human disease. [117–119]

In wild-type strains of *C. difficile*, *tcdA* and *tcdB* are expressed only in late-logarithmic and stationary growth phases.[109, 120] Toxin production is under the immediate control of 2 other genes on the PaLoc, *tcdR* and *tcdC*, which serve as positive and negative regulators, respectively. [109, 120–122] BI/NAP1/027 strains have been associated with nonsense mutations in *tcdC* that lead to a truncated, dysfunctional TcdC and thus TcdB production at all phases of growth, but these *tcdC* mutations are found in many other non-epidemic lineages.[25, 123] There is also *in vitro* evidence that BI/NAP1/027 strains produce more toxins than wild-type strains, although many other lineages including the type stain VPI 10463 make substantially more toxin than epidemic lineages.[121, 122, 124]

C. difficile also has potential virulence factors outside the PaLoc, including a binary toxin, encoded by two genes *cdtA* and *cdtB*, which is also prevalent among BI/NAP1/027 strains.[125–127] Evidence for a role of binary toxin in CDI, however, is limited by the observation that CDT+TcdA−TcdB− strains are incapable of reproducing CDI in the hamster model and that these strains have not yet been associated with human disease.[128] Preliminary evidence also points to increased adherence to intestinal mucosa mediated by mutations in *slpA*, a surface layer protein, in BI/NAP1/027 strains of C. *difficile*.[129]

BI/NAP1/027 strains may have an enhanced sporulation capacity compared with wild-type strains, but this finding has not been reproduced in later studies which have noted substantial variation within the epidemic lineage.[130, 131] Antibiotic resistance, particularly to clindamycin, macrolides, fluoroquinolones, and rifampin, is also more common in the BI/NAP1/027 strains and leads to potentiation of the spread of the organism within hospitals where such antibiotics are in common use. [21, 81, 132]

The clear association of antibiotic use with CDI has focused attention on the relationship of the human microbiome to CDI risk. Using culture-independent 16S rRNA phylogenetic analysis of microbial components of stool flora, it was shown that the stool flora of patients with recurrent episodes of CDI were significantly less diverse than that of patients with initial episodes of CDI whose stool flora was, in turn less diverse than control subjects.[133] In a subsequent study, pure culture of a single species of the Lachnospiraceae cultivated from mice that survived *C. difficile* challenge were able to confer partial colonization resistance in a germfree mouse model, although the mechanism for this colonization resistance was not determined.[134]

The role of conjugated primary bile salts (taurocholate and glycocholate) in recovering *C. difficile* in the clinical microbiology laboratory has been known for some time;[135] early

investigators hypothesized that the effect of antibiotics on the transit of these bile salts to the human large intestine after antibiotic use might be significant in the pathogenesis of CDI.[9, 10] The significance of bile salts in both the germination (by primary bile salts) and inhibition of *C. difficile* outgrowth by chenodeoxycholate has recently been confirmed,[136, 137] and subsequent studies in the mouse model of CDI correlated antibiotic challenge in mice with a shift from a predominance of deoxycholate, which inhibits *C. difficile* outgrowth, to taurocholate, which is essential to germination, in the murine cecum.[138] Microbiome analysis of patients colonized with *C. difficile* has suggested that *Clostridium scindens*, a bile acid 7α-dehydroxylating intestinal bacterium, is associated with resistance to CDI through its production of secondary bile acids,[139] a finding that awaits confirmation in formal trials of this candidate probiotic organism.

Laboratory Diagnosis

The laboratory diagnosis of CDI has undergone considerable upheaval since the first characterization of CDI. Cell culture cytotoxicity neutralization assays (CCCNAs) (see Fig. 2) were the only available clinical tests for CDI following the discovery of CDI; these tests recapitulate the process used to discover the disease. For CCCNAs, patient stool is filtered and incubated with human fibroblast cells with and without *C. difficile* (originally *C. sordellii)* antitoxin for up to 72 hours, and if the antitoxin-free well shows a cytopathic effect and the antitoxin well does not, the presence of *C. difficile* toxin in stool is confirmed (Fig. 2). CCCNA is technically demanding, takes 2–4 days to turn around a negative result, and is generally only performed by large clinical laboratories with the capacity to maintain cell cultures. Compared with identification of toxin-producing *C. difficile* using anaerobic culture (termed toxigenic culture, see **Microbiology**), CCCNA was observed to be 67% to 78% sensitive,[140, 141] but the extent to which either CCCNA or toxigenic culture could be considered a reference standard was never established. Both CCCNA and toxigenic culture identify patients with colonization and are therefore susceptible to false positives for the endpoint of CDI. Eventually, CCCNA came to be regarded as a clinical gold standard in its own right to which more rapid immunoassays (EIA) were compared as they were introduced into clinical use in the 1980s.

Cell culture cytotoxicity assays were replaced by most clinical microbiology laboratories in North America and Europe by EIAs for TcdA or TcdA+TcdB because of their ease of use and rapid turnaround time of approximately 2 hours. Compared to CCCNA as a reference standard, EIA has a comparatively low sensitivity of 80% to 90%,[141–143] and it is even less sensitive when compared with toxigenic culture. Clinicians frequently sought to overcome the perceived sensitivity issue of immunoassays for CDI through use of repeated testing. Submission of multiple samples from the same patient within days became a common strategy for both CCCNA and immunoassays, but these were observed to have a marginal return.[144–147] Various 2-step testing algorithms incorporating a rapid antigen test for *C. difficile* glutamate dehydrogenase (GDH) activity followed by reflex testing of positives to confirm toxin production by EIA or cell culture cytotoxicity were also devised,[148–150] but sensitivity of GDH screening varied by *C. difficile* lineage, casting some doubt on this strategy.[151, 152]

The introduction of nucleic acid testing for *C. difficile* into clinical practice in 2008 (Table 6) initially held significant promise for improving the sensitivity of testing relative to EIA platforms and substantially reducing the turn-around time and technical complexity of CCCNA. The latest generation of molecular tests has emphasized the detection of *tcdB*, although platforms that are designed to detect only *tcdA* are still able to detect toxinA-/B+ strains because of a conserved fragment of *tcdA* present in these lineages. Almost all currently approved platforms incorporate sample lysis, DNA extraction, target amplification, and interpretation of internal controls into a single sample vessel or cartridge, substantially reducing the technical complexity of the tests compared to earlier generations of real-time PCR instruments. Assays based on toxin gene detection exclude false-positive results from detection of nontoxigenic strains of *C. difficile*. The sensitivity of GDH antigen, PCR, and toxigenic culture were all recently shown to be decreased substantially by empiric therapy, with 45% of patients converting initially positive tests to negative within three days of therapy in one cohort of CDI patients.[153] The impact of empiric therapy has been poorly accounted for in most prior diagnostic test evaluations for CDI.

Recent studies of *C. difficile* testing performance have focused on strategies to incorporate clinical outcome or symptoms into test performance given increasing concerns about the lack of specificity of laboratory diagnostics for CDI. Dubberke and colleagues performed a prospective study of 150 patients being tested for CDI, including prospective evaluation of tested patients' symptoms using blinded physician interviews as part of a reference standard for evaluating 2 different EIAs, 3 nucleic acid amplificiation tests, CCCNA, and toxigenic culture.[154] Among the 150 patients, 44 were positive by toxigenic culture, but only 35 of these had clinically significant diarrhea.[154] Using a composite reference standard of ≥4 assays positive, 50/150 patients were positive, but only 40 of these patients had clinically significant diarrhea. As expected from prior studies, CCCNA had the lowest observed sensitivity regardless of reference standard used (55.0–62.9%) while the nucleic acid tests had very high sensitivity (100%). Somewhat unexpectedly, the sensitivity of varying EIAs was actually higher than CCCNA, and the inclusion of clinical symptoms into the reference standard increased the specificity and positive predictive value of all tested assays. The authors observed that 15 patients whose diagnosis of CDI was rejected by EIA but positive by the composite reference standard (mostly by nucleic acid tests) had no diagnosis of CDI within 60 days despite 10/15 patients having clinically significant diarrhea and 4/15 patients receiving empiric therapy for CDI, suggesting that less sensitive tests like EIA may actually predict typical CDI features more reliably.[154] Planche and colleagues conducted a much larger multicenter prospective observational study of 12,420 fecal samples from 6,522 inpatient episodes of diarrhea.[155] Mortality was observed in 72/435 (16.6%) patients with positive CCCNAs compared to 20/207 (9.7%) positive by toxigenic culture with negative CCCNAs and 503/5880 (8.6%) patients negative by both methods.[155] The authors also observed that patients with negative CCCNA but positive nucleic acid tests did not differ in their observed mortality, length of stay, or peak leukocyte count from patients negative on both assays; given that CDI is not 100% fatal this observation is not clear-cut evidence that CCCNA remains the most relevant clinical standard for diagnosis, nor could algorithm combining EIA, GDH antigen, and nucleic acid testing fully replicate the sensitivity and specificity of CCCNA itself in their cohort.[155] Finally, Polage and colleagues conducted a

prospective cohort study at a single center of 1416 hospitalized adult patients tested for CDI using EIA and nucleic acid testing (PCR) during which the PCR results were withheld from ordering physicians.[156] In this study 293/1416 (21%) were positive by PCR while 131/1416 (9.3%) were EIA-positive.[156] The authors observed that the EIA-/PCR+ patients had shorter duration of diarrhea than in EIA+/PCR+ patients despite minimal empiric treatment in the former group, and no CDI complications (attributable death, colectomy, ICU stay) were observed vs 10 complications in patients with concordant tests.[156] Recurrent positive *C. difficile* testing was observed in only 5 (3.1%) EIA-/PCR+ patients vs 14 (10.7%) of EIA +/PCR+ patients; deaths due to recurrent CDI occurred in both groups, but at significantly lower rates in the EIA-/PCR+ group.[156] The study did not include prospective use of CCCNA or toxigenic culture as a reference standard, however, which may have misclassified patients in the EIA-/PCR− group with mild CDI as control group patients, but overall this study adds to a body of evidence that nucleic acid tests may substantially over-diagnose CDI. The available evidence does not yet support that a return to EIA as the principal diagnostic test for CDI would not result in significant loss of sensitivity for diagnosis of patients with mild CDI, however. The need for laboratory correlates of colonization versus infection remains acute, and CDI will remain partly a clinical diagnosis until additional diagnostics are developed (see **Differential Diagnosis**).

Treatment, prognosis, and long-term outcome

Prior to recent shifts in observed incidence and severity after 2001, CDI was often regarded as a self-limited disease so long as the inciting antimicrobial agents could be stopped. Early therapeutic trials sometimes contained placebo arms, and head-to-head comparisons of the 2 main *C. difficile* antimicrobials, enteral vancomycin and metronidazole, failed to show superiority for either.[157] Metronidazole was generally recommended by expert guidelines as first-line therapy for reasons of cost and out of concern that widespread use of vancomycin would promote acquisition of vancomycin-resistant enterococci.[158] A randomized, double-blind trial of metronidazole versus vancomycin showed superior response rates for vancomycin in a subset of patients with severe disease, however.[159](Metronidazole was also found to be inferior to metronidazole?) for the endpoint of clinical success (defined as cessation of diarrhea for ≥ 2 days) in all CDI patients in a multisite blinded, randomized trial with 72.7 vs 81.1% success rates observed, respectively.[160] Fidaxomicin, a new macrocyclic antimicrobial, was observed to be non-inferior to vancomycin in three randomized trials, but it did not demonstrate superiority for the endpoint of clinical success; while much has been made of the observation that fidaxomicin has a ~10% reduced risk for recurrence compared to vancomycin, this was not consistent across studies and not tested in patients with multiple CDI recurrences, who were excluded from these trials.[161–163] Current clinical guidelines stress the use of vancomycin monotherapy for patients observed to be at risk for severe disease based on varying clinical criteria, although metronidazole use in patients with milder CDI is still recommended.[164, 165]

The debates regarding which anti– *C. difficile* antimicrobial is preferred in CDI management have been largely eclipsed by the observation of high rates of success for management of CDI, particularly multiply recurrent cases, with fecal microbiota transplantation (FMT).[166–171] In contrast to defined probiotic formulations, high-quality evidence has

emerged showing that FMT is superior to vancomycin in management of recurrent CDI (Table 7). The regulatory status of FMT with the Food and Drug Administration remains uncertain, however, and the risk of short and long-term donor-derived infection, particularly in immunosuppressed CDI patients, remains a concern for this treatment modality. The optimal dose, route of delivery, donor source for FMT have yet to be determined in well-controlled randomized trials, as is the use of FMT for first episodes of CDI. The risks of non-disclosure of sexual risk-taking and self-reported diarrheal illness in paid FMT donors as well as the use of small numbers of FMT donors for large numbers of recipients for the sake of improved patient access to this treatment modality may prove to be short-sighted in assessing the long-term risks of FMT in transmission of undiscovered pathogens. Nonetheless, FMT has emerged as the most effective single therapy for management of patients with recurrent CDI.

The prognosis for most patients with CDI remains favorable, but adverse event rates for CDI (colectomy/death) reached as high as 44 of 253 (17.3%) for inpatients with hospital-acquired CDI at the University of Pittsburgh during a 2-year period.[82] A 30-day attributable mortality of 6.9% was observed in 20 hospitals in Quebec subsequently.[81] For patients who require colectomy, all-cause mortality is 50%.[67] Recently surgical interventions have been devised that allow for delivery of vancomycin to the cecum of patients with severe, fulminant CDI using a loop ileostomy technique; this has a substantially improved observed success rates compared to historical controls who underwent colectomy. [172]

Differential diagnosis

The high prevalence of *C. difficile* colonization among inpatients and more modest colonization among outpatients often obscures a number of infectious and non-infectious causes of diarrhea and gastroenteritis when a positive *C. difficile* diagnostic test is generated, resulting in substantial diagnostic test bias. Among outpatients presenting with acute gastroenteritis, particularly with vomiting, norovirus remains the most prevalent etiology. Most new molecular diagnostic platforms that are replacing stool cultures for routine enteric pathogens in clinical microbiology laboratories are incorporating tests for not only *C. difficile* but also norovirus, astrovirus, sapovirus, and others. The vast majority of patients without traditional risk factors for CDI who test positive for both a viral etiology and *C. difficile* are probably colonized with the latter, and treatment for *C. difficile* in these patients should be avoided. Treatment with vancomycin has been observed to prolong carriage with toxigenic *C. difficile* and, in one instance, to actually provoke CDI in a patient with previously asymptomatic carriage.[173]

Among inpatients who present with diarrhea >3 calendar days after admission, CDI is widely cited as the most prevalent infectious cause. With the increasing focus on early and aggressive diagnosis of CDI, however, it is often overlooked that non-infectious causes of diarrhea predominate, particularly post-antibiotic diarrhea not associated with *C. difficile*. Osmotic diarrhea from enteral feeding formulations, malabsorption related to ischemic colitis, and inflammatory diarrhea from ulcerative colitis and Crohn's disease should always be considered in the differential diagnosis for diarrheal illnesses presenting in hospitalized patients. Even laxative use has been observed in 20% of hospitalized patients being tested

for CDI, however,[154] and the rate of diagnoses other than CDI was >90% in the largest diagnostic testing cohort yet reported,[155] indicating that CDI remains an uncommon finding among hospitalized patients with diarrhea. Patients with any of these causes of diarrhea are frequently colonized with *C. difficile* and are found on diagnostic testing, and to date there is no laboratory assay that can distinguish colonization from CDI. Fecal leukocytes, fecal calprotectin levels, and C-reactive protein have all been proposed as biomarkers to make this distinction, for example, but none of these can distinguish CDI from inflammatory bowel disease flares.[174, 175] Thus, the diagnosis of CDI remains partly a clinical one. The absence of known risk factors, intact serum albumin levels, normal peripheral leukocyte counts, and a failure to exhibit any improvement after 10 days of antimicrobial therapy for CDI should raise suspicion that a patient with a positive *C. difficile* stool test does not have CDI.

In the era of FMT, making the distinction between colonization and CDI is particularly important; patients with alternate causes of diarrhea are highly unlikely to benefit from FMT based on available data. In one outpatient study of 117 patients referred 2013–2014 to an FMT clinic, 29 patients (25%) were determined to have an alternative diagnosis, most frequently irritable bowel syndrome(IBS).[176] Because many patients with a history of multiple positive *C. difficile* tests may have had inappropriate tests of cure while experiencing IBS after recovery from CDI, careful history-taking must distinguish patients who exhibit a pattern of worsening symptoms days to weeks after cessation of CDI antibiotics from those who have “refractory CDI” regardless of their treatment status. While common in clinical parlance, “chronic,” “treatment-refractory,” and “treatment-resistant” CDI is unlikely to exist based on available evidence, and the preponderance of the of patients in CDI clinical trials who “fail” vancomycin and fidaxomicin (~10% in most recent trials) are probably among the patients who did not have CDI given the low observed mortality among these patients.

Immunity and reinfection

The most troubling aspect of CDI is the high relapse rate of 10–35% after a first episode, ~40% after a first recurrence, and 60–100% following 2 or more recurrences.[177] Patients with multiple CDI have been strongly associated with a failure to demonstrate an anamnestic IgG response against TcdA.[178–182] Besides age and immune senescence, continued exposure to antibiotics is a powerful predictor of CDI relapse.[183] Efforts to devise a C. *difficile* toxoid vaccine have met with initial success in individuals with multiple relapses,[184] but to date there are no published trials using vaccines for primary prevention of CDI. The debilitating effects of multiple CDI relapses were the principal drivers of the development of FMT, which has emerged as the only reliable treatment modality to date. A randomized, placebo-controlled trial of passive immunotherapy for recurrent CDI using monoclonal antibodies to TcdA and TcdB showed an 18% reduction in the absolute risk of recurrence.[185] Two phase 3 evaluations of these antibodies revealed that the antibody to TcdB (bezlotoxumab) was the active component, reducing recurrence rates by 10% compared to placebo.[186, 187] Bezlotoxumab is available only parenterally, must be used with standard antimicrobial therapies for CDI, and is likely to be substantially more costly than (and likely less effective than) FMT in managing recurrent CDI, however, and its role in clinical care of CDI patients has yet to be established.

Summary

CDIs have emerged as one of the principal threats to the health of hospitalized and immunocompromised patients. Nucleic acid testing for *C. difficile* toxin genes has eclipsed traditional clinical diagnostics for CDI in sensitivity and is now widespread in clinical use, but preliminary evidence suggests that this may have come at a cost of substantially reduced positive predictive value. The importance of *C. difficile* colonization is increasingly recognized not only as a source for false positive clinical testing but also as a source of new infections within hospitals and other healthcare environments. In the last five years, fecal microbiota transplantation has emerged as the most effective treatment for patients with multiply recurrent CDI. The increasing understanding of the microbiome and colonization resistance as one of the host defenses against CDI will likely result in improved therapeutics for CDI in the next decade.

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KEY POINTS

- *Clostridium difficile* is widely distributed in nature but concentrated within healthcare environments
- Asymptomatic carriage of humans with *C. difficile* is common, posing a significant challenge for the laboratory diagnosis of *C. difficile* infection (CDI)
- CDI poses a substantial threat to the health of hospitalized patients and other sub-groups of immunocompromised patients.
- The collateral protective effect of an individual's microbiome may be as important as host immune defenses for CDI.
- Fecal microbiota transplantation (FMT) is emerging as the most effective treatment for patients with recurrent CDI.

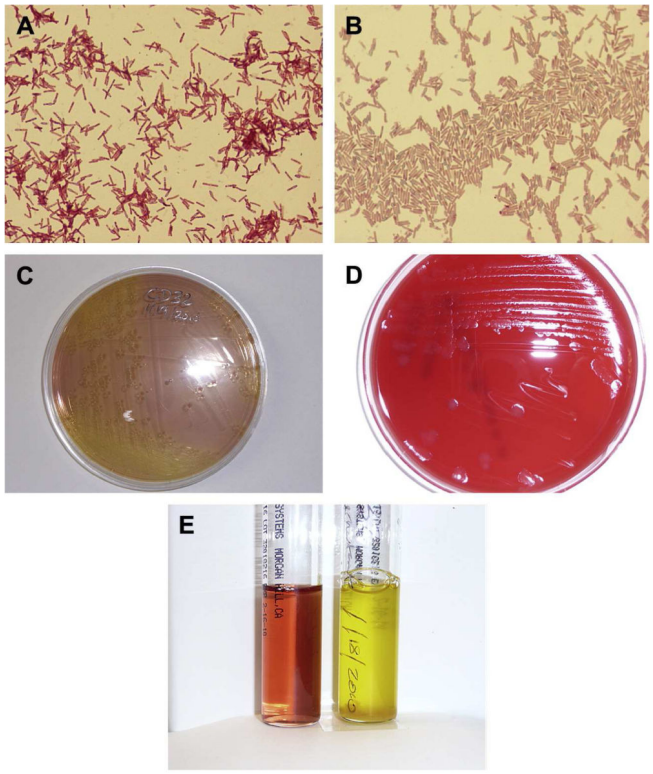

Fig. 1.

(A) Gram stain of C. *difficile* from 24-hour growth on trypticase soy agar with 5% sheep blood. The vegetative cell bodies are often gram negative during early growth; note the abundant subterminal endospores that do not swell the parent cell. (B) Malachite green stain of 48-hour growth of C. difficile. The s afranin counterstain renders vegetative cells pink, whereas the endospores stain green, revealing their ovoid shape. (C) A 48-hour growth of C. difficile on typical selective agar medium, C. difficile basal agar with moxalactam and norfloxacin, CDMN, that uses norfloxacin and moxalactam as selective antibiotics to allow primary isolation from stool specimens. Other media use combinations of cycloserine and cefoxitin as selective antibiotics (cycloserine cefoxitin fructose agar). Neutral red is turned yellow by C. difficile growth. (D) Typical appearance of C. difficile colonies on trypticase soy agar with 5% sheep blood at 48 hours. Colonies are nonhemolytic. (E) A selective broth medium for isolation of C. difficile, cycloserine cefoxitin mannitol broth with taurocholate and lysozyme. The tube at right shows turbidity and growth of *C. difficile* at 24 hours, with obvious alkalinization of the neutral red indicator. The tube at left is a negative control.

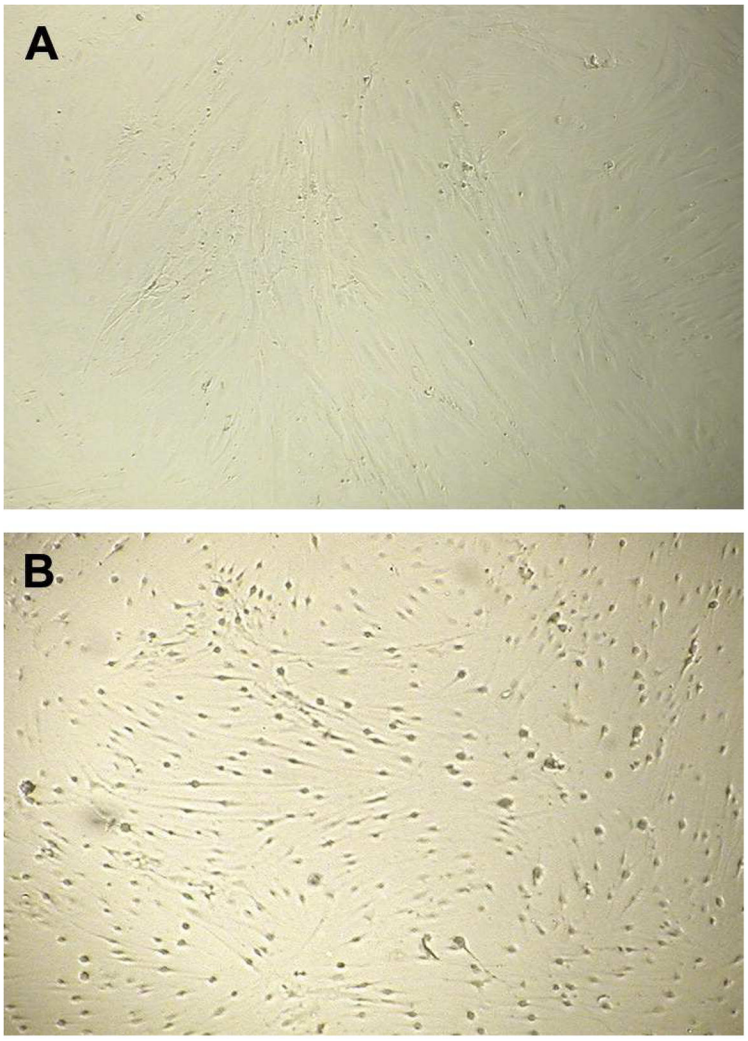

Fig. 2.

(A) Negative cell culture cytotoxicity assay. Human fibroblasts remain spindle-shaped and in contact with each other. (B) Positive cell culture cytotoxicity assay revealing cytopathic effects of *C. difficile* toxin B causing cell rounding and separation. (Courtesy of Ray Hariri, PhD.)

Table 1

Laboratory typing methods for CDI

Method	High-throughput	Portable	Determines Lineage	Discriminatory capacity	Reference
Toxinotyping	No	Yes	No	Low	188
Pulsed field gel electrophoresis (PFGE)	No	No	Yes	Low-moderate	22
Restriction endonuclease analysis (REA)	No	No	Yes	Moderate-high	27
PCR ribotyping	Yes	No	Yes	Low-moderate	23
Multilocus sequence typing (MLST)	Yes	Yes	Yes	Low	24, 189
tcdC genotyping	Yes	Yes	Yes	Low	25, 26
Multiple locus variable number of tandem repeats analysis (MLVA)	Yes	Yes	No	High	29, 30
Whole genome sequencing	No	Yes	Yes	High	28, 190

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Table 2

Prevalence of toxigenic C. *difficile* from sampling of various sources in South Wales.

Source	**N**	**toxigenic *C. difficile* (%)**
Domestic animals	200	3 (1.5)
Farm animals	524	4 (0.8)
Fish	107	0
Soil	104	9 (8.6)
Hospitals	380	72 (18.9)
Nursing homes	275	4 (1.5)
Houses	350	3 (0.9)
Dorms	200	3 (1.5)
Water*	110	36 (32.7)
Vegetables	300	5 (1.7)
Total	**2580**	**140 (5.4)**

Adapted from al Saif N, Brazier JS. The distribution of Clostridium difficile in the environment of South Wales. J Med Microbiol 1996;45(2):133–137; with permission.

Table 3

Prevalence of *C. difficile* in the human food supply.

Prevalence (%)	95 % CI	Region	Product sampled	Reference:
37/88 (42.0)	31.6 to 53.1	Tucson, AZ	ground meat	[191]
8/500 (1.6)	0.69 to 3.1	Netherlands	retail meats	[192]
5/111 (4.5)	1.5 to 10.2	Ontario, CA	Vegetables	[193]
0/46 (0)	0 to 7.7	Switzerland	ground beef	[194]
26/203 (12.8)	8.5 to 18.2	Ontario, CA	retail chicken	[195]
3/100 (3)	0.6 to 8.5	Switzerland	ground meat	[196]
3/50 (6)	1.3 to 16.6	Pennsylvania	ground veal	[197]
2/82 (2.4)	0.3 to 8.5	Sweden	ground meat	[198]
4/32 (12.5)	3.5 to 29.0	Texas	poultry meat	[199]
3/40 (7.5)	1.6 to 20.4	Texas	ground meat	[200]
2/102 (2.0)	2.4 to 6.9	Pennsylvania	ground meat	[36]

Table 4

Clinical risk factors for *Clostridium difficile* infection (CDI) and recurrent CDI

Any episode of CDI	Recurrent CDI
Age ≥65	Age 65
Length of inpatient stay	Number of prior CDI episodes
Antibiotic exposures:	Use of non CDI antibiotics after CDI diagnosis
• Clindamycin	
• Cephalosporins	
• Fluoroquinolones	
Proton pump inhibitors	Fluoroquinolone use
	Renal insufficiency
	Female gender
	NAP1/ribotype 027/BI/MLST1/*tcdC*1 lineage

Table 5

Populations at exceptional risk for *Clostridium difficile* infection.

Population	Prevalence (%)	Ref
ulcerative colitis	3.7	201
Crohn's disease	1.1	201
kidney transplant	4.7	202
pancreas transplant	3.2	202
lung transplant	10.8	202
liver transplant	9.1	202
heart transplant	5.2	202
solid organ transplant overall	7.4	202
allogeneic BMT	8.4	203

Table 6

Selected FDA-approved clinical diagnostic nucleic acid testing platforms for diagnosis of CDI

Device	Manufacturer	Approval	Platform	Gene target(s)
Cobas	Roche	5/2015	RT-PCR	tcdB
BD MAX	BD Diagnostics	4/2013	RT-PCR	tcdB
Verigene	Nanosphere	12/2012	PCR + nanoparticle hybridization	*tcdA/tcdB/tcdC/cdt*
Simplexa	Focus	4/2012	RT-PCR	tcdB
Illumigene	Meridian	7/2010	LAMP	tcdA
Xpert	Cepheid	7/2009	RT-PCR	tcdB/tcdC
Progastro CD	Prodesse	4/2009	RT-PCR	tcdB
BD Geneohm	BD Diagnostics	12/2008	RT-PCR	tcdB

Table 7

Key trials of fecal microbiota transplantation (FMT) for treatment of recurrent CDI.

N=	Design	Comparator	Dose (estimated stool mass)	Route	Followup	Observed successes (per protocol)	Comments	Reference
42	Randomized open label	Vancomycin +/− laxative	500 ml (~50g)	Nasoduodenal tube	10 weeks	13/16 (81%) single dose 15/16 (94%) overall 7/26 (27%) patients in comparator arms	3 patients required 2nd dose	169
20	Open label	None	48g	Oral capsules (n=30)	8 weeks	14/20 single dosing 19/20 2nd dose	15 capsules × 2 days=“1 dose”	170
20	Randomized nastogastric tube vs. colonoscopy	None	90 ml (41g)	Nasogastric tube Colonoscopy	8 weeks	8/10 colonoscopic 6/10 nasogastric tube	All unrelated donors Non-significant difference in success	171
80	Retrospective multicenter case series	None	varied	Colonoscopy	12 weeks	62/80 (78%) single dose 70/80 (89%) multiple doses	First series to report inclusion of immunocompromised (n=19 organ transplants, 3 HIV+)	167
43	Case series, directed fresh donors vs. standardized frozen donors	None	250 ml (50g)	Colonoscopy	8 weeks	7/10 directed 30/33 stoolbank p=0.12	30% inflammatory bowel disease Unrelated donors from stool bank	166
46	Randomized controlled double-blind	Autologous stool (recipient’s)	300 ml (64g)	Colonoscopy	8 weeks	22/22 (90.9%) donor FMT 15/24 (62.5%) autologous FMT	IBD and age ≥75 patients excluded 90% success for autologous stool reported at a single center	168
219	Randomized, double-blind frozen vs fresh FMT dose	None	50 ml (12.5g)	Retention enema	13 weeks	76/91 (83.5%) frozen FMT 74/87 (85.1%) fresh FMT	Single dose response rates much lower (62–63%)	204