The Emerging Threat of *Clostridium difficile* Infection:
New Insights into Diagnosis and Disease Management.

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Introduction

Healthcare-associated diarrhea has plagued hospitalized patients for decades. For many years, antibiotic-associated diarrhea was attributed to *Staphylococcus aureus* (3). Although the organism *Clostridium difficile* was described by Hall and O’Toole in 1935 (36), it was not until 1977 that Bartlett and colleagues identified *C. difficile* as the causative agent of “antibiotic-associated pseudomembranous colitis” (14). Today, concern about the rise in the rate of *Clostridium difficile* infections (CDI) now rivals concerns about methicillin resistant *Staphylococcus aureus* infections. *C. difficile* infections, particularly healthcare-associated diarrhea, have been increasing in incidence and severity, and are associated with an increase in lengths of hospital stay, costs, morbidity, and mortality among patients (23).

![Figure 1. Pseudomembranous colitis (44)](image)

Endoscopic images of pseudomembranes show characteristic yellow plaque.

Disease Presentation

*C. difficile* is a gram-positive, spore-forming, obligate anaerobic bacillus that is found in a variety of environmental niches (19, 57, 82). Toxigenic (toxin-producing) forms of the bacillus are pathogenic for humans. The organism’s spores are very resilient to heat and desiccation and can remain viable in the hospital environment for weeks to years. Based on a recent APIC (Association for Professionals in Infection Control and Epidemiology, Inc.) study, current U.S. estimates suggest that CDI affects 7,178 inpatients on any given day, and causes the deaths of about 300 patients per day (39). These staggering statistics have brought *C. difficile* to the forefront as one of the major challenges that healthcare facilities are addressing today.

CDI can range from mild diarrhea, defined as three or more loose stools (those that take the shape of the container) within a 24-hour period, to severe life-threatening disease (47). The more severe forms of diarrhea, particularly pseudomembranous colitis, can cause dehydration and tremendous discomfort for the patient. The most serious disease entity, although rare, is the syndrome known as toxic megacolon, in which the bowel mucosa becomes so inflamed that peristalsis stops, fecal impaction occurs, and emergent surgery, including bowel resection (colectomy), is required.
Epidemiology

Several large outbreaks of CDI in Montreal starting in 2000 ushered in a new era of *C. difficile* investigations and research (54, 73). The number of *C. difficile* cases in North America has been expanding dramatically over the past several years, in part due to the emergence of novel strains and to prolonged outbreaks of disease (see Figure 2) which facilitates the continuing spread of the organism (43, 67). As shown in Figure 2, data from the Department of Health & Human Services and other sources show a significant increase in CDI discharges since 2001 (82, 86). Although CDI has traditionally been seen in elderly inpatients and those recently released from healthcare facilities, a recent study carried out in North Carolina showed that only 42% of greater than 1000 patients with CDI had onset of their infection in a healthcare facility (50). In fact, 34% of the cases were acquired in the community and had no healthcare-associated risk factors. As noted in the case study “Pregnancy and *C. difficile*” on page 10, young, healthy adults, including pregnant women, are also among those now at risk for CDI. This change in the epidemiology may well be related to the emergence of novel strains of *C. difficile* (48, 53, 54, 59).

The alarming prevalence of CDI in the United States was reinforced in November 2008, when APIC issued the results of a survey regarding CDI infections (59). APIC asked infection preventionists to report laboratory data from patients in their facility that were tested for *C. difficile* on a single day sometime during the period of May to August 2008. A total of 648 hospitals in 47 states (i.e., 12.5% of all the hospitals in the United States), sent in their data. The average number of facilities participating per state was 14.3. Although the respondents were primarily acute care hospitals, healthcare institutions of all sizes and types (including pediatric, cancer, chronic care, and cardiac specialties) from public to private to county facilities participated. Among the 110,550 inpatients who were tested, 1,362 were infected (based on signs and symptoms) and an additional 192 patients were colonized (59). The data indicated that more than 12 of every 1,000 patients in the U.S. healthcare system are infected (i.e., experiencing symptoms) with this organism, and an additional 5.6% are colonized with an organism now known to be the major cause of healthcare-associated diarrhea.

Patients in healthcare institutions are most at risk of acquiring the organism, which becomes a component of their gut flora (47). In a key prevalence study, Johnson et al. reported that weekly surveillance cultures using rectal swabs obtained from inpatients in their hospital revealed that >20% of patients who remained in the facility longer than one week and up to nine weeks became colonized with *C. difficile* (41). Although it was widely held that *C. difficile* was a small component of virtually everyone’s bowel microbiota and gained ascendency when antimicrobial agents disrupted the normal bowel flora, the data from Johnson et al. suggest that this is not the usual pattern of disease progression (41). Overwhelmingly, patients acquire a new strain of *C. difficile* at some point after they have received antimicrobial agents. In fact, some patients who are already colonized with their own non–toxin B producing strain before they enter healthcare may be protected from acquiring a pathogenic version of *C. difficile* (59). The new focus on toxin B producing isolates (58) should facilitate better control measures, since detection of the toxigenic strains is both the key diagnostic factor and the basis of appropriate interventions.

CDI has become an increasingly common and serious threat to hospitalized patients, many of whom are elderly. Its initial presentation often goes unrecognized until the patient is toxic, has an elevated WBC count and a marked ileus. Since these frail patients may have other causes for these findings, an accurate, rapid test would allow targeted and earlier specific therapy, save exposure to unneeded antimicrobials and assure an improved clinical response.

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Director: R.M. Alden Research Laboratory, Santa Monica, CA
Recurrent *C. difficile* infection

**Case Study #1**

**A 76-Year-Old Man With Recurrent *Clostridium difficile***

Associated Diarrhea

This year, a typical case involving recurrent CDI was reported in the Journal of the American Medical Association. The patient was present at the discussion of his case at the Beth Israel Deaconess Hospital in Boston, a special type of clinical rounds which allows physicians-in-training a glimpse into the feelings and experiences of the patient during his treatment. The patient gains the benefit of hearing the differing opinions of many doctors and the expert recommendations that arise from such an in depth discussion of his particular case.

**Presentation:** The patient, who was 76 years old, had been the recipient of two previous kidney transplants (10 years apart) and suffered from spinal stenosis, neurogenic bladder, arthritis, and benign prostatic hypertrophy. He had an indwelling foley catheter and was primarily confined to a wheelchair, which made the prospect of having 3 bowel movements per hour each day, and often during the night, a daunting and painful activity. Unfortunately, several months earlier after receiving initial antimicrobial therapy (ceftriaxone and ciprofloxacin) for a pseudomonal urinary tract infection, he developed diarrhea. His physician treated him for CDI empirically with metronidazole without testing the stool, and the patient improved. Shortly thereafter, his diarrhea returned. Several tests for *C. difficile* were negative before the patient was readmitted to the hospital where another laboratory test revealed *C. difficile* toxin. The patient who was already frail lost ~15 pounds over the next several months due to his diarrhea. During the case presentation, the patient said “*C. difficile* is one of the most terrible things that I’ve been dealing with in my whole life…” Considering this patient’s previous clinical history, the impact of CDI can be fully appreciated.

**Outcome:** The patient received several courses of antimicrobial chemotherapy, including a 6-8 week course of vancomycin, after which he relapsed (46). In fact, approximately 20% of patients treated with vancomycin or metronidazole can be expected to relapse (6). At some point he required additional antimicrobial agents for another urinary tract infection, which exacerbated his diarrhea. At the time of the case discussion, he was taking metronidazole plus rifamixin, which seemed to control his symptoms to a tolerable level. The senior physician commenting on the case suggested that if additional treatment is needed for this patient, he would suggest a tapering and pulsing vancomycin regimen, perhaps in association with a probiotic at the end of the antimicrobial therapy.

Given the increasing prevalence and severity of CDI, more accurate and rapid diagnostic methods are essential for optimum patient management and infection control. In our experience, nucleic acid amplification methods compare favorably to anaerobic toxigenic culture and are emerging as tests of choice.

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The next major strain contributing to the global increase of CDI is known as 027/NAP1/BI (toxinotype III). This strain has become one of the predominant causes of severe C. difficile disease across the United States and in other parts of the world (26, 45, 53, 54, 67, 70, 88). There are several reasons why this strain demonstrates a selective advantage in our healthcare environment.

First, the organism is resistant to fluoroquinolones (59). Fluoroquinolone use, both in hospitals and in community settings (such as for lower respiratory tract disease), has been implicated in outbreaks of C. difficile in several reports (15, 28). Fluoroquinolones may contribute to CDI by inducing increased spore and cytotoxin production (85). A recent report by Kallen et al. demonstrated a significant decline in C. difficile cases when a complete restriction of fluoroquinolones was initiated in a hospital after the failure of other interventions to reduce CDI cases (43). The 027/NAP1/BI strains also produce binary toxin, whose role in virulence, while under investigation, was associated

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New C. difficile strains

The name given to a strain of C. difficile often depends on the molecular typing method used for identification (see Table 1). Thus, the same strain can have multiple names. As many as seven typing systems are currently used for C. difficile isolates (48). The current epidemic strain is called 027/NAP1/BI because three different typing methods [PCR-ribotyping/pulsed-field gel electrophoresis (PFGE)/restriction endonuclease analysis (REA)] have been used to characterize it.

The changing epidemiology of CDI may well be related to the rise of new, epidemic strains that have spread rapidly across major geographic areas during the last decade. The first of these, called the “J” strain (now known as 001/NAP2/J), was initially recognized in 1999 (42). This novel strain of C. difficile was clindamycin-resistant and the receipt of clindamycin by patients for a variety of infectious diseases was identified as a key risk factor for healthcare–associated diarrhea.
with more severe disease in studies of CDI in Europe (32). Other studies point to increased spore production of the 027/NAP1/BI strain which, because it increases environmental contamination, probably facilitates the spread of the strain throughout hospitals. The role of the environment in *C. difficile* transmission has been noted in investigations of CDI in both Western Europe and the United States (3). A recent study by Dumford et al. demonstrated how broadly *C. difficile* spores are disseminated. During an outbreak of the 027/NAP1/BI strain in their hospital, *C. difficile* spores were detected on computer keyboards, nursing stations, and 31% of physician work areas (39). The 027/NAP1/BI strain also has a genetic deletion at base pair 117 in the *tcdC* gene, which is a regulator of toxin A and toxin B expression. This change likely results in higher levels of toxin production compared with toxinotype 0 (wild type) strains of *C. difficile* (99). The presence of the binary toxin genes (*cdtA* and *cdtB*) and the deletion of a single base pair in *tcdC*, when present together, serve as excellent markers of the 027/NAP1/BI strain (99). A recent case control study by Sundram et al. reported that early mortality from CDI was four times higher with ribotype 027 strains than with the next most common strain (ribotype 106) in their hospital. Ribotype 027 strains constituted 45% of the *C. difficile* isolates in their hospital (88).

Another emerging strain of *C. difficile* is the 078/NAP7/BK strain (toxinotype V), which has been associated with both food animals and humans in Europe (54, 84) and more recently in the United States (40, 84). This strain is also binary toxin positive. Several different strains of *C. difficile*, including 078, have been isolated from retail meats in Canada (84) and the United States, further complicating the epidemiology of CDI. However, to date there have been no outbreaks of CDI specifically linked to the consumption of food.

Molecular epidemiological methods depend on having the organism isolated in pure culture from the patient’s specimen. However, despite its reliability and sensitivity, culture for *C. difficile* is rarely performed now in the United States or in Europe, due to its slow turnaround time and technical difficulty (10).

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### Table 1. Common typing methods for characterizing *C. difficile* strains

<table>
<thead>
<tr>
<th>STRAIN TYPING METHOD</th>
<th>EXPLANATION</th>
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<tbody>
<tr>
<td><strong>Restriction endonuclease analysis (REA)</strong></td>
<td>Frequent cutting restriction enzymes that cleave DNA at specific nucleic acid sequence sites along the bacterial chromosome are used to subdivide the bacterial genome into hundreds of fragments. The fragments are separated by agarose gel electrophoresis using a continuous electric current in a single direction. Smaller DNA fragments migrate further down the gel than larger ones. The banding patterns formed by the DNA fragments from multiple isolates are recorded and compared for relatedness. Groups of patterns that appear to be highly related (indistinguishable or very similar patterns) are named starting with letters, or a combination of letters followed by numbers (examples are J35, BI, BI8, and A5).</td>
</tr>
<tr>
<td><strong>Pulsed field gel electrophoresis (PFGE)</strong> (See Figure 3)</td>
<td>Restriction endonucleases (enzymes) similar to those used for REA, but with far fewer binding sites in the bacterial chromosome, are used to cleave the bacterial genome into 10-20 DNA fragments. In order to separate these larger fragments, the organisms are first embedded in agarose plugs, lysed, and the DNA is cleaved in situ. The DNA fragments are separated in an agarose gel, but unlike REA, the electric current is pulsed in alternating directions to “wiggle” the very large fragments of DNA through the gel. The fragment patterns are much simpler to interpret than those of REA, although software programs are typically used to record and analyze the patterns. Clusters of <em>C. difficile</em> that are similar by PFGE are named for the region in which they were characterized (examples are North American PFGE type NAP1 and United Kingdom type UN9).</td>
</tr>
<tr>
<td><strong>PCR-Ribotyping</strong></td>
<td>Nucleic acid sequences from genes coding for ribosomal RNA of <em>C. difficile</em> are amplified by PCR and the amplified products are separated by agarose gel electrophoresis. The number of bands is typically between 5 and 8. Related organisms exhibit similar patterns and thus strain types can be differentiated. These groups are named with numbers only as in 001 and 027.</td>
</tr>
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Figure 3. PFGE patterns of four *C. difficile* isolates, Lane 1, *Salmonella brandei* standard; Lanes 2 and 3, *C. difficile* isolates with NAP1 profile; Lane 4 and 5, *C. difficile* isolates with NAP1 profile. Courtesy of Dr. Richard Goering, Creighton University, Omaha, Nebraska.
Laboratory diagnosis

For many years, the cell culture cytotoxin neutralization test was considered the gold standard for diagnosis of CDI. However, both older published results and the conclusions of newer studies have shown that this test lacks adequate sensitivity for detection of toxin-producing strains when compared with the revised gold standard, commonly referred to as toxigenic culture (anaerobic culture for *C. difficile* followed by detection of toxin B production by the isolate in cell culture cytotoxin assay) (77, 87). One of the first publications to recognize this was by Peterson et al. in 1988 (76). These investigators compared the results of EIA, cell culture cytotoxin neutralization, and culture for detection of *C. difficile* from stool samples. Compared with culture, the cytotoxin EIA was only 66.7% sensitive. A recent study again reiterated that a commercial cell culture cytotoxin neutralization assay was only 67% sensitive (87).

The sensitivity of a diagnostic method is based on the reference to which it is compared; thus, when a test or assay is compared with cell culture cytotoxin neutralization, it may appear to be very sensitive, but the data can be misleading due to the sensitivity of the “gold standard” (the cell cytotoxin assay versus toxigenic culture). This difference is demonstrated in Table 3, which compares various testing methods against both cytotoxicity assays and toxigenic culture assays. Most laboratories today perform the simple, rapid, EIA tests for toxins A and B, either in broth microdilution format or as a solid matrix immunoassay (20). Although toxin B is the determinant of pathogenicity (58), testing for both toxins can add sensitivity, due to the differential lability of toxins in feces.

As shown in Table 3, many EIA assays lack adequate sensitivity for the detection of *C. difficile* in stool. The perceived lack of sensitivity of *C. difficile* tests on the part of physicians has often resulted in the receipt of multiple stool samples from the same patient for testing. As noted by Peterson and Robicsek (77), Cardona and Rand (79), and Aichinger et al. (2), the utility of testing more than a single sample a week with a low sensitivity test is poor. In these instances, the likelihood of a false positive result in successive samples begins to exceed the likelihood of a true positive result. Thus, using a highly sensitive and specific test at the outset of testing yields the most reliable results (77).

Glutamate dehydrogenase (GDH) is a cell-associated enzyme antigen (protein) found on most isolates of *C. difficile* and other *Clostridium* species. It is relatively stable in the feces, and because of its apparent ubiquity on isolates of clostridia, has been proposed by several groups as a sensitive but non-specific screening test for *C. difficile* in stool samples (79, 89). The need for the GDH antigen step was to overcome the false negative EIA toxin assay results due to sample dilution prior to testing or to toxin degradation that is known to occur during fecal transport and storage. Due to its low specificity, the GDH test is often used as part of a two-step algorithm in which a positive GDH test result is followed by a more specific test, such as an EIA, cell culture, or a molecular assay which identifies toxin producing genes (31, 79, 87, 89). In a recent study, Gilligan reported that two commercial EIA tests were not sensitive enough for GDH confirmatory testing and suggested that cell culture cytotoxin neutralization testing, although slow (up to 48 hours) would be more appropriate for diagnosis (31). However, Novak and colleagues recently reported that the sensitivity of the GDH assay, when used as a primary screening tool for CDI, did not compare favorably with toxigenic culture (68). In fact, the only rapid tests that come close to the sensitivity of the slow, complex toxigenic culture for *C. difficile* are the real-time polymerase chain reaction-based assays (10, 68, 86, 87, 90). Because the genes encoding for *C. difficile* toxins are quite stable in feces and because the gene encoding toxin B is necessary for virulence, the toxin B gene (*tcdB*) has become the primary target for commercial *C. difficile* PCR tests. Recent reports suggest...
Table 2. Common methods for detection of *C. difficile* (48)

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>Overview</th>
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<tbody>
<tr>
<td>Toxigenic culture (Gold Standard)</td>
<td>Fresh stool is inoculated directly or is treated with heat or alcohol to enrich for spores before inoculation onto selective cycloserine cefoxitin fructose anaerobic agar (CCFA) plates, sometimes containing taurocholate to encourage spores to germinate. The stool also is inoculated into a tube containing an anaerobic broth, such as a chopped meat carbohydrate. If the initial plates fail to grow the organism, a sample of the broth culture is plated onto a second selective agar plate. Isolated colonies of <em>C. difficile</em> are grown in fresh anaerobic broth from which the supernatant is removed and tested for toxin B production, typically using a cell culture assay. Results may not be complete for up to a week.</td>
</tr>
<tr>
<td>Nucleic acid amplification tests</td>
<td>Polymerase chain reaction or another nucleic acid amplification method is used to detect the presence of toxin genes or regulatory genes in the bacterial chromosome. Various targets include the toxin B gene (<em>tcdB</em>), the regulatory locus (<em>tcdC</em>), and/or the binary toxin genes <em>cdtA</em> and <em>cdtB</em>.</td>
</tr>
<tr>
<td>Cell culture cytotoxin neutralization assay</td>
<td>Fresh stool (unformed) is diluted in buffer and centrifuged to pellet the solid materials. The supernatant is removed and added to a healthy monolayer of human or animal cells in culture, with and without an antitoxin specific for toxin B. The antitoxin is usually prepared with <em>C. sordellii</em> toxin, which also neutralizes toxin B of <em>C. difficile</em>. If the cultured cells show a cytopathic effect (CPE) (i.e., they round up and die) and the CPE is inhibited by antitoxin, this indicates the presence of toxin B and the stool is reported as positive for <em>C. difficile</em> toxin. Results are available from 24-72 hours after inoculation of the cells.</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (GDH) detection</td>
<td>GDH is a cell-surface protein of certain clostridial species (including <em>C. difficile</em>) that is more stable in stool than toxins A and B, and is a more sensitive marker of the presence of <em>C. difficile</em>. However, because it is present in other species and is not universally detectable in stools from patients with CDI, a confirmatory test is required for positive samples. The test is performed currently using a solid matrix or a lateral-flow type enzyme immunoassay.</td>
</tr>
<tr>
<td>Toxin A and B enzyme immunoassay (EIA)</td>
<td>Toxin antigens (usually both A and B) are detected using an antibody to capture the toxin and a second antibody bound to an enzyme to detect the first antigen-antibody complex. The reaction can be performed in microtiter plates or on a solid phase matrix, such as a membrane. More recent tests favor a lateral flow system where the specimen wicks across a membrane and the toxin antigen encounters the specific antibody at a certain point on the membrane. Once the toxin binds to the fixed enzyme-antibody complex, the substrate for the enzyme is added and the reaction yields a colored end product. Problems include instability of toxin in stool and lack of sensitivity. Additionally, some strains have mutations that reduce or eliminate toxin A production.</td>
</tr>
</tbody>
</table>
### Table 3. Product performance data for selected tests used for laboratory diagnosis of CDI when compared with cell culture cytotoxin neutralization (CCCN) and/or toxigenic culture (TC)

<table>
<thead>
<tr>
<th>TYPE AND NAME OF TEST</th>
<th>SENSITIVITY (%)</th>
<th>SPECIFICITY (%)</th>
<th>PREDICTIVE VALUE OF POSITIVE RESULT (%)</th>
<th>PREDICTIVE VALUE OF NEGATIVE RESULT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCCN</td>
<td>TC</td>
<td>CCCN</td>
<td>TC</td>
</tr>
<tr>
<td>Cell culture cytotoxin neutralization (10, 32, 87)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-house cytotoxin</td>
<td>76</td>
<td>100</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>TechLab®/Wampole</td>
<td>64-67</td>
<td>99</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>EIA for toxins (31, 64, 86, 90)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Meridian AB</td>
<td>96-99</td>
<td>48-58</td>
<td>94-97</td>
<td>95-98</td>
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<tr>
<td>TechLab® AB</td>
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<td>87-99</td>
<td>96</td>
<td>92</td>
</tr>
<tr>
<td>Xpect® Remel</td>
<td>96</td>
<td>48</td>
<td>99</td>
<td>84</td>
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<tr>
<td>Lateral-flow for toxins (31, 64, 80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TechLab®</td>
<td>36-80</td>
<td>99</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>Immunocard Meridian</td>
<td>96</td>
<td>48</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>GDH (31, 76, 80)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TechLab®</td>
<td>83-94</td>
<td>97</td>
<td>83-88</td>
<td>97-98</td>
</tr>
<tr>
<td>PCR* (10, 32, 68, 87)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ProGastro™</td>
<td>85-92</td>
<td>77</td>
<td>95-96</td>
<td>98</td>
</tr>
<tr>
<td>GeneOhm™</td>
<td>84-96</td>
<td>84-94</td>
<td>95-96</td>
<td>98</td>
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<tr>
<td>Xpert®</td>
<td>94</td>
<td>84-96</td>
<td>73-84</td>
<td>99</td>
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* Also referenced from Prodesse Product Insert and Cepheid clinical trial data

> When I realized that our physicians were not waiting for our laboratory results when we used the 2 day cytotoxicity tissue culture assay tissue to confirm positive GDH EIA results, but were resorting to performing colonoscopy procedures to confirm the diagnosis of *C. difficile* colitis, then the decision to change testing methods was easy. The cost-benefit ratio of having rapid result that delivers the best possible sensitivity and specificity which also can detect the more virulent 027/NAP1 strain is very attractive to our clinicians and they are interested in having this test as it will allow a rapid diagnosis resulting in faster treatment and implementation of infection control measures in addition to reducing the use of unnecessary testing such as colonoscopy. A rapid negative result allows the clinician to pursue testing for other diagnoses, saves the patient from having to take unnecessary antibiotics and the hospital the costs of isolation.

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Pregnancy and C. difficile

Case Study #2

A case reported a few years ago in Morbidity and Mortality Weekly Reports is illustrative of the emerging, non-healthcare facility related problems associated with Clostridium difficile infection (CDI), a major cause of healthcare-associated diarrhea worldwide (18).

Presentation: A 31-year old woman, 14 weeks pregnant with twins, went to a local emergency department after three weeks of intermittent diarrhea, followed by three days of cramping and watery, black stools 4-5 times per day. Her stool specimens were positive when tested for C. difficile toxin and the patient was admitted to the hospital. Her only antimicrobial exposure during the preceding year was a prescription for trimethoprim-sulfamethoxazole (for a urinary tract infection) approximately three months before admission.

Outcome: She was treated with metronidazole and discharged but was readmitted the next day when her symptoms worsened. She remained hospitalized for 18 days with severe colitis, during which time she received metronidazole, cholestyramine, and oral vancomycin. She improved on vancomycin and was allowed to return home. However, four days later she was readmitted with diarrhea and hypotension. She spontaneously aborted her fetuses. Despite aggressive treatment including a subtotal colectomy, intubation, and inotropic medication to stimulate the heart, the patient died on the third hospital day.
Therapy

Once a patient has been diagnosed with CDI, two simultaneous actions should occur: the patient should be treated for the disease and interventions to prevent spread of the organism should be initiated by the infection control service.

The first step in CDI therapy is to discontinue the antimicrobial therapy that led to the disease, if possible. Supportive therapy, including rehydration, is also critical. In considering therapeutic intervention, in vitro studies have reported that most non–027 C. difficile isolates are susceptible to fluoroquinolones, glycopeptides, lincosamides, macrolides, metronidazole, and rifampin (56) although resistance to macrolides, clindamycin, fluoroquinolones, and rifampin has been reported, especially among 027 isolates for the latter two drugs (22, 42, 59). Reduced susceptibility to metronidazole also has been reported but the phenotype is unique to ribotype 001 strains (8, 49). Heteroresistance to metronidazole also has been reported, but has not, to date, been supported by population analysis data (10). Vancomycin resistance has not been documented in C. difficile isolates.

CDI is typically treated with either metronidazole (500 mg, 4 times daily) or vancomycin (125 mg, 4 times daily). There is a potential cost benefit to using metronidazole, which on average, is reported to cost $2/day versus $70/day for vancomycin, although other factors, including decreased length of stay with vancomycin therapy, may mitigate the higher cost of vancomycin (19). Vancomycin has a Food and Drug Administration indication for CDI, while metronidazole does not, although this is not considered a barrier to its usage. Bartlett has made the case for using vancomycin as the preferred agent for treating severe C. difficile disease, defined as the presence of pseudomembranes, or at least two of the following conditions: age >60 years, serum albumin level <2.5 mg/mL, peripheral leukocyte count >15000 cells/mL, and temperature >38.3°C (19). The relapse rates for the two antimicrobial agents in mild to moderate disease are similar. However, data from prospective clinical trials favor vancomycin use (49, 99). In addition, McFarland et al. have reported that tapered vancomycin therapy or pulsed dosing regimens of vancomycin may produce significantly improved responses for recurrent C. difficile disease (69). Earlier reports that ribotype 027 outbreaks and infections were associated with higher relapse rates (92) may in fact have been due to reinfection rather than treatment failure (74). The possibility that vancomycin use will encourage the development and spread of vancomycin–resistant enterococci is an important consideration in many medical centers, which still favor the use of metronidazole. There are new data that the 027/NAP1/BI strain is intrinsically more difficult to treat with fidaxomicin and vancomycin than other C. difficile strains (65). Additional in vitro data suggest that the ribotype 001 strains are less susceptible to metronidazole. Spiral gradient MICs of 4.16 mg/L for ribotype 001 isolates versus MICs of 0.8–1.03 mg/L for ribotypes 027 and 106, and 0.21 mg/L for other ribotypes were recently reported (86). Some reports of rifampicin/rifamixin resistance appearing preferentially in 027 strains have now surfaced; if such resistance is confirmed and found to be widespread, this could have treatment implications since rifamixin is one option used to treat recurrent infections (86).

Other antimicrobial agents that show activity against C. difficile isolates in vitro include oritavancin (9, 30), fidaxomicin (also known as OPT–80) (90) and nitazoxanide (69). Nitazoxanide was evaluated in a prospective, randomized, double blind, clinical trial by Musher et al. and compared favorably to metronidazole both in resolution of symptoms and the proportion of patients who had a sustained response at 31 days (69). Fidaxomicin showed encouraging results as a treatment for CDI in a phase 2 clinical trial (69). More recently, in a Phase 3 randomized, blinded, prospective trial, fidaxomicin showed a major reduction in recurrence rates for all strains except the 027/NAP1/BI strain when compared to vancomycin (90). Although oritavancin has been evaluated in a human gut model system, clinical trials of the agent have yet to be reported.

Several non–antimicrobial strategies have been used to treat CDI including probiotics, toxin binding agents, and immunotherapeutics (68). The goal of non–antimicrobial strategies is to spare or replenish normal bowel flora to exclude C. difficile strains. As noted by Pillai et al., there is insufficient evidence, at this time, that probiotics aid in the treatment of CDI (78). The toxin binding resin tolventamer, which was evaluated in both the United States and Europe, also proved to be inferior to standard antimicrobial therapy for bringing about resolution of symptoms (68). A more unusual approach to restoring bowel flora, using fecal transplants from healthy donors to re-establish colonic flora in C. difficile patients, has shown success in some patients in reducing symptoms and reducing recurrences, but controlled trials have yet to be completed (6).
There is no single, universally effective intervention to prevent spread of \textit{C. difficile} once it has become established in an institution \cite{62}. In fact, recent data strongly suggest that multiple interventions (bundles) are necessary to interrupt transmission \cite{43, 67}. The Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America have issued joint practice recommendations on strategies for control of \textit{C. difficile} \cite{46}. Surveillance and effective diagnostic testing are at the top of the list of actions recommended. The authors recommend that only diarrheal stools be tested (stool that takes the shape of its container) and that children less than one year of age not be tested. This is because children may harbor a toxigenic strain of \textit{C. difficile} asymptptomatically for up to one year after birth. The reason for this seeming paradox is unknown. Once identified by a positive laboratory test, patients should be placed on contact precautions. Cohorting of appropriate patients is also suggested. Beyond the individual patient, a structured prevention program should be initiated, which includes education, emphasis on hand washing, and proper disinfection and cleaning of the environment \cite{59}. In some cases, hospital–wide antimicrobial restrictions may be necessary \cite{66}. This is particularly important for containment of the 027/NAP1/BI strain of \textit{C. difficile} \cite{43, 66}. In fact, multiple interventions still may not stop an ongoing outbreak of this highly successful strain.

In a report of a longstanding \textit{C. difficile} outbreak from a Pennsylvania medical center, both an antimicrobial stewardship program, specifically restricting the use of fluoroquinolones, and a change in the environmental cleaning company were required to control an outbreak, which had persisted despite multiple infection control interventions (see Figure 4) \cite{43}.

Cleaning the hospital environment is critical to controlling \textit{C. difficile} outbreaks. Housekeeping personnel need to be monitored to insure that they follow room cleaning protocols explicitly \cite{43}. Most hospital cleaning solutions do not kill the spores of \textit{C. difficile}. The best approach currently is to use 10% bleach or other hypochlorite solutions \cite{53}, although these liquids can corrode metal and other surfaces and the resulting fumes are quite noxious. Given the liquid nature and high volumes of the diarrhea in most \textit{C. difficile}–infected patients, it is also difficult to find all the reservoirs in the patients’ rooms. The tiny indentations of textured bedrails, cracks in telephones, and other hiding places are perfect areas for spores to persevere even after thorough cleaning \cite{54}. In fact, not surprisingly, one study found that a dog used in a pet therapy program acquired \textit{C. difficile} in his fur after a visit to the hospital \cite{52, 77}.

One novel environmental disinfection method reported by Boyce and colleagues \cite{66}, uses dry aerosolized hydrogen peroxide to reduce viable spores. Some authorities are also advocating changing the materials on high–touch surfaces, such as bedrails, to something less hospitable to spores and vegetative organism survival, such as copper. It is clear that multiple and compounded interventions will become the norm as this ubiquitous pathogen extends its reach.
Summary

_Clostridium difficile_ has evolved from an uncommon healthcare-associated pathogen that primarily affected elderly patients, to a global healthcare concern affecting not only the elderly, but otherwise healthy young adults and children both in healthcare and community settings. Factors contributing to this dramatic increase include widespread use of broad-spectrum antimicrobials and increased resistance of _C. difficile_ isolates to many antimicrobial agents, which enhances their spread in communities and institutions. Control of outbreaks of CDI, especially those caused by the 027/NAP1/BI strain and other emerging epidemic strains, is difficult and requires significant infection control efforts and judicious antimicrobial stewardship. The availability of rapid and accurate _C. difficile_ tests represents an important advancement in addressing this growing epidemic. Immediate identification of CDI enables clinicians to administer appropriate therapy sooner, and supports immediate and prompt infection control measures to reduce the risk of this dangerous infectious agent spreading within an institution.

"Clostridium difficile continues to be a major opponent in the battle of healthcare-associated infections. With the emergence of the BI (027) outbreak strain, rates significantly increased in Pittsburgh and across the world. Today, in some settings, as many as 5% of patients admitted to hospitals contract _C. difficile_. Preventing acquisition and spread is key to control. Spread can be significantly reduced when interventions like hand hygiene, gowns, gloves, and bleach cleaning are consistently utilized. This can only be achieved when patients with _C. difficile_ are quickly and accurately identified. Historically _C. difficile_ testing methods were either insensitive or labor intense with long turn around time. Rapid test results can also help to quickly identify a jump in rates and potential outbreaks. This is important so that additional interventions can be considered."

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