Hot Topic

Laboratory Diagnosis of Clostridium difficile: Why So Difficult?
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Disclosures

- None

I have no relevant disclosures.
Let's start with some test utilization messages. As you view this presentation, consider the following important points regarding laboratory testing for *Clostridium difficile*.

- Various testing methodologies are available,
- Laboratories may use more than one testing platform in reflexive or algorithmic approaches in order to reach a diagnosis.
Burden of *Clostridium difficile* Disease

- *Clostridium difficile* is the most commonly reported pathogen causing healthcare-associated infections in US hospitals\(^1\)
  - Mandated reporting of *C difficile* infections (CDI) in United States
- Accurate and rapid diagnosis of *C difficile* infection (CDI)
  - Treat with appropriate antimicrobial agent
  - Discontinue the precipitating antimicrobial agent
  - Institute infection control precautions

*Clostridium difficile* has received a lot of attention in the last 2 decades due to the increasing burden it has been placing on our patients and our healthcare system. *C difficile* is the most commonly reported pathogen causing healthcare-associated infections in US hospitals, recently surpassing methicillin-resistant *Staphylococcus aureus*, or MRSA. Since 2013, the National Healthcare Safety Network (NHSN) has mandated reporting of *C difficile* infections (CDI) for hospitals participating in the Centers for Medicare and Medicaid Services (CMS) program.

Accurate and rapid diagnosis of CDI is important for the patient and the healthcare environment. Upon diagnosis, providers begin therapy with an appropriate antimicrobial agent such as metronidazole or oral vancomycin and discontinue antimicrobial agents that may be predisposing to CDI. Infection control precautions are instituted in order to curb spread of the spores.
C difficle disease presents with a range of clinical findings from diarrhea to pseudomembranous colitis to toxic megacolon. Pictured are pseudomembranes in the colon as seen on flexible sigmoidoscopy in a patient with CDI. Pseudomembranes are adherent thick layers of inflammatory cells and mucus.

The most significant risk factor for CDI is antibiotic exposure. Although clindamycin, broad-spectrum cephalosporins, and ampicillin have most often been implicated in CDI, any antibiotic can cause this. When a patient takes antibiotics, beneficial bacteria in the intestine are destroyed or impaired for a period of time, increasing the likelihood that C difficle can lead to infection.
**Pathophysiology**

- Spore-forming Gram-positive rod, obligate anaerobe
- Originally named *Bacillus difficilis*
- Spores are ubiquitous!
  - Spread by fecal-oral route
  - Present on environmental surfaces and on hands of caregivers
  - Resistant to alcohol gels and many hospital disinfectants
  - Persist on inanimate surfaces for several months

*C difficile* is an obligate anaerobe that is a spore-forming Gram-positive rod. In the 1930s, it was originally named *Bacillus difficilis* due to difficulty isolating this bacterium in the laboratory.

*C difficile* spores are ubiquitous in the natural environment including seawater, rivers, and soil. The organism is spread by the fecal-oral route. When spores are ingested from the environment, they can then germinate in the intestine and produce toxins. In hospitals, spores are present on many environmental surfaces such as commodes and bed curtains and on the hands of caregivers. They have even been found in pet animals, but there has been no evidence to date of zoonotic transmission from animals to humans.

The spores are resistant to alcohol gels and many hospital disinfectants and can persist on inanimate surfaces for several months if inadequately cleaned.
The epidemiology of *C. difficile* has been changing since 2000, with a rise in incidence and severity.

One factor associated with this evolving epidemiology is recognition of the NAP1/BI/027 strain, also referred to as the hypervirulent strain. NAP1 stands for North American pulsed-field gel electrophoresis type-1 and 027 refers to PCR ribotype number. Compared to non-NAP1 strains, this strain has been more often associated with severe disease, has shown higher rates of fluoroquinolone resistance, and produces more toxins A and B. In addition, NAP-1 strains were found to produce binary toxin. Although fluoroquinolone resistance does not affect management of CDI, because this class of antimicrobials is not used for CDI treatment, resistance to fluoroquinolones may provide the NAP1 strain with a survival advantage over susceptible strains in healthcare facilities where these antibiotics are commonly used. However, non-NAP1 strains have also been associated with high severity and production of binary toxin.

*C. difficile* has also been increasingly reported outside of acute care facilities in nursing homes and community home settings.
There are several *C difficile* toxins. Toxin A (encoded by the *tcdA* gene) is an enterotoxin that causes fluid accumulation in the bowel. Toxin B (encoded by the *tcdB* gene) is cytopathic to (causes distortion of) cells when cultured in the laboratory. Genes encoding for toxins A and B are present on the pathogenicity locus. The *tcdC* gene regulates toxin A and B production.

Genes *cdtA* and *cdtB* are located at an unknown distance from the pathogenicity locus and encode the binary toxin.
Asymptomatic carriage or colonization can occur with nontoxigenic, or nontoxin-producing, strains as well as toxigenic strains. This colonization state complicates the clinical diagnosis of infection due to *C difficile*.

Colonization with nontoxigenic strains ranges from 0.4% to 6.9% of adults (2% of nonhospitalized adults are colonized). Infant intestinal cells do not appear to have receptors for toxins A and B; therefore, a much higher percentage of neonates may have detectable *C difficile* in their stools, but do not manifest with disease.

Therefore, only unformed stools should be tested when assessing infection due to *C difficile*. Dr. Stephen Brecher coined CDI submission guidelines for stools shown in the table. Formed stools may be tested, however, in cases of ileus or toxic megacolon when stool is often not passed.
Enzyme immunoassays, or EIAs, may detect either toxins A/B and/or glutamate dehydrogenase (GDH).

Toxin A/B EIAs are rapid tests and take minutes to perform. However, toxin EIAs are somewhat less sensitive than other methods in diagnosing infection.

GDH EIAs detect the enzyme that is produced by both toxigenic and nontoxigenic strains of *C difficile*. GDH is produced at much higher levels than toxins A and B. Advantages of the GDH test include rapidity, and recent metanalyses have shown high sensitivity for these assays. The disadvantage is that this test cannot be used as a stand-alone test for CDI. A confirmatory test for the presence of toxin is needed. These assays have been used in algorithmic approaches to diagnosis in combinations of testing involving toxin EIA, molecular assays, or cytotoxicity assays.

An EIA is also available that simultaneously detects *C difficile* GDH, and toxins A and B, and the sensitivity of this assay is high.
Culture is a highly sensitive method of recovering the organism when selective culture media is used. Recovery of strains allows for further molecular typing studies (such as for comparison of relatedness of strains) or for antimicrobial susceptibility testing when indicated.

However, nontoxigenic strains can be recovered in culture, so further testing is required to confirm toxigenicity. Time to results is typically 24 to 48 hours. The chromogenic medium CHROMagar C difficile (bioMérieux) is utilized by Mayo Clinic Rochester for culture upon request, which is a recent addition to our testing capabilities. C difficile colonies will grow on this media and will fluoresce under ultraviolet light, which is pictured. Other bacteria will be inhibited from growing or will not fluoresce.
Nucleic acid amplification tests (NAATs) are molecular-based assays that detect the genes encoding for the toxins rather than the toxins themselves. Various NAATs are available to laboratories. At Mayo Clinic Rochester, *C. difficile* PCR targeting the *tcdC* gene is used. NAATs are more sensitive than enzyme immunoassays.
Fidaxomicin

- Macrolide antimicrobial agent
- Approved for treatment of CDI
- Bactericidal
- Oral administration leads to high fecal concentrations
- Mayo Clinic offers metronidazole and vancomycin susceptibility testing for *C difficile* from intestinal sources

A macrolide agent, fidaxomicin, was FDA-approved in May 2011 for the treatment of CDI. It was the second agent after vancomycin to be approved by the FDA for CDI.

It is bactericidal, and oral administration leads to high fecal concentrations that exceed the minimum inhibitory concentrations.

Mayo Clinic offers metronidazole and vancomycin susceptibility testing for *C difficile* from intestinal sources.
We'll wrap up this talk with general testing guidelines.

Repeat testing for use as a test of cure is not acceptable. Toxins can be detected in stool as long as 30 days after resolution of symptoms.

Formed stools should not be tested when assessing for *C difficile* infection. Testing should not be performed on children under 1 year of age.

Laboratories may use more than one testing platform in reflexive or algorithmic approaches when assessing *C difficile* diagnosis.
References


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