Developments in Diagnosing the Most Common Hospital-Acquired Infection: C. difficile

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Clostridium difficile (also known as C. diff or CDF) is a gram-positive anaerobic bacterium. It exists in both a bacterial form (also known as a vegetative form) and in spore form. The spores can mature into the bacterial form in the human colon. Once in the bacterial form, some species of C. diff secrete potent enterotoxins which damage the colonic mucosa and cause a secretory diarrhea. Most cases of CDF occur in individuals using antibiotics, where the normal colon flora have been destroyed and C. diff has a survival advantage, says Frank Friedenberg, MD, MS (Epi), professor of medicine, Temple University Hospital, Philadelphia, PA.

The most common symptoms of C. difficile infection (CDI) are watery diarrhea (grossly bloody stools are rare), abdominal pain, nausea, loss of appetite, cramping and fever. “Ironically, some of the most severe CDI cases will present with ileus, with a distended, tender abdomen, low blood pressure and ‘toxic’ appearance, but no diarrhea,” says Stuart Johnson, MD, deputy ACOS for research, Hines VA Hospital, and professor, Loyola Stritch School of Medicine, Chicago, IL. The peripheral white blood cell count is usually quite high and a radiographic picture of the abdomen may show marked bowel dilation and/or thickening.

The toxins may cause paralysis of the nerves and muscles of the colon leading to massive dilation of the bowel, i.e., toxic megacolon. This presents as severe abdominal pain and distention. The patient may become unstable and require urgent surgery to survive, says Dr. Friedenberg.

“The spectrum of disease runs the gamut from asymptomatic colonization to death in three days,” summarizes Stephen M. Brecher, PhD, director of microbiology, VA Boston Healthcare System, West Roxbury, MA, and assistant professor of pathology, Boston University School of Medicine, Boston, MA.

Rates Increase, Including Those Without Common Risk Factors

Rates of CDI across the United States increased dramatically between 2000 and 2001. This increase has coincided with the spread of an epidemic strain of C. difficile, referred to as BI/NAP1/027 by three of the most common typing systems used for epidemiological studies of C. difficile. In addition to the increase in rates of CDI, Dr. Johnson says there has been an increase in the number of severe cases and deaths. This phenomenon was most dramatically reported for the multiple hospital outbreaks in Montreal, Quebec, Canada, where it was estimated that more than 2,000 people died between 2003 and 2004 directly as a result of CDI due the epidemic BI/NAP1/027 strain.

There is some suggestion that the rates of CDI may have leveled off in the last several years. However, this disease is still the most common hospital-acquired infection across the United States, causing more incident cases than methicillin-resistant S. aureus (MRSA). The other aspect of C. difficile epidemiology that has received recent attention is the recognition of CDI cases that arise in the community, not in the hospital or other long-term care facility. Many of these “community onset” CDI cases can be attributed to recent hospitalization, but Dr. Johnson says there are clear-cut community-associated CDI cases without any link to hospitals or other chronic care-facilities.

“Anecdotally, many of the community-associated cases that I see are precipitated by patients given clindamycin for dental infections or procedures,” Dr. Johnson says. C. difficile forms hardy spores outside of anaerobic environments such as the colon, and it has been recovered from multiple environmental reservoirs. However, hospitals and long-term care facilities provide the best opportu-
nity for susceptible patients, *e.g.*, elderly patients treated with antibiotics, to come into frequent contact with *C. difficile* spores that are shed by other patients with CDI. The major public health problem of CDI is one of nosocomial (hospital-acquired) infections.

Although the major risk factors for obtaining CDI are hospitalization, advanced age and use of antibiotics (which render a patient susceptible through effects of these agents on the “normal” resident bacterial flora of the colon which provide an important line of defense for the host [colonization resistance]), CDI has occurred in other populations that weren’t considered at risk in the past. These include young, peripartum women, children (usually age 2 and older), non-hospitalized patients (particularly those on high-risk antibiotics such as clindamycin) and rarely, patients who have not taken antibiotics. Presumably patients who develop CDI without a history of antibiotics have been exposed to some other agent, *e.g.*, a chemotherapy agent, that disrupts the normal bowel flora or have abnormal gut flora by virtue of some other means, *e.g.*, inflammatory bowel disease. Of considerable controversy is the potential of proton pump inhibitor agents to precipitate CDI. If they are a risk, that risk is much less a risk than antibiotic agents, says Dr. Johnson.

According to Dr. Bercher, the antibiotic metronidazole is the typical treatment for a mild case of *C. diff*. Historically, this treatment had a 4 to 5 percent failure rate, but that has increased to a 10 to 15 percent failure rate and a 20 percent relapse rate. For a more severe infection, the treatment of choice is oral Vancomycin which is more predictively effective. Recurrences, however, occur at a similar rate with vancomycin as with metronidazole. If one recurrence occurs, the rate of a second recurrence increases to 45 percent and the rate of a third recurrence is 65 percent.

**Diagnosing CDI**

The historic laboratory test for diagnosis of CDI was the cell cytotoxin assay that relied on identification of cytopathic effects of stool specimens on a cultured eukaryotic cell line, such as fibroblasts or CHO cells. The specificity of the test was demonstrated by neutralization of the toxic effect by specific antisera, explains Dr. Johnson.

Currently, most clinical laboratories have switched to an enzyme immunoassay kit to detect both major *C. difficile* toxins, toxin A and toxin B. The first generation of immunoassays detected only toxin A, but naturally-occurring strains that were toxin A-negative, B-positive were missed by these assays and, contrary to original assumptions, these strains were fully capable of causing clinical disease. The major limitation of the toxin immunoassays are their relative insensitivity, which may range from 60 to 80 percent or lower in some instances, Dr. Johnson says.

A separate enzyme immunoassay has been developed to identify the presence of glutamate dehydrogenase in the stool which is also a marker of toxigenic *C. diff*. Unfortunately, this antigen is common to many colonic bacteria and, therefore, this test is non-specific, Dr. Friedenberg says.

The clinical laboratory provides a very important role in confirming the presence of *C. difficile* toxin in the stool or the presence of a toxin-producing *C. difficile* organism in a symptomatic patient. “The optimally sensitive test, culture and toxin testing on the recovered isolate is probably not practical in the majority of clinical labs. Therefore, the relative insensitivity of the tests offered has to be considered by the clinicians ordering the test,” Dr. Johnson says. This lack of sensitivity by the toxin immunoassays is not overcome by repeated testing and there are no data that support “stool toxin test x 3” to rule out the diagnosis of CDI.

Another helpful step by the laboratory is to limit stool *C. difficile* testing to liquid or soft stool specimens, *e.g.*, specimens that take the shape of the container, Dr. Johnson says. There is no role for “test of cure” assays or testing patients who do not have clinical symptoms consistent with CDI. This requirement may be particularly important if the laboratory decides to use one of the recently available polymerase chain reaction (PCR) assays for *C. difficile*.

Growth of *C. difficile* in the laboratory requires inoculation of the stool specimen on selective agar media and incubation under strict anaerobic conditions. With experience, most clinical laboratories are able to perform culture for *C. difficile*, but because of the investment in anaerobic hoods, media, turnaround time for reporting results and the fact that many patients in a hospitalized setting may be colonized without symptoms, most clinical laboratories have relied on detection of *C. difficile* toxin in clinical specimens, Dr. Johnson reports.

**Expected Advances in Detection**

Several PCR assays have now been approved for use in the clinical lab and all of them target a portion of the toxin B gene at a minimum. According to Dr. Bercher, these include Franklin Lakes, NJ-based Becton, Dickinson and Company’s GeneOhm *Cdiff*
These assays appear to overcome much of the sensitivity problems of the toxin immunoassays and retain specificity. It should be remembered, however, that these tests detect the presence of a toxin gene-carrying strain of *C. difficile*, *e.g.*, pathogenic strain. The problem of PCR testing on patients who do not meet a clinical definition of CDI, *e.g.*, no diarrhea, will likely be magnified considering the background of hospitalized patients who may be colonized with *C. difficile*, but don’t have CDI, Dr. Johnson says.

If shown to be highly accurate, Dr. Friedenberg predicts that PCR-based testing will replace enzyme immunoassay testing. Quality control, prevention of surface and equipment contamination will be of critical importance to labs performing these studies.
1. **Clostridium difficile (C. difficile)** is a gram-positive anaerobic bacterium that exists in both bacterial and spore forms.
   - A. True
   - B. False

2. Which is not a common symptom of *C. difficile* infection (CDI)?
   - A. grossly bloody stools
   - B. abdominal pain
   - C. nausea
   - D. loss of appetite

3. Regarding *C. difficile*, the spectrum of disease runs the gamut from asymptomatic colonization to death in five days.
   - A. True
   - B. False

4. Which statement is false regarding *C. difficile* and its increasing rates?
   - B. An increase in the early 2000s coincided with the spread of an epidemic strain of *C. difficile*, referred to as BI/NAP1/027 by three of the most common typing systems used for epidemiological studies of *C. difficile*.
   - C. There has been an increase in the number of severe cases and deaths.
   - D. During multiple hospital outbreaks in Montreal, Quebec, Canada, it was estimated that more than 4,000 people died between 2004 and 2005 directly as a result of CDI due to the epidemic BI/NAP1/027 strain.

5. Although there is some suggestion that the rates of CDI may have leveled off in the last several years, CDI is still the most common hospital-acquired infection across the United States, causing more incident cases than methicillin-resistant *S. aureus* (MRSA).
   - A. True
   - B. False

6. Specific populations that were not considered at risk for CDI in the past include:
   - A. Hospitalized patients
   - B. Elderly persons
   - C. Individuals who use antibiotics
   - D. Children under age 2

7. The historic laboratory test for diagnosis of CDI was the cell cytotoxin assay; however, most clinical laboratories have switched to an enzyme immunoassay kit to detect both major *C. difficile* toxins, toxin A and toxin B.
   - A. True
   - B. False

8. Ideally, laboratories should limit stool *C. difficile* testing to firm stool specimens.
   - A. True
   - B. False

9. Several polymerase chain reaction (PCR) assays have now been approved for use in the clinical lab and all of them target a portion of the toxin B gene at a minimum.
   - A. True
   - B. False

10. Which statement is false regarding PCR assays?
    - A. Current available PCR assays include Becton, Dickinson and Company’s GeneOhm Cdiff assay; Prodesse’s ProGastro Cd assay; and Cepheid’s Xpert® *C. difficile* test which runs on the GeneXpert® System
    - B. The assays appear to overcome much of the sensitivity problems of the toxin immunoassays and retain specificity.
    - C. These tests detect the presence of a toxin gene carrying strain of *C. difficile*, e.g., pathogenic strain.
    - D. None of these statements is false.