EDUCATIONAL COMMENTARY - CLOSTRIDIUM DIFFICILE

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Learning Outcomes

Upon completion of this exercise, the participant should be able to:

- explain the difference between colonization and infection with *Clostridium difficile*.
- discuss three recent changes in the epidemiology of *C. difficile*–associated disease.
- discuss the advantages and limitations of methods used to diagnose *C. difficile* infection.

First recognized in 1978, *C. difficile* is a common nosocomial pathogen and a frequent cause of diarrhea in patients undergoing therapy with antibiotics or anti-cancer drugs. *C. difficile*–associated disease (CDAD) is usually mild, with non-bloody, watery diarrhea, sometimes accompanied by abdominal cramping. However, severe disease resulting in complications such as toxic mega-colon, paralytic ileus, sepsis, and death may occur.

Strains of *C. difficile* may be either pathogenic or nonpathogenic. Pathogenic strains produce exotoxins that are termed ‘toxin A’ and ‘toxin B’. Some pathogenic strains produce both toxins, whereas others produce only toxin A or toxin B. Nonpathogenic strains of *C. difficile* do not produce toxin A or toxin B.

Epidemiology

Toxin-producing strains of *C. difficile* do not always cause disease. Persons who harbor the organism, but have no symptoms, are said to be colonized. Persons who have symptoms are said to be infected. Up to 70% of infants may be colonized with *C. difficile*, but rates of colonization quickly decrease until two years of age, to about 3% (the same as in adults). Despite the higher rate of colonization in young children, the incidence of disease in children is lower than in adults. Risk factors for developing CDAD include: antibiotic therapy, anti-cancer drugs, possibly proton-pump inhibitors, gastrointestinal surgery, long stays in a healthcare environment, serious illness, a compromised immune system, and advanced age.

In recent years, researchers have noted three changes in the epidemiology of CDAD. First, the frequency of CDAD is increasing, and the incidence of community-associated *C. difficile* infection may be higher than previously thought. In a recent survey of data from six hospitals in North Carolina, Kutty and colleagues found that 20% of all cases of *C. difficile* infection were community acquired. Studies in Sweden, the United Kingdom, and Canada have yielded similar results. Second, the severity of the disease is increasing, a development thought to be due to the emergence of a new strain of *C. difficile*
that produces 16 times more toxin A and 23 times more toxin B than other strains. Third, CDAD is becoming more difficult to treat because some strains are now resistant to metronidazole, the drug of choice for treating mild disease.

**Laboratory Diagnosis**

*C. difficile* should always be suspected as the cause of diarrhea in an adult with a history of antibiotic therapy, even if the diarrhea occurs several months after treatment ends. Also, only watery stools should be tested for the organism. This is because a positive result from a normal stool sample only indicates that the person is colonized. It does not prove that the person is infected. In the laboratory, methods used to detect *C. difficile* fall into three categories: anaerobic stool culture, assays detecting *C. difficile* toxins, and assays detecting *C. difficile* antigen.

**Culture**

Anaerobic culture is the most sensitive test for detecting *C. difficile*. It is the method least used, however, because culture is labor-intensive, costly, and has a long turnaround time of 48 to 96 hours. Also, culture alone cannot determine whether a particular *C. difficile* isolate is a pathogen. For this, an assay to detect toxins in the stool sample or the isolate must be used. Culture for *C. difficile* is most useful in investigating outbreaks, because isolates can be used for molecular typing.

Specimens submitted for culture should be processed within two hours of collection. Although spores can survive refrigeration for several days, the number of viable cells will likely decrease substantially. The specimen should be plated onto cycloserine-cefoxitin-fructose agar (CCFA) and incubated anaerobically at 35°C to 37°C, for 18 to 24 hours. On CCFA, colonies of *C. difficile* fluoresce chartreuse. When colonies are examined under a dissecting microscope, they appear flat, creamy yellow to grayish-white with a ground-glass texture, and have a characteristic odor of horse manure.

**Assays for Toxin**

Assays that detect *C. difficile* toxins include enzyme-linked immunosorbent assays and immunochromatographic assays. These tests are easy to perform, and they can provide results in two hours or less. However, they vary in sensitivity. In general, the enzyme-linked immunosorbent assays are more sensitive than the immunochromatographic assays, with reported sensitivities of 80% to 95% and 60% to 85%, respectively. These assays also vary in the toxins they detect. Some assays detect both toxin A and toxin B, but others detect only toxin A, or only toxin B. Also, because the toxins degrade rapidly, specimens should be tested within two hours of collection. If the assay cannot be performed immediately, the specimen can be stored at 4°C for up to three days. If testing will be delayed longer than three days, store the specimen at –70°C.
Assays for Antigen

Assays for C. difficile antigen include latex agglutination tests and membrane enzyme immunoassays to detect glutamate dehydrogenase (GDH), an antigen found in all C. difficile strains. Antigen detection methods are useful for screening because they have a high negative predictive value. They are easy to perform and have the shortest turnaround time of usually less than one hour. However, these methods cannot distinguish toxin-producing strains of C. difficile from non–toxin-producing strains. They also cannot differentiate among strains that produce only toxin A, only toxin B, or both toxins A and B. For this reason, specimens that test positive for GDH must subsequently be tested for toxins A and B. Finally, antigen detection methods can cross-react with other anaerobes, thus causing a false-positive result.

In addition to the methods discussed, two other less-often-used methods can detect C. difficile toxin. Tissue cytotoxic assays are considered the “gold standard” because they have the highest sensitivity (94%-100%) and specificity (99%) of all tests that detect toxin B. These assays, however, are costly and difficult to perform, and the turnaround time is 24 to 48 hours. Recently developed molecular methods detect C. difficile toxin genes directly in stool specimens. Until recently, molecular methods were used almost exclusively in research laboratories, but they are now increasingly used in clinical laboratories due to test kits that have been developed. The PCR method (polymerase chain reaction), a specific type of molecular method, is highly sensitive (94%-98%) and specific (91%-96%), and can yield results in as little as one hour. In addition, molecular methods may detect but not differentiate the NAP1 strain (which produces high levels of toxin) from other toxigenic C. difficile strains.

Conclusion

C. difficile is an important pathogen for persons undergoing antibiotic therapy, and for those who are admitted to a healthcare environment for an extended period of time. Some strains have recently become more virulent and more difficult to treat, and the incidence of community-acquired CDAD may be underestimated. For these reasons, it is important for laboratories to develop testing protocols that distinguish between colonization and infection, and choose test methods that optimize detection of pathogenic strains.

Suggested Reading


EDUCATIONAL COMMENTARY - CLOSTRIDIUM DIFFICILE (cont.)


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