Case 26

The patient was an 80-year-old female who 10 days previously had had a cystocele repair performed. At the time of that hospital admission, a urine culture was obtained that revealed >100,000 CFU/ml of an *Escherichia coli* strain that was susceptible to all antimicrobial agents against which it was tested. Postoperatively, she began a 7-day course of oral cephalexin. She was discharged after an uneventful postoperative course of 3 days. Ten days postoperatively, she presented with a 3-day history of diarrhea. The patient noted multiple, watery loose stools without blood, crampy abdominal pain, and vomiting. She presented with a temperature of 38.2°C, pulse rate of 90/min, respiratory rate of 20/min, and blood pressure of 116/53 mm Hg. Her white blood cell count was normal, but a large number (53%) of immature polymorphonuclear cells were seen. Physical examination, electrolytes, liver enzymes, and lipase were all within normal limits.

A methylene blue stain for fecal leukocytes is shown in Fig. 1. Cultures for *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter* spp. were all negative. An enzyme immunoassay (EIA) test which was positive for the presence of a bacterial toxin in the stool established the patient's diagnosis.

1. What organism was causing this woman's diarrhea? How would you confirm this diagnosis? Why was toxin detection rather than culture used to establish the diagnosis of this patient's illness?

2. What in her history was a predisposing factor for her development of this infection? How did it predispose her?

3. Describe the disease spectrum seen with this organism.

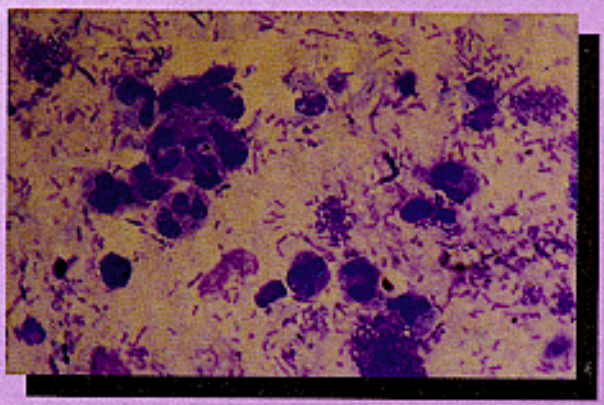

Figure 1

4. What virulence factors does this organism produce, and what roles do these factors play in the pathogenesis of disease?

5. Why is this organism particularly problematic as a nosocomial pathogen?

6. Why are treatment failures and disease relapses thought to occur frequently with this organism?

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Case Discussion

1. This woman had *Clostridium difficile*-associated diarrhea. *C. difficile* is the most common infectious cause of diarrheal disease in hospitalized patients and in patients receiving antimicrobial agents. The pathophysiology of the disease is due to the biologic activity of two exotoxins produced by the organism (see answers 3 and 4 for further details). The diagnosis of this disease is established by detecting one or both of the toxins produced by the organism in feces of infected individuals.

Several techniques have been developed for detection of *C. difficile* toxin. The reference method is a tissue culture cytotoxicity assay. This assay is very sensitive and highly specific but is also time-consuming (24 to 48 h) and laborious. As an alternative, EIA testing is frequently used. This method is not as sensitive as tissue culture (80 to 90%), but it is highly specific (97 to 99%) and rapid (2 h).

The etiology of most infections is established by detection of the infectious agent by culture, not by assaying for the organism's virulence factor(s). Why is *C. difficile* different? First, some strains of *C. difficile* fail to produce toxin. These strains are avirulent. Second, 3% of healthy individuals and 20% of individuals receiving antimicrobial agents excrete toxigenic organisms in feces asymptomatically. Finally, the presence of toxin correlates more closely than does culture with the presence of disease. It is for these reasons that toxin detection is preferable to culture for detection of *C. difficile*-associated disease.

2. Almost all patients who develop *C. difficile*-associated diarrhea have recently received or are currently receiving antimicrobial agents. This patient had just completed a course of oral cephalosporin when her diarrhea developed. Essentially all agents with antimicrobial activity, including agents not usually thought of as antimicrobial such as the anticancer agent methotrexate, can induce this disease. Animal and in vitro studies indicate that organisms found in normal gut flora can suppress the growth of *C. difficile*. When antimicrobial therapy is given, it may alter the gut flora in such a way as to eliminate the *C. difficile*-suppressive elements. With their elimination, *C. difficile* which either was an intrinsic component of bowel flora or was recently obtained from the environment can grow and produce toxin, resulting in disease.

3. *C. difficile* causes a broad spectrum of disease. Patients can be asymptomatic carriers. They can have mild diarrhea, often associated with concurrent antimicrobial therapy, which resolves on cessation of that therapy. The patient can have more severe diarrhea accompanied by nonspecific inflammatory changes in the intestinal tract. This manifestation may require specific antimicrobial therapy. The most severe manifestation of *C. difficile* disease is pseudomembranous colitis. In this disease, the intestinal mucosa, which is severely damaged, is overlaid by a pseudomembrane composed of fibrin, bacteria, cellular debris, and white and red blood cells. This pathologic lesion has a characteristic appearance when observed during colonoscopy, and the diagnosis of pseudomembranous colitis is usually made in this way. Pseudomembranous colitis is a life-threatening condition which can be complicated

by perforation and toxic megacolon. It must be aggressively treated with oral vancomycin or metronidazole. Oral and not intravenous therapy is used because intestinal drug levels are much higher when drugs are given orally. In patients unable to take drugs orally, these drugs can be administered per rectum.

4. Two exotoxins produced by *C. difficile* have been well characterized. These toxins are biochemically and immunologically distinct. One, an enterotoxin referred to as toxin A, is believed to be responsible for most of the pathologic events which occur with this disease. When purified toxin is given to experimental animals, most of the pathologic effects observed in humans can be reproduced. The second toxin is a cytotoxin called toxin B, which appears to play a minor but apparently synergistic role in the *C. difficile*-induced disease process. Other virulence factors have been identified, but their role in pathogenesis is not well understood.

5. *C. difficile* is a spore-forming bacterium. Spores can remain viable for months in a hospital environment and are much more resistant to disinfectants than are vegetative cells. These spores are frequently found throughout the rooms of infected individuals. They may remain present, capable of infecting other patients, weeks after the infected patient has left the room.

6. It is estimated that as many as 20% of individuals treated for *C. difficile* disease may suffer treatment failure or disease relapse. Spores are resistant to the activity of antimicrobial agents, and it is this resistance which is thought to play a central role in disease relapse. Studies have shown that when *C. difficile*-infected animals are treated with antimicrobial agents the number of vegetative *C. difficile* cells in feces declines dramatically while the number of spores increases. When antimicrobial agents are withdrawn, these spores develop into vegetative cells. In infected patients, similar events probably occur. If a *C. difficile*-suppressive microbial flora is still present in the gut at the completion of *C. difficile* therapy, it can prevent the spores from vegetating, thus preventing the cells from dividing, producing toxin, and causing relapse. If suppressive flora is not present, the spore can vegetate and cells can divide, producing toxin and a relapse of diarrhea. Some patients actually have multiple relapses and require multiple antimicrobial courses before the infection finally resolves.

References

1. **Bartlett, J. G.** 1994. *Clostridium difficile*: history of its role as an enteric pathogen and current state of knowledge about the organism. *Clin. Infect. Dis.* **18**(Suppl. 4):S265-S272.
2. **Peterson, L. R., and P. J. Kelly.** 1993. The role of the clinical microbiology laboratory in the management of *Clostridium difficile*-associated diarrhea. *Infect. Dis. Clin. North Am.* **7**:277-293.