PEARLS OF LABORATORY MEDICINE

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TITLE: **Clostridioides difficile**

PRESENTER: **Margaret Newman McCort**

Slide 1:
Hello, my name is Margaret Newman McCort. I am an infectious disease doctor at the University of Chicago. Welcome to this Pearl of Laboratory Medicine on "**Clostridioides difficile."**

Slide 2: Intro to CD
Clostridioides difficile, formerly known as *Clostridium difficile*, or C. diff (CD), is an anaerobic, spore-forming gram-positive rod that was first isolated in the stool of infants in 1935 and was identified as a cause of diarrheal infection in 1977. It was originally called *Bacillus difficilis* as it can be very difficult to culture without strict anaerobic environments. CD is an ubiquitous organism found in the environment; it has been isolated from the stool of many mammals. From its initial discovery as a pathogen, *C. difficile* incidence has increased exponentially, and is now considered one of the most important nosocomial infections in the world.

Slide 3: Pathogenesis of CD
C. difficile is acquired when an individual ingests endospores, which travel through the acidic gastric environment before germinating in the intestine. When antibiotics or other insults change the balance of an individual's gut bacteria, germinated *C. difficile* begins to overgrow and colonize the large intestine. However, many people with CD colonization do not get sick, for reasons that are still not yet clear. *C. difficile* causes disease through the actions of two toxins, A and B. Toxins produce enzymes that disrupt the structure of the gut epithelium, leading to breakdown of the mucosal barrier and subsequent inflammation and secretory diarrhea. The cytotoxic inflammatory response of colonic mucosa promotes the development of a pseudomembrane on the colon wall, which also perpetuates symptoms of diarrhea. Several studies have shown that an individual's intestinal biodiversity is inversely correlated to the likelihood of developing *C. difficile* disease. This is likely how systemic antibiotic use increases the risk of CDI.

Slide 4: Current Epidemiology of CD
Between 2-5% of healthy adults are colonized with *C. difficile*. Epidemiologic studies estimate that 3-26% of hospitalized patients are colonized with *C difficile*, but the majority will not develop

CD infection. These asymptomatic carriers of the bacteria contribute to CD transmission and CDI rates in healthcare institutions. CDI occurs in <1% of hospitalized patients in the US, but the incidence has doubled in the past 3 decades. CD is now the most common nosocomial pathogen. CDI incidence is higher in immunosuppressed populations such as solid organ or stem cell transplant recipients. *C difficile* is especially prevalent among individuals with frequent healthcare exposure, including nursing home residents and dialysis patients. Although CD has been identified in patients in the community, the infection remains much more prevalent in the hospitalized patient population. CD has now been identified in most countries of the world.

Slide 5: Symptoms of CD infection (and distinction from CD colonization)
CD colonization, or asymptomatic CD, is defined as a positive CD diagnostic assay without symptoms. A CD infection, however, is the presence of a positive test in a symptomatic patient. The symptoms of *C. difficile* infection are variable among individuals, ranging from mild diarrheal illness (with more than three bowel movements per day) to potentially fatal fulminant colitis. In addition to diarrhea, patients with CDI may experience abdominal pain, nausea, fever, stool incontinence, and decreased appetite. Physical exam may show abdominal distension or tenderness. Laboratory evaluation often displays marked leukocytosis, though immunosuppression and concomitant infections may obscure this finding. In severe CDI, patients can develop lactic acidosis and septic shock.

Slide 6: Risk factors for CDI
Several risk factors have been identified for CDI, including antibiotic use, prolonged healthcare exposure (including hospital length of stay as well as residence in a healthcare facility), and immunocompromised status. Age over 65 years has been particularly associated with increased risk of CDI. Some studies have also shown increased risk of CDI in patients on proton pump inhibitor therapy, though more recent studies refute this. Antibiotic use remains the main intervenable risk factor for CDI. Certain categories of antibiotics have been found to be more frequently associated with CDI risk, including cephalosporins, clindamycin, and fluoroquinolones. Recurrence of CDI is extremely common. Up to 25% of individuals who are treated for CD infection will develop recurrent symptoms.

Slide 7: CD Diagnosis
There are multiple diagnostic tests available for CD detection. CD was historically difficult to grow even in strict anaerobic culture, with a sensitivity around 67-75%. A selective media (cycloserine-cefoxitin-fructose agar) has facilitated culturing CD, but this method still requires several days and a separate toxin confirmation assay to make a diagnosis of CDI. Cell cytotoxicity neutralization assays (CCNAs) detect toxin directly in the stool but are also labor and time intensive. In the 1990s, enzyme immunoassays, or EIAs, began to replace toxigenic culture for the diagnosis of toxigenic CD. Toxin EIAs use monoclonal antibodies to detect either of the *C. difficile* toxins (A or B). Alternatively, an immunoassay for the glutamate dehydrogenase (GDH) enzyme utilized by *C. difficile* detects the presence of the bacteria itself. GDH EIA has a sensitivity between 81-100% and a specificity of 82-92%, whereas toxin EIA is only 58-96% sensitive but 95-100% specific. Two rapid immunoassay tests may be combined to optimize the sensitivity and specificity of toxigenic CD detection. Nucleic acid amplification testing (NAAT) detects multiple genetic targets of toxigenic *C difficile* with a sensitivity of 93-98% and specificity of 98-100%. This type of testing often gives the most rapid result, but is prone to false positives.

Slide 8: Controversies in CD Diagnostics
CCNA testing has both high sensitivity and high specificity but is not practical in the era of rapid diagnostics, except to compare newer diagnostic assays. The decision to use toxin- or NAAT-based testing has been a controversial topic for several years in the infectious disease community. The algorithm here explains these options for CD diagnostic testing and the pros and cons of each testing type. Because asymptomatic carriers can have a positive GDH EIA or NAAT test even when there is an alternative cause of diarrhea, and because tests are sometimes ordered in the absence of clinical symptoms, some experts advise against using these as single-step tests to the risk of false positives among colonized individuals. When used initially, low-sensitivity toxin-based EIAs may miss true diagnoses of toxigenic CDI in patients with intermittent toxin in the stool. Thus, the high negative predictive value of NAAT or GDH EIA testing makes these appropriate initial tests in a two-step algorithm, followed by toxin EIAs to confirm the diagnosis.

The IDSA/SHEA guidelines recommend that, when available, institutions implement either such a two-step toxin-based testing protocol, or single step NAAT-based testing if there is approved-upon institutional criteria for CD testing appropriateness, such as a requirement that stool be liquid for CD testing to be performed. All CD assays have the potential to be misinterpreted by providers who do not understand such nuances, especially in hospitalized patients with other potential causes of diarrhea.

Slide 9: CD Infection treatment
Several drugs have been found to be effective in the treatment of CD infection, including metronidazole and oral vancomycin. In more recent past, a novel macrocylic antibiotic called fidaxomicin was designed specifically to treat CD. Fidaxomicin has been proven to be as effective as oral vancomycin in treatment of acute CD infection and may decrease the risk of recurrence. Bezlotoxumab, a monoclonal antibody that binds and neutralizes CD toxin B, has been approved as a single dose adjuvant treatment for CD infection. It decreases the risk of recurrent infection when used in combination with vancomycin or fidaxomicin courses.

Several expert societies, including the Infectious Disease society of America, Society for Hospital Epidemiology, and American Society of Transplantation have recently updated their guidelines and recommend treating first episode of mild to moderate *C difficile* with either 125mg every 6 hours of oral vancomycin or 200mg every 12 hours of oral fidaxomicin. These are also the recommendations for treatment of first recurrence of CD infection. For severe or fulminant CD infections, a 500mg dose of oral vancomycin given with intravenous metronidazole is recommended. Other medications such as tigecycline and rifaximin have been shown to have activity against CD but are not recommended as primary treatment.

Slide 10: FMT
Fecal microbiota transplantation, or FMT, has been shown to be a safe and effective treatment for recurrent *C. difficile* infection, though this treatment is often reserved for patients who have failed alternate treatment options since this typically requires endoscopic administration. Additionally, the few commercially available FMT formulations can be expensive and not very standardized. Together with stopping systemic antibiotics, FMT has been shown to be the most effective treatment for recurrent CDI. Infection may recur in patients treated with FMT if they are exposed to antibiotics again, emphasizing the importance of the gut microbiota in the pathogenesis of CDI.

Slide 11: CD Complications
In some cases of severe fulminant CD infection, toxic megacolon can develop, which carries an associated risk of intestinal perforation. Patients with a high burden of infection may develop bacteremia or reactive arthritis. Dehydration and hypoalbuminemia related to severe CD diarrhea can lead to acute kidney injury, sepsis, and death. The mortality rate for CDI is 5%, though this may be up to 30% in intensive care units. Mortality is increased in patients with immunosuppression, older age, multiple comorbidities, and kidney failure.

Slide 12: Prevention of CD
CD is a uniquely challenging pathogen in the healthcare environment because it is very difficult to eradicate. CD endospores are resistant to commonly used quaternary ammonium cleaning agents and 70% ethanol hand sanitizer and can persist on environmental surfaces for months. Hand washing is vital to reducing transmission of CD. Healthcare workers are prominent agents of transmission in outbreak settings. The implementation of isolation precautions for patients with CD infection is recommended by multiple guidelines to reduce CD transmission in healthcare facilities. Such precautions often include private patient rooms, mandatory hand washing, and disposable gowns and gloves when contact with any portion of the patient environment is expected.

Hospital rooms where CD patients have been treated should be terminally cleaned to reduce risk of CD transmission to future occupants. This should include sodium hypochlorite solution (or, chlorine-based bleach) at strength of 5000 parts per million left in place for at least 10 minutes.

Antibiotic prophylaxis with either oral vancomycin or oral fidaxomicin may decrease CDI rates in patients at high risk for such infections, such as those on systemic antibiotics.
There is not enough data to recommend the use of probiotics to prevent or treat CDI.
Reducing or avoiding antibiotics when able has consistently been shown to be the most cost-effective intervention to prevent CD infections in patients at risk for these infections.

Slide 13: Future Directions for CD Research

Current research in CD focuses on the gut microbiome and its role in protecting against CDI. Although FMT may replace gut biodiversity, this is impractical as prevention. Other areas of research include bile salt conjugation process and its role in CD toxin production in the colon, as targeting this pathway could lead to vaccine development. It remains unclear why some individuals who are colonized with CD do not get sick, so detailing the host response to CD at the genetic and molecular level is also of interest.

In conclusion, *C difficile* represents an important pathogen that continues to cause significant disease among some of the most vulnerable patients. Please remember your role in preventing *C difficile* infections through antibiotic stewardship and regular hand washing, and only treat a positive CD result if the patient is symptomatic!

Slide 14: References

1. Curry SR. Clostridium difficile. Clin Lab Med 2017; 37:341-369.

2. Czepiel J, Drozdz M, Pituch H, et al. *Clostridium difficile* infection: a review. Eur Journ Clin Micro & Infect Dis 2019;
3. McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis 2018; 66(7): e1-e48.
4. Mullane KM, Dubberke ER, AST ID Community of Practice. Management of Clostridioides (formerly Clostridium) difficile infection (CDI) in solid organ transplant recipients: guidelines from the American Society of Transplantation Community of Practice. Clin Transplant 2019. e13564.
5. Wilcox MH, Gerding DN, Poxton IR, et al. Bezlotoxumab for Prevention of recurrent *Clostridium difficile* Infection. NEJM 2017; 376 (4): 305-317.
6. Feher C, Munez Rubio E, Merino Amador P, et al. The efficacy of fidaxomicin in the treatment of Clostridium difficile infection in a real-world clinical setting: a Spanish multi-center retrospective cohort. Eur J Clin Microbiol Infect Dis 2017. 36: 295-303.
7. Dubberke E, Burnham CD. Diagnosis of *Clostridium difficile* infection: treat the patient, not the test. JAMA Intern Med 2015. 175(11): 1801-2.
8. McFarland LV, Mulligan ME, Kwok RY, et al. Nosocomial acquisition of *Clostridium difficile* infection. NEJM 1989; 320: 204-10.
9. Curry SR, Muto CA, Schlackman JL, et al. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in Clostridium difficile transmission. Clin Infect Dis 2013; 57(8):1094-102.
10. Mamo Y, Woodworth MH, Want T, et al. Durability and Long-term clinical outcomes of fecal microbiota transplant treatment in patients with recurrent Clostridium difficile infection. Clin Infect Dis 2018; 66(11): 1705-11.
11. Kachirimanidou M, Tegou Z, Chasampalioti M, et al. A two-step approach improves the diagnosis of *Clostridium difficile* infection. *Journ of Microbiol Methods*, 2017. 143:17-19.

Slide 15: Disclosures

I have no financial disclosures.

Slide 16: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Clostridioides difficile**."

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PEARLS OF LABORATORY MEDICINE

Pearl Title: *Clostridioides (Clostridium) difficile*

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DOI:

CCTC Clinical Chemistry Trainee Council

Clostridioides difficile: an introduction

- Formerly known as *Clostridium difficile*
- Spore forming, gram positive rod
- Discovered in 1935, linked to infection in 1977
- Associated with hospitalization and antibiotic use
  → most common nosocomial infection in US
- Importance of infection control

C. difficile: the pathogen

- Ingest endospores
- Effects of antibiotics on gut flora
- Overgrowth of CD bacteria
- Toxins cause epithelial damage
- Inflammation, diarrhea, and pseudomembranes

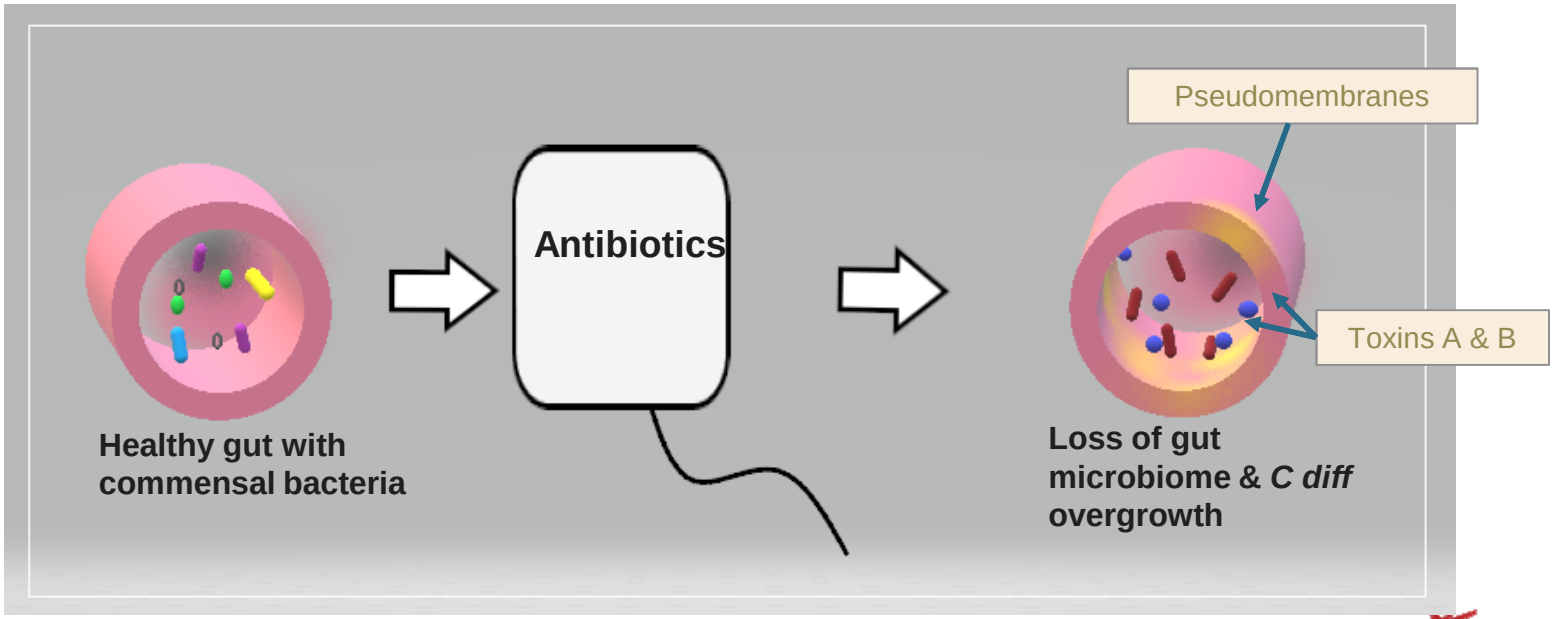


Figure 1: *C. difficile* in the gut. Author's own.

C. difficile Epidemiology

- 2-5% healthy adults colonized
- 3-26% hospitalized patients are colonized
- <1% hospitalized patients with CDI
  - Incidence is higher in immunocompromised

- Healthcare-Associated vs Community acquired

Clinical Presentation

C. difficile infection (CDI) = diarrhea + positive test

C. difficile (CD) colonization = positive test *without* symptoms

- Diarrhea
  - Spectrum of severity, but typically >3 BMs/day
- Fever
- Abd pain
- Leukocytosis
- Severe: development of ileus, distension, sepsis

Risk Factors for *C. difficile* Infection

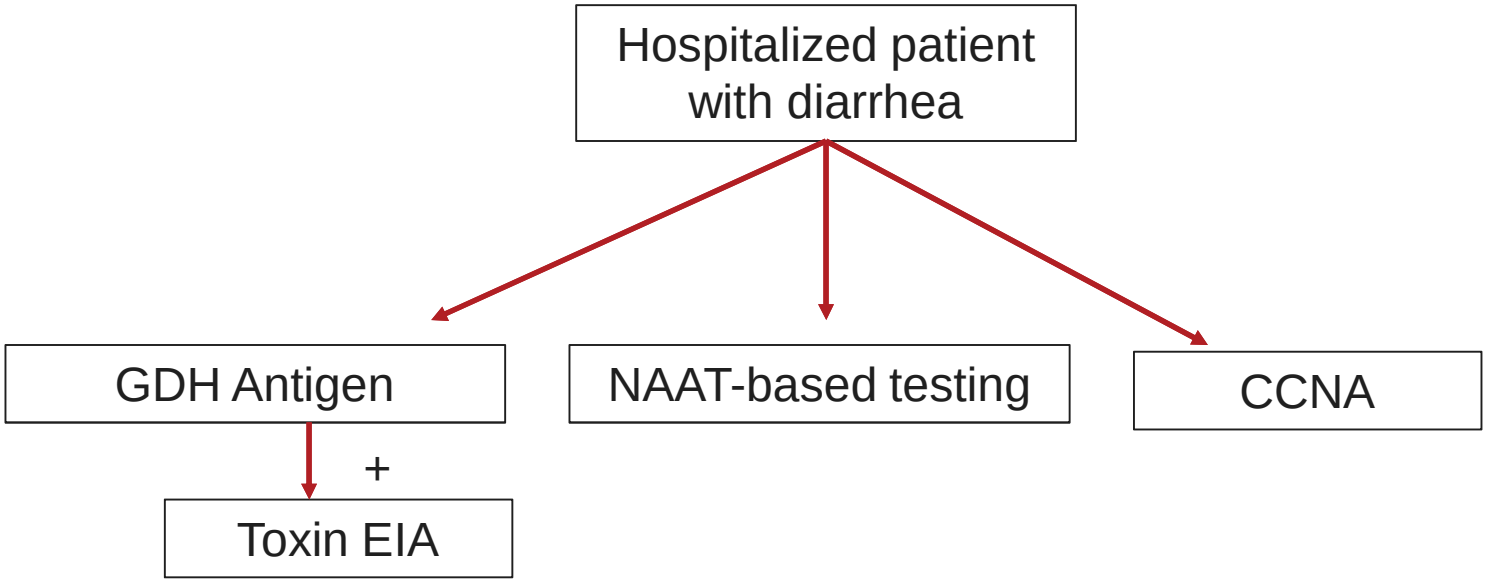
- Prolonged healthcare exposure
- Older age
- Antibiotic exposure
  - Fluoroquinolones
  - Clindamycin
  - Cephalosporins
- Proton Pump Inhibitor (PPI) use
- Immunocompromise

CD Diagnosis

Test Name	Time to diagnosis	Pros	Cons
Anaerobic toxigenic culture	4-5 days	Highly sensitive	Labor intensive Must confirm toxin assay Selective media* Not very specific
Cytotoxic Cell Neutralization Assays (CCNA)	3-4 days	Very sensitive Very specific	Labor intensive Time consuming Lacks standardization
Toxin Immunoassay (EIA)	Rapid	Moderately specific	Not very sensitive Variable performance
GDH EIA + toxin EIA	1-2 days	Very sensitive	Difficult to interpret Expensive Low specificity
NAAT-based test	Rapid	Very sensitive Moderate specificity	False positives in colonized

** Selective media = cycloserine-cefoxitin-fructose agar*

Controversies in *C. difficile* diagnosis



Potential false negatives

Potential false positives

C. difficile Treatment

Treatment type	Indication
Vancomycin (PO)	First episode mild/moderate CDI; first recurrence CDI; high-dose for severe
Fidaxomicin (PO or IV)	First episode mild/moderate CDI; recurrent CDI
Bezlotoxumab (IV)	**Reduce risk of recurrent CDI** when used as adjuvant to vancomycin or fidaxomicin course
Metronidazole (IV)	**Severe CD infection** (or mild/moderate without enteral access)
FMT	**Treat recurrent CDI**
Surgery	Toxic megacolon, colon perforation

C. difficile Treatment: Fecal Microbiota Transplant

- Indication: Recurrent CD, *when combined with antibiotic discontinuation*
- Administration: enema, pill, or endoscopic
- Proposed method of action: restore gut microbiome
- Risks:
  - No standardized formula
  - Expensive
  - CD may recur if antibiotics given after FMT

CD Complications

- Toxic megacolon
- Colon perforation
- Dehydration
  - Kidney injury
- Sepsis / Shock
- Bacteremia

C. difficile Prevention

Spores are easily spread, not easily killed

- Handwashing with soap & water
- Contact isolation
  - Gown
  - Gloves
  - Private room
- Cleaning the environment
  - Sodium hypochlorite (5000ppm chlorine bleach) solution x 10 min

Future Directions for CD Research

- Gut biodiversity & microbiome
- Bile salt conjugation and toxin production
- Host response to CD

Remember: to prevent CDI, think twice before prescribing antibiotics & always wash your hands!

Remember: only treat CDI if there is a positive test **and** symptoms present!

CDI= positive test + symptoms

References

- Curry SR. Clostridium difficile. Clin Lab Med 2017; 37:341-369.
- Curry SR, Muto CA, Schlackman JL, et al. Use of multilocus variable number of tandem repeats analysis genotyping to determine the role of asymptomatic carriers in Clostridium difficile transmission. Clin Infect Dis 2013; 57(8):1094-102.
- Czepiel J, Drozdz M, Pituch H, et al. *Clostridium difficile* infection: a review. Eur Journ Clin Micro & Infect Dis 2019;
- Dubberke E, Burnham CD. Diagnosis of *Clostridium difficile* infection: treat the patient, not the test. JAMA Intern Med 2015. 175(11): 1801-2.
- Feher C, Munez Rubio E, Merino Amador P, et al. The efficacy of fidaxomicin in the treatment of Clostridium difficile infection in a real-world clinical setting: a Spanish multi-center retrospective cohort. Eur J Clin Microbiol Infect Dis 2017. 36: 295-303.
- Mamo Y, Woodworth MH, Want T, et al. Durability and Long-term clinical outcomes of fecal microbiota transplant treatment in patients with recurrent Clostridium difficile infection. Clin Infect Dis 2018; 66(11): 1705-11.
- McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). CID 2018; 66(7): e1-e48.
- McFarland LV, Mulligan ME, Kwok RY, et al. Nosocomial acquisition of *Clostridium difficile* infection. NEJM 1989; 320: 204-10Mullane KM, Dubberke ER, AST ID Community of Practice. Management of Clostridioides (formerly Clostridium) difficile infection (CDI) in solid organ transplant recipients: guidelines from the American Society of Transplantation Community of Practice. Clin Transplant 2019. e13564.
- Wilcox MH, Gerding DN, Poxton IR, et al. Bezlotoxumab for Prevention of recurrent *Clostridium difficile* Infection. NEJM 2017; 376 (4): 305-317.

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**:
- **Consultant or Advisory Role:**
- **Stock Ownership:**
- **Honoraria:**
- **Research Funding:**
- **Expert Testimony:**
- **Patents:**

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