Clostridium difficile: Infection-Impact-Intervention

Pre-Survey Questions

for

"Clostridium difficile: Infection-Impact-Intervention"

Presented by

J. Michael Miller, PhD, D(ABMM)

Director, Microbiology Technical Services, LLC

Dunwoody, Georgia

1. Which of the following statements is false:

A. *C. difficile* is responsible for 15-25% of cases of antibiotic-associated diarrhea

B. *C. difficile* spores are susceptible to treatment with both metronidazole and vancomycin

C. Virtually all cases of antibiotic-associated pseudomembranous colitis are caused by *C. difficile*

D. *C. difficile* is easily spread within the hospital environment

2. Ethanol is an effective disinfectant for *C. difficile.*

A. True
B. False

3. The laboratory test of choice for diagnosing *C. difficile* toxin disease is:

A. Enzyme immunoassay for Toxin A and Toxin B
B. Enzyme immunoassay for the glutamate dehydrogenase (GDH) of *C. difficile*
C. A combination of the GDH and toxin assays
D. PCR alone or GDH + toxin assay that reflexes to PCR

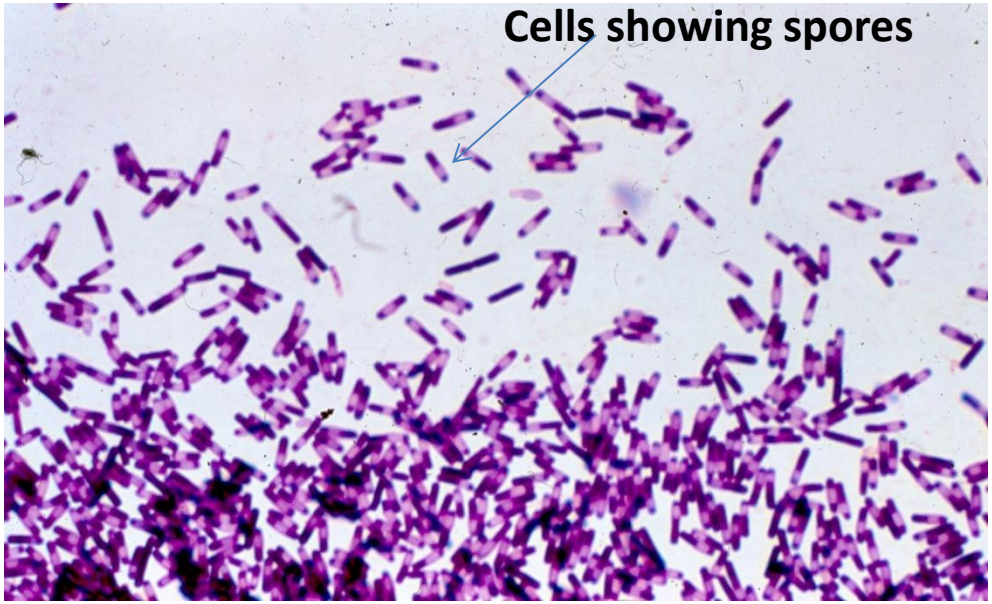
4. Which of the following statements is true:

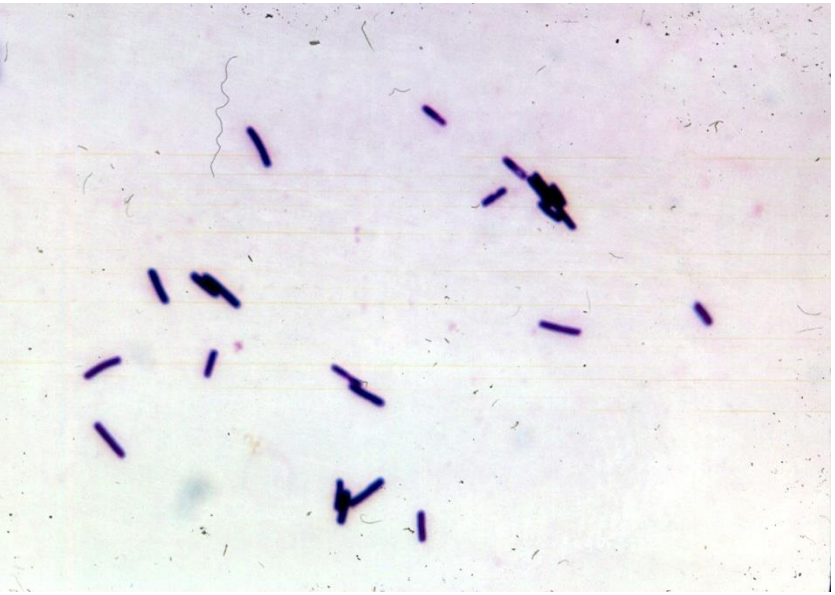
A. The NAP1 strain of *C. difficile* must be treated with a combination of Vancomycin and a fluoroquinolone
B. Patients who are only colonized with *C. difficile* exhibit only mild diarrhea
C. *C. difficile* diagnostic tests should not be used as a test of cure
D. As a safety measure, all stool specimens should be screened for *C. difficile* toxins

Introduction

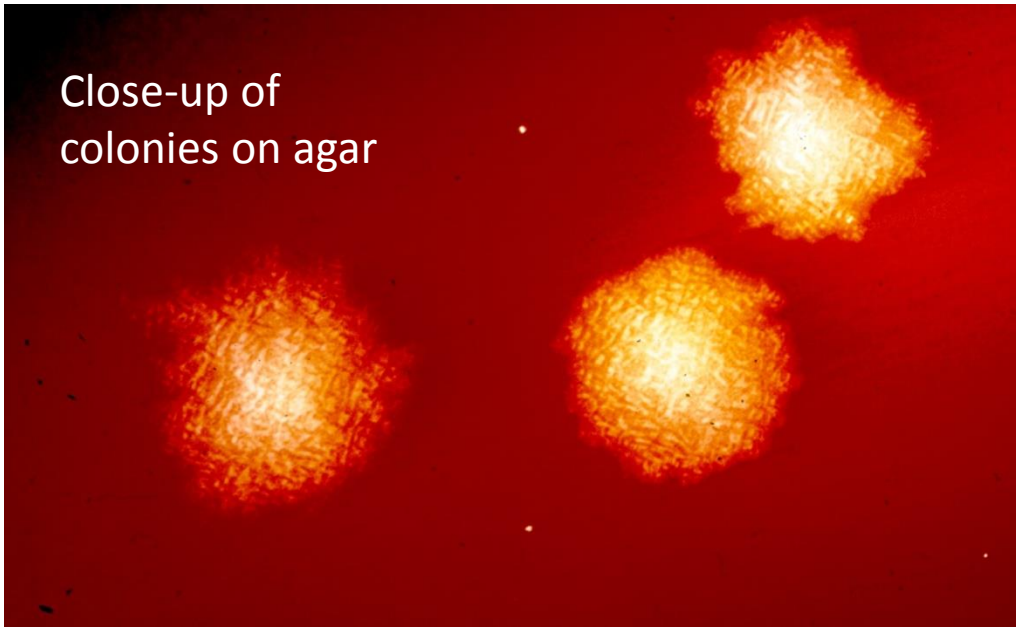
- Organism first described in 1935 - isolated from feces of normal infants and named *Bacillus difficilis* because of difficulty in isolation
- Only recognized as a pathogen in late 1970s
- Diarrhea is efficient method of spreading spores of *C. difficile*. *Team this up with "hands" and you get a GREAT way to spread infection.*

C. difficile





Microscopy at 1000X



What is “C. diff” ?

- A capsule-shaped bacterium that causes inflammation of the colon – colitis
- Produces two toxins – A and B
- *C. difficile* that causes disease is found in the feces
  - May be normal in about 3% of healthy adults
- Spores can survive for long periods in the environment (surfaces in a room) and be spread by hands that touch contaminated surfaces

BIOLOGICAL ACTIVITIES OF TOXIN A AND B OF *C.DIFFICILE*

Activity	Toxin A	Toxin B
Cytotoxicity	++	++++
Lethality	++	++
Hemagglutination	+	-
Enterotoxicity	++++	+/1

Toxin B may be more important than Toxin A. Toxin B causes the opening of the tight junctions of intestinal epithelial cells, to increase vascular permeability and induce hemorrhaging. Toxin A leads to diarrhea by damaging villous tips that disrupt the brush border membrane, cell erosion, fluid leakage.

Clin. Microbiol. Rev. **1** (1): 1–18.

C. difficile Epidemiology

How Do You Get *C. difficile* Disease?

- People taking antibiotics, and the elderly, are at greatest risk
  - 80% of infections are hospital associated. 20% of infections are community acquired
  - *Many commonly prescribed antibiotics can lead to C. diff disease, especially ampicillin, amoxicillin, cephalosporins, erythromycin, and quinolones like ciprofloxacin and levofloxacin*
- Some disease can be picked up from touching feces-contaminated surfaces

Where do infections come from?

- **C. Diff Infections Not Always Acquired from Symptomatic Patients.**
  - Researchers at the University of Oxford found that over half of infected patients did not acquire their infection from other patients, but rather from outside sources such as water, pets, farm animals, or food. They performed whole gene sequencing on 957 isolates obtained from symptomatic patients

    (Eyre DW et al. *N Engl J Med* 2013;369:1195-1205).
- **Asymptomatic Carriers a Major Source of Infection.**
  - Researchers at the University of Pittsburgh found that 29% of infected patients contracted their disease from asymptomatic carriers.

    (Clin Infect Dis. 2013 Oct;57(8):1094-102. doi: 10.1093/cid/cit475. Epub 2013 Jul 23)

C. difficile Epidemiology

- Responsible for 20-30% of cases of antibiotic-associated diarrhea (AAD) and for virtually all cases of antibiotic-associated pseudomembranous colitis (PMC) SHEA-IDSA Guideline-2010
- leading cause of Hospital-associated infectious diarrhea in adults and can be responsible for large outbreaks SHEA-IDSA Guideline-2010
- >500,000 illnesses in the U.S. annually. ~350,000 hospitalized. 29K deaths in 2011. ~$1 billion/yr in U.S.
- Up to 20% of patients get sick again
  - Initial infection not fully cleared
  - Reinfected with a different strain
  - After 1 recurrence, further recurrence up to 65% SHEA-IDSA Guideline-2010
    - Older than 65
    - Taking other abx while being treated for *C. difficile*
    - Co-morbid conditions – kidney disease, IBD, liver disease

Epidemiology

- Shed in feces and can be transmitted person-to-person
- Nosocomial *C. difficile* infection results in an increased length of stay in hospital ranging from 8 to 21 days
- Patients can be contaminated from any environmental surface, shared instrumentation, hospital personnel hands and infected roommates ***(essentially fecal contamination)***
- *C. difficile* may be spread rapidly throughout the hospital environment where spores may persist for months

Measures that are effective in reducing incidence of *C. difficile* infections and cross-infection

- accurate and rapid diagnosis
- appropriate treatment
- implementation of enteric precautions for symptomatic patients
- reinforcement of hand-washing
- daily environmental disinfection
- a restrictive antibiotic policy

– Barbut F, Petit JC. , Epidemiology of Clostridium difficile-associated infections. Clin Microbiol Infect. 2001 Aug;7(8):405-10.

C. difficile Infection (CDI)

Summary Definition of *Clostridium difficile* Infection (CDI)

- A case definition of CDI should include the presence of symptoms (usually diarrhea) and either a stool test result positive for *C. difficile* toxins or toxigenic *C. difficile,* or colonoscopic findings demonstrating pseudomembranous colitis.

SHEA-IDSA Guideline 2010

Colonization vs Infection

- Colonization
  - Patient exhibits NO clinical symptoms
  - Patient tests positive for the organism. Non-toxigenic *C. difficile* present.
  - More common than *C. difficile* infection
- Infection
  - Patient exhibits clinical symptoms
  - Patient tests positive for the organisms and/or its toxin

Symptoms of *C. difficile* infection

- Watery diarrhea (3+ bowel movements per day for two or more days)
- Fever
- Loss of appetite
- Nausea
- Belly pain/tenderness
- Causes pseudomembranous colitis
- Must be diagnosed and treated immediately or risk serious complications like toxic megacolon, perforations of the colon, sepsis, death (rarely)

Sequence of events leading to *C.difficile* infection

- Alteration of normal gut flora (leaves a commensal flora ”vacuum”)
- Nosocomial infection by *C.difficile*
- Growth in gut and production of toxins
- Tissue damage by toxin A, exacerbated by toxin B
- Diarrhea and colitis

Guidelines for the Diagnosis, Treatment, and the Prevention of *Clostridium difficile* infections

Diagnostic Tests

1. **Only stools from patients with diarrhea should be tested**
2. **NAATs such as PCR for *C. difficile* toxin genes are superior to toxins A+B testing alone as a standard diagnostic test for CDI**
3. **GDH screening tests for *C. difficile* can be used in 2-3 step screening algorithms with subsequent toxin A+B EIA testing, but the sensitivity may be lower than NAATs alone**
4. **Repeat testing should be discouraged**
5. **Testing for cure should not be done**

Am J Gastroenterol advance online publication. 26 February 2013; doi:10.1038/ajg.2013.4

Relapse or New Infection?

- **Is recurrence associated with the same strain or a different strain?**
- **Of patients with second episodes within 8 weeks, 88% (75/85) had the same strain[1]**
- **Of patients with second episodes > 8 weeks, 65% (32/49) had the same strain[1]**
- **Similar results from Figueroa et al[2]**
- **Diarrhea after an initial episode of CDI may not be CDI[3]**

1. Kamboj, M. et al. 2011. Clin Infect Dis.53: 1003-1006

2. Figueroa, I et al. 2012. Clin Infect Dis. 55: S104-S109

3. Polage, CR et al. 2012. Clin Infect Dis. 55: 982-989

Changing Face of *C. difficile*

- U.S. is seeing an increase in number and severity of cases.
- Who is affected?
  - Elderly, especially those taking antibiotics
  - Otherwise healthy individuals (community acquired)
  - Peripartum women
- New strain of *C. difficile* called NAP1 or PCR ribotype 027.
  - NAP – North American Pulsed Field type
  - Pittsburgh (2000), Atlanta (2001-2), and Montreal (2003)
  - More toxin produced, 16x Tox A. 23x Tox B, plus a binary toxin*
  - More resistant to fluoroquinolones, although these drugs are not recommended for treatment

*Warny M, Pepin J, Fang A, et al. Increased toxins A and B production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet 2005;366:1079--84.

Laboratory Diagnosis of *C. difficile* Infection

Laboratory Tests for *C. difficile*

- Culture – bacteria will grow on special media in the lab but takes 48-96h for results. Does NOT determine if toxin is present or not.
- Genetic tests – tests for the bacterial gene that produces toxin B. They are sensitive and specific but expensive. May take 1-3 h.
- Antigen detection – rapid, <1h. Commonly used. Often combined with toxin or genetic tests.
- Toxin testing – tissue culture or enzyme immunoassay for toxin A and/or B.

Are toxin detection kits sufficiently accurate to be used as single tests to detect *C. difficile* toxins ?

- The currently available kits for detection of *C. difficile* toxins have variable performance
- Currently available kits may miss about 1 in 5 to 1 in 10 cases of CDI and will falsely identify 1-2 out of 10 cases as positive when they are not
- The poor positive predictive values of toxin detection kits, especially in the context of widespread testing, and the possibility of missing true positives mean that there are limitations to using these as single tests for the laboratory diagnosis of CDI

http://www.pasa.nhs.uk/PASAWeb/NHSprocurement/CEP/CEPproducts/CEP+catalogue.htm

http://www.pasa.nhs.uk/pasa/Doc.aspx?Path=%5bMN%5d%5bSP%5d/NHSprocurement/CEP/CEP08054.pdf

TABLE 3. Comparison of commercial *C. difficile* detection assays with results of cytotoxigenic culture

Assay	No. of samples with the following assay result:				Cytotoxigenic culture result	No. of samples tested	Sensitivity (%) (95% CI)[a]	Specificity (%) (95% CI)[a]
	Positive	Negative	Equivocal	Invalid				
Cytotoxin test	108	17	0	0	Positive	125	86.4 (79.1–91.9)	99.2 (97.9–99.8)
	4	471	0	0	Negative	475		
Premier toxin A+B	101	24	0	0	Positive	125	80.8 (72.3–87.3)	97.5(95.5–98.6)
	12	463	0	0	Negative	475		
GA *Clostridium difficile* antigen	86	39	0	0	Positive	125	68.8 (59.9–76.8)	91.4 (88.4–93.7)
	41	454	0	0	Negative	475		
Ridascreen toxin A/B	75	50	0	0	Positive	125	60.0 (50.9–68.7)	95.6 (93.2–97.2)
	20	434	1	0	Negative	475		
Techlab toxin A/B II	100	25	0	0	Positive	125	80.0 (71.9–86.6)	96.0 (93.7–97.5)
	19	456	0	0	Negative	475		
Remel ProSpecT	102	23	0	0	Positive	125	81.6 (73.7–87.9)	93.3 (90.6–95.4)
	32	443	0	0	Negative	475		
Vidas *C. difficile* Tox A/B	100	16	9	0	Positive	125	80.0 (71.9–86.6)	97.3 (95.2–98.5)
	2	462	11	0	Negative	475		
Remel Xpect	86	31	8	0	Positive	125	68.8 (59.9–76.8)	99.4 (98.2–99.9)
	3	472	0	0	Negative	475		
Techlab Tox A/B Quik Chek	93	32	0	0	Positive	125	74.4 (65.8–81.78)	98.9 (97.6–99.7)
	3	470	1	1	Negative	475		
Premier Immunocard A + B	86	29	0	10	Positive	125	68.8 (59.9–76.8)	93.0 (90.4–95.2)
	2	442	0	31	Negative	475		
Techlab C. diff Chek-60	92	13	0	0	Positive	105	87.6 (72.4–93.0)	94.3 (91.7–96.2)
	26	433	0	0	Negative	459		
BD GeneOhm *C. difficile*	92	11	0	1	Positive	104	88.5 (80.3–93.6)	95.4 (92.9–97.0)
	16	433	0	5	Negative	454		

GDH testing (i)

All *C. difficile* strains appear to produce the cell wall-associated enzyme glutamate dehydrogenase antigen (GDH). GDH appears to be highly conserved among *C. difficile* ribotypes.

- GDH tests have been reported to have high sensitivity (but poor specificity) for the detection of toxigenic *C. difficile*
- GDH assays have been widely used a screening tests for *C. difficile,* as recommended in US / European guidelines

Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH; Society for Healthcare Epidemiology of America; Infectious Diseases Society of America. *Infect Control Hosp Epidemiol* 2010;31:431-55.

Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ, (ESCMID). *Clin Microbiol Infect* 2009;15:1053-66.

GDH testing (ii)

- There is some uncertainty regarding whether GDH assays detect some *C. difficile* strains better than others. Data are contradictory

Tenover FC, *et al*. Impact of strain type on detection of toxigenic *Clostridium difficile*: comparison of molecular diagnostic and enzyme immunoassay approaches. *J Clin Microbiol* 2010;48:3719-24.

Goldenberg SD, *et al*. Lack of effect of strain type on detection of toxigenic *Clostridium difficile* by glutamate dehydrogenase and polymerase chain reaction. *Diagn Microbiol Infect Dis* 2011;70:417-9.

Carman RJ, *et al*. Glutamate dehydrogenase is highly conserved among *Clostridium difficile* ribotypes. *J Clin Microbiol* 2012;50:1425-6.

Table 3. Sensitivity and Specificity of individual assays and algorithms compared with cell cytotoxin assay – Training dataset (n = 6761)

	Single assays-Manufacturers' cut-offs				Two stage assays-Manufacturers' cut-offs				
	GDH EIA	NAAT	Toxin EIA 1	Toxin EIA 2	GDH EIA	GDH EIA	GDH EIA	Toxin EIA 1	Toxin EIA 2
					Toxin EIA 1	NAAT	Toxin EIA 2	NAAT	NAAT
Sensitivity % (95% CI)	95.9 (93.4-97.6)	96.9 (94.7-98.4)	69.2 (64.3-73.8)	82.3 (78.9-85.9)	67.4 (62.4-72.1)	94.6 (91.9-96.6)	80.4 (76.2-84.3)	68.9 (64.0-73.6)	82.0 (77.8-85.7)
Specificity % (95% CI)	92.1 (91.4-92.8)	94.9 (94.3-95.4)	99.4 (99.2-99.6)	98.8 98.5-99.1)	99.7 (99.5-99.8)	95.6 (95.5-96.5)	99.6 (99.4-99.7)	99.7 (99.6-99.8)	99.6 (99.4-99.8)
PPV% (95% CI)	42.7 (39.4-46.1)	54.0 (50.2-57.7)	87.4 (83.0-91.0)	80.8 (76.5-84.5)	93.1 (89.2-95.7)	59.3 (55.3-63.1)	91.8 (88.2-94.4)	93.9 (90.2-96.3)	93.0 (89.6-95.4)
NPV% (95% CI)	99.7 (99.5-99.8)	99.8 (99.6-99.9)	98.1 (97.8-98.5)	98.9 (98.6-99.1)	98.1 (97.7-98.4)	99.7 (99.5-99.8)	98.8 (98.5-99.1)	98.1 (97.8-98.5)	98.9 (98.6-99.1)

Davies KA et al. The largest ever study to define a testing algorithm to optimise the laboratory diagnosis of *C. difficile* infection. 22nd ECCMID, 2012. http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=151898&XNSPRACHE_ID=2&XNKONGRESS_ID=161&XNMASKEN_ID=900;

NAATs for CD toxin genes (i)

- **Potential Achilles heel for NAATs because they do not detect the presence of fecal toxin, which is considered as *sine qua non* for CDI.**
- Elderly in-patients typically have *C. difficile* toxigenic culture positive rates of 10-20%.
  - 14% of 271 patients were colonized with *C. difficile* on admission, including 18 who were asymptomatic carriers
  - A further 47 patients (17%) became colonized during their hospital stay, 19 of whom remained asymptomatic.
- Colonization by *C. difficile* is protective against the development of CDI when accompanied by an antitoxin antibody response.

Kyne *et al*. NEJM 2000;342:390–7.

NAATs

- RT-PCR is highly sensitive
- Detects toxin genes and not active toxins; cannot distinguish CDI from carriage
- If rate of CDI is <10% of samples submitted; the PPV is 71%
- If rate of CDI is 10-20%, PPV would be 78%
- If rate of CDI is >20%, PPV becomes 93%, still with false positive results

Clin Infect Dis. 2011;53(7):e81-e90

Clin Gastroenterol Hepatol 2013; 11(10): 1216-1223

NAATs for CD toxin genes (iii)

- ~25% of primary CDI cases have a recurrence of symptoms following treatment.
- Long-term *C. difficile* gut colonization is also frequent; in an endemic CDI setting, 56% of patients were asymptomatic *C. difficile* carriers 1-4 weeks after treatment cessation.
- **The significance of a positive *C. difficile* NAAT after a primary episode of CDI is therefore unclear.**

These examples emphasize the importance of appropriate specimen selection & clear clinical details.

Without these, accurate interpretations of test results cannot be made.

Sethi *et al*. *Infect Control Hosp Epidemiol* 2010;31:21–27.

If the First PCR is Negative Should I Order Another PCR?

- **Of 406 tests from 293 patients with a prior negative PCR[1]**
  - **396 negative (97.5%)**
  - **10 positive (2.5%)**
    - **Only 3+ in <7 days (0.7%)**
- **Exceptions**
  - **Severe clinical changes**

No

1. Luo RF, Banaei N. *J Clin Microbiol*. 2010;48:3738-3742.

What others are seeing

Site	Method	% Positive
Wilmington, NC	EIA	8.1
Oakland, CA	Algorithm	12
Atlanta. GA	NAAT	12.1
Los Angeles, CA	NAAT	14
Temple, Tx	NAAT	14.3
Stanford, CA	NAAT	15
Oceanside, CA	NAAT	15
Louisville, KY	Algorithm	17
Cincinnati, OH	NAAT	17
Tallahassee, FL	Algorithm	17
Wilmington, NC	NAAT	17.3
Hollywood, CA	NAAT	17.5
Jacksonville, FL	NAAT	19.5
Atlanta, GA	NAAT	19.7
Nashville, TN	NAAT	20.1

NAAT Average = 16.5 Algorithm average = 15.3

Recommendations 2015

- Acceptable strategies
  - EIA for GDH and/or toxins A/B with a molecular assay for discrepant results
  - A molecular test with or without a confirmatory toxin assay
- Unacceptable
  - A stand-alone EIA for toxins A/B

How is *C. difficile* treated?

- In about 20% of patients, infection will resolve within 2-3 days of discontinuing the antibiotic that the patient is taking
- Treatment (usually 10 days) in uncomplicated cases with:
  - Metronidazole (Flagyl)(oral) or
  - Vancomycin (oral) or
  - Fidaxomicin

Other Treatments for Severe Disease

- Fecal Transplants:
- The European Society for Clinical Microbiology has released an updated algorithm for the treatment of various types of *C. difficile* infections which include the use of probiotics and fecal transplants for cases with multiple recurrences
- Researchers from the University of Calgary have used pills made up of feces from the family members of the afflicted patient.
  - Of the 27 patients tested, all were successfully treated and experienced no recurrences of disease.
  - Only the bacteria were extracted from the feces, which was encased in a triple layer of gelatin capsules to help survive the acid conditions of the stomach before it reaches the small intestine.

Infectious Diseases Society of America (2013, October 4). Fecal transplant pill knocks out recurrent C. diff infection. *ScienceDaily*. http://www.sciencedaily.com /releases/2013/10/131004105253.htm

Fecal Transplants

- Investigational agent
  - June 2013: IND enforcement by FDA is discretionary (Investigational New Drug)
  - March 2014: Draft guidance. More IND limits
- In these cases, physicians may proceed without filing an IND, provided
  - They have received the appropriate informed consent from the patient indicating that FMT is an investigational therapy,
  - The donor source is known
  - Donor screening by a licensed laboratory/physician

Fecal Transplants

- Evidence (five studies)
  - 89% cure in immunocompromised
  - 87% of 536 CDI patients improved
  - 81% cure in randomized trial – duodenal infusion
  - 85% cure in 48 patients, double-blind study
  - 95% cure in Rhode Island study for 1-2 transplants
- Billing:
  - CPT 44705 (Preparation)
  - CPT 43235 (installation)
  - CHPCS G0455 (Prep and instill)
- See: www.Openbiome.org

Information Sources

- Centers for Disease Control and Prevention website for *C. difficile* infection
- Infectious Disease Society of America guideline on gastrointestinal disease
  - **"Clinical Practice Guidelines for Clostridium difficile Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA)"**

2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings

C. difficile

Contact precautions for the duration of the illness.

Discontinue antibiotics if appropriate.
Do not share electronic thermometers;
ensure consistent environmental cleaning and disinfection.

Best testing strategies - IDSA

- Testing for *C. difficile* or its toxins should be performed **only on diarrheal (unformed) stoo**l, unless ileus due to *C. difficile* is suspected **(B-II).**
- **Testing of stool from asymptomatic patients is not clinically useful**, including use as a test of cure. It is not recommended, except for epidemiological studies. **(B-III)**
- **Stool culture is the most sensitive test** and is essential for broad epidemiological studies **(A-II).**
- Although stool culture is not clinically practical because of its slow turnaround time, the sensitivity and specificity of stool culture followed by identification of a toxigenic isolate (ie, toxigenic culture), as performed by an experienced laboratory, provides the standard against which other clinical test results should be compared **(B-III).**
- **Enzyme immunoassay (EIA) testing** for *C. difficile* toxin A and B is rapid but is less sensitive than the cell cytotoxin assay, and it **is thus a suboptimal alternative approach for diagnosis (B-II).**

Best testing strategies - IDSA

- **Toxin testing is most important clinically**, but is not sensitive. One potential strategy to overcome this problem is a 2-step method that uses EIA detection of glutamate dehydrogenase (GDH) as initial screening and then uses the cell cytotoxicity assay or toxigenic culture as the confirmatory test for GDH-positive stool specimens only. Results appear to differ based on the GDH kit used; therefore, until more data are available on the sensitivity of GDH testing, this approach remains an interim recommendation. **(B-II)**
- **Polymerase chain reaction (PCR) testing appears to be rapid, sensitive, and specific** and may ultimately address testing concerns. More data on utility are necessary before this methodology can be recommended for routine testing. **(B-II)**
- **Repeat testing during the same episode of diarrhea is of limited value and should be discouraged (B-II)**.

Summary and Conclusions

- ***C. difficile*** **testing has improved dramatically in the past 3 years**
- **Practice Value-effective rather than Cost-effective microbiology**
- **Limit testing to at-risk patients with clinically significant diarrhea**
- **Eliminate repeat testing unless clinically necessary**
- **Do not perform a test of cure**
- **Create a CDI Team**

Hospital Internal Medicine Conference

Post-Survey Questions
for

"Clostridium Difficile: Infection-Impact-Intervention"

Presented by

J. Michael Miller, PhD, D(ABMM)
Director, Microbiology Technical Services, LLC
Dunwoody, Georgia

1. Which of the following statements is false:

A. *C. difficile* is responsible for 15-25% of cases of antibiotic-associated diarrhea

B. *C. difficile* spores are susceptible to treatment with both metronidazole and vancomycin

C. Virtually all cases of antibiotic-associated pseudomembranous colitis are caused by *C. difficile*

D. *C. difficile* is easily spread within the hospital environment

2. Ethanol is an effective disinfectant for *C. difficile.*

3. The laboratory test of choice for diagnosing *C. difficile* toxin disease is:

A. Enzyme immunoassay for Toxin A and Toxin B
B. Enzyme immunoassay for the glutamate dehydrogenase (GDH) of C. difficile
C. A combination of the GDH and toxin assays
D. PCR alone or GDH + toxin assay that reflexes to PCR

4. Which of the following statements is true:

A. The NAP1 strain of C. difficile must be treated with a combination of Vancomycin and a fluoroquinolone

B. Patients who are only colonized with *C. difficile* exhibit only mild diarrhea

C. *C. difficile* diagnostic tests should not be used as a test of cure

D. As a safety measure, all stool specimens should be screened for *C. difficile* toxins