Controversies in the diagnosis of *C. difficile* infections (CDI)

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Questions?

- **Who** should you test?
- **How** should you test?
- **When** should you test/re-test?

*Clostridium difficile*

- Anaerobic, Gram-positive, spore-forming, bacilli, causes pseudomembranous colitis
- Pathogenic form produces 1-2 exotoxins
  - A enterotoxin, B cytotoxin
- Rarely "normal flora" - 1-3% normal adults
- *But is it normal flora in children?*

**NICU**

- *Clostridium difficile* toxin detected in the feces of 10% of normal newborn infants and 55% of neonates in the newborn ICU.
  - Donta and Myers J Peds 1982

*C difficile* colonization by age

Jangi and Lamont, JPGN 2010

What about older children?

- 306 outpatients (2 weeks to 16 years old) cultured for *C. difficile*
  - *C. difficile* from 7% of patients with diarrhea (12 of 171) ….
  - and 15% of controls with non-diarrheal illnesses (20 of 135).
  - Ped Inf Dis 1982 (GR Fleisher)
< 3: Hard to know who is colonized and who has an infection

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>No. of Positive Cases (%)</th>
<th>No. of Positive Controls (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEC EIA positive</td>
<td>9 (35)</td>
<td>0</td>
<td><strong>0.9 (0.5–1.9)</strong></td>
<td>= 0.004</td>
</tr>
<tr>
<td>C. difficile all ages</td>
<td>14 (5.5)</td>
<td>28 (6.2)</td>
<td>0.9 (0.5–1.9)</td>
<td>= 0.004</td>
</tr>
<tr>
<td>C. difficile &lt;36 months old</td>
<td>9 (5.2)</td>
<td>26 (8.8)</td>
<td>0.6 (0.3–1.3)</td>
<td></td>
</tr>
<tr>
<td>C. difficile ≥36 months old</td>
<td>5 (6.3)</td>
<td>2 (1.3)</td>
<td><strong>4.6 (0.8–27)</strong></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>110 (43.3)</td>
<td>1 (0.22)</td>
<td><strong>60 (22–127)</strong></td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

CSI/CDI

- Not all children carrying toxigenic C. difficile are sick. The clinical laboratory can place the perpetrator (C. difficile) and weapon (usually toxin B) at the scene of the crime, but only the clinician can presumptively determine whether a crime (CDI) has taken place.

Controversy #1-Who?

- A positive test does not mean CDI in newborns and infants
- Testing for CDI in newborns and infants is like picking your nose in public – if you get something, what are you going to do with it? Better decide in advance (or not do it).

Clostridium difficile epidemiology (1970’s vs Now)

- Before: Transmission almost exclusively in health care facilities – “at risk patients”
- Now: More outbreaks; more frequent CDI in both “at risk” and not at risk patients
- Increase in community acquired CDI (adults and children).

Epidemic – holding steady

Antibodies to Toxin A and B in 50-70% of School Age Children

Epidemic – holding steady

Epidemic – holding steady
Healthcare Cost and Utilization Project (HCUP). 1997-2006 (89% of children in US pop)

Agency for Healthcare Research and Quality, Rockville, MD.
www.hcup-us.ahrq.gov/databases.jsp.

Nylund et al, APAM, 2011

Total Number of Pediatric Discharges
ICD-9-CM principal diagnosis code 008.45

<table>
<thead>
<tr>
<th>Year</th>
<th>Median Charges ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>6,257</td>
</tr>
<tr>
<td>2000</td>
<td>7,351</td>
</tr>
<tr>
<td>2003</td>
<td>9,946</td>
</tr>
<tr>
<td>2006</td>
<td>11,068</td>
</tr>
</tbody>
</table>

Median Charges ($) Hospitalized Children with CDI

Colectomy in Children with CDI

Colectomies

Colectomy rate/1000

Community Acquired CDI: Increasing in children?:

Community Acquired CDI: When to think of it?

- Old CW: Inpatient, antibiotic exposure
- New CW: Inpatient + Community Acquired
  - NO hx of antibiotic exposure
  - Have chronic gastrointestinal conditions e.g., IBD - (Mezoff et al JPGN 2011)
  - History of previous CDI, aka relapse.
Controversy #2 – Who?

- (Inpatient) prevalence of *C. difficile* in children is increasing.
  - I have a low threshold for testing in the inpatient setting and a lower threshold for IBD patients admitted for flare.
- The presence of a positive stool test does not mean CDI is the cause of the symptom.
  - I generally treat if positive. Colonoscopy and biopsy can help, but we need a better way to diagnose infection vs colonization.

Current Testing Options

- Cytotoxin Neutralization Assay/Toxigenic Culture
- Microwell EIA/Rapid Cartridge EIA
- Glutamate Dehydrogenase Based Combination Procedures (common antigen screening to increase sensitivity)
- Molecular Procedures

Tarnished Gold Standard

- First diagnostic test surrounding the in 1978 by C. sordellii toxin (B) or *C. difficile* toxin.
- No longer sensitive and specific.
- Requires 24–48 h turnaround time (TAT), is labor intensive and expensive, and many laboratories do not perform tissue culture.

Problem

- Optimal laboratory diagnosis of CDI remains controversial/uncertain.
- Multiple tests with different *C. difficile* targets (the bacterium, toxin(s), toxin gene, glutamate dehydrogenase enzyme (GDH)) reflect and contribute to this uncertainty.

Current Testing Options

- Single toxin detection is inadequate to diagnose *Clostridium difficile* diarrhea in pediatric patients.
  - Kader et al (CHOP) (Gastroenterology 1998 115:1329)
  - Of 1061 specimens from patients with diarrhea 276 (26.8%) were positive for *C. difficile* toxin(s).
  - Toxin A 51 18.5%
  - Toxin B 133 48.2%
  - Both A+B 92 33.3%

Toxin A+B EIA!
“Fast is fine but accuracy is everything.”
—Wyatt Earp

Need to know 95% CI around Sensitivity and Specificity and Frequency of Occurrence to determine PPV and NPV

Even a blind squirrel finds an acorn once in a while

http://www.medcalc.com/bayes.html
Molecular Based Tests

- BD GeneOhm™ Cdiff Assay
- Gen-Probe Prodesse® ProGastro™ Cd
- Cepheid® Xpert C. difficile
- Meridian illumigene™ C. difficile

BD GeneOhm™ Cdiff assay

- BD GeneOhm™ Cdiff assay is a qualitative in vitro diagnostic test for the rapid detection of C. difficile toxin B gene (tcdB) in human liquid or soft stool specimens

<table>
<thead>
<tr>
<th>Assay Performance*</th>
<th>Dataset</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>93.8%</td>
<td>95.5%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100.0%</td>
<td>97.7%</td>
</tr>
</tbody>
</table>

* BD GeneOhm™ CDiff Quanta kit, BD Diagnostics 2005
  *(T)B: Detection of tcdB gene by tcdB target. TP= 22 of 22 positive, TN= 22 of 22 negative.*

Gen-Probe Prodesse® ProGastro™ Cd

- 3 hr PCR for tcdB

<table>
<thead>
<tr>
<th>OXYSUM ASSAY</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>TOTAL</th>
<th>AGREEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProGastro Cd</td>
<td>Positive</td>
<td>95</td>
<td>27</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>6</td>
<td>852</td>
<td>858</td>
</tr>
</tbody>
</table>

Cepheid® Xpert C. difficile

<table>
<thead>
<tr>
<th>Assay Parameter</th>
<th>C. difficile</th>
<th>TcdB</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TcdB</td>
<td>391</td>
<td>391</td>
<td>93.8%</td>
<td>94.8%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95.6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>96.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

45 min
Epidemic 027 Strain
Toxin B, Binary Toxin, and tcdC

PPV 0.76
NPV 0.99
LAMP - Illumigene

- The illumigene® C. difficile DNA molecular assay is based on loop-mediated amplification technology, which uses specifically designed primers to the PaLoc pathogenicity locus.
- Not tested in pediatrics but should detect Toxin A, B or A+B strains.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Speed</th>
<th>Convenience</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxin neutralization</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Toxigenic Culture</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Toxin EIA</td>
<td>+</td>
<td>+/+++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>GDH based algorithm</td>
<td>+++</td>
<td>+++/++++++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Molecular assay (PCR, LAMP)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

Controversy #3

- No test is perfect.
- Molecular tests are fast, likely to identify a true positive and there are very few false negatives. A negative test strongly suggests this is NOT C. difficile.

Repeat/Multiple Testing: YES for:

- Endocarditis
- TB
- Parasitic disease
- BUT for CDI: NO demonstration that you need three negative tests or that the yield significantly increases with further testing.

Value of Repeat Testing

<table>
<thead>
<tr>
<th>Reference</th>
<th>Test</th>
<th>No. of patients or colonies tested</th>
<th>No. of positive or positive results</th>
<th>No. of negative or negative results</th>
</tr>
</thead>
</table>

Conclusion: “little value of repeat testing for C. difficile by RIA or PCR.”


Value of Repeat Testing
C. difficile testing conclusions

- Many C. difficile toxin testing options
- Molecular assays perform very well
- Test only patients with diarrhea
- Repeat testing for toxin within 7 days of little value
- Only diarrheal stools should be submitted for testing
- Only a single specimen should be tested
- Used for diagnosis only and not “test-of-cure” – especially molecular approaches
- Children < 1 year old and maybe up to age 3 – higher threshold for testing - ?interpretation of positive results.

Questions?

- **Who** should you test?
  - Children >1-3 with (persistent) diarrhea.
- **How** should you test?
  - Use molecular tests
- **When** should you test/re-test?
  - One specimen, no repeat and no test of cure