Clostridium difficile-Associated Diarrhea

Robert Schlaberg, MD, MPH
Case

• 73 year-old female, diabetes, peripheral vascular disease
• Below-knee amputation
• Post-operative day 2
  – Low-grade fever, diffuse abdominal pain, cramps
  – Leukocytosis
• Post-operative day 5
  – *C. difficile* toxin
• Antibiotic therapy
• Post-operative day 7
  – Respiratory/cardiovascular failure, mental status changes, patient expires
• Postmortem
  – Pseudomembranous colitis (entire colon and rectum)
**Clostridium difficile**

- Anaerobic, gram-positive rod
- Toxigenic vs. non-toxigenic strains
- Spores
  - Resistant to heat, acid, antibiotic
- Vegetative forms
  - Toxin producing
Toxins

• Potent exotoxins
  – Receptors on intestinal epithelial cells
  – Mucosal injury, fluid secretion, inflammation -> colitis + watery diarrhea
  – Toxin A ("enterotoxin")
  – Toxin B ("cytotoxin")

• Stool toxin levels ~ disease severity

• PaLoc
  – Includes tcdA, tdcB

• Most strains: toxins A and B
  – Variant toxin expression

Nature. 2010 Oct 7;467:711
J Clin Microbiol 2000; 38:1696
J Clin Microbiol 2002;40:2079
Ann Intern Med 2001;135:434
NAP1/BI/027 Strain
Brief History of CDI

• 1935
  – Anaerobic, GPR, ‘normal flora of neonates’

• 1978
  – *C. difficile* as common cause of antibiotic-associated colitis
  – Clindamycin

• 1989-1992
  – Outbreaks with J strain, highly clindamycin R

• Association with other antibiotics
  – Penicillins, cephalosporins, fluoroquinolones...

• Since ~2002
  – NAP1/BI/027 (increased incidence/severity)
  – Fluoroquinolone R
  – Global, community associated, younger patients

• Any antimicrobial

Gastroenterology; 75(5):778-82
Am J Clin Pathol; 137(1):10-5
CMAJ; 171(5):466-72
Clin Infect Dis; 53(7):e81-90
Pathogenesis

- Fecal-oral colonization (spores)
- Antimicrobial therapy
- Disruption of normal intestinal flora
- Expansion of *C. difficile*
- Exotoxins A and B
- Intestinal epithelial disruption, ulcer
- Release: serum proteins, mucus, inflammatory cells
- Pseudomembranes
- Antibiotic-associated pseudomembranous colitis
Risk Factors

• Antibiotic use
• Hospitalization
• Advanced age
• Severe illness
  – Gastrointestinal surgery
  – Cancer chemotherapy
  – Hematopoietic stem cell transplantation
• Gastric acid suppression (PPI)
• Enteral feeding
• None (children, postpartum women)
Symptoms

• **Case definition of CDI**
  – Symptoms (diarrhea x3/24h)
  – Stool test or pseudomembranous colitis

• **Colitis with watery diarrhea**
  – Asymptomatic, pseudomembranous colitis, toxic megacolon
  – Abdominal pain, low-grade fever, leukocytosis (~15,000)

• Onset classically during/after antibiotic therapy
• Median onset 2-3 days after colonization
• Nosocomial vs. community acquired
• Recurrence in 10-25% (relapse > re-infection)
Adult *C. difficile*–related Hospitalizations

![Graph showing the number of cases per 10,000 population over years, with different age groups and overall trend lines increasing.]
Nosocomial

• Common healthcare-associated infection (HAI)
  – ~1% of hospitalized patients
• Increasing incidence, severity
• Carrier rate
  – Low in healthy adults
  – Up to 20% (hospitalized adults), 50% (long-term care)
  – Up to 50% in infants
  – Asymptomatic shedding
• Highly transmissible
  – Fomites (hands, clothing, stethoscopes)
  – Can be aerosolized

Am J Infect Control. 2009 May;37(4):263-70
Curr Opin Infect Dis, 25, 405-411
Infect Control Hosp Epidemiol;31(5):431-55
Prevention of HAI with *C. difficile*

- **Effective**
  - 20% reduction over ~21 months in 71 hospitals
- **Contact isolation**
  - Gloves, gowns
  - Hand hygiene
  - Soap, water during outbreaks/increased prevalence
  - Individual rooms or cohorting
- **Environmental cleaning (sporocidal)**
- **Antimicrobial stewardship**
- **Screening for carriers not recommended**
Community-Associated

- Increasing incidence
- Younger, healthier, less likely on antibiotics
- Less common severe infections
- Emerging sources
  - Food products
  - Domestic animals
Treatment

• **Antibiotic**
  – Metronidazole (PO, IV)
  – Vancomycin (PO)
  – Fidaxomycin (PO)

• **Under investigation**
  – IVIG, monoclonal anti-toxin antibodies
  – Intestinal microbiota transplantation
  – Vaccination
Diagnosis

- Indication for testing
  - 2 days of significant **diarrhea** (3+ stools/d)
  - 1 day of 10-15 stools/d, fever/nocturnal diarrhea
  - Exception: ileus

- Recurrence: same as initial episode

- No indication
  - Asymptomatic, i.e. **formed stool specimens**, unless **ileus** is suspected
  - No test of cure

Infect Control Hosp Epidemiol 2010; 31(5)
Laboratory Testing

• Toxigenic culture
  – Stool -> culture -> isolate -> cytotoxin detection

• Cytotoxin assay
  – Stool -> cytotoxin detection

• Toxin A/B EIA
  – Stool -> toxin EIA

• GDH + toxin detection
  – Stool -> GDH -> if positive: toxin detection

• NAAT
  – Stool -> NAAT
Cytotoxin Assay

- Fresh stool sample
  - Dilute, buffer, filter
- Inoculation of cultured cells
  - Human (foreskin) fibroblasts
- Incubate
- Cytotoxic effect (rounding)
  - Filtered sample (cytotoxic)
  - Preincubated with neutralizing antibody (normal)
- Sensitivity 67%-100%
- TAT ~ 24-48h
Toxigenic Culture

- Anaerobic stool culture
  - CCFA agar (cycloserine, cefoxitin, fructose)
  - No distinction: toxigenic vs. non-toxigenic strains
- Testing of *C. difficile* isolates for toxin production
  - Isolate suspension
  - Cytotoxicity assay
- Most sensitive
- TAT ~ 2-3 days, up to 9 days
- Surveillance (provides isolates)
Toxin A/B EIA

• Direct detection of toxin A/B
  – Filtered stool sample

• Toxin A-only assays not recommended
  – Variant and/or toxin A-non-expressing strains

• Rapid TAT

• Insufficient sensitivity
  – No significant improvement with early repeat testing
  – Performance varies between kits

• Sensitivity 63%–94%
GDH Screen + Toxin Detection

• EIA for glutamate dehydrogenase (GDH)
  – Produced by all *C. difficile* strains
  – Sensitivity 85%-95%

• Screening as part of multi-step algorithms
  – Detects toxigenic and non-toxigenic *C. difficile*
  – Separate conformation of toxin production
  – E.g. cytotoxicity assay, NAAT, toxin A/B EIA...

Infect Control Hosp Epidemiol;31(5):431-55
Example Algorithm

A)

GDH assay

positive

negative = Negative for CDI

Toxin A/B assay

positive = Positive for CDI

negative

PCR assay

positive = Positive for CDI

negative = Negative for CDI
Nucleic Acid Amplification Tests

• Methodologies
  – Real-time PCR
  – Isothermal amplification (LAMP and others)
• High sensitivity
• High specificity
• Rapid
  – Treatment, infection control
• More expensive, less experience
• Limited PPV with prevalence <10%

# FDA-Cleared NAAT

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manufacturer</th>
</tr>
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<tbody>
<tr>
<td>BD GeneOhm C. diff Assay</td>
<td>BD Diagnostics, Inc.</td>
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<tr>
<td>Illumigene C. difficile Assay</td>
<td>Meridian Bioscience, Inc.</td>
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<tr>
<td>ProGastro Cd Assay</td>
<td>Gen-Probe, Inc.</td>
</tr>
<tr>
<td>Simplexa C. difficile Universal Direct Assay</td>
<td>Quest Diagnostics</td>
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<tr>
<td>Verigene C. Difficile Nucleic acid Test</td>
<td>Nanosphere, Inc.</td>
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<tr>
<td>Xpert C. difficile</td>
<td>Cepheid</td>
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<tr>
<td>Xpert C. difficile/Epi</td>
<td>Cepheid</td>
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</table>

http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial
FDA-Cleared/Approved Molecular Diagnostic Tests (AMP, July 5<sup>th</sup>, [www.amp.org/FDATable/FDATable.doc](http://www.amp.org/FDATable/FDATable.doc))
Considerations

• Assay comparison is difficult
  – No single accepted reference test
  – Lack of standardized protocols for reference tests
  – Regional differences in strain types

• Repeat testing
  – Not indicates (7 days commonly used)

• Test of cure
  – Not indicated (2-4 weeks commonly used)
Laboratory Testing - Summary

• Optimal strategy has not been determined

• Reference methods
  – Cytotoxicity (tissue culture) assay: specific, slow, requires cell culture, not standardized
  – Toxigenic culture: most sensitive, provides isolate, slow, requires cell culture, not standardized

• Frequently used
  – Toxin A/B EIA: rapid, inexpensive, insensitive
  – GDH + toxin A/B EIA: rapid, high negative predictive value, sensitivity variable (GDH) and limited by toxin A/B EIA
  – NAAT: rapid, sensitive, more data needed

Infect Control Hosp Epidemiol. 2010 May;31(5):431-55
## Test and Algorithm Comparison

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EIA only</th>
<th>GDH + EIA</th>
<th>GDH + EIA + cytotoxin&lt;sup&gt;b&lt;/sup&gt;</th>
<th>GDH + Xpert&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Xpert only&lt;sup&gt;d&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>No. of specimens</td>
<td>432</td>
<td>432</td>
<td>431</td>
<td>432</td>
<td>428</td>
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<tr>
<td>Sensitivity</td>
<td>58.3 (42/72)</td>
<td>55.6 (40/72)</td>
<td>83.1 (59/71)</td>
<td>86.1 (62/72)</td>
<td>94.4% (68/72)</td>
</tr>
<tr>
<td>Specificity</td>
<td>94.7 (341/360)</td>
<td>98.3 (354/360)</td>
<td>96.7 (348/360)</td>
<td>97.8 (352/360)</td>
<td>96.3 (343/356)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>88.7 (383/432)</td>
<td>91.2 (394/432)</td>
<td>94.4 (407/431)</td>
<td>95.8 (414/432)</td>
<td>96.0 (411/428)</td>
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<tr>
<td>PPV</td>
<td>68.9 (42/61)</td>
<td>87.0 (40/46)</td>
<td>83.1 (59/71)</td>
<td>88.6 (62/70)</td>
<td>84.0 (68/81)</td>
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<tr>
<td>NPV</td>
<td>91.9 (341/371)</td>
<td>91.7 (354/386)</td>
<td>96.7 (348/360)</td>
<td>97.2 (352/362)</td>
<td>98.8 (343/347)</td>
</tr>
</tbody>
</table>

### Comparison to CYT and DPCR results<sup>b</sup>

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>No. of specimens</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% PPV (95% CI)</th>
<th>% NPV (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>CYT (tissue culture)</td>
<td>Positive</td>
<td>47</td>
<td>58.8 (47.8–68.9)</td>
<td>100</td>
<td>100</td>
<td>94.9 (93.0–96.4)</td>
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<tr>
<td></td>
<td>Negative</td>
<td>33</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GDH-Q</td>
<td>Positive</td>
<td>69</td>
<td>86.3 (76.9–92.3)</td>
<td>92.7</td>
<td>60.5</td>
<td>98.1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>11</td>
<td></td>
<td>96.6 (96.3–99.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-step GDH-Q/AB-Q</td>
<td>Positive</td>
<td>26</td>
<td>32.5 (23.2–43.4)</td>
<td>99.7</td>
<td>92.9</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>54</td>
<td></td>
<td>76.3 (76.3–99.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three-step GDH-Q/AB-Q/DPCR</td>
<td>Positive</td>
<td>67</td>
<td>83.8 (74.0–90.4)</td>
<td>99.7</td>
<td>97.1</td>
<td>97.9</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>13</td>
<td></td>
<td>89.4 (89.4–98.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
General Testing Recommendations

- Detection of *C. difficile* toxins by EIA is not recommended as a stand-alone test.
- Only test diarrheal (e.g., unformed) stool (>2 loose stools/day for 1-2 days).
- Non-diarrheal stool should only be tested with suspected fleas due to *C. difficile*.
- Testing of asymptomatic patients and test of cure is not clinically useful.
- Repeat testing during the same episode of diarrhea is not recommended.

1 Modified from “Clinical Practice Guidelines for *Clostridium difficile* infection in Adults. 2010 Update by SHEA and IDSA” and “A practical guidance document for *C. difficile* toxin laboratory testing” (ASM, 6/24/2010)

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**RECOMMENDED SINGLE TEST**

**TURNAROUND TIME ≤24 HRS**

ORDER

*Clostridium difficile* toxin B gene (tcdB) by PCR

(nucleic acid amplification test)

- Positive: Toxigenic *C. difficile* detected
- Negative: Toxigenic *C. difficile* not detected

**RECOMMENDED ALTERNATIVE**

**TURNAROUND TIME DEPENDS ON RESULT**

ORDER

Glutamate Dehydrogenase Antigen (EIA)

Detects toxigenic and non-toxigenic *C. difficile*

- Positive: *C. difficile* antigen not detected
- Negative: Toxigenic *C. difficile* detected

**REFERENCE METHOD**

**TURNAROUND TIME 48-96 HRS**

ORDER

*Clostridium difficile* Culture with Reflex to Cytotoxin Cell Assay

- Positive: Toxigenic *C. difficile* not detected
- Negative: Toxigenic *C. difficile* detected

The following testing may be considered when high level of clinical suspicion:

- *Clostridium difficile* Culture with Reflex to Cytotoxin Cell Assay
- Endoscopy

**The following testing may be considered when high level of clinical suspicion:**

- *Clostridium difficile* toxin B gene (tcdB) by PCR
- *Clostridium difficile* Culture with Reflex to Cytotoxin Cell Assay
- Endoscopy

**The following testing may be considered when high level of clinical suspicion:**

- *Clostridium difficile* toxin B gene (tcdB) by PCR
- Endoscopy

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http://www.arupconsult.com/Algorithms/CDAD.pdf
**SHEA, IDSA Guidelines**

- Test only diarrheal stool (exception: ileus)
- Don’t test if asymptomatic (test of cure)
- Culture most sensitive, not clinically practical
  - Reference test if performed by experienced lab
- Toxin A/B EIA suboptimal (rapid, less sensitive)
- GDH screening + cytotoxicity/culture
  - Sensitivity varies by kit, interim recommendation
- NAAT rapid, sensitive, specific
  - More data on utility necessary
- No repeat testing during same episode
Thank you for your attention