Clostridium difficile: Overview

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Clostridium difficile

- Anaerobic spore-forming bacillus
- Pseudomembranous colitis, toxic megacolon, sepsis, and death
- Fecal-oral transmission through contaminated environment and hands of healthcare personnel
- Antimicrobial exposure is major risk factor for disease

Clostridium difficile

- Microbiology
- Epidemiology
  - Transmission of disease
- Pathogenesis
- Clinical manifestations of disease
- Diagnosis
Clostridium difficile: the Organism

- An anaerobic, spore forming gram positive rod first described as a member of the normal flora of healthy infants in 1935
- Due to the difficulty in cultivating the organism it was originally called Bacillus difficilis. Given its current name 3 years later
- Closely related to C. sordellii but not to other toxigenic clostridia like C. perfringens, C. botulinum or C. tetani.
- Can be grown on selective media (CCFA)

Microbiology and Ecology

- Vegetative vs spore forms
C. difficile
Dual Modes of Existence
- **Vegetative form**
  - Found in the gut, survives in the environment for 4-6 hours
  - Susceptible to gastric acid, antibacterial soaps, alcohol based hand foams
  - Neutralization of stomach acid may allow the vegetative form to become pathogenic
- **Spore form**
  - Found in the gut and in the environment
  - Spore formation induced by stressing the organism: exposure to the environment, exposure to antimicrobials, sublethal concentrations of microbicidal cleaning products
  - Resistant to stomach acid, antibacterial soaps and alcohol based hand foams
  - Can survive for years on surfaces

Toxin Production
- 5-25% of strains do not produce toxins and don’t cause disease
- **Toxin A**
  - 308 kd enterotoxin and cytotoxin
  - Chemoattractant for neutrophils
  - Activator for macrophages and mast cells
- **Toxin B**
  - 269 kd potent cytotoxin
  - Disrupts the actin cytoskeleton
  - Found to be a potent necrotizing enterotoxin
  - Probably acts synergistically with toxin A

Resistance to Disinfectants
Acquisition of the Organism

- In healthy adults intestinal carriage rates of toxigenic *C. difficile* are 5% or less
- Carriage in healthy adults thought to be transient
- High prevalence in newborns- up to 60-70% - all asymptomatic
- 20% hospitalized adults asymptomatic carriers
- Carriage rate in long term care facilities up to 50%
- In hospitalized patients colonization begins upon admission in patients given antimicrobials
  - 13-20% within the first week
  - 50% in patients hospitalized for more than 4 weeks
- Even relatively minute amounts of antimicrobials can predispose to colonization

Environmental Sources of *C. difficile*

Are Animal Reservoirs Important in Human Disease?

- 20-40% carriage rate
Changing Epidemiology of CDI

- Increasing incidence and severity
  - Based on CDC data, national hospital discharge data, reports from healthcare systems, death certificate data

- Recent outbreaks of severe disease caused by epidemic strain of *C. difficile* with increased virulence, antibiotic resistance

- Although elderly are still most greatly affected, more disease reported in "low-risk" persons
  - Healthy persons in community, peripartum women

Rates of CDI Nearly Tripled in U.S. Hospitals between 2000 and 2005

![Graph showing rates of CDI discharges per 100,000 population from 1996 to 2005.](http://www.hcup-us.ahrq.gov/reports/statbriefs/sb50.pdf)

National Estimates of US Short-Stay Hospital Discharges with *C. difficile* as First-Listed or Any Diagnosis

![Graph showing number of discharges with CDI from 1997 to 2006.](http://www.hcup-us.ahrq.gov/reports/statbriefs/sb50.pdf)
Yearly *Clostridium difficile*-related Mortality by Listing on Death Certificates, United States, 1999–2004.

CDI Rates and Mortality Increase with Increased Patient Age

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>CDI Rate per 1000 Admissions</th>
<th>Attributable 30-Day Mortality Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>3.5</td>
<td>2.6</td>
</tr>
<tr>
<td>41-50</td>
<td>11.2</td>
<td>1.2</td>
</tr>
<tr>
<td>51-60</td>
<td>20.0</td>
<td>3.2</td>
</tr>
<tr>
<td>61-70</td>
<td>24.4</td>
<td>5.1</td>
</tr>
<tr>
<td>71-80</td>
<td>38.3</td>
<td>6.2</td>
</tr>
<tr>
<td>81-90</td>
<td>54.5</td>
<td>10.2</td>
</tr>
<tr>
<td>&gt;90</td>
<td>74.4</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Loo et al NEJM 2005;353:2442-9

Current Epidemic Strain of *C. difficile*

- BI/NAP1/027, toxinotype III
- Historically uncommon, now epidemic
- Current strain more resistant to fluoroquinolones
- Carries extra toxin known as binary toxin
- Polymorphism in toxins A and B regulatory gene (*tcdC*) and increased toxin production *in vitro*
Increased Toxin B Production \textit{In Vitro}

In vitro production of toxins A and B by \textit{C. difficile} isolates. Median concentrations and IQRs are shown. \textit{C. difficile} strains included 25 toxinotype 0 and 15 NAP1/027 strains (toxinotype III) from various locations.


CDI in Previously Low-Risk Populations

- 10 Pregnant women
- 23 Generally healthy persons in the community
- Cases without precedent antimicrobial use


\textit{C. difficile}: Asymptomatic Carriers a Reservoir for Transmission?

Rationale to consider extending isolation beyond duration of diarrhea


Outcomes of CDI in Setting of Endemic Disease

- Excess costs
  - $2,380 to $3,240 per index hospitalization
  - $3,797 to $7,179 inpatient costs over 180 days of follow-up
- Other outcomes
  - 2.8 days attributable excess length of stay
  - 19.3% attributable readmission (180 days)
  - 5.7% attributable mortality (180 days)
  - More likely discharged to long-term care

Dubberke ER, et al. 17th Annual Meeting of The Society for Healthcare Epidemiology of America (SHEA), April 14-17, 2007; Baltimore, MD.
Unpublished data.

C. difficile Associated Diarrhea: Risk factors

- Established Risk Factors
  - Antibiotic use
  - Hospitalization
  - Advanced age
  - Severe illness
- Possible Risk Factors
  - Gastric acid suppression (PPI)
  - Enteral feeding
  - GI surgery
  - Cancer chemotherapy
  - Bone marrow transplant
<table>
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<tr>
<th>Risk Factor by Multivariable Analysis</th>
<th>OR (95% CI)</th>
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<tbody>
<tr>
<td>C. difficile-associated disease pressure</td>
<td>Reference</td>
</tr>
<tr>
<td>&lt;0.03</td>
<td></td>
</tr>
<tr>
<td>0.3-1.4</td>
<td>2.9 (2.1-4.2)</td>
</tr>
<tr>
<td>&gt;1.4</td>
<td>4.0 (2.9-5.6)</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>Histamine-2 blockers</td>
<td>2.0 (1.6-2.5)</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>1.6 (1.3-2.1)</td>
</tr>
<tr>
<td>Fluoroquinolones, &gt;7 days</td>
<td>2.5 (1.8-3.5)</td>
</tr>
<tr>
<td>IV vancomycin, &gt;7 days</td>
<td>1.9 (1.3-2.7)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>0.5 (0.3-0.6)</td>
</tr>
</tbody>
</table>

CI=confidence interval; IV=intravenous; OR=odds ratio.

C. difficile-Associated Disease Pressure

- **Unit A**: Fewer patients with active CDI = lower risk of acquiring CDI.
- **Unit B**: More patients with active CDI = higher risk of acquiring CDI.

**CDAD pressure**

\[ \text{CDAD pressure} = \text{number of days in unit} \]

**Antimicrobial agents that may induce clostridium difficile diarrhea and colitis**

<table>
<thead>
<tr>
<th>Frequently associated</th>
<th>Occasionally associated</th>
<th>Rarely associated</th>
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<tbody>
<tr>
<td>Fluoroquinolones</td>
<td>Macrolides</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Trimethoprim</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td>Penicillins (broad spectrum)</td>
<td>Sulfaonnides</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>Cephalosporins (broad spectrum)</td>
<td></td>
<td>Metronidazole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vancomycin</td>
</tr>
</tbody>
</table>
**Prerequisites for CDI**

- Antimicrobial therapy
- Disturbed colonic microflora
- Acquisition of toxigenic *C. difficile*
- Toxin A & Toxin B production

- Advanced age
- Underlying illness

**Pathogenesis of *Clostridium difficile* diarrhea**

- Antibiotic therapy
- Disruption of colonic microflora
- *C. difficile* exposure and colonization
- Release of toxin A ("enterotoxin") and toxin B ("cytotoxin")
- Mucosal injury and inflammation

**C. difficile: Pathogen Induced Inflammatory Response**

- Toxin A is a potent enterotoxin (causes fluid loss) and a very active white blood cell attractant.
- Toxin B is a potent cell cytotoxin (kills cells)
- Fecal Lactoferrin, Interleukin-1beta, and Interleukin-8 are elevated in patients with severe CDI. Clin Diagnostic Lab Immuno 1997;6:719-722
- Cyclooxygenase (Cox)-2 expression and prostaglandins are elevated in the presence of Toxin A. JID 2001;184:648-52
C. Difficile: Role of Host Factors

- Toxin A antibody production
  - Asymptomatic carriers have higher levels of IgG
  - Colonization with nontoxigenic strains is protective
- Interleukin (IL)-8 levels
  - Impaired humoral response and increased susceptibility to CDAD
- Intestinal toxin receptors
  - Absence of receptors in neonates protective

Antibiotic-associated osmotic versus Clostridium difficile diarrhea

Pathogenesis of C. difficile Infection (CDI)
**C. difficile Infection: Clinical Disease Spectrum**

- Asymptomatic colonization more common than clinical illness of any type
- Mild diarrhea, minimal discomfort
- Typical CDAD:
  - Crampy abdominal pain
  - Profuse diarrhea with mucoid, greenish watery stool with a characteristic odor
  - Low grade fever and leukocytosis
- More severe disease with high fever, marked leukocytosis
C. difficile Infection (CDI): Clinical Presentation

- Collitis can be throughout colon, but usually most severe in distal colon and rectum
- Localized right sided disease can present a diagnostic challenge
- Fulminant disease with ileus, toxic megacolon
- Uncommonly extraintestinal disease
- Reactive arthritis
Normal Colon and Pseudomembranous Colitis (PMC) as seen at Colonoscopy

Normal Colon

Colon with PMC due to Clostridium difficile Infection

Pseudomembranous colitis

Gross appearance of the colon from a patient with pseudomembranous colitis. The pseudomembranes are yellow or off-white raised plaques 0.2 to 2.0 cm in diameter, which are scattered over festoon normal-appearing intervening mucosa. Courtesy of J Thomas Laffon, MD.

Pseudomembranous colitis

Endoscopic appearance of Clostridium difficile-induced pseudomembranous colitis. Left panel: scattered pseudomembranes are visible on top of the mucosa, being separated by areas of relatively normal mucosa. Some of the lesions have a red base (arrows). Right panel: yellow pseudomembrane circumferentially covering the entire colonic mucosa. Courtesy of James B. McGee, MD.
Histological Appearance of Pseudomembranous Colitis (PMC)

Colon Autopsy Specimen of a Patient who Died of Severe CDI: Confluent PMC is Evident
C. difficile: Diagnosis

- Definition of diarrhea
  - 3 or more loose (unformed) stools in 24 or fewer consecutive hours
  - Many stools (10-15) with fever or nocturnal diarrhea even if duration less than 24 hours
  - Patients with ileus may not have frequent stools
  - Patients on bulk forming agents may not have loose stools

- Definition of unformed stools
  - Stool takes the shape of the container

- Testing of asymptomatic pts not indicated

C difficile Diagnosis

- Endoscopy looking for pseudomembranes
  - Quick, and specific
  - Pseudomembrane formation usually starts on the right side
  - Pseudomembrane formation a late manifestation

- Culture – Gold Standard
  - Takes several days
  - Need cytotoxic assay of organism after culture
  - Immunoassays
  - PCR

Methods Evaluated for Detecting

<table>
<thead>
<tr>
<th>Diagnostic Assays Investigated</th>
<th>Number of Studies (n)</th>
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<tbody>
<tr>
<td>Toxin A and B Immunoassays</td>
<td></td>
</tr>
<tr>
<td>Premier Toxin A&amp;B, Meridian</td>
<td>7</td>
</tr>
<tr>
<td>Tox A/B II, TechLab</td>
<td>6</td>
</tr>
<tr>
<td>Tox A/B QUIK CHEK, TechLab</td>
<td>4</td>
</tr>
<tr>
<td>ImmunoCard A&amp;B, Meridian</td>
<td>7</td>
</tr>
<tr>
<td>Xpect Toxin A/B, Remel</td>
<td>4</td>
</tr>
<tr>
<td>ProSpecT Toxin A/B, Remel</td>
<td>2</td>
</tr>
<tr>
<td>VIDAS C. diff Tox A/B, bioMerieux</td>
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</tr>
<tr>
<td>Gene Detection Tests</td>
<td></td>
</tr>
<tr>
<td>GeneOhm, Becton Dickinson</td>
<td>3</td>
</tr>
<tr>
<td>GeneXpert, Cepheid</td>
<td>2</td>
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C. difficile: Summary

- Causes antibiotic associated colitis but manifests spectrum of disease from asymptomatic to fulminant toxic megacolon
- Colonization occurs via fecal oral route and requires disruption of normal intestinal flora by antimicrobial therapy but not always
- Increase in frequency, severity and refractoriness to therapy attributed to hypervirulent NAP1/B1/027 strain
- Diagnosis based on lab tests detecting toxin producing strains by EIA or PCR or endoscopic identification of pseudomembranes
- Challenges for Infection Control
  - Early diagnosis
  - Resistance of spores to disinfection,
  - High levels of environmental contamination,
  - Persistent shedding beyond duration of diarrhea
  - Overuse of antibiotics
  - Increase in community associated infection