Straight Poop about *Clostridium difficile*
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Many Interesting Aspects
- History
- Pathology
- Bacteriology and cell biology
- Epidemiology
- Clinical manifestations
- Drug therapy
- Alternative therapies

Pseudomembranous Colitis (PMC)
- Organism first isolated from healthy infants in 1935
- PMC described decades ago (Penner & Bernheim, Arch. Pathol., 1939; Kay, Brit. J. Surg., 1958 and even before that!)
- Speculation as to etiology included infection (possibly viral), antibiotics, ischemia, and toxins

Pathology of Pseudomembranous Colitis
- Pathologists can grade severity but with far advanced disease:
  - complete structural loss of the mucosa with thick covering of fibrin, mucous & inflammatory debris that constitutes the pseudomembrane

Endoscopic Appearance
**Staphylococcal Enteritis**

- 1950s: some noted heavy growth in stools of those who appeared to have the disease
- But seldom had pseudomembranes, patients usually had mild diarrhea and self-limiting
- Furthermore, heavy growth of Staph. in patients with no GI sx's, esp. in those receiving abx
- Animal models unsuccessful.
- Organism recovered no more frequently than anticipated by chance alone.
- By 1977, had been debunked as cause (Gorbach & Bartlett, JID, 1977)

**PMC linked to antibiotic use**

- Associated with clindamycin use (Cohen, JAMA, 1973; Tedesco, AM, 1974)
- Nearly all antibacterial antibiotics have been implicated
- In hamster model, sulfonamides do not provoke

**Discovery of Role of Toxin Produced by Clostridium difficile**

- Toxin described in 5/6 patients in UK with PMC (Larson, BMJ, 1977)
- Tissue cultures inoculated with fecal suspensions developed toxic effect overnight
- No viruses detected
Larson (cont)

• Determined to be heat-labile substance with MW > 6,000
• Not found in stools from a wide variety of other intestinal disorders
• “If it is proved to be caused by a bacterial toxin then it may become possible to treat it with specific antibacterial measures.”

Hamster Model

Hamsters & Clostridium difficile

• Using key search terms "Clostridium difficile" AND "hamster" yielded 224 articles (PubMed accessed 6/14/2011)
• "Clindamycin-induced enterocolitis in hamsters" Lusk et al, J. Infect. Dis. 1978
• Still used as model to understand biology & for drug development

Clostridium difficile in Culture

Characteristics of Colonies

• On anaerobic blood agar: large, white, circular, matte to glossy, convex, opaque, nonhemolytic, horse stable odor, fluoresces yellow-green
• On cycloserine-cefoxitin-fructose agar: yellow, ground glass

C. diff. Photomicrograph
**Bacteriology**

- Gram-positive, spore-forming rod
- Strict anaerobe
- Virulence factors
  - Toxin A, primarily an enterotoxin, weak cytotoxin
  - Toxin B, potent cytotoxin, 1,000 x more potent
  - Binary toxin
  - Adhesin factor
  - Hyaluronidase
  - Spore formation

**Structure of *C. diff.* toxin**

**Structure of *C. diff.* toxins**

**C. diff. Immunology**

- Asymptomatic carriers of *C. diff.* have high levels of IgG Ab to toxin A & decreased risk of developing *Clostridium difficile* infection CDI compared to noncarriers
- Vigorous serum Ab response to toxin A during initial episode is associated with protection vs. recurrent disease
  (Gould & McDonald, Critical Care, 2008)

**C. difficile Epidemiology**

- Found in stools of 25% to 80% healthy infants
- 3% healthy adults in community colonized.
- Leading cause of nosocomial diarrhea. Good percentage of patients became colonized during hospitalization (1-13% < 1 wk, 50% > 4 weeks)
- All antibiotics are risk factors incl. some anticancer drugs
Adjusted Hazard Ratio for C. diff. by Antibiotic Received (Pepin, C.I.D., 2005)

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>AHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolones</td>
<td>3.44</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Up to 1.89</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>1.77</td>
</tr>
<tr>
<td>Beta-lactam/beta-lactam inhibitors</td>
<td>1.88</td>
</tr>
<tr>
<td>Macrolides</td>
<td>1.65</td>
</tr>
<tr>
<td>Narrow spectrum penicillins</td>
<td>1.37</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Additional Risk Factors for CDI

- Advanced age
- Severe underlying disease
- Immunosuppression
- Gastrointestinal surgery
- NG tubes
- Possibly gastric acid suppression
  (Gould & McDonald, Critical Care, 2008)

C. difficile Epidemiology

- Patient to patient transmission
- Organism found on environmental surfaces in rooms of infected patients, electronic thermometers on hands, clothing and stethoscopes of HCWs
- Outbreaks in hospitals and ECFs
- Infection adds 3.6 days to LOS and $3,669 to hospital costs. Up to $1.1 BILLION per year
- Recent incr. in young, healthy individuals from the community and in pregnant and peripartum women due to the NAP1/027 strain

C. diff Mortality USA per Million

Severe C. difficile Colitis

- Incidence increasing recently
- Canadian study 1991-2003
  - Incidence 1991 35.6/100,000 vs 2003 156.3/100,000
  - Complicated: toxic megacolon, perforation, shock, colectomy or death
  - Overall incidence complicated Clostridium difficile infection (CDI) increased from 7.1% in 1991 to 18.2% in 2003, 30 day mortality from 4.7% to 13.8%
- Factors associated with complicated CDI include age > 65 yr, WBC > 20K, elevated creatinine

Manifestations of Clostridium difficile Infection

- Asymptomatic carriers
- Mild to moderate colitis with acute watery diarrhea, abdominal pain, fever
- Severe C. diff. colitis with WBC > 15K, creatinine >/= 1.5 baseline, hypoalbuminemia
- Severe complicated C. diff: ileus, toxic megacolon, perforation, need for colectomy or death


New Epidemic Strain of C. diff. B1/NAP1/027

- Restriction endonuclease analysis group B1, N. Am. pulse-field type 1, PCR ribotype 027
- Overproduces toxins A & B related to deletion in tcdC regulatory gene
  - 16-fold higher concentrations of toxin A & 23-fold higher conc. of toxin B in vitro (Warney, Lancet, 2005)
- Also carries binary toxin
- Fluoroquinolone resistant, FQ use predominant risk factor for CDI in some studies
- US study 51% isolates in outbreaks caused by this strain (NEJM 2005; 353:2433)

More C. diff. Epi.

- Projected 450,000 to 750,000 cases per annum in USA 2010
- Ribotype 027 has persisted as dominant C. diff. epidemic strain in USA
- By July 2008, 027 describes in outbreaks in 16 European countries, although Dutch have recently experienced decrease in 027 CDI

Community Acquired CDI

- CDI acquired outside health care facilities
- CDI with onset in community or within 48 hrs of admission who has not been in health care facility (HCF) in previous 12 weeks
- 2006 study Connecticut 60% of community onset CDI met definition of community acquisition
- In Netherlands, among toxin positive, 65% had not been admitted to HCF in previous year and 42% had not used antibiotics in 6 months previous, 26% had neither risk factor
Diagnosis

• Tissue culture assay for toxin
• Anaerobic culture with detection of toxin in isolated C. diff.
• ELISA for toxin A &/or B

Diagnosis

• ELISA for glutamate dehydrogenase
• PCR based assays for C. diff. toxin B gene
  – Sensitivity 93%, specificity 94%
• Sigmoidoscopy can miss 10% in right colon only
• Positive toxin assays may remain positive after successful treatment.

Antimicrobial Therapy of CDI

• Metronidazole for 10 days previously equivalent to vancomycin for 10 days in resolving symptoms and risk of relapse
• When do you expect them to get better?
  – Retrospective study, mean duration of symptoms was significantly shorter in those treated with vancomycin (3 days) vs those treated with metronidazole (4.6 days) Wilcox, JAC, 1995
• 95% generally resolve symptoms with original therapy but approx. 20% relapse
• Metronidazole had been preferred because of much lower cost and avoidance of selective pressure for vancomycin resistance (e.g. VRE)
• Recent studies
  – Vancomycin 5-fold lower odds ratio of complicated disease
  – RCT showed better response to initial vancomycin (97%) vs metronidazole (76%) in severe disease

<table>
<thead>
<tr>
<th>Disease severity</th>
<th>No. of patients cured/ no. of patients treated (%)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Mtz group 37/41 (90) Vs Vm group 30/40 (98)</td>
<td>.36</td>
</tr>
<tr>
<td>Severe</td>
<td>Mtz group 29/36 (76) Vs Vm group 30/31 (97)</td>
<td>.02</td>
</tr>
<tr>
<td>All</td>
<td>Mtz group 66/79 (84) Vs Vm group 69/71 (97)</td>
<td>.07</td>
</tr>
</tbody>
</table>

NOTE: Mtz, metronidazole; Vm, vancomycin.
* $P$ values were calculated using Fisher’s exact test.

Treatment

• 41% failure rate of patients treated with metronidazole who remained on antibiotics

Latest Guidelines Initial Therapy of CDI

• 1. Mild disease- metronidazole
• 2. Severe disease - vancomycin
• 3. Pregnant women, children - vancomycin
Houston VA Experience

- Prospective study of 207 pts with C. diff, all treated with metronidazole at least 1.5 gm/day x at least 7 days
- Only 50% had complete clinical cure without relapse at 3 mos. if/u
- 22% were refractory to therapy
- 28% had recurrence within 90 d
- 21% mortality among those who were cured vs 33% mortality among those who did not respond P < 0.05

Quebec Experience with Metronidazole

- Recurrence rates were 21% now 47%
- Recurrence rates now 58% if over 65 years

Fidaxomicin-It’s New!

- Macrocyclic antibiotic more active in vitro than vancomycin by 8 x, is bactericidal, narrow spectrum with minimal activity on GNR
- RCT Phase 3 fidaxomicin 200 mg bid noninferior to vanco 125 mg qid x 10 days for clinical cure. Excluded pts with fulminant disease, IBD, or more than one occurrence in past 3 months before start of study
- Fidaxomicin had significantly fewer relapses than vancomycin, 15% vs 25%, but difference in relapse seen only in patients not infected with 027
- May 31, 2011, FDA granted approval

Fidaxomicin/Dificid

- Among non-027 strains
  - Fidaxomicin 7.8% recurrence vs 25.5% recurrence with vancomycin
- 69% reduction in recurrences
- 3.3 times relative risk of recurrence for those treated with vanco vs fidaxomicin

Fidaxomicin (Dificid) Structure

May 31, 2011, FDA granted approval
Mean Fecal Concentrations of Fidaxomicin (OPT-80) and Primary Metabolite, OP-1118, on Day 10 of Dosing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Subjects</th>
<th>Mean Fecal Conc. (ug/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OPT-80</td>
</tr>
<tr>
<td>100 mg/day</td>
<td>11</td>
<td>256</td>
</tr>
<tr>
<td>200 mg/day</td>
<td>9</td>
<td>442</td>
</tr>
<tr>
<td>400 mg/day</td>
<td>13</td>
<td>1433</td>
</tr>
</tbody>
</table>

Fidaxomicin (Dificid)

- Prolonged post-antibiotic effect
- Minimal effect on normal gut flora
- This might maintain colonization resistance, inhibiting reemergence of *C. diff*?
- Stool concentrations thousands of times higher than MIC

Rx of Severe, Complicated *C. diff*.

- Higher dose po vanco 500 q 6hr
- +/- iv metronidazole
- Vanco enemas 500 mg in per rectum q 6 hr
- Subtotal colectomy esp. with toxic megacolon or perforation

Predictors of Those Requiring Surgery & Predictors of Mortality

- Early predictive factors for those requiring surgery: age > 65 yrs, WBC >/= 20K, serum lactate 2.2-4.9 meq/L
- Perioperative mortality increased substantially when serum lactate >/=5 or WBC >/= 50K

Recurrent CDI

- First recurrence, treat as for initial episode, metronidazole or vanco depending on severity
- In Phase III trial, fidaxomicin not significantly better than vancomycin for those with previous episode of disease (MITT P = 0.30)
- Second recurrence, vanco in tapered and/or pulse regimen-vanco 125 mg qid x 10-14 d
  - Then 125 mg bid x 7 d
  - Then 125 mg qd x 7 d
  - Then 125 mg q 2 or 3 days x 2-8 weeks

More Recurrent CDI

- No evidence that adding cholestyramine or rifampin to treatment regimen decreases risk of further recurrence
- Uncontrolled series multiple recurrences CDI, oral rifaximin 400 mg bid x 2 weeks cured 7 of 8 when started immediately after last course of vanco
- Administration of *Sacharomyces boulardii* (Florastor) 500 mg bid x 30 days with vancomycin appeared to decrease the number of recurrences. Not covered by insurance, costs approx. $100
Alternative Therapies

- Nitazoxanide
- Rifaximin
- Oritavancin
- Ramoplanin
- Tigecycline
- Rifalazil
- Immunoglobulins
- Monoclonal antitoxin antibody
- Fecal bacteriotherapy

Nitazoxanide

- Licensed by FDA for Rx of giardiasis and cryptosporidiosis
- 500 mg bid x 7 days or 10 days noninferior to metronidazole x 10 days. No significant difference in median time to resolution
- Nitazoxanide vs vancomycin similar response rates 77% vs 74% but #s too small to prove noninferiority
- Salvage therapy for those who failed metronidazole with persistent sx or mult. relapses. 54% response rate with no relapses over 60 d f/u
- High cost

Rifaximin

- Approved for traveler’s diarrhea and for hepatic encephalopathy
- Rifamycin drug, inhibits bacterial RNA polymerase
- Ess. not absorbed, fecal concentrations 8,000 mcg/g
- One tiny trial (N=20) response rates similar to vancomycin
- Recurrent CDI-used as a “chaser” x 2 weeks following a course of vanco, resolved CDI in 7 of 8 patients who previously had suffered unremitting recurrent CDI. Dose 400 mg to 800 mg daily.
- Second small series use to treat 6 pts with recurrent CDI-dosed 400 mg tid x 14 d followed by 200 mg tid x 14 d. 5 of 6 had resolution

Oritavancin

- Semisynthetic lipoglycopeptide
- Oritavancin rapidly reduced numbers of both vegetative and spore forms
- Inhibition of outgrowth of 027 spores observed even after drug removed by washing
- Could translate into lower likelihood of symptomatic relapse?

Ramoplanin

- Glycodepsipeptide-inhibits peptidoglycan synthesis but cross resistance unlikely with vancomycin different target sites
- Nonabsorbable, high fecal concentrations, low MIC
- Effective in hamster model
- In phase III trials for CDI

Tigecycline

- Minocycline derivative
- Low MIC 90s vs C. diff., 0.06 to 0.25
- Median fecal concentrations 5.6 mcg/ml
- Series of 4 patients with severe, complicated CDI
- All 4 had clinical resolution within 7 days, no relapses within 3 mos. f/u
Rifalazil

- Rifamycin
- T ½ 100 hrs
- Poorly absorbed
- In hamster model superior to vancomycin
- May be more effective preventing relapse
- AEs HA, myalgia, back pain, dizziness, fever

Monoclonal Antibodies vs Toxins A & B

- Large trial N = 200
- Rx with either vancomycin or metronidazole
- Recurrence rate 7% for Ab group vs 25% for placebo group P < 0.001
- However no significant difference in duration of illness or length of hospitalization
- Anticipated high cost
  - Lowy, NEJM, 2010

Fecal Bacteriotherapy

“Colonization Resistance”

- Production of antimicrobial factors
- Competing for binding sites on epithelium
- Utilize nutrients more efficiently
Changes in Colon Bacteriology in CDI

• Elderly pts with CDI have markedly reduced numbers & diversity of *Bacteroides*, *Prevotella*, and *Bifidobacterium* vs healthy controls
• Strategy that restores microbial diversity could be used to resolve CDI when antibiotic treatment fails

Fecal Bacteriotherapy

• First described as therapy in 1958 (Eiseman, Surgery, 1958)
• Summary of 13 published reports from 1958 to 2008:
  – N 100
  – 24 via upper GI tract, 76 via lower GI tract
  – 89% cure rate
    (Bakken, Anaerobe, 2009)

Mechanics of Fecal Bacteriotherapy

• Recurrent or refractory CDI should be documented by testing
• Suitable donor, usually a relative with whom patient lives
• Screen donor for contagious diseases
• Route of administration
• Preparation of patient prior to procedure
  – Vancomycin x 4 to 7 days prior plus omeprazole evening before and morning of procedure if administered via upper GI route
• Preparation of the donor sample

Preparation of Donor Stool

<table>
<thead>
<tr>
<th>Time of collection</th>
<th>Upper GI tract</th>
<th>Lower GI tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning of procedure</td>
<td>25 to 30 gm</td>
<td>100 to 300 gm</td>
</tr>
<tr>
<td>Diluent</td>
<td>0.9 N NaCl</td>
<td>0.9 N NaCl or 4% milk</td>
</tr>
<tr>
<td>Volume</td>
<td>50 ml</td>
<td>250 to 1500 ml</td>
</tr>
<tr>
<td>Dose instilled</td>
<td>25 ml</td>
<td>250 to 1500 ml</td>
</tr>
<tr>
<td>Need for repeated instillations</td>
<td>No (but you can)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Prevention of CDI

• Judicious antibiotic prescribing, restrictions on certain types of antibiotics esp. in outbreaks
• Contact precautions- hand hygiene, gown and gloves, private room with private bathroom or cohort with other CDI patient
• Single use disposable or patient dedicated noncritical equipment, eg use disposable thermometer
• Cleaning with chlorine containing compound eg 1:10 dilution of 6% hypochlorite solution-drawbacks must be prepared daily and can be damaging to hospital equipment
• Vaccines under investigation