Objectives

At the conclusion of this presentation, the attendee will have learned about the:

• History of antibiotic associated diarrhea and Clostridium difficile.
• Current problems with C difficile testing
• Potential future directions for detection of C. difficile disease

Disclosures

I have received research grants and honoraria from:
– Curetis, Inc., Germany
– Accelerate Diagnostics, Inc. Tucson, AZ
– Abbott Molecular, Abbott Park, IL
– GenePOC, Quebec, CA
First Pathological Description of Pseudomembranous Colitis (PMC)

- 1893 Finney reported first description of PMC
- Patient was a 22 y.o. woman described by William Osler as a "miserable, emaciated creature in wretched physical condition".
- She underwent resection of a gastric tumor
- Bloody diarrhea on the 10th post-operative day and died 5 days later
- Autopsy: "diphtheritic membrane" involving ileum and colon

Early Studies on the Role of Antibiotics in Lethal Colitis

- 1950s- colitis increased as a complication of antibiotic use
  - Suspected pathogen: Staphylococcus aureus
  - Oral vancomycin proved effective
- 1974- landmark paper by Tedesco
  - Prospective study of 200 pts. treated with clindamycin at Barnes Hosp. (St. Louis)
  - 42 patients (21%) developed diarrhea—all were endoscoped
    - 20 patients (10%) had PMC
  - FDA required Upjohn to issue a warning—disease became known as "clindamycin colitis"
  - Important observation: absence of S. aureus
  - First documentation of nosocomial epidemic of C. difficile colitis

On the trail of the pathogen and its toxin...

- 1935-Hall and O'Toole described Bacillus difficilis in normal colonic microbiota in newborns
- 1974-Hafiz (Univ. Leeds) PhD thesis
  - C. difficile widely distributed in nature
  - Found in stools of many animals
  - Most strains produced toxin—quantity varied
- 1974 Green inoculated intestinal contents from guinea pigs with penicillin cecitis into tissue culture cells—cytotoxic changes mistakenly attributed to a virus
- 1977 Larsen, et al. demonstrated that a cytotoxin could be detected in feces of 5/6 patients with PMC
Establishing the Link between Lesion, Toxin and Organism

- 1975-1980—several groups involved in early work
- 1977—Bartlett and colleagues established hamster model and demonstrated that clindamycin-associated colitis was due to toxin producing strain of *Clostridium*
  - Te-Wen Chang characterized cytopathic assay
  - Nancy Taylor described toxin A and toxin B
- 1978—Discovery of *C. difficile* in human cases of PMC


C. difficile Cytotoxin Assays

![C. difficile Cytotoxin Assays](image)


Two "gold standards" emerged
- Cell culture with neutralization—detects presence of free toxin in stool
- Anaerobic culture—detects presence of bacteria can produce toxins
  - must be used with a toxin test
  - not practical
Cell Culture Cytotoxicity Neutralization Test

- Cell culture neutralization assay became the standard reference method for *C. difficile* diagnosis.
- 100% sensitive in the hamster model
- In humans, false negatives were seen due to procedural problems such as over-dilution
- False "positives"
  - Procedural errors—filtration and dilution steps
  - Asymptomatic patients who harbor toxin-producing strains
- Never standardized for use in clinical labs
  - Not easy to perform
  - Not rapid

Enzyme Immunoassays

- Use monoclonal or polyclonal antibodies to detect toxin
- EIA development began soon after discovery—10 fold less sensitive than cytotoxin testing and hence not pursued!
- First-antibody based *C. difficile* assay was marketed in 1986: Culterette brand rapid latex test (Marion Scientific, Inc.)
  - Designed to detect toxin A in 30 min.
  - Actually detected glutamate dehydrogenase (GDH)—common antigen found in toxin positive and toxin negative *C. difficile*
  - Cross reacted with other anaerobic species
- Other commercial assays followed: most detected toxin A only

Laboratories Compensate for Suboptimal EIA Performance with Repeat Testing


- Study performed JHH-268 non-oncology patients
- EIA only
  - 72% (31/43)—diagnosed with 1st specimen
  - 84% (36/43)—diagnosed with 2nd specimen
  - 93% (40/43) diagnosed with 3rd specimen
- Institutional practice became: “stools for C diff X 3”
Toxin A/B EIAs Revisited

- Series of complaints prompted re-evaluation of C difficile EIA test in use in the JHH lab: sensitivity had fallen to 38%!
- Literature from others followed:
  Gilligan P. J Clin Microbiol 2008; 46:1523: EIA sensitivity 43%-69%
  Erb S. Clin Microbiol Infect 2015; 21:998.e9: EIA sensitivity 57%
- Reviews of 6 Tox A/B tests
  - None met acceptability criteria: sensitivity 90%; false positives < 3%
- Enzyme immunoassays – suboptimal!

Emergence of Epidemic Toxin Variant Strains of Clostridium difficile

- US-8 facilities/6 States reported outbreaks in 2001
- 50% of 187 isolates were clonal--PFGE (NAP-1)/ ribotype 027/ ST 1/REA group BI
- Fluoroquinolone resistant
- Quebec study--12 hospitals
  - 6.5% patients required intensive care; 1.9% required colectomy
  - 30-day attributable mortality was 6.9% (1.5% baseline)

The Quest for a Better Test: JHH Experience

- Between 2005 and 2010 we searched for the optimal test
- Evaluated multiple commercial EIAs
- We re-implemented cell culture cytotoxin neutralization assay
- Resurrected culture to facilitate epidemiological studies
- Two and 3 step algorithms were evaluated
- Evaluated and implemented nucleic acid amplification tests (NAATs)

Glutamate Dehydrogenase Assays

- Detect highly conserved “common” antigen
- Improvements in assay development
- Two formats
  - Solid phase microtiter plate assay
  - Lateral flow devices, some combined with toxin tests
- Incorporated into multi-step algorithms
- Meta-analyses show > 90% sensitivities

TechLab, Inc., Blacksburg, VA


Two step Clostridium difficile Testing Algorithm

<table>
<thead>
<tr>
<th>GDH negative</th>
<th>GDH positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Report as: C difficile antigen not detected</td>
<td>C difficile antigen detected. The presence of antigen may not correlate with disease. Toxin assay will be performed.</td>
</tr>
<tr>
<td>Negative Reported: C difficile toxin assay negative</td>
<td>Positive Reported: Positive by C difficile neutralization assay</td>
</tr>
</tbody>
</table>

Molecular Testing for Clostridium difficile Diagnosis

- Early reports using PCR appeared in the literature in 1991
  - End detection
  - Cumbersome extractions
  - Cross reactivity with other Clostridium species
- Decade later
  - DNA extraction methods from fecal samples improved
  - Real-time platforms became available
  - Analytical sensitivity compared to CCNA (10-100 times more sensitive ~ 10 genome copies per PCR)
  - Analytical specificity also improved
- First FDA-cleared platform became available in 2009

Examples of FDA-Cleared Nucleic Acid Amplification Tests for *C. difficile* Detection

- BD GeneOhm C. diff Assay
- Prodesse ProDiagnost CD Assay
- bioMerieux NucliSENS easyMag
- Realtime PCR Analysis on the SmartCycler®
- bioMerieux Simplexa C. difficile
- Focus Technologies Portrait Analyzer Toxigenic *C. difficile* assay Great Basin
- Meridian illumigene™
- Prodesse ProGastro CD Assay
- Nanosphere Verigene® C diff
- AmpliVue C. difficile Assay
- Quidel Inc.

Nucleic Acid Amplification Tests (NAATs) for Diagnosis of *C. difficile*: Caveats and Questions

- **Practical concerns**
  - Assays detect genes encoding toxins and not toxin itself—can they reliably predict disease?
  - Impact on CDI rates when switching from EIA to PCR
- **Theoretical concerns**
  - Genetic drift of *tcdB* or other gene targets in regions of primer/probe binding
  - Lack of toxin gene expression
- **Other questions**
  - Will use of NAATs lead to reductions in *C. difficile* transmission?
  - What will be the impact on patient care?


[Graph showing healthcare associated *C. difficile* infections from 2007 to 2015]
3-Step Algorithms

Modified from Swinkels, et al. JCM 2010;48:608

Sharp, JCM 2010;48:2082

- 3 step neg. $11.50 (88%)
- 3 step pos. $45 (12%)
- PCR alone $33

Culbreath K., et al. JCM 2012; 50:3073

- 3 step vs. stand-alone PCR
  - $90,000 savings

Importance of Clinical Symptoms in Assay Interpretation


- Evaluation of 9 assays including three NAATs
- Prospective patient interviews/physical exams
- Analysis: 4 reference standards
  - Toxigenic culture with and without symptoms
  - 4 test methods positive, with and without symptoms
- Results:
  - NAATs had highest sensitivity (100%); lowest specificity (<86%)
  - Specificity, PPV for all assays increased when clinical symptoms were ignored
  - 36% of patients did not have clinically significant diarrhea
  - 20% of patients were on a laxative (JHH rates—44%)


- Multicenter observational study of CDI: validation of tests based on clinical outcomes (N=12,420 stool samples)
- Compared 4 commercial assays (EIAs, GDH, PCR) results to 2 reference methods (CCTA and toxigenic culture)
- Assessed outcomes for 6522 patients in 3 groups
  - CCTA positive (group 1)
  - Toxigenic culture positive and CCTA negative; PCR positive and CCTA negative (colonized group 2)
  - Negative by all tests (group 3, neg. controls)
- Observations
  - All cause 30-d mortality markedly higher in group 1 (16.6%) than group 2 (9.7%, p=.022) or 3 (8.6%, p<.0001); mortality in group 2 was not different from that of the control group (8.6%, p=0.530)
  - Multivariate analysis confirmed mortality between groups
UK Study: Planche T, et. al. (cont.)
*Lancet Infect Dis 2013;13:936*

- Other clinical outcome measures
  - Toxin positive group had:
    - Longer inpatient stay
    - Higher markers of severity (WBC, albumin, creatinine)
- Conclusions
  - CCTA best diagnostic indicator of *C. difficile* disease (poor outcome)
  - Patients who are cytotoxic culture positive (NAAT alone positive) may be an infection risk to others
  - 2 and 3 step combination tests provide best options for patient care

Do Toxin Negative PCR positive Patients Require Treatment?

Prospective observational study of 1416 patients-only EIA toxin results were reported
- 131 Tox+/PCR+, 162 Tox-/PCR+, 1123 tox-/PCR-
- 55% of pts. C diff PCR pos. lacked toxin by EIA
- Tox-/PCR+ pts had milder, shorter duration of symptoms; 58.7% were not retested; only 13% had full course of Rx
- 18/19 C diff related complications and deaths occurred in toxin positive patients only
- Conclusions: 50% of patients with positive molecular tests do not experience adverse events without treatment

Toxin Identification and Clinical Outcomes among Patients with CDI: Systematic Review and Meta Analysis

Bunnell K, et al Poster P0608, ECCMID, April 2016
- 426 studies identified — 7 included (2617 patients)
- Risk ratio of all-cause mortality in pooled analysis:
  - 1.27 (95% CI 1.01-1.61) for pts. with detectable toxin compared to pts. with no detectable toxin (e.g. PCR pos. only)
- Recurrent disease meta-analysis
  - 4 studies, 599 patients
  - Risk ratio for recurrent disease among pts. with detectable toxin was 1.89 (95% CI 1.25-2.87) by random-effects models
- Conclusion: Pts with CDI and detectable toxin had increased risk of recurrence and a trend toward higher all cause mortality compared to the no fecal toxin group
Lack of Correlation of Toxin Positivity with Disease Severity

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaltsas A, et al. J Clin Microbiol 2012;55:1303</td>
<td>128 cancer patients; 56 PCR pos. only; 72 pos. by toxin and PCR</td>
<td>No difference in severity or mortality between groups. Toxin pos. pts had higher recurrence rates</td>
</tr>
<tr>
<td>Erb S, et al. Clin Microbiol Infect 2015;21:998.e9</td>
<td>480 immunocompromised patient; pts. met clinical criteria for CDI</td>
<td>EIA pos. and EIA neg, but TC pos. pts. had similar outcomes: transfer to intensive care, recurrent disease and CDI attributable mortality</td>
</tr>
</tbody>
</table>

Should C. difficile be Part of Multiplex NAAT GI Panels?

- FDA-cleared multiplex panels that include C difficile
  - Luminex, Toronto CA 11 pathogens
    - 7 bacteria, 2 viruses, 2 parasites
  -FilmArray GI panel (BioFire, Inc. SLC, UT) -23 pathogens
    -14 bacteria, 5 viruses, 4 parasites
- C. difficile was most common pathogen detected in study by Khare et al (J Clin Microbiol 2014; 52:3667)
- Another study reported high rate of co-infection with C difficile (53%) Buss SN, et al (J Clin Microbiol 2015; 53:915)
- Studies to date lack clinical data
- Targeted populations likely to be at low risk for C. difficile disease

What Can Laboratories Do To Ensure Appropriate Use of C diff tests?

- Limit testing to loose or soft stools that take the shape of the container
- Bristol Stool Form Scale type 6 or 7
- Discourage repeat testing within 7 days
- Computer decision support
For an initial negative test, there is a hard stop for duplicate tests within 7 days. If there is a positive test within 14 days, there is a hard stop.

### Studies Demonstrating Impact of IT Interventions

<table>
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<tr>
<th>Study</th>
<th>Patient Population</th>
<th>Intervention</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otto JCM 2015</td>
<td>Immunocompromised</td>
<td>BPAs for repeat pos, neg at order entry</td>
<td>Overall significant decrease (31.9%, p=0.028) in repeat positive tests</td>
</tr>
<tr>
<td>Nicholson ICHE</td>
<td>Young children</td>
<td>BPAs and education</td>
<td>115 fewer tests/year post intervention; $12,880 saved</td>
</tr>
<tr>
<td>Truong JCM 2017</td>
<td>Hospitalized pts</td>
<td>Electronic pt. data tracking (diarrhea and laxatives) to enforce intervention testing criteria; testing cancelled by lab</td>
<td>C diff tests decreased from 208.8 to 143/10,000 pt. days, oral vanco days decreased by 4.4/10,000 pt. days</td>
</tr>
<tr>
<td>Mizusawa ASM 2017</td>
<td>Hospitalized pts</td>
<td>EPIC hard stops-pts on laxatives, repeat pos within 14 d, neg within 7 d</td>
<td>15% fewer tests; no adverse patient outcomes</td>
</tr>
</tbody>
</table>
New Methods: Digital ELISA

- Uses antibody-coated paramagnetic capture beads and biotinylated detection antibodies to detect and quantify toxins A & B in stool
- Beads are loaded into arrays containing femtoliter-sized wells that detect bound molecules
- Limit of detection ~20 pg/mL; high clinical specificity and sensitivity compared to a cytotoxicity assay

Diagnostic Assays in Development

- Chemiluminescent immunoassay (LIAISON™DiaSorin)1-3
  - Available in Europe-automates GDH and reflex toxin A, B
  - Studies show sensitive GDH component compared to other GDH assays (98.7%-100%); sensitivity of toxin test comparable to low end EIA (60%-69%)
  - Technical problems observed in Benedek study
- Proximity ligation assay coupled with PCR4
  - Detects proteins so indicates presence of toxins
  - Analytical sensitivity better than standard EIAs
  - No available clinical data yet

Burden of C difficile Disease

CDC Emerging Infections Program

- Study period Jan-Dec 2011
- Active population and laboratory based surveillance across 10 USA geographic areas
- Estimated # incident cases-453,000; # deaths, 29,300
- Recurrence rates 14%-21%


Summary

- Since its discovery, C. difficile continues to cause substantial disease burden.
- The diagnostic test pendulum has swung from toxin tests to molecular assays with a call to reinstitute toxin testing.
- Clinicians should partner with labs to ensure appropriate test utilization regardless of the test used.
- Newer, novel methods are needed to move us out of diagnostic uncertainty.

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