Update on *Clostridium difficile* Research: Hospital Reservoir, Diagnosis of CDI and Harnessing Intestinal Microbiota

TDSHS Sponsored Research

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Additional appointments as Professor:
University of Texas and Baylor Graduate Schools of Biomedical Science
MD Anderson Cancer Center
University of Houston
Conflicts

- Grants for clinical studies:
  Sanofi Pasteur, Takeda, Seres Health, Riobitex
  Salix-Valeant
Disinfection Methods

- Ultraviolet light plus standard treatment (quaternary ammonium solution)
- 10% bleach (Dispatch)
- Hydrogen peroxide/Peracetic acid (Oxycide)
- Quaternary ammonium solution only as a control group

<table>
<thead>
<tr>
<th>Study Floor</th>
<th>Method of Decontamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floor 1</td>
<td>Ultraviolet light plus quaternary ammonium solution (odd numbers)</td>
</tr>
<tr>
<td></td>
<td>Quaternary ammonium solution ONLY (control) (even numbers)</td>
</tr>
<tr>
<td>Floor 2</td>
<td>10% Bleach (Dispatch)</td>
</tr>
<tr>
<td>Floor 3</td>
<td>Hydrogen peroxide plus peracetic acid (Oxycide)</td>
</tr>
</tbody>
</table>
C. difficile Identification
3 Hospital Floors Studied

- Environmental sponge swabbing patient room and bathrooms and stools samples from patients with diarrhea were cultured for C. difficile using and confirmed by PCR
- Molecular typing method used was to look for clustering suggesting hospital-acquired infection vs single clones suggestive of community acquisition
Study 1 Design

- 3 hospital floors with a total of 85 rooms housing 126 adult patients were sampled.
- Swabs were from high touch areas of patient room and in bathroom around toilet while rooms were cleaned daily.
### C. difficile Identified from Rooms of 3 Study Units Using Different Cleaning Methods July 2014 through June 2015

<table>
<thead>
<tr>
<th>Clean Method</th>
<th>N</th>
<th>Room with C. difficile Identified</th>
<th>Bathroom with C. difficile Identified</th>
<th>Total Rooms with C. difficile Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O₂ &amp; peracetic acid (OxiCide)</td>
<td>802</td>
<td>17 (4%)</td>
<td>51 (13%)</td>
<td>60 (7%)</td>
</tr>
<tr>
<td>Quaternary Ammonium</td>
<td>468</td>
<td>5 (2%)</td>
<td>20 (9%)</td>
<td>23 (5%)</td>
</tr>
<tr>
<td>UV plus QA</td>
<td>548</td>
<td>3 (1%)</td>
<td>11 (4%)</td>
<td>12 (2%)</td>
</tr>
<tr>
<td>10% Bleach</td>
<td>996</td>
<td>13 (3%)</td>
<td>35 (7%)</td>
<td>44 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>2814</td>
<td>38 (3%)</td>
<td>117 (8%)</td>
<td>139 (5%)</td>
</tr>
</tbody>
</table>

QA = Quaternary Ammonium, UV = UltraViolet

P value = 0.0003, when comparing C. difficile contamination rate in the hospital environmental with different cleaning methods
All strains of *C. difficile* isolated from surface of study rooms were typed by MLST* to identify clones.

Molecular fingerprinting can identify in-hospital transmission of strains.

*Multi-locus sequence typing*
70% Similarity

<table>
<thead>
<tr>
<th>Floor (Positive/Total)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (6/67=9%)</td>
<td></td>
</tr>
<tr>
<td>1 (6/39=15%)</td>
<td>0.0061</td>
</tr>
<tr>
<td>2 (15/47=32%) √</td>
<td></td>
</tr>
<tr>
<td>3 (53/67=79%) √</td>
<td></td>
</tr>
<tr>
<td>1 (12/39=31%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>2 (26/47=55%)</td>
<td></td>
</tr>
<tr>
<td>3 (8/67=12%)</td>
<td></td>
</tr>
<tr>
<td>1 (21/39=31%) √</td>
<td>0.0001</td>
</tr>
<tr>
<td>2 (6/47=13%)</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions Study 1 and 2

- Ideal cleaning is quaternary ammonia + UV light plus which poses challenges because of time with room not in use.
- It may be that any cleaning method will work if hospital housekeepers spend sufficient time cleaning.
- Much of hospital-associated CDI is acquired regionally in hospital units where clones of spores can be found.
- Multiple clones are seen throughout the hospital from multiple entry points.
**Clostridium difficile – a Unique Hospital Problem**

- Hands of 24% of health care workers positive for spores vs. 0% for hospital workers without patient exposure\(^1\)
- *C. difficile* spores are shed from both symptomatic/asymptomatic patients
- 4%-13% inpatients without diarrhea are colonized by *C. difficile*\(^2,3\)
- Study found that contact isolation of carriers admitted from the ED led to significant reduction in CDI in the hospital\(^2\)
- Spores can survive months without killing by standard disinfectants

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\(^1\)Landelle C et al. Infect Control Hosp Epi 2014;35:10-15
\(^2\)Longtin Y et al. JAMA Intern Med 2016 Epub
Traditional Methods of Infection Control

- Hand washing reduces person-to-person contact spread of microbes in hospital but is difficult to enforce.
- This does not address infections being carried in by patients which assure a hospital reservoir even with total cleanliness and high levels of hygiene.
- Cleaning of rooms is done by poorly trained people who are told to turn the rooms over quickly.
- 50% of surface areas not cleaned by routine cleaning.
Modern Methods of Infection Control

- We have to focus on both endogenous and exogenous pathogens
- Room cleaning needs to be performed by dedicated and trained personnel
- UV radiation, gaseous plasma (hydroxyl and hydroproxyl free radicals) or hydrogen peroxide vapor may help
- Antibiotic stewardship offers major payoff
- The Hospital Microbiome Project\(^1\) takes an ecological approach to improve intestinal colonization resistance to reduce environmental transmission of pathogens and antibiotic resistance

\(^1\)Shogan BD et al. Stds Genomic Sciences 2013;8:571-9
How to Modernize Hospital Infection Control

- Require an accurate diagnosis of CDI and stop treatment if found negative
- Assume all patients and hospital personnel are *C. difficile*-positive
- Require that room cleaning be effective and that frequent and effective hand washing with soap and water are performed
- Stay tuned to ongoing studies of disinfection of hospital environments
Study 2 Difficulty in Diagnosing CDI

Making the diagnosis: diarrhea plus positive fecal test for *C. difficile* toxin(s):

- EIA lacks sensitivity;
- Toxigenic culture and Tissue culture cytotoxicity assay takes 3 days
- PCR overly sensitive and picks up carriage
- Two step methods have been developed (e.g. glutamate dehydrogenase + EIA or PCR)
- Fecal CD toxin test plus finding inflammatory markers in stool suggests; Finding pseudomembranous colitis by endoscopy confirms the diagnosis
When in May 2011 our 700-bed university hospital in Houston changed from cytotoxicity assay to PCR the rate of CDI doubled from 13.4 to 27.0 patient days/10,000.

PCR+ *C. difficile* toxin(s) found in 13% of asymptomatic patients in the hospital.

10% of patients in our hospital with AAD are C diff toxin positive.

Most antibiotic associated diarrhea in our hospital with positive PCR test for *C. difficile* toxin is a false positive.

Making an Accurate Diagnosis of CDI

- Most patients with antibiotic-associated diarrhea don’t have CDI
- Colonization of C. diff is seen in between 5-15% of inpatients
- Considering these most PCR positive C. diff’s are false positives
- We are looking at 170 patient stools positive for *C. difficile* for presence of inflammation markers (calprotectin and lactoferrin)
Outcomes Studied

- With a gold standard diagnosis of CDI we can educate doctors to treat those and avoid false positives
- Laboratories may perform PCR plus neutrophil inflammation marker reporting ++, +, --
The packed microbes (10 times the number cells in the body) prevent growth of antibiotic resistant bacteria and disease causing bacteria (e.g. *Salmonella* & *C diff*) because of space the healthy bacteria occupy & organic acids they produce.
Unhealthy Diet and or Recurrent or Prolonged Antibiotic Exposure

Sparse Intestinal Microbiota = Dysbiosis

Blooms of unhealthy Proteobacteria

Few bacterial species giving room for harmful bacteria to grow
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Blooms of unhealthy Proteobacteria

Few bacterial species giving room for harmful bacteria to grow

With exposure to antibiotics and exposure to hospital spores, *C. difficile* can grow unchecked leading to severe potentially fatal diarrhea

*Clostridium difficile* infection
Unhealthy Diet and or Recurrent or Prolonged Antibiotic Exposure

Sparse Intestinal Microbiota = Dysbiosis

Blooms of unhealthy Proteobacteria

Few bacterial species giving room for harmful bacteria to grow

With exposure to antibiotics with dysbiosis, *E. coli* can grow unchecked, leading to antimicrobial resistant intestinal microbiota, resulting in difficult to treat infections and antibiotic resistance.
Unhealthy Diet and or Recurrent or Prolonged Antibiotic Exposure

Sparse Intestinal Microbiota = Dysbiosis

Blooms of unhealthy Proteobacteria

Few bacterial species giving room for harmful bacteria to grow

With exposure to antibiotics and exposure to hospital spores, *C. difficile* can grow unchecked leading to severe potentially fatal diarrhea

With exposure to antibiotics with dysbiosis *E. coli* can grow unchecked lead antimicrobial resistant intestinal microbiota leading to difficult to treat infections

*Clostridium difficile* infection

Antibiotic resistance

Fecal Microbiota Transplantation (FMT) can reverse dysbiosis
For Depletion of Diversity of Microflora - Fecal Microbiota Transplantation (FMT)

Donor Screening and Preparation & Delivery of FMT Product

- Donor selection and screening process
- ≥ 100 g of stool is collected and mixed in stomacher and filtered twice to remove fecal material
- Study 1: fresh, frozen or lyophilized (freeze-dried) bacterial product dispensed via colonoscope
- Study 2: frozen product given by enema and lyophilized by oral capsule
- 90% successful with one treatment
Fecal Microbiota in Recipients Before and after Fecal Microbiota Transplantation

Recipients After $\geq 3$ Bouts of CDI
Fecal Flora Diversity in 8 Donors and 41 Recipients Before and After Fecal Microbiota Transplantation

Recipients After ≥ 3 Bouts of CDI
Our Patients Remind of Us of the Miracle of FMT

- We have treated more than >120 patients with recurrent C diff infection by FMT
- Success rate 90%; failures receive second FMT from a different donor
- Our patients will show you how grateful they are

FMT # 1-9
“Thank you!, Thank you!, Thank you! For the 1st time in a year I can hold a grandchild”

FMT # 1-69
Chairman, Department Head, sent the university his resignation, cured by FMT, rescinded his resignation, “you saved my life”

FMT 2 #10
20-yr old lost 40 lbs (down to 88 lbs), was cured by FMT and organized fund raising event to pay for medical expenses

FMT 1 #69
19-yr old college football player with ulcerative colitis and 4 episodes of C diff infection with 90 lbs of weight loss was cured by FMT and he is back playing football
Partial List of Conditions With Unhealthy Dysbiosis of Intestinal Flora

- Clostridium difficile Infection
- Inflammatory bowel disease & Irritable bowel syndrome
- Type II diabetes and metabolic syndrome
- Rheumatoid arthritis
- Chronic neurologic diseases (e.g. MS, Parkinson’s disease)
- Allergic disorders and asthma
- Allogeneic hematopoietic stem cell transplantation
- Obesity
- Hospitalized patients & resistant microbiota
Control of Current Epidemic of Antibiotic Resistance

- Key factors: avoid unnecessary antibiotics (Stewardship)
- Use narrow spectrum antibiotics when can
- Maintain a healthy gut microbiota with diet
- Fecal microbiota transplantation may reverse MDR colonization
Conclusions and Summary

- Careful cleaning of hospital rooms is critical to controlling C diff infections: UV light plus conventional cleaning is effective but more rigorous standard cleaning may work.
- Being aware of the problem of false positive testing for CDI should lead to better antibiotic stewardship by focusing on those with clinical disease.
- A gold standard diagnostic test is needed.
- FMT is a powerful low-tech physiologic means of reversing dysbiosis that is being(harnessed to treat non GI diseases & potentially prevent endogenously acquired infections from gut microbiota and reduce the reservoir of antibiotic resistance.

Support Bacteria
They are the Only Culture
Some Folks Have!