Clostridium difficile Infection (CDI):
Discovering the need for new treatment algorithms and care pathways
CDI recurrence: The importance of intestinal microbiome
Microbiome as the flip side of C. difficile infection

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| In the past 2 years I have been an employee of: | University of Calgary, Self Employed |
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| In the past 2 years I have held investments in the following pharmaceutical organizations, medical devices companies or communications firms: | NA |
| In the past 2 years I have been a member of the Scientific advisory board of: | Pfizer, Merck |
| In the past 2 years I have been a speaker for: | Cubist, Optimer |
| In the past 2 years I have received research support (grants) from: | Cubist, Optimer, Actelion |
| In the past 2 years I have received honoraria from: | Cubist, Merck, Optimer, Pfizer |
| I agree to disclose approved and non-approved indications for medications in this presentation: | YES |
| I agree to use generic names of medications in this presentation: | YES |

There are no relationships to disclose
Establishing the role of components of the microbiome for the prevention and treatment of C. difficile infection.

Rec
CDI

Fecal microbiome transplantation
whole stool slurry by enema,
endoscope, nasojejunal

Modified FMT..pure
microbial preps,
microbe ID and
recovery

Modified FMT via
subcomponents of
microbiota alone or in
combination

Specific microbial ‘probiotics’ that
restore colonization resistance in
a preventative manner in
susceptible populations

Discovery of functional role
of microbial Genus /
species in host defence /
colonization resistance
CDI occurs in association with a
• $10^{-1}$ to $-3$ CFU/g reduction of Firmacutes
• $10^{-2}$ to $-6$ reduction of Bacteroidetes
• $10^2$ to $3$ increase in coliforms

- **Comparison**: 81.1% vs 72.7%
- **P-value**: 0.0203
- **Response to treatment**: 210/259 vs 202/278

*Vanco* (vancomycin) vs *Metro* (metronidazole)
Increasing risk of recurrence of CDI after repeated recurrences; by-product is dependence on vancomycin

McFarland et al, ICHE 1999; 20: 43,
Gerding, Curr Top Microbiol Immunol 2000; 250: 127
Louie, 2011 NEJM
Van Nood, Kuijper and Keller, NEJM 2013

Serial damage to microbiome? VS increased adherence/persistence, biofilm, spore density?
Treatment of 1000 cases of Clostridium difficile infection with metronidazole or vancomycin: effect of cumulative response patterns on total episodes of disease

1000 new cases CDI

- 800 "cured"
  - 624 sustained cure
  - 176 recCDI
    - 109 sustained cure
    - 67 recCDI
      - 27 sustained cure
      - 40 recCDI
        - 8 sustained cure
      - 32 recCDI
    - 28 sustained cure
    - 17 recCDI
      - 7 sustained cure
      - 10 recCDI
      - 3 sustained cure
  - 200 failed
    - 190 "cured HiDoseRx"
      - 145 sustained cure
      - 45 recCDI
        - 28 sustained cure
        - 17 recCDI
        - 10 recCDI
        - 7 recCDI
Treatment of 1000 cases of Clostridium difficile infection with metronidazole or vancomycin: effect of cumulative response patterns on total episodes of disease

1000 new cases CDI

800 "cured"

624 sustained cure

200 failed

190 "cured HiDoseRx"

145 sustained cure

1000 new cases CDI results in 1394 episodes of CDI

176 recCDI

109 sustained cure

67 recCDI

27 sustained cure

40 recCDI

8 sustained cure

32 recCDI

28 sustained cure

45 recCDI

28 sustained cure

17 recCDI

7 sustained cure

10 recCDI

3 sustained cure

7 recCDI
Effect of Vancomycin on Bacteroidetes, C. coccoides [XIV], C. leptum and C. difficile counts during and after treatment of CDI in Genzyme 301 patients.
Changes in 3 main component normal microbiota in patients randomized to metronidazole 375 mg QID as treatment of CDI in the Genzyme 301 study
Rates of cure of recurrent CDI. Van Nood, JJ Keller, NEJM Jan 2013

![Bar chart showing rates of cure without relapse](chart.png)
Figure 3. Microbiota Diversity in Patients before and after Infusion of Donor Feces, as Compared with Diversity in Healthy Donors.

Microbiota diversity is expressed as Simpson’s Reciprocal Index of diversity in fecal samples obtained from nine patients before and 14 days after the first infusion of donor feces, as compared with their donors. The index ranges from 1 to 250, with higher values indicating more diversity. The box-and-whisker plots indicate interquartile ranges (boxes), medians (dark horizontal lines in the boxes), and highest and lowest values (whiskers above and below the boxes).
qPCR analysis of the gut microflora in normal donors and C. difficile infected patients. CFU, colony forming units, * p<0.05, LLOD 10^{3-4}
qPCR analysis of the gut microflora in *C. difficile* infected patients pre and over time post bacteriotherapy. Pre therapy microflora levels are compared to (A) 1 week, (B) 1 month, (C) 3 months and (D) up to 1 year post bacteriotherapy. CFU, colony forming units,* p<0.05.

N=28; 800 ml fecal slurry x 1, or 12-17 ml of pelleted and resuspended fecal microbes orally x1.
Lawley et al, Plos Pathogens, October 2012. Ribotype 027 persists post infection in C57BL mice, whereas ribotype 012 [630], and 017 [toxAneg] clear spontaneously. 027 is a special strain type. All strains were clindamycin R/ermB pos.
Table 3. Antagonism of *Clostridium difficile* in gnotobiotic mice inoculated with various indigenous bacteria

<table>
<thead>
<tr>
<th>Flora of gnotobiotic mice</th>
<th>Days after inoculation with <em>C. difficile</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>N</em> = 5</td>
</tr>
<tr>
<td><em>E. coli</em> + faeces of CV mice</td>
<td>&lt; 3·0</td>
</tr>
<tr>
<td><em>E. coli</em> + CHF faeces of CV mice</td>
<td>&lt; 3·0</td>
</tr>
<tr>
<td><em>E. coli</em> + faeces of LF mice</td>
<td>&lt; 3·0</td>
</tr>
<tr>
<td><em>E. coli</em> + clostridia (F strains)</td>
<td>7·1 ± 0·32 (5)*</td>
</tr>
<tr>
<td><em>E. coli</em> + lactobacilli (L strains)</td>
<td>7·7 ± 0·11 (5)</td>
</tr>
<tr>
<td><em>E. coli</em> + bacteroides (B strains)</td>
<td>7·5 ± 0·32 (5)</td>
</tr>
<tr>
<td>(C. difficile monoassociation)</td>
<td>8·4 ± 0·16 (5)</td>
</tr>
</tbody>
</table>

*Mean count (log₁₀) ± SD of *C. difficile* per gram of faeces when present; values in parentheses refer to the number of mice yielding the organisms.*
Lachnospiraceae spp limits severity of disease in CD challenged mice.
Table 1 Composition of stool substitute (RePOOPulate)

Closest species match, inferred by alignment of 16S rRNA sequence to GreenGenes database

<table>
<thead>
<tr>
<th>Species</th>
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</thead>
<tbody>
<tr>
<td>Acidaminococcus intestinalis</td>
<td>Eubacterium ventriosum</td>
</tr>
<tr>
<td>Bacteroides ovatus</td>
<td>Faecalibacterium prausnitizii</td>
</tr>
<tr>
<td>Bifidobacterium adolescentis (two different strains)</td>
<td>Lachnospira pectinoshiza</td>
</tr>
<tr>
<td>Bifidobacterium longum (two different strains)</td>
<td>Lactobacillus casei/paracasei</td>
</tr>
<tr>
<td>Blautia producta</td>
<td>Lactobacillus casei</td>
</tr>
<tr>
<td>Clostridium cocleatum</td>
<td>Parabacteroides distasonis</td>
</tr>
<tr>
<td>Collinsella aerofaciens</td>
<td>Raoultella sp.</td>
</tr>
<tr>
<td>Dorea longicatena (two different strains)</td>
<td>Roseburia faecalis</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Roseburia intestinalis</td>
</tr>
<tr>
<td>Eubacterium desmolans</td>
<td>Ruminococcus torques (two different strains)</td>
</tr>
<tr>
<td>Eubacterium eligens</td>
<td>Ruminococcus obeum (two different strains)</td>
</tr>
<tr>
<td>Eubacterium limosum</td>
<td></td>
</tr>
<tr>
<td>Eubacterium rectale (four different strains)</td>
<td>Streptococcus mitis</td>
</tr>
</tbody>
</table>

Repopulate: Emma Allen-Vercoe and Elaine Petrof, U Guelph and Queens U, respectively. Microbiome 2013.
Log$_{10}$ CFU/gm Feces of Major Cultivable and Noncultivable Genera of the Normal Fecal Microbiota; n=20 (Fidaxomicin phase 3 [003] protocol)

**Bacteroides/Prevotella group**

- **P** = 0.0001
- 0.0001
- 0.03
- 0.04

**C. coccoides group**

- **P** = 0.001
- **P** < 0.03

**C. leptum group**

- **P** < 0.03

Off label use of fidaxomicin

- Use as a fidaxomicin ‘chaser’, following vancomycin [Johnson & Gerding, CID Jan. 2013]
- Treatment of multiple recurrent CDI. Will 10 days BID work? Longer? Taper dose?

68 y.o. M with 5 recurrence, treated with 10 days of FDX.
Strategies to maintain colonization resistance against C. difficile.

Nutrients → Consumption, Conversion eg SCFAs → C. difficile → Bacterial products MAMPs

Bacterial metabolism

Host metabolites, bile acids

Microbial products bacteriocins

Antimicrobial peptides

IgA

cytokines

Trends in Microbiology 2012 Jul, Britton & Young
CamSA, a cholate-meta-benzene sulfonic derivative blocks taurocholate stimulation of germination. Infected mice are prevented from illness. JID 2013 E. Abel – Santos, UNLV
Establishing the role of components of the microbiome for the prevention and treatment of C. difficile infection.

- Fecal microbiome transplantation: whole stool slurry by enema, endoscope, nasojejunal
- Modified FMT (pure microbial preps, microbe ID and recovery)
- Modified FMT via subcomponents of microbiota alone or in combination
- Discovery of functional role of microbial Genus / species in host defence / colonization resistance
- Specific microbial ‘probiotics’ that restore colonization resistance in a preventative manner in susceptible populations
Figure 2. Phylogeny-based Principal Component Analysis.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0059260