A NOVEL ECOSYSTEM THERAPEUTIC FOR THE TREATMENT OF *CLOSTRIDIUM DIFFICILE* INFECTIONS

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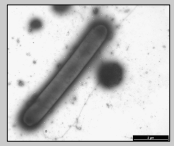
Introduction

- *Clostridium difficile* infection (CDI) is the #1 cause of hospital-acquired diarrhea[1], which in severe cases can result in death[2]
- Antimicrobial use is the most important risk factor for CDI[3], causing an imbalance in the gut microbiota, and allowing pathogenic *C. difficile* to overgrow
- The most prevalent PCR ribotype in the US and Canada is 027 (NAP1), with 078 being identified in increasing numbers in hospitals, in the community, and in food[4]
- Standard antibiotic therapies are not always effective, often leading to recurrent CDI[5]
- Intestinal microbiota transplantation is an alternative therapy that repopulates the gut with indigenous species from healthy donor stool: it has been shown to be 92% effective[5]
- Intestinal microbiota transplantation can be problematic
  - for patient acceptance
  - in composition: preparations are undefined and despite screening protocols may still harbour pathogenic microorganisms

Recurrent CDI



MTZ: metronidazole
VAN: vancomycin

C. difficile infection → MTZ or VAN Treatment → 65% infection cleared

MTZ or VAN Treatment → ≤ 35% improvement followed by recurrence of symptoms → 35% infection cleared

≤ 35% improvement followed by recurrence of symptoms → ≤ 65% chronic recurrence pattern = 'Recurrent CDAD'

≤ 65% chronic recurrence pattern = 'Recurrent CDAD' → MTZ or VAN Taper = 31% recurrence | MTZ or VAN Pulse = 14% recurrence*

A suitable alternative to these treatments is a necessity.

*Adapted from Gough, E. *et al.* 2011

Hypothesis

A Defined Ecosystem Therapeutic can be used to effectively cure recurrent CDI and persist in the human gut

Making a Defined Ecosystem Therapeutic "RePOOPulate"

Isolating Strains

- Stool sample from a healthy 41 year-old female with average BMI was cultured. Donor had very little antibiotic use (1 course in 10 years)
- Sample was cultured on various media types and incubated in both aerobic and strict anaerobic conditions. Resulting growth was streaked to purify colonies
- Strains were identified by 16S gene sequencing and characterized based on antibiotic susceptibility profiles. Only Imipenem and Vancomycin sensitive strains were included in the formulation
- The final formulation was used to inoculate an *in vitro* chemostat model of the distal human gut to test for stability of the community

Selecting Strains

- A closely matched profile from MetaREP was used (metagenomic database collection of stool sample datasets from healthy donors)
- Profile was used as a guide for relative abundance for each species
- Measured using microbiological loops to estimate biomass ratios

Assembly

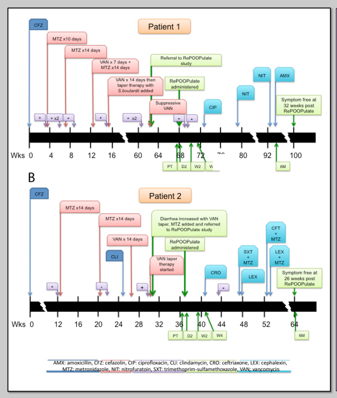
RePOOPulate*

Acidaminococcus intestinalis	*Faecalibacterium prausnitzii*
Bacteroides ovatus	*Lactobacillus casei*
Bifidobacterium adolescentis (x2)	*Lactobacillus paracasei*
Bifidobacterium longum (x2)	*Parabacteroides distasonis*
Collinsella aerofaciens	*Raoultella* sp.
Dorea longicatena (x2)	*Roseburia faecalis*
Escherichia coli	*Roseburia intestinalis*
Eubacterium eligens	*Ruminococcus torques* (x2)
Eubacterium limosum	*Streptococcus mitis*
Eubacterium rectale (x4)	Likely novel species (x5)
Eubacterium ventriosum	Likely novel genus & species (x1)

"Taken together, the stability, prevalence and potential functionality [of the *Lachnospiraceae* family] support the fact that the identified core phylogroups are pivotal in maintaining gut homeostasis and health."[6]

*In some cases 16S rRNA gene sequencing could not resolve identity beyond genus and that closest match does not infer definitive speciation

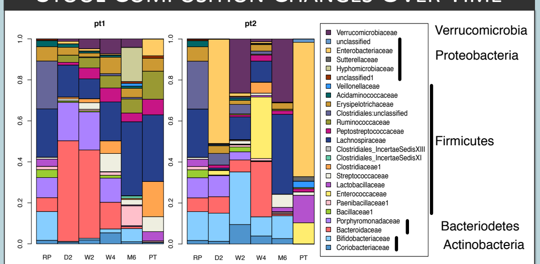
Patient Timelines & Outcomes



Patient 1
B
Patient 2
RePOOPulate administered
Symptom free at 26 weeks post RePOOPulate
Wks
AMX: amoxicillin, CFZ: cefazolin, CIP: ciprofloxacin, CLI: clindamycin, CRO: ceftriaxone, LEX: cephalexin, MTZ: metronidazole, NIT: nitrofuratoin, SXT: trimethoprim-sulfamethoxazole, VAN: vancomycin

- 2 patients suffering from recurrent CDI (078) were treated
- Antibiotic therapy withheld 2 days prior to procedure
- Colon-cleansing prep
- RePOOPulate administered by colonoscopy
- 50mL deposited in region of the cecum/proximal ascending colon
- 50 mL drizzled through transverse colon as colonoscope was removed
- Stool samples taken to monitor bacterial community profiles over time

Stool Composition Changes Over Time



pt1
pt2
RP
D2
W2
W4
M6
PT
Verrucomicrobiaceae
unclassified
Enterobacteriaceae
Sutterellaceae
Hyphomicrobiaceae
unclassified1
Veillonellaceae
Acidaminococcaceae
Erysipelotrichaceae
Clostridiales:unclassified
Ruminococcaceae
Peptostreptococcaceae
Lachnospiraceae
Clostridiales_IncertaeSedisXIII
Clostridiales_IncertaeSedisXI
Clostridiaceae1
Streptococcaceae
Lactobacillaceae
Enterococcaceae
Paenibacillaceae1
Bacillaceae1
Porphyromonadaceae
Bacteroidaceae
Bifidobacteriaceae
Coriobacteriaceae
Verrucomicrobia
Proteobacteria
Firmicutes
Bacteriodetes
Actinobacteria

RP: RePOOPulate; D: Day; W: Week; M: Month; PT: Pre-Treatment

The composition of RePOOPulate persisted in both patients over time and seemed to protect against further CDI relapse. Both patients remain symptom free at 32- and 26-weeks post-RePOOPulate.

References & Acknowledgements

1. Bartlett JG. Ann Intern Med **2006**; 145(10):758–764. **2.** Badger, V. O., Ledeboer, N. A., Graham, MB and C. E. Edmiston, Jr. *JPEN J Parenter Enteral Nutr*, online 10 May 2012. **3.** Freeman J, Wilcox MH. Microbes Infect **1999**; 1(5):377–384.
4. Rupnik M, Widmer A, Zimmermann, O, Eckert C, Barbut F. J Clin Microbiol 2008, 46(6):2146. **5.** Gough, E., Shaikh, H. and A. R. Manges. Clinical Infectious Diseases. 2011. 53(10):994–1002. **6.** Sekelja, M., Berget, I., Næs, T. and Knut Rudi. ISME J 2011; 5: 519–531.

The majority of the work described here from: Petrof, E., Gloor, G., Vanner, S., Weese, S., Carter, D., Daigneault, M., Brown, E., Schroeter, K. and Emma Allen-Vercoe. Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: "RePOOPulating" the gut. Microbiome. 2012. Accepted in press.

This work was supported by the Southeastern Ontario Academic Medical Organization (SEAMO) and Physicians' Services Incorporated (PSI) Foundation

The authors would like to acknowledge the following people who contributed to the study:
Clinical investigators (collected data, provided and cared for study patients): Lawrence Hookey[3], Cathy Lowe[3], Mark Ropeleski[3], Gerald Evans[3], Christopher A. Smith[3], Robert Bechara[3]
Laboratory and technical assistance (processed samples and collected data): Curtis Noordhof[3], Adriana Breen[3], Karen Solomon[1], Christian Ambrose[1]
Bioinformatics assistance (helped with QIIME analysis and barplot production): Jean Macklaim[2]