

BCHM 421/422 – 2018/2019

Project Outline: *Clostridium difficile* is a pathogenic spore-forming bacterium and a major cause of hospital-associated diarrheal infections (*C. difficile* infection; CDI). Rates have been increasing in the last decade, causing increase costs in healthcare each year. There are some risk factors associated with this infection, including antibiotic treatments, proton pump inhibitors, long-term hospitalizations, and age. In particular, the use of antibiotics provokes alterations in the normal microflora in the gut. These changes promote the growth of many opportunistic bacteria such as *C. difficile*.

In this situation, *C. difficile* is able to proliferate and release its toxins, including two major toxins: toxin A (TcdA) and toxin B (TcdB). These toxins are proteins that can induce physiological damage to the colonic mucosa causing inflammatory responses. They have been related to affect cytokine production, increase epithelial permeability, and neutrophil infiltration [1]. All these responses provoke a direct damage on the intestinal epithelium, generating ulcerations in the colon and causing pseudomembranous colitis [2]. The common treatment is the use of antibiotics such as vancomycin, clindamycin, or metronidazole. However these treatments in many cases fail generating relapses and resistance in many patients.

As a method of treatment for recurrent *C. difficile* infections, reintroducing healthy gut flora through a fecal microbiota transplant (FMT) has proven successful [3]. However it is necessary to do screenings of the healthy donors and follow safety protocols for an effective treatment. Currently, there is a great interest to find a less invasive yet effective treatment to re-establish the microflora in patients with CDI. The Petrof Laboratory has developed a defined microbial community of protective native gut bacteria, Microbial Ecosystem Therapeutic-1 (MET-1) isolated from a healthy volunteer. This mixture had showed promissory results to be used as a potential treatment to control CDI [4]. The protective effect of individual isolates and subsets of MET-1 bacteria are still being explored (in chemostatic (i.e. “RoboGut”), cellular, and animal models) to learn about their contribution to the effectiveness of MET-1.

Here we propose to continue the study of defined microbial communities to better understand the mechanisms underlying the success of its use as a treatment for CDI.

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Project Title: Study of human, defined microbial communities for the treatment of *Clostridium difficile* infections.

Keywords (3-5):

- 1. Pathogen**
- 2. Microbiome**
- 3. Translational**
- 4. ELISA**
- 5. DNA sequencing**

Project Goals:

1. Determine the protective effect of MET against *C. difficile* infections:
 - a. Quantify toxin levels post-MET treatments.
 - b. Evaluate the toxin's activity post-treatment.
 - c. Determine the microbial shifts post-MET and *C. difficile* treatments.

Experimental Approaches:

- ELISA assays will be performed to quantify *C. difficile* toxins within mice models post-MET treatments.
- DNA isolations and sequencing protocols will be used to evaluate the microbial shifts within tested groups.
- Histological techniques will be used to determine the intestinal damage after *C. difficile* infections.
- Cell toxicity assays will be conducted to evaluate the activity of the *C. difficile* toxins isolated from the mouse stool.

References:

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2. Ofosu A (2016) Clostridium difficile infection: a review of current and emerging therapies. Ann Gastroenterol 29 (2):147-154. doi:10.20524/aog.2016.0006
3. Lofland D, Josephat F, Partin S (2013) Fecal transplant for recurrent Clostridium difficile infection. Clin Lab Sci 26 (3):131-135
4. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E (2013) Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. Microbiome 1 (1):3. doi:10.1186/2049-2618-1-3