

# Diarrheal Dysbiosis and Treatment

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Diarrhea is one of the leading reasons for clinical evaluation in both inpatient and outpatient clinical settings<sup>1</sup>. Among hospitalized patients the leading identifiable cause of diarrhea is *C. difficile* infection, resulting in annual costs to the US health care system in excess of \$1 billion<sup>2</sup>. However the majority of diarrheal cases have no identifiable cause. Significant strides have been made towards understanding the role of the gastrointestinal microbiome in diarrheal diseases, and recent evidence suggests its modulation may be harnessed for therapy. One of the most notable examples is treatment for *C. difficile*-associated diarrhea with fecal microbiota therapy (FMT)<sup>3</sup>. These studies have highlighted the important role of dysbiosis, or an imbalance of microorganisms, in these conditions, but have not addressed its role in diarrheal illness more broadly. Identifying potential novel pathogens and elucidating the dysbiotic changes associated with diarrheal illness is an initial step towards understanding the pathogenesis of diarrheal illness and may provide opportunities for therapeutic intervention.

The two available sample collections with which we will address the topic of diarrheal dysbiosis are:

1. 800 samples collected from patients with diarrhea through the Massachusetts General Hospital (MGH) Clinical Microbiology laboratory (Drs. Traverso and Begun). A subset of only 25% of these samples tested positive in the standard practice *Clostridium difficile* culture test. Besides stool culture data, clinical patient and treatment data will also be available for the timespan prior and post diarrhea. For patients with recurrent diarrhea, multiple samples will be available;
2. Dr. Hohmann, a physician in the Department of Infectious Diseases at Massachusetts General Hospital, has an active IND (IND 15199), and Institutional Review Board approval to investigate the use of fecal microbiota transplantation (FMT) in recurrent *Clostridium difficile* colitis. Together with Drs. Sauk and Youngster, she performs procedures on a weekly basis. For each patient that they have handled so far (n=30), samples were collected from the donor, as well as pre-transplant, and multiple post-transplant timepoints from the recipient, adding up to a total of 200 samples.

The planned metagenomic sequencing activities per collection are:

	16S-seq	WGS DNaseq	RNAseq
Hospital Diarrhea (n=800)	all samples	200	100
<i>C. diff</i> treatment (n=200)	all samples	100	50

With the obtained dataset, we will address the following questions:

On the samples from hospital patients with diarrhea:

- Can a culture-independent sequence-based metagenomic approach help identify a microbial infectious agent (bacterial or viral) in samples with no identifiable cause (*C. diff* negative)?
- Using metagenomics, can we obtain a more detailed characterization of the *C. diff* strains with regard to toxicity and antibiotic resistance?
- How does the microbiome in dysbiosis differ from the healthy one, and what are the different dysbiosis states?
- Can we link dysbiosis states to the pre-diarrhea treatment or primary disease?
- Can we predict drug treatment outcome from microbiome composition?

On the samples from patient with recurrent *C. diff*, treated with FMT:

- What fraction of donor fecal microbiota is transmissible and persistent in the recipient?
- Does the composition of the recipient's microbiota before FMT, and the infecting *C. diff* strain impact the modulation of the microbiome?
- Is there any constituent of the recipient's microbiota before FMT that persists after the therapy?
- Can a culture-independent sequence-based metagenomics approach enable a more comprehensive safety assessment of pre-screened donor stool?

1. Peery AF, Dellon ES, Lund J, et al. Burden of Gastrointestinal Disease in the United States: 2012 Update. *Gastroenterology* 2012;;1–12.
2. McGlone SM, Bailey RR, Zimmer SM, et al. The economic burden of *Clostridium difficile*. *Clin Microbiol Infect* 2012;18(3):282–9.
3. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* 2013;368(5):407–15.