Title: Metagenomic and metabolomic shifts associated with successful fecal microbiota transplantation in patients with recurrent *Clostridioides difficile* infection

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Clostridioides difficile (*C. difficile*) is a Gram-positive, spore-forming, anaerobic pathogen responsible for *C. difficile* infection (CDI), a healthcare-associated infection that causes significant morbidity and mortality. Recurrent CDI (rCDI), or the reoccurrence of symptoms after initial treatment with an antibiotic, affects approximately 40% of patients. This cycle of antibiotic treatment and recurrence is often associated with a continued disruption of the gut microbiota and loss of colonization resistance, resulting in the loss of key microbial taxa and metabolites. Fecal microbiota transplantation (FMT) has emerged as an effective therapy for rCDI, with cure rates exceeding 90%. However, many of the underlying mechanisms which make FMT a successful therapy remain poorly understood. We hypothesize that there will be unique structural (taxonomic) and functional (metabolomic) shifts in the feces of patients undergoing FMT for rCDI.

We enrolled patients undergoing FMT for rCDI at the University of North Carolina Medical Center in Chapel Hill in a prospective registry. The registry collected clinical data and patient stool samples two weeks pre-FMT (n=16), and two weeks (n=11), two months (n=10), and six months (n=8) post-FMT. Shallow shotgun sequencing was performed by CoreBiome and untargeted metabolomics was performed by Metabolon on stool. The bioBakery suite of tools developed by the Huttenhower lab were used to analyze shotgun sequencing data, determining relative taxonomic abundance and identifying relevant genes within reads. Linear modeling with Bonferroni q-value correction was performed to determine the relationship between taxonomic abundance, microbial genes, and metabolites.

As expected, there was an increase in alpha diversity post-FMT compared to pre-FMT, with minimal variation between the two week, two month, and six month post-FMT timepoints. Pre-FMT samples had higher levels of Enterobacteriaceae, while post-FMT samples had increased levels of Lachnospiraceae. Using random forest analysis, the most important metabolites between pre- and post-FMT samples were classified as lipids, specifically acylcarnitines and bile acids.

Linear modeling revealed significant differences in genes associated with enzymatic reactions impacting these metabolites, such as carnitine-CoA ligase, choloylglycine hydrolase (BSH), and 3α-hydroxysteroid dehydrogenase (HSDH). Most of the carnitine-CoA ligase reads mapped to *Escherichia*, while choloylglycine hydrolase (BSH) and 3α-hydroxysteroid dehydrogenase (HSDH) reads mapped largely to *Blautia* and *Fusicatenibacter,* respectively. This research sheds light on the structural and functional shifts associated with successful FMTs and holds promise for the development of more targeted therapies to treat rCDI.