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TITLE: A NOVEL ROUTE TO CONTROLLING CLOSTRIDIOIDES DIFFICILE GROWTH VIA SHORT CHAIN FATTY ACID AND BILE ACID MODULATION

AUTHORS (FIRST NAME, LAST NAME): Julie A. McDonald[1], Benjamin H. Mullish[1], Alexandros Pechlivanis[1], Jia V. Li[1], Jeremy K. Nicholson[1], Elaine Holmes[1], Mark R. Thursz[1], Julian Marchesi[1, 2]

ABSTRACT BODY:

Abstract Body: Background: Antibiotic perturbations of the gut microbiota have been associated with Clostridioides difficile infection (CDI). Fecal microbiota transplantation (FMT) treats recurrent CDI, however there are concerns regarding its safety, reproducibility, composition, and stability. Mechanisms of FMT have not yet been elucidated, and mechanistic studies are challenging in vivo. Samples from CDI patients prior to FMT are collected shortly after stopping antibiotics, meaning researchers see bacteria/metabolites increasing post-FMT that may not be important for resolving the disease. We used an in vitro model to study the effects of FMT and identify bacterial metabolites that are important in CDI pathogenesis.

Methods: A CDI chemostat model was used to test the effects of FMT under strictly controlled conditions (n=3). In each experiment 2 vessels were inoculated with feces and allowed to stabilise. Then, C. difficile spores were introduced and the vessels dosed with clindamycin for 7 days. Next, microbial communities were again allowed to stabilise. Then, we tested a single dose of FMT preparation into one vessel against saline in the other vessel. The following analyses were performed: C. difficile total viable counts (TVC), C. difficile spore counts, 16S rRNA gene sequencing, 16S rRNA gene qPCR, liquid chromatography mass spectrometry-based bile acid profiling, and ^{1}H-NMR spectroscopy. We performed batch culture experiments to directly investigate how specific metabolites affected C. difficile growth.

Results: FMT resulted in a 93.6% reduction in C. difficile TVC (p=0.025) and an 85.8% reduction in spores (p=0.034). During antibiotics C. difficile TVC had strong positive correlations with taurocholic acid (r=0.68, p=6.29x10^{-4}) and cholic acid (r=0.61, p=0.003), and strong negative correlations with deoxycholic acid (r=-0.75, p=8.86x10^{-5}), and lithocholic acid (r=-0.76, p=6.36x10^{-5}) (Figure 1). After stopping antibiotics, bile acids recovered to pre-antibiotic levels and were not affected by FMT. Batch culture experiments showed taurocholic acid is required for C. difficile germination but has no effect on vegetative growth. During antibiotics C. difficile TVC negatively correlated with valerate (r=-0.59, p=0.005) and positively correlated with 5-aminovalerate (r=0.54, p=0.01) (Figure 2). Following FMT C. difficile TVC had a strong negative correlation with valerate (r=-0.67, p=0.0001) and a strong positive correlation with 5-aminovalerate (r=0.76, p=6.07x10^{-6}). Batch culture experiments with C. difficile ribotypes 010, 012, and 027 showed valerate decreased vegetative growth.

Conclusions: Our data shows that manipulating the levels of key bile acids during antibiotic exposure could inhibit C. difficile spore germination and prevent CDI. Valerate may also be a useful intervention that could be given to patients to inhibit C. difficile vegetative growth.