Restoration of the Intestinal Microbiota of Individuals with Severe *Clostridium difficile* Disease. By B. O. Anonye[1], M. D. Stares[1], A. W. Walker[2], K. Cannon[3], J. Parkhill[1], T. J. Louie[3] and T. D. Lawley[1], *[1]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, UK, CB10 1SA*, *[2]The Rowett Institute, University of Aberdeen, AB21 9SB, UK* *[3]University of Calgary, 2500 University Dr. NW, Calgary, Alberta, Canada, T2N 4J8*

Introduction

The human intestinal microbiota is inhabited by a diverse and abundant community of microorganisms, which plays vital functions in health and disease. Pathological imbalances in the intestinal microbiota caused by antibiotics, and pathogens such as *Clostridium difficile*, have profound effect on the microbiota's composition and function (1). *C. difficile*, an anaerobic spore-forming bacterium, is the main cause of infectious diarrhoea in hospitalized patients. Treatment with standard antibiotics often leads to recurrence of disease in 20-35% of patients. Recurrent disease occurs as a result of a decrease in or elimination of health-associated bacteria by antibiotics, therefore allowing *C. difficile* to overgrow without competition. An alternative treatment for persistent, recurrent *C. difficile* infection involves the restoration of the indigenous microbiota with faecal microbiota transplantation (FMT). FMT involves the instillation of homogenized faecal suspension from a healthy donor to an individual with recurrent *C. difficile* disease and about 90% success rates have been observed (2, 3). We examined the faecal microbiota of individuals with recurrent *C difficile* before and after FMT and observed restoration of the intestinal microbiota after FMT.

Methods

In the present study, 454 sequencing of the 16S rRNA gene was used to define the bacterial taxa in faecal samples from 12 pairs of healthy donors and recurrent *C. difficile* infected patients before and after FMT. Briefly, DNA was extracted directly from the faecal samples using the FastDNA Spin Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions. The V3-V5 regions of the 16S rRNA gene were amplified using barcoded primers adapted with linkers to enable multiplexing and deep sequencing of the samples. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, UK), and quantified using the Qubit® dsDNA HS Assay Kit (Life Technologies, UK). These purified PCR products were sequenced on the Roche 454 FLX-Titanium platform. The resulting sequence reads were trimmed, filtered and pre-processed using the mothur software (4). The species diversity in each sample was measured by calculating the Shannon diversity index (SDI), which takes into account both species richness and relative proportional abundance. Sequences with 97% phylogenetic similarity were regarded as belonging to the same operational taxonomic units (OTUs). The OTUs were given a taxonomic assignment using the Ribosomal Database Project classifier (5). These OTUs were

then used to generate principal component analysis (PCA) using Unifrac to compare the microbial community of the different samples (6).

Results and Discussion

Analysis of the OTUs obtained from the healthy donor samples indicated a greater proportional abundance of the Firmicutes, Bacteroidetes and Actinobacteria. In contrast, OTUs belonging to the phylum Proteobacteria was more abundant in the patient samples before FMT (pre-FMT). Members of the *Lachnospiraceae* and *Ruminococcaceae* families from the Firmicutes phylum dominated the donor's microbiota. Some of these members have been identified in a previous study as being associated with a healthy intestinal microbiota (7). The family *Enterobacteriaceae* (52%) were abundant in the pre-FMT samples and this family is known to harbour opportunistic bacteria.

Since our study included a longitudinal sampling of the patients' sample post-FMT, we were able to examine the dynamics of microbiota change in response to disease resolution. At the phyla level, there was a significant increase in the relative abundances of Actinobacteria and Firmicutes ($p < 0.05$) and a significant reduction in the Proteobacteria phylum ($p < 0.05$).

Next, we were interested in examining the changes in microbial diversity after FMT in patient samples. UniFrac analysis uses phylogenetic information to compare the microbial community structure of samples. The phylogenetic trees generated by UniFrac were examined using principal component analysis (PCA) to cluster samples before and after FMT and determine the similarity in community structure with the donor (Figure 1). Using weighted and unweighted UniFrac distances, the clustering was found to be significant and this was supported by the parsimony test ($p<0.001$). From the PCA plot, the pre-FMT samples clustered separately from the donor samples indicating difference in community structure. After FMT, the patient samples clustered together with the donor samples indicating a shift to a healthy microbiota with increased diversity (Figure 1). This increase in bacterial diversity was also observed in the Shannon diversity index at various times post-FMT marked by a significant increase in the *Lachnospiraceae* and *Ruminococcaceae* families and a reduction in the *Enterobacteriaceae* family ($p < 0.05$).

Conclusion: FMT restored the intestinal microbiota of the patients with no recurrence of *C. difficile* infection. Current research in progress is geared towards identifying and validating specific bacterial species that confer colonization resistance against *C. difficile* infection. Hence, this work should lead to the rational design of a defined mixture of beneficial bacteria that can be used to treat recurrent *C. difficile* infections.

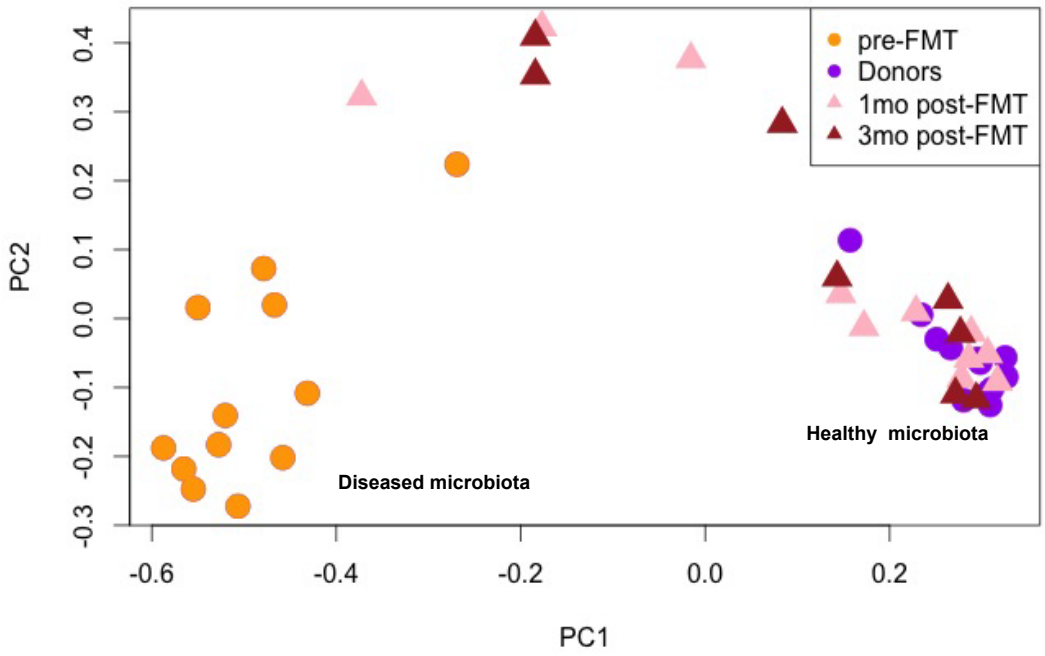

Figure 1. **Principal component analysis of the samples before and after FMT.** The pre-FMT samples clustered closely together indicating similar microbial community associated with antibiotic use leading to intestinal dysbiosis. Treatment with FMT led to a shift from a diseased microbiota to a healthy one by clustering with the healthy donors across the various time points. Principal components 1 and 2 account for ~46% of the variation in the samples.

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