Serial Microbiome Analysis in a Patient with Multiple Failed Fecal Microbiota Transplantations

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BACKGROUND

- Fecal microbiota transplantation (FMT) is recommended to treat refractory or recurrent cases of *Clostridioides difficile* infection (CDI) through restoration of a healthy intestinal microbiome.[1]
- The procedure has reported success rates of 90% or higher for CDI,[2-4] but several risk factors for FMT failure have been identified.[5,6]
- Here we present a case of a patient failing four FMTs over a two-year period, with accompanying microbiome and metagenomic analyses.

METHODS

Sample collection:

- Leftover stool samples were collected from the clinical microbiology laboratory and clinical information collected from the medical chart.

Microbiology and ribotyping:

- Stool samples were incubated under anaerobic conditions for 48 hours for *C. difficile* growth as previously described.
- Strain typing was done using PCR-based ribotyping method
- Multiple colonies were picked from each culture to assess for mixed ribotypes.

Multidrug resistant organism (MDRO) screening:

- After growth from stool on selective media, antimicrobial resistance genes were determined by PCR including vancomycin-resistant Enterococcus (vanA, vanC2/3), methicillin-resistant *S. aureus* (mecA), carbapenem-resistant Enterobacteriaceae (KPC, NDM1, and OXA48), and Candida species.

DNA extraction and whole genome sequencing:

- DNA was extracted using the AnaPrep automated DNA extractor (BioChain), quantified by NanoDrop (ThermoFisher) and Qubit (ThermoFisher), and DNA quality was assessed using a BioAnalyzer (Agilent).
- The generated fastq files were trimmed using Trimmomatic 4 and sequencing quality was examined by software FastQC.
- The presence of known antimicrobial resistance genes was determined from cleaned reads using the ARG-ANNOT database 5 and SRST2 pipeline 6.
- For whole-genome SNP analysis, cleaned sequence reads were mapped to the R20291 reference genome (GenBank accession number FN545816) using the RedDog pipeline according to the developer's guidelines.
- Phylogenetic trees were created in FigTree and heat maps were generated using R.

Minimum inhibitory concentration (MIC) analysis:

- MICs were determined by broth microdilution in 0.1% sodium taurocholate BHI.
- Cultures of C. difficile were prepared by inoculating one isolated colony on blood agar plate to BHI medium. Cultures were diluted 1:100 to approximately 106 CFU/mL in fresh media and doubling dilutions of each antibiotic.

16s microbiome analysis:

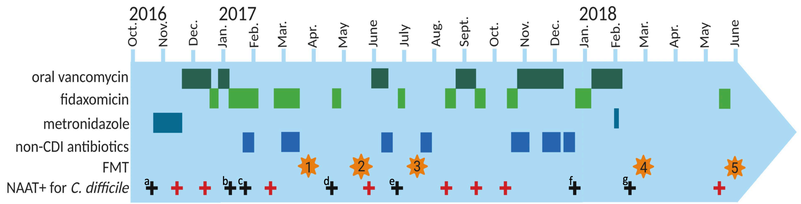
- Stool sample microbiome characterization was performed by sequencing the V3-V4 region of the 16S rRNA gene in Illumina MiSeq platform followed by bioinformatics analysis related to microbial composition, diversity, and community structure.
- Raw sequences were quality filtered and minimum of 15,000 reads per sample was used for the downstream analysis. Sequences were clustered bases on similarities with 97% or higher for Operational taxonomic units (OTUs). The representative sequences from each cluster was searched against NCBI database (September, 2018) for taxonomy assignment. Calculation of the alpha diversities using Chao and Shannon metrices, and beta diversities using weighted Unifrac distance metric was performed in QIIME 1.9.0. RStudio 1.1.456 was used for the visualization of the analysis

RESULTS

- 42-year-old female with various comorbidities, including systemic lupus erythematosus.
- The vancomycin MICs of infecting *C. difficile* strains increased with cumulative exposure. (**Figure 1**)
- Multidrug-resistant organisms were detected in stool, including Enterococcus spp., MRSA, and *Candida glabrata*.
- The first five of the seven strains were ribotype (RT) 078-126, one was mixed RTs 002 and 054, and one was RT 002.
- The analysis of 16S rRNA gene sequences demonstrated that microbial diversity was never restored after FMT procedures. (**Figure 2**)

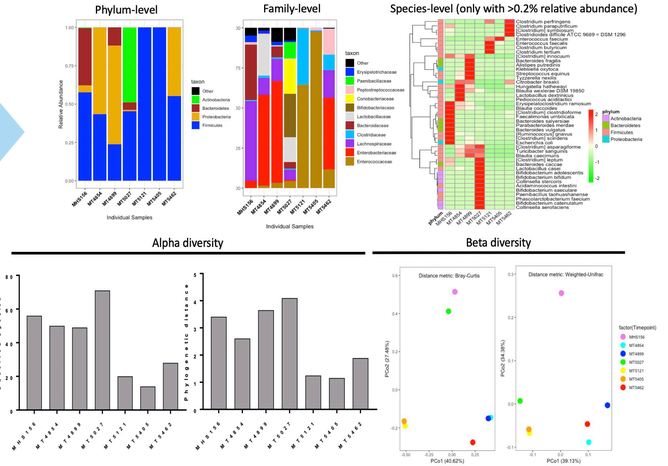
Figure 1. Comprehensive timeline of events.

***Letters a-g correspond to samples included in the chart.**



Sample	[a]MHS156	[b]MT4854	[c]MT4899	[d]MT5027	[e]MT5121	[f]MT5405	[g]MT5462
Ribotype	F078-126	F078-126	F078-126	F078-126	F078-126	F002/F054	F002
MIC (24 hr)							
Colonies tested (no.)	1	6	6	ND	8	2	1
vancomycin	0.5	1.0-2.0	0.5-2		0.5-1	4	2
metronidazole	0.125	0.25-2	0.125-4		0.125-0.5	2	0.5
fidaxomicin	0.016	0.03125-0.016	0.016-0.0625		0.016	0.0625	0.016-0.03125
eravacycline	0.03125	0.016-0.0625	0.016-0.0625		0.016-0.03125	0.03125	0.016
levofloxacin	2	2.0-4.0	2.0-4.0		2.0-4.0	4	2
meropenem	8	8	8		8	8	8
MDROs Isolated							
VRE		Yes	Yes			Yes	
MRSA							Yes
Candida glabrata		Yes			Yes		
Candida tropicalis					Yes		

Figure 2. Microbial composition and diversity analysis using 16S V3V4 amplicon data



CONCLUSION

- **A number of systems biology changes were observed in a patient with persistent CDI despite multiple FMTs.**
- **The lack of FMT engraftment was most likely due to continuous broad-spectrum antibiotic exposure in an immunocompromised patient.**

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