Effects of intraperitoneally administered phage therapy on resident gut microbiota

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Introduction

- Antimicrobial resistance is a growing global health threat that necessitates the development of alternative treatments beyond current antibiotics
- Bacteriophage (phage) therapy is a potential alternative treatment to antibiotics that has largely been utilized for compassionate use but it's use is ongoing in clinical trials
- Phage therapy utilizes viruses that specifically kill individual bacteria species or strains as opposed to antimicrobials which target conserved mechanisms
- There are large assumptions currently held regarding the effects of phage therapy on non-target species present in microbial communities
- Few studies have thoroughly assessed changes in microbial community composition following phage administration
- To fully evaluate the impact of this therapeutic modality, it is important to understand the potential effect of phage on non-target species and the composition of the gut microbiota

What is the impact of phage therapy on the composition of the gut microbiome?

Model

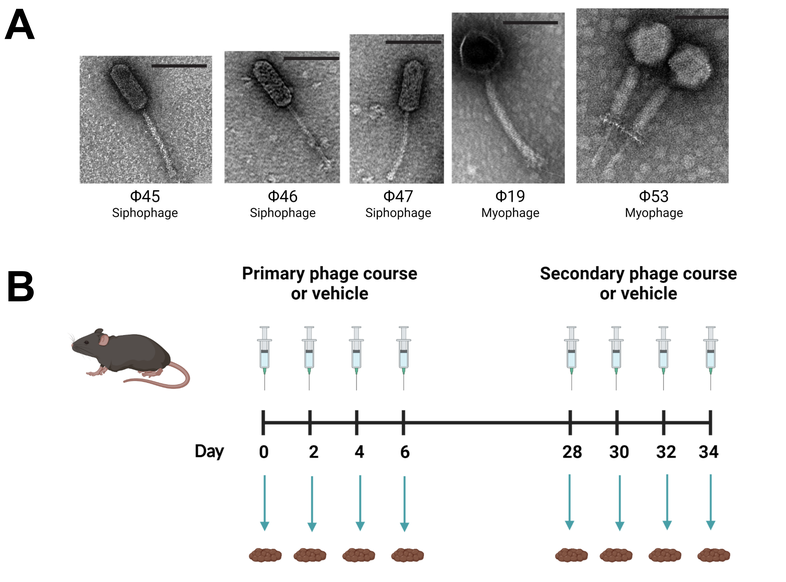


Figure 1: Murine model to assess phage cocktail administration on gut microbiota
A. Five phage from two different families (Siphoviridae and Myoviridae) were chosen for the cocktail. All five phages target vancomycin-resistant enterococcus strain SF28057, which was not detected in initial gut microbiota samples. Images taken using immunogold labeling EM.
B. Wildtype C57Bl/6 mice were treated via intraperitoneal injection (IP) with a five-phage cocktail ($5x10^9$ PFU/day) curated to target VRE strain SF28057 or vehicle (water). Stool samples were collected every day prior to injection and used to assess microbiota present in GI tract.

Methods

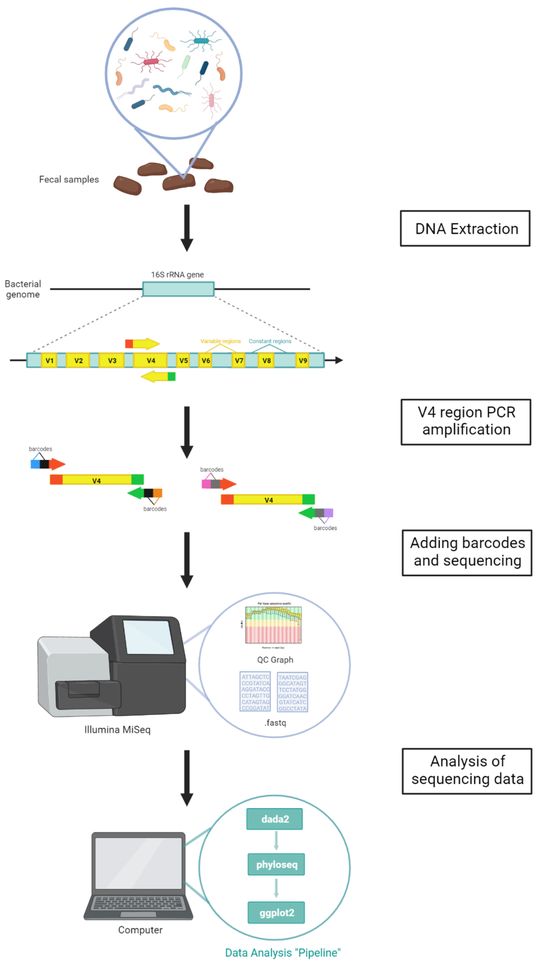
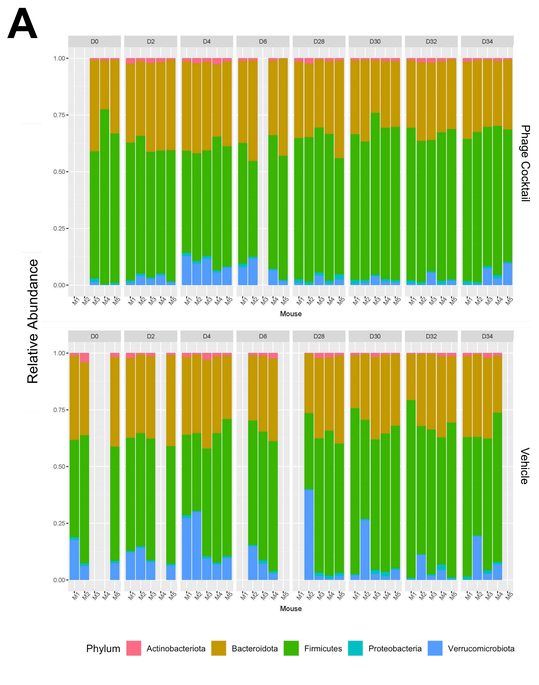


Figure 2: Process overview for obtaining microbiota profiles from collected fecal samples
Mouse fecal pellets were obtained from four cages each housing five mice for n=5 biological replicates per treatment group per duration of treatment. Stool was stored according to individual sample in a 96-well plate and sent to University of Michigan Medical School Microbiome Core where DNA extraction, PCR amplification, and 16S V4 amplicon sequencing was performed using the Illumina MiSeq platform. The returned sequencing profiles were analyzed using the dada2, phyloseq, and ggplot2 packages in the RStudio platform.

Results and Discussion



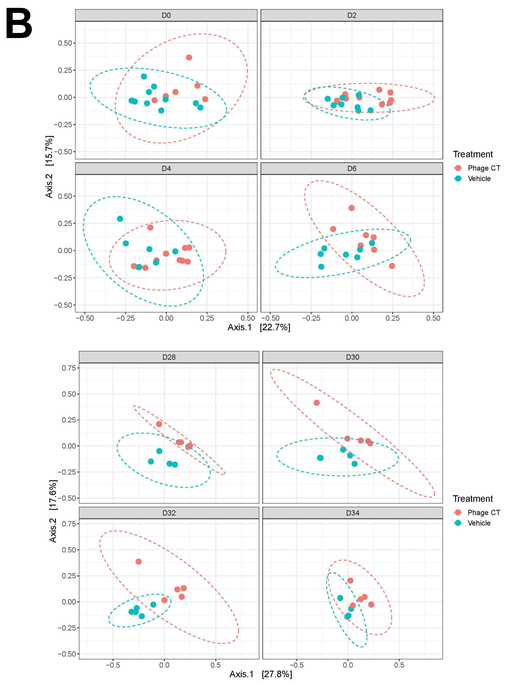


Figure 3: Relative abundance and diversity of microbiota profiles over treatment course
A. The top five most prevalent phyla in microbial communities profiled from mouse fecal samples stratified by date of collection and treatment received for mice treated with two courses of phage or vehicle injections. Samples with missing data are result of inability to collect fecal pellet at time of sampling or read depth not exceeding the standard set of >700 reads per sample. Phyla not displayed constitute proportions of <1% of total phyla in sample. Microbial composition notably does not change consistently over period of treatment with phage therapy compared to treatment with vehicle control, suggesting microbial profile is not significantly impacted by administration of phage therapy.
B. Beta diversity graphed using Bray-Curtis statistics to quantify compositional dissimilarity between treatments. Each point represents a mouse microbial community sampled on the given timepoint. Different treatment groups were housed in different cages. Mice receiving treatment as part of the two-course treatment model were pooled with mice receiving a single course of treatment for a total of n=10 datapoints on days 0-6.

Future Directions

- Extend duration of treatment administered and repeat protocol for profiling microbial community composition
- Explore different routes of administration such as intravenous injection, subdermal, and inhalation
- Collect metatranscriptomic data from microbial communities before, during, and after phage therapy administration

Acknowledgements and Disclaimers

Figure images generated using BioRender. Research supported in part by the University of Michigan Medical School Microbiome Core. Opinions expressed in this presentation are the author's own and do not reflect the view of the Food & Drug Administration, the Department of Health and Human Services, or the United States government. Funding: FDA/CBER. This project was supported in part by an appointment to the Research Participation Program at CBER/OVRR/DBPAP/LMPCI U.S. Food and Drug Administration, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and FDA