Combating *Clostridium difficile* infection with an optimised bacteriophage cocktail

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**Background**
- *Clostridium difficile* infection (CDI) remains a global health challenge due to insufficient treatment options. Bacteriophages or phages (viruses that specifically target and kill bacteria) can provide alternative therapies for CDI.
- We have developed a novel 4-bacteriophage cocktail, that can completely eliminate *C. difficile* in pure cultures and biofilms, and reduce colonisation in hamster and wax moth larva CDI models.
- Here, we describe the activity of the cocktail in a batch fermentation CDI model spiked with combined freshly voided fecal slurries obtained from four healthy volunteers covering diverse ethnic and age groups as the source of human gut microbiota.

**Methods**
- Four fecal samples were collected from healthy adults: British-born (70 yr) and British-ethnic (70 yr) and from African adolescents (17 yr) and African adults (40 yr).
- Samples were inoculated for bacterial load then mixed in equal proportions to ensure inoculum.
- Mixed fecal slurries were treated with a cocktail of four bacteriophages and/or bacteria at various time points as shown in Table 1.

**Aims:**
1. To determine the efficacy of an optimised 4-phae cocktail (Fig. 1) to clear cultures of a clinically ribotype 014/020 isolate under competitive pressure from the human gut microbiomes.
2. To determine the potential synergistic or antagonistic effects of phage therapy on other components of the human gut microbiome.

**Result 1: Phages are effective at clearing *C. difficile* in the gut model**
- Viability assays showed that a diverse range of microbiota was contributed by the donors (Fig. 2).
- We observed 6 and 1 log reductions in *C. difficile* counts in the prophylaxis and remedial regimens respectively within the first 5 h post-infection, and complete eradication of the bacteria at the 24th hour in both regimens (Fig. 3).
- *C. difficile* remained undetected from the 5 h time point, until the experiment was terminated at the 72h hour (Fig. 3).

**Result 2: Phages exert no antagonistic effect on other culturable microbiota and promote colonisation of specific components of the gut microbiome**
- The commensal Bifidobacteria, Enterococci, Lactobacillales, total Anaerobes and Enterobacteriaceae were not affected by either the prophylactic or remedial regimens (Figs. 4A-E, Table 1).
- However, the phage control (Table 1) showed ~2 log increase of the total Anaerobes and Enterobacteriaceae counts compared to the two regimens and the bacterial/untreated controls at the 24-72 h time points (Fig 4D and E, Table 1).

**Result 3: Strong correlation between viability assays and metagenomic data at 24 h**
- The percent reads mapped to bacteria, Archaea and viruses are shown in Table 2.
- Although the individual groups of bacteria remained consistent in all treatments, their abundances varied considerably in the vessels (Table 2, Fig. 5).
- Consistent with our viability assays, we observed that percent bacterial abundances of Enterobacteriaceae (marked in red boxes), and Bifidobacteriaceae, Lactobacillales were considerably high in vessel 3 from the metagenomics data (Fig. 5).

**Conclusions and future work**
This data supports the application of the phage cocktail to prevent/treat CDI. The elevated levels of specific commensals in the phage-treated control (vessel 3) could prevent colonisation of *C. difficile* and provide protection from the infection. Further genetic work is ongoing to produce therapeutically acceptable phages for CDI.

**References**