Therapeutic Use of Bacteriophage Cocktails for the Treatment of Antibiotic Resistant *Acinetobacter baumannii* Infections

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Antibiotic Resistance Crisis

- Antibiotic Resistant (AR) infections represent a global health crisis, as nearly 50% of prescribed antibiotics prove ineffective
- In the US, antibiotic resistant bacteria are responsible for 2 million infections annually, and draw $20 billion in treatment costs and novel drug development (*Figure 1*)
- In 2013, the CDC declared *Acinetobacter baumannii* as one of the ESKAPE Pathogens

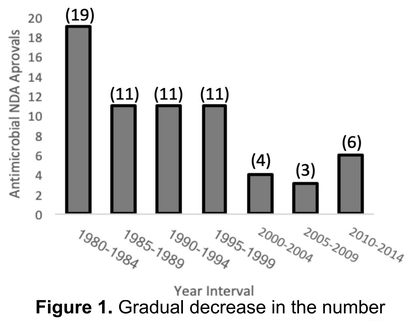


Figure 1. Gradual decrease in the number of antimicrobial new drug applications (NDA) approvals

Research Objective

- Develop a bacteriophage-based therapeutic cocktail as an alternative treatment against antibiotic-resistant infections
- Compare bacteriophage therapeutics to conventional treatments and determine synergistic effects with antibiotics

Bacteriophage Therapeutics

- Bacteriophages are a type of virus that exclusively infect bacteria. They are extremely abundant, with an estimated 10^{32} total virus particles. The mechanism of action is summarized in *Figure 2*

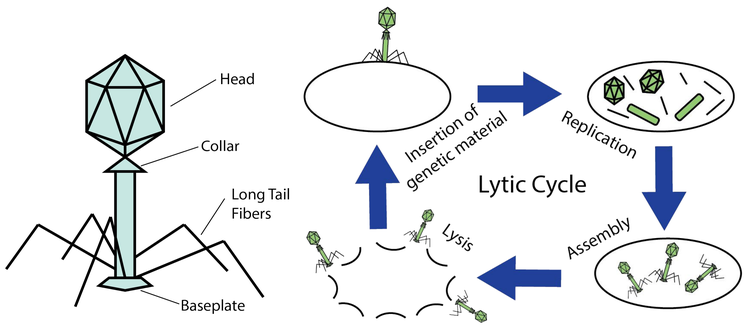
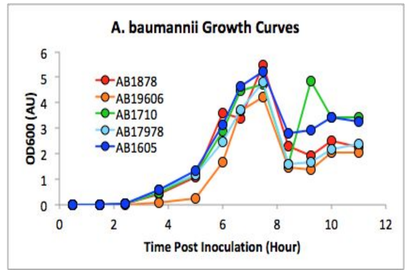


Figure 2. Bacteriophage structure and Lytic Cycle summary

- Phage therapy is a promising yet insufficiently researched tool for solving the antibiotic resistance crisis. Phage therapeutics offer the following advantages:
  - Coevolution allows phage to target resistant bacteria
  - Lower rate of toxicity caused by release of endotoxins
  - Penetration of biofilms in medical and food safety settings
  - Phage cocktails, or mixtures, enhance treatment effectiveness

Bacteria Characterization

Figure 3. Growth curves for five strains of AR *A. baumannii* executed in a 24 well microplate reader. The bacteria overshoot the carrying capacity of the flask and a correction is observed at 8 hours post-inoculation to achieve stationary phase.



Coevolution

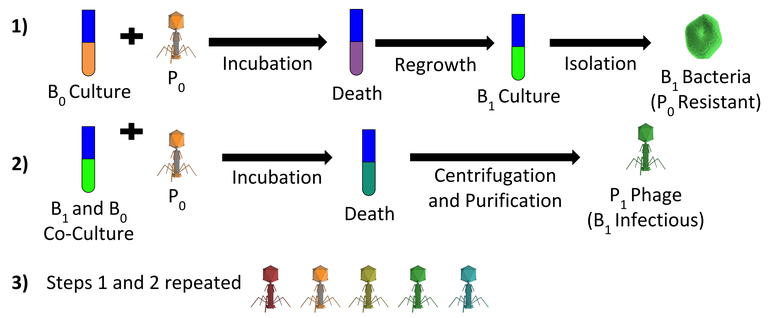
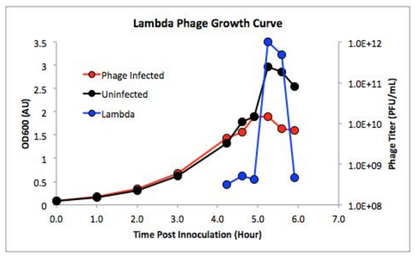


Figure 4. This figure shows the process of coevolution between the bacteriophage and its bacterial target. Through several cycles of this process, a library of bacteriophages is generated.

Phage Single Step Growth Curve

Figure 5. Reduction of bacterial optical density is preceded by a surge in phage titer. Mid-log cultures of *E. coli*-pGlo were inoculated with phage at an MOI of 0.5 PFU/CFU. A background phage titer of ~8.5 PFU/mL corresponds to infected bacteria which were plated during titering. Reabsorption of phages to the remaining viable bacteria was observed after the initial burst.



Results

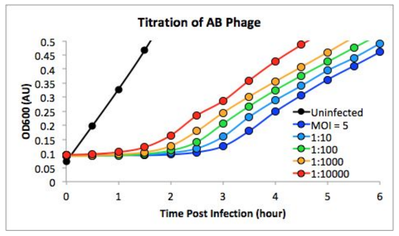


Figure 6. Growth curve for *A. baumannii* showing greater lag periods as the multiplicity of infection (MOI) is increased. Uninfected bacteria showed far less lag in growth than all MOIs.

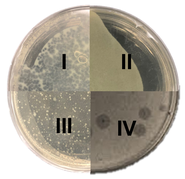


Figure 7. I shows phage plaques while II shows no plaques indicating the absence of phage. III shows phage resistant colonies. IV shows individual plaques.

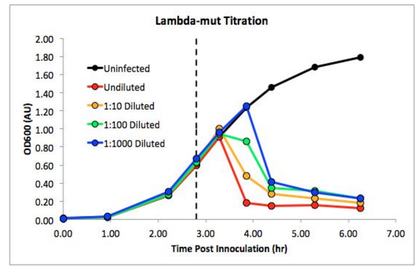


Figure 8. Bactericidal action of λ-mut shows dependence on phage concentration. Mid-log cultures of *E. coli*-pGlo were infected with the indicated dilution of λ-mut stock. The optical density of infected flasks decreased significantly 45 minutes post infection. The undiluted flask was completely lysed at this time.

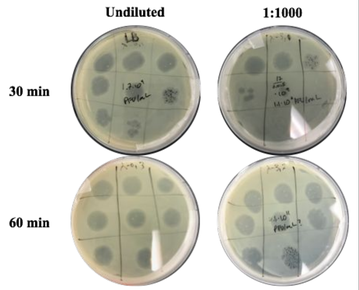


Figure 9. λ-mut titer increase before a decrease in optical density is observed. At 30 minutes post inoculation the phage titer in each flask increases by slightly more than 2 log units and increased by a further 4 units after 60 minutes.

Future Research Goals

Prove viability of coevolution using *A. baumannii* and *E. coli*:

- Phages in conjunction with antibiotics--show that bacteria treated with phages become susceptible to non-harmful antibiotic treatments
- Parasitic bacterium--*Bdellovibrio bacteriovorus* is a bacteria that attacks other gram-negative bacteria
- Expansive phage library--show that with more phages evolved there is greater effectiveness against bacteria

Acknowledgements and Citations

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