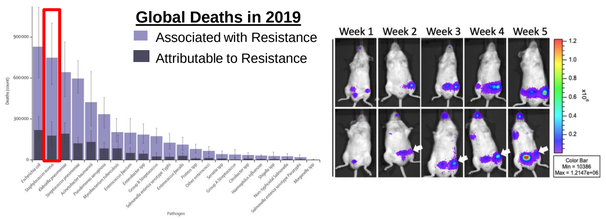
The Isolation, Characterization, and Optimization of Bacteriophages Targeting Clinical Isolates of *Staphylocccus aureus*

Hamza Nagarwala, Michael Hadjiargyrou, Bryan Gibb

BACKGROUND

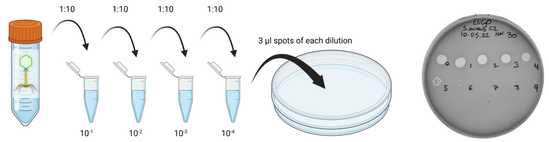
- *Staphylococcus aureus* is one of the leading causes of global mortality due to bacterial infection. Antibiotic-resistant forms such as methicillin-resistant *S. aureus* (MRSA) are more difficult to treat, which poses an increased risk of complications to patients.
- Multiple organizations predict that by 2050, antibiotic-resistant bacterial infections could cause 10 million deaths a year. Therefore, developing effective treatments for antibiotic-resistant infections is urgently needed.
- Bacteriophages may be a viable alternative treatment for MRSA



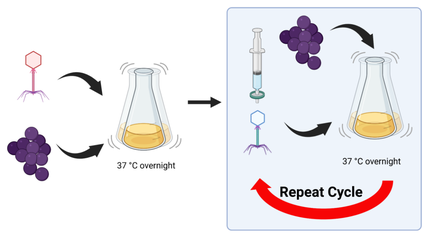
- Collaborators at Washington University of St. Louis engineered a clinical isolate of MRSA USA300 to be bioluminescent and fluorescent and used this strain in a mouse infection model for monitoring course of infection non-invasively over time
- **Goal: Isolate bacteriophages capable of infecting the MRSA host for *in vivo* testing of phage efficacy in mouse infection model system**.

METHODS

- Two viable phages were isolated from Georgian commercial phage cocktails
- Both showed infectivity at 30°C, but poor infectivity at 37°C.

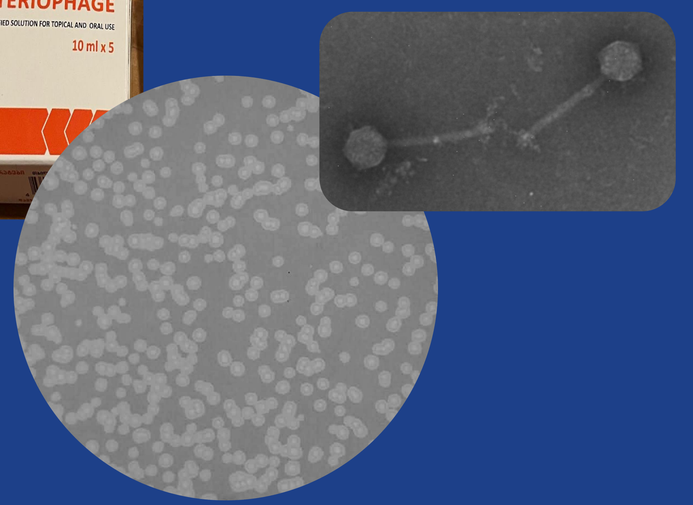


- Phage was repeatedly cultured at 37°C to select for heat resistant variants. Each round was evaluated by spot titer assay



- DNA was extracted from the original and evolved phage by phenol-chloroform extraction.
- Purified DNA was sequenced by Illumina MiSeq.
- DNA reads were assembled using Unicycler and annotated with pharokka. Additional comparative analysis was conducted using tools provided by BV-BRC

Directed evolution of phage enhances infectivity of MRSA

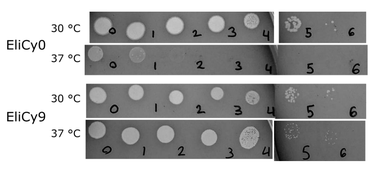


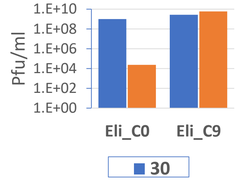
QR code for references, and pdf copy of this poster

NEW YORK INSTITUTE OF TECHNOLOGY

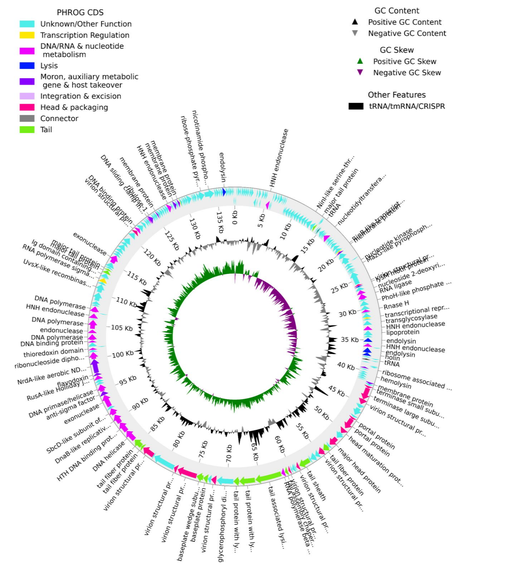
RESULTS

- Phage Eli was successfully isolated from a commercial Staphylococcal phage cocktail from the Eliava Institute of Tblisi.





- After nine rounds of propagation at 37°C, the efficacy of plaquing of Phage Eli improves by 10^5 relative to the original isolated phage.
- The isolated phage is a mutated version of *Staphyloccus aureus* phage Sb-1

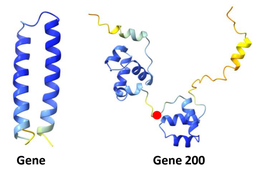


Gene #	length aa	Function	Pos	Change	Amino Acid Change	Mut type
200	148	DNA Binding Protein	121496	217C>A	Arg73Ser	missense
		intergenic region	28606	G>T		intergenic
28	60	hypothetical protein	7800	31G>T	Glu11*	nonsense

There are 3 SNPs in phage EliCy9

- Gene 28: mutation introduces a premature stop codon at amino acid 11. The gene has no clear function, but alphafold shows it as a helix-loop-helix motif
- Gene 200: mutation from arginine to serine in helix-turn-helix motif of a putative DNA binding domain. Location of mutation shown with red dot.
- Last change occurs in an intergenic region upstream of a putative gene predicted to be a phosphate starvation-inducible protein PhoH with an ATPase domain

Alphafold predictions



FUTURE DIRECTIONS

- Begin *in vivo* experiments of phage efficacy in mouse infection model system
- Investigate the mechanism of improved infectivity. What mutation(s) are responsible? How?
- Continue attempts to isolate phages that can infect our test strain