Biology Directed Studies Proposal

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Title: Isolation of Bacteriophages from Sewage Water Samples and their Effectiveness in Combating Multi-drug Resistant Bacteria

Introduction:

The emergence of antibiotic resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019 (CDC, 2021). Antibiotic resistance is when bacteria that cause illness/infections become resistant to the antibiotic drugs that are used to treat them. This can further threaten the ability to treat even common infectious diseases. There are two main ways with which bacterial cells can acquire antibiotic resistance. One is through mutations that occur in the DNA of the cell during replication and the other way is through horizontal gene transfer (insert). Although antibiotic resistance has been apparent since penicillin resistance after World War II in the 1950s, there has been a recent rise in the cases of antibiotic resistance (Ventola, 2015). Alongside the known ways of acquiring antibiotic resistance (mutation and selection), the misuse and overuse of antimicrobials, lack of access to clean water, sanitation and hygiene, poor infection and disease prevention and control are synergistically increasing the presence of antibiotic resistant bacteria.

To combat antibiotic resistance, in the past, the usage of a different antibiotic may help but, that may also have certain drawbacks. For example, the new antibiotic may have more side effects or risk promoting even more resistance. Other times, there is no treatment.

Bacteriophages are viruses that infect bacteria and have been successfully used as an alternative therapy in treating multi-drug resistant bacteria. In a preliminary study conducted in our lab, Aman Galymov, Muhammad Rehan and Dr. Naowarat Cheeptham (Ann) isolated three strains of bacteriophages (EC1KELHOS, EC3KAMCTY, and EC1KELCTY) which were successful in infecting two clinical strains of multi-drug resistant *Escherichia coli.* The bacteriophages were isolated from the municipal and hospital sewage of Kamloops and Kelowna in the Interior of British Columbia, Canada, during the summer.

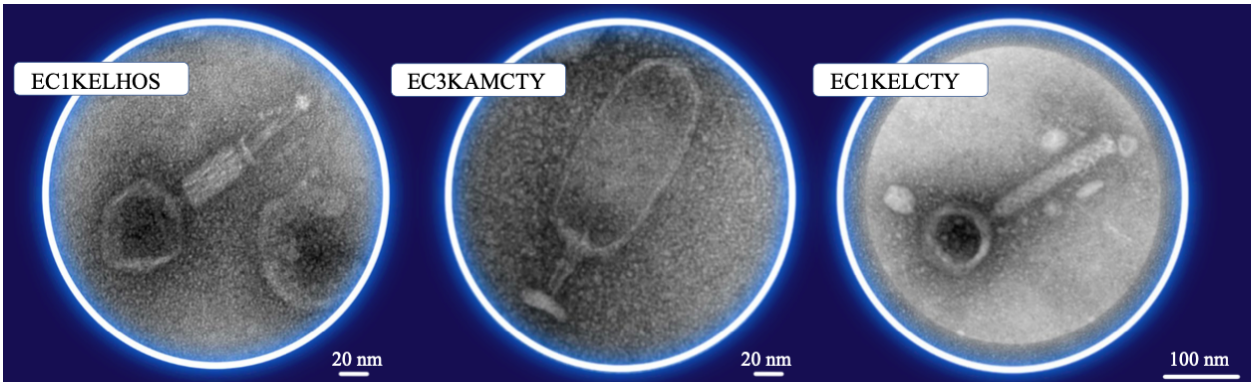

Figure 1. Transmission Electron Microscopy images of the three bacteriophages isolated in the preliminary study (UBC Bioimaging Facility RRID:SCR_021304).

Additionally, last year, we specifically tested the effect of bacteriophages isolated from local sewage plants that were obtained during the winter season. Taking both studies into account, we found that we were able to successfully isolate bacteriophages during both the winter and summer seasons and the bacteriophages present were able to effectively kill multi-drug resistance *Escherichia coli* bacteria. Therefore, we appreciate that bacteriophages can be isolated year-round, are successful in combating antibiotic resistance, and are not dependent on the season.

Objectives:

The objective of the proposed project is that we will repeat the experiment which was done last year to confirm the presence of similar strains of bacteriophages and their effectiveness on *Escherichia coli*. We will further investigate the effect of the three known and previously isolated bacteriophages (EC1KELHOS, EC3KAMCTY, and EC1KELCTY) on additional multi-drug resistant bacteria (e.g., *Pseudomonas* and MRSA bacteria.), as well as look for additional bacteriophages to further prove that phage therapy is an effective procedure in overcoming antibiotic resistance.

Materials and methods:

We will use the standardized methodology that was developed in our lab (Aman Galymov, Muhammad Rehan and Dr. Naowarat Cheeptham). A double-layer agar method will be used to screen, purify, and propagate bacteriophages from the local sewage samples. Then these bacteriophages will be mixed with the bacterial sample and poured over an agar plate. These will be allowed to solidify and incubate. A phage buffer will be poured onto the agar plate and swirled. Thirty minutes will be given for the phages to elute into the phage buffer. The buffer and phages will be siphoned into a sterile tube and the top agar will be scraped for collecting any remaining phages.

Expected results

We expect that this experiment will confirm the presence of the three strains of bacteriophages in the collected water samples and that they will be effective against *Escherichia coli* strain and the additional bacterial strains we chose. Furthermore, we also expect that we will find some additional bacteriophages effective against additional multi-drug resistant bacteria.

Timeline

January: Obtaining samples/supplies and prepping all reagents and samples so they are ready for the trials.

February: Conducting the various trials with the different bacterial samples.

March: Writing my report and disseminating my findings at various conferences (ex: SUPER).

Early April: Report submission.

Literature cited:

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Ghosh S, Persad E, Shiue T-Y, Lam C, Islam A, Mascibroda LG, Sherman MB, Smith T, Cheeptham N. Explorative Study on Isolation and Characterization of a Microviridae G4 Bacteriophage, EMCL318, against Multi-Drug-resistant Escherichia coli 15-318. Antibiotics. 2018; 7(4):92. https://doi.org/10.3390/antibiotics7040092

Ventola, C. L. (2015, April). *The antibiotic resistance crisis: Part 1: Causes and threats*. P & T : a peer-reviewed journal for formulary management. Retrieved September 13, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4378521/