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

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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. 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, Dr. 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.

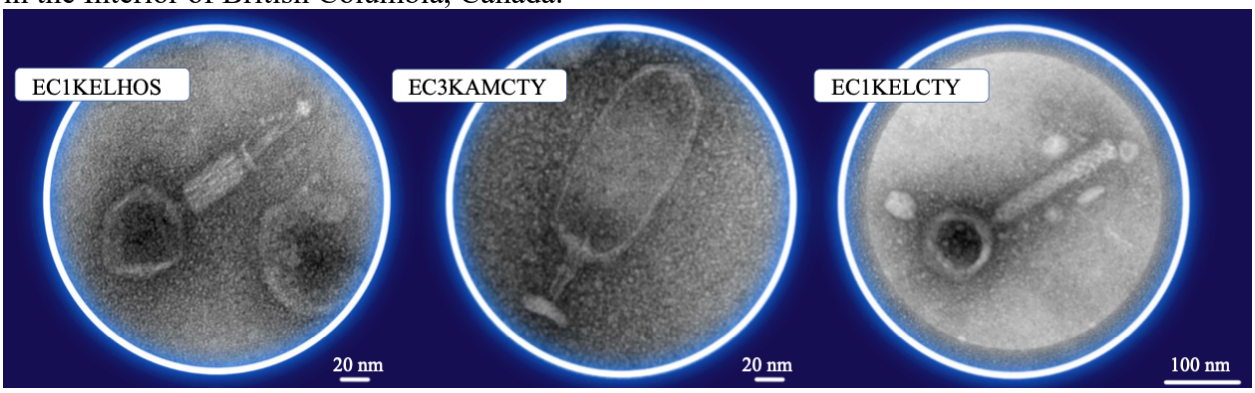


Figure 1. Transmission Electron Microscopy images of the three bacteriophages isolated in the preliminary study (UBC Bioimaging Facility RRID:SCR_021304).

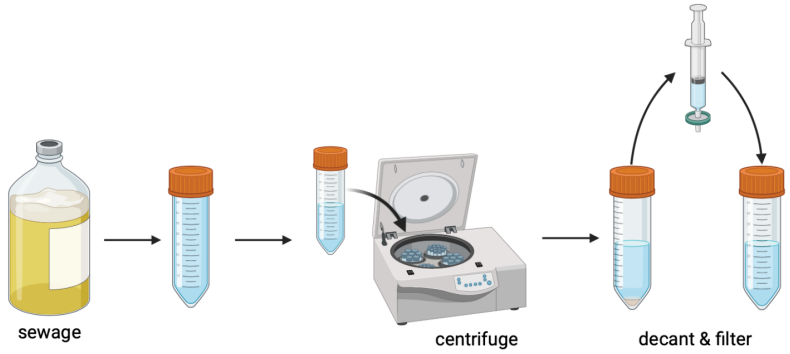
Objectives:

The objectives of the project were to

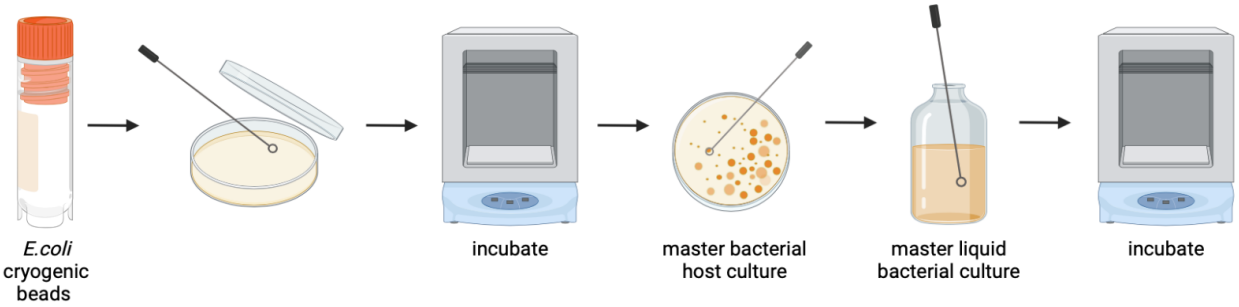
- Repeat the experiment to confirm the presence of the three strains of bacteriophages and their effectiveness on *Escherichia coli*.
- Investigate the effect of these three bacteriophages on additional multi-drug resistant bacteria, as well as, look for additional bacteriophages to further prove that phage therapy is an effective procedure in overcoming antibiotic resistance.
- Investigate the temporal distribution pattern of bacteriophages in the interior of British Columbia and how their effectiveness in combating multi-drug resistant bacteria differs due to it.

Methods:

1. *Sewage sample collection:* Sewage samples were collected from the Kamloops Sewage Treatment Centre, Vernon Water Reclamation Center, and the Kelowna Wastewater Treatment Facility.
2. *Sewage Sample Preparation:* Sewage samples were filtered and screened into conical tubes after using a floor top centrifuge at 7000 g for 15 minutes. Supernatant was decanted and filtered.

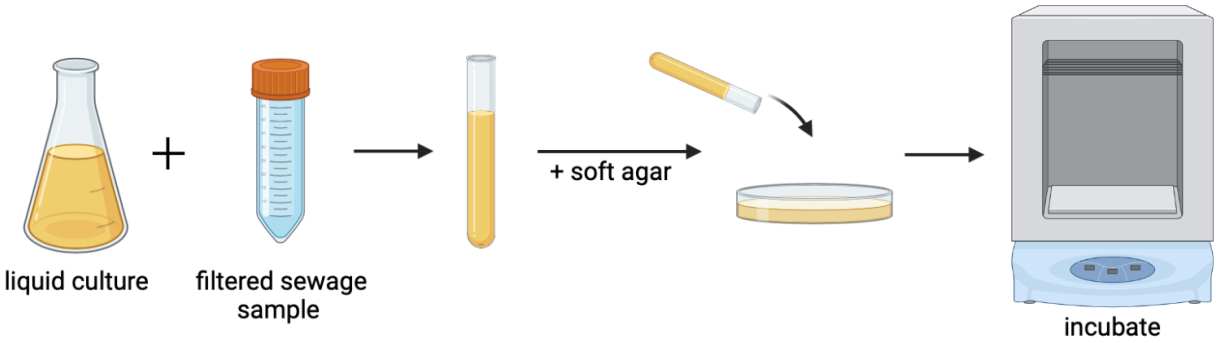


3. *Cultivating Bacterial Hosts and Liquid Bacterial Cultures*: Master culture stock plates of potential bacterial hosts were created using *E.coli* #1, #2, and #3. Beads from the cryovials were streaked for isolated colonies on nutrient agar plates and incubated at 37 °C. From culture, one plaque was picked into sterile nutrient broth and incubated overnight at 37 °C.



4. *Screening for bacteriophages*: The filter sterilized sewage samples containing possible bacteriophages were mixed with the liquid bacterial host sample and poured over an agar plate. These were allowed to solidify and incubate according to the bacterial hosts' optimal growth conditions. The appearance and the amount of the plaques (inhibitory

clear zone) were observed. The bacteriophages then will be further isolated and purified for identification.



We used the standardized methodology that was developed in our lab (Galymov *et al*., 2022). A traditional double-layer agar method was used to screen, purify, and propagate bacteriophages from the local sewage samples.

Results:

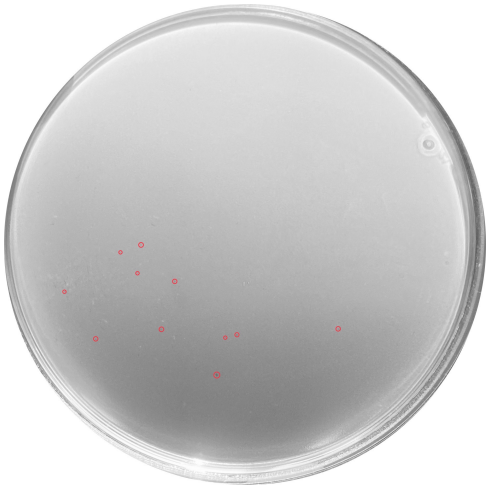


Figure 3. Plaques formed by bacteriophages from the Vernon sample in the double layer agar assay with the MDR bacterial host E. coli #3.

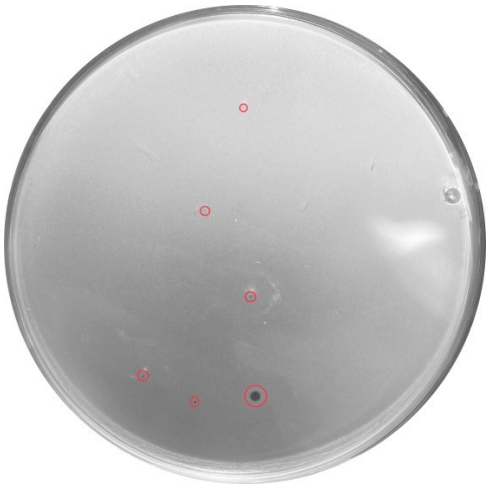


Figure 4. Plaques formed by bacteriophages from the Vernon sample in the double layer agar assay with the MDR bacterial host E. coli #2.

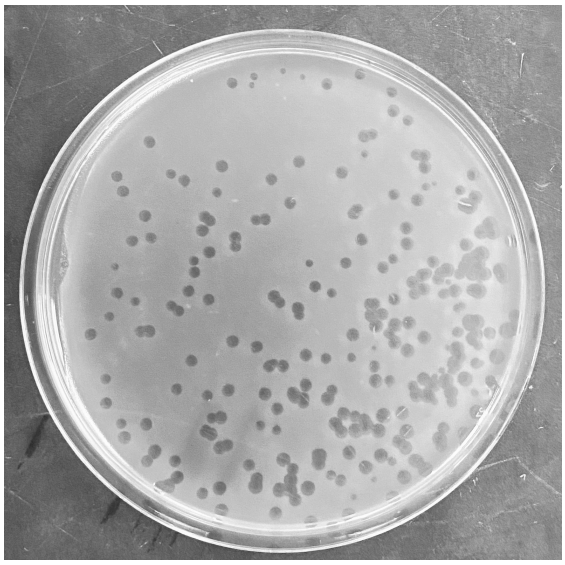


Figure 5. Plaques formed by the isolated bacteriophages from Figure 3 in the double layer agar assay with the MDR bacterial host *E. coli* #2.

- Bacteriophages isolated from sewage samples (Vernon, Kamloops, and Kelowna) were successful in infecting all three clinical strains of MDR *Escherichia coli (E.coli #1,* #2, and #3*).*
- Upon isolation of plaques and inoculation onto new nutrient agar plates, a more concentrated plate was formed. This plate showed a denser lawn of productive lysis (Figure 3).

Discussion:

- The sewage samples collected (Vernon, Kamloops, Kelowna) were each able to display plaques on one of the MDR *Escherichia coli* strains *(E.coli #1,* #2, and #3*).*
- This confirms the presence of bacteriophages in each sewage sample.
- In comparison to the preliminary study, this study showed lysis from samples taken from the Vernon Water Reclamation Center. This could be due to the difference in the season in which both samples were obtained. The change in the population diversity of the bacteriophage due to the change in temporal pattern confirms that bacteriophages are season specific.
- Additionally, unlike the previous study, plaques in *E. coli #2* were seen confirming that, during the winter, bacteriophages were found that were able to lyse *E. coli #2* bacteria.

Conclusion:

- This study shows that bacteriophages could be potential candidates for the use in phage therapy. There is still an abundance of uncharacterized bacteriophages that could be used as an alternative and more targeted solution to battle MDR bacteria, given the specificity between the bacteriophage and its bacterial host.

Future Work

- Since this is an ongoing project, we plan to isolate the bacteriophages and specify the strains prevalent in each sewage sample.
- We would also like to test the effect of these bacteriophages on *Pseudomonas* and MRSA bacteria.
- We would also like to see if these strains of bacteriophages have a similar effect on Gram-positive bacteria.

References

- Galymov, A., Rehan, M., Urban, J., and Cheeptham, N. (2022). Identification and characterization of bacteriophages isolated from sewage water samples effective against two clinical strains of multidrug-resistant *Escherichia coli*. Poster Presentation Abstract Submitted and accepted to the Canadian Association for Clinical Microbiology and Infectious Diseases (CACMID_AMMI) Annual Conference. Sheraton Wall Hotel, Vancouver. April 5 to 8, 2022.
- 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
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- The methodology diagrams were created with the help of BioRender.com