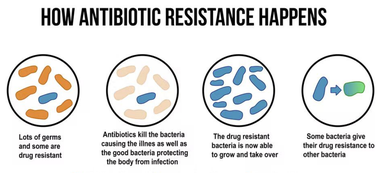
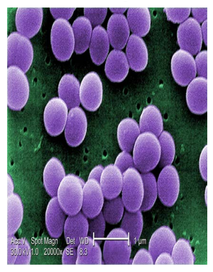
INTRODUCTION

- Bacteriophages are viruses that infect bacteria. From water sources to the soil, phages are everywhere on earth. They treat bacterial infections and diseases that are antibiotic resistant
- *Staphylococcus aureus*, *or* S. *aureus* is a bacteria, present in the environment and normal human flora, that can cause infections in humans. Infection acquired from hospitals and other community settings.
- *Methicillin Resistant Staphylococcus Aureus* or also known as MRSA is a drug-resistant staph bacteria. According to the CDC, infections caused by MRSA most likely result in skin infections, and if left untreated it leads to sepsis, or an extreme reaction to infection.

This is an image of MRSA



This is an image of *S. aureus*



- Wastewater has a great abundance of S. *aureus* phages, making it a good source for sampling.
- We hypothesized that bacteriophage that is able to infect S. *aureus* is present in the wastewater samples we selected. By isolating, purifying and amplifying our located phage, we hope to image them and identify the characteristics of the phage. With this information, we can discover potential benefits of using the phage, such as a tool in phage therapy.

METHODS

- Six students extracted phages from sewage water in the past three years from NYIT waste matter.
- 8 samples were used to conduct experiments on the sewage waste water samples: 04/01/2019, 06/04/2019, 09/08/2019, 09/20/2019, 10/08/2021, 09/01/2021, 02/09/2022, and 03/02/2022.
- Methods used to identify and isolate phage consisted of plaque assays, webbed plates, lysate collection from flooded webbed plates, spot titers, full plate titers, and eventually imaging using TEM. These methods tested different dilutions of the phage lysate on different strains of S. *aureus* to see which strain is most susceptible to infection by our isolated phage.

Our Story & Results

- Began in mid February 2022, students sampled parts of their own bodies, such as the nose and armpit in hopes of finding phage of S. *aureus*, but were unsuccessful as depicted in Figure 1.

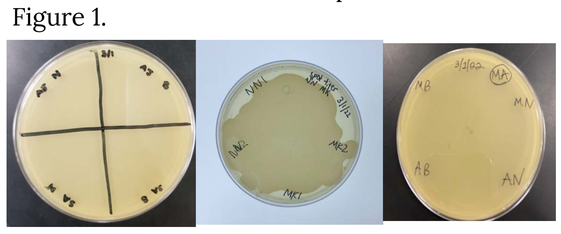


Figure 1: Skin samples that do not have any staph.

- We then decided to test 8 different samples of wastewater that had been collected from NYIT in the past three years. The samples were tested on three different clinical strains of S. *aureus*, labeled Lab, C2, & C3.

Lab vs C2 vs C3 Strains:

- C2 and C3 are clinical strains of S. *aureus* that are resistant to infection, obtained from a previously done study. The cause of resistance is unknown. C2 and C3 were isolated from a pediatric study done in Missouri, while the Lab strain was made in the laboratory.
- The Lab strain of S. *aureus* that had been made in the laboratory that does not show resistance to infection by bacteriophage.

After conducting the spot titers, the results were compared for each strain, shown in table 1. C2 and C3 strains were both infected by sewage sample 1, 3, and 4.

Figure 2: Spot Titer Results with Lab, C2, and C3 strains of S.*aureus*.

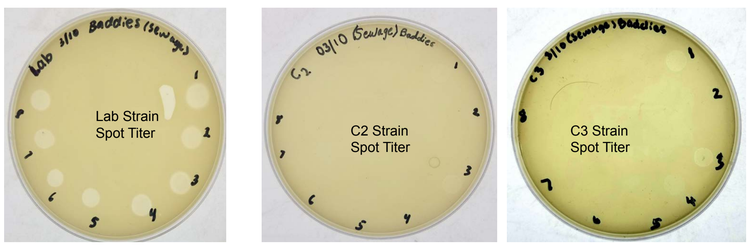


Table 1: Results for Lab, C2, and C3 Spot Titer.

Sample No.	Dates	LAB (+/-)	C2(+/-)	C3(+/-)
1	04/01/19	YES	YES	YES
2	06/04/19	YES	NO	NO
3	09/20/19	YES	YES	YES
4	09/08/20	YES	YES	YES
5	09/01/21	YES	NO	NO
6	10/08/21	YES	NO	NO
7	02/09/22	YES	NO	NO
8	03/02/22	YES	NO	NO

In Figure 2, the different numbers around the plate represent different samples of the waste water that were tested, according to the dates on the Table 1.
The data from the spot titers shows phage hits only the Lab strain, with little to no hits on the other two strains of the bacteria.

- After conducting serial dilutions and plaque assays on the samples 1, 3 and 4, the results were analyzed and found that sample 1 had no clearings on C3. Due to the lack of clearings we decided to pursue Sample 8. Samples 3, 4, and 8 had clearings on at least 2 out of the 3 strains.

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Finding Bacteriophage in NYIT Fecal Matter Samples

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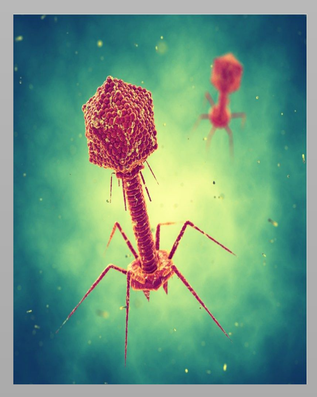

FUTURE WORK

- Transmission Electron Microscopy (TEM) is a technique that is used to form the image of the phage. TEM is used to view tiny specimens, which helps us determine the different characteristics of the phage, such as size and shape.
- Genome sequencing is used to determine what exactly the phage is. This will allow us to see the exact organism and its cell type. Thus, we will be able to have a deeper understanding of disease causing mutations associated with this phage.

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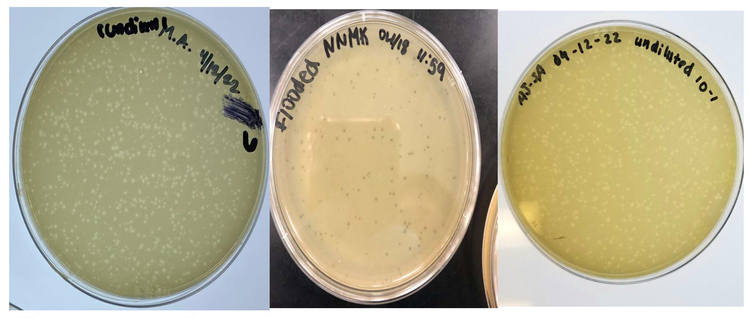
RESULTS

Lysate Collection:
To obtain proper lysates, webbed plates from plaque assay need to be flooded. The lysates collected were named Momoney, Normex, and Princess.

Figure 3: Plates used for Titer Calculation and Flooding.



The images in Figure 3 represent three plates in which lysate were collected from. These lysates were used in different full plate and spot titers to test for phage amounts.

Table 2: Results of Titer Calculation

TABLE 2	
SAMPLE	TITER(pfu/ml)
Momoney	6 x 10^7
Normex	N/A
Princess	5 x 10^7

We used the plates in Figure 3 to calculate the titer for our phages as shown in Table 2 but the titer calculation for Normex could not be calculated due to time constraints and we could not perform the experiment to create webbed plates for Momoney and Princess.

Full Plate Titers:
We performed full plate titers of undiluted phage with amounts of 10 µl and 100 µl, along with diluted phage samples of 10^{-1} and 10^{-2} dilutions. From these results, we found that only the plate that had 100 µl of undiluted phage sample had any phage hits, and the rest of the plates had no phage hits at all. The reason that this happened is still unknown, but it is obvious that the different bacterial strains have different effects on the phage.

Figure 4 depicts four different full plate titers that were done, in which there was only phage hits on the undiluted 100 ul plate, but not on any other plates.

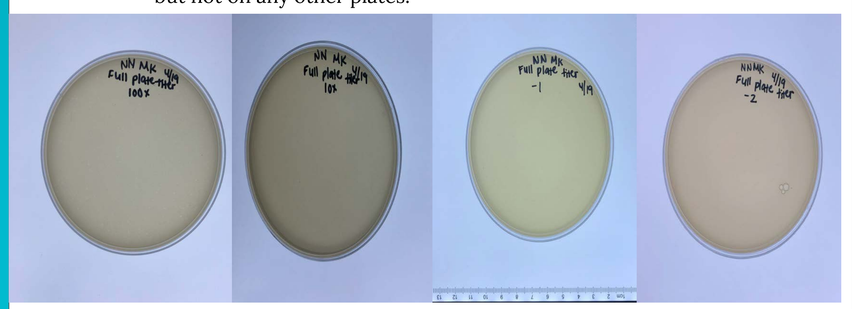


Figure 4: Full Plate Titer Results

Serial Dilutions:
Serial dilution involves the dilution of the phage with buffer. These dilutions are then pipetted onto a spot titer to study the titer of the phage. The titer represents the number of phages on the plate, and it is good to have lots of phages on a plate, usually to a titer of 10^{-9}.

Phage Isolation:
We proceeded to isolate phages from samples 3, 4, and 8. We did three rounds of isolations on each phage and because of time constraints were not able to reach webbed plates to form a high titer sample.

Plaque assay: Made full plate titers for each sample and from the plate with the highest number of plaques, one plaque was selected at a time to ensure that one phage is being identified at a time.

Experimenting with 5 different strains:
In this experiment we compared the lysates Princess, Momoney, and Normex to five different strains of S. *aureus* including the Lab, C2, C3, BW, and FD strains.
This image shows the result from this, in which there were only hits on the lab
strain, and only for Momoney and Princess. Sample 4, or Normex only had results with the undiluted sample as shown in Figure 4.

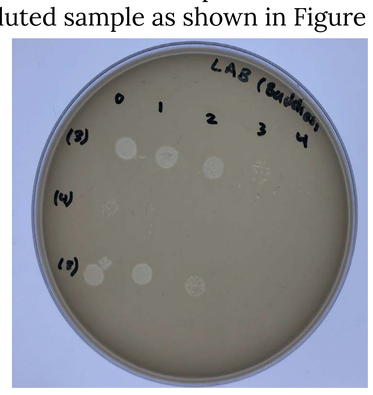


Figure 5: Lab Strain Spot Titer: 3 ,4, and 5 represent the lysate sample used. 0, 1, 2, 3, and 4 represent the dilution level

CONCLUSIONS

- We obtained lysates that contain bacteriophage, which could infect S. *aureus*. However, we weren't able to obtain a high titer lysate from our chosen plaque isolations as the lysate was unable to infect the specific strain of staph tested. We also were unable to image our phage via TEM.
- After conducting experiments to see if our lysate was able to infect different strains of staph, we concluded that our lysates were ineffective in infecting staph strains.
- Thus, we believe the bacteria has a defense mechanism that protects it from infection of the obtained bacteriophage present in the lysates. The strains C2 and C3 are still a big mystery as to what the bacteria's defense mechanism is or if the bacteria is employing a defense mechanism.
- We plan to do more future research on MRSA as it plays a key role in antibiotic resistance. This will help us understand the mechanism by which clinical strains C2 and C3 are resistant.