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The Study of *S. aureus* Bacteriophages found in Antibiotic-resistant Fitness Centers

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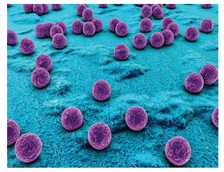
INTRODUCTION

- ***Staphylococcus aureus*** is a common bacteria found on the human body and in the environment. Infections are not caused by *S. aureus* being present but when it gains entry to different tissues. For example, it can gain entry through the skin in the form of a cut and cause a skin infection. There are millions of Staphylococcus infections in the United States every year with some being even life threatening.



Left: Example Bacteriophage
Right: *S. aureus*

- The go to treatment method for these infections is antibiotics. Antibiotics are drugs that can kill bacteria by inhibiting their function. However, with continued use, bacteria can evolve and develop resistance to antibiotics making them ineffective. There are a limited number of antibiotics, so it is important to find new treatment methods for these infections. These antibiotic resistant strains can spread throughout the community through person to person contact or contact with contaminated surfaces,
- Bacteriophages are specialized viruses that infect certain bacteria without infecting humans. They are specialized killers that can inhibit bacterial growth and kill them. Because they are viruses they can evolve with the bacteria which limits the potential for the bacteria developing resistance. Bacteriophages need their host to function and replicate, so the most likely place to find them is near high levels of their host bacteria.
- Phage Therapy is the clinical use of bacteriophages to treat bacterial infections and issues. There is also a large amount of bacteriophages in the world, 10^31 different phases, so they present an abundant supply.
- Our goal was to find and isolate bacteriophages in fitness centers. (Figures below) Fitness centers have been shown in previous studies to have been contaminated with high levels of *S. aureus*, including drug resistant forms. This is especially true in high touch areas such as equipment surfaces and bathrooms. Out of all gym types, community fitness centers were seen to have the highest levels of antibiotic resistant bacteria. These gyms possess the dangerous potential to spread these strains throughout communities due to the large amounts of activity, touch, and shared surfaces in the gyms.

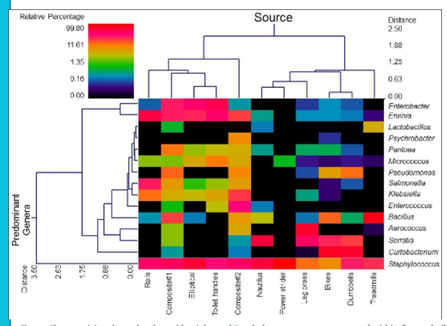
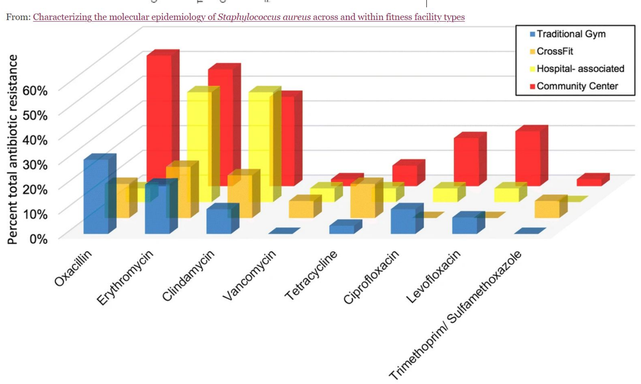


Figure 1 (Left): Prevalence and identity of Microbes found on high touch areas of Fitness Centers. (Bottom Row is Staphylococcus) (Mukherjee et al.)

Figure 2 (Below): Presence and levels of antibiotic resistant strains of *S. aureus* across fitness center. (x-axis shows different antibiotics) (Dalman et al.)



- **Based on this, we decided to search for bacteriophages in local community fitness centers. We did this with the intention that we would be able to find, isolate, and study a phage that infects a drug resistant strain of *S. aureus* found in the gym. We chose to sample three surfaces: the handle of a barbell, the top surface of a toilet seat, and the edges of a shower drain.**

OUR WORK

Sampling of Fitness Center Surfaces and Enrichment of Samples

- Our first steps were to sample surfaces our local community fitness centers. The gyms we sampled from were Retro Fitness in Syosset and LA FItness. The two surfaces sampled from Retro Fitness were the handles of a barbell and the exterior of a shower drain. The remaining surface sampled from LA Fitness was a toilet seat. Samples were taken by rolling a transport swab on the surfaces.

Figure 3: Surfaces Sampled from Fitness Centers. *Left*: Gym Toilet Seat, *Middle:* Exterior of Gym Shower Drain, *Right*: Handles of Gym Barbell

- These samples contained different microorganisms and phages so further work needed to be done to filter out any bacteria or other molecules.To amplify and bias the presence of *S. aureus* bacteriophages, our samples were enriched by seeding them with *S. aureus*, our host bacteria. The enriched samples were the then centrifuged and filtered to isolate the phages from the *S. aureus.*

Screening of Enrichments Against DIfferent *S. aureus* strains

- Enrichments were screened for phage that can infect *S. aureus* by spot assay on three different strains of *S. aureus*: Lab, C2, C3. The Lab strain or strain NCTC 8325 is typically used for genetic manipulation. The Clinical Strains C2 and C3 were strains isolated from pediatric patients. C2 and C3 are very similar strains with slight structural differences.

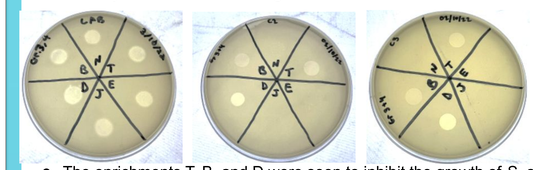


Figure 4: Spot Assays against different *S. aureus* strains. *Left:* Lab Strain, *Middle*: C2 strain, *Right*: C3 Strain.
*T= Toilet, B=Barbell, D=Drain, *N/J/E= other potential S. aureus phage enrichments**

- The enrichments T, B, and D were seen to inhibit the growth of *S. aureus* on the plates (circular clearings). To follow up on this result, we did an experiment to confirm that the phages within the enrichments could infect and replicate within the three *S. aureus* strains. Our enrichments were able to infect on replicate on plates grown with the Lab strains but not on the C2 and C3 strains. This indicates that our enrichments which inhibited the growth of C2 and C3 were exhibiting killing without: some phage byproduct is killing the bacteria (killing without infection). The enrichments were able to infect and replicate on the Lab strains of *S. aureus*. This can be by the plaques or small dots on the plates. These plaques indicate a plaque forming unit or phage present on the plate.

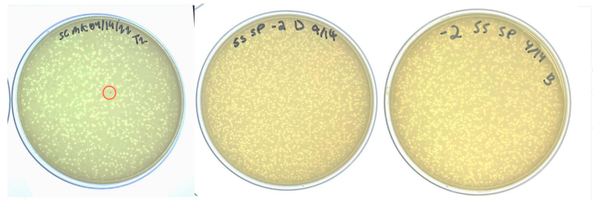


Figure 5: Infection and Replication of Enrichments on *S. aureus* Lab strain plates. *Left:* Toilet sample, *Middle*: Drain Sample, *Right*: Barbell Sample.

These results were not seen on clinical strains

- Through further testing and experimentation, our enrichments were confirmed to inhibit the clinical strains by exhibiting killing without infection.

Figure 6: The inhibition and infection abilities of three enrichments: T, B, and D on the three strains: Lab, C2, and C3.

	Lab strain		C2 strain		C3 strain	
	Inhibition	Infection	Inhibition	Infection	Inhibition	Infection
T	+	+	+	-	+	-
B	+	+	+	-	+	-
D	+	+	+	-	+	-

Current Work and Short Term Goals

- We are going to continue isolating our phage from the fitness center samples and will be trying to prepare the phage for Transmission Electron Imaging for visualization.
- This will be done by testing the host range of our phage by infecting multiple strains of *S. aureus*, either lab or clinical, which will help us further understand the infection capabilities of our phage.
- Next, we are going to be collecting our phage lysate by filtering our phage from plaques on our plates, which are areas where the phages lysed *S. aureus.* We can calculate the titer which is the density of plaque forming units (pfu) of our lysate.
- Then, using this lysate of known titer, we can start making webbed plates which are plates with a very high density of plaques.
- Lastly, these results will be able to go to a lab for Transmission Electron Imaging, which would give us a cross section of our phage and help us visualize its structure. These images will then allow us to further characterize our phage and determine the type of phage it is and the mode of infection.

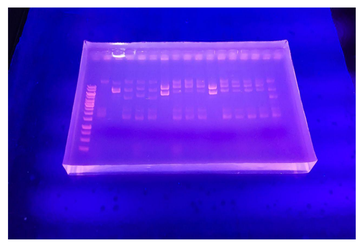
Future Studies and Long Term Goals

- We are going to be collecting our high-titer phage lysate that we have isolated from our fitness center samples.
- We will be doing whole genome sequencing and analysis to further understand our phage. This will be done by isolating and extracting DNA from our phage.
- Further characterization of our phage will be done by using restriction enzyme digests and gel electrophoresis to cut our phage genome into multiple fragments based on its DNA sequence and analyze them, respectively.
- If our phage has clinical significance, we can observe the method of infection propagation and we should also observe whether the *S. aureus* can develop a resistance to the phage over time or the phage will adapt to the evolving *S. aureus.*
- We can experiment and look into the factors that influence *S. aureus* to secrete biofilms to see if those can be mitigated in a clinical environment.
- We can further study the mechanism of the enrichments inhibiting the growth of *S. aureus* without infection. We could try to identify and understand what is being secreted or produced within the enrichments that is preventing growth of *S. aureus.*
- Once we have fully characterized and understood our phage then we will be able to name our phage and add it to the database of known phages others have been able to isolate.

CONCLUSIONS

- We were able to find and gather phages for *S. aureus* from our fitness center samples that were able to inhibit the growth of *S. aureus* for the lab and clinical strains.
- We determined that some phage byproduct produced in the enrichment and infection of the lab strains was able to kill the clinical strains which is called killing from without infection.
- Our enrichments were able to infect and replicate on the lab strains of *S. aureus*.

Transmission Electron Imaging



Gel Electrophoresis