Research Article

Methods to Isolate Possible Bacteriophage for *Micrococcus luteus* and *Acinetobacter baumannii*

Amanda M. Hillis[1,2]*, Rachel Hulse[1], and Peter P. Sheridan[2]

[1]*Department of Medical Laboratory Sciences, Idaho State University, Pocatello ID, United States.*
[2]*Department of Biological Sciences, Idaho State University, Pocatello ID, United States.*

Abstract: The increasing prevalence of antibiotic-resistant strains of bacteria has led to a crisis in treatment options. *Acinetobacter baumannii* is an example of a bacterium that has developed a dangerous level of multidrug resistance. Not only does it have genes allowing for the resistance to antibiotics, but it also produces a biofilm that protects it. In recent years, *A. baumannii* has become a major contributor to nosocomial infections making it critical to develop new treatment methods. *Micrococcus luteus*, while typically not thought of as a pathogen, is also developing a resistance to antibiotics. *M. luteus* is capable of forming a biofilm on its own making it worrisome as it has increasingly been noted as an opportunistic pathogen.

One potential new treatment of antibiotic resistance is the development of bacteriophage therapy, using bacterial viruses to target the infection and treat it. This study examines methods for isolating novel bacteriophage from dairy cattle feces, specifically for the biofilm producers *A. baumannii* and *M. luteus*.

Keywords: Antibiotic resistance, *Acinetobacter baumannii*, *Micrococcus luteus*, Bacteriophage therapy.

1. Introduction

One of the rising concerns within the medical health profession is the increase in cases of multidrug-resistant Bacteria. Multidrug-resistant organisms (MDROs) exhibit resistance to more than one antibiotic, leading to difficulties in finding treatment that is effective. One Gram-negative bacterium that is of huge concern is *Acinetobacter baumannii*, in which some strains are showing resistance to all available antibiotic agents [1].

A. baumannii is a Gram-negative, aerobic, non-fermentative, coccobacillus bacterium that is considered ubiquitous and can survive very harsh environments, including surfaces in healthcare facilities [2-5]. This is problematic because of the growing antibiotic resistance co-occurring with this organism [3,6-9]. *A. baumannii* has been categorized as an opportunistic pathogen but in the past few years, it has caused many infections in both immunocompromised and healthy individuals [6,7,10]. It is responsible for hospital-acquired (nosocomial) pneumonia, urinary tract infections (UTI), bloodstream infections, and wound infections [11-14]. The ability of *A. baumannii* to become drug resistant has led to its classification as a 'critical' organism by the WHO [7].

Biofilms likely play a role in the ability of *A. baumannii* to survive long periods of time in environments that lack moisture, or even in the presence of disinfectants. This allows the organism to cling to and survive on otherwise sterile surfaces, as in clinical environments [6]. Biofilms are one of the major factors determining whether antibacterial treatments fail, along with genes the bacteria contain. It is hard for antibiotics to get through the thick polysaccharide matrix of the biofilm and make it to the bacterial cells [8,15-19].

Biofilms have been associated with infections causing endocarditis, UTI, chronic otitis media, chronic bacterial prostatitis, and respiratory tract infections seen with cystic fibrosis patients [6]. In some instances, the organisms capable of forming biofilm that may cause infections from medical equipment may be normal flora of the skin, oral cavity, urinary tract, reproductive system, and gastrointestinal tract [6,16]. This is the case in the instances of infection by *Micrococcus luteus*.

Micrococcus luteus has been designated normal flora of the oral cavity. It is a gram-positive bacterium that group in tetrads [20,21]. Like *A. baumannii*, *M.*

luteus is ubiquitous in the environment, however, it is rarely the source of disease. Because of the classification as normal flora, it is often considered a contaminate when seen in cultured specimens. This can delay treatment in instances where *M. luteus* is the opportunistic pathogen and the source of the infection. As a pathogen *M. luteus* has typically been seen in cases of immunocompromised individuals, however, it is increasingly being seen in relation to biomedical procedures [22]. It has been associated with septic arthritis, meningitis, endocarditis, intracranial abscesses, and pneumonia [21,23]. Typically, these infections have been traced back to medical equipment such as indwelling intravenous lines, continuous ambulatory peritoneal dialysis fluids, ventricular shunts, and prosthetic valves [21,22].

M. luteus has several ways of avoiding antibiotics even though there have rarely been associated with antibiotic resistance. One way, which is like *A. baumannii*, is its ability to form a biofilm. A second and more unique method is dormancy. The cells of *M. luteus* can decrease their metabolism in order to survive conditions that would be otherwise fatal to the cells [24]. These cells can survive three to six months in this dormant state and still return to an active metabolic state and begin to divide to increase the population [20]. This dormant behavior prevents most antibiotics from acting on the organism [25]. Without actively dividing cells antibiotics that target peptidoglycan production are ineffective, and without normal metabolic processes occurring antibiotics that target metabolism also fail to work [24,25].

Recently there has been interest in looking at bacteriophage as a new method of treatment for these harder to treat infections [6,11,16,26-30]. Bacteriophage was discovered in 1896 by and were first described for their antibiotic properties [16,30]. Phage are viruses that specifically target and lyse bacterial cells. They can be isolated from nearly everywhere sea water, sewage water, sludge pods, feces, food, etc. and they typically utilize the bacteria that are already present in the sample as their hosts [27]. Bacteriophage presents a new form of treatment because of their ability to multiply exponentially, their mode of action, the ability to move through protective biofilms, and their selective nature [6,11,16,26-30].

Because phage only targets specific bacterial species or even specific strains, they are more likely to be safe for use on plants and animals [2]. Their ability to filter through biofilms implies that they can function in conditions where antibiotics are unable to reach [6,11,26]. Phage injects their genetic material into bacterial cells, including dormant cells, and hijack the cells own mechanics to replicate the phage particles. This is completely different than the mode of action of antibiotics and means that they can be used against multidrug-resistant strains of bacteria [3,6,11,16,26,30].

This study looks at three slightly varying methods of isolating lytic bacteriophage for *A. baumannii* ATCC 19606 and *M. luteus*. These bacteria were chosen because of their ubiquitous nature and their ability to form biofilms. Dairy cattle feces was utilized as the source to look for novel bacteriophage, due to the ubiquitous nature of these bacteria and the fact that dairy cattle feces has not readily been studied for bacteriophage isolation, yet the close interaction of these animals with human pathogens has. The feces may be a good source for possible bacteriophage for *A. baumannii*, *M. luteus*.

2. Material and Methods

2.1. Method 1: One-week incubation

The first method utilized to isolate bacteriophage involved two separate week-long incubations. 5 grams of dairy cattle feces was added to 50 mL of Luria-Bertani (LB) broth. The broth contained 10mM of $CaCl_2$, which facilitates phage attachment [27]. 1 mL of a turbid *A. baumannii* or *M. luteus* culture was added to the feces and LB broth. The *A. baumannii* culture was grown overnight *M. luteus* was grown for 24-36 hours as it had a slower growth rate than *A. baumannii* and thus took longer to get to a turbid culture. These cultures were grown for a week to allow phage to interact with the bacterial cells and replicate within them. The *A. baumannii* and feces culture was incubated at 37°C for the week, whereas the *M. luteus* and feces culture was incubated at room temperature (RT) for the week, due to their varying growth conditions.

After the week incubation, the cultures were centrifuged at 10,000 rpm for 5 minutes. The supernatants were then filtered through a .45 μm filter and again through a .22 μm filter to ensure that no bacteria would be present in the phage enrichment solution. 1 mL of the enrichment was added to fresh 50mL of LB, $CaCl_2$ along with 1 mL of fresh pathogen culture (grown the same as previous). These cultures incubated for another week in the same conditions as they were in the first round of incubation.

Once the incubation was complete the cultures were once again centrifuged and then the supernatant was filtered in the same manner as the previous incubation. The second-round enrichment was then combined with fresh pathogen and plated in an overlay method. This involves mixing 300 μL of enrichment with 300 μL of pathogen in 2.5 mL of soft agar (.5% agar instead of 2%). This solution was then poured on LB, 10mM $CaCl_2$ thick poured agar plates. The liquid was gently swirled until the entire surface of the plate was covered. The plates were left undisturbed on a flat surface for an hour to allow the soft agar to solidify before they were moved for incubation. The plates containing the *A. baumannii* plus enrichment were

incubated overnight at 37°C while the *M. luteus* plus enrichment plates were incubated for 36 hours at RT.

Once the bacterial lawns had grown the plates were examined for clearings in the lawn. When symmetric clearings are formed by virus particles they are referred to as plaques. If plaques are present they can be utilized for further isolation of the phage, by two different methods to be discussed later.

Controls were done using 300 μL of sterile water with 300 μL of pathogen in 2.5 mL of soft agar. No plaques should be seen on the control plates, only smooth bacterial lawns.

2.2. Method 2: Overnight incubation

The second method used to isolate bacteriophage was done very similarly to the first. The main difference being that instead of each incubation being done for a week, they were only allowed to incubate for 12-48 hours depending on the pathogen. The same measurements of broth, feces, and pathogen that were used in the initial incubation were used in this experiment. The solutions were filtered the same between each step and the final enrichment was plated in the same soft agar overlay method. The plates were incubated the same as in the first method, and each plate was again examined for plaque formation. Controls were also done in the same manner as in the previous method.

2.3. Method 3: Overnight incubation without pathogen

The third method utilized for phage isolation changed a little bit more from the first two. Due to the large quantity of left over enrichment the measurements were decreased for the incubating samples. The measurements instead were 5 mL of broth inoculated with .5 g of feces that were left to incubate overnight. There was no addition of pathogen in the initial incubation. The following day the enrichments were centrifuged and filtered as before then used in the second incubation. 5 mL of broth were combined with 100 μL of turbid pathogen culture and 100 μL of enrichment. The cultures were incubated in the same fashion as the first two methods before being centrifuged, filtered, and plated.

Control plates were done in the same fashion as the previous two methods.

2.4. Phage Isolation

When plaques are seen on the plates they need to be further isolated to ensure that a single phage is present in an isolated culture. Because the initial enrichment came from a mixed culture it is possible that there may be more than one phage present on the plates. This is done in one of two ways; the first is to core a selection of isolated plaques. Typically, this procedure is done with at least 5 separate plaques. The extraction was done by extracting a 1 mm diameter

section of plate that contains a single plaque, using a pasture pipette for ease of transfer. This agar piece was placed in 1 mL fresh LB, 10mM $CaCl_2$ and placed in a 4°C refrigerator overnight to allow the phage to elute out of the agar and into the broth without allowing for bacterial growth. The next day the solutions were plated using the soft agar overlay method. After an overnight incubation, the plates were examined for plaques.

The second method of phage isolation was done when the plaques are too close together to be extracted individually using the pipette method. Instead, an overlay of 10 mL of LB, 10mM $CaCl_2$ broth was added to the top of the soft agar plates and they were left on a flat surface overnight. The overnight incubation at RT acts in the same manner as the 4°C incubation, it allowed for the phage to elute out of the agar and into the broth. The broth was then centrifuged and filtered given that bacterial cells can grow at RT. The sample was then diluted from 10^1 to 10^{10} and plated in the soft agar overlay method and incubated overnight. The plates were then examined for plaque formation.

3. Results

3.1. Control plates

Growth on the control plates was typically a uniform bacterial lawn showing few if any deformations of the agar (Fig. 1). There was an issue with some of the *A. baumannii* control plates in that they showed a swirl pattern characteristic of biofilm formation. Within the swirls, clearings could be seen that were similar to plaque formations, but this did not occur on every control plate (Fig. 2). *M. luteus* control plates all showed the same uniform bacterial lawn growth across the soft agar surface.

3.2. One-week incubation

The plates made from the enrichments that were left to incubate for a weeks' time showed no visible plaques. Each plate, for both *A. baumannii* and *M. luteus*, showed uniform bacterial lawns that looked no different than the control plates.

3.3. Overnight incubation

The plates made from the enrichments that were left to incubate overnight showed plaque like clearings on the *A. baumannii* plates (Fig. 3). No plaque like clearings were seen on the *M. luteus* plates, and a weak lawn was noted after 18 hours. Plates were looked at again at 36 hours. They showed a much thicker and uniform lawn but no plaque-like clearings were noted.

3.4. Overnight incubation without pathogen

The plates made from the enrichment that lacked pathogen in the first round showed plaque-like clearing zones for both *A. baumannii* and *M. luteus*. The *M. luteus* plates were left to grow for 36 hours to show adequate thickness of the lawn. The plates showed 5-10

plaque-like clearings per plate (Fig. 4), plaques were cored from one plate and a broth overlay was performed on another.

3.5. Plaque Isolation

Because of the issue seen with the *A. baumannii* control plates neither coring or broth overlay were completed with these samples. The cored samples for

M. luteus showed few possible plaque-like clearings, but the broth overlay dilutions showed a great reduction in bacterial growth (Fig. 5,6). Bacterial cell density was taken from the same turbid culture in the same measurements across all 10 dilution plates. The 10^{10} dilution showed not only a reduction in bacterial growth but plaque-like clearings as well (Fig. 7).
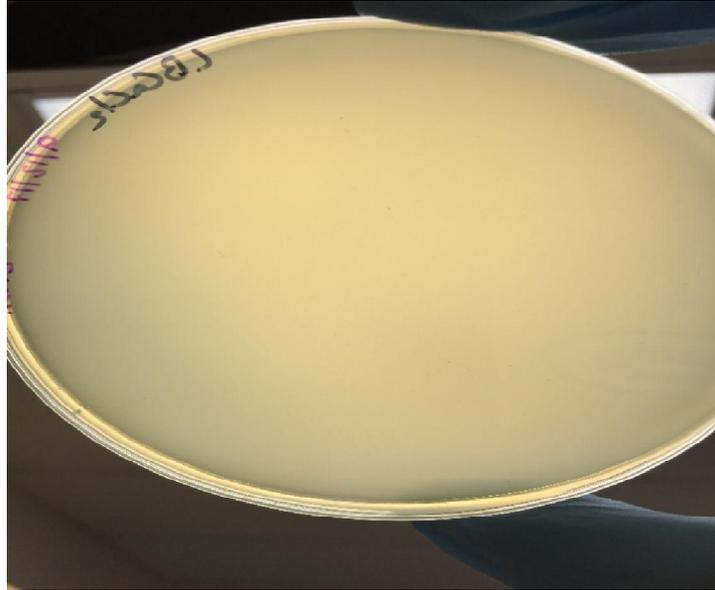

Fig. 1. Photograph of a control plate for *M. luteus* after 36 hours incubation at RT, showing a uniform bacterial lawn.
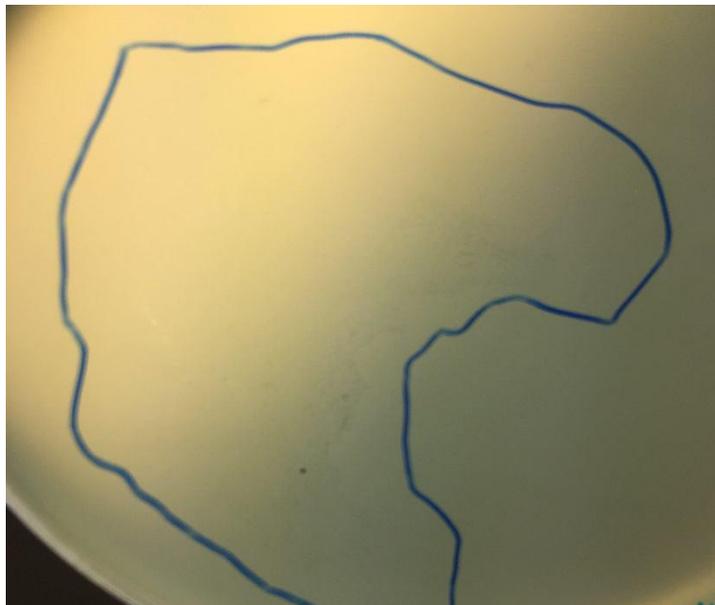

Fig. 2. Picture of a control plate for *A. baumannii* after 18-hour incubation at 37˚C, showing the swirl pattern containing plaque-like clearings.
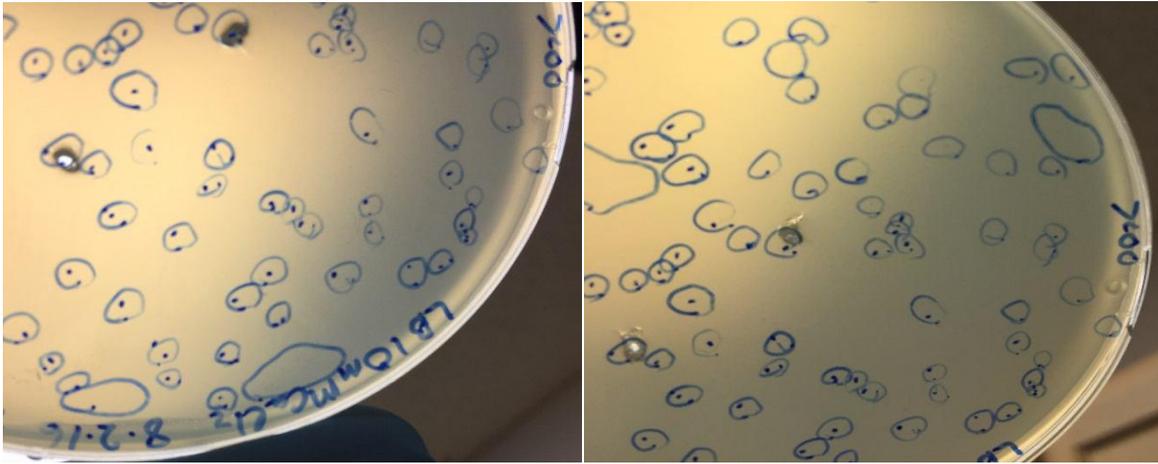
Fig. 3. Photographs of soft agar overlay plates for *A. baumannii* after 18-hour incubation at 37°C, showing scattered clearings consistent with possible plaque formation. The large holes in the agar are from coring isolated plaques for further testing.

Fig. 4. Photographs of soft agar overlay plates for *M. luteus* after 36-hour incubation at RT, showing cored holes with a few plaque-like clearings (left) and circled plaque like clearings in the agar (right).

Fig. 5. Photographs of *M. luteus* broth overlay plating after 36 hours of incubation at RT, showing decreasing bacterial growth with increasing dilution of broth overlay stock solution. A thick lawn for the 10^8 dilution (left), thinner lawn for the 10^9 dilution (middle), thin lawn with plaque-like clearings for the 10^{10} dilution (right).
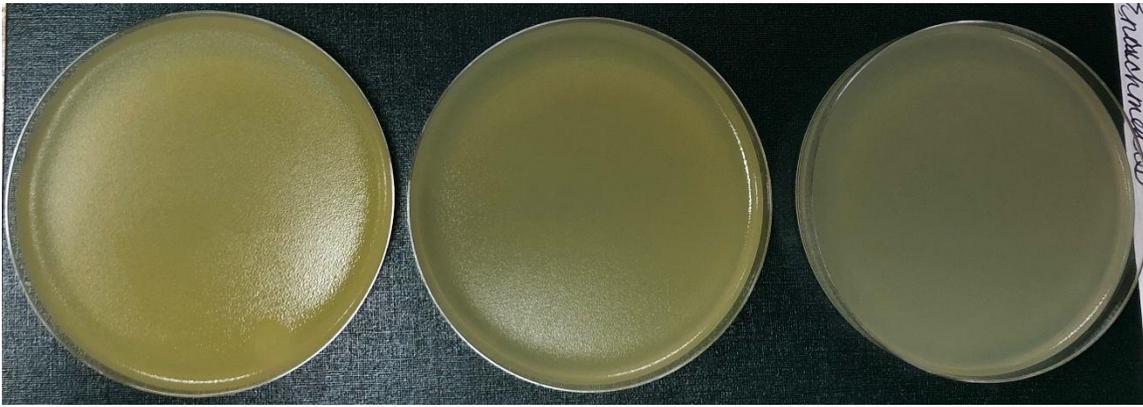
Fig. 6. Photograph of *M. luteus* broth overlay plating after 36 hours of incubation at RT, showing decreasing bacterial growth from dilution 10^8 (left) to dilution 10^{10} (right).
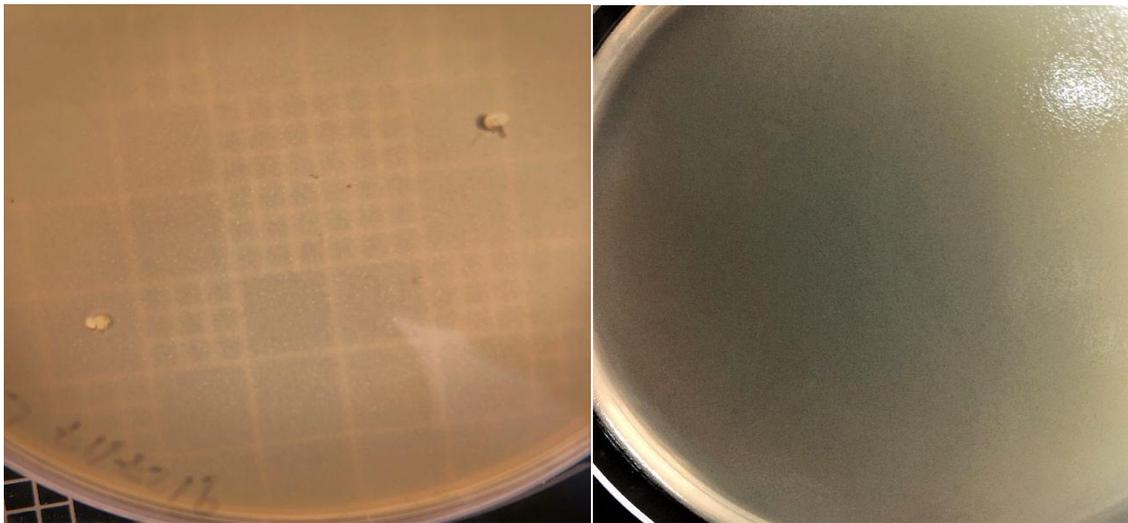

Fig. 7. Photograph of *M. luteus* broth overlay plating after 36 hours of incubation at RT, showing phage-like plaques on a light box for the dilution 10^{10} (left), and a view using a dark background (right).

4. Discussion

Three slightly varying methods were used to try and isolate novel bacteriophage from dairy cattle feces, with *A. baumannii* or *M. luteus* as their hosts. The first method of enriching showed no isolation of phage. The lack of any visible plaques on the plates could be from the conditions in the media after a weeks' worth of growth. Bacteriophage are relatively delicate; they have a small range of pH and temperature that they remain viable in. Once the bacteria have reached stationary phase and start lysing or dying off the cellular components they release into the culture may reduce the viability of the phage in the solution.

The extended exposure to bacterial cells also increases the likelihood that the cells that remain in the culture may have developed a resistance to the phage present. Bacteria have innate defense against the attachment or injection of the phage genome and after a period of time, many of the cells in the solution may be showing those resistance. One of the most well-known

defense mechanisms in bacteria is the CRISPR system and it has been a target of study in recent years [31]. The resistant cells would then become the more prevalent cells in the culture and reduce the likelihood of recovering phage.

The results of the one-week incubation lead to the changes to overnight incubation method. The bacterial cultures were still in the log phase in an overnight culture and thus may have actively dividing phage in the culture as well. The addition of the pathogen in the initial incubation would allow for any phage that utilizes the pathogen as a host to replicate in the initial enrichment. This was possibly seen with *A. baumannii* since plaque-like clearings were seen on the sample plates. However, it can't be said for certain that this was the cause of phage for *A. baumannii*, given the plaque-like clearings that were noted on some of the control plates as well.

There is the possibility that the clearings seen on the control plate, and thus on the experimental plates, may have been caused by lysogenic phage (prophage)

being triggered to enter the lytic cycle of their division. Something within the environmental conditions of the incubating plates may have initiated the lysing of cells by phage that otherwise remains dormant within the bacterial genome [16,30].

The clearings could also be caused by the presence of biofilm formation in the starter culture. The polysaccharide polymers may cause the swirl patterns seen on the control plate and may block bacterial growth from some sections of the plate. One way to combat the possibility of biofilm formation in the starter culture causing the issues seen in the control plates would be to do a growth curve assay on the *A. baumannii* strain used. This would ensure that the starter culture was in the log phase of growth and that biofilm formation was at a minimum. This was the suspected cause of the issues with phage isolation for *A. baumannii* in this study as the appearance of the swirl patterns and clearings were only seen periodically on the control plates.

Another way to verify that what was seen on the experimental plates may have been phage is for a spot test to be performed. A spot test is done by creating a bacterial lawn on a plate using soft agar containing the pathogen that has been allowed to solidify. The phage enrichment samples are then spotted onto the soft agar surface in increasing dilutions. Once dry the plates are incubated and then examined for clearings that reduce with each dilution [32].

These swirl patterns were not seen with *M. luteus*, but neither were any plaque-like clearings. Further adjustments to the methods were then done to facilitate a larger number of possible phage in the enrichment. Instead of adding pathogen to the first incubation the feces was incubated overnight on its own. This increased the likelihood of the phage present in the sample would be amplified before pathogen was added to it. The second incubation included the pathogen to further amplify any phage that were present that could use it as a host. It was with this method that plaque-like clearings were seen with *M. luteus*.

Single phage cores were taken and re-plated from these initial samples and the initial results were inconclusive, showing plaque-like clearings but not in the increased number that was expected or showing no plaques at all. It is possible that the diffused phage number was too high in these samples, as they were not diluted out and re-plated. Diluting the cored samples and plating may offer more conclusive results.

Broth overlays were also done on the initial samples and the serial dilutions of the broth overlay showed a reduction in growth of the bacterial lawn. The 10^{10} dilution also showed an abundant number of small plaque-like clearing zones. While these results do not conclusively indicate that phage has been isolated for *M. luteus* they do make a strong suggestion that this is the case. Further dilution of the broth overlay would need to be done to get better spacing between plaque-like clearings so single phage coring could be done to ensure isolation.

This study did not conclusively show the isolation of bacteriophage from both *A. baumannii* and *M. luteus*, but it did suggest that novel phage may be present in the samples. Future research needs to be done to verify that what was seen is in fact bacteriophage acting on the bacteria present, but it is reasonable to conclude that the methods used within this study can be used to isolate bacteriophage. This approach was used to look for novel phage from dairy cattle feces, an area that has previously not been looked at for novel phage, to identify phage that may be candidates for use in bacteriophage therapy. The discovery of novel bacteriophage is important given the sharp increase in MDRO being seen in both nosocomial and community-acquired infections. New therapies offer a different way to combat severe infections that may otherwise kill patients when conventional antibiotics don't work. Bacteriophage also can move through biofilms increasing their activity at clearing infection when compared to antibiotics alone.

References

[1]. Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*:268-281.

[2]. Peng, F., Mi, Z., Huang, Y., Yuan, X., Niu, W., Wang, Y., Hua, Y., Fan, H., Bai, C., Tong, Y. (2014). Characterization, sequencing and comparative genomic analysis of vB_AbaM-IME-AB2, a novel lytic bacteriophage that infects multidrug-resistant *Acinetobacter baumannii* clinical isolates. *BioMed Central Microbiology, 181*(14):1-14.

[3]. Jin, J., Li, Z.J., Wang, S.W., Wang, S.M., Huang, D.H., Li, Y.H., Ma, Y.Y., Wang, J., Liu, F., Chen, X.D., Li, G.X., Wang, X.T., Wang, Z.Q., Zhao, G.Q. (2012). Isolation and characterization of ZZ1, a novel lytic phage that infects *Acinetobacter baumannii* clinical isolates. *BioMed Central Microbiology, 156*(12):1-8.

[4]. Chang, K.C., Lin, N.T., Hu, A., Lin, Y.S., Chen, L.K., Lai, M.J. (2011). Genomic analysis of bacteriophage φAB1, a φKMV-like virus infecting multidrug-resistant *Acinetobacter baumannii*. *Genomics, 97*:249-255.

[5]. Chen, L.K., Liu, Y.L., Hu, A., Chang, K.C., Lin, N.T., Lai, M.J., Tseng, C.C. (2013). Potential of

bacteriophage φAB2 as an environmental biocontrol agent for the control of multidrug-resistant *Acinetobacter baumannii*. *BioMed Central Microbiology, 154*(13):1-10.

[6]. Yele, A.B., Thawal, N.D., Sahu, P.K., Chopade, B.A. (2012). Novel lytic bacteriophage AB7-IBB1 of *Acinetobacter baumannii*: isolation, characterization and its effect on biofilm. *Archives Virology*, 157:1441-1450.

[7]. Malaka De Silva, P., Kumar, A. (2017). Effect of Sodium Chloride on Surface-Associated Motility of *Acinetobacter baumannii* and the Role of AdeRS Two-Component System. *Journal of Membrane Biology, 251*:5-13.

[8]. Bardbari, A.M., Arabestani, M.R., Karami, M., Keramat, F., Aghazadeh, H., Alikhani, M.Y., Bagheri, K.P. (2018). Highly synergistic activity of melittin with imipenem and colistin in biofilm inhibition against multidrug-resistant strong biofilm producer strains of *Acinetobacter baumannii*. *European Journal of Clinical Microbiology & Infectious Diseases, 37*:443-454.

[9]. Bouvet, P.J.M., JeanJean, S., Vieu, J.F., Dijkshoorn, L. (1990). Species, Biotype, and Bacteriophage Type determinations compared with cell envelope protein profiles for typing Acinetobacter Strains. *Journal of Clinical Microbiology, 28*(2):170-176.

[10]. Farmer, N.G., Wood, T.L., Chamakura, K.R., Everett, G.F.K. (2013). Complete Genome of *Acinetobacter baumannii* N4-Like Podophage Presley. *Genome Announcements, 1*(6):1-2.

[11]. Dubrovin, E.V., Popova, A.V., Kraevskiy, S.V., Ignatov, S.G., Ignatyuk, T.E., Yaminsky, I.V., Volozhantsev, N.V. (2012). Atomic Force Microscopy Analysis of the *Acinetobacter baumannii* Bacteriophage AP22 Lytic Cycle. *PLOS One, 7*(10):1-9.

[12]. Sato, Y., Unno, Y., Ubagai, T., Ono, Y. (2018). Sub-minimum inhibitory concentrations of colistin and polymyxin B promote *Acinetobacter baumannii* biofilm formation. *PLOS One. March 19, 2018*: 1-18.

[13]. Tiwari, V., Patel, V., Tiwari, M. (2018). *In-silico* screening and experimental validation reveal L-Adrenaline as anti-biofilm molecule against biofilm-associated protein (Bap) producing *Acinetobacter baumannii. International Journal of Biological Macromolecules, 107*:1242-1252.

[14]. Jeon, J., Kim, J.W., Yong, D., Lee, K., Chong, Y. (2012). Complete Genome Sequence of the Podoviral Bacteriophage YMC/09/02/B1251 ABA BP, Which Causes the Lysis of an OXA-23-Producing Carbapenem-Resistant *Acinetobacter baumannii* isolate from a Septic Patient. *Journal of Virology, 86*(22):12437-12438.

[15]. Feng, J., Liu, B., Xu, J., Wang, Q., Huang, L., Ou, W., Gu, J., Wu, J., Li, S., Zhuo, C., Zhou, Y.

(2018). *In vitro* effects of N-acetylcysteine alone and combined with tigecycline on planktonic cells and biofilms of *Acinetobacter baumannii. Journal of Thoracic Disease, 10*(1):212-218.

[16]. Parasion, S., Kwiatek, M., Gryko, R., Mizak, L., Malm, A. (2014). Bacteriophages as an Alternative Strategy for Fighting Biofilm Development. *Polish Journal of Microbiology, 63*(2):137-145.

[17]. Hughes, K.A., Sutherland, I.W., Jones, M.V. (1998). Biofilm susceptibility to bacteriophage attack: the role of phage-borne polysaccharide depolymerase. *Microbiology, 144*:3039-3047.

[18]. Farshadzadeh, Z., Taheri, B., Rahimi, S., Shoja, S., Pourhajibagher, M., Haghighi, M.A., Bahador, A. (2018). Growth Rate and Biofilm Formation Ability of Clinical and Laboratory-Evolved Colistin-Resistant Strains of *Acinetobacter baumannii. Frontiers in Microbiology, 9*:1-11, doi: 10.3389/fmicb.2018.00153.

[19]. Feng, L., Li, X., Song, P., Du, G., Chen, J. (2014). Physicochemical properties and membrane biofouling of extra-cellular polysaccharide produced by a *Micrococcus luteus* strain. *World Journal of Microbiology and Biotechnology, 30*:2025-2031.

[20]. Mukamolova, G.V., Yanopolskaya, N.D., Votyakova, T.V., Popov, V.I., Kaprelyants, A.S., Kell, D.B. (1995). Biochemical changes accompanying the long-term starvation of *Micrococcus luteus* cells in spent growth medium. *Archives of Microbiology, 163*:373-379.

[21]. Seifert, H., Kaltheuner, M., Perdreau-Remington, F. (1995). *Micrococcus luteus* Endocarditis: Case Report and Review of the Literature. *Zentralblatt für Bakteriologie, 282*:431-435.

[22]. Peces, R., Gago, E., Tejada, F., Laures, A.S., Alvarez-Grande, J. (1997). Relapsing bacteraemia due to *Micrococcus luteus* in a haemodialysis patient with a Perm-Cath catheter. *Nephrology Dialysis Transplantation, 12*:2428-2429.

[23]. Fosse, T., Peloux, Y., Granthil, C., Toga, B., Bertrando, J., Sethian, M. (1985). Meningitis Due to *Micrococcus luteus. Infection, 13*:280-281.

[24]. Kaprelyants, A.S., Kell, D.B. (1993). Dormancy in Stationary-Phase Cultures of *Micrococcus luteus*: Flow Cytometric Analysis of Starvation and Resuscitation. *Applied and Environmental Microbiology, 59*(10):3187-3196.

[25]. Mali, S., Mitchell, M., Havis, S., Bodunrin, A., Rangel, J., Olson, G., Widger, W.R., Bark, S.J. (2017). A Proteomic Signature of Dormancy in the Actinobacterium *Micrococcus luteus. Journal of Bacteriology, 199*(14):1-15.

[26]. Kang, H.W., Kim, J.W., Jung, T.S., Woo, G.J. (2013). wksl3, a New Biocontrol Agent for *Salmonella enterica* Serovars Enteritidis and Typhimurium in Foods: Characterization,

Application, Sequence Analysis, and Oral Acute Toxicity Study. *Applied and Environmental Microbiology, 79*(6):1956-1968.

[27]. Li, L., Zhang, Z. (2014). Isolation and characterization of a virulent bacteriophage *SPW* specific for *Staphylococcus aureus* isolated from bovine mastitis of lactating dairy cattle. *Molecular Biology Reports, 41*:5829-5838.

[28]. Shin, H., Lee, J.H., Kim, H., Choi, Y., Heu, S., Ryu, S. (2012). Receptor Diversity and Host Interaction of Bacteriophages Infecting *Salmonella enterica* Serovar Typhimurium. *PLOS One, 7*(8):1-11.

[29]. Warner, C.M., Barker, N., Lee, S.W., Perkins, E.J. (2014). M13 bacteriophage production for large-scale applications. *Bioprocess Biosystems Engineering, 37*(10):2067-2072.

[30]. Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Ikeuchi, M., Tani, T., Fujieda, M., Wakiguchi, H., Imai, S. (2005). Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *Journal of Infection and Chemotherapy, 11*:211-219.

[31]. Cady, K.C., Bondy-Denomy, J., Heussler, G.E., Davidson, A.R., O'Toole, G.A. (2012). The CRISPER/Cas Adaptive Immune System of *Pseudomonas aeruginosa* Mediates Resistance to naturally occurring and Engineered Phages. *Journal of Bacteriology, 194*(21):5728-5738.

[32]. Ghajavand, H., Esfahani, B.N., Havaei, A., Fazeli, H., Jafari, R., Moghim, S. (2017). Isolation of bacteriophages against multidrug resistant *Acinetobacter baumannii*. *Research in Pharmaceutical Sciences, 12*(5):373-380.