Phage Therapy as a MRSA Treatment

Miriam Sussman
Miriam Sussman is accepted to Touro Winthrop Joint degree PA school, for January 2017

Abstract
This paper seeks to review how previous research on bacteriophage therapy can be utilized to improve the treatment of MRSA infections. Due to the growing phenomenon of antibiotic resistance, scientists are looking to utilize the natural antibacterial qualities of bacterial viruses called phages to fight MRSA infections. In order to improve the therapeutic methods of combatting MRSA, one must first understand the mechanics of how phages infect bacterial cells and lyse them with their proteins. The narrow host range of bacteriophages causes the infection of only the pathogenic bacteria and maintains the state of the normal flora. Research suggests that the combination of antibiotics and bacteriophage therapy is a more effective means of combatting MRSA than the utilization of each individual treatment on its own. The next step in developing this treatment option is isolating and classifying each specific bacteriophage and determining its host range. Matching the phages and their corresponding bacteria may seem like a daunting process; however, researchers discovered that phages are naturally located near the bacterial strain that they infect. An additional aspect of the ongoing research is the determination of what causes some phages to be more effective than others. Scientists note that not only does the phage have an inherent strength which is derived from its genetic makeup but, its strength in combating infections is also affected by the host's immunity. New findings lead researchers to believe that there may be a more direct method of treating MRSA infections. Although the phage in its entirety can be used to lyse bacterial cells, scientist have isolated a protein found within the phages that can lyse bacterial cells. Scientists hope to harness its power for a more sinuous treatment of infections.

Abbreviations
CDG- Chronic Granulomatous Disease
ICA- Inter-Cellular Adhesion
MRSA- Methicillin Resistant Staphylococcus Aureus
PAS- Phage-Antibiotic Synergy
SA- Staphylococcus Aureus
VRSA- Vancomycin Resistant Staphylococcus Aureus

Introduction
One of the growing challenges in healthcare is the rising occurrence of antibiotic resistance. Many pathogens have developed resistance to an array of drugs; the discovery of new antibiotics has not been keeping up with the rate of the evolving antibiotic resistant bacteria. Many are concerned that the medical world may return to the times when small infections will be lethal.

For example, Methicillin Resistant Staphylococcus Aureus (MRSA) is an example of a bacterial strain that is resistant to every antibiotic except for Vancomycin. This bug is responsible for most hospital acquired infections., and these bacteria can colonize anywhere from surgical sites, lungs, blood, heart, skin and even brains. (Jensen et al., 2015). Unfortunately, there has been a recent discovery of Vancomycin Resistant Staphylococcus Aureus (VRSA). The emergence of VRSA and its dangers have lead scientists and medical doctors to worry about the future of medicine.

Felix d'Herelle co-discovered bacteriophages in the early 1920s. Bacteriophage therapy utilizes the killing powers of nature's predators for bacteria; bacteriophages. Interest in phage therapy, and its associated research diminished once Flemings discovered the curative powers of penicillin. However, Russia and many Eastern European countries have continued studying this topic. The growing need for alternative treatments for bacterial infections gives rise to a resurgence of interest in this novel topic (Pincus et al., 2015).

A bacteriophage is a virus that binds to receptors that are only found in bacterial cells. Bacteriophages infect bacterial cells, reproduce and then lyse their host bacteria. Then the original phage's offspring then spreads and infects more bacterial cells. Bacteriophages have a specific receptor site and are thus antibacterial to a narrow range of bacterial cells. In addition, their specificity prevents them from infecting human cells. Bacteriophage therapy seeks to harness these powers and use them to treat human infections (Jensen et al., 2015).

Scientists are hopeful that bacteriophage therapy will successfully treat infections caused by many pathogens, specifically the hard to treat MRSA infections. Since research on this topic is fairly new, clinical trials need to be run prior to its institution in standard medicine. This paper will review some of the different experiments and their outcomes. The author wishes to determine how these experiments will impact the future of phage therapy.

Phage Infection Mechanism
Bacteriophages are viruses that infect bacterial cells. They are found all over the planet. The phages infect bacterial cells by binding the tips of their tails to receptor molecules on the bacterial cell. The viral DNA is then injected into the host cell. The virus now directs the creation of more phages, which can be created in less than thirty minutes. The phage bursts out of the bacterial cell, thereby killing the bacteria. The released phage's offspring continue to infect more bacterial cells (Pastagia et al., 2013).

Bacteriophages produce two proteins in order to lyse the cell: Holin, creates a pore in the bacterial cytoplasmic membrane, and Lysin interacts with the peptidoglycan in the cell wall. Lysin cleaves to the peptidoglycan bonds and ruptures the cell wall. Unable to withstand the osmotic pressure, the cell wall bursts (Wang et al., 2000). The released phages can then continue the cycle of infecting bacterial cells.

Narrow Host Range

As a bacterial virus, bacteriophages can be extremely host specific. Consequentially, they exhibit some major advantages in comparison to broad spectrum antibiotics. For instance, using phages to rid the body of infections does not pose a problem to the normal flora, whereas antibiotic treatments impact normal flora. The downside to the phage specificity is the rapid ability of the bacterial viruses to develop resistance, thus increasing the risk that the therapeutic effect is merely transient.

One way to circumvent this problem is to create a personalized phage cocktail specifically for each patient. This would entail culturing every infection, and then creating a specific mixture of bacteriophage from a phage library. This would ensure that the specific viruses could be combined in accordance with the different infectious pathogens present in the patient. In order for this to be a viable treatment option, the hospital would need to have a broad range of bacteriophage present at all times. One group of researchers studied how probable it is to have a wide resource of phages to treat infections.

The researchers purchased many strains of antibiotic resistant bacteria from both a lab and a hospital. In order to isolate the bacteriophages, they filtered sewage waste and then centrifuged the supernatant. Finally, the bacterial viruses were purified three times by plating and re-plating the plaques.

After multiple attempts, the researchers discovered that only one out of 117 strains of bacteriophages were effective against MRSA. After obtaining more samples of the bacterial viruses from other sources, the researchers still had difficulty isolating effective phage. The study resulted in the realization that it is not feasible to treat MRSA infections on demand; isolating effective strains presents significant challenges. In order to treat these infections, pre-made wide range cocktails would have to be available. It is important to note that on demand discovery of phages does seem possible for other bacterial pathogens such as Pseudomonas aeruginosa, Salmonella, and E. coli. Treating these infections with personalized cocktails is a viable option (Mattila et al., 2015).

Combination of Phage and Antibiotic Therapy

One way to use bacteriophage to combat infections is to combine phage therapy with antibiotic therapy. The combination of phages and antibiotics so far seems to be synergistic; the interactions between the two antibacterial agents provide a combined effect that is greater than the sum of the individual effects. One phenomenon that has been noted is called phage-antibiotic synergy (PAS). Research shows that administering sub-lethal doses of antibiotics brings about this effect, regardless of whether the pathogen is a multi-resistant or naïve bacteria or whether an old or new antibiotic is used.

Evidence suggests that a high dosage of antibiotics leads to a tremendous evolutionary advantage for antibiotic resistant pathogens. So, combining phage and antibiotics may be the solution to the medical dilemma. A lower dosage can be used to target naïve bacteria, and the bacteriophage can simultaneously inhibit the growth of the antibiotic resistant strains (Torres-Barceló et al., 2016).

Concerns in PAS Treatment

There are some concerns about the negative effects that can occur as a result of this two-fold treatment. Although there is no research that provides a basis for these fears, in order to ensure the complete safety of all patients, it is imperative to keep these concerns in mind. One major concern is regarding the possibility of breeding double resistant strains of a pathogen. This fear is an outgrowth of a similar phenomenon that has been noted for some antibiotic combination therapy (Torres-Barceló et al., 2016).

Researchers must also be mindful of specific antibiotic-phage combinations that inhibit the effectiveness of each individual component. For example, phages can block the bacteria's ability to absorb the antibiotic, or the antibiotic may affect the host in a way that limits the phage's ability to infect the bacteria or to produce sufficient offspring. Thorough screening of all the various combination before treatment can easily eliminate this concern (Torres-Barceló et al., 2016).

Finally, researchers caution that PAS may increase the virulence factors of certain infections. This phenomenon can be seen in the instance of quorum sensing, the communication between different bacteria cells, which can coordinate the production of some virulence factors. It has been observed that certain exposure to either phages or antibiotics has produced increased virulence. However, the bacterial viruses that target quorum sensing receptors can inhibit this response. Although there is little or no research indicating that these concerns are obstacles to the use of bacteriophage therapy, proper research must be done before combining antibiotics and phage therapy in treatment of infections (Torres-Barceló et al., 2016).

Isolation and Host-Range Determination of Bacteriophage

n order to harness the therapeutic powers of phages, the therapeutic index must be carefully studied and documented. One group of researchers bought bacterial cells from labs and public facilities. They isolated the methicillin resistant strains on agar plates. The next step required that they isolate the virulent phages. Upon obtaining samples from the environment, the researchers purified the samples by filtration and centrifugation three times (Jensen et al., 2015).

In the study they discovered 12 bacteriophages that exhibit lytic activity against SA and MRSA. In order to determine the host range, they took spectrophotometric assays of the cultures of phage-treated bacteria and performed spot testing. After further testing the cocktails on fabric and on glass, they found that the bacteriophage viruses were able to reduce the colony count on cultures of fabric and glass. Interestingly, the phage cocktails showed greater lytic activity against human MRSA then against non-human Staphylococcus Aureus (SA) and against methicillin-susceptible SA. In conclusion, they isolated 6 unique lytic phages that show strong lysis of MRSA (Jensen et al., 2015). The information gathered from this research now opens the door to further studying of these specific bacteriophages. In order to advance the applications of this study in medicine, the efficiency of these phages need to be clinically tested in humans.

Phage Treatment Viability

In order for the bacteriophage to be therapeutically useful, there must to be a way to store it in pharmacies. In order to examine phage viability, researchers stored phage in the freezer, refrigerator, and at room temperature. They concluded that the phage was most stable at room temperature, and was almost 100% viable after 60 days (Pincus et al., 2015). This is a positive discovery for the future of phage therapy, as the shelf life seems to be suitable for pharmaceutical storage and sale.

Isolation of Phages and Host Range Determination

Another area of life in which MRSA affects humans is its ability to infect livestock. Generally, animals are not harmed by these infections. However, they can be responsible for the transfer of infectious bacteria to humans, and this transmission has been documented. In particular, farmers and vegetarians who experience greater exposure to livestock are at a higher risk for this kind of infection. One group of researchers isolated three bacteriophages that were found in pig farms and tested the effectiveness of their antibacterial qualities (Kraushaar et al., 2013).

The methods used in the experiment are as follows. Researchers purchased bacterial strains, specifically MRSA CC398 strain 10-1355, from a laboratory. The phages were taken from a variety of sources including dust swabs, nasal swabs, and fecal swabs from four German pig farms. They then centrifuged, filtered, and tested the phages on MRSA lawn. The host range was tested on agar plates of 86 different MRSA strains CC398 strains and 34 other MRSA strains. The researchers also analyzed the DNA of the phages. None of the analyzed phages contained known virulence or resistant genes. Hence, the study could be conducted without concern that the phages would eventually cause more harm than good to the human host.

The MRSA strains belonging to complex 398 are commonly found on pig farms. These researchers aimed to combat livestock-associated MRSA infections by discovering the phages that naturally inhibit this pathogen. They screened 91 samples. Twenty percent of the samples exhibited lytic activity. An interesting phenomenon was noted in the course of this process. One of the farms was negative for both MRSA and anti-MRSA bacteriophages. This discovery is important, because it connotes that phages are found in proximity to the bacteria that they infect. Thus, researchers can assume that if they have a bacterial pathogen it is likely that phage can be isolated from that environment (Kraushaar et al., 2013). This can simplify the process for future research as the knowledge of where to find the right phages is now known.

Genetics' Effect on Phage Efficiency

The researchers studied three phages that are genetically related. The three PSa phages interestingly infect the same wide range of bacteria. Twenty- four MRSA strains were infected by these phages. These phages all have a receptor that researchers believe may be lipoteichoic acid. Of the three PSa types, type two and type three reduced the numbers of a specific MRSA strain more efficiently than type one. This aspect was observed with wider clearings on agar plates, but the plaques produced by type two and type three were ten times more reductive that the plaques produced by type one. The genotyping of the three phage's genes indicates that this property is a result of the differences in the lysis genes (Kraushaar et al., 2013). Further examination of the genes that favor greater bacterial lysis would be the next step in producing antibacterial cocktails for human medicine.

Another interesting result of this experiment was the discovery of mutant bacteria that were resistant to the phages used in the study. Even when the three different phages were combined in one cocktail, they still did not harm the mutant cells. This discovery is important as it highlights the necessity of combining more diverse phages in a cocktail to prevent resistance to similar phages (Kraushaar et al., 2013). This is another area of research that can be expounded upon based on the findings of this experiment.

Phage Therapy and Host Immuntiy

An important aspect of phage therapy research is the determination of specific bacteriophage's therapeutic index. Additionally, it is vital to explore if the bacterial virus is equally effective in all human hosts or if different factors contribute to the optimal productivity of the bacteriophage. One study explored the efficiency of phage SATA-8505 and its ability to prevent and treat infections caused by the MRSA strain USA300. The therapeutic index was examined in vitro with human cells and in vivo in mice. This specific strain was selected due to earlier research that proved its efficiency in combating MRSA USA300 (Pincus et al., 20).

All materials for the experiment, MRSA strain USA300, bacteriophage SATA-8505, and the phages were obtained from laboratories. Using agar plates, the researchers were able to confirm that phage SATA-8505 has the ability to kill MRSA USA300. This phenomenon was seen when they observed the colony morphology of culture plates two to four hours after phage inoculation.

Once it was clear that the phages were effective killing agents of MRSA, the researchers inoculated the mice with MRSA pathogens immediately after they injected bacteriophages into the skin. They then measured the diameters of the skin lesions for six consecutive days. The mice that were treated with an equal concentration of phages and bacteria displayed smaller skin lesions than the control group. The researchers also used Chronic Granulomatous Disease (CDG) immuno-deficient mice. CDG patients are more susceptible to MRSA infections due to their problematic neutrophil response. Interestingly, the CDG mice developed bigger skin lesions than the healthy mice; usually CDG patients develop MRSA infections on deep tissue, such as the liver. (Pincus et al, 2015). This could be a positive sign, for despite the fact that the skin lesions were big, the infections resembled those of healthy patients. One may think that due to the large lesion size, the CDG mice did not benefit from the phage therapy. Upon further examination, one may conclude that these mice improved relative to their own individual states of immunity and infection.

Unfortunately, the phage treatment does not seem to be a magical injection. Both groups of mice exhibited an increased lesional inflammatory cytokines. The fact that the actual lesion size was reduced while the bacterial count and cytokine response did not decrease led the researchers to hypothesize that although the administered dose of phage reduced the ability of the bacteria to inflict toxin-generated damage, it did not affect the viability of USA300 (Pincus et al., 2015). These researchers did a complete project. In order to completely understand this phenomenon, they repeated the experiment while changing the phage dosage and the original bacterial count. They discovered that an increase in phage administration brought about an increase in the inflammatory response in the CDG mice. This may indicate why bigger lesions that are produced when a higher dosage of phage is administered.

This discovery highlights the importance of phage dosage, especially in immunocompromised patients. The scientists noted that although the phage worked at certain concentrations, when the dosage was increased, negative side effects, such as an inflammation of the infectious site, were observed. Further research is required in order to configure the optimal dosage for treating patients while incurring minimal or no harm. The researchers make a point in warning clinicians that before phage therapy is used on any immuno-deficient individual, the dosage must be reconfigured. Extrapolating data from healthy individuals would not work for the CDG patients who were more affected by increases in dosage.

Once researchers noted that the phages induced an inflammatory response in mice, they investigated whether a similar response could be noted in human cells. When human blood cells were exposed to dosages of phage, the human cells did not produce inflammatory responses. The response of human keratinocytes was also investigated. When compared to the diluent treatment, phage induced a small, yet significant, cytokine response (Pincus et al 2015). This is a potential roadblock in the implementation of phage therapy. The only way this treatment could work is if the scientists find a way to inhibit the human body's negative response to the phage itself.

The next step involved investigation of the impact of phage SATA-8505 on human blood in both healthy and immuno-deficient individuals. Unfortunately, the phage did not seem to affect bacterial growth or colony morphology. The bacteria cells that were exposed to phage in blood and were then re-cultured grew just as well as bacteria that were unexposed to bacteriophage. The researches further investigated the phage and bacterium used and found that the bacterial cells were killed by the phage in a culture plate. This negates the possibility that a phage-resistant strain of MRSA was used to inoculate the blood sample. In order to elucidate why the bacteria is unaffected by phage in the presence of human blood, further research is necessary.

The last part of this research included the study of the efficiency of SATA 8505 on Vancomycin Resistant Staphhylococcus Aruerus (VRSA). The researchers determined that this phage is incapable of killing out the VRSA strains (Pincus et al., 2015). This observation highlights the strain-specificity aspect of phage therapy, since the phage worked fairly well on the MRSA but did not affect the VRSA growth.

Strain Specificity

The strain specificity of phage has both positive and negative aspects to it. While it is true that phage therapy successfully avoids destroying normal flora, one must first determine the exact strain of the infection prior to beginning the treatment. Unfortunately, this determination often takes time, and while treating MRSA infection, time is often of essence. Therefore, phage therapy may not yet serve as the first line treatment against infections (Pincus et al., 2015).

Thus, possibility of using phage cocktails holds much promise. Similar to multivalent vaccines, phage cocktails combine

a few different strains of bacteriophage. This would increase the chances that one of the strains would cover the unknown pathogen in the human host. Although this solution seems scientifically sound, there is a financial drawback. Each phage used in the cocktail must be separately tested in isolated therapy. Then research must be conducted on the combination to ensure that the combined effects are positive or synergistic. The added cost of all this research may be an issue (Pincus et al., 2015).

Phage Lysins: Pathogen Directed Treatment

One major drawback to phage therapy is the bacteria's ability to develop resistance to phage attachment. Recently researchers have developed ways to purify the lysins from the bacteriophages. The lysins can be used on their own as an antibacterial agent by causing bacterial lysis on contact. The use of lysins is generally limited to bacterial organisms with a cell wall, although researchers are searching for lysins with Gram-negative bacterial activity (Pastagia et al., 2013).

Lysin Mechanism

The lysins have two main functions: substrate recognition and enzymatic hydrolysis. The N-terminal domain enzymatically severs specific peptidoglycan bonds. This can be accomplished in multiple ways. They can hydrolyze the glyosidic bonds in the glycan strand of the cell wall, or the enzymes can hydrolyze the cross-bridge. The enzymes may also cleave the amide bond which is located between the glycan moiety and the main peptide. There is a level of specificity of lysin binding which is based on binding ligands and the specificity of cell wall binding domains.

Additionally, the lysins bind to their substrates with an affinity constant similar to antibodies (Ka = 6x108). This suggests that lysins do not disengage from the receptors after they bind and cleave, just like antibodies. This also explains the speed of lysin action. Their high affinity constant accounts for their speedy identification of their target bacterial cells (Pastagia et al., 2013).

Lysin Clinical Potential

The primary benefit of phage lysins is that, to date there has been no documentation of bacterial resistance to phage lysins. Additionally, bacteria with thick capsules and bacteria in biofilms do not inhibit lysin activity. These reasons are enough to aim research toward harnessing bacteriophage lysin antibacterial potential.

Lysins generally exhibit bactericidal activity against the bacterial host of the phages they were harvested from. Like bacteriophages, lysins are strain specific, and therefore do not affect normal flora. One specific lysin, chimeric lysin (ClyS) has been proven to kill MRSA strains along with other staphylococci. Some lysins have been proven to affect a broader range of bacterium (Pastagia et al 2013). These kinds of lysins would be a great first defense method against infections in which the exact strain of pathogenic bacteria is unknown.

Phage Lysin and MRSA Biofilm Eradication

Phage lysins have potential to fight biofilms, a serious health problem. All biofilms are harder to treat than planktonic bacteria; specific biofilms of antibiotic resistant pathogens, such as MRSA are even more difficult. Biofilms exhibit antibiotic resistance up to 1000 times more than planktonic bacterial cells (Chopra et al., 2015). These biofilm infections develop in patients that have prosthetic objects implanted in their bodies. Phage lysins seem to be the optimal way to combat MRSA infections because of the speed of their bactericidal activity, the small chances of bacterial resistance, and the low probability of it affecting the normal flora. Additionally, the usage of lysins allows for all the positive aspects of phage therapy without the negative possibilities such as resistant mutations.

Lysins generally exhibit bactericidal activity against the bacterial host of the phages they were harvested from. Like bacteriophages, lysins are specific to bacterial strains and therefore do not affect normal flora. One specific lysin, chimeric lysin (ClyS), has been proven to kill MRSA strains along with other staphylococci. Some lysins have been proven to affect a broader range of bacterium (Pastagia et al 2013 and sources within). These kinds of lysins would be a great first defense method against bacterial infections when exact strain of the pathogenic bacterium is unknown.

Research was conducted on the efficiency of phage lysins in eradicating MRSA biofilms. The object of this study was to evaluate the success of a phage lysin in eradicating old and new biofilms alone and in conjunction with antibiotics. The MRSA bacteria strain and the antibiotic, Minocycline, were obtained commercially. The phage lysin was extracted from phage MR-10 that had been previously been characterized by the same research group. In order to isolate and confirm their results, the team followed a published method for isolating phage enzymes (Chopra et al., 2015). This method of obtaining the phage lysin left some room for potential error. The research team may have made some errors in isolating the original phage, and the methods that they used originally are not documented in their paper. Thus, the whole project could have operated with impure or misnomered materials. Conversely, the method they utilized to isolate the enzyme leaves little room for error because they followed a proven method of isolating the endolysin. They did not rely solely on their observations and research. Additionally, they confirmed their results with SDS-PAGE analysis after every purification step (Chopra et al., 2015).

The biofilms were grown in the lab, and their morphological characteristics were identified by scanning with electron microscopy. The biofilms were developed on coverslips. Two different types of biofilms were formed; ica-positive and ica-negative. For the first part of the research, both types of biofilms were treated with the endolysin. The ica-negative biofilms were initially treated with 9 µg/ml. After three hours no significant reduction was observed. Even once they increased the duration of the treatment, there was no significant reduction. However, when the ica- negative biofilms were treated with 18µg/ml of lysin a statistically significant reduction was observed after six hours. This seemed to be the highest effective dosage for any increase in phage lysin concentration did not create a significant decrease in bacterial count. Contrarily, the ica-positive MRSA biofilm showed a reduction in bacterial cell count with an application of 36µg/mL of phage lysin after six hours. Increasing the duration to 24 hours did not make a difference in the bacterial reduction (Chopra et al., 2015). Although the two biofilms were very similar, the difference in the intra-cellular adhesion clearly affected the optimum concentration of lysin needed to eradicate the biofilms.

Phage Lysin and Antibiotics

The next step in this research was to examine the combined effects of phage lysin with antibiotics in treatment of biofilms. They were subjected to both minocycline (a tetracycline antibiotic,) and MR-10 endolysin. A significant decrease was observed in the first three days of the treatment. After that there was no more significant reduction in both forms of the biofilms The researchers also conducted this experiment on old biofilm This strong unit did not exhibit significant reduction in the cell count. They researchers theorize that this observation is a result of the two antibiotic agents acting primarily on the top layer of the thick biofilm. Other literature clearly states that antibiotics cannot penetrate deep into the biofilm because of the complicated nature of the biofilm matrix. Since the lysins are one time use enzymes, it is possible that they acted on the same cells as the antibiotic at different receptor sites. This could be the reason for the lack of reduction in the very thick biofilms (Chopin et al., 2015). Based on this theory, one way to combat thick biofilms would be continuous application of lysins. Once they kill out the upper layers, the new lysins can kill the cells in the deeper layers of the matrix.

The next step of this research team was to study how sequential treatment of phage lysin in conjunction with minocycline would affect the biofilms. There were two different sequences studied with different results. The first time the researchers applied endolysin for six hours and then added minocycline before incubating overnight. The next time they applied the minocycline first and then the phage endolysin. In comparison to both the controls and each other, the second method of sequential treatment delivered the strongest significant decrease in bacterial cell count. Figure 1 below compares the difference in total bacterial cell concentration from the control group and the two separate sequential treatments. Using the Live/Dead backlight kit, the researcher's stained the biofilms to see the total live and dead cells in the biofilm. After the second sequential treatment the staining confirmed that the majority of the live cells were killed (Chopin et al., 2015).

The positive results of biofilm treatment with three hours of minocycline followed by overnight incubation with endolysin MR-10 has a two-fold explanation. First, the antibiotic, minocycline eradicated the metabolically active cells. According to literature, the active cells are in the upper layers in the biofilm. Since the antibiotics cannot penetrate the layers and it is only affective on active cells, using it first ensured that there were active cells in the biofilm that the antibiotics could access. Next, the endolysins were applied. These lysins are small, low molecular weight proteins. Additionally, they act on both growing and stationary cells. Therefore, adding the lysins second enabled the smaller molecules to penetrate the biofilm and act on the metabolically inactive cells (Chopin et al., 2015). This reasoning also explains why the first sequential treatment was not nearly as successful. The endolysins probably killed the uppermost cells first, so subsequent treatment with antibiotics did not have a very strong additive benefit. By the time the antibiotic was applied there was not much more metabolically active cells present in the uppermost layer where the antibiotics were able to access. Therefore, they did not do as much as they could when they were applied first.

MRSA biofilms are another manifestation of the dangers posed by this antibiotic resistant bacterium strain. Instead of using the whole bacteriophage, this study provided solid research that supports the idea that an isolated enzyme from the phage will work in eradicating these biofilms. Since the lysins attack the peptidoglycan of the bacterial cell, it is a great solution for the hard to treat metabolically inactive cells found in biofilms. With the foundation of this research, many more studies can be conducted in order to isolate other endolysins for all sorts of biofilm infections.

Conclusion

Although many are discouraged by the growth of MRSA infections and the lack of treatment options, research suggests that bacteriophages may serve as the solution to this medical dilemma. Researchers have explored multiple aspects of bacteriophages and they have classified numerous strains of anti-MRSA phages. In order for the medical world to harness the natural powers of these viruses, more clinical studies must be

performed. Scientists must first continue to experiment on animals, in order to rule out all possibilities of the occurrence of negative side effects of phage inoculation. After the preliminary experimentation on animals has been completed, human trials can establish phages as tried and true medicinal option. If scientists continue to diligently explore this topic, there may be reason to believe that the world will be rid of the inherent dangers of multi-resistant pathogens.

References

Chopra S, Harjai K, Chhibber S. Potential of sequential treatment with minocycline and S. aureus specific phage lysin in eradication of MRSA biofilms: an in vitro study. Microbiol Biotechnol Applied Microbiology and Biotechnology. 2015;99(7):3201-3210. doi:10.1007/s00253-015-6460-1.

Jensen KC, Hair BB, Wienclaw TM, et al. Isolation and Host Range of Bacteriophage with Lytic Activity against Methicillin-Resistant Staphylococcus aureus and Potential Use as a Fomite Decontaminant. PLOS ONE PLoS ONE. 2015;10(7). doi:10.1371/journal.pone.0131714.

Kraushaar B, Thanh MD, Hammerl JA, Reetz J, Fetsch A, Hertwig S. Isolation and characterization of phages with lytic activity against methicillin-resistant Staphylococcus aureus strains belonging to clonal complex 398. Arch Virol Archives of Virology. 2013;158(11):2341-2350. doi:10.1007/s00705-013-1707-6.

Mattila S, Ruotsalainen P, Jalasvuori M. On-Demand Isolation of Bacteriophages Against Drug-Resistant Bacteria for Personalized Phage Therapy. Front Microbiol Frontiers in Microbiology. 2015;6. doi:10.3389/fmicb.2015.01271.

Pastagia M, Schuch R, Fischetti VA, Huang DB. Lysins: the arrival of pathogen-directed anti-infectives. Journal of Medical Microbiology. 2013;62(Pt_10):1506-1516. doi:10.1099/jmm.0.061028-0.

Pincus NB, Reckhow JD, Saleem D, Jammeh ML, Datta SK, Myles IA. Strain Specific Phage Treatment for Staphylococcus aureus Infection Is Influenced by Host Immunity and Site of Infection. PLOS ONE PLoS ONE. 2015;10(4). doi:10.1371/journal.pone.0124280.

Torres-Barceló C, Hochberg ME. Evolutionary Rationale for Phages as Complements of Antibiotics. Trends in Microbiology. 2016;24(4):249-256. doi:10.1016/j.tim.2015.12.011.

Wang I-N, Smith DL, Young R. Holins: The Protein Clocks of Bacteriophage Infections. Annual Review of Microbiology Annu Rev Microbiol. 2000;54(1):799-825. doi:10.1146/annurev.micro.54.1.799.