Investigating the effectiveness of bacteriophage therapy on antibiotic resistant bacterial infections

Thousand Oaks High School

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INVESTIGATING THE EFFECTIVENESS OF BACTERIOPHAGE THERAPY ON ANTIBIOTIC RESISTANT BACTERIAL INFECTIONS

Abstract

In recent years, antibiotic resistant pathogens have been labeled as a paramount threat to public health and safety. As a result, alternative antimicrobials such as bacteriophages have gained attention. Bacteriophages are bacteria-specific viruses that infect and destroy their host bacteria. This study investigates bacteriophage therapy and its effectiveness in treating drug-resistant bacterial infections. The objective of this study was to determine whether bacteriophages are effective in the inhibition of bacterial infections. Data was collected using databases such as EBSCOhost, PubMed, PLOS, NCBI, and a variety of other journals to perform meta-analysis. Data was collected in tables and phage treatment groups were compared to untreated control groups using a risk difference analysis. It was found that the use of bacteriophage therapy results in greater bacterial inhibition of drug-resistant bacterial infections compared to untreated groups. Bacteriophage therapy was on average 90.6% more effective at treating resistant *S. aureus* infections ($p<0.001$) and 60.3% more effective at treating resistant *E. coli* infections ($p<0.001$) than untreated control groups using a fixed effects model. Using a random effects model, bacteriophage therapy was on average 92.9% more effective at treating resistant *S. aureus* infections ($p<0.001$) and 72.8% more effective at treating resistant *E. coli* infections ($p=0.003$). During a time of growing antibiotic resistance, bacteriophage therapy is a promising alternative antimicrobial option.

Key Terms: Bacteriophage, bacteriophage therapy, antibiotic-resistant bacteria, MRSA, *S. aureus, E. coli*

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Introduction

On September 21, 2016, the United Nations General Assembly convened to discuss the problem of antibiotic resistance and deemed it "the greatest and most urgent global risk" (Lin et al., 2017). Antibiotic resistant bacteria are a major problem in today's medical field. The Center for Disease Control (CDC) estimate antibiotic-resistant infections result in 2 million illnesses and at least 23,000 deaths a year; deaths from complications by antibiotic resistant infections cost the United States $55 billion annually (Lin et al., 2017). Antibiotic resistance is caused by the misuse of current antibiotics, especially through the excess use of prescriptions and their use as prophylactic agents in animals. This has increased the number of resistant strains of pathogens, such as methicillin resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis*. These bacteria present a major health issue and developing new antibiotics to counter the rise in antibiotic resistance has become costly. The spread of pathogenic bacteria that have developed resistance to antibiotic treatment threatens to reduce the efficacy of medicine to a level similar to the pre-antibiotic era (Matsuzaki et al., 2003). This created interest into alternative antimicrobials, notably bacteriophage therapy. Bacteriophages are viruses that target specific species types of bacteria and cause lysis of the cell membrane. Bacteriophage therapy involves using bacteriophages to treat infections that do not respond to antibiotic treatment. When bacteriophages were first discovered, they were tested as a treatment option for bacterial infections; however, with antibiotics being more effective, research into phages stalled (Lin et al., 2017). Due to the epidemic of antibiotic resistant bacteria, there is a rekindled interest in bacteriophage therapy.

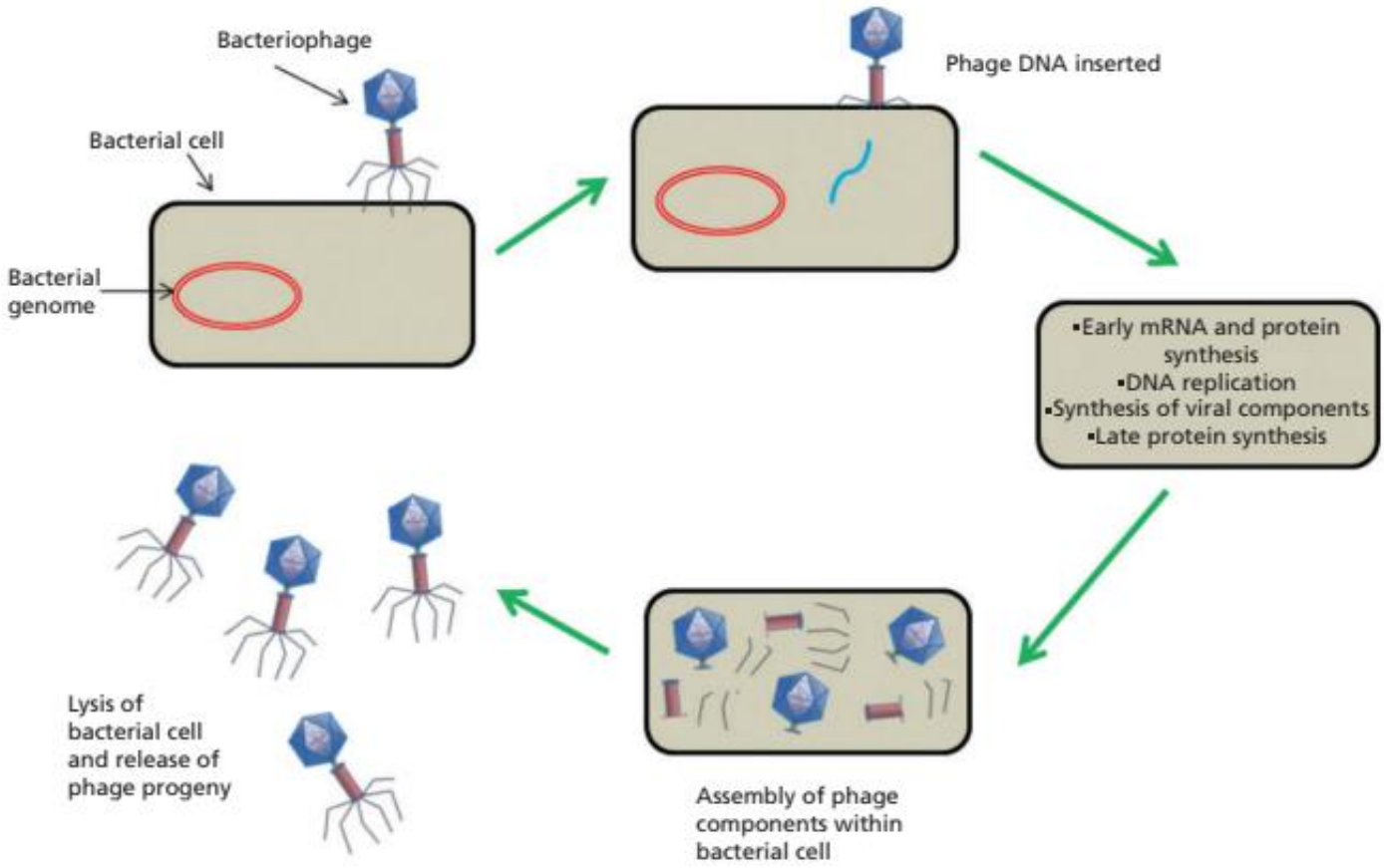

Figure 1: Lytic cycle of a bacteriophage infecting a bacterium (Ryan et al., 2011).

The introduction of bacteriophages as antibacterial agents began in the 1920s after their discovery by Dr. Felix d'Herelle. d'Herelle demonstrated their efficiency by administering anti-dysentery phages to patients suffering with severe dysentery, all of whom began to recover within 24 hours of treatment (Golkar et al., 2013). However, most early attempts at phage therapy were difficult because of inadequate delivery systems and protocol for purifying and storing phages (Lin et al., 2017). Penicillin, one of the earliest antibiotics, replaced phage therapy due to its effectiveness. (Lin. et al., 2017). The purpose of researching bacteriophage therapy is to find alternative methods of treating bacterial infections so medical treatments are not reliant on antibiotics. The rapid emergence of antibiotic resistant genes encoding for bacterial resistance pose a great threat to current medical treatments by making them ineffective. Although new antibiotics could be developed to aid in the treatment of resistant pathogens, pharmaceutical companies face several obstacles in the development of novel antibiotics. Antibiotics are generally less profitable than other drugs because they are curative treatments with brief

regimens, and the growth of antibiotic-resistance shortens the lifespan of new antibiotics, making pharmaceutical companies less keen to invest in their development (Ryan et al., 2011). Bacteriophages or drugs derived from them could have potential as alternative methods of treating infections.

Bacteriophages have several advantages over conventional antibiotics. Once phages are amplified through infection of the host organism and exclusive bacterial parasites, they become powerful antimicrobial agents. Phages are ubiquitous in the environment and can be isolated from human and animal subjects and later refined (Oliveira et al., 2009). Furthermore, phages have high specificity, meaning they only kill only their host bacterium. This minimizes the possibility for secondary infections and, in contrast to antibiotics, leaves the body's natural microbial biome unharmed (Ryan et al., 2011.). Phages replicate through lysis of the bacterial pathogen, so the simultaneous destruction of the pathogen combined with the release of additional bacteriophages causes phages to concentrate at the site of infection (Dabrowska et al., 2005). A review by Loc-Carrillo et al. highlighted the advantages of bacteriophages and their properties as therapeutic agents. These properties include the bactericidal effects of phages, host-specificity, minimal disruption of normal flora, low toxicity, and lack of cross resistance to antibiotics (Loc-Carrillo et al., 2011). Phages have also shown the ability to disrupt biofilms made by bacteria (Malik et al., 2017). The advantages of phages and their ability to replicate and kill pathogenic strains of resistant bacteria make them viable options for treating bacterial infections.

Despite its advantages, there are a number of limitations to the approach of bacteriophage therapy. Hyman et al. discusses the development of phage resistant mutants in bacterial strains. Phage resistance develops at a similar rate to antibiotic resistance and can make it difficult to develop lytic phages suitable for therapy. (Hyman et al., 2010). In addition, bacteriophage therapy requires a precise bacteriological diagnosis before therapy can begin to ensure that the proper phages are used in treatment (Ryan et al., 2011). However, phage therapy can be used synergistically with antibiotic treatment to circumvent phage resistance. Valério et al. conducted a study in 2017 to investigate the effects of the combined use of antibiotics and bacteriophages to inactivate *Escherichia coli* bacteria. The synergy between antimicrobials was tested in vitro, and the combination of antibiotics and phages resulted in high bacterial inactivation efficiency (Valério et al., 2017). Ultimately, phage therapy does not need to reduce bacterial infections to

zero, but instead decrease the number of bacteria to a level that allows immune defenses to overcome the remaining bacteria (Levin and Bull, 2004).

Delivery routes are a key factor in the success of phage treatment, as phage therapy will fail if bacteriophages cannot reach the site of infection. Many early trials of bacteriophage therapy were unsuccessful because of limited understanding of interactions between phages and the patient's body (Lin. et al., 2017). For example, the role of the patient's innate immune system in removing active phages and diminishing the efficacy of phage therapy was only observed recently (Hodyra-Stefaniak et al., 2015). Certain phages can be intolerant to conditions in the gastrointestinal tract or be inactivated by the immune system; therefore, it is of utmost importance to understand phage dissemination and proper delivery routes so that phages can reach the site of infection (Oliveira et al., 2009). In 2011, Ryan et al. conducted a study investigating recent advances in bacteriophage therapy, focusing on delivery routes for bacteriophages as well as formulation and concentration of phage stocks used for treatment. This study was conducted in order to determine the most effective routes for bacteriophages as antibacterial agents. This article investigates parenteral delivery, oral delivery, local delivery, inhalation, and topical administration of phages. Parenteral delivery of phages was tested by isolating the *Escherichia coli* bacterium from infected chickens, inoculating healthy chickens with *E. coli*, and administering the phage preparation. *E. coli* produced almost 100% mortality in newly hatched chickens that were not given phage treatment. Higher concentrations of phage preparation provided greater protection from infection. This study investigated other similar trials of phage therapy to determine the most successful route of administration for different types of infection. The most effective delivery route for the treatment of systemic infections was via the parenteral route. Oral delivery was most successful in treating gastrointestinal infections, inhalation of phage preparations was most successful in treating lung infections, and local delivery was most effective for topical infections. The review concluded that although phage therapy is an excellent alternative for the treatment of bacterial infections, optimization of formulations and long-term stability data is required before it can be widely used within a clinical setting (Ryan et al., 2011). Optimization of delivery routes is a necessary step to ensure maximum efficacy and the overall success of phage therapy.

The encapsulation of phages has also been proposed to ensure phages reach the site of infection. Phage encapsulation involves the process of coating doses of phages in an appropriate

hydrocolloid in order to segregate and protect the viruses from the surrounding environment (Mortazavian et al., 2007). A review done by Malik et al. investigates the clinical needs and challenges for treatment acute and chronic infections, as well as the drivers for phage encapsulation. These drivers for bacteriophage encapsulation include increasing the shelf life of phages through improved storage, ensuring reproducible dosages, and more effective delivery. Encapsulated phages also have the advantage of increasing the circulation time in the treatment of systemic infections and allow for controlled or sustained release at the target site of infection. The review concludes by looking at promising new approaches for micro- and nanoencapsulation of phages and how these may address gaps in the field of phage therapy. (Malik et al., 2017). The survival of bacteriophages in the acidic conditions of the digestive tract is oftentimes questionable, and encapsulation technology is an effective way to protect bacteriophages from adverse conditions. Choińska-Pulit et al. conducted a study investigating effective methods of encapsulating bacteriophages to protect them from these conditions. Bacteriophages can either be encapsulated in core shell capsules, which involves forming a protective coating made of substances such as calcium carbonate ($CaCO_3$) over a core of phages, or immobilized in matrix capsules, which involves trapping cores within a protective matrix of similar substances. (Choińska-Pulit et al., 2015). Developing new, effective methods of phage encapsulation is essential to the success of bacteriophage encapsulation, especially in the treatment of gastrointestinal infections such as *E. coli*.

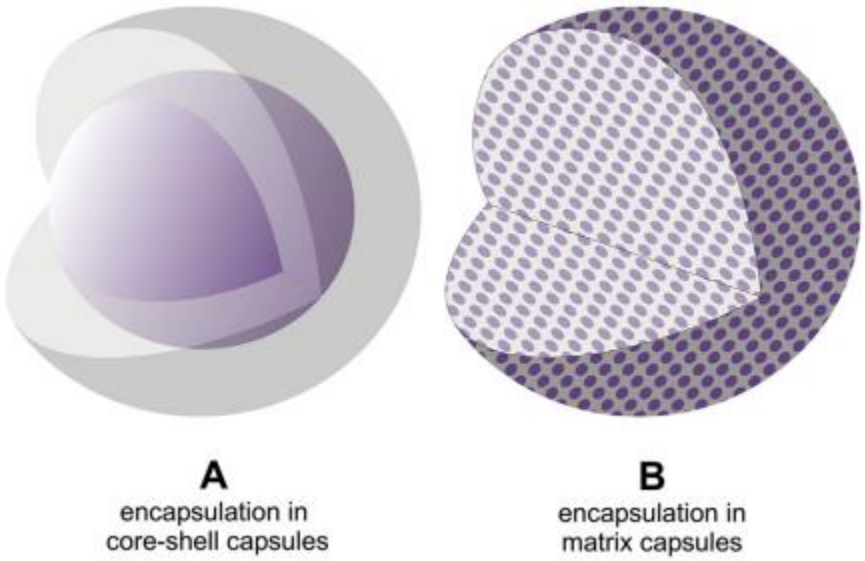

Figure 2: Differences between core-shell encapsulation of bacteriophages (A) and immobilization of phages in matrix capsules (B) (purple = core/phages, grey = shell).

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Escherichia coli is a bacterium that is one of the most frequent causes of common bacterial infections (Wernicki et al., 2017). In addition, *E. coli* is a major issue in the livestock industry: it can cause severe systemic infections in animals and is often associated with significant economic losses (Oliveira et al., 2009). Due to restrictions on the use of antibiotics in livestock and the growth of antibiotic resistant bacteria, phages are currently being used on animals as targeted therapy (Wernicki et al., 2017). As a result, several studies have been conducted to investigate using bacteriophages to treat and control *E. coli*.

Amarillas et al. conducted a study in 2017 to investigate phiLLS, a strictly lytic phage targeting the *E. coli* bacterium that does not carry genes associated with virulence factors or allergen proteins. Phage bacteriolytic activity was determined *in vitro* against antibiotic resistant *E. coli* O157:H7. Water samples were tested for the presence of bacteriophages designated phiLLS. The bacteriophage has shown promising results for biocontrol under *in vitro* condition, suggesting that it could be an alternative agent for the control of foodborne pathogens. However, toxicity testing must be done to ensure safety before the phage is used (Amarillas et al., 2017). Combining research done on delivery methods and *E. coli*-specific phages, Rastogi et al. conducted a study focusing on the transdermal delivery of the T4 bacteriophage both ex-vivo and in-vivo using microemulsion as delivery carrier. Microemulsions were prepared by mixing oil, surfactants, and aqueous phase containing phages. This was tested in rats infected with *E. coli*: rats that received phage treatment survived while significant mortality was observed in those that did not receive treatment. Microemulsion based delivery is a promising approach to treat *E. coli* infections but must be investigated further in terms of efficacy and safety (Rastogi et al., 2017).

Several commercially available products designed as biocontrol agents use bacteriophages to eliminate *Listeria monocytogenes,* a foodborne bacterium that causes listeriosis. Sadekuzzaman et al. conducted a study investigating the effectiveness of commercially available bacteriophage products designed to kill *L. monocytogenes.* The efficacy of ListShield, a bacteriophage biocontrol agent against *L. monocytogenes* biofilms, was evaluated. *L. monocytogenes* biofilms were established on lettuce, stainless steel, rubber, and an MBEC biofilm device and exposed to the ListShield phage preparation for 2 hours. ListShield proved to be an effective tool for the inactivation of *L. monocytogenes* biofilms, as biofilms were significantly reduced in all cases (Sadekuzzaman et al., 2017). Another study of a similar manner done by Gutiérrez et al. assessed the effectiveness of the bacteriophage preparations Listex P100

and ListShield against *Listeria monocytogenes* from food origins. ListShield was effective in lysing 100% of *L. monocytogenes* strains examined, whereas Listex P100 lysed only 64% of the same strains. Both products were effective at removing 72-hour old biofilms formed on stainless steel surfaces. These results suggest that phage-based products can be useful for biocontrol of *L. monocytogenes* in food contact surfaces (Gutiérrez et al., 2017).

Currently, MRSA infections pose a major threat to health and safety. *Staphylococcus aureus* is a pathogen that can cause inflammatory diseases, food poisoning, and toxic-shock syndrome, and it is also a major causative agent for opportunistic infections and is often associated with a high mortality rate (Matsuzaki et al., 2003). *S. aureus* infections can induce a diverse range of life-threatening infections and has exhibited exceptional behavior in developing resistance to new antibiotics (Rasool et al., 2016). Phage therapy is an alternative method of treating these infections that has shown promising results. Rasool et al. conducted a study in 2016 aiming to find indigenous phages in Pakistan and investigate their potential as antibacterial agents against MRSA. Bacterial isolates *S. aureus* were collected and identified using standard microbial procedures. Phages were taken from sewage water and enriched using *S. aureus* as a host organism. The antibacterial effects of these phages were determined in-vivo using a rabbit wound model. Phages exhibited lytic activity, which peaked in three to six hours of phage infection. The rabbits' wound healing exhibited a positive trend in all of the groups tested except for the bacterial control group (Rasool et al., 2016). Bacteriophages can be further found and characterized and appear to be a promising candidate for phage therapy against MRSA.

Bacteriophage therapy could prove to be a novel method of treating antibiotic-resistant strains of bacteria. Infections caused by drug-resistant pathogens are a major cause of fatalities, and if a new method to treat these infections is not discovered we could return to an age in which simple infections claim many lives. Currently, we take many medical procedures, such as surgery and radiotherapy, for granted because of antibiotics. Resistant strains of bacteria threaten to end the safety net of antibiotics. Bacteriophage therapy is a promising method of treating bacterial infections that do not respond to antibiotic treatment, and therefore could be a solution to the epidemic of drug-resistant bacterial infections.

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Purpose

The purpose of this research is to determine whether phage therapy is an effective method of treating antibiotic resistant bacterial infections, as it is important to find alternative methods of treating bacterial infections due to the rise bacteria that are not eliminated by antibiotics.

Research Question

How effective is the administration of bacteriophages in the inhibition of antibiotic resistant bacterial infections?

Hypothesis

Alternative Hypothesis

The treatment with specific phages will result in significant bacterial inhibition of the treated groups compared to the control groups.

Null Hypothesis

There will be minimal to no difference bacterial inhibition after the treatment of specific phages compared to the control groups.

Methods

Research was conducted through meta-analysis. Data was obtained from other researchers' published peer-reviewed articles.

Sample of Studies

Literature related to bacteriophage therapy and its use in the treatment of antibiotic-resistant strains of bacteria were collected through several online databases. These include EBSCOhost, California State University of Channel Islands' library databases, PLOS Journal, Google Scholar, ScienceDirect, ResearchGate, American Society for Microbiology, and National Center for Biotechnology Information (NCBI). A broad range of research was collected,

including research on trials of phage therapy against multiple pathogens, reviews highlighting the advantages and disadvantages of phages as antimicrobials, and research on delivery methods and phage encapsulation. After collecting and identifying the core body of reports and reviews, the references of these reports and reviews were investigated to find additional pertinent studies.

Selection Criteria

Studies were included in the systematic review based on the following criteria:

1. Only studies that involved the treatment of bacterial infections using individual phages or phage cocktails were included. Experiments testing bacteriophage therapy combined with another form of antimicrobial were omitted.
2. Studies were taken from post 1998. Studies ranged from the years 1998 to 2017.
3. Studies report that the bacterial infection being treated displayed antibiotic resistance. Studies that did not treat antibiotic resistant bacterial infections were omitted.
4. Studies reported bacterial inhibition or the survival rates of animals treated with bacteriophages.
5. Studies included a control group so that the results of phage treatment can be compared to the corresponding control group.

Data Collection

Studies were narrowed down into two categories based on the host bacteria: *S. aureus* infections and *E. coli* infections. Data collection included the animal infected with the host bacteria, infection type, initial bacterial concentration measured in colony forming units (CFU), initial phage concentration used in treatment measured in plaque forming units (PFU), phage type, and bacterial inhibition. The majority of studies did not directly state bacterial inhibition, so bacterial inhibition was calculated using the survival rates of animals treated (survivors/total animals treated). For each experiment, the data from the corresponding control group was collected. Control groups were administered equal concentration of CFU but did not receive phage treatment. Bacterial inhibition of treated animals was compared between those that had received phage treatment and control groups that did not receive treatment.

Statistical analysis was performed using MedCalc software. Risk difference analysis was performed in order to compare the bacterial inhibition values of treated and control groups and determine the significance of collected data. Risk difference was taken with confidence intervals of 95%. Both a fixed effects model and a random effects model of the data were used so that results could be compared for consistency. A fixed effects model assumes that all studies are observing the same treatment effect, while a random effects model accounts for differences in treatment effects and sampling variance. Data was considered statistically significant when $p<0.05$.

Results

Treatment of S. aureus infections

Table 1A: Treatment data from phage therapy trials against antibiotic resistant *S. aureus*. Four trials were examined to collect data. Data collected included the infected animal, the infection type, the bacterial concentration of the infection (CFU), the phage concentration administered as treatment (PFU), the bacterial inhibition, and the phage type administered. Phage cocktails consist of multiple species of bacteriophage. Average bacterial inhibition was calculated using the collected values for bacterial inhibition.

Ref.	Animal	Infection type	Bacterial concentration (CFU)	Phage concentration (PFU)	Bacterial Inhibition	Phage Type
Capparelli et al., 2007	Mouse	Systemic	10^8	10^9	29/30 (93%)	Single
Wills et al., 2005	Rabbit	Wound	8x10^7	2x10^9	7/8 (87.5%)	Single
Matsuzaki et al., 2003	Mouse	Systemic	8x10^8	2x10^11	10/10 (100%)	Single
Rasool et al., 2016	Rabbit	Wound	1.5x10^8	10^8	3/3 (100%)	Phage Cocktail
				Average Bacterial Inhibition	49/51 (96.07%)	

Table 1B: Control groups for phage therapy trials against antibiotic resistant *S. aureus*. Control groups were administered saline solution rather than a phage suspension. Average bacterial inhibition was calculated using the collected values for bacterial inhibition.

Ref.	Animal	Infection type	Bacterial concentration (CFU)	Phage concentration (PFU)	Bacterial Inhibition
Capparelli et al., 2007	Mouse	Systemic	10^8	0	0/30 (0%)
Wills et al., 2005	Rabbit	Wound	8x10^7	0	0/8 (0%)
Matsuzaki et al., 2003	Mouse	Systemic	8x10^8	0	1/10 (10%)
Rasool et al., 2016	Rabbit	Wound	1.5x10^8	0	0/3 (0%)
				Average Bacterial Inhibition	1/51 (0.019%)

The potential efficacy of specific phages to inhibit drug-resistant strains of *S. aureus* was evaluated by analyzing survival rates of infected animals that were administered phage treatment. Four trials of phage therapy were analyzed, taken from Capparelli et al., Wills et al., Matsuzaki et al., and Rasool et al. Data concerning antibiotic resistant *S. aureus* infections was collected and displayed in Tables 1A and 1B, with 1A being data on phage treatment and 1B being on corresponding control groups that received no treatment. Bacterial inhibition was much higher for groups that received phage treatment. The average bacterial inhibition of the phage therapy trials was 96.07%, while the average bacterial inhibition of the control groups was 0.019%.

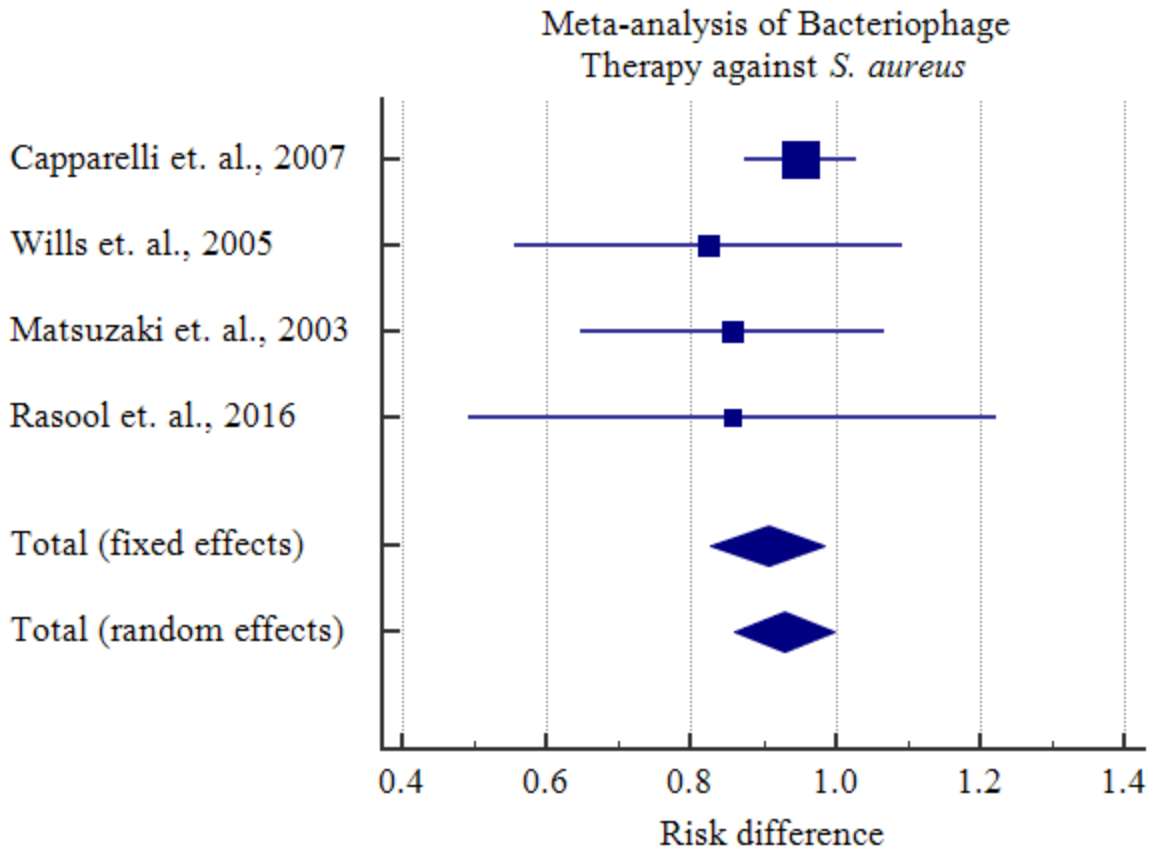

Figure 3: Risk difference analysis for data collected on *S. aureus* infections and shown in Tables 1A and 1B. Forest plot shows the summary estimate (center of the square/diamond) and its 95% confidence interval (width of line/diamond). Risk difference using a fixed effects model was 0.906 ($p<0.001$), while risk difference using a random effects model was 0.929 ($p<0.001$).

Risk difference analysis was performed on collected data in order to determine the absolute effect of bacteriophage therapy against *S. aureus* infections, as well as the significance of the data. Risk difference using a fixed effects model was 0.906, meaning that the actual difference in effectiveness between the treated groups and control groups was 90.6%. Observed p-value was less than 0.001: therefore, data is significant and the null hypothesis should be rejected. Using a random effects model, risk difference was 0.929, meaning that the difference in effectiveness between treatment and control groups was 92.9%. Observed p-value was less than 0.001: therefore, data is significant and the null hypothesis should be rejected.

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Treatment of E. coli infections

Table 2A: Treatment data for phage therapy trials against antibiotic resistant *E. coli*. Four trials were examined to collect data. Data collected includes the infected animal, the infection type, the bacterial concentration of the infection (CFU), the phage concentration administered as treatment (PFU), the bacterial inhibition, and the phage type administered. Phage cocktails consist of multiple species of bacteriophage. Average bacterial inhibition was calculated using the collected values for bacterial inhibition.

Ref.	Animal	Infection type	Bacterial concentration (CFU/mL)	Phage concentration (PFU/mL)	Bacterial Inhibition	Phage Type
Barrow et al., 1998	Chicken	Meningitis	10^3	10^8	13/15 (86.7%)	Phage Cocktail
Barrow et al., 1998	Chicken	Septicemia	10^6	10^6	15/15 (100%)	Phage Cocktail
Tanji et al., 2005	Mouse	Gastrointestinal	10^9	10^9	6/6 (100%)	Single
Huff et al., 2002	Chicken	Pulmonary	5.6x10^5	4.6x10^7	21/30 (70%)	Phage Cocktail
				Average Bacterial Inhibition	55/66 (83.3%)	

Table 2B: Control groups for phage therapy trials against antibiotic resistant *E. coli*. Control groups were administered saline solution rather than a phage suspension. Average bacterial inhibition was calculated using the collected values for bacterial inhibition.

Ref.	Animal	Infection type	Bacterial concentration (CFU/mL)	Phage concentration (PFU/mL)	Bacterial Inhibition
Barrow et al., 1998	Chicken	Meningitis	10^3	0	2/15 (13.3%)
Barrow et al., 1998	Chicken	Septicemia	10^6	0	0/15 (0%)
Tanji et al., 2005	Mouse	Gastrointestinal	10^9	0	0/6 (0%)
Huff et al., 2002	Chicken	Pulmonary	5.6x10^5	0	12/30 (40%)
				Average Bacterial Inhibition	14/66 (21.2%)

Four trials concerning antibiotic resistant strains of *E. coli* were also analyzed to determine the efficacy of phage therapy. Two trials of phage therapy were taken from Barrow et al., one from Tanji et al., and one from Huff et al. Data concerning phage treatment of *E. coli* infections and untreated control groups are shown in Tables 2A and 2B respectively. The average bacterial inhibition of phage treatment was 83.3%, while the average bacterial inhibition of the control groups was 21.2%.

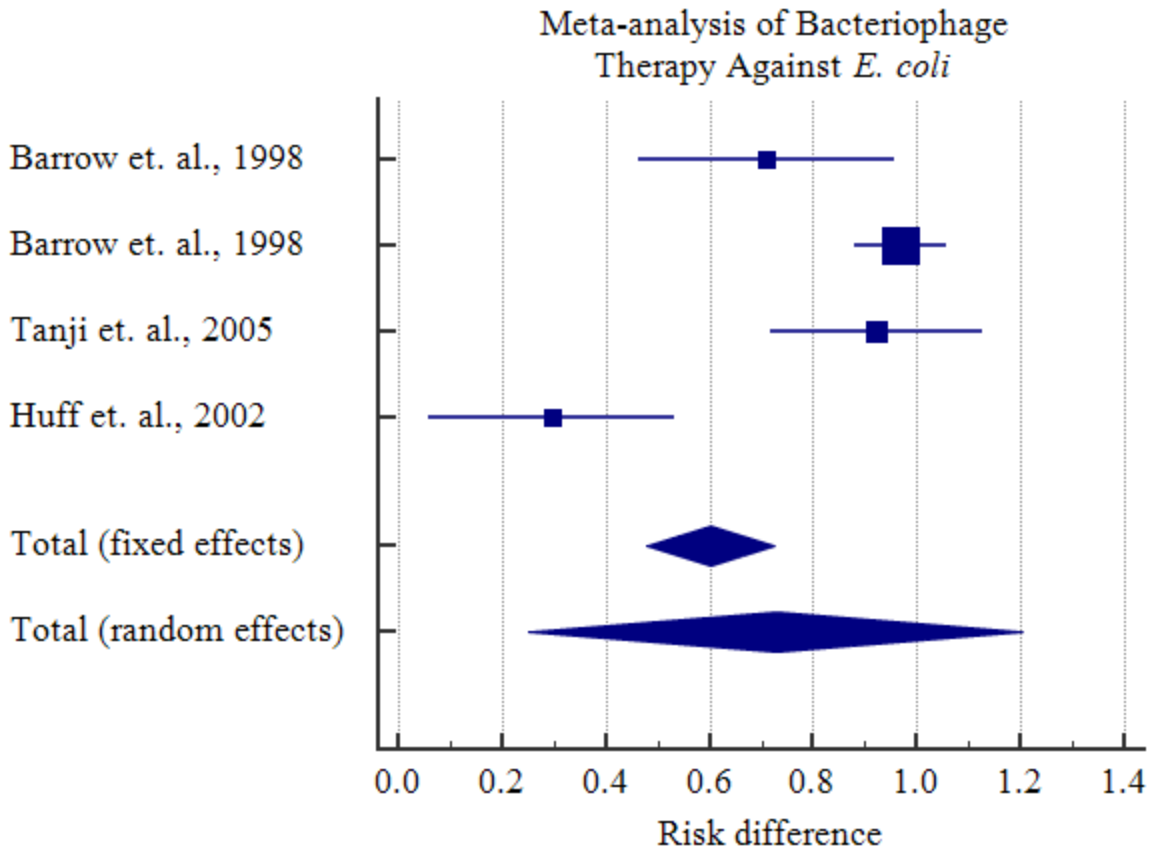

Figure 4: Risk difference analysis for data collected on *E. coli* infections and shown in Tables 2A and 2B. Forest plot shows the summary estimate (center of the square/diamond) and its 95% confidence interval (width of line/diamond). Risk difference using a fixed effects model was 0.603 ($p<0.001$) while risk difference using a random effects model was 0.728 ($p=0.003$).

Risk difference analysis was performed on collected data in order to determine the absolute effect of bacteriophage therapy against *E. coli* infections, as well as the significance of the data. Risk difference using a fixed effects model was 0.603, meaning that the actual difference in effectiveness between the treated groups and control groups was 60.3%. Observed p-value was less than 0.001: therefore, data is significant and the null hypothesis should be rejected. Using a random effects model, risk difference was 0.728, meaning that the difference in effectiveness between treatment and control groups was 72.8%. Observed p-value was 0.003: therefore, data is significant and the null hypothesis should be rejected.

Discussion

The treatment of *S. aureus* infections has become increasingly difficult due to the rapid emergence of antibiotic resistance and the presence of staphylococcal infections that resist multiple antibiotics. Drug-resistant bacteria such as MRSA pose a serious threat to current medical practices that rely on antibiotics to stop opportunistic infections. Bacteriophages provide a possible substitute treatment for these bacterial infections.

The present study shows the effectiveness of bacteriophage therapy in treating antibiotic resistant staphylococcal infections. Review of four trials indicates that the average bacterial inhibition of antibiotic resistant *S. aureus* was 96.07%, compared to the control group with an average bacterial inhibition of 0.019% (Tables 1A and 1B). According to the risk difference analysis, phage therapy was 90.6% more effective at treating drug resistant *S. aureus* infections when compared to untreated control groups in a fixed effects model ($p<0.001$) and 92.9% more effective using a random effects model ($p<0.001$). Results from a fixed effects model and a random effects model are consistent in showing that phage therapy has a positive effect. These results confirm the alternative hypothesis that groups treated using specific phages have higher bacterial inhibition than untreated control groups. Therefore, it can be concluded that phage therapy is effective in treating antibiotic resistant *S. aureus* infections.

The use of phage therapy against MRSA was only tested against systemic infections and wound infections. Thus, further research is necessary to determine whether phage therapy is effective against other types of *S. aureus* infection, such as pulmonary infections. Based on the results of the data collected, bacteriophages could be a promising new antimicrobial weapon against systemic and wound infections of MRSA.

Foodborne diseases are a major cause of illness and mortality worldwide, and incidences of antibiotic resistance pathogens has increased in recent years (Amarillas et al., 2017). This study investigated the effectiveness of phage therapy against *E. coli*, one of the most common foodborne illnesses. It was determined from the data collected that the average bacterial inhibition of drug resistant *E. coli* was 83.3%, compared to the control group with an average bacterial inhibition of 21.2% (Tables 2A and 2B). Bacterial inhibition in untreated control groups was much higher compared to trials done using *S. aureus*, most likely because bacterial doses in the control groups were lower. Barrow et al. administered control group chickens a bacterial

dosage of 10^3 CFU and Huff et al. administered control group chickens a bacterial dosage of $5.6x10^5$ CFU; the bacterial inhibition for these groups were 13.3% and 40% respectively. These trials had the lowest concentration of *E. coli* administered, which most likely contributed to higher rates of bacterial inhibition in control groups as the immune system was able to successfully fight off the smaller concentration of pathogens. According to the risk difference analysis, the application of *E. coli* specific phages was 60.03% more effective at treating antibiotic resistant strains of *E. coli* when compared to untreated control groups using a fixed effects model ($p<0.001$), and 72.8% more effective using a random effects model ($p=0.003$), showing that phage therapy is an effective treatment option for drug resistant *E. coli* infections. From the data collected, it can be inferred that the difference between the fixed and random effects model resulted from the trial done by Huff et al., which had the lowest difference between the rate of inhibition in treatment and control groups. Because the trial was done on a larger group of animals (30 animals), the fixed effects model weighted this study more, while the random effects did not. As a result, the fixed effects model is more accurate in showing the effectiveness of phage therapy as it accounts for the size of the treatment groups.

One of the primary keys to the success of phage therapy is proper delivery routes. Improper delivery of phage treatment can lead to failure in treating infections, as shown in a trial done by Stanford et al. in which phage treatment was administered to control *E. coli* in cattle. Phage therapy did not result in a significant reduction in *E. coli* CFU because the acidic nature of the cattle's gastrointestinal tract disabled the phages before they could reach the site of infection (Stanford et al., 2010). Encapsulation of phages may provide a solution to this in the future by protecting phages from harmful environments within the body.

One of the common concerns with phage therapy is the appearance of phage resistant mutants. If bacteria become refractory to phage treatment, the problem of resistant bacterial infections remains. Phage resistance was monitored in trials done by Capparelli et al., where they observed the frequency of phage-resistant *S. aureus* bacteria was $1.3x10^{-8}\pm4.16x10^{-9}$ in a culture of 10^8 CFU. (Capparelli et al., 2007). The above results indicate that phage resistance is an uncommon event, perhaps even more uncommon than antibiotic resistance. Even if bacteria acquire phage resistance, new lytic phages can be isolated quickly due to mutations in phages (Matsuzaki et al., 2003). Each trial of phage therapy analyzed in this study used different types of phages or phage cocktails, showing that there a variety of available lytic phages for

treatments. Phage resistance is less of a threat due to the abundance of lytic phages available for isolation and purification (Lin et al., 2017). Isolating novel bacteriophages is much easier than creating novel antibiotics, and circumnavigating phage resistance will likely be a more straightforward task than solving the current antibiotic resistance crisis.

In all trials that were investigated, authors reported antibiotic resistance in bacterial infections. This study demonstrated that antibiotic resistance did not inhibit the effectiveness of phage therapy. This study supports the conclusion reached by other researchers that phage resistance and antibiotic resistance are independent, which makes it is highly unlikely that bacterial infections would exhibit cross resistance (Loc-Carrillo et al., 2011). Synergistic use of antibiotic and phage treatment may have potential in treating cross-resistant infections; however, further research must be conducted. Overall, phage therapy looks to be an effective method of treating antibiotic resistant bacterial infections.

Conclusion

The increasing acquisition of antibiotic resistance by bacteria necessitates new strategies for the treatment of drug resistant bacterial infections. The available literature on the usage of phages to treat antibiotic resistant pathogens shows promise for phage therapy. Phages have several advantages over conventional antibiotics, including host-specificity, concentration at the site of infection, low toxicity, and a lack of cross-resistance with antibiotics. Phage therapy has large room for improvement through the isolation and amplification of new lytic phages and development of phage encapsulation to improve delivery methods. The development of phage preparations may prove to be one of the most potent methods of treating bacterial infections in both animals and humans in the future.

Further work

Further work can be done through analyzing more trials evaluating the effectiveness of bacteriophage therapy against antibiotic resistant bacteria, especially trials concerning different types of infections that were not investigated in this paper (such as pulmonary *S. aureus* infections). Trials could also be done investigating novel delivery methods for bacteriophages. Research on the encapsulation of drugs in microspheres or nanospheres could be applied to the field of bacteriophage therapy. Encapsulation of phages looks to be a promising delivery method: different barriers could be tested to determine the most effective method of preserving phages and delivering them to the site of infection. Phage therapy must also undergo clinical trials to determine the safety and efficacy of treating antibiotic resistant infections in humans. Safety and toxicity testing are especially important to ensure that phage treatment does not have adverse effects on patients. The synergistic effects of phage therapy combined with other antimicrobials could also be further investigated in vivo to determine its effectiveness.

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