Antibiotic Resistance Crisis Spurring Phage Therapy Research

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Antibiotic Resistance Crisis

Antibiotics have a clinical history almost a century long. Over that century, the burden of communicable diseases on the world population has diminished largely due to the widespread use of antibiotics. Though antibiotics greatly reduced deaths due to infectious disease, they also created new challenges in the form of antimicrobial resistance (AMR). Multiple drug resistant (MDR) pathogens are increasingly common and are considered grave threats to global health.

Penicillin was the first antibiotic to be effectively used on a large scale. Alexander Fleming discovered penicillin by accident while studying *Staphylococcus*. One of his petri dishes became contaminated with a mold, and he noticed the bacterial growth was inhibited around the mold colony. He investigated this observation further and identified penicillin as the antibiotic agent produced by the mold. Fleming reported his finding in 1929, and briefly mentioned the possibility of using penicillin to treat localized infections (1). Fleming's colleagues Ernst Chain and Howard Florey worked in collaboration with him over the next decade to develop methods for purifying penicillin and harvesting it in of sufficient amounts for human clinical trials (2). During the second world war, the United States and Britain jointly supported what became known as "The Penicillin Project." Prior to the United States' entry into the war, there was only enough penicillin available to treat less than 100 patients. By September 1943, there was enough penicillin for the demands of the United States armed forces and its allies (3). Penicillin undoubtedly saved many soldier's lives during World War II and sparked the age of antibiotics. Subsequently, Fleming and his colleagues were awarded a Nobel Prize in 1945 for their discovery.

The period following the discovery and success of penicillin is considered the golden age of antibiotics (Figure 1). The majority of antibiotics discovered during this time were isolated from natural sources. The primary sources of these antibiotics were actinomycetes, fungi, and other bacteria. Antibiotic producing bacteria are identified through a process called antibiotic screening. In the 1940's Selman Waksman screened soil bacteria for antimicrobial properties through several culture-based techniques. These methods are based on the same principle: inhibition of a test strain over a closely cultivated indicator strain. The test strain is the strain suspected to produce an antibiotic compound targeting the indicator strain. The cross-streak technique is performed by creating horizontal streaks of the test and indicator strain that cross each other in the center of the plate. If the test strain produces an antimicrobial compound against the indicator strain, the growth of the indicator strain will be inhibited at the point that the horizontal streaks cross. Techniques such as cross-streaking have been used to create catalogues of antibiotic-producing microbes (Figure 2) (4).

During the golden age between the 1940s and 1970s, twenty new classes of antibiotics were discovered(4). This coincided with a significant decrease in deaths due to infectious diseases. During the twentieth century, the leading cause of death in the United States gradually changed from communicable diseases like tuberculosis and pneumonia to non-communicable diseases like cardiac ischemia and cancer. At the beginning of the twentieth century, 46.4% of deaths were due to infectious disease. By the end of the century, the leading infectious diseases accounted for only 4.5% of deaths (5).

However, the battle against infectious diseases is not over. Bacteria can develop resistance mechanisms that prevent antibiotics from interfering with cellular processes. These mechanisms include modification of the antibiotic target, efflux pumps, and the secretion of enzymes that degrade antibiotics. Modification of the antibiotic target occurs when there is a change in structure of that target. This change in structure is often due to mutations within the bacterial genome. The structural change of the target prevents the antibiotic from binding and the drug is no longer effective. Bacteria also employ efflux pumps that move toxins such as antibiotics from inside the cell to outside the cell. This prevents the antibiotic from reaching a critical concentration within the cell. Another mechanism of resistance is the secretion of enzymes targeting the antibiotic. These enzymes break down the antibiotic, so they are not effective (5).

Antibiotic resistance was observed in pathogens shortly after the advent of antibiotics, and its dangers were anticipated by Alexander Fleming. During his Nobel prize acceptance speech in 1945, he foreshadowed the dangers of antibiotic resistance (5):

> "But I would like to sound one note of warning… The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily under dose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant."

Antibiotic resistance was first reported soon after the discovery of penicillin. A penicillin resistant strain of *E. coli* was identified in 1940 and many penicillin resistant strains of *S. aureus* were identified three years later (6, 7). Antibiotic resistance spread quickly in *S. aureus*, and by 1960, 80% of all *S. aureus* infections were penicillin resistant (8). During the antibiotic golden age, scientist and pharmaceutical companies stayed ahead of resistance by continually discovering and producing new classes of antibiotics such as aminoglycosides, tetracyclines, and quinolones. These new classes of drugs targeted a variety of cellular processes including cell wall synthesis, protein synthesis, and DNA replication. This arms race against infectious disease resulted in the use of more potent broad-spectrum antibiotics and unfortunately produced multiple drug resistant (MDR) pathogens. MDR's are considered to be one of the largest threats to global health. Extensively drug resistant tuberculosis was first identified in 2005, but has now been reported in 92 countries (5). The Centers for Disease Control (CDC) released a list of what is considered to be the biggest global health threats in 2015 and listed 18 drug-resistant pathogens as urgent, serious, or concerning threats. The report also states that about 2.8 million people in the US are infected with antibiotic resistant pathogens resulting in an estimated 35,000 deaths annually. The World Health Organization (WHO) also considers MDR an urgent threat. In a 2015 report on antibiotic resistance, WHO listed MDR *E. coli* and tuberculosis among the top threats. According to WHO, 3.6% of all new and 20.2% of previously treated TB cases exhibit MDR.

In Europe, a direct correlation was found between the amount of antibiotics prescribed and the levels of reported antibiotic resistance. Countries like France prescribe antibiotics more liberally and also report more cases of antibiotic resistance. Countries like the Netherlands prescribe far fewer antibiotics and also report lower levels of antibiotic resistance (9). This trend is likely due

to unnecessary antibiotic use. Antibiotics are often prescribed to patients with viral illnesses or based on patient requests. In these situations, there is little evidence to support administering antibiotics to a patient. A 2016 study of prescribing practices in the United States determined that 30% of oral antibiotic prescriptions were inappropriate. In cases of acute respiratory infections, the researchers deemed more than 50% of antibiotic prescriptions were unnecessary (10). Furthermore, a study in Finland found that a countrywide reduction of outpatient antibiotic prescriptions correlated to a significant decrease in the prevalence of erythromycin resistant Staphylococci (11).

In addition to treating humans with bacterial infections, antibiotics are also used extensively in agriculture and livestock farming. Antibiotics serve several purposes in agriculture. They act as growth promoters by decreasing the microbial burden on the animal. A decreased microbial burden makes animals more efficient at absorbing nutrients from their food, which also makes them grow much faster (12). Antibiotics are also used to prevent the spread of disease among animal populations. High density farming practices encourage the rapid spread of microbial diseases, and these diseases pose a significant threat to the world's food supply. In 2012, 2 billion dollars in losses were attributed to *S. aureus* infections in dairy animals alone (13). To reduce the spread of disease, low doses of antibiotics are mixed with livestock food supplies. Animal consumption now accounts for about 80% of the total antibiotic use in the United States. This measure has helped ensure stable food supplies and that animal meat is safe for human consumption. However, it has also created a reservoir for the spread of antibiotic resistance. It is now believed that many multidrug resistance pathogens originated in animal food supplies (12, 13).

Multi-drug resistant pathogens are an increasing concern because there are few antibiotics able to kill these pathogens, and soon there may be none. During the age of rapid antibiotic development, new drugs were flooding into the market that could kill pathogens resistant to older drugs. Nowadays, however, the pharmaceutical industry has all but ceased antibiotic research and development, leaving little hope for a new wave of drugs to treat current MDR pathogens (5, 14). Several factors contribute to the diminished incentive for pharmaceutical companies to continue antibiotic discovery. These issues include the fact that the costs associated with drug development are enormous. A 2016 study estimateed the cost to bring a new drug to market in the United States was between about 1.3-2.5 billion dollars(15). The high costs associated eith drug development has led many pharmaceutical companies to switch to developing drugs for chronic diseases with large patient populations, which promise a large return on investment. For example, Humira is a monoclonal antibody drug used to treat chronic inflammatory disorders such as rheumatoid arthritis and Crohn's disease. These chronic diseases represent large patient populations and have made Humira the highest selling drug by a wide margin for many years. Antibiotics, unlike Humira, are short term treatments and the potential patient population for new antibiotics is currently small(14). There are several proposed avenues for slowing the spread and reducing the risk of antibiotic resistance. These approaches include renewing antibiotic research and development in order to provide new treatment options for current multidrug resistant pathogens. Additionally, reevaluating current prescription practices and reducing or eliminating the broad use of antibiotics in agriculture could also help to slow slow the spread of drug resistant pathogens.

The antibiotic resistance crisis poses a significant threat to global health and the global economy. One study estimated the economic cost of a "worst-case scenario" for the AMR crisis. In this scenario, all bacteria eventually become MDR and antibiotics are no longer effective. The authors of this study found that in the US alone, healthcare cost tied to AMR would increase by 20 billion dollars annually. They also estimated that a loss of productivity would account for another 35 billion dollars. If the AMR crisis continues to worsen, it could cost the US 55 billion annually, which the authors stated was "an underestimate"(16). This results of this study emphasize the importance of solving the AMR crisis. Alternative treatments for microbial diseases may be required to address this crisis. Phage therapy, the use of viruses to treat bacterial infections, is one possible alternative. Phage therapy is being researched and tested with renewed interest because it is a promising solution to antibiotic resistance.

Phage Biology

Viruses are obligate intracellular parasites and cannot replicate independently of a host organism Viruses lack DNA and RNA polymerases and ribosomes which are necessary for replicating the viral genome and creating proteins. For this reason, viruses must infect a host cell and use its machinery to create to replicate. Bacteriophages, or simply phages, specifically infect bacteria. Phages replicate within a host bacteria cell through either the lytic or the lysogenic life cycle. In the lytic replication pathway, the phage attaches to receptors on the cell surface and gain entry to the cell. The phage will then release its genome and replicate using bacterial machinery for transcription, translation, and genome replication. Once new phages have been assembled, they escape the host via lysis. Phages can lyse a cell many ways (i.e., inhibiting production of peptidoglycan or through the use of lytic enzymes)(17). The cycle continues as the viral progeny are released to infect new bacterial hosts. Lysogenic phages also begin the replication cycle by attaching and then inserting its genome. However, once inserted, the viral genome integrates in the host genome. At a later time, the viral genome will remove from the host genome and enter the lytic cycle. The activation of the lytic cycle is caused by physiological or environmental stressors (Figure 3) (18).

Phage Therapy:

Viruses are highly diverse and can infect and kill all species of bacteria. Every species of bacteria is likely susceptible to one if not many species of phage. Phages' ability to kill bacteria suggest they may have clinical potential. The use of phage as a treatment for bacterial infection is known as phage therapy. Phage therapy has been employed by many countries in the past with some success but has been largely ignored by most of the Western world until recently.

Both lytic and lysogenic phages can be used for phage therapy. Lytic phages are preferred for phage therapy because they lyse bacteria more quickly and reliably. Lysogenic phages would be less effective because their lytic action would be difficult to predict. There are concerns that both lytic and lysogenic phages could have negative side effects. A highly lytic phage may lead to the release of bacterial endotoxins that could worsen the condition of a patient. Lysogenic bacteria

also pose a risk. They could carry virulence or antibacterial genes that may be conferred to bacteria when the viral genome integrates with the host(19).

There is immense potential in phage therapy due to the way phages infect and kill bacteria. First, they are highly abundant and highly specific (17, 20). Each phage strain usually infects one type of bacteria, although some phages can infect several strains (21). Phage specificity could have reduced side-effects compared to antibiotics. Antibiotics affect broad ranges of bacteria, which increases side effects for patients. For example, broad acting antibiotics can reduce bacterial diversity in the gut microbiome and contribute to diseases such as Crohn's disease (22). Furthermore, these antibiotics increase the development of antibiotic resistance by targeting many species of bacteria. Because phages only target bacteria within a narrow host range, phage therapy would contribute less to the development of resistance than antibiotics. Phages acts on fewer species of bacteria; therefore, fewer bacterial species have the ability to develop resistance. Horizontal gene transfer also contributes to the spread of resistance among bacteria. Antibiotics act on many species of bacteria so many species are incentivized to acquire antibiotic resistance genes through horizontal gene transfer. Phage therapy produces less selective pressure for bacteria to acquire these genes through horizontal gene transfer. For example, *S. aureus* would not be incentivized to acquire resistance for a phage that only infects *E. coli,* thus reducing the spread of resistance among different species of bacteria and shrinking the potential reservoir for AMR. The kinetic properties of phage therapy are also an appealing quality. Phages are self-amplifying. A few phages may infect a cell, but after replication hundreds to thousands of new phages will be released. Due to phage's self-amplifying nature, the amount of phage can grow over time rather than decrease over time as with antibiotics. Hypothetically, smaller and less frequent doses of phage would be sufficient to clear an infection (17). These qualities make phage therapy an appealing alterative to antibiotics in the midst of the antibiotic resistant crisis.

History of Phage Therapy:

Phages were discovered by English bacteriologist Frederick Twort in 1915 (23). French-Canadian Félix D'Herelle was the first to describe plaque assays in 1917 (24). During D'Herrelle's investigation, he conducted plaque assays on stool samples from dysentery patients. He noted the phage titer was low at the beginning of the illness and highest following recovery. He concluded that the development of phage specific for pathogenic bacteria was responsible for patient recovery. This conclusion, though not entirely accurate, sparked a period of enthusiastic research into phage and their clinical potential (25, 26). D'Herelle continued investigating phage and conducted clinical studies in both humans and animals. He was known to determine the safety of his phage treatments by first administering phage to himself and then family members and coworkers. During the 1920's, D'Herelle conducted several studies and reported positive results. In one such study, D'Herelle treated four patients with the bubonic plague by injecting phage into their buboes. The patients then displayed an impressive recovery from the plague. They conducted another study on cholera patients. The treatment group of 12 patients was given oral phage and only one patient died. The control group was given no phage and 12 of the 18 patients died (26, 27). In its early days, phage therapy was displaying success. Unfortunately, the enthusiastic beginnings of phage therapy did not last.

This period was believed by many at the time to be overly optimistic. Some scientists began to point out flaws in phage clinical studies and the general lack of knowledge regarding phage biology. In 1934, American doctors Stanhope Baynes-Jones and Monroe Eaton released a highly critical review of phage therapy clinical studies. Eaton and Baynes pointed out the absence of controls in the studies being conducted. At the time, there was no consistency in the way phage was being prepared, dosed, or administered. There was also no established method for analyzing results. Many of the human studies were conducted on self-limiting and non-fatal diseases making it impossible to distinguish with certainty the role phage played in those patients' recovery. Eaton and Baynes suggested that phages must first be proven effective *in vitro* and then in animal models before human studies occur. In the end, Eaton and Baynes believed the phage therapy movement was disorganized and lacked the ability to explain or prove the actions of phage *in vivo* (28).

The Eaton-Baynes report tempered much of the enthusiasm surrounding phage therapy in the United States. Following its publication, the general view of phage therapy became more negative. The West mostly turned its attention to antibiotics, which were effective and mass produced easily following World War II. Countries in the East like Georgia and the Soviet Union continued to use phage therapy. In Georgia, phage cocktails were developed to treat various ailments. These cocktails contained many different phages that could target a variety of pathogenic bacteria. Some of these cocktails were available to the public without prescription. One such cocktail, "Pyophage," was applied to wounds and also came in the form of a bandage that would slowly release phage over time. In the Soviet Union, phage therapy was focused on treating ailments of war like diarrheal and gangrene infections. Though the Eastern world had long, successful experiences with phage therapy, much of their practices were kept secret prior to the collapse of the Soviet Union. It wasn't until the 1990's, when scientists were gaining access to the Russian and Eastern European literature that interest in phage therapy began to gain momentum. At the time, the growing threat of antibiotic resistance was also driving the search for alternative treatments (25).

While Eastern countries were embracing phage therapy, the Western world had all but ceased clinical studies in the field. Some scientist continued to examine phage therapy in animal models, and only a handful of human studies occurred. During the 1960's the World Health Organization sponsored a clinical trial in Pakistan. In the study, acutely ill cholera patients were given high doses of anti-cholera phage. The patients given phage treatments did not recover any faster than patients given the typical antibiotic treatment. A subsequent study was conducted with a larger patient population and more controls. The results were similar to the primary study (29). The phage treatment group showed no improvement over the normal treatment group. (26).

Current Studies in Phage Therapy:

Research in the field of phage therapy is now actually booming as the search for antibiotic alternatives continues. Many safety studies have been conducted in recent years to evaluate the tolerability of phage therapy. These studies have evaluated various phages and subjects with various ailments. A study in Australia examined the safety of a phage treatment in 13 subjects suffering from serious *Staphylococcus aureus* infections. They were treated with myoviridae phage intravenously twice daily for 14 days. The subjects were monitored for fever, tachycardia, hypotension, hepatic dysfunction, and other systemic effects. The subjects exhibited no adverse effects. The study concluded that myoviridae phage administered intravenously for patients with *Staphylococcus aureus* infections, endocarditis, and septic shock is safe. The study, however, made no assessment of the phage treatment's efficacy (30). Another safety study evaluated the tolerability of phage cocktails in subjects with recurring sinus infections caused by *Staphylococcus aureus* that were unsuccessfully treated by other means. This Phase I trial examined 9 subjects in 3 cohorts receiving ascending phage doses. The phage was administered by intranasal irrigation twice daily for 14 days. The patients exhibited no fever and no significant changes in vital signs. Only minor side effects associated with the intranasal irrigation were reported. The study concluded that phage administered by intranasal irrigation was safe and showed preliminary signs of efficacy (Figure 4). 2 of the 9 patients showed evidence of *Staphylococcus Aureus* eradication. (31).

Scientist are also searching for new phages that may be effective therapeutics. These studies have been conducted both *in vitro* and in animal models. An exploratory study out of India published in 2019 characterized the efficacy of various phages against *E. coli.* Samples of blood, stool, urine, and sputum were collected from various medical facilities, and phages with lytic activity against *E. coli* were isolated. Over 50 phages were isolated and characterized by host range and other metrics such as adsorption rate. One of the phages, myPSH1131, was then selected to conduct further *in-vivo* testing with *G. mallonella* larvae, which are often used to model bacterial infections. myPSH1131 was found to be effective against five strains of *E. coli* both *in vitro* and *in vivo*. The study suggests that myPSH1131 could potentially go on to safety and clinical trials (21). Another study published in 2019 tested phages against antibiotic resistant strains of *E. coli, K. pneumonia*, and *E. aerogenes.* Phages were isolated from the Ganges river in India. Three of the isolated phages were identified for further testing. These were *E. coli* virus myPSH 2311, *Enterobacter* virus myPHS1140, and *Klebsiella* virus myPSH 1235. The phages were tested *in vitro* to determine latency period, adsorption rate, and burst size. The three phages were combined as a phage cocktail and tested in vitro against the antibiotic resistant bacteria. Bacterial growth declined after 2 hours until after 24 hours there were no viable cells. The cocktail was effective *in vitro* against the resistant strains of bacteria (21). These exploratory studies will help establish phage banks for future development of phage therapies.

One specific area of interest in current phage therapy research is modulation of the gut microbiome. The gut microbiome is a crucial part of the overall health of the host. It helps protect against pathogenic infections, modulates the immune system, and influences homeostasis of the gut itself (32, 33). Dysbiosis of the gut microbiome has also been implicated in several diseases including obesity and Crohn's disease (34, 35). Phages naturally play an important role in modulating the gut microbiome. Patients with Crohn's disease were found to have lower

phage diversity in their microbiomes than healthy persons (36). This suggests that phages could be used to treat diseases associated with gut microbiome dysbiosis.

There have been several clinical studies investigating the efficacy of phage therapy as a gut microbiome modulator. A safety study conducted in 2018 aimed to determine the tolerability of a phage cocktail targeting *E. coli* in 32 healthy adults with mild to moderate gastrointestinal distress. The subjects in this randomized, double-blinded, placebo-controlled study consumed a capsule containing a cocktail of four phages daily for 28 days. The subjects underwent comprehensive metabolic panels and answered health questionnaires over the course of the study. The mean values of the patient's metabolic levels remained within acceptable values and no adverse health effects were observed. The study concluded that the phage cocktail was safe and tolerable for the human population (37).

Results from a clinical trial using the same phage cocktail was published in 2019 and examined how phage treatment affected the overall gut microbiome. The study was conducted on 36 subjects with self-reported gastrointestinal issues. It was found that the phage treatment had little effect on the overall composition of the microbiome but that it actually reduced inflammation. This study was randomized, double-blinded, and placebo controlled. The subjects took daily capsules of the phage cocktail over the course of two 28-day treatment periods. Stool samples were collected and DNA was extracted to measure changes in the gut microbiome composition. Plasma was also drawn to measure levels of inflammatory signaling. ELISA tests were run on 13 cytokines and chemokines. The study found that the treatment subjects exhibited a 71% decrease in *E. coli* population compared to pretreatment levels (Figure 5). The control subjects exhibited a 47% reduction of *E. coli*. The overall microbiome composition was largely unchanged due to the phage treatment. Only a few species of bacteria showed population changes correlated with reduction of *E. coli* in the microbiome. This is advantageous because antibiotic treatment often leads to significant decreases in the gut microbiome diversity and produces dysbiosis (22). The treatment group also showed a mean 10% decrease in IL-4 signaling from t=0 to t=28 while the placebo group showed a 2% decrease (Table 1). The authors suggest this decreased inflammatory signaling could be due to lower levels of circulating LPS resulting from the phage killing of *E. coli* (38). These studies have produced largely positive results. None of the studies report and significant adverse effects. These results bode well for the future of phage therapy.

Challenges to Phage Therapy:

While phage therapy has shown some success in recent studies, there are several considerations that must be addressed before it can advance as a treatment. One challenge is standardization of methods. In the past, dosing practices have been inconsistent across the field. The inconsistency is in part because phages present challenges to the conventional dosing practices used for antibiotics. Antibiotic dosing strategies are more simplified due to single-hit bacterial killing kinetics. Phage therapy dosing is more complex due to the nature of phages. As stated previously, phages are self-amplifying which is not the case with pharmaceutical drugs. Different phages kill bacteria at different speeds. Critical densities of phage must be achieved at the infections site. Phage efficacy is also influenced by the density of the bacterial population. Due to these factors, determining phage therapy dosing is a difficult task (20, 39, 40).

Another complicating factor are interactions between the immune system and the phage. These interactions must be well understood when evaluating phage therapy efficacy and safety. Drugs can often enhance the immune response in a positive way, but they can also have adverse effects. Phages have been shown to enhance the innate immune response by controlling bacterial density and displaying adjuvant activity. Phage therapy has been associated with increased cytokine signaling leading to a more effective immune response. Phage therapy has also been shown to be dependent on the immune system to fully clear a bacterial infection (41). A 2017 study using mouse models verified the importance of phage-immune system interactions. Mice with pneumonia caused by *P. aeruginosa* were treated with phage. Mice with fully functioning immune systems were able to clear the infections while neutrophil and lymphocyte deficient mice were unable to clear the infection. This suggests that phage therapy is dependent on the immune system to completely eliminate a bacterial infection (42). In other cases, the immune system interactions present differently. Other studies have shown little influence on cytokine signaling (43). These varying degrees of immune system interactions suggest that the relationship between phages and the immune system may be case specific. These interactions must be well understood before the therapies can be considered safe, predictable, and effective.

Another challenge to the success of phage therapy are bacteria's ability to acquire phage resistance. Bacteria have many antiviral mechanisms like clustered regularly insterspaced short palindromic repeats(CRISPR). CRISPR is a gene editing system in bacteria that creates a memory of past viral infections by incorporating portions of the viral genome into the bacteria genome. Bacteria also utilize constantly evolving surface receptors that prevent viral attachment and restriction-modification systems that can destroy foreign DNA. Phage can also evolve to overcome these antiviral mechanisms (44). The coevolution between bacteria and phage is an important consideration for phage therapy efficacy. Studies have found that the speed of resistance is highly variable. In one human trial, bacteria developed phage resistance in anywhere from 17% (*S. aureus*) to 85% (*E. coli*) of treatments (45). Often the phage will have reduced the bacterial population enough that the immune system can clean up any phage resistant bacteria. The concern is the potential selection for bacteria containing these antiviral genes. Subsequent horizontal gene transfer could potentially spread these genes within the bacterial population (45). The spread of phage resistance could be reduced by the use of phage cocktails. These cocktails are made up of several phages that in combination are broad acting and target several bacterial mechanisms. Phage cocktails reduce the chance that resistant bacteria survive (46). Reducing the threat of phage resistant bacteria will be an important consideration for the field of phage therapy moving forward.

Conclusion:

Phage therapy has had a long and winding history. The past decade has seen phage therapy return to scientific prominence as the search for antibiotic alternatives continues. The field has gained significant momentum and the human trials have shown largely positive results. These encouraging results will spur continued research and development. Phage therapy could potentially minimize the threat of multi-drug resistant pathogens, and it may not be long before phage therapy is a common treatment for a variety of bacterial infections and diseases.

Figure 1 Timeline of antibiotic discovery

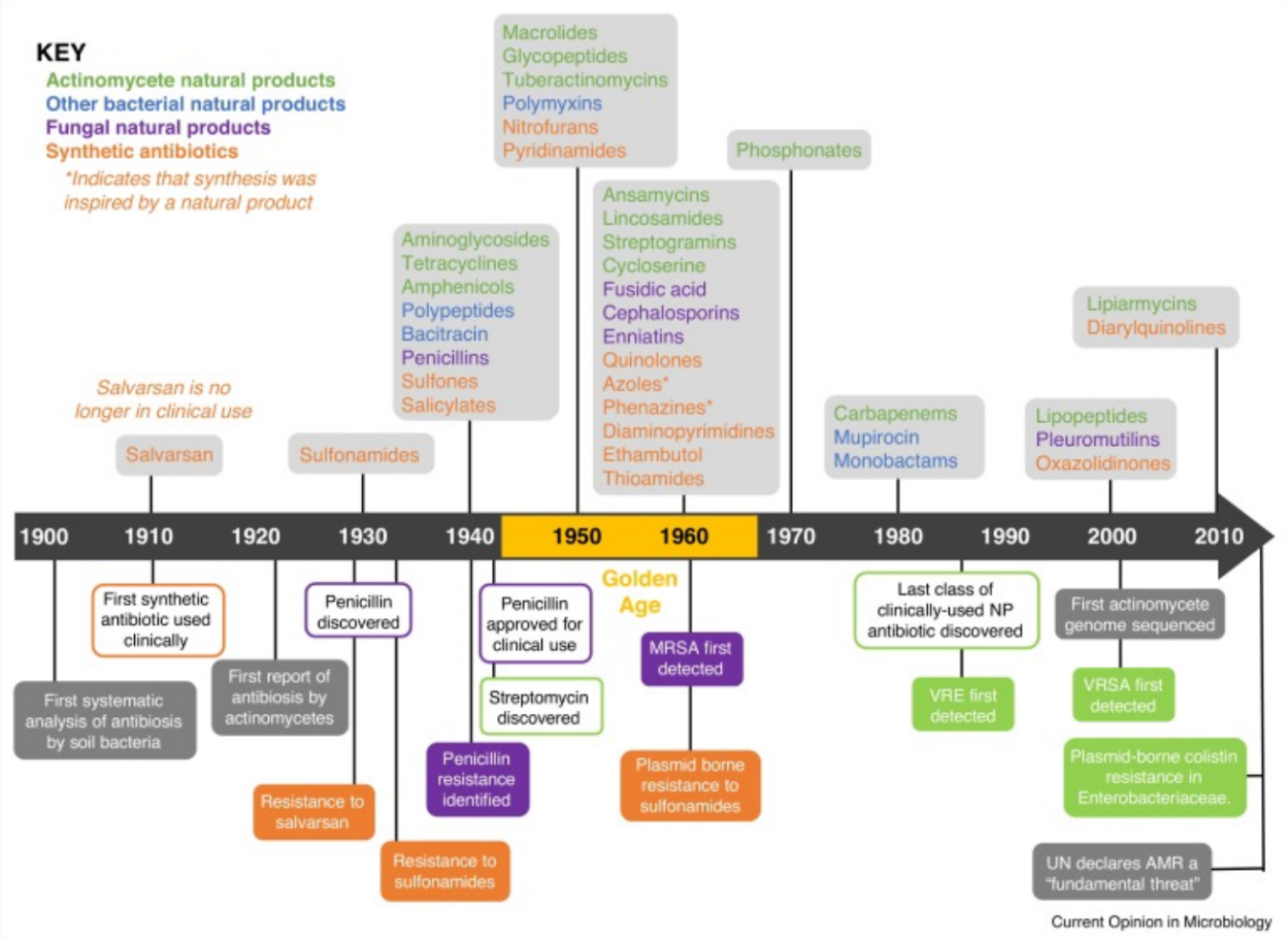

Figure 2: Culture based techniques for screening antibiotic producing microbes

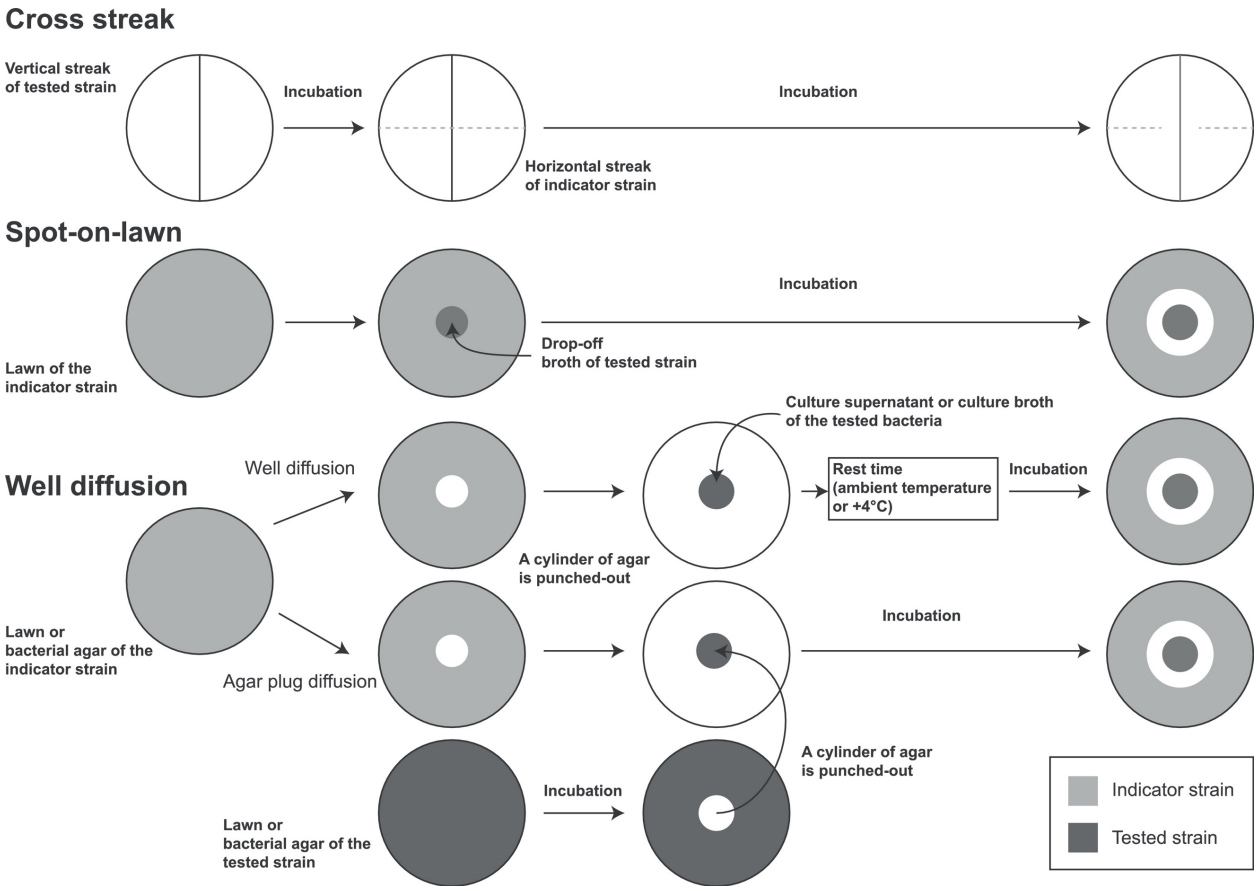

Figure 3: Phage Lifecycles-Lytic and Lysogenic

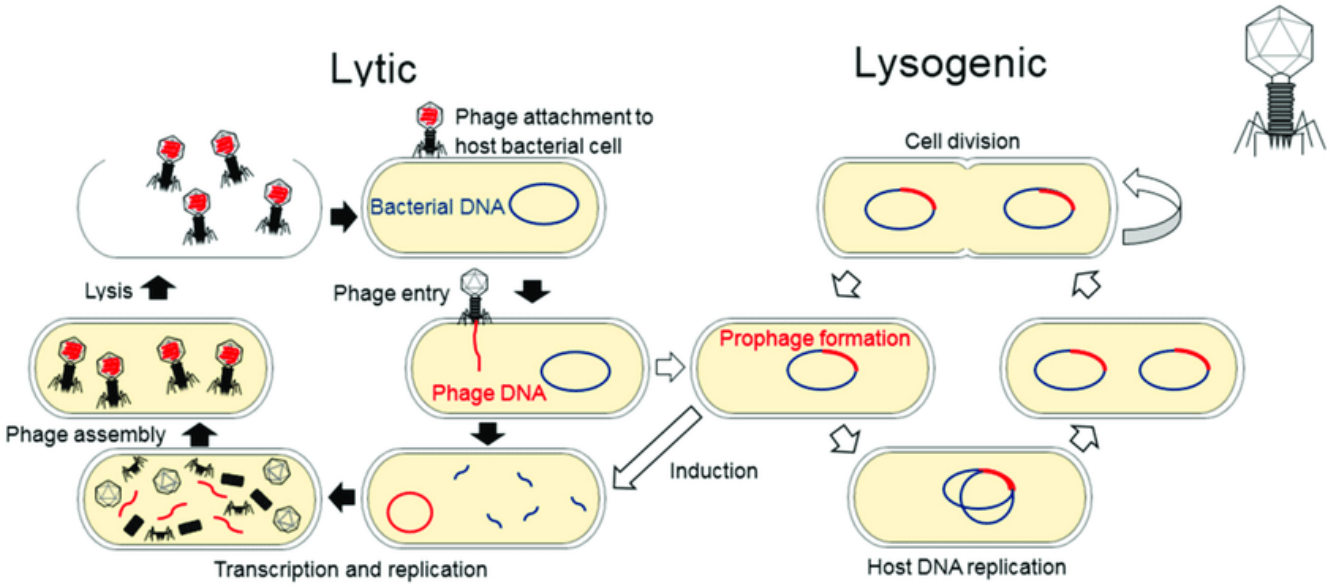

Figure 4: For the Lund-Kennedy Score (LKS), scores range from 0-20, with higher scores indicating worse endoscopic disease; Sino-Nasal Outcome Test-22 (SNOT-22), scores range from 0-110, with higher scores indicating worse symptoms; and visual analog scale (VAS), scores range from 0-100, with higher scores indicating worse symptoms.

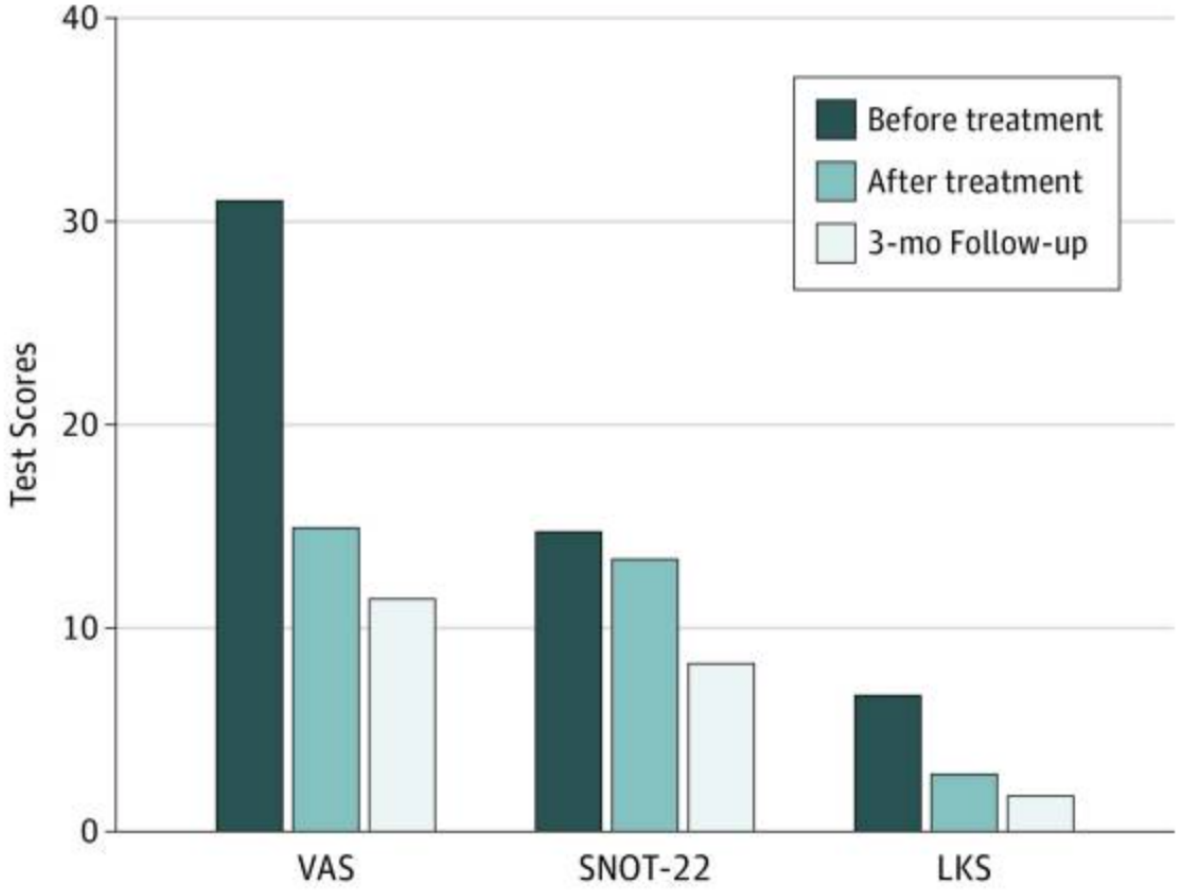

Figure 5: Change in *E. coli* levels from baseline values after treatment or placebo consumption. Values based on percent of total reads represented by amplicon sequence variants (ASVs)

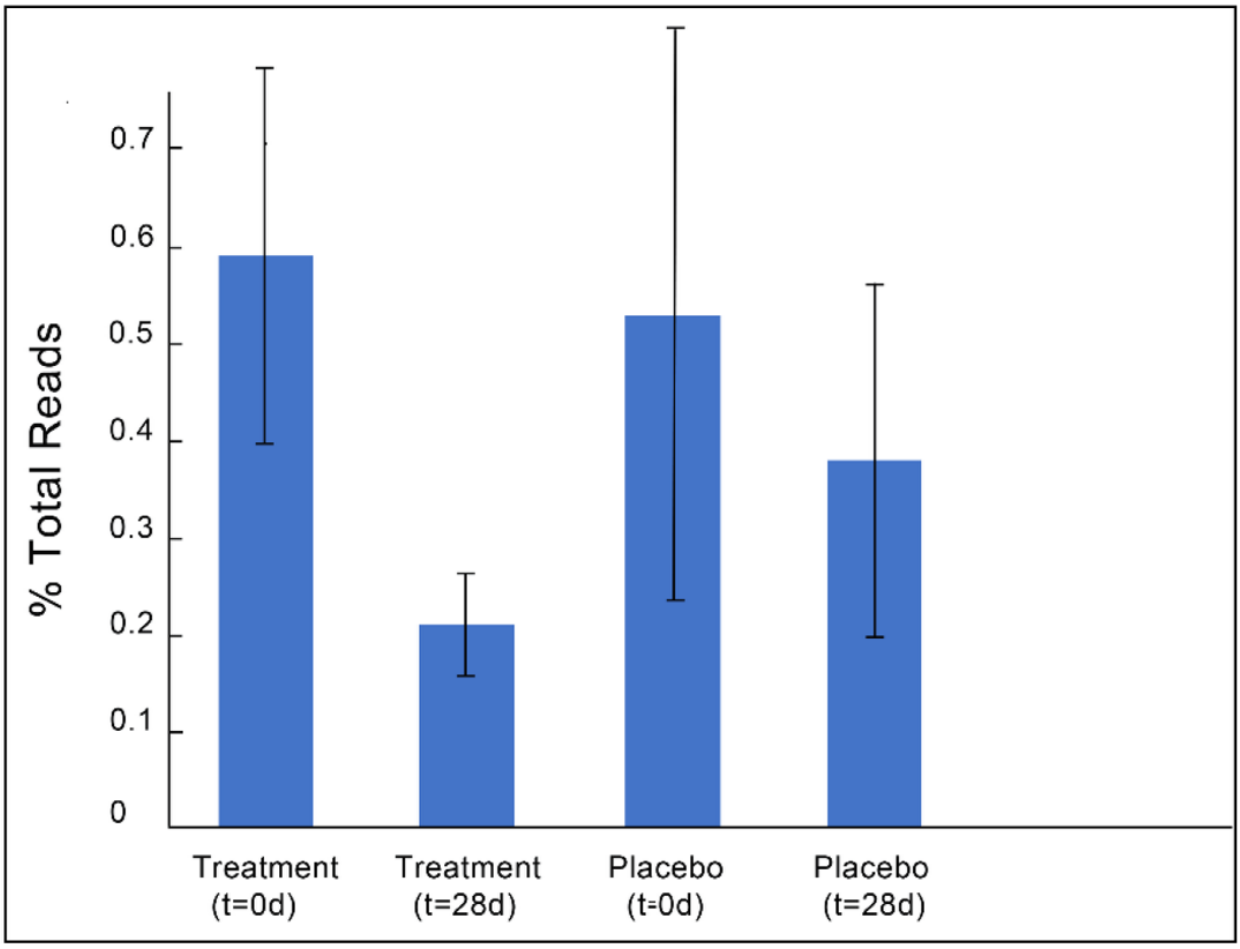

Table 1: Reduced IL-4 Inflammatory Signaling Following Treatment with Phage Cocktail

Table 2. Plasma C-reactive protein (CRP) and cytokines.

	Treatment (*t* = 0)	Treatment (*t* = 28)	Placebo (*t* = 0)	Placebo (*t* = 28)
hsCRP (mg/mL)	1.76 (±0.51)	1.79 (±0.52)	1.56 (±0.41)	2.45 (±0.68)
GMCSF (pg/mL)	80.69 (±9.99)	80.54 (±10.35)	83.17 (±9.78)	80.59 (±10.10)
IFN-γ (pg/mL)	12.67 (±1.12)	12.29 (±1.02)	14.21 (±1.86)	13.81 (±1.89)
Il-10 (pg/mL)	24.13 (±3.30)	23.53 (±2.85)	26.03 (±3.75)	24.83 (±3.84)
Il-12 (pg/mL)	3.57 (±0.33)	3.57 (±0.32)	3.75 (±0.35)	3.55 (±0.37)
Il-13 (pg/mL)	23.05 (±5.53)	22.16 (±5.71)	25.39 (±6.06)	24.5 (±5.73)
Il-1β (pg/mL)	1.76 (±0.13)	1.71 (±0.10)	1.85 (±0.13)	1.73 (±0.12)
Il-2 (pg/mL)	2.24 (±0.14)	2.15 (±0.18)	2.46 (±0.28)	2.36 (±0.31)
Il-4 (pg/mL)	69.48 (±5.75) [a]	59.83 (±4.43) [b]	63.79 (±4.95) [a,b]	61.71 (±3.88) [a,b]
Il-5 (pg/mL)	8.16 (±3.26)	5.55 (±1.35)	7.85 (±2.71)	6.38 (±1.57)
Il-6 (pg/mL)	3.37 (±0.33)	3.43 (±0.33)	3.76 (±0.38)	3.82 (±0.41)
Il-7 (pg/mL)	13.37 (±1.07)	13.39 (±1.14)	13.91 (±1.16)	13.35 (±1.12)
Il-8 (pg/mL)	4.14 (±0.70)	4.24 (±0.77)	4.51 (±0.83)	4.45 (±0.81)
TNFα (pg/mL)	4.45 (±0.29)	4.18 (±0.25)	4.23 (±0.24)	4.09 (±0.26)

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