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# Microscopic Saviors: The Use of Phages in Medicine

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## ABSTRACT

The influenza virus has plagued humans for centuries. Recently antiviral medications, which shorten the duration of the flu, have been introduced into society. These medications along with vaccinations, which try to give the body immunity before the virus strikes, help to stop the flu before it attacks the host. The virus, however, replicates using host cells and can slightly change itself with each replication, which over time could lead to a strain immune to the current antiviral medication and vaccines. However, using more preventative measures could help slow the changing strains of the flu virus. Using vaccines to stop the virus at the host before it mutates and using antiviral medication before the flu starts replicating inside patients are both examples of preventative measures. In the future, research must be focused on creating vaccines to limit the need for antiviral medication to slow the evolution process of the influenza virus overall.

**KEYWORDS:** phage, phage therapy, antibiotics, drug resistant bacteria, isolation method

## INTRODUCTION

In an age where medicine has evolved so quickly, the diseases that plague us have been forced to adapt, growing stronger as we took our advancements for granted. This has led disease-causing bacteria to adapt to modern medicine, creating superbugs that are resistant to most antibiotics. It is estimated that if left unchecked, superbugs could cause around 10 million deaths per year by 2050.<sup>1</sup> However, while the bacteria have evolved to fight off medicine, they have been chased by another group of microscopic killers. Bacteriophages, better known as phages, are microscopic viruses that target and attack bacterial cells. Phages are found everywhere that bacteria live and are highly specific, meaning a species of phage tends to attack only one specific bacterial strain or species.<sup>2</sup>

Due to the unprecedented growth of drug resistant bacteria, humans have turned to other methods in hopes of fighting these superbugs, and an option being investigated is phage therapy. Phage therapy is a medical process in which bacteriophages are administered to a patient in order to attack bacteria and rid the body of infection.<sup>3</sup> Since it is a relatively new field, there has not been enough research done to introduce the concept on a wide scale. However, enough work has been done to provide detailed isolation and selection methods for specific phages as well as demonstrate high success rates in trials run on drug resistant bacteria. This research could lead to phage therapy becoming a viable treatment alternative in medicine.

## **ISOLATION AND SELECTION METHODS**

Phages act differently than antibiotics because while antibiotics tend to be broad and can attack both harmful and helpful bacteria, phages specifically target a certain bacterial species or strain, which makes them highly specific. This specificity is one of the main driving factors as to why phage therapy is successful. Before administration of the phage therapy to patients, however, the necessary phages must be gathered and tested to guarantee that the correct phages were retrieved to fight a certain strain of bacteria. As such, those involved in the development of treatment search areas where phages are believed to reside. Since phages lack a uniform distribution, they are often found near hospitals, sewage, and natural sources like cow and sheep feces or river and lake water due to the large quantity of bacteria living in those areas as well.<sup>2, 4-7</sup> After collection, the samples go through sterilization by filtering and incubating, as is demonstrated using phage 1513, one example out of millions of phages.<sup>2, 4-5, 7-8</sup> Researchers centrifuge the sample containing phage 1513 to remove unnecessary particles, add bacteria to the remaining liquid, and incubate the mixture.<sup>4</sup> Following this, one of five procedures are done to confirm phage presence: the plate (double layer) method, enrichment method, spot method, electron microscopy method, or the colorimetric method; however the most commonly used procedure is the plate method.<sup>2, 5-8</sup> The plate method is done to test which phage is present in the sample by placing the liquid sample on a plate of agar containing bacteria, preferably an infectious strain, and allowing plaques to form, thus showing which phages attacked the bacteria.<sup>2, 4, 9</sup> As an added measure if the plate method fails, the colorimetric method is based on the tendency of bacteria to reduce a molecule called tetrazolium to formazan, which produces a dark red color as the process is carried out.<sup>2</sup> The colorimetric test functions by having the phages destroy the bacteria, preventing the reduction and demonstrating a lack of color, showing what phages are present and active depending on which bacteria was killed and stopped producing color.<sup>2</sup>

Nonetheless, any of the other tests can be done to confirm the results of the plate method.<sup>6</sup> The isolation process is shown in Figure 1.

Once isolation of the specific phage has been completed through one of the five previously mentioned tests, the selection method can take place. This process allows for the genome, as well as other characteristics, of the phage to be determined, functioning as an identification of the isolated phage. An example of this is shown with phage 1513, where growth of the phage during isolation testing shows that the phage grows and works best at a neutral pH and low temperatures up to normal body temperature, displaying some characteristics found during selection.<sup>4</sup> An important factor to determine selection of a phage is whether a phage is lytic, meaning that the phage must be able to break open the infected bacterial cell to kill it.<sup>2, 10</sup> Therefore, the genome of such phages must be sequenced if this characteristic, and many other preferred ones are found to have a function in phage therapy.<sup>2</sup> Researchers must also be certain the newly sequenced phage is different or unique enough from previously catalogued phages to avoid having lacking effects on chosen bacteria, as similarities between the phages may negatively impact treatment.<sup>9</sup> Some other possible characteristics that researchers search for include affinity to certain bacteria, whether a phage can cause the bacterial cells to activate uncontrolled apoptosis or cell death when certain conditions are met to make the bacteria kill itself, or how well a phage can lyse bacterial cell walls.<sup>2, 8-11</sup> During this stage of selection, the genome of the phage can also be modified to better fit the objectives of the researchers.<sup>12</sup> For example, the genome of an isolated phage can be modified to manipulate the genome of the target bacteria by inserting genes that make the cells more vulnerable to antibiotics.<sup>12</sup> However, this process would serve better as a preemptive measure to make the bacteria more susceptible to antibiotics prior to infecting a human.<sup>12</sup> Even so, it is possible that it could serve as a way to allow better synergy between phage cocktails and antibiotics during treatment.

## PHAGE THERAPY CASE STUDIES

### *Animal-Based Case Studies*

Once the phages are isolated and selected, they are stored until needed to treat specific infections. While the treatment is still experimental, tests have been run on both animals such as mice, rats, and moth larvae, as well as trials on humans with advanced infections and no other remedies with high success rates and promising results.<sup>4, 11, 13-15</sup> Tests run on mice infected with lethal multidrug resistant *K. pneumoniae* were given single phage doses of phage 1513, which was selected to treat *K. pneumoniae*, intranasally.<sup>4</sup> Following

phage therapy, the survival rate went from 0% all the way up to 80% depending on the dose given.<sup>4</sup> The therapy was also shown to improve tissue damage and reduce the effects of infection such as weight loss.<sup>4</sup> An upgrade from single phage doses was researched through tests on rats with *P. aeruginosa*, which introduced the use of phage cocktails.<sup>14</sup> The cocktails used various phages to widen the range of bacteria that could be targeted, as well as introduced the possibility of synergizing phage therapy with antibiotic treatment to counteract the mutations of bacteria developing phage resistance.<sup>1, 14</sup> A cocktail of 12 phages were isolated to fight 33 strains of *P. aeruginosa*, killing off all but 2 phage resistant strains.<sup>14</sup> However, the phage resistant strains were far less infectious than the other strains, and they were treated by the antibiotics that were administered alongside the phage cocktail.<sup>14</sup>

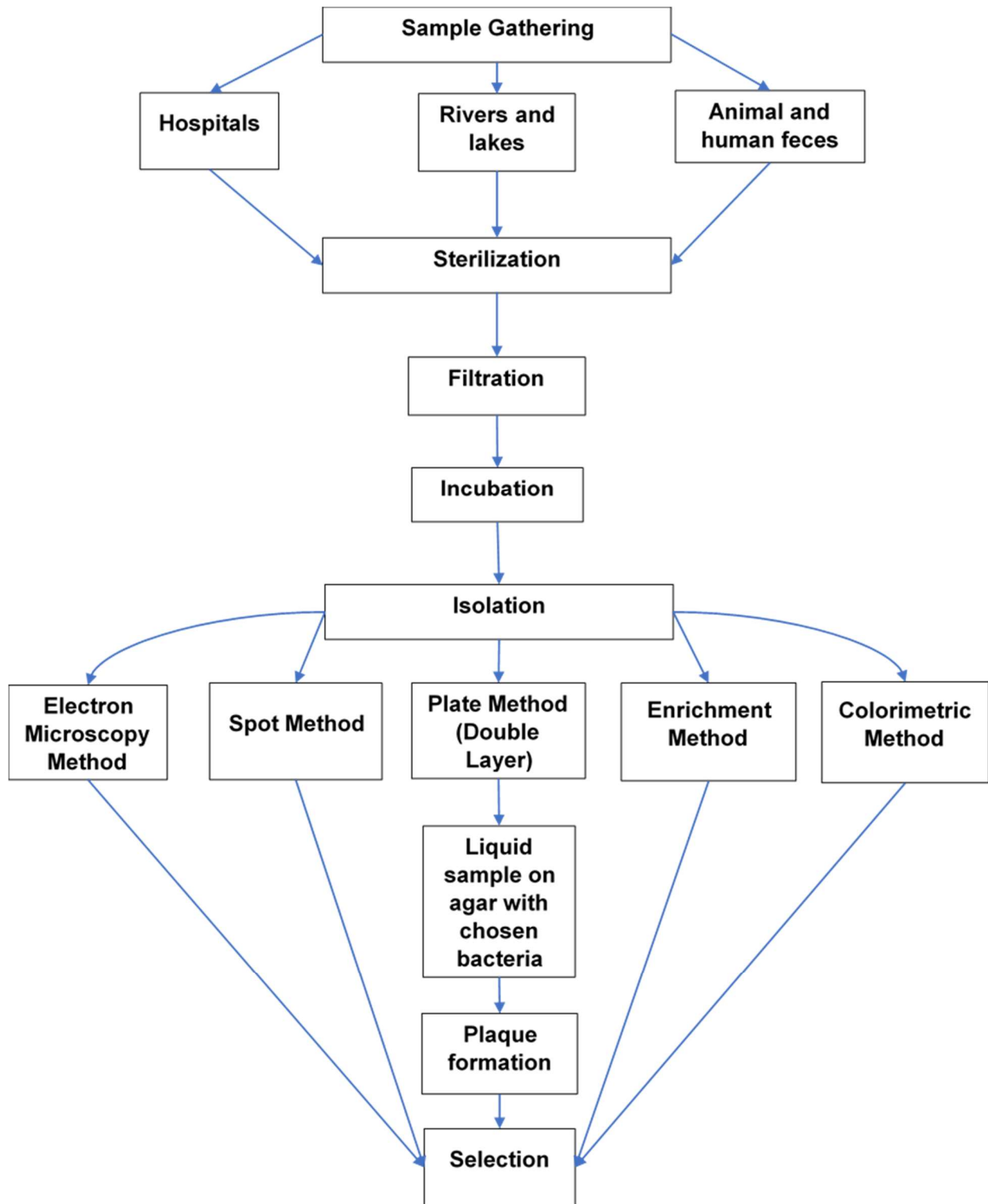
### *Human-Based Case Studies*

Due to a lack of overall awareness and FDA approval, phage therapy is currently seen as an experimental last resort in medicine. As such, human trials are usually done on patients that have severe late stage infections where no other form of viable treatment has helped improve their condition. In the selected human trials, both patients had contracted serious drug resistant infections, as one had developed necrotic sores and septicaemia from *P. aeruginosa* and the other had a worsening pancreatitis condition caused by *A. baumannii* that led to a coma.<sup>13, 15</sup> Both patients were administered phage cocktails specifically made to treat their infection, which improved their conditions drastically through repeated administration of treatment.<sup>13, 15</sup> The patient with the *P. aeruginosa* infection had an immediate change in his blood cultures, as they returned negative results when tested for presence of bacteria, kidney function had returned, and the heavy fever had dissipated.<sup>13</sup> The patient with the *A. baumannii* infection was able to successfully wake up from his coma and his condition gradually improved over the course of treatment administration.<sup>15</sup> A separate in vitro trial took samples from diabetic patients that had developed septic wounds and analyzed the pus from the wounds for signs of bacterial infections, finding drug resistant strains of *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, and *E. coli*.<sup>6</sup> The necessary phages were isolated and applied to the infected samples, which were then observed to lower bacterial population density between the first 4 to 14 hours of incubation.<sup>6</sup> However, after the 14 hours, researchers noticed that the bacterial populations began to increase once more, which they attributed to either growth of phage resistant bacteria or the inactivation of the phages.<sup>6</sup> Although phage therapy is a viable alternative that has high success rates in both human and animal trials, there is still a risk of the targeted bacteria mutating to develop

resistance to the phages that attack them as demonstrated previously. Various tests on both animals and humans have shown that phage therapy can lead to mutant bacteria developing resistance to the phages, which can be fatal if left untreated.<sup>1</sup> Even so, the development of phage resistance in bacteria does not come without drawbacks. The development of phage resistance causes bacteria to lose virulence and resistance to antibiotics, which can greatly benefit patients as the bacteria cannot infect as well as they could previously.<sup>1</sup> This development of phage inactivation and phage resistance, as well as the costs of developing phage resistance, should lead to more tests that examine the administration of antibiotics alongside the phage therapy to maximize potential treatment in the case of a repeat scenario like what happened in this study.

## **CONCLUSION**

Phage therapy, while still experimental and lacking some research, has proven to be extremely effective when treating bacterial infections that were resistant to antibiotics.<sup>2-4, 9, 11, 13-15</sup> In vitro and in vivo trials have shown excellent results, especially when combined to make phage cocktails rather than single phage doses.<sup>2-3, 11, 13, 15</sup> Treatment can be further improved when combining phage cocktails with antibiotics to target any remaining bacteria that mutated phage resistance.<sup>14</sup> Phage therapy, if given the funding and attention needed for more research into collection, testing, and effects on humans and safety, could easily become a perfect alternative for treating bacterial infections that have become resistant to multiple types of antibiotics.



**Figure 1: General process of gathering and isolating phages before selection for phage therapy.** Phages are gathered in samples from sources containing bacteria such as natural water sources, sewage, or near hospitals.<sup>2, 4-7</sup> The samples are then filtered and

incubated to remove unwanted particles and bacteria, then the phages are isolated using one or more of the listed methods before selection.<sup>2-11, 13-15</sup> The listed tests allow for researchers to isolate phages that target their selected superbug or bacterial strain by testing which phages attack the bacteria, such as forming plaques during the plate method.<sup>2-11, 13-15</sup>

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