# Phages implications on Controlling antibiotic resistance and future biotechnology in Plants and Animals disease: a review

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## SUMMARY

Phages are bacterio-specific viruses. Involved in the origin of life and evolution, constituting a major part of the biosphere, they are promising as a sustainable, ecological and intrinsically cheap antibacterial. They have been proposed as alternatives to antibiotics for many antibiotic resistant bacterial strains. Phages can be used as biocontrol agents in agriculture and petroleum industry. Moreover phages are used as vehicles for vaccines both DNA and protein, for the detection of pathogenic bacterial strain, as display system for many proteins and antibodies. Bacteriophages are diverse group of viruses which are easily manipulated and therefore they have potential uses in biotechnology, research, and therapeutics. The aim of this review article is to enable the wide range of researchers, scientists, and biotechnologist who are putting phages into practice, to accelerate the progress and development in the field of biotechnology. Details given above give a glimpse of the large range of applications of phages in the field of biotechnology and medical science. There is the hope that phages could be useful to humans in many ways. By making a cock tail of phages it would become easy to treat a wide variety of bacterial infections that are otherwise resistant to the latest generations of antibiotics. Due to the rapid progress in the fields of biotechnology and molecular biology it is hoped that these entities (phages) which are present abundantly in the biosphere could answer many questions human beings are having.

**Keywords:** Phage therapy, Antibiotics resistance, Vaccine, Biocontrol, Biotechnology

## INTRODUCTION

Bacteriophages are the most abundant entities on earth. These bacterial viruses have genetic material in the form of either DNA or RNA, encapsulated by a protein coat. The capsid is attached to a tail which has fibers, used for attachments to receptors on bacterial cell surface. Most of the phages have polyhedral capsid except filamentous phages. Phages infect bacteria and can propagate in two possible ways; lytic life cycle and lysogenic life cycle. When phages multiply vegetatively they kill their hosts and the life cycle is referred to as lytic life cycle. On the other hand some phages known as temperate phages can grow vegetatively and can integrate their genome into host chromosome replicating with the host for many generations. If induction to some harsh conditions like ultraviolet (UV) radiations occurs then the prophage will escape via lysis of bacteria. After the discovery of bacteriophages in early 20$^{th}$ century many researchers thought about their (phages) potential of killing bacteria, which could undoubtedly make them possible therapeutic agents. But after World War II when antibiotics were discovered, this natural potential therapeutic agent got little attention and was only considered as a research tool for many years *(*Haq *et al.,* 2012).

Bacteriophages have contributed a lot to the field of molecular biology and biotechnology and are

still playing its part. Many mysteries of molecular biology are solved by bacteriophages. Today when everything is much more advanced than ever before, bacteriophages are getting enormous amount of attention due to their potential to be used as antibacterial, phage display systems, and vehicles for vaccines delivery. They have also been used for diagnostic purposes (phage typing) as well.

Bacteriophages are accepted as natural antimicrobial agents to fight bacterial infections in humans, animals or crops of agricultural importance. Moreover, bacteriophages are easily accessed, cheap and safe for humans. On the other hand, resistance mechanisms to antibiotics and bacteriophages are different. Bacteria have different complex mechanisms against antibiotics, which are difficult to overcome; yet resistance to bacteriophages has not been commonly reported, and in case there is resistance, it will be via different mechanisms. This means that antibiotic resistant bacteria remain sensitive to bacteriophages (Rahmani *et al.,* 2015)

The Phage Therapy Center in Georgia reports that there are fewer side effects with their treatment than with antibiotics, since phages are more specific and do not disrupt the natural bacteria in the body. As with antibiotics though, there is still the possibility that phages could kill too many bacteria too fast, causing the bacteria to release dangerous levels of endotoxins which then make the patient sick. One of the main problems with phage therapy, however, is the cost. While it is easier to develop a new phage than it is a new antibiotic, treatment at the Phage Therapy Center in Georgia ranges from $2,500 US dollars for outpatient care to $20,000 for inpatient treatment, in addition to travel costs. In addition, if the bacterium that the patient has is resistant to the phages the center has, there is an extra charge to develop a specific, unique phage for that person. This brings up another problem with bacteriophages – bacteria can become resistant to them just like antibiotics. The difference, however, is that even if a bacterium is resistant to one phage, the phage can respond to the bacteria's resistance and evolves to infect it again in a new way (Mcclean, 2010).

**Bacteriophage Life Cycle**

Recent publications have provided interesting evidence that challenge the notion that viruses are non-living. In a recent publication by Erez *et al.* (2017) communication between viruses has been identified. These reported findings indicate a unique small-molecule communication system that controls lysis–lysogeny decisions in a temperate phage. Another study reported the assembly of a nucleus-like structure during the viral replication of phage 201Φ2-1 in *Pseudomonas chlororaphis* suggesting that the phage has evolved a specialized structure to compartmentalize viral replication. These findings indicate that viruses may be parasitic organisms similar to bacteria and fungi that rely on hosts to complete their life cycles.

These microscopic phages have beautifully diverse and complicated structures when observed through transmission electron microscopy. For stance, two main features of tailed phage (order *Caudovirales*) include a capsid that encloses genetic material in the form of either DNA or RNA and a tail that varies in size among different bacteriophage (Figure 1).

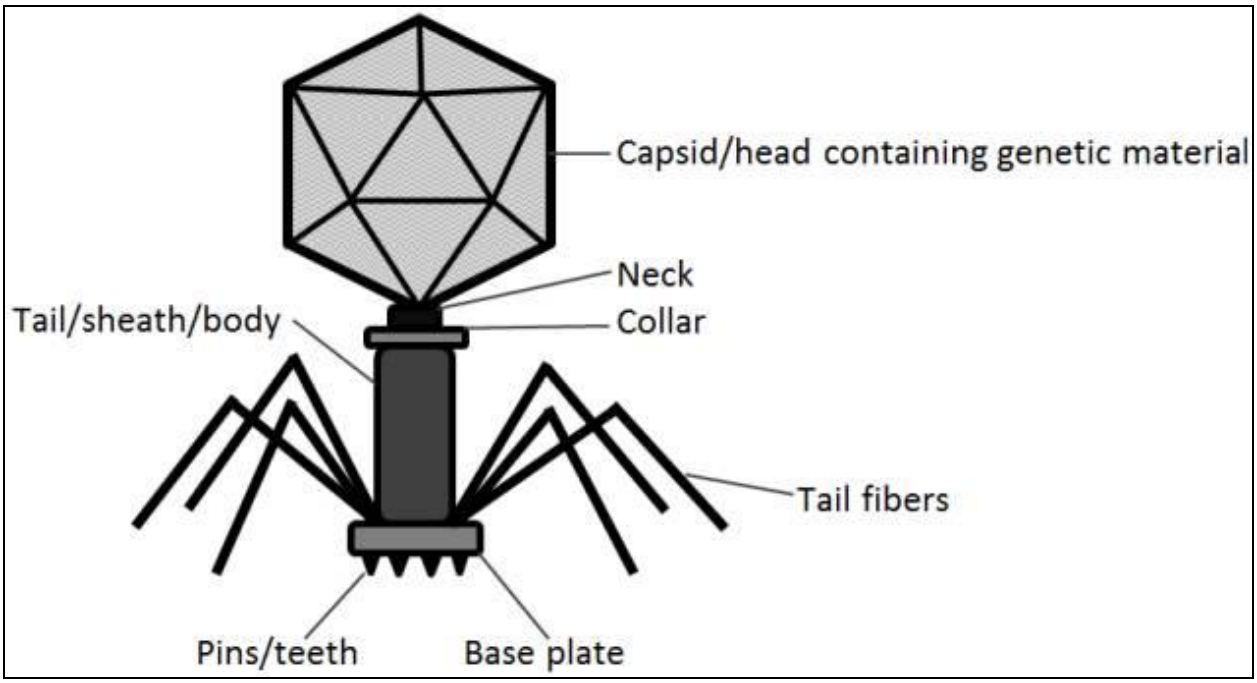


**Figure 1:** Morphological features of tailed phage (order *Caudovirales*) (Mcclean, 2010)

**The anatomy of a tailed bacteriophage of the order *Caudovirales***

The induction signals vary among bacteriophage but prophage is commonly induced when bacterial SOS responses are activated due to antibiotic treatment, oxidative stress, or DNA damage. Once the lysogenic cycle is terminated, expression of phage DNA ensues and the lytic cycle starts (Mcclean, 2010) (Figure 2).

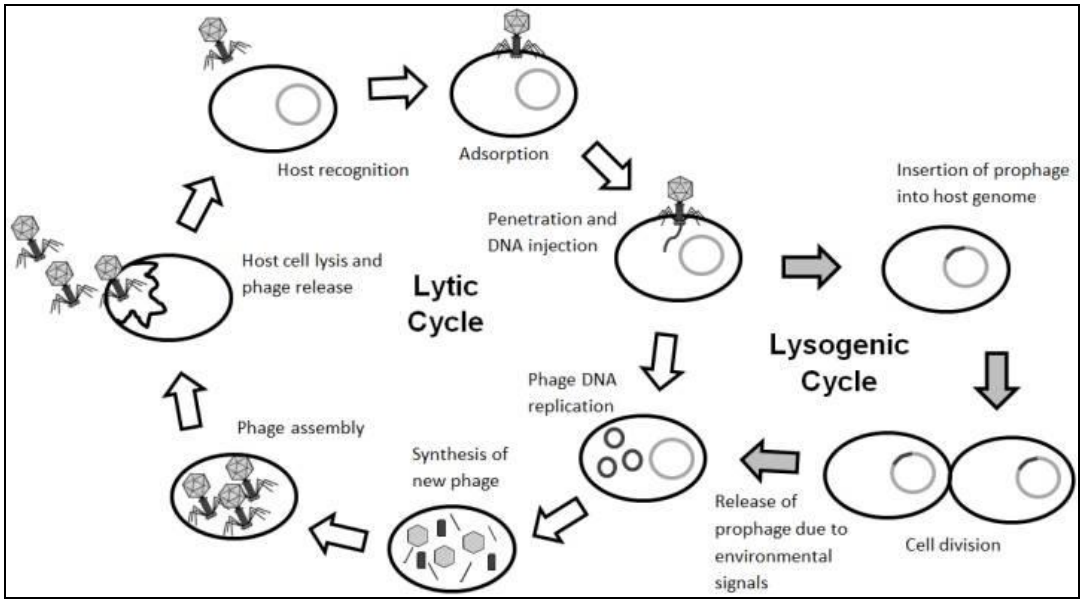


**Figure 2:** Induction signals in bacteriophage (Mcclean, 2010)

## Phage therapy

Phages as therapeutic agents in humans were first used in 1919 just when they were discovered. Phage therapy started back in 1896 when Ernest Hankin first reported the existence of antibacterial activity against Vibrio cholera the causative agent of cholera which was considered one of the deadliest peril humans had faced. In 1915, Frederick Twort hypothesized that antibacterial activity could be due to the virus (phage), but he did not pursue his discovery, therefore bacteriophages were discovered by Fe'lix d'He'relle in 1917. In 1925 d'He'relle reported treatment of plague (four types) by antiplague phages which drew attention towards phage therapy. Later on he visited India and worked on phage therapy of plague at the Haffkine Institute, Bombay(Mumbai). In west the concept of phage therapy died out in 1940 due to the emergence of antibiotics, but in former Soviet Union it was used and is still in practice. The Eliava institute in Tbilisi Georgia is considered the pioneer in this regard where phage therapy is extensively studied and applied. West has remained reluctant to use phage therapy due to the unreliable early trials of phage therapy. But still phage therapy got attention in USA. William smith and his fellows reported the successful use of phages against E.coli in mice. But today it is accepted that the main reason behind the failure was poor understanding of phage biology and some other issues like quality control during preparation of therapeutic stocks. Phage therapy has been used in animals, plants, and humans with different degree of success (Haq *et al.,* 2012).

## Phage Therapy in Plants

New phage are constantly being discovered and utilized in many different applications, including the use of phage therapy in agriculture. The renewed interest in treating diseased crops with phage therapy has yielded promising results, including the treatment of some antibiotic-resistant infections involved in bacterial blight on soybeans . Several phage have been approved by the United States Food and Drug Administration (FDA) for use on crops destined for human consumption. Use of phage therapy in plants is still in the early stages. Companies and organizations are focused on discovery, isolation, and marketing of bacteriophage products to control bacterial pathogens in environmental, food processing, and medical settings in the hope of reducing product loss and production costs. In food processing, phage-based products have been used for the decontamination and elimination of pathogens from food sources to reduce food-borne illness caused by bacteria. Several different products have been created that target common foodborne pathogens such as *Listeria monocytogenes*, *Salmonella*, and *E. coli* O157:H7 (Kalatzis *et al.*, 2018).

In addition to phage cocktails, proteins produced by and isolated from bacteriophage have been investigated as alternatives to using complete and intact phage to treat crop infections. Phage lysozyme is a protein that can fragment the bacterial cell wall, and has been isolated from phage $\phi$Xo411. Although the host of this phage is *Xanthomonas oryzae* pv. *oryzae*, the lysozyme (Lyse411) was tested against several other bacterial strains. Lyse411 effectively reduced bacterial concentration in *Xanthomonas* and other organisms (Barekz *et al.*, 2017).

## Advantages of Phage Therapy

Phage therapy has a number of advantages over traditional antibiotic therapy. The isolation of phage is fast, relatively simple, and inexpensive .Resistance to phage develops about ten times slower than antibiotic resistance. These qualities indicate that phage therapy as opposed to traditional chemical antibiotics may require fewer or limited administrations while performing as well or better than conventional treatments. Most phage isolated to date has a relatively high level of specificity for their host. This advantage of phage reduces the risk of harming the natural microbiota of the human body and eliminating the side-effects associated with chemical antibiotics.

## Challenges Facing Phage Therapy

However, there are some important factors that present a challenge to the use of phage therapy as a mainstream antimicrobial. One drawback is the

use of phage therapy against intracellular pathogens such as *Salmonella* species. Intracellular pathogens have the advantage of surviving inside the host cells, where they would presumably be inaccessible to phage due to the inability of phage to enter eukaryotic cells. Currently, few studies have investigated this problem, although one study did find phage therapy to be effective against salmonellosis, indicating the potential use of phage therapy against intracellular pathogens.

Although phages are not direct pathogens of eukaryotic cells, the human immune system may recognize phage as foreign antigens and respond by producing phage-neutralizing antibodies. Another characteristic of phage that may be a disadvantage in phage therapy is their ability to pick up genetic material through horizontal gene transfer. The use of phage therapy could lead to the transfer of genes that increase the bacterial host's virulence through general or specialized transduction mechanisms. Especially concerning is the possibility of transferring antibiotic-resistance genes and virulence factors; evidence suggests that genes for antibiotic resistance have been found in the genomes of some phage. Infection with the CTX Φ prophage increases the virulence of *V. cholera*. Some phage have the ability to pick up genetic elements through horizontal gene transfer that allow the phage to produce bacterial toxins, such as enterotoxins and exfoliating toxins . In order to avoid this problem, phage that are unable to package host DNA would be ideal in phage therapy . One method to determine if a phage carries any of these genes is to perform polymerase chain reaction (PCR) before their use in phage therapy. As an alternative, horizontal gene transfer can be exploited to directly transfer lethal genes or genes that can make bacteria susceptible to certain antibiotics. M13 is a non-lytic filamentous bacteriophage that is not frequently used in phage therapy. M13 has been genetically modified to have multiple insertion sites that can accommodate different genetic fragments. Interestingly, Moradpour et al. constructed a modified M13-derived phage with a lethal catabolite gene activator protein, and demonstrated that the number of *Escherichia coli* O157:H7 in contaminated cow milk and Luria–Bertani media could be reduced when treated with their designer phage. This customization of phage genomes can allow for extended versatility in the employment of designer phage against pathogenic bacteria.

However, the high phage concentrations used in phage therapy are likely to be greater than the concentrations of any single type of phage found in nature. If the phages are introduced into the environment at higher than normal concentrations, this might create an imbalance that would impact ecological communities. Unlike antibiotics, phage does not simply degrade over time. They are stable over a wide range of temperatures and they can multiply indefinitely if host bacteria are consistently available.

Although phage resistance has been shown to occur in laboratory studies, it has not been reported to be a substantial problem in clinical studies. However, it is still one of the major concerns about phage therapy, because this would limit the usefulness of phage therapy against MDR pathogens. Phage resistance occurs through a variety of mechanisms; for example, through CRISPR-Cas systems or through a change in the surface receptors to which phage must attach. Since phage and bacterial hosts coevolve, new phage can theoretically be re-isolated from the environment. This is an advantage of phage discovery in comparison to the extensive time required for the development or discovery of many chemical antibiotics. In this regard, phage resistance would not be as problematic as drug resistance.

Another issue that arises with phage therapy is the possible downstream effects of lysing bacteria. When Gram-negative bacteria are lysed, the cellular components such as endotoxin may be released. This is a major problem currently associated with the use of certain antibiotics. If a large amount of endotoxin is released into the body, fever or septic shock can occur, which could lead to death. One technique to circumvent this issue is to engineer phage that is lysis-deficient. There are unique phage proteins that play roles in the lysis of bacterial cells: endolysin,

holing, and virion-associated peptidoglycan hydrolase (VAPH). Endolysins are enzymes produced by the bacteriophage that mediate the release of progeny phage at the end of the lytic cycle through the degradation of peptidoglycan. Holins are small proteins that accumulate in the cytoplasmic membrane of the host and allow endolysin to escape to degrade peptidoglycan. In contrast, VAPH proteins degrade peptidoglycan to aid in host penetration when initially infecting bacteria and are not as strong as the endolysin enzymes in therapeutic uses. An endolysin-deficient phage engineered to lyse the bacterial cell and prevent a harmful immunological response from the endotoxins would be ideal. Endolysin gene disruption has been performed on phage such as T4, whose host is *E. coli*, and P954, whose host is *Staphylococcus aureus*. Despite not releasing progeny, the endolysin-deficient phage retained their lethality by creating a hole in the inner membrane of the bacteria via the holin.

Using phage proteins instead of whole phage potentially avoids many of the problems of using a constantly reproducing particle, such as horizontal gene transfer and environmental containment issues. In general, phage cocktails, combinatorial therapy, or phage protein products may provide promising alternatives to antibiotics (Barekzi *et al.,* 2017).

**Targeted gene delivery through Phages**

Phages are the potential therapeutic gene delivery vehicles. The rationale of using phages for targeted gene delivery is similar to that of using phages for DNA vaccines delivery in which the phage coat protects the DNA inside from degradation after it has been injected.But conceptually both are different. Phages ability to display foreign proteins on their surfaces enable them to target specific cell types which is a prerequisite for successful gene therapy . Phage display and artificial covalent conjugation are used to display targeting and processing molecules on the surfaces of phages. For the delivery of phages, targeting sequences such as fibroblast growth factor have been used to the cells having the appropriate receptors. Enhancing the uptake and endosomal release of phages, proteins sequences such as penton base of adenovirus which mediates entry, attachment and endosomal release are used. The protein transduction domain of human immunodeficiency virus (HIV) tat protein and the simian virus 40 (SV40) T antigen nuclear localization signal have also been used to enhance the uptake and nuclear targeting of phages like lambda that have been modified. Other displayed peptides that can facilitate gene delivery via phages include integrin binding peptides which enhance binding and uptake and DNA degradation reducing DNase II inhibitor. To screen the ability of phages for targeting specific cells and tissues, phage display libraries have been used in mice many times and every time phages were found in specific tissues. For instance isolating phages that target liver, mice were inoculated with phage display libraries and phages were isolated after extracting the livers. Similar in vitro strategy is used for the isolation of phage displayed peptides that enhanced cytoplasmic uptake into mammalian cells. So again phages proved themselves to be versatile by making it possible to target specific tissues either by screening phage display libraries randomly or by rational design. Phages as vehicles for vaccines delivery Phages have been used as vehicles for the delivery of vaccines. Phage particles can be used directly carrying the vaccine antigens expressed on their surfaces. But in case of DNA vaccines the sequences that are essential for the vaccine antigen synthesis are incorporated into the phage genome and the phage would then act as vehicle for the delivery of DNA vaccine.

Phage display libraries can be screened with specific antiserum to detect novel antigens and mimetopes. Mimetopes are the peptides that mimic the antigenic properties and secondary structures of protective protein, lipid or carbohydrate, although having different primary structure.Phage display libraries can also be screened against the serum of convalescents for the identification of potential vaccines against specific diseases. There are some cases in which whole phage particles that displayed antigenic peptides have been used as vaccines in animal models.

## Phages as biocontrol and bacteriophage bioprocessing

Phages could be used as predators of pests (bacteria) found in association with plants, fungi or their products. Phage mediated biocontrol of plant pathogens has successfully been attempted against Xanthomonas pruni associated bacterial spot of peaches to control infections of peaches, cabbage and peppers. Phages have also been used to control Ralstonia solanacearum of tobacco. Bacteriophages in bioprocessing are used to reduce the bacterial load in foods usually in the minimally processed foods to avoid cooking associated flavor or texture.

## Perspectives and conclusion on Phage Therapy Today

Wherever bacteria thrive, so do predatory phages. During 2017, we celebrated 100 years from the discovery of the bacteriophages and the idea of using specific bacteriophages as a weapon to biologically control pathogenic bacteria. Phage therapy approaches against bacterial infections have been revived, primarily due to major problems with antibiotic-resistant bacteria we are facing as a result of excessive usage of antibiotics. In addition, the increasing temperature in the oceans, the fatal effects of vibriosis on the global aquaculture industry, as well as a plethora of different vibrios that may trigger the disease has further emphasized the need for exploring the potential of phages to control vibrio pathogens. Additionally, the combinatory usage of bacteriophages, together with another ecologically friendly alternative such as probiotic bacteria, constitutes a strategy that would be expected to be highly effective against bacterial diseases. Combining biological approaches with different targets and modes of action may minimize the risk of future resistance development, as has been seen in human medicine, where combined drugs are successful in antibacterial and antiviral treatment (Middelboe *et al*.,2018).

Details given above give a glimpse of the large range of applications of phages in the field of biotechnology and medical science. The applications of phages range from the diagnosis of the disease, through phage typing, and its prevention (phage vaccine), to the treatment (phage therapy). There is the hope that phages could be useful to humans in many ways. By making a cock tail of phages it would become easy to treat a wide variety of bacterial infections that are otherwise resistant to the latest generations of antibiotics. A phage can be used individually to treat a bacterial infection by lysing the bacterial cell as it is having the lytic potential. At the same time the versatility of phages would allow us to use the antibodies against the bacteria that have been displayed on the phage surface. Similarly a protective antigen could be delivered as a DNA or phage display vaccine. So a mixture of phages that are modified genetically would be more helpful in addressing all these problems. Phages have also been good to cope with the food spoilage problem, and to treat the bacterial infection of plants and fruits. Due to the rapid progress in the fields of biotechnology and molecular biology it is hoped that these entities (phages) which are present abundantly in the biosphere could answer many questions human beings are having.

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