Phage Therapy – A New Approach in Science

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**ABSTRACT**

Since the advancements in the field of medical science , bacteriophages have been at the zenith of interest to scientists . They have the potential to be used not only as tools to understand basic molecular biology but also as the absolute vectors of novel therapeutic agents and horizontal gene transfer. After a long time , it is finally understood , that , by finding out the secret mystery of the biology of phages and their relationship with hosts, we may be finally figure out the key to understand microbial systems and their part in it. In this review, we describe the different types of phages, how their modification is done, their application in clinical and research field is done. This review also shows how modeling of phages is done and how they have been administered via different routes. All the data provided here , are being consolidated together to provide unparalleled insights into these tiny but vital constituents of the microbial world. This very narrative review , actually highlights the current understanding of phages and also the new strategies for a phage revolution in the field of future health care .

**Keywords** – Antimicrobial resistance; Bacteriophages; cocktails; Antibiotics

**I. INTRODUCTION**

Till now, the beneficial aspects of **viruses** in terms of human health is unknown . But , as days pass , with more and more experiments , we are now steadily discovering the importance of our viral companions. With that note , here we have tried to introduce an overlooked section of the microbial world - the **virome** (or the World of viruses). Though , the role of bacteria in terms of human health is well known in the field of medical science, but we are a long way from finding out the beneficial relationship of viruses in this aspect . But , the good news is , it is now firmly accepted , that without our **ally microorganisms** we would not be able to thrive normally and that brief hope of finding new cures , medical science has fixed its eyes on the horizon, straining to give meaning to something that’s hidden and puplexing, presently beyond the grasp of humankind. This is what will be the next challenge of human , finding out the interactions and roles of viruses in our daily lives .

In present day, scientists take the **virome** to be the most dynamically diverse and probably the biggest part of themicrobial world . As the most of the viruses in our guts are bacteriophages , which is based on the idea that , *Where there are bacteria, there will be the bacteriophages in abundance*. **As per various data collected , it can be stated that Phages are the most abundant life forms on our planet , more or less kind of omnipresent . Some freshwater sources may contain phages up to 10 billion per [milliliter].** [1]

How bacteriophages work is that , first they infect bacteria . Then they steer their cell machinery and use it to replicate their own genetic material. It is now abundantly clear that gut bacteria of human , influence health in our lives . So viruses that infect gut bacteria should have a notable influence in our lives too.

**II. BACTERIOPHAGES IN LEGENDS AND MYTHS**

Sometimes, often a question rises, why does the river water doesn’t get extensively polluted, despite of so many pollutions done by man? Or, as a matter of fact why isn’t out own Ganga river, is said to purify even the impurities? What’s the real concept among the stories and what’s the real science behind it?

The Ganges water actually never deteriorates . Too , there is no insect remains in this river. People have done many savageries on the Ganges **time and time again**. Drains, dead bodies and garbage was thrown in her, but yet nothing happened in the Ganges water. [2]

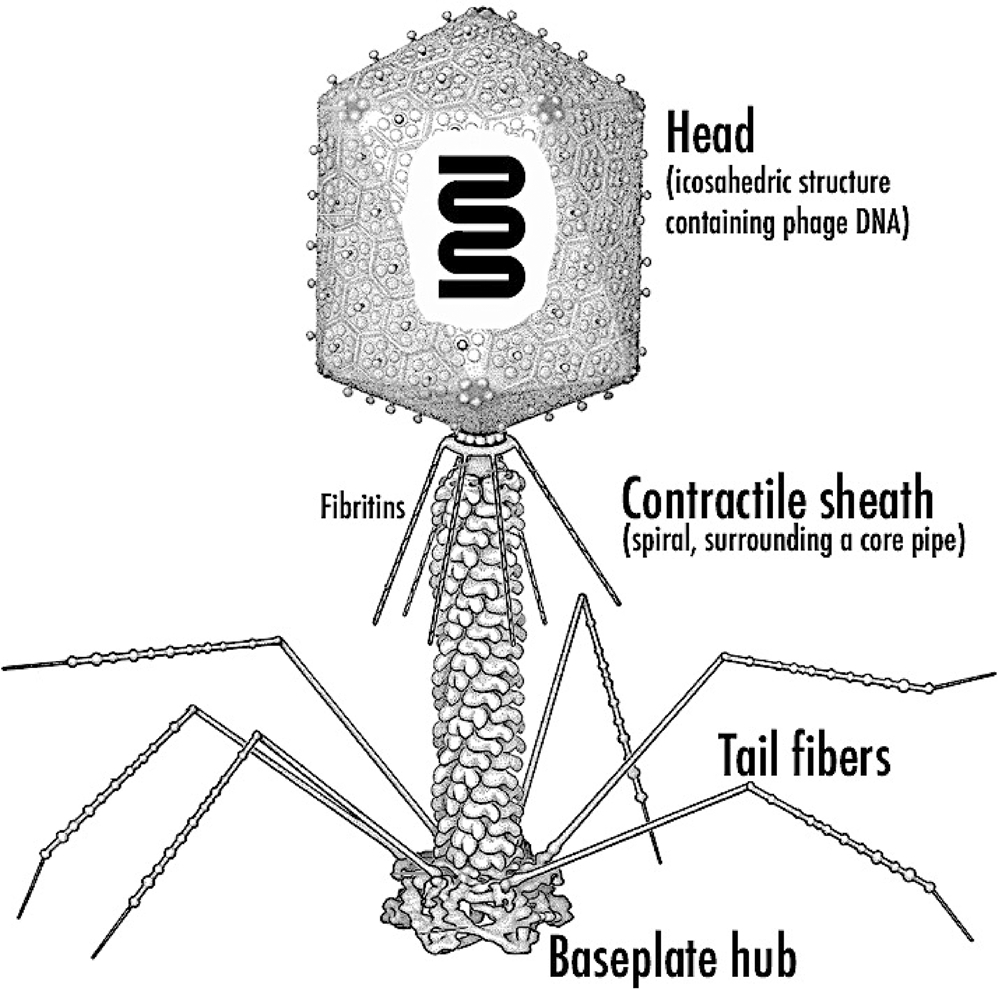
Actually , the real secret is the virus (bacteriophage). This is the very the reason why water of this river never gets spoiled. These **bacteria killing** viruses prevents rotting of the river water . The famous British scientist **Ernest Hankin** researched the Ganges water in the 1890s because a water borne disease known as **cholera** was spreading at that time. In the early days , people used to throw the dead bodies in the River Ganges . Hankin , being a researcher , feared that the other people who bathed in that water might also get infected with the **cholera pathogen** . But why it did not happen , perplexed him . Hankin was really surprised about this and started doing research on it. Then , after finding the results , in 1896, one of the first works on Ganges water was published by Ernst Hankin . It wholly demonstrated antibacterial property of the Ganges water against the pathogen known as *Vibrio cholera*. Then days passed and after 20 years, another French origin scientist took the research forward. It was then found that the viruses that were mixed in the Ganges water were penetrating into the bacteria (which spread cholera) and were eliminating them. Due to this virus, the Ganges water remained pure.

For centuries, Ganges, has been esteemed for its special healing and self-cleansing properties. Still , more than 450 million people depend on the waters of Ganges for various work purposes. [3]

In one study , the abundant presence of lytic bacteriophages against seven most commonly found bacteria were studied during Kumbh-Mela. The host-specific bacteriophages against *Escherichia coli (E. coli B and E. coli K12*), *Vibrio cholerae, Enterococcus faecalis, Staphylococcus aureus, Salmonella typhimurium and Pseudomonas aeruginosa* were analysed at specially selected bathing sites during the event. [4] The data obtained from there showed promising results , creating a possibility of treating bacterial gut infections with phage cocktails if necessary.

**III. STRUCTURE OF BACTERIOPHAGES**

Bacteriophages are the natural predators of bacteria. They are actually self-replicating, intracellular and obligatory parasites, that are biochemically lifeless in extracellular surroundings. The biosynthetic machinery of the host bacterium is controlled by them in order to produce various proteins (viral) of their own . They are ever-present creatures, in the direction of various atmosphere in the way that soil, water etc. Usually, bacteriophage makeup exhibits a three spatial makeup. The ancestral material is encircled in a protein capsid icosahedral head, a tail and surface receptor proteins.



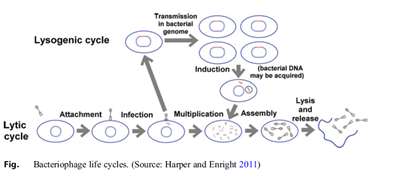
**Figure 1. Schematic representation of a bacteriophage .** [**Rossmann et al. (2005)**](#_bookmark191)

**IV. WHAT IS PHAGE THERAPY AND WHY THE TIME FOR USING PHAGES HAS COME?**

A phage is a virus, neither alive nor dead . Their head is kind of like an icosahedron – a dice, with 20 faces and 30 edges. It contains the genetic material of the virus . It sits on a long tail that has leg like structures called fibres. There are more phages on earth than every other organisms combined and they are probably everywhere where living things exists. Although, they do commit mass killing for breakfast, they only kill bacteria. Approximately , 40% of all bacteria in the oceans are killed by them every single day.[6]

But phages also have major flaws. Phages too , need a host to survive and reproduce. They are not much more than genetic material in a hull and they usually chose a specific bacteria or maybe some of its close relatives to prey on.

Like a cruise missile, phage only hunts and kills members of very specific unlucky family of bacteria. After the virus particles are assembled, in the final step they produce endolysin – a powerful enzyme that punches a hole the bacterial membrane. The lysine goes through the membrane and cleaves the bonds in the peptidoglycan. Then after the genetic material is inserted, new phages are created inside the bacterium. The pressure becomes so high, that the pressure in the bacterium is greater than the external environment. As a result the organism explodes, releasing the phage progenies, in the environment to start a new cycle. This whole process is phage therapy – using a phage and its cycle to kill a bacteria.



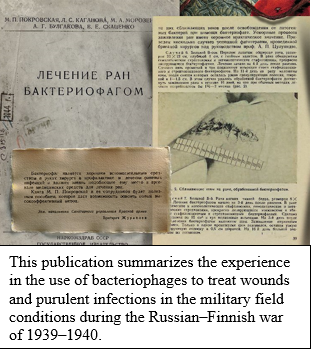
### **Figure 2: Bacteriophage life cycles. [**6**]**

Also in another process, using a technique called 5 phase lysine where using only Lysine itself as a standalone molecule, that can be used from the outside to punch a hole in the bacteria, causing the explosion process to kill the bacteria. Phage combinations are able to treat dysentery, pneumonia, rhinosinusitis and UTI before antibiotics were discovered.

But there are some issues regarding this therapy. First is the cultural barrier. It’s very difficult to culture a virus. They are biological entities , not small molecules like antibiotics , that means scalability is potentially an issue because every one of them is unique in its own right and may have specific ways in which it has to be made .The second barrier is , the regulatory approval . Like, whether or not it is fit to deal with a biologic like this, as a medicine.

Recently in many experimental studies we have started looking into them by injecting millions of bacteriophages into patient’s bodies, because we are sort of getting desperate, when MDR showed in certain bacterial strains.

In the history, we have seen , that a single cut from the wrong thing could kill us. Bacteria were like **our phages**. They were like tiny monsters that hunted us mercilessly. But then, about 100 years ago, we found a solution in nature .By accident we found fungi that produced compounds that killed bacteria – the Antibiotics.

Suddenly, we got a powerful super weapon. Antibiotics were so effective that we stopped thinking of bacteria as monsters.. So, we used antibiotics more and more for less serious causes. But bacteria are living things that evolve, and one by one they started to become immune against our antibiotics .This continued, until we had created what are now called as **superbugs** – mutant bacterial species , which are immune to almost every antibiotics we have. This immunity of bacteria is spreading across the world as we speak. In a study it is seen that by 2050, superbugs could kill more humans than the cancer. The days, when a cut or a bladder infection, or a cough could kill us , are coming back. In US alone, more than 23000 die alone from resistant bacteria each year. But it turns out, phages, our tiny killer virus robots, could save us .We could inject them into our bodies to help cure infections.[7]

But how? Actually phages are specialized killers of bacteria. They are so specialized that humans are completely immune to them. We are too different. We encounter billions of phages every day and we just politely ignore each other.

Antibiotics are like carpet bombing, killing everything, even the good bacteria in our gut and intestines that we don’t want to harm. Phages are like guided missiles that only target what they are supposed to. But the question arises, if we use phages to kill bacteria, wont bacteria develop ways of defending themselves? Well, it’s more complex than that. Phages evolve too. For billions of years there has been an arms race between the phages and the bacteria and till now they are doing great. This makes phages the smartest of all weapons that are getting constantly better at killing bacteria . But even if bacteria were to become immune against our phages, we still might be able to win. It seems that, in order to become resistant to even just a few species of phages, bacteria have to give up their resistance to antibiotics. Afterall , we might be able to trap them in a play of **catch 22**.This therapy has already has already been successfully tested with patients who had no hope left. The bacteria *Pseudomonas Aeruginosa*, one of the most feared bacteria, infected a man’s chest cavity. This specific strain of bacteria is mostly resistant to most antibiotic and can even survive an alcoholic hand gel.

After years of suffering, a few thousand phages were directly inserted into his chest cavity, together with antibiotics that bacteria were immune to. In 48 hours the patient woke up from his coma. After a few weeks the infection had completely disappeared. In another case study, a man was diagnosed with pancreatic pseudo cyst, filled with *Acinetobacter baumannii*, which was treated with a phage cocktail.

Phages are ubiquitous .In earlier days, France and former Soviet union had various experiments regarding this therapeutic agents. Bacteriophage had been kind of brought back from the shelf as a potential new approach to therapy.[7]

But they are not simple to use and we have to develop a cocktail for each patient’s own isolate as they seem to be relatively safe together. But the problem is they are difficult to develop from both the research and regulatory perspective.

However , the good news is that we have awfully , good tools now – robotics , and much more sophisticated molecular tools that enable this to be done , which 10 – 15 years ago would have been impossible to contemplate .

Unfortunately, this treatment is experimental and pharmaceutical companies are still reluctant to invest the necessary billions in a treatment that has no official approval yet.

But things are finally changing , In 2016 the largest phage clinical trial to date began , which showed that phages are getting more and more attention , and we better get used to it because the era in which antibiotics have been our super weapon is about to meet an end . It might be a weird concept, but injecting the deadliest being directly in our bodies could save millions of lives.

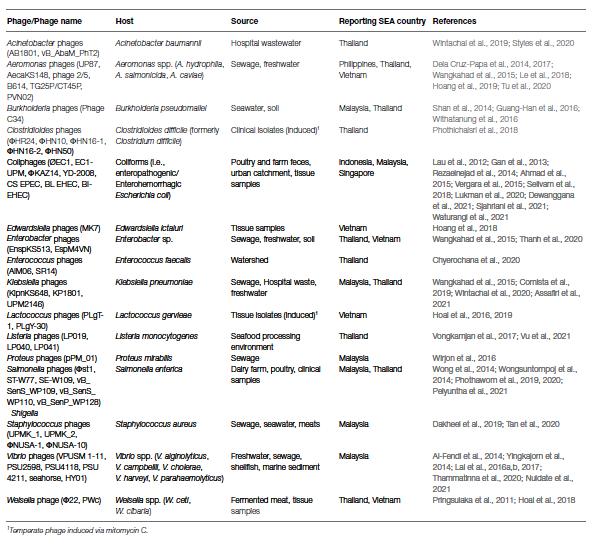
**V. BRIEF ON PHAGE THERAPY – HISTORY OVERVIEW**

In 1896 Ernest Hanbury Hankin discovered bacteriophages showing antibacterial properties against *Vibrio cholerae* from the water of Indian river [4]. Phages were discovered first in 1915 and in 1917 by Fredrick William Twort and Felix d’Herelle respectively. It was Felix d’Herelle, a French Canadian microbiologist, who first coined the name “Bacteriophage” (viruses that kill bacteria) for the first time. After his discovery, he suggested that phages could serve as a therapeutic tool against bacterial infections. He used phage preparations to successfully treat Cholera outbreak in India and *Shigella* dysentery patients in France. Since then phage therapy was considered as an eminent therapeutic tool and the exquisite treatment for bacterial infections [5].

Phage therapy use viruses to attack a specific gram-positive and gram-negative bacterial pathogen to treat specific bacterial infections. These viruses are called phages or bacteriophages, their target is selective and destroys the target either by lysis or lysogeny or pseudolysogeny, without harming the host beneficial microflora of the gut, hence minimizing the complication of phage therapy.

Phages, natural parasites of bacteria have a capsid that encloses their genetic material, in some cases, they have a proteinaceous tail. Tailed phages of the class *Caudoviricetes* include myovirus, podovirus, and siphovirus [8], and the polyhedral *Microviridae* family are usually associated with the applications of phage therapy [9,10]. Phages are ubiquitous and can be found in feces, seawater, sewage, soil, sludges, and anywhere and everywhere bacteria grow [11,12].

**Table 1: Shows the discovered phages, having potent biomedical applications from the year 2011 to 2021 [37].**



**VI. EVOLUTION OF PHAGE THERAPY**

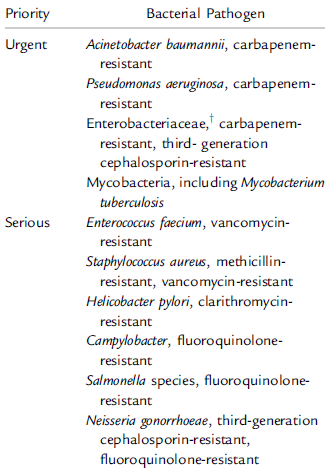
The 20th century marked the implementation of phage therapy to decrease the incidence of several disease-causing bacterial pathogens. In 1919, D’Herelle administered an oral dosage of anti-shigella phages to four patients who were suffering from dysentery, caused by *Shigella* bacteria, and they showed recovery within a day [13]. A new era began on 1945 called the golden age of antibiotics, which lead to the taking off of phage products from the market. Shortly after, penicillin resistance became a clinical problem, and in response to that new classes of antibiotics and modifications of older ones were being developed [14]. Today, multiple drug resistance (MDR) bacteria are making the available antibiotics harder to survive in the market [15]. Phage therapy is back again being considered an important means to treat bacterial infections not amenable to antibiotics. In March 2016, a 30 years old woman severely injured her leg in a suicide bombing at Brussels airport. Even after the administration of antibiotics, her wounds did not heal due to the presence of a resistant *Klebsiella pneumoniae* strain. Antibiotic treatment in turn caused several side effects but failed to clear the infection. It was in the year 2018, she was finally treated with the phage in combination with antibiotics. Within weeks, her condition improved and the badly damaged femur started to heal (*Nature Communications*, doi.org/hdbt). In 2019, the third leading cause of death globally was AMR behind ischaemic heart attacks and strokes. The more conservative estimate means that AMR killed more people that year than AIDS. By 2050, deaths due to AMR are predicted to rise to 10 million.

Bill Bryson’s book [The Body: A Guide for Occupants](https://www.theguardian.com/books/2019/sep/26/the-body-guide-for-occupants-bill-bryson-review)***(2019)*** says: “At the current rate of spread, antimicrobial resistance is forecasted to lead to ten million preventable deaths a year”. Then, [an article from Chemistry World](https://www.chemistryworld.com/features/the-antibiotic-countdown/3008544.article), reports: “Already, drug-resistant bacterial infections kill 700,000 people every year and this figure may rise to 10 million by 2050.” An April 2019 report by an UN interagency group stated: “Drug-resistant diseases already cause at least 700,000 deaths globally a year, including 230,000 deaths from multidrug-resistant tuberculosis, a figure that could increase to 10 million deaths globally per year by 2050 under the most alarming scenario if no action is taken” . [Again, in an analysis](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5127510/) done in PLoS Medicine, referring to the 10 million figure, observes: “The scenario that seems to be underlying the most often quoted line entails a sharp initial rise of current resistance rates by 40 percentage points, after which rates remain stable until 2050, and doubled infection rates.” [16]

When we hear that there could be 10 million deaths in 2050 from antimicrobial resistance, we should take the warning of the threat very seriously. When scientists tell us that we must take [far-reaching and unprecedented changes in society](https://www.cnn.com/2018/10/07/world/climate-change-new-ipcc-report-wxc/index.html) to avoid particularly disastrous levels of climate change, we must achieve at least to a minimum level of reduction in that timeframe. This is even as [2030](https://insideclimatenews.org/news/27082019/12-years-climate-change-explained-ipcc-science-solutions) is more of a critical benchmark in the timeline of humanity. Journalists should provide the full context expected possibilities of future events so that their audience can understand just what the claim is. In this case, the AMR Review highlights – there will be 10 million deaths annually by 2050.[17]

The situation is critical for most difficult-to-treat, community-acquired, healthcare-associated, and nosocomial infections caused by ESKAPEE pathogens[40].

**Table 2: The global priority pathogen’s list of antibiotic-resistant bacteria by the World Health Organization in 2017 [4]**



**VII. PHAGE SELECTION**

Phages ideal for therapy should be obligately lytic to ensure the killing of the target pathogen [9,18,19]. The phage should have a high rate of adsorption to the target pathogen, a short generation time [20], and species-specific activity [11]. As in the case with typical antimicrobial agents, scientists also recommend profiling the phages in a phagogram [21]. Phagogram continuously tests the efficacy of the phage particle against a defined collection of pathogens called a pathogen library [22,23] and hence will ensure that the therapeutic phages are specific to their target pathogen.

**VIII. PHAGE FORMULA**

Clinically phages are typically administered as a cocktail of viral strains [24,25, 26,27.] A single phage strain will precisely target a specific bacterial strain [28,29.] The drawback of the approach is it requires a very careful definition of the etiologic bacterium before therapy. It is only applicable for proof of the concept, checking of efficacy and tolerability in vitro. However, the benefits linked with the cocktail approach are increasing the target bacterial strain spectrums, targeting multiple species in one go, increasing the dose potency by multiple phage strains attacking the same bacterial cell, and limiting resistance by forcing the target bacterium to evolve resistance to multiple phages simultaneously to survive [30-34.] The only drawback individual phages generally require a reduction in concentration when mixed into a single dose and the phages need to compete with one another for the same bacterial cell surface receptor and drive cross-resistance [35].

**IX. PHAGE RESISTANCE**

Bacteria can thwart phage attacks through various antiviral mechanisms, which are spontaneous chromosomal mutations (major problem), the ability to block the entry of genetic material of the phage particle, DNA restriction-modification enzymes present, abortive infection, and lastly CRISPR-Cas adaptive immunity of the bacteria. Switching to new phages with different binding sites or orders of exposure can be used to improve the efficacy of phage therapy [36] Maintenance of bacterial antiviral defense mechanisms comes with a cost [37,38]. However, phages also have a defense system to counteract bacterial immunity [39,40] and counter-adapt to reinfect resistant bacteria [41,42] which are among the sweet benefits.

**X. PHAGE ADJUVANT**

Phage adjuvants are active compounds not affect bacterial growth in isolation and help to block phage resistance or enhance phage activity when administered in combination with the phages. The combination of synergistic antimicrobials is an adjuvant as they boost phage production. For example, phage-infected *Burkholderia cenocepacia* cells produce higher phage particles in the presence of sub inhibitory concentrations of ciprofloxacin, tetracycline, and meropenem [43]. Tetracycline causes cell clustering, which may promote an increase in phage infections by minimizing lateral travel between the two adjoining cells, hence increasing contact with phage receptors on the cell surface of uninfected cells. The increase of phage production is unaltered when bacteria are antibiotic resistant. Combining phages with sub inhibitory concentrations of ciprofloxacin or meropenem may inhibit the regrowth of the resistant phage mutants in a murine endocarditis model and hence improve phage therapy outcomes. Phages in combination with antibiotics may provide a chance for the discovery of newer antibiotics and increase the pressure on the development of phage therapy. The major drawbacks are in most cases phage antibiotic interactions are unknown and even though in vitro studies of synergy justify the combination of phage antibiotic therapy, they are further not pursued within animal models [44].Apart from this, bacterial biofilms act as a major hindrance in the fight against bacterial infections because they are inherently refractory to various antibiotics. DNAs are a potent adjuvant that will degrade extracellular DNA, which plays various roles in both aggregations of bacteria and interaction of the resulting biofilm with polymorphonuclear leukocytes during the inflammatory response [45]. Other phage adjuvants can be sugar alcohols such as xylitol, sorbitol which inhibits bacterial growth by diffusing through the biofilm and accumulating as a toxic substance, nonmetabolizable sugar alcohol phosphate. These findings suggest that phage adjuvants may help to improve the efficacy of bacterial killing during the treatment.

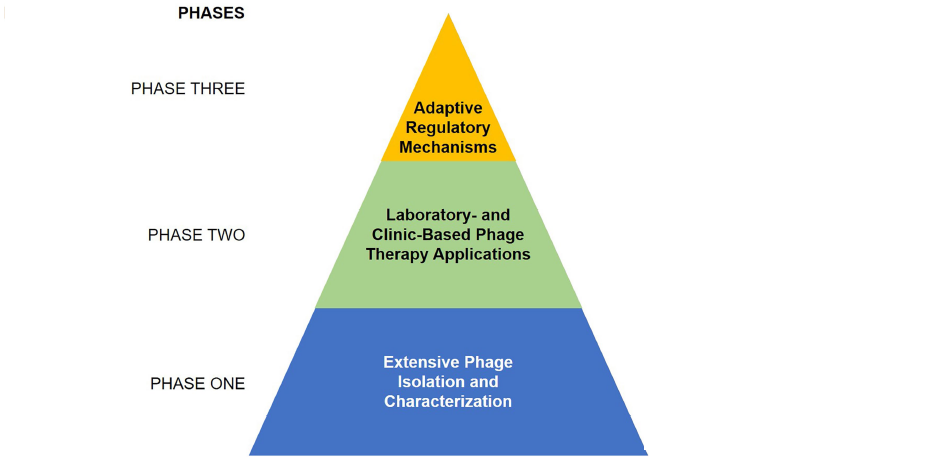
**XI. THREE-PHASE PHAGE STRATEGY**

A structured research and implementation approach are necessary for the use of phage therapy against multidrug-resistant infections, which is divided into three phases, which are as follows:[49]

Phase One: It includes isolation, characterization, and finally , the matching of phages to their target pathogen, which is to be guided by the existing knowledge of phage biology.

Phase Two: While this phase includes , designing of appropriate models, both laboratory and clinical-based, to implement relevant phage therapy rules.

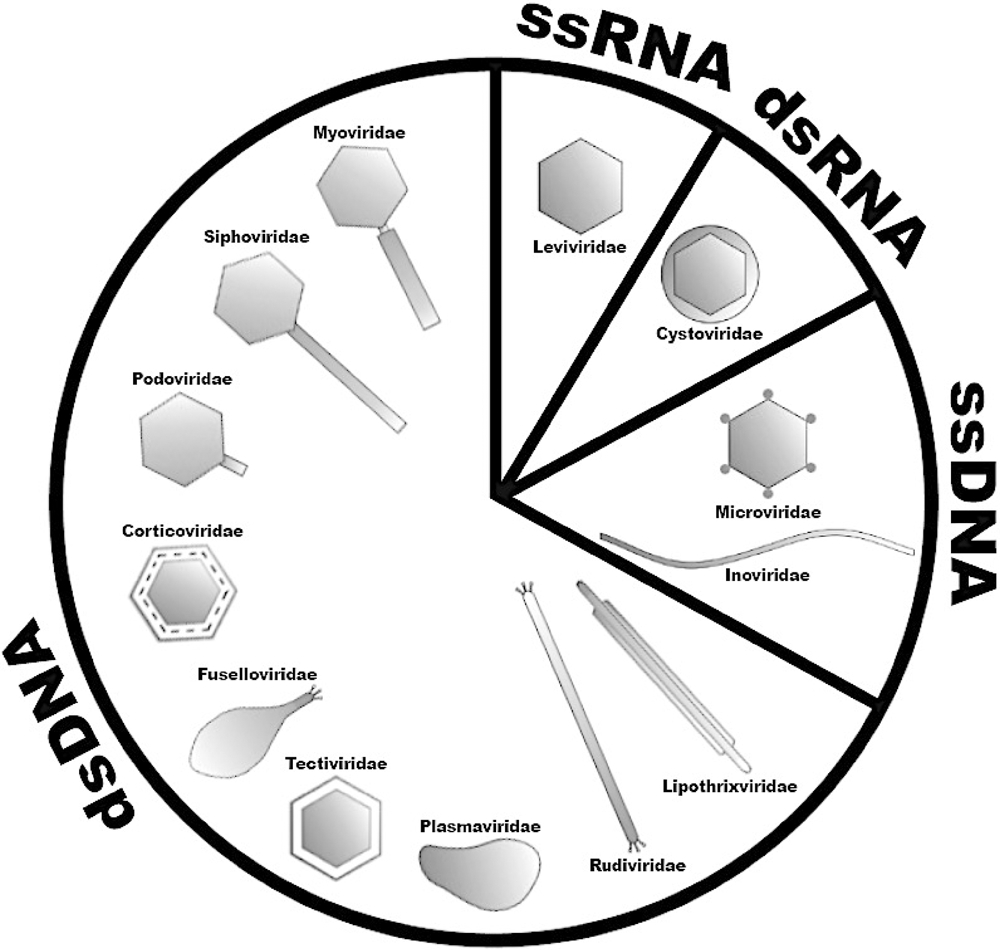
Phase Three: It requires establishing regulatories and specific standards for phage therapy.



**Figure 3: Three-phase phage strategy to combat multiple drug resistance (adopted from Carascal, B., M., et al., 2022.)**

**XII. TYPES OF PHAGES GENERALLY FOUND**

Over the last ﬁfty years, more than 5100 bacteriophages have been identiﬁed and studied, with more than 90% of them belonging to the *Siphoviridae , Myoviridae* and *Podoviridae* families . In relation to the type of genetic material they have within the capsid's core, bacteriophages can be divided into four major groups (see): single stranded DNA phages (ssDNA), single stranded RNA phages (ssRNA), double stranded DNA phages (dsDNA), and double stranded RNA phages (dsRNA). [50]



**Figure 4: Classiﬁcation of bacteriophages according to their morphology, genetic material and major characteristics.**

**XIII. STRATEGIES OF REPLICATION AND POSSIBLE FUTURE COMMERCIALIZATION**

In general terms, Bacteriophages or phage cultures require host cells in which they multiply. Cultures are grown by infecting bacterial cells with bacteriophages. The phages can be isolated then from the resulting plaques in a lawn of bacteria on a microbial plate.[48]

**XIV. THEORETICAL MODELS FOR BACTERIOPHAGE PRODUCTION**

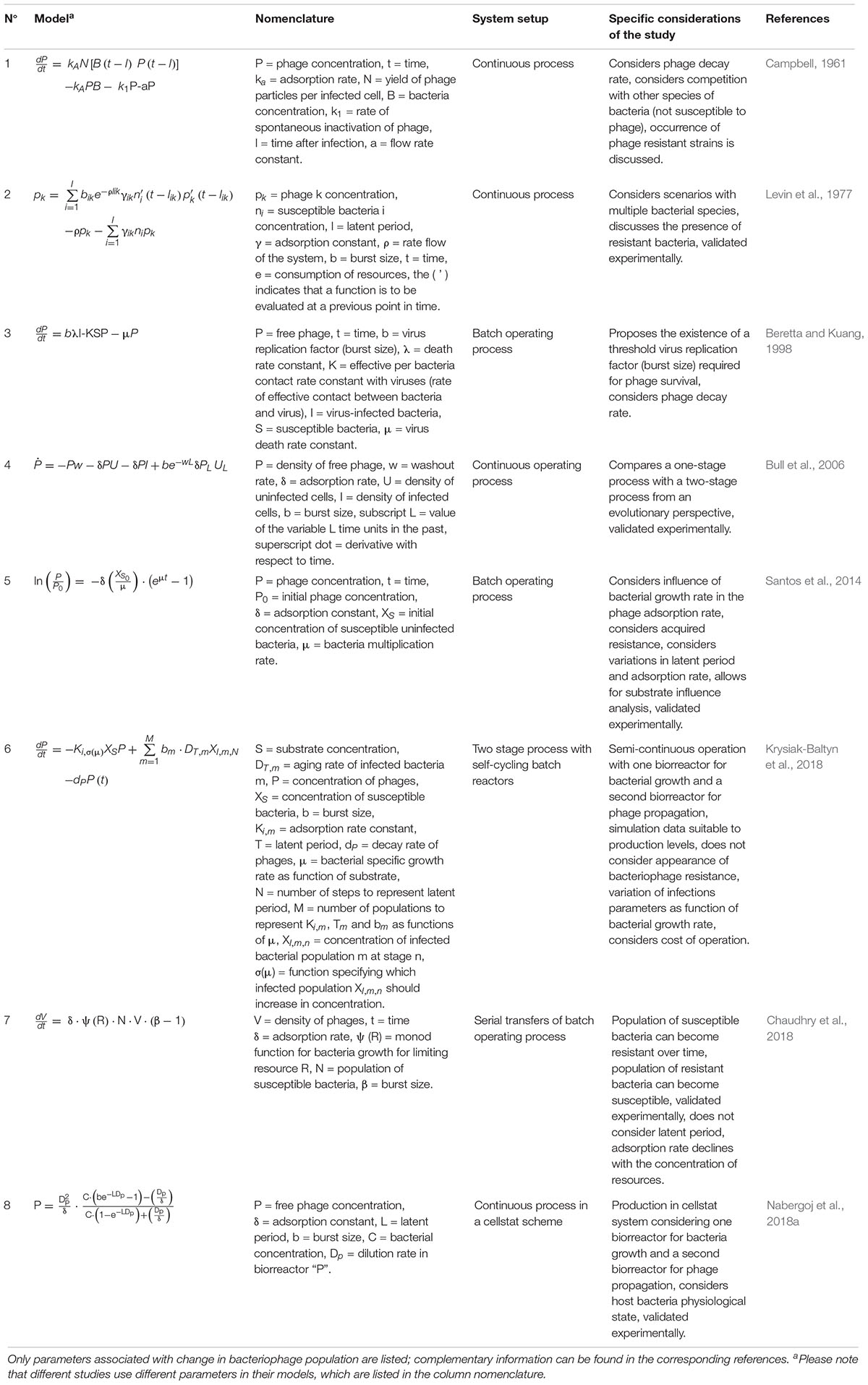
The populations of phage-infected bacteria, susceptible uninfected bacteria and free phages are the 3 basic parameters for phage production . Following this , different models have included additive variables like **resistant uninfected bacteria**. They are controlled by several kinetic parameters , which are associated with **bacterial growth and phage infection**. Based on the nomenclature used by other authors , we generally use the Greek characters to name the different kinetic parameters in phage reproduction. Likewise , **Burst size can be symbolized by β, eclipse time by ε, phage decay rate by λ and adsorption rate by δ. *The only exceptions is phage concentration, which is commonly indicated as “P,” and latency time, as “L***.” The uniformity in this mathematical language is the only thing that makes the understanding and data mining in realm , easy for future reviewers.

Many efforts were made to describe models of phage production, describing its population behavior under several conditions. **Table 1.** summarizes different phage production models, given as differential and integral equations .

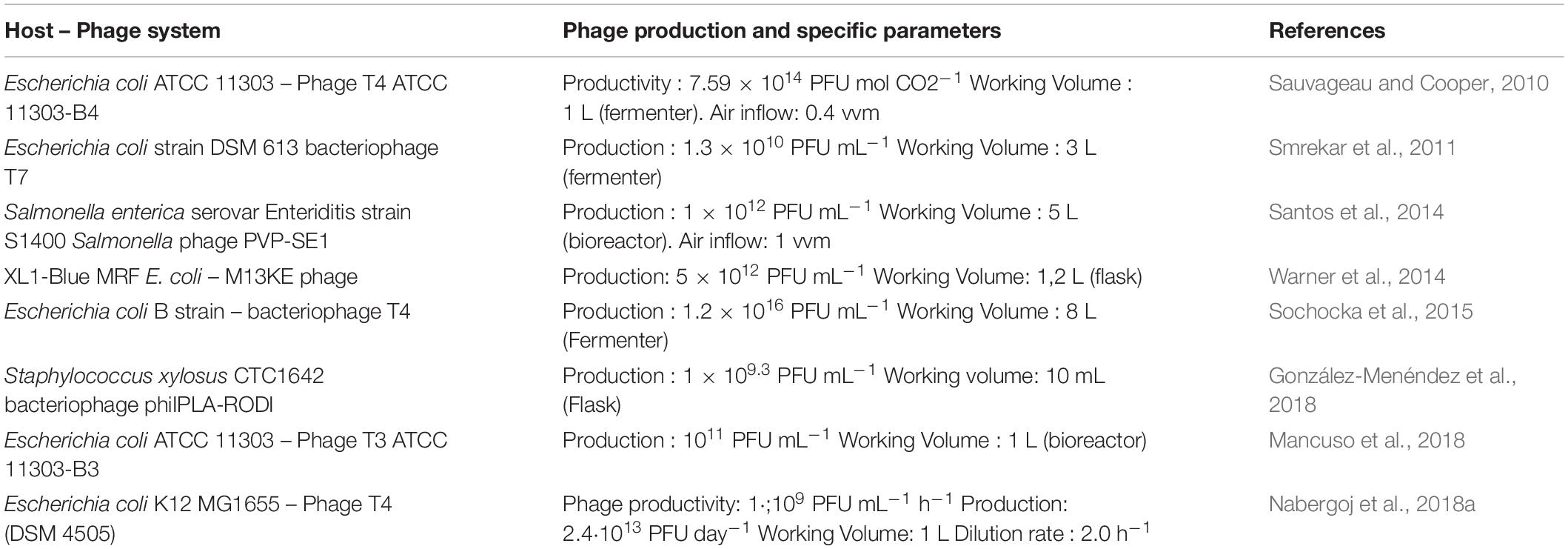
Phage production models generally describe population changes of phages over time. This may be represented as a kinetic change in plaque forming units (PFU) per unit of time. Several models proposed by Campbell (1961) and Beretta and Kuang (1998) are consistent in balancing phage particles .These models are useful due to their extensive simplicity .One interesting model proposed by Santos et al. (2014) considers the influence of bacterial growth rate with respect to the phage adsorption constant . Many other models have also explored the the occurrence of bacterial resistance .Another interesting analytical study of Krysiak-Baltyn et al. (2018), which also described variable infection parameters while estimating operational cost and productivity in a two-stage process.

Bacteriophage evolution must be considered in terms of a commercial process, since the phages might increase their efficiency to infect bacteria over time. This idea could be represented as infection rates in host-range enhancement experiments, where methods for host-range expansion can be achieved for phage therapy applications which in turn may find a better way to solve the MDR problems. [47]

**Table 3. Models of bacteriophage production**



**Table 4. Production data available on bacteriophage production cases evaluated experimentally**



**XV. PHAGE ABUNDANCE AND DIVERSITY**

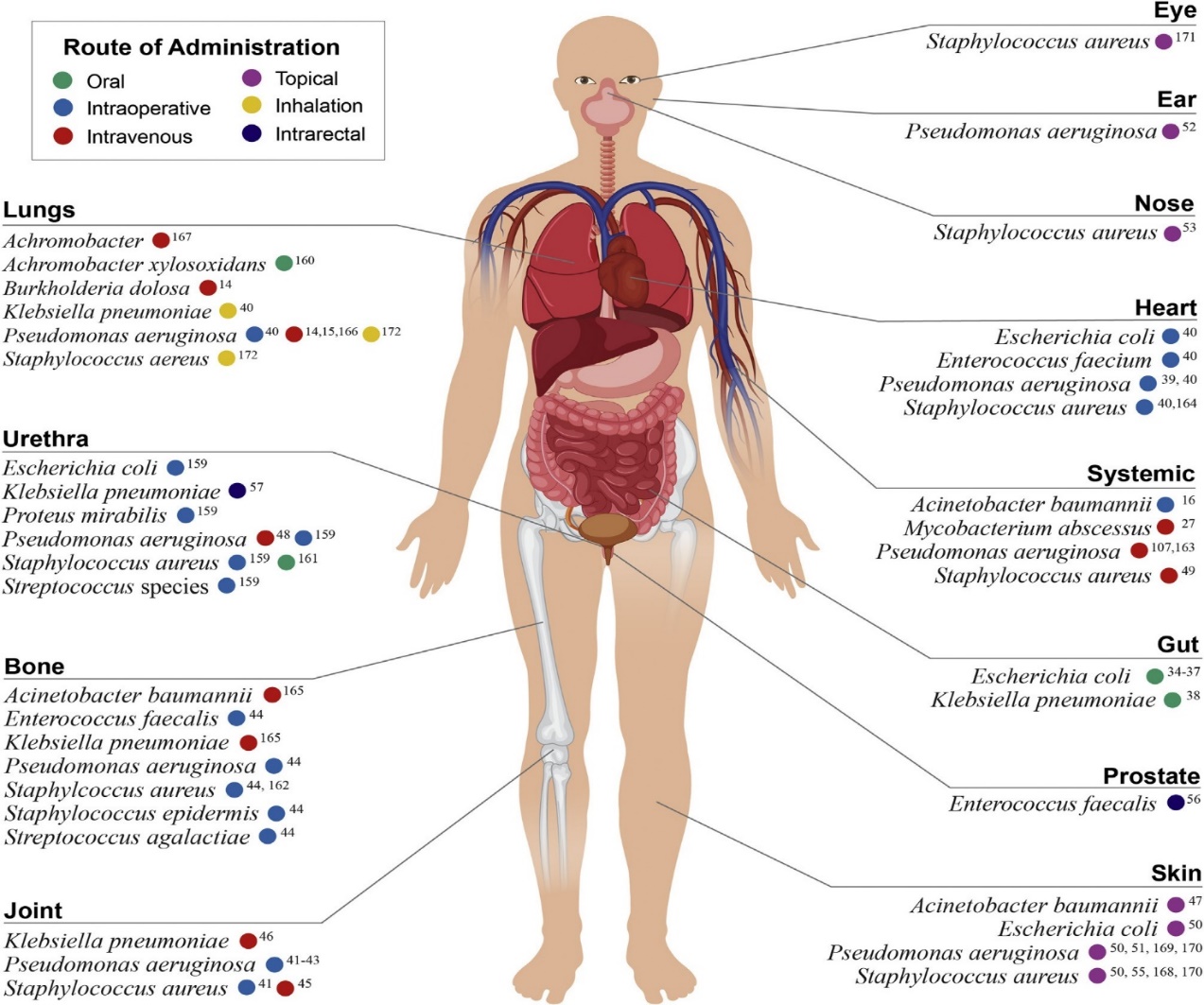
Considering the possible phage life cycles, it is quite logical to review where phages are found, and how they are characterized. Specific phages and their characterization are being in the case studies. The first approaches which led to the realization of phage abundance were based on epifluorescent microscopy followed by DNA staining which suggested that, in sea water there are approximately around 10 phages in existence for each bacterial or archaeal cell. Hence, to make sense of phage abundance, one must establish where the majority of their hosts exist. Most of the Earth’s Bacteria and Archaea are found in the open ocean, in ocean sediments and in terrestrial sub-surfaces, where, an estimation of 1.2 x 1029, cells can be found respectively. Bacteria and Archaea are often associated with humans and animals, who provide many **niche** environments within them, where these micro-organisms become an essential symbiont. Although not significant, bacteria are of essential importance when associated with humans, particularly either in a disease, or as a food producing context, which can fall prey to the bacteriophage attack. Therefore, in terms of human impact, a study of the roles of bacteriophages which infect these bacteria is of immense importance. [46,47]

**XVI. SOME METHODS TO GENETICALLY ENHANCE PHAGE & THEIR CAPABILITY**

Although, there are a lot of naturally occurring phages in the environment, still they can be enhanced using genetic engineering approaches. These techniques allow researchers to increase phage killing efficacy and introduce other desirable properties in them. One of the most widely used approaches for phage engineering is **homologous recombination**. Yeast-based and in vitro phage genome assembly methods have been developed to avoid the possible toxic effect of phage replication. Lastly, cell-free transcription-translation systems can create the phage virus-like particles from DNA. The Noireaux laboratory has developed cell-free systems to successfully synthesize, replicate and assemble **MS2, T7, phiX174** and **T4** phages.[48,49]

**XVII. ADMINISTRATIVE ROUTES AND REAL LIFE EXAMPLES OF PHAGE COCKTAILS IN PATIENTS**

Since phage therapy is a very new scientific approach in health care , every patients , who are being treated with phage therapy, are treated empirically . This means adequate routes of administration, duration, dosing, and antibiotic compatibility of phages is just in a trail and error scenario. **Fig. 5**, briefly summarize the **selected phage therapy clinical trials** and the single-patient reports in between 2005 and 2020 in terms of *routes of administration*. [49,50,51] With limited clinical trial data available , the experience , gathered from various case studies , we have showed the potential data of routes and their pathway of threat possession , in the following figure .



**Figure 5. A summary of the clinical trials of phage therapy (case reports ranging between 2005 - 2020). Reports are grouped by the target pathogen and site of infection . The color coding, represents the primary route of phage administration.**

**XVIII. APPLICATIONS OF BACTERIOPHAGES IN REAL LIFE:**

**A. Therapeutic potential Enhancement**

The use of synthetic phages (genetically modified) can extend the therapeutic potential of phage therapy. Like in one study , Zhang and colleagues removed genes associated with lysogeny to make the phages insensitive to repression to enhance lytic growth and extend the host range.[52 ,53] They demonstrated its efficacy in killing vancomycin-resistant *E. faecalis ,* within a biofilm of course ! [54]. Also , recently the first clinical application of engineered bacteriophages was used to treat a patient with cystic fibrosis with a *Mycobacterium abscessus* infection. 9 days after the enactment of phage therapy, the patient was able to leave the hospital. [55].

**B. Host Range enhancement for broad spectrum attacks**

Till now , phages actually has the potential to infect a limited range of bacterial species [56]. This narrow specificity is kind of an advantage , as due to this , phages are not capable to disrupt commensal bacteria of the host. But it is a disadvantage too , as this therapy is costly. So, to bypass this limitation, multiple phage populations can be mixed into a cocktail to ensure a broader range of activity of the therapy. Also, phages can be genetically altered to expand their host range [57,58,59]. Yehl and colleagues developed a method to widen host-range , using targeted mutagenesis of specific regions of the tail fiber , which plays an active role in host recognition. This approach ensured a large diversity. The mutant phage library with expanded host range , has reduced the out coming of phage- resistant bacteria [60].

**C. Removal of Antibiotic Resistance**

The phage components separately , can also be modified to carry payloads to enhance the bactericidal activity of antibiotics. In one such study , Lu and Collins modified a lysogenic phage M13mp18 which repressed the SOS DNA repair system [61]. They demonstrated, the effectiveness of this method in an in-vivo mouse model where mice treated with antibiotic and modified phage had an 80% chance of survival , which is very lucrative [62]. In another example, Edgar and colleague engineered, the phage λ to carry wild-type versions of the rpsL and gyrA genes [63].

Several other groups of scientists have also engineered phages to carry CRISPR-Cas systems to disrupt antibiotic resistance [64]. Citorik and colleagues designed several phage-based fabricates while targeting β-lactamase genes, which gives resistance to β-lactam antibiotics. [65]

**D. Animal Gut Modification**

The use of bacteriophages to alter the gut microbial community has already been attempted in various animal models . In one study , Hsu and colleagues tested the effect of phages on germ-free mice . The mice was pre-comprised with bacterial species known to colonize the human gut. They targeted each member individually with phages. Within two days, 28% of the *E. faecalis* population were seen to be phage resistant [66]. This work demonstrates the importance of the development of resistance is a very impactful concern for the potential use of phages to alter the microbiome of the gut .

**E. Delivering of new Antimicrobial agents**

The remarkable ability of phages to target specific bacteria opens the doorway for more targeted delivery of new antimicrobials. In one study , Yacoby and colleagues used specific filamentous phages as targeted drug carriers to eliminate pathogenic bacteria [67,68]. They did that by modifying the p8 coat protein of the bacteriophage to display a bacterial-specific peptide. An inactive form of antibiotic - chloramphenicol, was conjugated to the phage. The modified phage were successful to bind the target bacteria. This approach enhances the concentration of the drug at the target site, thereby increasing the potency and decreasing the toxicity [69,70].

**F. Targeted CRISPR Editing by Phages**

CRISPR editing paves the pathway to inactivate any gene in a bacterial population. In one study , Selle and colleagues used genetically modified phage ΦCD24-2 , encoding a self-targeting CRISPR to redirect the type I-B CRISPR-Cas3 system in *C. difficile* towards the bacterial chromosome [71]. After the infection took place , the phage-delivered CRISPR activated the endogenous Cas3 protein to digest the chromosomal DNA of the bacterial host. The results showed that the modified phage, was more effective at killing *C. difficile* [72].

**G. Disrupting of Biofilms for better antibacterial environments**

Biofilms actually provide tolerance to antimicrobial agents by putting up a physical barrier [73]. Phages which infect bacterial strains , produce depolymerases- enzymes that degrade polysaccharides. In one study , Lu and Collins developed a T7 phage which produced the biofilm-degrading enzyme - dispersin B , during infection. The modified phage reduced the bacterial biofilm cell counts by 99% [74]. Phages can also be bio-engineered to deactivate the quorum-sensing molecules. T7aiiA application , reduced the quorum sensing of *Pseudomonas aeruginosa* in a mixed biofilm resulting in a reduction in biomass by 75% [75].

**H. Killing of bacteria with Endolysins – the phage enzymes**

Several group of scientists focused on the cell wall degrading endolysins encoded by phages, rather than using the whole phage. Near the end of the replication cycle, phage endolysins degrade the host peptidoglycan, which causes the cell lysis. After this, release of the phage particles takes place in the process [76]. In one study , it was seen that , an endolysin was able to kill *Gardnerella* bacteria without disrupting the vaginal microbiome in 13/15 patient samples [77].

In several other studies , lysocin PyS2-GN4 was also seen to be sterilizing high concentrations of *Pseudomonas*.

**I. Delivering of Drugs in association with phages (*In terms of Eukaryotic Applications*)**

The ability of bio-engineered phages to deliver drugs to specific cancer cells is like a boon of wish to minimize the toxicity and side effects of cancer therapies [77,78,79]. Phages can be attached to drugs that have low solubility , which may allow for a lower dose. In one study , Bar and colleagues reported an improved potency of hygromycin (carried by phages) compared to free drug treatment of the human breast carcinoma [80].In another study, Du and colleagues did phage coupling , which were targeting the human hepato-carcinoma cell line with doxorubicin and observed a reduction in tumor growth [81]. Modified phages have also been designed to induce targeted killing of cancer cells [82]. Also treatment of brain disorders like tinnitus, **Parkinson’s, and Alzheimer’s disorders** are being carried out with phage applications.

**J. Phages acting as Sensors**

Sensors generally have at least two functional components. First , is the recognition element for a target and the Second is transducing for reporting , after the recognition element detects the target. After , the receptor binding proteins detects the target, for example a bacterium, then the genome of the phage acts as the reporter, creating more phages as a plaque after it enters the target cell. Therefore, in this way, bacteriophages have extraordinary ability to recognize their target like a biosensor. [82,83,84].Another method for detecting bacteria depends on bacteriophage proteins that have specificity for binding with bacteria[84,85]. Each of them binds to specific molecules on the surface of a bacterium. The reporter then binds to the bacterial surface[86,87]. In one study , Poshtiban and colleagues [88] used the putative RBP of a *Campylobacter jejun*i phage to functionalize paramagnetic beads. The beads were used to concentrate C. jejuni from food samples, which in turn served as a detector.

**K. Tissue Construction by Genetically Bio-Engineered Phages**

Phage display can also be used to create phages containing cell binding peptides , which inturn can do tissue construction. Phages can self-assemble into 2-D and 3-D structures [89,90] and can be used in 3-D printing to create scaffolds for cells to grow on [91,92]. The best choice for this are the filamentous phages containing more than one protein , which can be used for display [93]. Finally, phages are modified both chemically and genetically [89] and if required , can be cultured at various scales [94].

**L. Eukaryotic cell gene delivery**

Interest in using phage vectors is increasing day by day , due to various benefits offered by phages in terms of targeting, safety and cargo capacity [95,97]. Bacteriophages have a large cargo capacity . Also , they can be easily engineered to express eukaryotic cell targeting. Bacteriophages might also be safer than mammalian viral vectors as they lack natural tropism for eukaryotic cells . Also, siRNAs can be delivered using phages [96-98]. In one study , Hajitou and colleagues developed a viral vector named - **AAVP,** in which, a chimeric genome containing an adeno-associated virus (AAV) cassette was initiated into the phage genome and packaged in a M13 phage particle. Also, the international team at Imperial College London showed promising results while using bacteriophages to target cancer cells in the brains of mice. Using that specific approach, they were able to deliver targeted therapy directly to cancer. Also, Antitumor activity of bacteriophage T4 in a mouse B16 (melanoma model) was seen in a study.

Finally, as CRISPR Cas systems are emerges to edit the genetic information, in one study , Qazi and colleagues used **P22 VLPs** to create a programmable delivery vehicle for Cas9.[99]

**M. Phages in making Vaccines**

The natural immunogenicity of some specific phages makes them useful as excellent vaccine delivery vehicles [99,100]. The gene therapy of phages can be applied to DNA based vaccines [101,102] with the another lucrative output of them being the nucleic acid cargo deliverer.[99,103,104]. T4 bacteriophages have been used to display various antigens for HIV, foot-and mouth disease virus (FMDV) and anthrax toxin. [105] The results of these preliminary studies demonstrate the ability of phage-based vaccines to evoke both cell and antibody mediated responses.

# XIX. CONSTRAINTS OF PHAGE THERAPY

Phage therapy can also show some cons.

1. Since , every creature evolves and adapts . Bacterial evolution in respect to phage attacks may hinder the therapy . New mechanisms developed by host bacteria to digest extrinsic bacteriophage DNA (restriction modification) one such example of the evolution .

2. Adsorption blocking and production of extracellular barrier matrix by some bacteria present another problematic side to this therapy .

3. With the mechanism of super injection exclusion (Sie) bacteria can impair genome injection done by phages.

Replication of phage genome can also be inhibited by (BREX) systems , present in bacteria .

4.Pharmacokinetics of phages is much more complex than we thought . Like , phage therapy in animals can elicit immune system to produce antibodies of bacteriophages .

6. All bacteriophages are not good therapeutic agents. The challenge for phage application is their stability and their competency to reach and lyse the bacterial host.

**XX. CONCLUSION – AN END AND A BEGINNING**

While combating bacterial in­fections , the available data on the use of bacteriophages , specifically those of **multidrug-resistant bacteria**, shows promise for the new revolution of phage therapy , as either a sup­plement to antibiotics or as an excellent alternative to it . However, inconsistencies in recent findings on the potential for horizontal gene transfer and the host range , makes it very clear that we need a far better understanding of the interaction between phage, the human host and the microbiome, before doing a large scale implementation of phage therapy . Here , comes the Phage endolysins , which may thus be a much more practical therapeutic tool for their immunological potential and other ***ease of access*** benefits . Finally , these antibacterial bioagents when used in cocktails , like in association with antibiotics or modified mutant phage forms , showed that, this will be an absolute necessity for countering the rising problem of antibiotic-resistant infections as , till now it’s the only effective way to enhance its efficacy .

**XXI. FINAL THOUGHTS**

If there is a disease, the nature will always create a therapy for it. We just need to find it, like we did with the bacteriophages. In the biome it’s always a inter – relationships between a prey and a predator. So, what seems like a predator must be a prey to some other organism or microorganism.

Finally, if the findings of ours, in this review, can help even 1% of the population we would think this work of ours has reached its zenith of success.

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