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Review on phage therapy: A novel approach to combat antibiotic resistance

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Abstract
Almost a century before penicillin was discovered since then antibiotic resistance in bacterial pathogens became a substantial threat to the human race. However, the development of new classes of antibiotics has slowed in recent years due to the complex and lingering process of development and formulation of new antibiotics. There is now a strong need for new antimicrobial therapies. Phage therapy has emerged as a comparatively effective and faster-evolving practice for treating bacterial infections. Bacteriophages, are viruses that specifically kills bacteria and have been used since 1919 as an alternative way of treating infections like Shigella dysenteriae. The host range of lytic phages is practically determined by the fact that most phages are only infectious to bacteria that have their complementary receptor. Phages differ in their host specificity; some are only capable of infection a particular type of bacteria, while others are capable of infecting entire genera. Bacteria have developed an extensive number of defense mechanisms against lytic phage infection, and phages have harvested an equally remarkable range of defense mechanisms against bacterial resistance. This diversity is where phages excel compared to antibiotics. They can target specific bacterial strains, minimizing damage to beneficial bacteria in the body. As the threat of antibiotic resistance grows, phage therapy offers a promising alternative for treating bacterial infections. Phage therapy represents a promising avenue for combating antibiotic resistance and address the root cause of this issue.

Keywords: Antibiotic resistance, phage therapy, bacterial infections, lytic phages

Introduction
Penicillin was discovered by Alexander Fleming's in 1928 which revolutionized the field of medicine and marked the beginning of antibiotics globally. His discovery was a monumental breakthrough that paved the way for the development of many other life-saving antibiotics. In the dawn of antibiotic era. At that time, scientists made the prediction that overuse and misuse of antibiotics could result in antibiotic resistance, a condition in which bacteria develops defense mechanisms against the effects of antibiotics. This suggests that the germs reproduce swiftly and cannot be eliminated by the antibiotics. Treatments for resistant infections are also too much challenging and sometimes completely impossible. The development of resistant in Mycobacterium tuberculosis, the tuberculosis-causing bacteria, during early clinical trials questions upon the effectiveness of streptomycin, one of the first antibiotics used to treat tuberculosis [1]. This marked the beginning of a troubling trend: the emergence of bacteria that were resistant to the drugs designed to kill them. Despite these challenges, the discovery and development of antibiotics continued to flourish for 4 to 5 decades. Years from 1940s to 1960s were considered the golden period for antibiotic, when numerous new drugs were introduced into the clinical practices. These new antibiotics provided effective treatments for a wide range of bacterial infections and saved countless lives. However, by the late 20th century, the pace of antibiotic discovery began to slow-down, and alarming increase of antibiotic resistance became a cause of concern. The overuse and misuse of antibiotics became the primary cause of this crisis [2]. Time taken in the identification of causative microorganisms and unnecessarily use of wide spectrum antibiotics in addition to overuse and misuse of antibiotics leads to dramatic increases in resistance cases. When resistance bacteria related infections are handled with poor practices leads to disseminate to other person and environment [3]. Bacteriophage which are shortly known as Phages are bacteria-specific viruses that are used to treat bacterial infection in Phage therapy. Phage therapy works by utilizing bacteriophages and viruses that infect and destroy bacteria. Term “bacteriophage” was coined by French-Canadian microbiologist Felix

d'Herelle meaning bacterium eater [4]. Herelle noticed certain invisible bacteria that were "antagonistic" to bacteria in the stool filtrates of dysentery patients. He hypothesized that "ultraviruses," a filterable virus, were a contributing element to bacterial infection. He demonstrated that phage concentrations rise as the illness progresses and peak during healing. After those successes, d'Herelle start testing its safety and efficacy on humans. At first, on himself, his family and on his co-worker. Then lately on patients suffering from bacillary dysentery and cholera [5] which led to the discovery of phage therapy in 1917.

Due to the rise of antibiotic resistance at alarming rate phage therapy experienced a renaissance. This review aims to put a ray of light on phage therapy which can be a novel approach in combating antibiotic resistance.

Bacteriophage

Bacteriophages are the viruses which replicates by infecting bacterial cells. They are ubiquitous in the environment and the most primitive organisms on Earth. Their diversity in size, appearance, and genetic makeup is astounding. Bacteriophages comprise of nucleic acid genome covered in a capsid that has been encoded with phages, protecting the genetic information and facilitating its transfer in the host cell. Phages are immobile organisms and they reach their prey by Brownian motion. Similar to other viruses they exhibit strong host-species specificity, typically limiting their infection to a single bacterial species or even individual strains within it [6].

Types of Bacteriophages based on their Life Cycle

1. **Virulent Phages:** These causes the host cell to lyse (burst open), known as the lytic cycle. In lytic cycle mechanism, the bacterium is affected intracellularly, leading to the lysis induced by phage in bacterium. This is more common than compare with lysogenic cycle. The lytic cycle begins with the attachment of the phage to specific receptors present on the bacterial cell wall. Once attached, the phage begins to inject its genetic material into the host cell. The phage genome then takes over the host cell's machinery, directing it to produce phage components [7]. These components assemble to form new phages, which eventually lead to the lysis of the host cell and the release of the newly formed phages that will now infest other bacterium.
2. **Temperate phages:** these either causes host cell lysis or integrate their viral DNA into the bacterial host cell genome, known as the lysogenic cycle. Temperate phages can choose between the lytic and lysogenic cycles. In the lysogenic cycle, the phage DNA integrates into the bacterial chromosome, knows as prophage. The prophage replicate along with the bacterial DNA and passes on to daughter cells during cell division. Under the stressful conditions, the prophage can excise itself from the bacterial chromosome and enter the lytic cycle, leading to cell lysis [8].The integration of phage DNA into the bacterial DNA can have significant effects on the host cell. It can alter the gene expression leading to changes in cell phenotype. In some cases, the presence of a prophage can provide the host cell with new traits, such as antibiotic resistance or virulence factors, through a process known as lysogenic conversion.

Lytic phage replication cycle

During a lytic infection cycle (depicted in figure 1), a

bacteriophage (phage) initiates its attack on a bacterial cell by first attaching to specific receptors on the bacterial cell wall. These receptors are mostly proteins or sugars that the phage recognizes and binds irreversibly. Once attached, the phage injects its genetic material into the bacterium [9].Inside the bacterium, the phage hijacks the bacterial machinery for transcription, translation, and replication to begin producing new viral particles. The phage genome directs the synthesis of viral components, including structural proteins and enzymes, using the bacterial resources. This process essentially reprograms the bacterium to become a factory for producing more phage particles [10, 1]. As new phage particles are assembled, the bacterium undergoes lysis, a process in which the cell membrane ruptures, releasing the replicated phages into the surrounding environment continuing this cycle infection.

Bacteriophages mechanism of action

Bacterial lysis is necessary for the discharge of lytic phage offspring from infected bacterial cells. Amurins, which are single proteins, are used by certain lytic bacteriophages to prevent the formation of peptidoglycan [11]. To destroy the host cell, the majority of them, nevertheless, use two protein groups holins and endolysin and together they constitute the holin-lysin system [17, 31] (depicted in figure no. 2). Firstly, phages pierce the cytoplasmic membrane of the host bacterium, allowing endolysins to access through bacterial peptidoglycan. Holins act at a specific moment, they regulate the accessibility of bacterial murein for phage endolysins coordinating the holin-lysin system's activity in latter stages of the phage replication cycle [12, 31]. Phage endolysins (enzymatic proteins) are in charge of breaking down the bacterial cell walls by hydrolyzing bacterial peptidoglycan. By destroying murein, endolysins carry out the functions of endopeptidase, amidase, glycosidase, or lytic transglycosylase to eliminate bacterial cells[14]. Endolysins stimulate the release of virion offsprings at the end of the phage replication cycle [15, 16]. Distinct enzyme targets are targeted by different endolysins so for those targeting gram-positive and gram-negative bacteria have different architectures [30]. Gram-negative bacteria have an outer membrane around them, which limits external access to the cell wall. This appears to be the cause of the fact that endolysins that are meant for gram-negative bacteria are tiny globular proteins containing single domain Enzyme-Active Domain (EAD), whereas endolysins designed for gram-positive bacteria also have a Cell Wall Binding Domain (CBD) [17]. Gram-positive bacteria are the target of an enzyme that binds to their peptidoglycan surface via their CBD. CBD optimizes endolysin's hydrolytic activities by coordinating with EAD, which catalyzes an enzymatic protein function. Endolysin binds firmly to one peptidoglycan structural site during the process. The holin-lysin system is in charge of breaking the cycle of phage infection at a certain moment. The mechanism elucidated by the dual-start hypothesis. According to this hypothesis, the ratio of holin to antiholin, its antagonist, determines how long bacteria take to lyse. An open reading frame encodes both of them. By managing their translational expression, the ratio of holin to antiholin is tightly controlled. Loss of plasma membrane integrity occurs after an increase in the holin-antiholin ratio, allowing endolysin to enter the periplasm and start breaking down the host peptidoglycan [18].

Case study of Dr. Tom Patterson on Phage Therapy

While on vacation in Egypt, Dr. Tom Patterson, a psychiatry professor at UCSD School of Medicine, became infected with a fatal infection that was caused by MDR *Acinetobacter baumannii*. Antibiotics were unable to manage his infection. "There's nothing else that we can do," the physicians essentially told his wife Dr. Steffanie Strathdee, an associate dean of global health sciences at the University of California as well as co-founder and co-director of the Center for Innovative Phage Applications and Therapeutics. Additionally, you could see him deteriorating in front of us." She searched the internet frantically, hoping to find anything that would save Patterson before time ran out. She became interested in phage therapy after reading an article about it, and she brought up the concept to Patterson's doctors. They agreed to test it after receiving FDA approval. The team turned to researchers from Texas A&M University's Center for Phage Technology and experts from the U.S. Navy to find phages that could eliminate Patterson's A. baumanniiisolate. The hunt for new phages from sewage, barnyard manure, and even the bilges of Navy ships proved to be fruitful. The hunt involved searching through pre-existing phage libraries, which are collections of phages already isolated from varied sources. Following intravenous phage mix administration, Patterson showed improvement almost immediately. Nine months after being admitted to the hospital, he fully recovered and returned home (Figure-3). Their adventure was chronicled in a book called The Perfect Predator, which was released by Patterson and Strathdee four years later after this incident [19].

Clinical Studies on Phage Therapy

Phages have been used in medicine to treat a variety of infections since the early 1920s. The safety and effectiveness of this therapy were seriously questioned, though, due to the varied outcomes of phage experiments that were reported during the 1930s, the absence of controls, and the improper characterisation, manufacturing, and purification of phage preparations. As a result, phage therapy has only been used in a few Eastern European nations where research has shown that phages are effective in treating specific infections with no side effects [20]. However, the absence of validation in accordance with evidence-based medicine, such as clinical studies, continues to encourage regulatory bodies and Western physicians to be reluctant to employ phage therapy. To be considered as an effective substitute to antibiotics, phage treatment requires definitive efficacy data from randomized controlled clinical trials. Over the past few years, more clinical trials have been conducted to address this issue, although only a very small number have been finished as of yet. Wright *et al*., conducted phase I & phase II clinical trial under the supervision of Central Office for Research Ethics Committees (COREC) and the UK Medicines and Healthcare Products Regulatory Agency (MHRA) in 2009. The experiment was randomized, double-blind, and placebo-controlled. In order to evaluate the safety and effectiveness of a phage preparation made up of six phages for the treatment of otitis caused by antibiotic-resistant Pseudomonas aeruginosa, this experiment was conducted on 24 patients with chronic otitis. Only three of the twelve patients receiving phage treatment appeared to be healed by the time the trial ended on day 42, despite improvements in

all clinical indications (such as ulceration, inflammation, discharge type and quantity, and odor) in those treated with phages [21]. Notably, no severe side effects were mentioned. Rhoads and associates published a second randomized, a double-blind, controlled trial that looked at the safety rather than the effectiveness of a phage cocktail that targets *Escherichia coli, P. aeruginosa,* and *Staphylococcus aureus* in the treatment of venous leg ulcers (VLU)Forty-two VLU patients participated in the first phage therapy trial conducted in the United States. For a maximum of 24 weeks, patients received topically applied phage cocktail or saline solution (control) treatment for 12 weeks [22]. Phage treatment did not have any negative side effects, and there were no appreciable variations in the frequency and rate of healing between the phage-treated and control groups. This is not shocking because the phages' ability to infect the bacteria that cause the VLU was not examined. The researchers recommend that a phase II efficacy trial be conducted to assess the effectiveness of the phage preparation. The experiment should involve a bigger sample and wounds infected with bacteria that are receptive to the phage cocktail. PhagoBurn trial (2013) was the largest clinical trial on phage therapy carried out in Europe under good manufacturing procedures (GMP) and good clinical practices (GCP). 27 patients with burn wound infections were enrolled in this multicenter randomized controlled phase I/II clinical trial from hospitals in France and Belgium. The patients were randomly assigned to receive either standard care (1% sulfadiazine silver emulsion cream) or phage therapy (a cocktail of 12 lytic phages) in order to compare the tolerability and efficacy of both treatments in patients with *P.aeruginosa*infected wounds. Topically, both treatments were applied for seven days, followed by a 14-day recovery period. While there was a slower rate of improvement compared to the control group (normal care), overall the phage cocktail was effective to reduce the bacterial burden in burn wounds. In a positive aspect, the group who received phage treatment did not experience any negative side effects. The phage cocktail's limited efficacy was attributed to a notable decline in phage titre during GMP manufacture, which resulted in much lower phage concentrations for the participants than originally thought [23]. More significantly, prior to therapy, the wound bacteria's susceptibility to the phage mixture was not determined. It was eventually discovered that the bacteria in the patients whose phage therapy failed were resistant to low phage dosages.

Bacteriophages and Host Immune System

Considering phage therapy carries the potential for immunological reactions, research on the relationship between immunity and phages is crucial to the sensible application of this treatment. The location of the bacterial infection and the place at which therapeutic phages are injected determine the immune response against bacteriophages [24]. Certain phages are linked to diet and the eukaryotic component of the gut microbiota in physiological settings. The presence of anti-phage antibodies in the sera of diverse species, including humans, indicates that animals and people naturally come into contact with different types of phages on a frequent basis. Additionally, the generation of antibodies is induced by the oral administration of phages during phage therapy for bacterial infections caused by *Staphylococcus, Klebsiella, Escherichia, Proteus,* and *Pseudomonas*. After consuming significant doses of phages, there is no indication of immunological problems [25]. Furthermore, there have been no negative effects from phage topical treatments. The bloodstream and other internal organs, which are not phages' natural habitats, provide a different picture. Bacterial pathogens injected intravenously significantly enhance both innate and adaptive immunity. Research indicates that phages can enter the bloodstream through any mode of administration. Phagocytic cells quickly remove certain phages from the blood and internal organs if there are no host bacteria present. Investigation has demonstrated that individuals receiving phage therapy exhibited a decrease in fully developed neutrophils and an increase in neutrophil precursors in their peripheral blood. These findings suggested that phage preparations had the ability to trigger the innate immune response, which aids in the removal of bacterial infections [26]. Phages, however, can also influence the metabolic processes of immune cells. For instance, research indicates that bacteriophages reduce antibacterial innate immunity and significantly suppress ROS formation in response to harmful microorganisms. Phage therapy involves the induction of particular antibodies, known as neutralizing antibodies, against the target bacteria by the phages. This usually prevents the phages from effectively lysing the bacteria *in vivo.* As a matter of fact, antibodies that attach to the virion's critical epitopes for infection of the host cells are known as neutralizing antibodies and duration how long these particular antibodies will be in circulation is still unknown. Neutralizing antibody concentration is dependent on a number of factors, including 1st the mode of phage administration (oral and topical administration generate a little rise in antibodies) and 2nd the dose regimen [27]. Research has indicated that anti-phage neutralizing antibodies are likely one of the primary causes of the phage therapy's limited success. The kinetics of phage action are far faster than the host's manufacture of neutralizing antibodies, according to Sulakvelidze *et al.,* hence the creation of neutralizing antibodies shouldn't be a major issue during the first treatment of acute infections. Anti-phage antibodies, however, may be a problem if they persist when the second round of treatment is started. This problem can be resolved by repeated phage administration, raising phage concentration and utilizing other phages [28]. Aside from the humoral immune response, cellular immunity is also vital against phages. According to Langbeheim, guinea pigs who received subcutaneous injections of MS-2 phages experienced a severe hypersensitive reaction. *In vitro* studies have produced comparable outcomes [29]. Nevertheless, some other research suggested that the inactivation of phages is mostly independent of cellular immune responses. They demonstrated that T cell-deficient mice cleared the T_7 phage at a rate comparable to that of wild-type mice [30]. Further research is necessary on this problem in light of the inconsistent results. It's interesting to note that certain research has demonstrated the immunosuppressive potential of phages. In his investigation into the function of bacteriophages in the formation of transplantation tolerance, Górski noted that phages have the ability to prevent T cell activation. Furthermore, Kniotek showed that humoral immunity is also lowered following phage delivery. The collective findings indicate that testing each phage's immune response is crucial, especially if intravenous therapy is being explored. Phage treatment has

not caused any significant immunologic reactions in prior clinical or animal trials [31].

Therapeutic Potential of Phages Therapy

1. The ability to "self-replication" sets phages apart from traditional antibiotics and contributes to the effectiveness of phage therapy.
2. Certain phage species have depolymerases on their tail structures that can break down the extracellular matrix of bacteria that create biofilms.
3. Due to the phages' great host-specificity for bacteria, phages have little to no impact on human cells or the bacterial flora that exists in humans.
4. Phages no influence from antibiotic resistance mechanisms.
5. Bacteria have developed a wide range of defense mechanisms against lytic phage infection, and phages have developed an equally remarkable array of defense mechanisms against this resistance.
6. Even if somehow a bacteria developed a resistance for phage, then it will it's F-factor of antibiotic resistance.
7. Phages work against both treatable and antibiotic-resistant bacteria.
8. Phages are also effective against SUPERBUGS that are resistant to most, if not all antibiotics.

Precautions

The potential formation of bacteriophage-insensitive mutants (BIMs), which could compromise the efficacy of phage therapy, is one of the main issues with this treatment. The issue of bacterial resistance to phages has been the subject of numerous researches in recent years, showing that the generation of phage-resistant mutants is common and practically inevitable [32]. Bacteria employ numerous resistance mechanisms to counteract phage evasion. These mechanisms include elimination or alteration of bacterial receptors to prevent phage adsorption, phage dank entrance is prevented by super infection exclusion mechanisms, degradation of phage dank by crispr-cas systems or restriction-modification systems and other related systems (brex, disarm, etc.), using systems of abortive infection that prevent transcription, translation and replication of phage and anti-phage signaling systems based on cyclic oligonucleotides [33]. There are various ways to overcome bacterial resistance to phages. The most popular is a phage cocktail, which is a single preparation that combines many phages that have complimentary host ranges and selectively target distinct receptors. Such mixtures not only exhibit greater coverage against a specific bacterial species but also halt the emergence of BIMs [34]. These are the principal justifications for why phage cocktails are used in therapy instead of single phage preparations. Phage cocktails can be made specifically for a patient or they can have a fixed composition that covers a wide host range [35]. Since phages are abundant and diverse in nature due to their ongoing co-evolution with bacteria, this can be very straightforward for them, but it is difficult for antibiotics. Lastly, to prevent the emergence of bacterial resistance and to increase the effectiveness of treatment, phages can be used with antibiotics or other antimicrobial treatments [36] (Depicted in figure-4).

Discussion and Conclusion

Phages are highly selective and do not have any effect on beneficiary bacteria's which is an advantage over broad-spectrum antibiotics. Another advantage of phage therapy is its ability to get evolved together with bacteria which ensure its effectiveness over time. These key features of phages make phage therapy a potentially valuable tool in the ongoing battle against antibiotic resistance [37]. However, there are also challenges associated with phage therapy. One of the main challenges is the lack of standardized regulations and protocols for phage therapy. Unlike antibiotics, which are rigorously tested and regulated, phage therapy is still relatively uncharted territory in many parts of the world. Additionally, there are concerns about the potential of phages to trigger immune responses in patients. While phages are generally well-tolerated by the immune system, there is still a risk of adverse reactions, especially in patients with compromised immune systems. Despite these challenges, the potential benefits of phage therapy are significant. With antibiotic resistance on the rise, there is an urgent need for alternative treatments, and phage therapy offers a promising avenue for further exploration and development. As research in this field continues to advance, phage therapy could play a crucial role in the future of medicine, offering new hope for fighting against drug-resistant infections.

Future scope

Safety, efficacy, quality and shelf life of phages will be improved if there will be proper safety guidelines and standard protocols for the manufacturing and storage of phages[38]. There is also a task of minimizing the mutation rates in phage which will increase the selectivity and specificity[39]. Bacteria are typically present in the form of biofilms (self-produced matrix comprised of proteins, lipids, polysaccharides, and extracellular DNA) in both the natural world and the human body [40, 41]. Bacterial cells work together closely in biofilms as a means of surviving and persisting in hostile conditions, such as by increasing their resistance to antibiotics. One important component lessening a phage's efficacy is biofilm which act as a barrier for phage diffusion [42, 43]. Numerous phages have the ability to synthesize depolymerases which are the enzymes that break down the capsular polysaccharides of bacteria. This allows the phage to bind to its receptor on the surface of the bacterium and facilitate the entry of phages in the bacterial cells [44]. We can also genetically modify the phages to produce depolymerases to counteract the problem of biofilms (Depicted in figure-5). Quorum sensing, which uses extracellular signal molecules that detect population density to coordinate gene expression, is often responsible for controlling gene expression in biofilms [45]. Bacteria can employ quorum sensing to respond to phage infections by controlling the expression of phage receptors, CRISPR-Cas systems, and biofilm matrix development, among other mechanisms [46]. By encoding receptors for the bacterial quorum sensing molecules or, once inside the bacterium, producing their own extracellular signaling molecules, several phages have evolved techniques to take use of the bacterial quorum sensing system to direct their decision between lysis and lysogeny. Phage populations allow them to detect favorable or unfavorable conditions for lytic

development [47]. It is evident that phages have the ability to regulate biofilm infections. Before phage therapy becomes a viable treatment for infections linked to biofilms, additional research is necessary due to the intricacy and diversity of phage-biofilm interactions [48].

Table 1: Few case studies on phage therapy

Case Study	Outcomes
Age 42 male in Israel was surfing from Trauma related tibia infection caused by XDR *Acinetobacter baumannii* and *MDR Klebsiella pneumoniae*	Received intravenous phage dose, rapid tissue healing and eradication of positive cultures (8 months follow up) were observed.
Age 26 female in USA surfing from Cystic fibrosis infected by MDR *Pseudomonas aeruginosa*	Received intravenous phage dose, No recurrent pneumonia and cystic fibrosis exacerbations within 100 days of follow-up was observed.
Age 80 female in France suffering from Prosthetic joint infection (hip) infected by MDR *Pseudomonas aeruginosa* and penicillin-resistant *Staphylococcus aureus*	Local injection (joint cavity)
Age 63 female in USA suffering from Diabetic foot ulcer with osteomyelitis infected by Penicillin-resistant *Staphylococcus aureus*	Received phage dose by Local injection (soft tissue) complete resolution of the infected ulcer and the osteomyelitis (proved by follow-up 3 years later)
Age 17 female in USA suffering from Cystic fibrosis infected by MDR *Achromobacter xylosoxidans*	Received oral and inhaled phage - dyspnea resolved, cough reduced, lung function improved. From 54% to 84% after 12 months treatment.
Age 67 male in USA suffering from Recurrent pneumonia infected by MDR *Pseudomonas aeruginosa*	Received intravenous phage- resolution of pneumonia and improvement of respiratory status.
Age 68 male in USA suffering from Necrotizing pancreatitis caused by MDR *Acinetobacter baumannii*	Received intravenous dose of phage - clearance of infection and return to health.
Age 61 male in Belgium suffering from Septicaemia with acute knee injury infected by Colistin only sensitive *Pseudomonas aeruginosa*	Received intravenous phage dose - Blood cultures negative, fever disappeared, kidney function recovered.
Age 27 male in UK suffering from Burn wounds (50% of surface area) infected by *Pseudomonas aeruginosa*	Received topical phage dose - successful extensive grafting
Age 76 in USA suffering from Prosthetic vascular graft infection with associated fistula infected by *Pseudomonas aeruginosa*	Received local phage dose no signs of recurrence in 18 months

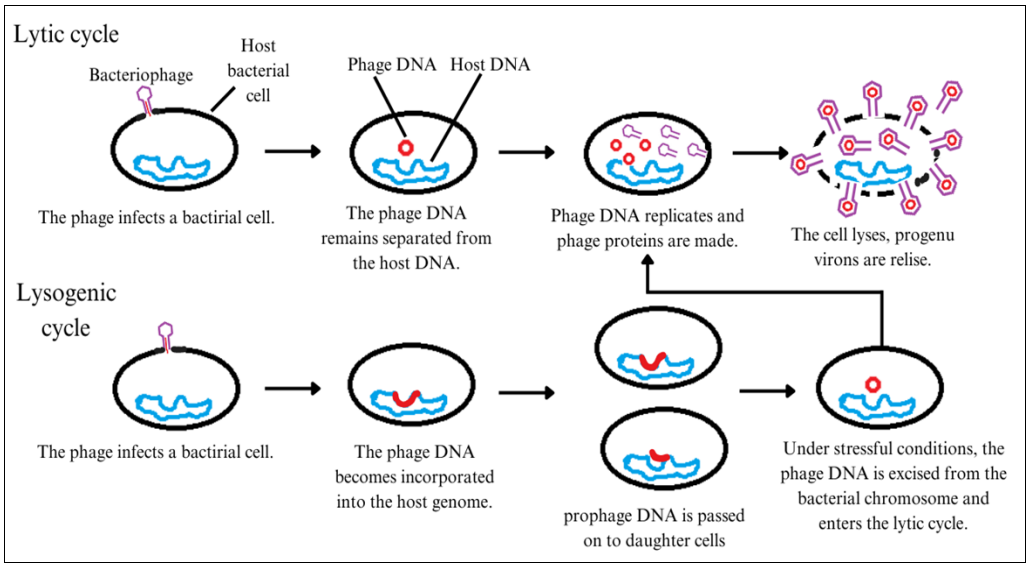

Fig 1: Replication cycles of Phages

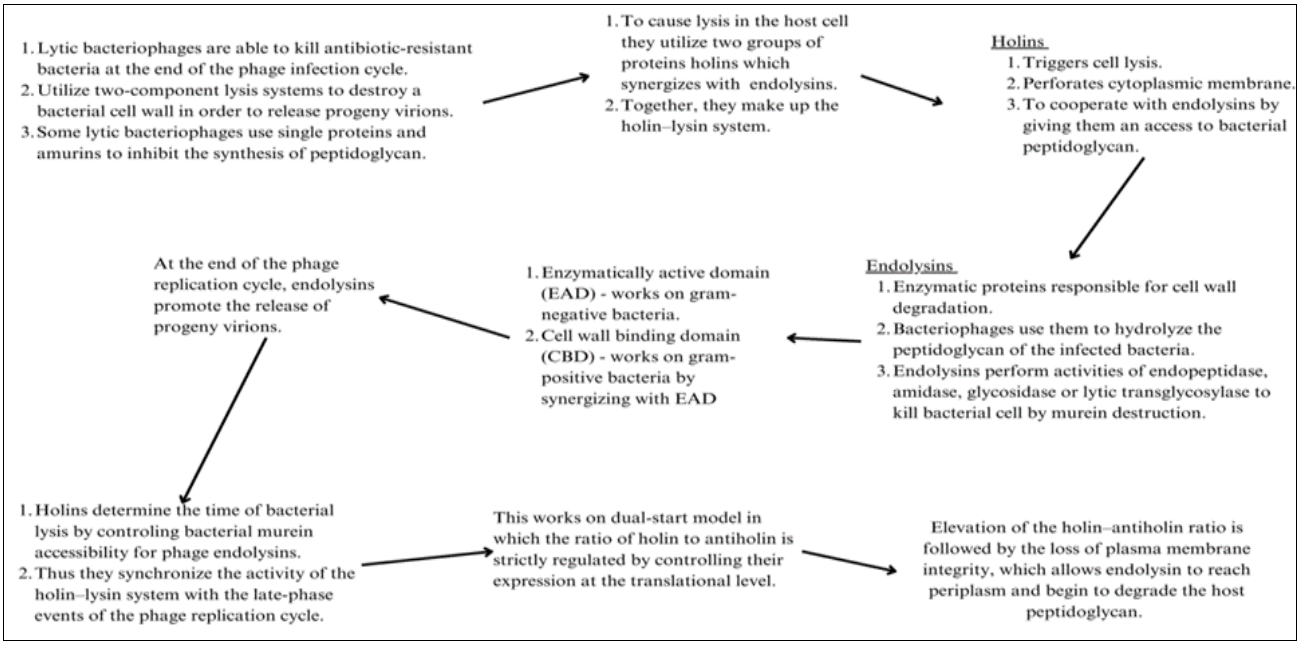

Fig 2: Holin-lysin system [17'31]

Fig 3: Image of Dr. Tom Patterson [19]

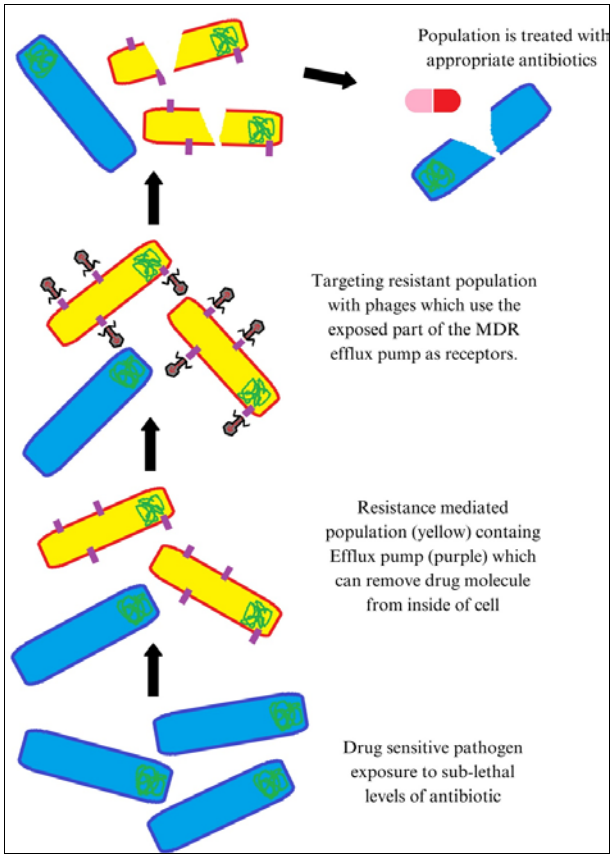

Fig 4: Phage and Antibiotics Working Together [36]

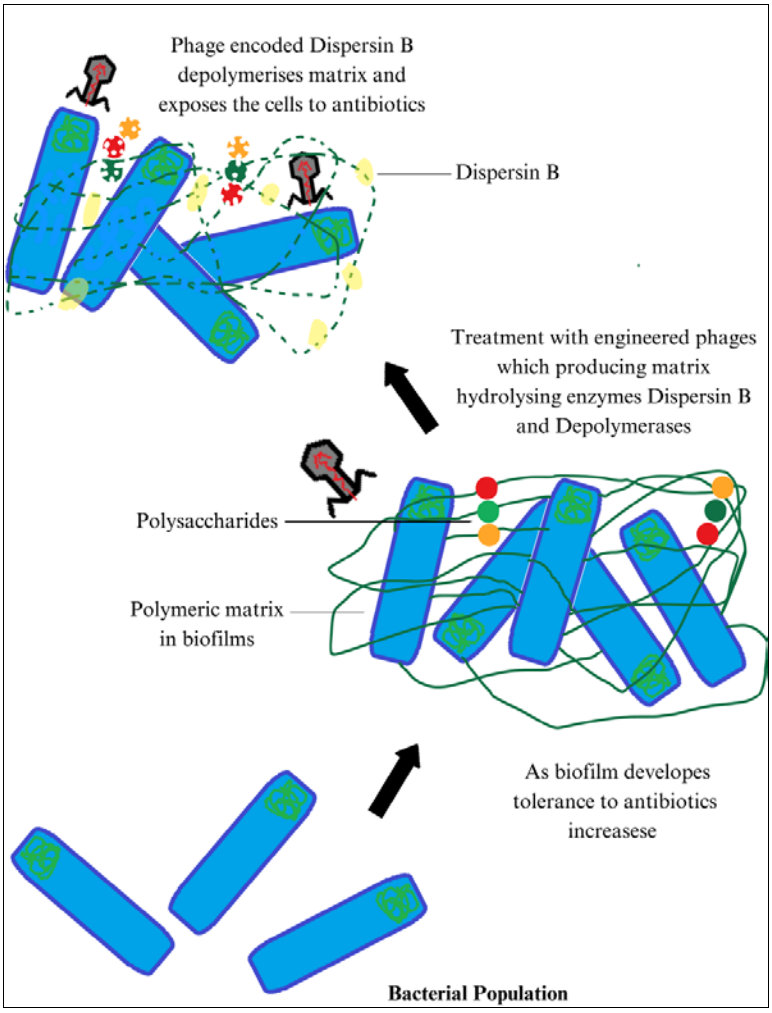

Fig 5: Phage-Biofilm Interactions

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