



Review on the use of Bacteriophages as a promising way of mitigating the crisis of Antimicrobial resistance

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ABSTRACT

Antibiotic resistance is considered as a major threat to therapeutics in this era. This resistance has occurred due to various actions that neglect the ethical use of antimicrobials and antibiotics ending up in the abuse of these drugs in clinical, veterinary or agricultural practices. As the number of resistant pathogens increase, more drugs are being produced to cope with the situation and many research methodologies have been carried out in search of an alternative antimicrobial to assuage the threat of antimicrobial resistance. Consequently, phage therapy was discovered and considered effective as well as an alternative way to control the problem of antimicrobial and antibiotic resistance. Bacteriophages are viruses that infect and lyse bacteria. They are commonly referred to as “phage”. They are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. The nucleic acids of phages often contain unusual or modified bases, which protect phage nucleic acid from nucleases that break down host nucleic acids during phage infection. Depending upon the phage, the nucleic acid can be either DNA or RNA but not both. Due to their unique characteristics they are considered more effective than other alternatives. Previous trials in the use of bacteriophages have proved that phage as therapeutics have the ability to target bacteria of certain strains or species, without any harmful effect on the rest of the bacterial microflora. Moreover, bacterial antibiotic resistance is not a barrier for phage therapy and they are more effective when combined with antibiotics. This paper briefly reviews the use of phage therapy as an effective alternative to mitigate the global anti microbial resistance problem we are currently battling.

Key words: Alternative therapy, Antibiotic resistance, Bacteriophage, Phage therapy

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1. INTRODUCTION

Antimicrobial resistance as defined by WHO, is a natural phenomenon which includes all forms of resistance to medicines on the part of viral, parasitic, fungal or bacterial infections [5]. Antibiotics are very important therapeutics that is used to control bacterial infections. They are considered as one of the major contributions to modern science. On the other hand, the emergence of bacterial resistance to antibiotics following extensive clinical, veterinary or agricultural usage has made antibiotics less and less effective [13]

In Addition, very frequent and inapt use of antibiotics, lack of educational awareness, lack of regulatory authority regarding antibiotic usage, production, and marketing makes the situation worse [56] The report by WHO on global surveillance of AMR revealed that antibiotic resistance is no longer a prediction of the future, it is happening now [54]. Treatable infections and minor injuries can once again be a problem and many recent medical advances are threatened [42].

In the developing world, there have been concerns about inappropriate drug promotion and the impact on public health for at least two decades. For this reason, the WHO produced a guideline for drug promotion entitled "Ethical Criteria for Drug Promotion" [53].

The problem caused by antibiotic resistance demands that new and improved efforts be made in search for antimicrobial agents that will be effective against pathogenic microorganisms without any fear of resistance development. Some of the new techniques or antimicrobial alternatives that should be considered include vaccines, probiotics, immune boosting trace elements and some bioactive photochemicals [33].

Another promising technique for overcoming antibiotic resistance includes phage therapy which involves using specific proteins from phages that lyse bacteria, which can be

isolated and used to treat bacterial infections. Phage therapy significantly increases the ability to combat antibiotic resistance of bacteria's that are known to cause variety of health problems like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* with 90% efficiency (Pirisi, 2000). Moreover, phages are effective against ten of the most antibiotic resistant strains of *E. coli* [37].

Hence, the main objective of this paper is:

To provide an insight on bacteriophage therapy, as an effective alternative yet promising way to diminish the problem of antibiotic resistance in the current era of multidrug resistant pathogens.

2. LITERTURE REVIEW

2.1. Drug Resistant Bacteria

One of the first and most resistant pathogen is *Staphylococcus aureus* which is a bacterium commonly found as commensals of the mucous membranes. It was one of the earlier bacteria in which penicillin resistance was found which now appears to be endemic in many urban regions [27].

Escherichia coli were found to be resistant to five fluoroquinolone variants in 1993. Additionally, *Mycobacterium tuberculosis* is now commonly resistant to isoniazid, rifampicin and sometimes universally resistant to the common treatments. *Pseudomonas aeruginosa* is a highly prevalent opportunistic pathogen, but with low antibiotic susceptibility [35].

Streptococcal organisms are other major culprits. There have been increasing incidence of their resistance to other antibiotics, including resistance of *Streptococcus pneumoniae* to penicillin and other beta-lactam, though all strains have remained relatively and uniformly sensitive to penicillin [27].

2.2. Mechanism of Drug Resistance

Drug resistance can be described in two ways, the first one is intrinsic or natural whereby

microorganisms naturally do not possess target sites for the drugs and therefore the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures. This is true especially for those drugs that require entrance into the microbial cell in order to initiate or effect their action. Resistance can also be acquired, whereby a naturally susceptible microbe acquires ways of not being affected by the drug. Acquired resistance mechanisms can occur through various ways but there are a few known mechanisms [14].

The few known mechanisms include:

- i. Drug inactivation or modification: Some microorganisms are capable of enzymatically inactivating drugs. This is seen in enzymatic inactivation of Penicillin G in some penicillin resistant bacteria including some strains of *Staphylococcus aureus* [33].
- ii. Alteration in binding sites: Some antibiotics have binding proteins on the cell walls of sensitive microorganism. Alterations in such proteins will bring about development of resistance against such microorganisms. Examples are the binding target site of penicillin in MRSA and other penicillin resistant bacteria and mutations at key sites in DNA gyrase or Topoisomerase that decreases affinity of cell wall to quinolones, thus decreasing the drug's effectiveness [8].
- iii. Alteration of metabolic pathway: Some sulfonamide-resistant bacteria do not require PABA, an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid. This makes it easy for such microorganisms to develop resistance [33].
- iv. Reduced drug accumulation: Decrease in drug permeability and/or increase in active efflux of the drugs across the cell surface

causes development of resistance [25]. This is seen in development of resistance against fluoroquinolone where efflux pumps can act to decrease intracellular quinolone concentration [40].

2.3 Bacteriophage

2.3.1. History of bacteriophage

Bacteriophages are viruses that infect bacteria. Their application has been investigated extensively, such as an indicator of fecal contamination (Endley *et al.*, 2003) and against antibiotic resistant bacteria [55].

The use of bacteriophages to treat pathological bacterial infections is called phage therapy. This therapy began in France in 1919. By the 1930s, phage therapy was applied in Europe and the United States [18].

Relatively soon after their discovery, the first use of phages as antimicrobial agents proceeded with the first phage therapy publication appearing in 1921. Approximately over the same period, D'Hérelle was observing a role for naturally occurring bacteriophages in the control of bacterial disease. The disease is only definitely overcome at a time when the virulence of the bacteriophage is sufficiently high to dominate the resistance of the bacterium. As the problem of antibiotic resistance became more apparent during the 1990s, numerous individuals as well as companies turned both to phage therapy and those institutions that still routinely practiced phage therapy. The result has been a growing interest in the potential to use phages as antibacterial agents within the context of medicine as well as veterinary medicine, agriculture and other circumstances [31].

During subsequent studies it was found that a single dose of specific *E. coli* phage reduced the number of target bacteria in the alimentary tract of calves, lambs and piglets infected with a diarrhea causing *E. coli* strain. The treatment also stopped the associated fluid loss, and all

animals treated with phages survived the bacterial infection [43,44,45].

One of the best known series of recent studies on the use of phages in veterinary medicine came from the laboratory of William Smith at the Institute for Animal Disease Research in Houghton, Cambridge shire, Great Britain. In one of their early papers, the authors reported the successful use of phages to treat experimental *E. coli* infections in mice [46].

2.3.2. *Biology of bacteriophage*

i. Structure

ii. Classification of phage

The first bacteriophage known to science was the Bacteriophagum intestinale described by Félix d'Hérelle [9], an enterobacterial phage or a mixture of phages that was considered by D'Hérelle as a single virus with many races [9].

In 1961 Eisenstark published the first list of phages, which included 111 phages with tailed,

Bacteriophages are viruses which have either DNA or RNA as their genetic material. They may appear as both single and double stranded forms. Their structure is similar to the living organisms found in the environments, with a polynucleotide chain consisting of a deoxyribose (or ribose) phosphate backbone to which are attached to a specific sequence of the four nucleotides adenine, thymine (or uracil), guanine, and cytosine. It is exceptional in single stranded phages where two complementary chains are paired together in a double helix [3]

cubic

or filamentous morphology [11]. A second phage list, published by Fraenkel Conrat in 1974, included 411 bacterial viruses and the dimensions and physicochemical properties of many of them [13]. At present, over 5000 bacteriophages have been studied by electron microscopy and can be attributed to 11 virus families [1].

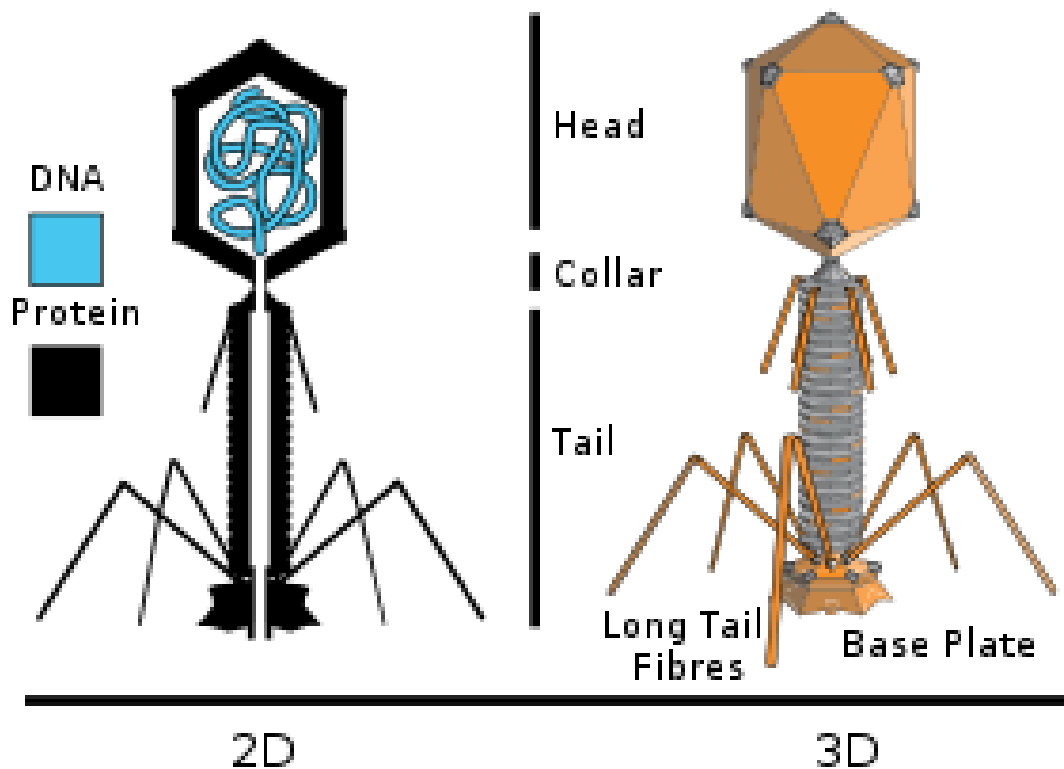
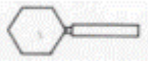
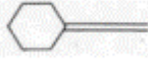







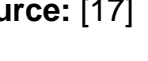
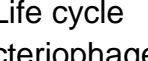


Figure 1: Structure of bacteriophage

Source: <https://en.wikipedia.org/wiki/Bacteriophage>

Table 1: Bacteriophage Families

Shape	Order or family	Nucleic acid, particulars, size	Member	Number
	Caudovirales	dsDNA (L), no envelope		
	<i>Myoviridae</i>	Tail contractile	T4	1312
	<i>Siphoviridae</i>	Tail long, noncontractile	λ	3262
	<i>Podoviridae</i>	Tail short	T7	771
	<i>Microviridae</i>	ssDNA (C), 27 nm, 12 knoblike capsomers	ϕ X174	38
	<i>Corticoviridae</i>	dsDNA (C), complex capsid, lipids, 63 nm	PM2	3?
	<i>Tectiviridae</i>	dsDNA (L), inner lipid vesicle, pseudo-tail, 60 nm	PRD1	19
	<i>Leviviridae</i>	ssRNA (L), 23 nm, like poliovirus	MS2	38
	<i>Cystoviridae</i>	dsRNA (L), segmented, lipidic envelope, 70–80 nm	ϕ 6	3
	<i>Inoviridae</i>	ssDNA (C), filaments or rods, 85–1950 x 7 nm	fd	66
	<i>Plasmaviridae</i>	dsDNA (C), lipidic envelope, no capsid, 80 nm	MVL2	5

Source: [17]

iii. Life cycle

Bacteriophages have the ability to interfere between lysogenic cycle and lytic cycle, which follow a unique pathway to control bacteria. In the lytic cycle, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterial DNA. In this case, they grow in high numbers in bacterial cells, leading to cellular lyses. At the end of the cycle, a release of newly formed phage particles is observed ([22]).

In the lysogenic pathway, the phage genome integrates as part of the host genome. It stays in a dormant state as a prophage for an extended period of time. Later, due to certain conditions such as adverse environmental condition of the host bacterium, the prophage will be activated, turning on the lytic cycle. At

the end, the newly formed phage particles are ready to lyse the host cell [26].

The life cycle of phages can be distinguished into four basic steps. First, is an extracellular stage during which the virion capsid protects the phage genome such as from nucleases. This is preceded by an infection stage during which a majority of phage physiological aspects are observed [4].

Infection ends with release, usually through phage-induced bacterial lyses, thus initiating the extracellular phase. The extracellular phase ends and infection begins in the course of what variously is described as attachment, adsorption, uptake, penetration, ejection, injection, and/or translocation [22].

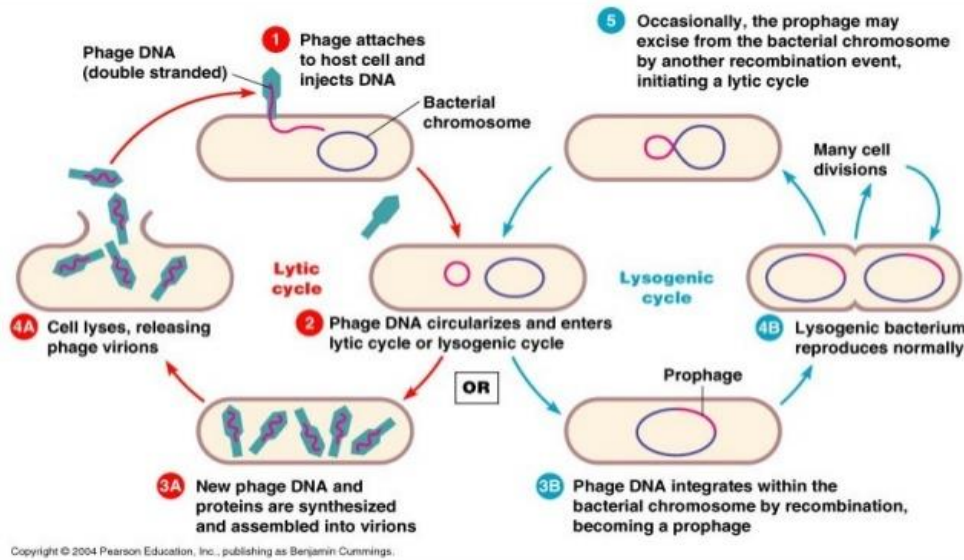


Figure 2: Two cycles of bacteriophage reproduction

Source: <https://image.slidesharecdn.com/lamdaphage-131130104245-phapp02/95/lamda-phage-6-638.jpg?cb=1385808200>

2.3.3. Mechanism of killing bacteria

The first step of phage infection is adsorption to the receptor, with a protein or sugar found on the bacterial surface. After adsorption, the phage injects its DNA into the bacterial cytoplasm. Then, the injected DNA is replicated, and multiple copies of DNAs are synthesized and taken into the capsid, which is constructed de novo during the late stage of phage infection. Descendant phage particles

are completed by the attachment of a tail to the DNA-filled head. Finally, the progeny phages are liberated by the coordinated action of two proteins, holin and endolysin (lysin), coded by the phage genome. Lysin is a peptidoglycan-degrading enzyme (peptidoglycan hydrolase). Holin proteins form a “hole” in the cell membrane, which enables lysin to reach to the outer peptidoglycan layers [50].

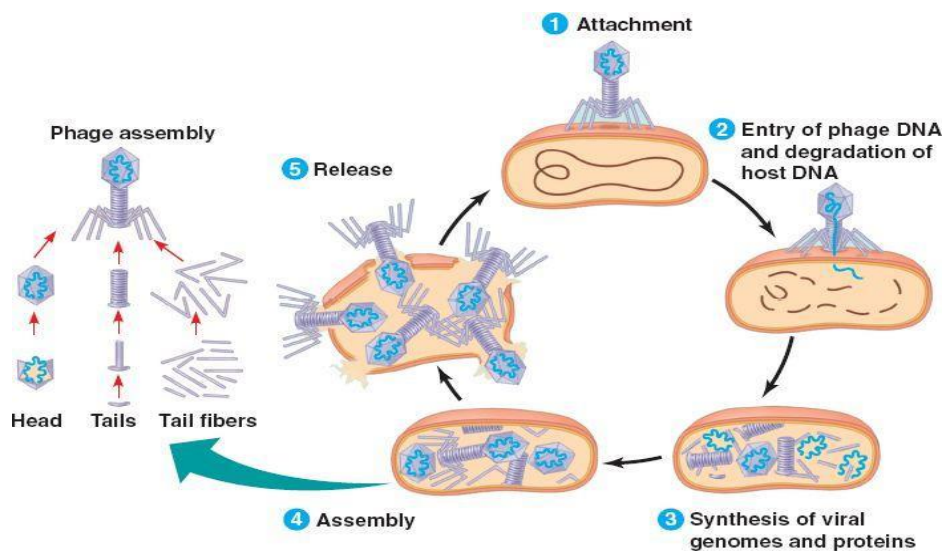


Figure 3: Bacteriophage killing targeted bacteria

Source: <https://www.studyblue.com/notes/n/bio-1404-study-guide-2012-13-dini-mcginley/deck/9724485>

2.4. Trends in Using Phage Therapy

While in principle all bacteria can be impacted by phages, in practice it is especially gastrointestinal afflictions, localized infections and otherwise chronic infections that are treated within a phage therapy context [47].

The actual practice of phage therapy is fairly straightforward. One or more phage types that are either thought to be effective against target bacteria or that have been shown to be effective following laboratory testing are administered in some manner to a patient. Ideally these phages can reach and then disrupt target bacteria. Disruption can be accomplished by killing bacteria, clearing biofilms and perhaps also by increasing bacterial susceptibility to existing host immunity. Indeed, it has long been postulated that phages may play roles as components of a body's normal microbiota as a natural defense against bacteria [22].

The therapeutic effect of the phages can be limited to a decrease in the pathogens population down to a point at which the immune system can effectively control its reproduction. Several current strategies to combat livestock associated pathogens such as toxinogenic *E. coli*, *Campylobacter* and *Salmonella* are direct extensions of classical phage therapy approaches in that they focus on targeting the bacteria in animals before slaughter. On the other hand, food contamination, for instance with *Listeria monocytogenes*, is more likely to occur during food processing, which consequently is the most reasonable time point for phage biocontrol of this pathogen [16].

Phages therapy trials have been carried out in USSR, France, Poland and USA for dysentery, wound infections, burns in children hospitals and infectious diseases hospitals [28].

Although human clinical trials for phage therapy do occur, most studies have been conducted on animals. An MDR strain of *E. coli* from a diabetic foot ulcer was used to isolate a phage,

TPR7 which was then tested in a mouse model of infection. One group of mice was treated once with TPR7 and the second group of mice was treated multiple times with gentamycin. Both conditions resulted in clearance of the bacterial infection on day two. In the untreated group, all mice were still infected on day seven, indicating that the phage treatment was as effective as multiple antibiotic dose treatments [38].

Another study utilized phage MR-10 as a co-treatment for MRSA in diabetic mice. The phage-treated mice showed improvement and decreased bacterial burden, redness and swelling compared to the control group. This result was similar to the conventional treatment of mice with the antibiotic linezolid. The most effective results were evident when the mice were treated with a combination linezolid and MR-10 treatment [7]. The results of these two studies indicate that phage therapy can be as effective as antibiotic therapy and that phage therapy may be considered as a possible alternative or concurrent treatment, especially when treating infections caused by MDR bacteria [10].

In the food industry, meat may become contaminated by pathogenic bacteria. Tainted chicken pork and beef have led to food poisoning and other food related diseases. For example, the transportation of pigs from the holding pens to the processing plant can result in the final product being contaminated by *Salmonella* species, this phenomenon was thoroughly investigated. Then, an anti salmonella phage cocktail was used to attempt to prevent bacterial infections. The use of phage cocktails in small pigs allowed the pigs to fight off *Salmonella enteric* serovar, *Typhimurium* infections and reduce *Salmonella* colonization [49].

This initial analysis in animals indicates the promise of phage therapy, phage cocktails, and use of combination therapy as a means to reduce the transmission of food borne illness

due to pathogenic bacteria. The fish and shellfish industry has also been investigated to determine if phage therapy can eliminate harmful bacteria. The demand for fish and shellfish has reached record highs, while regulations on wild catches have become more stringent. These two factors combined have resulted in increased aquaculture production. The impact of pathogenic bacteria on farm-raised marine and freshwater food sources can result in a huge financial burden if the products are killed, damaged or infected [39].

Investigations were made in the use of phage therapy against *Vibrio harveyi*, in abalone infections. It was reported that two *Siphoviridae* phage isolated from *V. harveyi* strains significantly improved the survival rate of abalone and that phage therapy is an effective treatment against vibriosis [51].

Furthermore, water samples from locations such as fish farms to isolate nine lytic phage against *Flavobacterium columnare*, which causes cottonmouth disease in fish. One of these phage, was used to treat a highly infectious strain of *F. columnare* that was injected into catfish. The phage therapy was prepared in three different ways; immersion in a bath containing a high titer of phage, intramuscular injection of phage and finally, orally administered phage therapy with phage impregnated food. It was reported that all of the fish tested survived and there was a significant decrease of *F. columnare* numbers on the fish [36].

This study was interesting and creative in the method of phage therapy administration and indicates the potential for phage treatment against fish contaminated with pathogenic bacteria. It also showed that where there are pathogens, there is a high chance of finding a phage that can kill that pathogen in the same location or environment. There have been some promising studies on the use of phage therapy against pathogens that cause bleaching and white plague like disease in

corals. Phage BA3, which is specific to the coral pathogen *Thalassomonas loyana*, was used to treat diseased corals. In the study, the progression of the white plague like disease and its transmission to nearby healthy corals was inhibited [2].

Recently, a study conducted in Japan has highlighted the protective effects of phages against experimentally induced bacterial infections of cultured fish (aquaculture) [32].

The versatility of phage therapy makes it attractive in uses for human health, agriculture and protection of fragile ecosystems. In the future, this alternative to antibiotics may play an important role in both aquatic and terrestrial environments [10].

2.5. Advantages of Phage Therapy Compared to Antibiotics

Bacteriophages replicate at the site of infection where they are mostly needed to lyse the pathogens, but antibiotics travel throughout the body and do not concentrate at the site of infection. No side effects have been reported during or after phage application, but resistant bacteria, allergies (sometimes even fatal anaphylactic reaction) and secondary infections are the common side effects of antibiotics treatment [46].

Bacteriophages main advantages as therapeutics are their ability to target bacteria of certain strains or species, without any harmful effect on the rest of the bacterial microflora, as well as their self limited propagation which is controlled by the availability of a sensitive host [41].

Bacteriophages are environmentally friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process compared to new antibiotic development, which may take several years, may cost millions of dollars for clinical trials and may also not be very cost effective [52].

Although bacteria can become resistant to phages, phage resistance is not nearly as

worrisome as drug resistance. Like bacteria, phages mutate and therefore can evolve to counter phage-resistant bacteria [19, 29]. Furthermore, the development of phage resistance can be forestalled altogether if phages are used in cocktails (preparations containing multiple types of phages) and/or in conjunction with antibiotics. In fact, phage therapy and antibiotic therapy, when co-applied, are synergistic [19, 23].

Moreover, unlike antibiotics, phages have the ability to remain in a bacterium in the form of a prophage and increase its adaptive potential, as well as to participate in the horizontal gene transfer between bacterial cells, preclude their use in therapy due to safety concerns. Factors that matter in the prediction of the remaining phages therapeutic efficacy include host range and killing potential, adsorption kinetics and propagation efficiency, stability during storage and under natural conditions, the ability to penetrate encapsulated cells or biofilms, easiness of purification [21].

2.6. Limitations of Phage Therapy

As an antibacterial therapeutic, phage therapy is not yet a fully established alternative to antibiotics. Some of the problems of using whole phages in therapy include the development of antibodies after repeated treatment with phages or for example, against phages used to treat enteric pathogen because of prior exposure. Other problems might include the rapid uptake and inactivation of phages by the spleen and the contamination of therapeutic phage preparations with endotoxin from bacterial debris [30].

Host specificity or the narrowness of phage host ranges will at a minimum place limitation on presumptive treatment [20]. Treatment courses that begin prior to the identification of the pathogen's susceptibility to antibacterials such as to specific phages. However, as phages can often be employed in combination with other antibacterial agents, including phage

cocktails, the lytic spectrum of phage products can be much broader than the spectrum of activity of individual phage types [15, 23, 24,].

2.7. Prospects of Phage Therapy

Infectious disease experts have warned that there is now a compelling need to develop totally new classes of antibacterial agents, ones that cannot be resisted by the same genes that render bacteria resistant to antibiotics. Phage therapy represents such a "new" class. It is believed that the impediments can be overcome, freeing up the phages so that their attributes can be used to great advantage [6].

i. Host specificity

While the host specificity is somewhat of a drawback, it also offers the great advantage that the phages will not kill other species of bacteria. Thus, phage therapy is not likely to kill off the healthy flora of the intestines, lungs or urogenital tract and is therefore unlikely to provoke the illnesses and deaths seen when antibiotics cause overgrowth of pathogens [6].

ii. Ideal candidate for combination therapy

The most successful phage therapy is the combined usage of bacteriophages and antibiotics. By using this technique, treatment has shown a promising increase in the reduction of bacterial infection and reducing the amount of bacteria evolving to develop antibiotic resistance. In the future, using both bacteriophages and antibiotics to fight infections may appease drug companies that seek the opportunity to continue producing their own medications, while increasing the effectiveness of phage therapy treatment [48].

iii. Genetic engineering

It is possible to genetically engineer phages to express new traits of potential value. In so doing, scientists will have to deal with the legitimate concerns of regulatory agencies concerning recombinant organisms. The regulatory obstacles may be well worth the

price, given the powerful engineering tools that are currently available [6].

3. CONCLUSION AND RECOMMENDATIONS

At present, antibiotics are considered as a limited precious resource, and like all limited resources we must conserve and value them. In doing so, we must consider bacteriophage and other alternatives as antimicrobial agents to reduce the current threat of AMR. Though other alternatives may be valuable, bacteriophages are efficient and their unique characteristics make them very much interesting and beneficial. Bacteriophages are environmentally friendly and host specific. Researches and previous trials of using phage therapy has shown promising results in reintroducing antibiotic susceptibility into antibiotic resistant bacteria, which has become an increasingly prevalent issue in the modern medical world. In addition to combating antibiotic resistance, phage therapy could eventually be used as a more effective alternative for bacterial infections. Expressing the host specificity attribute of phages and genetically engineering phages to express new traits of potential value can result in a bright future of phage therapy. Furthermore, consideration of combination therapy will not only increase the effectiveness of phages but will also reduce the amount of bacteria evolving to develop antibiotic resistance.

Therefore, based on the above conclusion, the following recommendations are forwarded

- Wide range of research should be enhanced to accelerate the use of phage therapy.
- Through advanced research, development of phages that are effective and don't result in the development of antibodies after repeated treatment must be made.
- The technology of bacteriophage production should be enhanced and

application must be adopted in developing countries like Ethiopia.

- Phages should be used synergistically with antibiotics as cocktails to obtain a promising effect and to eliminate the problem of antibiotic resistance.

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