INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 6.078
(Volume 6, Issue 3)
Available online at: www.ijariit.com

A review on Bacteriophages: Our saviour and weapon for post-antibiotic world

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ABSTRACT

The rate of Emergence of antibiotic-resistant bacteria has become exponential in recent times due to the irrational exploitation of antibiotics. If this trend continues, the world and human civilization as we know it would be pushed back to the dark pre-antibiotic age. Bacteriophages could hold the key to abate the antibiotic crisis. Bacteriophages are the viruses that specifically infect and kill prokaryotic bacteria sparing human eukaryotic cells and also has a limited effect on the human microbiome this dogma makes them suitable candidates to replace antibiotics in the near future. Bacteriophages, by using a novel mechanism than the conventional antibiotics would empower us to treat drug-resistant bacterial infections, they can also be used along with antibiotics to give a maximum effect to terminate bacterial infections. Phage therapy was already in use before the discovery of antibiotics however, discovery and development of antibiotics dampened their progress in modern therapeutics. In the current scenario, it seems vital to reinvent and reestablish phage therapy and employ them to treat infections by superbugs. Plenty of groundbreaking research has been done in the past few years regarding the usage of phages in modern therapeutics. Bacteriophages, if studied properly, has the potential to supplement or even substitute antibiotics. This review will provide information on the lytic mechanism of phages, summaries of successful research done by using phage therapy against bacteria in the animal model, and current human trials along with synergism between antibiotics and phages. Some of the key aspects of synthetic or bioengineered phages and their uses are also incorporated. Finally, a discussion on solutions for carving a path for the successful employment of phage therapy in the future is inserted.

Keywords— *Bacteriophage, Phage, Phage therapy, Antibiotic resistance, Multiple drug resistance, Lysin, Synthetic phage*

1. INTRODUCTION

Bacteriophages are one of the most abundant organisms found in nature. Being a virus, they are obligate parasites. Bacteriophage term was first used by Felix d'herelle meaning "bacterium eater". Hence, they are the viruses that specifically and particularly infect bacteria. For a bacteriophage or simply phage, bacteria is the host and a phage requires this host to perform basic biological functions and to reproduce, ultimately some of the phages end up killing the host. This "lysis" of the bacterium is one of the main principles which makes phages so interesting therapeutic agents for treating and eliminating bacterial infections. The antibiotic crisis is on a rise where the bacteria are becoming resistant to the conventional antibiotics and giving rise to multiple drug resistant bacteria or "superbugs".

It is being predicted by many experts around the globe that in near future the world will face a great shortage of new antibiotics that could eventually lead to a global health crisis giving rise to multitude of outbreaks by these resistant bacteria which were once easy to treat and control. Employing bacteriophages for eradication or decreasing pathogenic load could become one of the potential ways to tackle the enormous challenge of treating bacterial infections in "post antibiotic world".

This review will focus on various aspects of bacteriophages which makes them suitable agents against bacterial pathogens and what needs to be done in future to make phage therapy a success.

2. LIFE STAGES OF A BACTERIOPHAGE

Bacteriophages can be classified according to the life cycle which they follow one of which is called a "lytic" phage and other being a "temperate" phage.

Lytic phages or virulent phages induce lysis of the host bacterium which at the end of the life cycle effectively kills the host bacterium, this principle makes lytic phage efficacious for use to terminate bacterial infections. Additional information on mechanism of bacteriolysis is reviewed ahead in this article. Steps involved in this lifecycle are:

Adsorption: In this first step bacteriophages adsorb to the specific site on bacteria, to the specific a receptor which gives specificity to a particular phage for a particular bacterium.

↓

Penetration: After adsorbing to a host, phage makes a hole in bacterial wall through which it transfers its genetic material into the host bacterium

↓

Replication: Bacteriophage now hijacks hosts molecular machinery to produce its own structural components with the help of genetic code on the phage genome present in host.

↓

Maturation: During this stage the structural components and genetic material produced are assembled properly to give rise to new phages

↓

Lysis and release: In this final stage with the help of holin and lysin, bacterial cell is destroyed and multiple copies of phages are released kickstarting the cycle again.

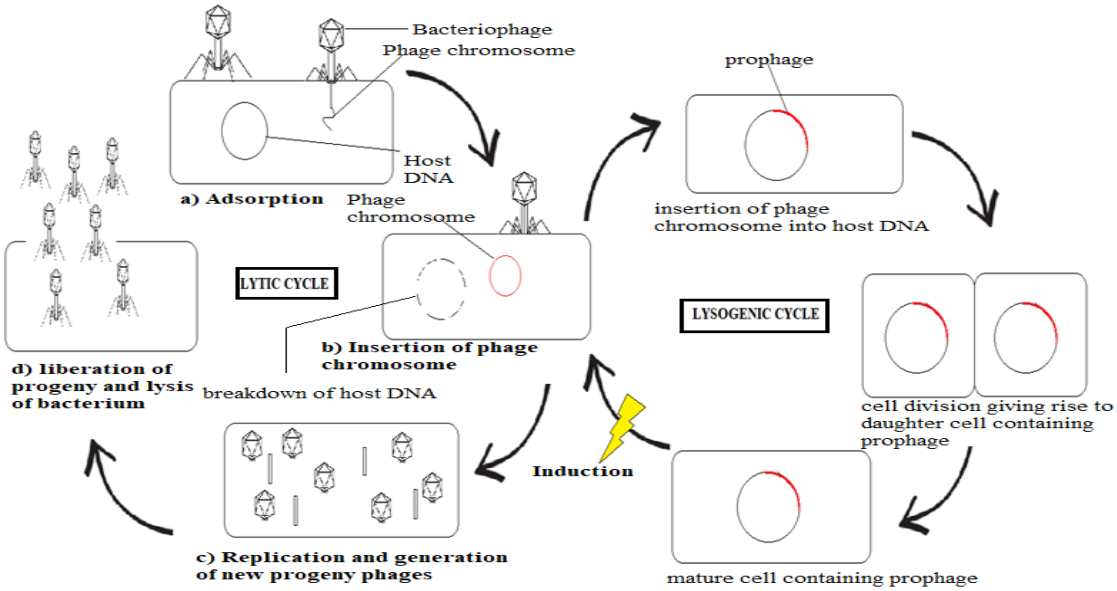

Fig. 1: Lytic and lysogenic life cycle of bacteriophages

In case of temperate phage adsorption and penetration step remains same however, after adsorption and penetration the phage genome is integrated into bacterial genome giving rise to prophage. This prophage can remain idle within a bacterium for number of generations, when bacterium undergoes cell division giving rise to daughter cells, these daughter cells contains prophage. However, under certain inducing stimuli like UV radiation or external stressors [1] induction occurs which separates phage genome from that of bacterium and phage enters lytic cycle, destroying its host.

Lysogeny can lead to lysogenic conversion in bacteria which has various effects on host bacterium. Two imperative points relevant clinically are first, phenotypic changes in host bacterium which confers the bacterium with certain genes that makes the infected bacterium resistant to infection from other phages or secondary infection.[2] For an instance, in case of lambda phage, prophage's CI repressor terminates the replication of other phage making the bacterium host immune to secondary infection.[2]

Secondly, lysogenic cycle can lead to transduction. Transduction is one of the mechanisms by which bacteria can acquire resistance to antibiotics by transfer or transduction of genes responsible for conferring a bacterial strain resistant to antibiotics. In addition, lysogenic conversion is responsible for conversion of a non-pathogenic bacterial strain to pathogenic one. Genes for Diphtheria toxin, botulinum toxin, cholera toxin, and erythrogenic toxin are transferred to bacteria during lysogenic cycle converting a non-pathogenic strain to a pathogenic one.[3]

3. EXPONENTIAL RISE AND GRADUAL DOWNFALL OF ANTIBIOTICS

From its advent in 1928 by the discovery of penicillin by Alexander Fleming and its widespread use from 1940s antibiotics have become magic bullets to cure off infections commonly found in clinical practice and have acquired a top tier position in many reforms and advances in medicine and surgery [4].

Antibiotics are compelling drugs used to treat infections caused by microorganisms. Antibiotics can be categorized into bacteriostatic antibiotics (inhibits the growth of invading pathogen) or bactericidal antibiotics (kills the invading pathogen) which by their use provides a better chance for the body's immune system to take over and eliminate the infectious organisms[5].There are

various means and mechanisms by which an antibiotic can act depending on its chemical structure such as inhibition of cell wall synthesis, inhibition of protein synthesis, cell death caused by damage to DNA, and various other site-specific actions.[6]

In the current world scenario, we can unequivocally say that the unprecedented success of clinical medicine is largely due to contributions made by the discovery and use of antibiotics. In addition to the medical world, many parallel advances have been achieved in the agriculture and poultry industry by successfully using antibiotics.

The 1950s by many is considered as "Golden age" of the antibiotic era as many new classes of drug with better efficacy and tolerable pharmacodynamic and pharmacokinetic profile with novel mechanism of actions had been introduced however the future in terms of use of antibiotics is grim as by the last 10-15 years the pipeline for the drug research and development for new antibiotics has ran dry due to multitude of factors and over the top of it, the emergence of resistance in bacterial population to the antibiotics is at an all-time high[5,7].

4. EXPLOITATION OF ANTIBIOTICS: THE CRISIS

The cautionary statement regarding antibiotic resistance came from Alexander Fleming himself he said, "The thoughtless person playing with penicillin treatment is morally responsible for the death of the man who succumbs to the infection with penicillin resistant organisms."

As seen with the case of *S. aureus* it was largely assumed that the infection caused by this organism was largely in control until penicillinase-producing *S. aureus* emerged in the mid-1940s. After the discovery of methicillin in 1959 it was used far and wide often relentlessly which led to the development and emergence of methicillin resistant *S. aureus* (MRSA), initially a nosocomial infection now has become community associated (CA-MRSA) [8]. There is also emergence in finding of vancomycin resistant *S. aureus.*[9]

Nowadays, bacterial strains which are resistant to one or more drug therapies are on a rise, often defined as multiple drug resistant [MDR], extremely drug resistant [XDR], pan drug resistant [PDR] and even total drug resistant [TDR].

A recent report published by WHO (2019) states that "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. Around 2.4 million people could die in high income countries between 2015 and 2050 without a sustained effort to contain antimicrobial resistance [10]".

Thus, as evident the world stands at the end of antibiotic era and if the trend to use these powerful drugs continues inexorably than we would be left with no weapons at our disposal to cure even a minor infection and the world as we see it world be reverted back to its century old version, a version dominated by microbes and human civilization at its knees.

5. PHAGE THERAPY: A REVIVAL

As the sun is setting on the antibiotic age the key to tackle infectious diseases in the future could be bacteriophage therapy or phage therapy. Bacteriophages are dead and inactive organisms that require a host to survive, perform metabolic functions, and reproduce. The host for a bacteriophage being a bacterium, this can be used to target drug-resistant pathogenic bacteria to eradicate infection caused by it, using this as a dogma, bacteriophage therapy could be a solution to our antibiotic crisis.

5.1 The mechanism of bacteriolysis

The basic requirement for a phage to be used in therapy is that the phage employed has to be a lytic phage, it should have selectivity towards the target used, and should have an efficacious response.[11]

Lysogenic phages are generally not used for phage therapy as they do not involve lysis or "killing" of a bacterial host, in addition to that lysogenic conversion of a bacteria can induce certain phenotypic changes in the host which confers the host immune to other lytic phages or can even be responsible to enhance the virulence of the host [11]. For instance, a filamentous lysogenic phage, CTXφ bacteriophage through lysogenic conversion is responsible for the conversion of non-toxic *V. cholerae* strain to pathogenic strain, this pathogenicity is rendered to *V. cholerae* by certain cholera toxin-producing genes carried by CTXφ bacteriophage [15].

The first and foremost step towards the lytic infection is adsorption of bacteriophages to several targeted receptors present on bacteria, these receptors are peptide sequences or polysaccharides in nature and located on cell walls of gram-positive or gram-negative bacteria, bacterial capsules, slime layer or on pili and flagella [12]. One or more than receptors can be involved in binding, which can be reversible or irreversible. Furthermore, phage resistance can be found if the receptors on the bacteria become inaccessible or the fit of the host receptor or phage receptor binding protein [RBP]is non-complementary to each other.[12]

After the adsorption of bacteriophage to the bacterial host, phage injects its DNA into the host cell and hijacks the bacterial host's molecular machinery to make multiple copies of its own DNA and cellular components for progeny phages which in later stages are assembled together to form a fully functional bacteriophage [13]. These newly generated bacteriophages are liberated from the host by the action of two proteins, holin and lysin, the genes for which are found on the phage genome. Holin forms pores in the bacteria's cell membrane which effectively allows lysin or endolysin a peptidoglycan damaging enzyme to reach the cell wall and break it apart which results in lysis of bacteria. Holins are also regarded as "clocks" for the lytic cycle [14]. The action of lysin is enough for a gram-positive bacterium however, in case of gram-negative bacterium which has an outer membrane spanins are required to catalyse fusion of outer and inner membrane for an effective lysis and in order to release progeny.[11]

Peptidoglycan layer is made up of alternating sequence of two sugars that are β-(1,4) linked *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). To the NAM unit amino acid side is attached which is further linked amino acid side chain of another adjacent unit by a peptide chain. This repeated sequencing of amino acid side chain, linking peptide chain and sugar forms a mesh like structure located outside of cell membrane. Lysin acts on various specific location of peptidoglycan layer and classified according to the location on which they act in peptidoglycan layer. Different types of lysin are:
(1) N-acetyl-*β*-D-muramidase,
(2) lytic transglycosylase,
(3) N-acetyl-*β*-D-glucosaminidase,
(4) N-acetylmuramoyl-Lalanine amidases
(5) endopeptidase [31]

However, for successful therapy, a specific correlation between phage and the selective host must be established by studies focused on their receptor interaction and foreseeing the signs of potential resistance against phage. Before using phage therapy to eradicate bacterial infection the genomic studies of the participant phage should be carried out to avoid transduction of pathogenic or antibiotic-resistant genes to the host.

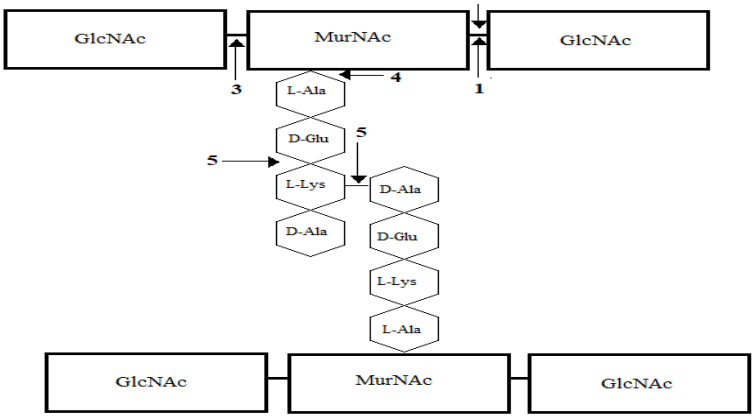

Fig. 2: Diagram showing action sites of lysin on peptidoglycan layer

5.2 Early findings and research

In 1896, Ernest Hanbury Hankin showed that the waters obtained from sacred rivers of Ganga and Jamuna in India contained an antiseptic principle which can destroy cholera forming bacteria, these findings were further consolidated by the evidence that the cholera disease was not found in populations inhabiting in the downstream area of these rivers [16].

In 1915, Frederic Twort, in an attempt to grow vaccinia virus on agar plates in order to develop a vaccine discovered a substance that was easily filterable and would particularly require bacteria to grow [17].

Two years later in 1917 Felix d'Herelle independently reported that he founded an invisible microbe obtained from the stools of recovering shigella positive patients which when added to the cultures of shigella the bacteria showed clear lysis, he further demonstrated that this antagonizing anti-shigella microbe requires shigella bacteria itself to grow [18].

Further progress was made in the coming years regarding the findings and implementations of bacteriophage therapy as a clinical tool which was later on dampened and led to abandonment due to widespread research and use of antibiotics. However, the research and implementation of bacteriophages as therapy continued in former USSR and eastern Europe and a huge amount of ground breaking research and reports were published by experiments done in Georgia and Poland, these papers were mainly in Russian and polish which due to language barriers remained confined to these areas, in addition to that the more acceptable and palatable antibiotics were a main focus and target for bacterial infections at that time.

5.3 Experiments done using an animal model

There are many examples which can show successful results obtained by employing phage therapy for commonly found clinical pathogens some of which are highlighted below:

Smith & Huggins in 1982 [19] inoculated mice with 018:K1: H7ColV+ strain of *Escherichia coli,* designated as MW strain, and employed anti K1 phage against them (the strain of phage more specific and effective towards the MW strain of *Escherichia coli).* The group of mice that received anti K1 phage as a treatment option showed decreased mortality. In addition to that, the group of mice that received intramuscular or intracerebral doses of anti K1 phage showed better mortality rates than the group of mice which received multiple doses of tetracycline, ampicillin, chloramphenicol or trimethoprim plus sulphafurazole.

Another study conducted by Smith & Huggins in 1983 [20] showed prominence of oral phage therapy for the cure of enteritis caused by *Escherichia coli* in calves, piglets, and lambs. By this study, they concluded that decreased deaths were observed in calves, piglets, and lambs that received oral phage therapy in comparison to the ones left untreated. The phage employed also showed excellent results when used in prophylaxis of diarrhoea caused by *Escherichia coli.*

Experiments by Soothill,1992 [21] demonstrated that when the mice were subjected to fatal doses of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, the mice groups subjected to bacteriophages active against these two pathogenic organisms showed a drastic survival as compared to the control group.

A study conducted by Biswas et al., 2002 [22] showed the effect of phage therapy on Vancomycin-Resistant *Enterococcus faecium* and observed that single intraperitoneal injection of anti-VRE phage rescued 100% of mice from bacteraemia, moreover good recovery was obtained when anti-VRE phages were injected after a delay or till the animals were moribund. They used cesium chloride density centrifugation to purify phages which led to the development of fewer side effects of therapy. A positive attribute obtained from this study is that even if antibody titer increased during the course of therapy for several months it was not associated with any adverse effects or anaphylaxis. In addition to that this study strongly implies that the survival of mice is a function of phage therapy and not due to any activation of the non-specific immune response

Matsuzaki et al., 2005 [23] observed the effect of phage ϕMR11 on *Staphylococcus aureus* by using an animal model which looks promising with no observed adverse effects. The MOI of phage ϕMR11 on *Staphylococcus aureus* strain used was at minimum 1 to yield a "fully protective effect", which suggests a strong bacteriolytic activity.

5.4 Employment of phage therapy on humans.

Recently many trials and research have been conducted on human subjects, mostly giving positive and favourable results, some of these studies and trials are summarized as below.

In 2009, a randomized, double blind, placebo controlled, phase I/II trial was conducted [24]
Which gives significant insight on the use of phage therapy on *Pseudomonas aeruginosa* infection that causes chronic otitis media. By using phage therapy on 24 patients significant decrease in pathogenic bacterial load was observed and a definitive clinical improvement was observed in group treated with phage therapy as compared to placebo group. In addition, there was no reportable side effects along with any local or systemic toxicity.

Bacteriophage cocktail therapy was used in 2017 to treat a patient suffering from necrotizing pancreatitis complicated by MDR *Acinetobacter baumannii* infected pancreatitis pseudocyst[25]. Patient was 68 years old male, and a diabetic patient and resistant to most of antibiotics, due to which phage cocktail therapy was initiated. Initially the cocktail was given percutaneously and afterwards switched to I.V, which was proved to be life-saving, his pressor requirements was diminished gradually he woke up from comatose state. However, minocycline which was sensitive to the pathogenic isolate was administered proving an interplay of antibiotics and phage therapy, which sparks interest to further elucidate the use of phage therapy along with antibiotics.

A case report by Jennes et al., 2017, [26] shows the effectiveness of bacteriophage monotherapy when used in case of a debilitated patient, 61 years old, male for the treatment of *Pseudomonas aeruginosa* septicaemia with acute kidney injury. The use of antibiotics in this case was subdued to further decrease the nephrotoxicity. When phage therapy was used, blood cultures of bacterial pathogenic load was found to be negative, CRP levels decreased, and kidney function was improved.

These studies show that phage therapy has tremendous potential for use in cases where effects of antibiotics are negligible or they can't be employed due to their side-effects.

Our research should be carried forward in terms of phage therapy to meet the crucial demand of finding ways to tackle the challenges in post antibiotic world to ward off bacterial infection, this can be achieved by increasing the research on animal models and finding out ways that these results can be safely examined and extrapolated to human subjects.

Table 1: Some of the papers published showing effectiveness of phage therapy

Bacterial pathogen	Model	Summary	Ref
Escherichia coli	Murine	Mice which received specific phage against causative agent showed decrease in mortality rates.	[19]
Escherichia coli	calves, piglets, and lambs	Animals which received phage treatment showed decrease in death rates. Prophylactic use also showed encouraging results.	[20]
Acinetobacter baumannii and *Pseudomonas aeruginosa*	Murine	Increase in survival rate in group which was treated by phage therapy.	[21]
Vancomycin-Resistant *Enterococcus faecium*	Murine	100% of mice were rescued from bacteraemia.	[22]
Staphylococcus aureus	Murine	Bacterial pathogenic load was cleared off.	[23]
Pseudomonas aeruginosa	Human	Phage therapy initiation was followed by a downward trend in pathogenic bacterial load. No side effects or toxicity was seen	[24]
MDR *Acinetobacter baumannii*	Human	Phage therapy demonstrated life-saving potential	[25]
Pseudomonas aeruginosa	Human	Phage therapy was followed by decrease in pathogenic load with decreasing signs of inflammation.	[26]

5.5 Phage-Antibiotic synergy (PAS)

An interesting phenomenon is observed when antibiotics and phage therapy is combined together to terminate bacterial infection. When a certain dose of antibiotic and phage is combined, a synergistic relationship is observed, which means that the overall effectiveness of phage therapy is increased when it is combined with antibiotics.

Comeau et al,2007[27] studied this phage-antibiotic synergy (PAS), according to them when they used a sub-lethal dose of antibiotics along with phage therapy, the production of phages increased along with the greater lytic activity. They used β-lactam

antibiotics (in low concentration then usually required) with phages. The sub-lethal dose of antibiotics was enough to stop the cell division of microbes but not so for killing them. In this case, they observed that this dose of antibiotic was responsible to increase biomass and biosynthetic capability of cells which when combined with phage therapy eventually resulted in increased production of phages which in turn will have greater lytic activity.

PAS was also observed with Methicillin-Resistant *Staphylococcus aureus* [28].MR-5 phage was used along with different classes of antibiotics. Protein synthesis inhibitors like linezolid and tetracycline when used in lower concentrations along with required phage showed that the plague size increased dramatically on agar plates. The parallel observation was also seen with a certain concentration of clarithromycin and telithromycin, with plague size increased.

The effect of this synergy was also observed in controlling colonies of *Pseudomonas aeruginosa* on biofilms [29].This study clearly shows that when different classes of antibiotics are used, to which organisms are still sensitive along with an appropriate phage(s) lower doses of antibiotics produce remarkable effects in controlling bacterial colonies. One of the important observation which was seen is that maximum effect is observed when phages are administered before the administration of antibiotics, rather than administering both the components at the same time.

A study conducted in mice with diabetic foot and with an infestation of MRSA [30] found that when MR-10 phage when combined with linezolid antibiotic, greater effect to control the infection was seen. This antibiotic-phage combination therapy was more effective in treating diabetic foot as compared to both the agents used alone.

Thus, this synergistic combination when properly used will lead to higher rates of treating an infection that is resistant to an antibiotic or if a higher dose of antibiotics can't be used in a patient. This synergy in an overall broad sense will lead to two imperative results, one of them being the reduction in dose of antibiotics which will ultimately result in decreased frequency of antibiotic resistance in clinically important pathogens and secondly, it will lead to decrease in treatment failure rates as both combination of antibiotic and phage therapy eventually results in higher treatment ratios.

6. BIOENGINEERED PHAGE: THE BETTER PHAGE

The arrival of synthetic biology age has changed many perspectives to look into biology and its complex molecular mechanisms. With the rise in bioengineering era many biological substances are now better understood and better used, with myriad of functions at our disposal. Synthetic phages or bioengineered phages has become more useful with improved quality and increased functions. They can be now better used by certain modifications to treat bacterial infections, can be used in diagnostics, gene manipulation, increased efficacy towards pathogenic bacteria, drug delivery systems and many more [32].

It is marvellous to think that so much could be achieved by using these tools. Many papers are published which can show the true potential of bioengineered phages, some of which are summarized below.

Table 2: Some of the examples of Bioengineered phage and their uses

Key Aspect	Use	REF
With the use of CRISPR-Cas system and lysogenic phages, antibiotic resistant genes could be modified or deleted.	Lysogenic phages by using rational CRISPR-Cas system, could act as a vector which can alter antibiotic resistant genes, making bacterial population sensitive to antibiotics.	[33]
PEGylation of bacteriophages	PEGylation with surface proteins of bacteriophage increased $t_{1/2}$ in blood and decreased cell mediated immune response towards phages	[34]
Attaching antibiotics to bacteriophages to form a delivery system	Antibiotics could be attached to antibiotic, which in turn will act a delivery mechanism for that particular drug particle. Yacoby, Bar & Benhar, 2007, used antibiotic chloramphenicol and attached it to a filamentous bacteriophage aiding in its delivery	[35]
Employment of engineered enzymatic bacteriophage for targeting biofilms.	Bacteriophages can be engineered to express certain enzymes which can target and disperse biofilms. Biofilm dispersible enzymes encoded in bacteriophage replicates within infected bacteria which upon the terminal stage of the lytic cycle is released effectively degrading the biofilm.	[36]
Enhancement of host range by modification of long tail fibres.	Tail fibres of bacteriophage has receptors for identification of complementary receptor on bacterial cell for its binding. Mahichi et al 2009, produced chimeric phages by homologous recombination of long tail fibre genes of IP008, a phage having broad host spectrum with T2 phage. It was observed that chimeric phages have similar broad-spectrum host range as that of IP008.	[37]

These examples show that "synthetic phages" has plethora of opportunities in field of therapeutics. Creative and novel ideas using these techniques could change the future in medical field with improved diagnostics and high-quality and efficacious therapeutics and diagnostic tools.

7. PROS AND CONS OF PHAGE THERAPY

Pros and cons associated with phage therapy are summarized as below [38]. There are a considerable of pros regarding the phage therapy which can easily outweigh its limitations. Disadvantages of bacteriophages at the moment looks like they can be easily

challenged and solutions to tackle them can be found out by collaborative efforts of researchers worldwide, which would give phage therapy a global recognition and make its science more acceptable and easier to use

7.1 Advantages

(a) Bactericidal action is seen with lytic phages.
(b) As they have different mechanism of action than antibiotics they can be used in resistant cases.
(c) Less chances of dysbiosis
(d) As they have bactericidal action, frequency for developing resistance is less.
(e) As they are able to replicate within lost and give rise to progeny dose adjustment is not necessary. They follow "auto dosing".
(f) They have single hit kinetics.
(g) Less chances of immune response against them specifically if phages are purified and free of endotoxins.
(h) They can be used with antibiotics giving rise to synergy called phage-antibiotic synergy (PAS)
(i) Synthetic phages or bioengineered phages can be used in variety of diagnostic and therapeutic applications.
(j) As phages can transfer from treated to untreated subjects, they an be used to control infection in poultry industry.
(k) Low cost of preparation than antibiotics

7.2 Disadvantages

(a) As bacteriophages are selective towards a host, phage-host relationship has to be established before employing them in therapeutics.
(b) Only lytic phages are useful. Lysogenic phages can cause transduction of resistant or toxic genes.
(c) Full genomic sequence of phage has to be studied to avoid transfer of harmful genes.
(d) As they are biological agents, they can evolve in course of time, rendering them toxic or inactive.
(e) Possibility of action with immune system.
(f) Lack of intellectual property rights.
(g) For empirical treatment phage cocktail has to be used or broad-spectrum phage has to be identified

8. A PATH FOR FUTURE: WHAT SHOULD WE DO NEXT?

Phage therapy has tremendous potential to combat multiple drug-resistant bacteria. Despite much ground-breaking research done in this field, we have to travel a long path ahead before phages can be used and employed for regular use. Future research ahead should focus on keeping patients need and palatability a frontier. An optimistic attitude toward finding a way to treat resistant bacteria will surely reach an acceptable destination. Below are some of the points which can be useful to carve a future path for phage therapy.

- Official libraries containing details of bacteriophage genome and its host relationship should be established. This can be done if all the researchers globally work in a unison. These libraries would be a great help in boosting research work and save time for the same.
- We have to increase our search for finding new phages and to determine their virulence, host spectrum and compatibility in vivo.
- Formulation of phage cocktail should be emphasized to find an immediate cure for empirical infections.
- By bioengineering manipulation of phages should be encouraged in an attempt to find creative and novel ways for drug delivery and to increase the host range of phages.
- Purification techniques should be developed to dampen immune response against residual endotoxins.
- Evolutionary patterns of phage should be checked so that a possible resistance or toxic genes could be found out and isolated.
- Intervention by biotechnology should be encouraged to increase lethality and selectivity of phages.
- Ways to formulate them in an acceptable dosage forms and their delivery routes should be established.
- Genomic sequencing of a phage should be done to find out toxic or unwanted genes.

9. CONCLUSION

The cases of antibiotic-resistant bacteria are increasing at an exponential rate which at any moment could become a perfect storm for human civilization. We have to be aggressive and assertive in our approach to solve this great threat before it's too late. Bacteriophages could, in the near future, be a part of the multifactorial approach in combating antibiotic-resistant cases. Ever-increasing researches showing a positive outcome of phage therapy towards multiple drug-resistant bacteria is evidence for it. The use of antibiotics in conjugation with phage therapy, commonly termed as phage antibiotic synergy, also has an overwhelming rate of success. However, there is a long way to cover before this novel therapy is used widely and globally. Well defined studies should be started immediately for a better understanding of phage therapy, evaluating its safety and its possible interaction with human physiology. With our positive outlook and unified attempt, there is a chance that bacteriophages could become metaphorically, the new age antibiotics**.**

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