RETRACING PHAGE THERAPY: KEY TO THE POST ANTIBIOTIC ERA

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ABSTRACT

We live in the era of superbugs which develop resistance to most of the antibiotics employed in practice today at an alarming rate. Consequently, this failure of antibiotics demands an alternative treatment option for multidrug resistant pathogens. Bacteriophage therapy, the process of tackling pathogenic bacteria using specific viruses, was first explained in 1915, thirteen years prior to the discovery of penicillin. Unfortunately, phage therapy wasn’t utilized to its full potential because of the golden era of antibiotics. However, since the reintroduction of phage therapy to clinical trials in 2015, bacteriophage is being recognized as a potential alternative to treat infectious diseases. The efficacy of bacteriophage against ESKAPE pathogens (Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas and Enterobacter spp.) is well established. The high specificity of bacteriophage offers major clinical advantage as the pathogenic bacteria is selectively destroyed, sparing the normal gut microbiome and host cells. Emergence of resistance, the biggest drawback of conventional chemotherapy, is less likely with the employment of bacteriophage. However, there is limited data available for the in-vivo susceptibility of pathogens to phages. Moreover, further research is required to evaluate the immunological response against bacteriophages. Although phage therapy is currently restricted to Poland, Georgia and Russia, with the recent promising results, bacteriophage therapy could be the vital key to the much anticipated post antibiotic era. In this review, we aim to discuss the history, recent developments as well as the scope of bacteriophage therapy in combating bacterial infections.

KEYWORDS: Bacteriophage; Infectious diseases; antibiotics; drug resistance.
INTRODUCTION
In the era of superbugs we live in, antibiotic resistance is emerging at a frightening rate. Currently, desperate measures are being taken for developing a super sensitive class of antibiotics, unfortunately with no promising results. Consequently, this failure of antibiotic agents demands an alternative treatment option for multidrug resistant pathogens. Bacteriophage therapy, the method of tackling pathogenic bacteria with the use of viruses, has been around for decades now. The concept of bacteriophage being first explained in 1915, that is thirteen years prior to the discovery of penicillin by Sir Alexander Fleming, has never been put to its full usage potential owing to the discovery of antibiotics, the golden era and various controversies. Introduction of antibiotics was indeed a revolution in the western medicine.

Penicillin was received globally as a “wonder drug”, particularly due to its extensive utilization in the Second World War. Following the discovery of prontosil in 1932 and the subsequent development of sulfonamides, came the golden era of antibiotics in the 1950s with the introduction of streptomycin, erythromycin, vancomycin, metronidazole and tetracycline. Although there was an increasing number of antibiotics being developed towards the 1950s, most of the products were slightly altered varieties of the preexisting antibiotics. The world witnessed a tremendous drop in the discovery of antibiotic classes following the 1960s.[1] Consequently, the scenario worsened after fluroquinolone and oxazolidinone classes were brought to the market. Until mid-1980s, the antibiotic markets were estimated to grow by 25% per year, however the end of the 20th century witnessed a marked decline of this growth to 6%.[2]

Recent estimates by the Centers for Disease Control and Prevention (CDC) suggest that over 2 million individuals acquire antibiotic resistant bacterial infections in the United States, and at least 23,000 of these individuals succumb to the same.[3] The mortality rate of infectious disease has shown an increase from 42% to 63% during a course of fifteen years from 1980 to 1995.[4] In India, the infectious diseases compose at least 30% of the overall disease burden of the country, owing to overexploitation of antibiotics, and consequent emergence of resistance.[5]

The reason for this alarming rate of resistance can be attributed to the sole fact that the number of antibiotics being brought to the market today is not at par with the degree of overutilization of the same by mankind. It is estimated that the global use of antibiotics
elevated by a margin of 40% in the first decade of 21st century.[6] This rate poses a serious threat to public health, and may continue to deteriorate unless drastic measures are taken. In India, the development of antibiotic resistance by both gram negative and gram positive bacteria have taken a new high, with 80% of the E. coli strains being resistant, often to the reserve antibiotics. The concept of methicillin resistance appeared shortly after its discovery in 1959 to combat resistance to penicillin resistant S. aureus [7] Vancomycin resistant strains, however were brought to public attention only after 2002, suggesting a slower development of resistance. Prevalence of MRSA continues to be a public health crisis, with a threatening resistance pattern and steady increase in the isolation rates.[8]

Role of physicians and the pharmaceutical industries in the resistance crisis.

Global Pharmaceutical market is expected to exceed a whopping 1.12 trillion dollars in the next five years. It is well established that the pharmaceutical companies promote extensive antibiotic usage so as to compensate for the overall expenditure on research and development. However, due to insufficient financing, academia and antibiotic research is coming to a standstill. Companies are forced to consider a lot of economic factors when it comes to the development of antibiotics, as emergence of resistance to a particular agent is immediately followed by a downturn in the overall profits.

Extensive research has been done in order to evaluate the rational prescription of antibiotics by the physicians, and it is not surprising to note that at least half of the antibiotic prescriptions in the United States are inappropriate.[9] Although several key factors play a significant role in the overall judgment of the physician in the selection of antibiotic, prescription of high end expensive antibiotics ultimately favor the growth of the industry. Fortunately, this is not always the case, owing to the reservation of newer antibiotics by infection control organizations as a measure to combat resistance. Consequently, this has a dwindling effect on the profits anticipated by the market. Thus, there appears to be a threeway relationship being shared between the pharmaceutical industry, the clinicians and academia, and these parties have to jointly implement a health policy that can tackle the ongoing predicament.[10]

Pharmaceutical pollution is a less discussed pathway by which resistance develops. Antibiotics ultimately end up in the environment, and pharmaceutical industries often contribute to such pollution. Microorganisms are exposed to a cocktail of antibiotics, and by the method of gene transfer, microorganisms can develop multidrug resistance and evolve to
become dangerous pathogens. Analysis of effluents from a certain plant in Hyderabad was found to contain fluoroquinolone antibiotics at concentrations of 31mg/L.\[11\]

**The historical development of bacteriophage: a brief outline**

Ernest Hanbury Hankin, in 1896 recognized that the Indian rivers Ganga and Yamuna contained a biological agent that destroyed cultures of *Vibrio cholerae*. This marked the beginning of the massive study on bacteriophage. In 1915, Frederick Twort, an English bacteriologist, identified a filter-passing transparent material which may lyse the bacterial culture, discovering phages. In 1917, Felix d’Herelle, a French microbiologist, investigated the nature of phages while examining patients suffering from bacillary dysentery and consequently suggested the therapeutic role of phages. He successfully introduced intravenous phage therapy for invasive infections. Sever years later, John Northrop concluded that phages get activated only inside a living host by the production of an inert protein.

In 1923, d’Herelle along with George Eliava, founded the Eliava Institute located in Georgia devoted solely to the future developmental research of phage therapy. The 1940s witnessed the Eli Lilly and company undertaking the marketing and commercialization of phage therapy in the United States.

Owing to the worldwide popularity earned by antibiotics and its widespread marketing, scientists all over the world lacked interest in conducting further research on phage therapy. However the Russian scientists, despite the antibiotic revolution, continued to develop and subsequently employed phages to treat the wounds of soldiers in army hospitals. This trend followed in the Second World War too, where bacteriophages were used by the Soviet Union to treat dysentery and gangrene. However, lack of scientific resources and subsequent growing popularity of the antibiotics led to the failure of this potential utility from proliferating all across the globe. The mechanism by which the phage lyases the bacterial hosts was not well understood, and this contributed to the disapprobation of phage therapy.

Lwoff demonstrated the ability of some phages to incorporate into the chromosomes of prophages i.e. the bacteria. It takes a few weeks or longer for the prophages to enter the lytic cycle and appear as plaques. It is also most likely that some trials had deleterious outcomes due to the unintended use of phage strains, but this issue was resolved by specifically using lytic phages. Many animal studies have been conducted in which phages proved to be a
promising utility in the treatment of several animal infections. Phage therapy appeared to be effective in preventing death from systemic infections in rats, prevented deaths in calves and chick from *E.coli* induced infections. Phage therapy was also found to be very effective in preventing *Pseudomonas aeruginosa* infections in rabbits as well as in swine.[15],[16]

**Recent advancements in the journey of phage therapy**

Ever since d’Herelle successfully used bacteriophages isolated from human stools to treat children with dysentery almost a century ago, military hospitals in Poland, Georgia and Russia have continued to prefer phages over antibiotics even today. Unfortunately, the clinical utility of bacteriophage is still restricted to Poland, Georgia and Russia. The recent advancements listed below may result in phage therapy establishing itself as valuable resource to combat the existing plethora of resistant microorganisms.

PhagoBioDerm (Phage International, Georgia) is a product marketed by Phage International Georgia, consisting of phages with activity against *E. coli*, *Pseudomonas* spp., *S. aureus*, *Proteus* and *Streptococcus* spp.[17] In 2006, the USFDA approved the use of phage preparations (ListShield™ and LISTEX™ P100) against meat contamination by *E. coli* and *Listeria monocytogenes*. Phage BioDerm, when used in patients for the treatment of ulcers and poorly healing wounds infected with resistant organisms, appeared to have a success rate of 70%. Other food borne pathogens like *Campylobacter jejuni*, has been proven to be susceptible to phage therapy and judicial use of the same can aid in reducing the high poultry carriage of the pathogen in the United Kingdom.[20]

The efficiency of phages in the treatment of MRSA induced osteomyelitis in animal models was demonstrated by Nath et al.[21] In another study conducted in Kuala Lampur, Phage C34, a purified strain originally isolated from sea water, was shown to possess antibacterial activity in mice infected with *Burkholderia pseudomallei*, a deadly drug resistant bacteria. The efficiency of phage therapy models against *P. aeruginosa*, *Yersinia pestis* and *Burkholderia cepacia* in mouse models has also been well established.[17],[22] A phase I safety trial which was performed to evaluate the safety of Bacteriophages in chronic venous leg ulcers, revealed that no side effects were attributed to the phage therapy.[23]

The first multicentric bacteriophage phase I/II trial was launched in Europe by French biotech firm, Pherecydes Pharma in 2015. It aims to assess the safety, efficacy and dynamics of two phage cocktails in wounds and burns infected with *E.coli* and *P.aeruginosa*. The Phagoburn
A clinical trial will be conducted in Military hospitals in France along with other burn units in France, Belgium and Switzerland. A San Diego based firm, AmpliPhi Biosciences, conducted a phase I/II trial to evaluate the safety and efficacy of phage therapy against burns and wounds infected with MRSA and other resistant *S. aureus* strains, in about 24 US soldiers. In 2017, Ampliphil biosciences announced that they received acknowledgement from the FDA to conduct Phase II trials, following positive results obtained from Phase I trials in 2016.[24] Ampliphil biosciences has also developed a phage cocktail against *Clostridium difficile*, whose antibacterial efficacy was confirmed in 2016.[25]

In 2017, a patient with a pancreatic pseudocyst infected with *Acinetobacter baumannii* was treated successfully in San Diego with a cocktail of four phages provided by Texas A&M and Ampliphil. In Wroclaw, Poland, a large number of patients undergo phage therapy for infections caused by various organisms including *Acinetobacter, Citrobacter, Enterobacter, Morganella, Salmonella, Serratia and Stenotrophomonas*.[26],[27]

In 2016, a superficial cell wall protein referred to as the “phage infection protein from *Enterococcus faecalis*”, was found to be potent factor in phage response.[28] Another interesting small scale clinical trial conducted by Aleshkin et al in 2016, demonstrated the high therapeutic efficiency of a phagebiotic cocktail against multidrug resistant strains in the intensive care unit.[29]

**Ethical issues, phage resistance and other challenges surrounding phage therapy**

Narrow host range of phages, presence of contaminants in phage preparations as well as poor stability, lack of understanding of lytic mechanisms, and ultimately the failure to establish concrete scientific evidence were the problems faced by early phage research.[30]

The increasing number of pathogenic bacteria becoming impervious to existing antibiotics and the drying up of antibiotic pipeline are the driving forces for the ongoing search for complementary antibacterial therapy. Western medical world does not introduce phage therapy due to four main obstacles. Firstly, the European regulators do not seem to approve the available past clinical data about the safety and efficacy of phage therapy. Secondly, a substantial investment is done on the development and marketing of conventional medicinal products by pharmaceutical companies that there is no proper pharmacoeconomic model for phage development. Thirdly, most countries prefer drugs having fixed chemical composition, and bacteriophages challenge this definition by being mutable. Lastly, there exist various
uncertainties about the consequences of unlimited phage therapy. Access to bacteriophage therapy for the patients in need remains highly problematic due to these obstacles. The question of phage therapy being ethically justified remains unanswered. However, certain criteria would bring the phage therapy under “Ethically Justified Medical Treatment”. The criteria are firstly, there has to a “just cause” or a good reason for subjecting a patient to phage therapy. Secondly, in a clinician- patient relationship, the clinician is ought to have “ethically proper intentions” only. Thirdly, the unapproved therapy needs to be the “last resort”. Phage therapy for instance can be considered the last resort when antibiotics being marketed today are no longer effective. Phage therapy when combined with traditional antibiotic therapy has shown synergic beneficial effects.[31]

A century has passed by since the technique of phage therapy was first explained to mankind, yet there is no credible evidence that proves the effectiveness of phage therapy in human beings. The specificity of phages to their bacterial counterparts eliminates the chances of developing microbial imbalance or super infections, however this can be burdensome when the exact pathogenic species is unknown or if the patient is suffering from multiple infections. Phages are larger than antibiotic chemical molecules; hence it is most effective for the treatment of wounds or infections at easily accessible sites. When the agents are located deep inside the human cells, they might often be inaccessible to the bacteriophages.[32]

Identifying phages that display good primary pharmacodynamics i.e. antibacterial virulence and minimal secondary pharmacodynamics i.e. least harm to patients in addition to good pharmacokinetics, the ability to reach target bacteria remains an uphill struggle for microbiologists. Even though bacteriophages seem to exhibit a lot of controversies in comparison to antibiotics, these obstacles pose relatively minor risks in reality.[33]

The development of a new phage is much easier than developing a new antibiotic drug moiety. However the cost of therapy afflicts the common man. In the Phage Therapy Centre at Georgia, phage therapy cost would range from $2,500 USD for outpatient care to $20,000 USD for in-patient treatment, in addition to the personal costs borne by the patient. The duration of treatment is around 6 weeks or longer.[34] In Poland, the estimated cost of therapy including consultation, diagnostic tests and phage typing sums up to around $800 USD to $1700 USD. An initial treatment of two weeks precedes the actual treatment, with the cost of the former ranging from $400 USD to $800 USD.[35] Jerome Gabard, chief executive of the
French biotech firm Pherecydes Pharma, claims that the treatment with their products will probably cost around $2500 to $8500 per treatment.[36]

Another significant barrier to phage therapy is the rapid development of phage-resistant bacteria. A variety of mechanisms are involved in the development of phage resistance including blocking bacteriophage absorption, inhibiting the injection of bacteriophage genomes, restriction-modification systems and abortive infection systems. According to the data gathered from in-vitro studies, phage resistance can develop in time ranging from few hours to several days in accordance with the mutation and growth rates. But here arises a question whether the evolution of phage resistance in-vitro is relevant to the in-vivo conditions where replication is slower and environmental conditions are more challenging.[37] Clustered Regularly Interspaced Palindromic Repeats (CRISPRs) are an array of prokaryotic DNA sequences that can induce a special form of acquired immunity to certain viral pathogens. In addition to all the mechanisms of phage resistance discussed above, a newly discovered CRISPR-mediated phage resistance appears to be quite interesting. Recent studies reveal that the bacterial cells incorporate the phage genetic material into CRISPRs as spacers and by acquiring these spacers the bacterial hosts develop resistance towards the phage that carry the incorporated sequence.[38]

Ultimately, the factors affecting phage therapy can be classified as extrinsic and intrinsic. Extrinsic factors are more problematic and unresolvable. Firstly, phage isolation for fastidious hosts is often challenging. Secondly, reaching intracellular pathogens is difficult for phages. Thirdly, phages can be cleared or neutralized by antibiotics. The lack of public awareness and regulatory acceptance are also categorized under extrinsic factors. Intrinsic factors like pH, temperature and moisture also affect the phage activity and its ability to lyse the targeted pathogens in-vivo.[39] Appropriate funding for the development of phages is crucial in order to conduct elaborate researches and human trials to analyze the safety and demonstrate the efficacy in controlled environments. Without sufficient clinical data, the stigma against medicinal use of bacteriophage in the United States and Europe is set to continue.

The future of phage therapy: beyond expectations and controversies.

The unnecessary overexploitation antibiotic therapy has led to an increased bacterial resistance and this is a deadly hazard to human health. Millions succumb to infections by multidrug resistant organisms every year.[40][41] Despite the rising demand for a newer class
of antibiotics, it would be very expensive to do the same, due to emerging multidrug resistance.\(^{[42]}\)

Bacteriophage is one such alternative which can be developed as a solution for the multidrug resistance crisis. Bacteriophages are mostly environment friendly and natural selection is the criteria to identify and isolate bacteria whereas antibiotic development is a more complex procedure. There are many ‘phage properties’ which make them an ideal alternative to antibiotics. Phages which are very specific to their host, do not alter the gut microbiota unlike antibiotics. Auto-dosing is one major factor, phages have the ability to proliferate specifically in the regions of high bacterial density.\(^{[43]}\)

Phages are relatively less toxic compared to antibiotics. Clinically, usage of bacteriophage is a therapeutic bliss considering the current failure of antibiotics. The efficacy of phage against ESKAPE organisms is well established. The phages used against these pathogens have cured 90% of the infection. Although bacterial resistance to phage is possible, the likelihood of this resistance being troublesome is narrow, owing to the ability of bacteriophages to get mutated. Presently, there is a need for more clinical trials and experiments to be conducted, before phages can replace the conventional antibiotic therapy against bacterial infections. Phage therapy has also contributed enormously to the field of modern biotechnology. Enigmas in the molecular biology are explained by the concept of bacteriophages. The pharmacological properties in addition to the recent advances, suggest the ascending of a post antibiotic era where bacteriophage could take over as the first line antimicrobial therapy.\(^{[44]}\)

Phage act as vehicles for vaccines delivery, by carrying the antigen of the vaccine superficially. In the case of DNA vaccines, the vaccine antigen sequences are integrated into the phage genome and thereby act as the vehicle.\(^{[45]}\) However, the sequences that are essential for the vaccine antigen synthesis are incorporated into the phage genome and the phage would then act as vehicle for the delivery of DNA vaccine. Phages are being evaluated for their roles in diagnostic purposes; phage typing is a broad concept which holds the key to the advancement of diagnostic procedures in the future.

Thus, phage therapy applications range from the diagnosis (phage typing), to prevention (phage vaccine), as well as the management (phage therapy) of bacterial diseases. Lytic phages can be utilized to tackle multiple present day issues, by aiding in the treatment of simple infections to preventing major outbreaks by MDR pathogens. Phage therapy can go on
to become a resourceful new chapter in the modern era of medicine and many more large scale clinical studies should be conducted to confirm the safety and efficacy of this therapy.

**Can Bacteriophage resolve the TB burden in India?**

The tuberculosis burden in India remains an unresolved hindrance to its developmental progress, accounting for 29% of the 1.8 million TB deaths worldwide and about 16% of the overall multidrug resistant cases.[46] The current predicament comprising of MDR and XDR strains raises interest in the vital role bacteriophage can play in the treatment of TB.

Mycobacteriophages which specifically target *Mycobacterium tuberculosis* and *Mycobacterium smegmati*, express marked genetic diversity.[46] Ever since the isolation of first Mycobacteriophage strain in the late 1940s, thousands of strains have been developed from *M. smegmati* alone, in addition to the isolation of phages against *M. tuberculosis* from the stool samples of tuberculosis patients.[47] The clusters identified so far share very less sequence similarity, with marked difference in the guanine plus cytosine content (GC %).[48]

Although the delivery of mycobacteriophages to the lungs is not a tedious process, there lacks sufficient in-vivo data to support the correct delivery of phages into the mycobacterial hosts present inside the granulomas. Phages can also be employed as prophylactic regimen to the high risk groups. However, the last decade observed a tremendous improvement in the study of mycobacteriophages, with over 285 complete genome sequences being determined.[49] This rich genomic data anticipates significant utility of mycobacteriophages in the future to combat the existing tuberculosis burden, particularly in developing countries like India.

**CONCLUSION**

With over 18 strains of pathogenic bacteria developing ultimate resistance to all the antibiotics being marketed today, there exists an augmenting demand for an alternative agent which can effectively combat the emerging global crisis. It is estimated that bacterial infections will result in the deaths of over 10 million people by the year 2050, if the current trend perpetuates. Resistance to antibiotics propels the advancement of bacteriophages and paves way for it clinical utility in the management of various infectious diseases. High pathogen specificity, ability to be at par with their rapidly mutating bacterial counterparts and relatively low toxicity to humans as well as gut microbiota are the promising features of phage therapy that distinguishes them from the conventional antibiotics. However, the current paucity of clinical data necessitates profound clinical trials to be conducted in order to obtain...
in-depth analysis and safety data, which are crucial for the worldwide acceptability of bacteriophages.

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