DOI: https://doi.org/10.36094/sc.v89.2024.Phage_Therapy_a_Renewed_Hope.Chakrabarti_Dutta.478

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Sci. and Cult. 90 (11–12) : 478-489 (2024)

PHAGE THERAPY: A RENEWED HOPE IN INFECTIOUS DISEASE MANAGEMENT UNDER THE THREAT OF ANTI-MICROBIAL RESISTANCE

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Infectious diseases are in limelight since the primitive days, because "Health is Wealth" remained a central theme in the history of human societies. In light of epidemiological landscape and the historical context of diseases, every millennium can be regarded as unique as marked by appearance of deadly diseases and their distinct features. The 21st century appears no longer an exception, the trend continued with fear of viral pandemics. However, beyond all other existing challenges, our world is now facing a novel problem: the primary agents used in the treatment of bacterial infections which are crucial for saving life in the healthcare system begun to exhibit ineffectiveness. This concern has come into attention predominantly over the last two decades, although warning issued shortly after the discovery of such agents, the antibiotics by Alexander Fleming, the inventor of penicillin. Ineffectiveness of antibiotics to treat a disease occurs due to the emergence of multi drug resistance (MDR) bacterial strains which is difficult to manage with the existing antibiotics. MDR bacterial strains are probably the most important concern of Antimicrobial Resistance (AMR). AMR occurs when microorganisms such as bacteria, viruses, fungi, and parasites change when they are exposed to antimicrobial drugs. As a result, the medications become ineffective and infections persist in the body, increasing the risk of spread to others. This can lead to severe health complications, prolonged illnesses, and even death. AMR is a global health issue that affects both humans and animals. Management of AMR is a crucial component of public health initiatives aimed at combating the rise of drug-resistant infections. Under this challenging AMR issues, there is a critical need to investigate and evaluate new treatment strategies. This paper will explore the reasons of AMR in context of bacterial infection and an optional treatment of MDR bacterial infection, the phage therapy.

Introduction

Modern medicine can be classified into two distinct eras: the pre-antibiotic era and the post-antibiotic era. The pre-antibiotic era, which covers the long history of medical practice before the discovery and widespread use of antibiotics, was characterized by limited treatment options for bacterial infections and a reliance on various traditional remedies, surgical interventions, and natural substances. Originally, diseases were believed to be caused by supernatural forces until the middle of the 19th century when scientific ideas began to shift. Louis Pasteur's research on fermentation demonstrated that life does not spontaneously arise but develops from germs[1]. Robert Koch's work on anthrax further solidified the understanding that contagious diseases result from microorganism infections, giving rise to the field of microbiology[2]. Before the discovery of antibiotics, the treatment of diseases and infections

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presented significant challenges due to limited understanding of their causes, where most of the treatments relied on empirical observations and old-fashioned remedies, often ineffective or harmful. Infectious diseases, such as bubonic plague, pertussis, diphtheria, tuberculosis, typhoid, cholera and smallpox, spread uncontrollably without vaccines or proper quarantine measures, resulting in high mortality rates[3]. These infections were fatal regardless of age or gender. Contagious diseases spread through poor hygiene, direct contact, or airborne particles caused widespread epidemics and pandemics. Diagnostic tools were limited, leading to misdiagnoses and inappropriate treatments. Society then faced scary challenges with unusual resources and ineffective therapies, resulting in high mortality rates and a constant struggle to improve patient outcomes.

With time, gradual advancements in biomedical research and innovation in the modern era have led to a deeper understanding of new microbes and associated diseases. This has initiated a new chapter in the field of biomedical sciences, with numerous ground-breaking discoveries that have transformed the healthcare system management[4]. The discovery of antibiotics can be regarded as one of the most significant discoveries of the last century[5]. Historically and unknowingly, antibiotics have been used for thousands of years to treat infections, even when the existence of bacteria was mysterious and even later when the connection between bacteria and infections wasn't properly understood[6]. Historical accounts reveal that the red soil in Jordan has antibiotic like qualities, which have been traditionally used and remain in use still today as a remedy for skin infection. Several bacteria capable of producing antibiotics has been identified in this soil recently[7]. Early civilizations like the ancient Egyptians used moulds and plant extracts to treat infections. In the late 19th century, Paul Ehrlich discovered that certain chemicals could selectively kill bacteria without harming other cells. In 1909, he found arsphenamine effective in treating syphilis, marking the first modern antibiotic. Alexander Fleming accidentally discovered penicillin in 1928 and its mass production during World War II saved many lives. Scientists in Oxford played a crucial role in developing the mass production process, leading to the first mass-produced antibiotic and the 1945 Nobel Prize in Medicine being shared by Howard Florey, Ernst Chain, and Alexander Fleming[8].

Soon after the ground-breaking discovery of penicillin, series of new antibiotics emerged to revolutionize the field of antibiosis which marked a significant turning point in medical history, enriching treatment options and significantly reducing mortality rates. Thus by the 1970s, during the "golden age" of antibiotics, effective treatment options became available for a wide range of bacterial infections. However, with time, the initial enthusiasm surrounding antibiotic discovery began to fade. The rich pipeline of antibiotics discovery gradually began to dry up, leading to concerns about the future effectiveness of these life-saving medications. Over the past four decades, the convectional approach of antibiotic discovery facing challenges to yield new antibiotics mainly due to a stringent penetration barrier and various mechanisms employed by bacteria to endure antibiotics exposure[9]. The overuse and misuse of antibiotics in both clinical and agricultural settings contributed to the rise of antibiotic-resistant strains of bacteria, posing a serious threat to public health worldwide. Consequently, the medical community faced the frightening challenge of finding new ways to combat this growing crisis and develop innovative treatment strategies to address the evolving threat of antibiotic resistance.

While this problem ongoing, the scientific community continuously searching for an alternative treatment option to fight against the MDR bacterial strains to combat the problem of AMR.

Shifting from the Golden Era of Antibiotics to the Gradual Transition to a Depleted Pipeline of Innovations

The post-antibiotic era marks a transformative period that began in the early 20th century with the discovery of penicillin by Alexander Fleming and subsequent developments in antibiotic therapy. Effective management of disease conditions became possible that were once considered deadly. The introduction of antibiotics revolutionized healthcare, leading to significant reductions in morbidity and mortality rates associated with bacterial infections. It opened new avenues for medical advancements, enabling more complex surgical procedures and treatments for various conditions, as the risk of postoperative infections was greatly diminished.

However, soon after his discovery of penicillin and application of antibiotics in medical fields, Alexander Fleming expressed his worry and issued a significant warning and cautioned that the excessive and inappropriate use of antibiotics could bring a new problem of antibiotics resistance where bacteria may evolve and adapt themselves to develop mechanisms to withstand the effect of antibiotics. Fleming's worry came true with the rise of multidrug-resistant (MDR) bacteria which has emerged alongside a diminishing pipeline for antibiotic development.

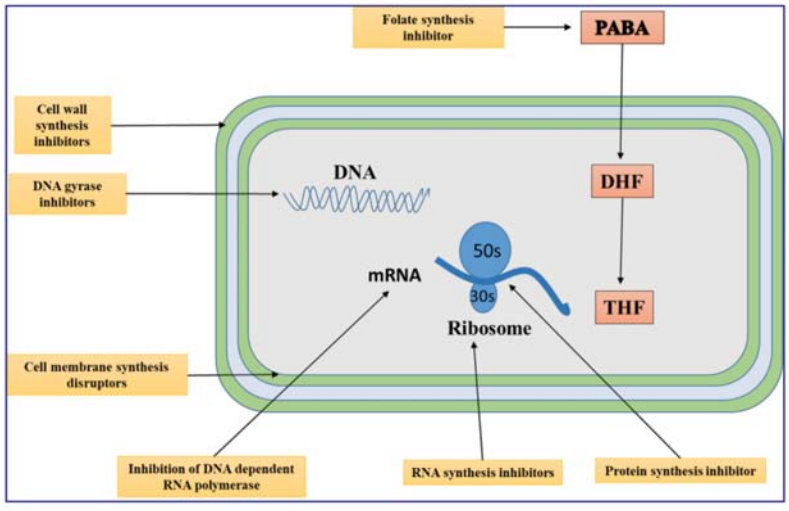

Fig. 1: Schematic presentation of mode of actions of antibiotics

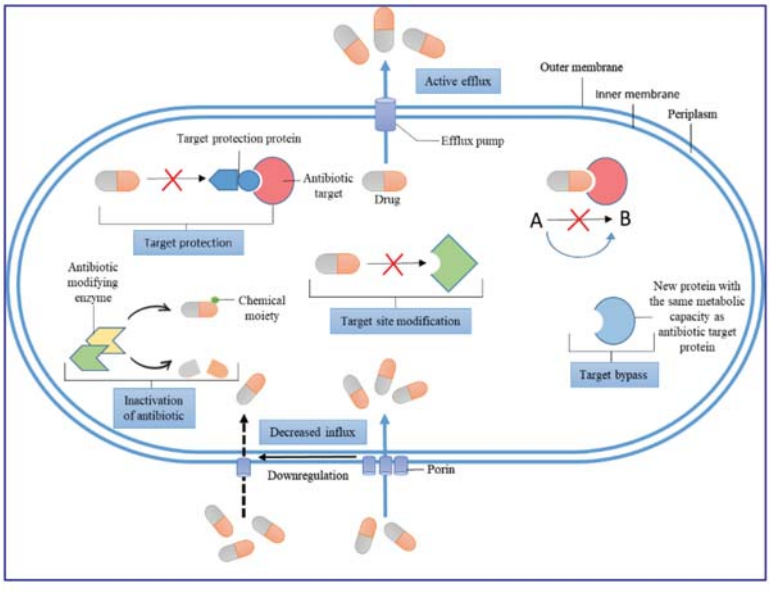

Fig. 2: Mechanisms of development of MDR bacterial strains

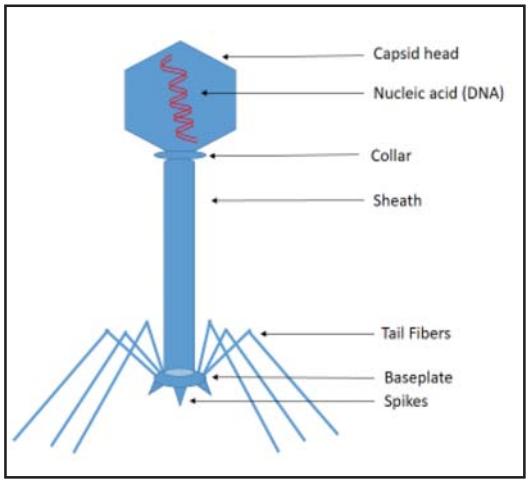

Fig. 3: Schematic Diagram of Bacteriophage

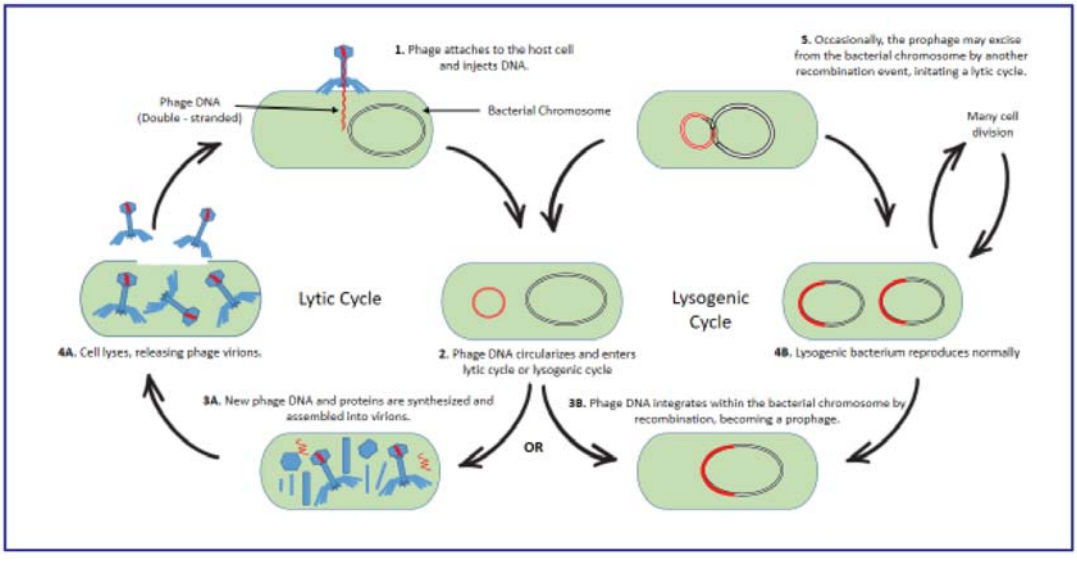

Fig. 4: Replication of bacteriophage using Lytic and Lysogenic cycle

At present, Antimicrobial resistance (AMR) is a critical global health threat, driven primarily by the overuse and misuse of antibiotics. World Health Organization (WHO) have identified AMR pathogens as a critical threat to public health[10,11]. Despite the lack of a unified international AMR surveillance system, reports estimate that over 2 million AMR infections occur annually in the U.S., leading to approximately 29,000 deaths and healthcare costs exceeding $4.7 billion. In Europe, AMR is responsible for more than 33,000 deaths and a loss of 874,000 disability-adjusted life years annually, contributing to direct and indirect costs amounting to $1.5 billion[12]. Developing nations, though lacking comprehensive economic data, face exacerbated challenges from AMR as communicable diseases remain the top causes of death, now compounded by emerging and re-emerging infections.

AMR genes, while naturally occurring in the environment, have proliferated due to the widespread use of antibiotics, particularly in the absence of rapid diagnostic methods to detect bacterial pathogens and AMR genes. This has driven the overuse of broad-spectrum antibiotics. WHO released a priority list of pathogens in February 2017 to steer research and development toward new antibiotics[13]. These pathogens have acquired resistance through genetic mutations and mobile genetic elements, rendering them resistant to a wide array of antibiotics, including last-resort treatments like carbapenems, glycopeptides, and polymyxins. Resistance to lipoglycopeptides, though rare, has recently been reported, likely due to their dual mode of action in inhibiting peptidoglycan synthesis and destabilizing bacterial membranes. The rise of such resistant strains has increasingly contributed to hospital-acquired infections[14].

Method of Action for Antibiotics

Antibiotics work by targeting key bacterial processes to stop their growth and survival. They can inhibit cell wall synthesis (like penicillins and vancomycin), protein synthesis (like tetracyclines, macrolides, and aminoglycosides), nucleic acid synthesis (like fluoroquinolones and rifampicin), disrupt cell membranes (like polymyxins), and inhibit metabolic pathways (like sulfonamides and trimethoprim)[15]. The schematic diagram (Figure 1) below illustrates the mechanism of action of antibiotics. These processes result in cell lysis, inhibition of protein synthesis, disruption of DNA replication, damage to the membrane, and interference with folic acid synthesis, ultimately culminating in the death of bacterial cells. (Fig. 1)

Development of MDR Strains: a Major arm of AMR

Over the time the bacterial strains gradually gained resistance against different antibiotics. AMR has emerged primarily due to the ability of bacteria to adapt and develop various mechanisms that enable them to prevent the effects of antibiotics. These resistance strategies not only complicate treatment regimens but also contribute to the persistence and spread of resistant infections. Different mechanisms employed by bacteria to achieve resistance property are described below (Figure -2).

One of the most recognized mechanisms of resistance is antibiotic inactivation, which involves the production of specific enzymes by bacteria. A prominent example is beta-lactamases, which are enzymes that target and hydrolyze the beta-lactam ring essential for the activity of many antibiotic classes, including penicillins and cephalosporins[16]. By breaking down the antibiotic's structure, these enzymes render the drug ineffective, allowing the bacteria to survive and proliferate even in the presence of antibiotics.

Bacteria utilize efflux pumps as another critical mechanism of resistance by effectively transporting antibiotics out of the bacterial cell using molecular pumps. By expelling the drug before it can exert its harmful effects, the intracellular concentration of the antibiotic is significantly diminished which allows the bacteria to survive despite exposure to antimicrobial agents.

Target modification serves as another means by which bacteria can resist antibiotic action. In this mechanism, genetic mutations occur that alter the drug's binding sites, which can include critical components such as ribosomes[17]. When bacteria modify these target sites, the affinity of the antibiotic for its target decreases, making it more challenging for the drug to inhibit essential cellular functions. As a result, the bacteria can thrive even in the presence of antibiotics that would typically be effective.

Reduced permeability is yet another strategy employed by bacteria to counteract antibiotics. Certain bacterial species can alter their cell membrane's characteristics, leading to a decreased uptake of antibiotics. This alteration creates a physiological barrier that limits the entry of antimicrobial agents into the cell, thereby decreasing their effectiveness and allowing the bacteria to evade the lethal effects of these drugs.

Notably, biofilm formation is a sophisticated tactic that provides a significant survival advantage to bacteria.

Biofilms are dense clusters of bacteria encased in a protective extracellular matrix. This matrix not only shields the bacteria from the direct action of antibiotics but also offers a defensive barrier where bacteria can exhibit a remarkable level of tolerance to antimicrobial agents, making infections associated with biofilms particularly challenging to treat[18].

Taken together, these mechanisms highlight the complex and dynamic ways in which bacteria have adapted to resist antibiotics. Understanding these various strategies is crucial for developing effective interventions to combat the growing threat of antimicrobial resistance.

Search for Alternatives of Antibiotics

The emergence of resistant strains of bacteria results from various factors and their uncontrolled spread has created an urgent necessity for the exploration and development of alternative treatments to antibiotics which could provide new avenues for effectively managing infections caused by AMR bacteria. Search for alternatives against AMR is the need of the hour. In light of this concerning trend, researchers and healthcare professionals are seeking innovative solutions to mitigate the impact of AMR. Addressing the challenges posed by AMR will require a multifaceted approach, combining scientific research, public health policies, and global cooperation to ensure that effective alternatives to traditional antibiotics are available.

While efforts are underway to develop novel natural antibacterial agents and investigate immunotherapy to strengthen the body's defences, fight diseases, nanotechnology developments, and Probiotic use as a possible area for study and advancement pathways, phage therapy offers a noteworthy possibility in combating antibiotic resistance (AMR)

Bacteriophages

Bacteriophages, or simply phages, are one of the beneficial viruses that can infect bacteria, and they play a crucial role in maintaining the balance of ecosystems by targeting harmful bacteria without arming the surrounding beneficial microbes. These tiny viruses help in preventing diseases by effectively killing off pathogenic bacteria instead of creating diseases, making them highly significant in the fight against bacterial infections[20]. They are unable to perform the majority of biological processes independently and depend on a living host for their replication. Therefore, they are classified as obligate parasites of their bacterial hosts and are present in nearly all environments where bacteria thrive. What's even more fascinating is that bacteriophages are the most abundant entities on Earth, and play a crucial role in the ongoing regulation of the diversity, richness, abundance, evolution, and physiology of microbial communities within specific habitats[21,22]. Outnumbering bacteria by a staggering ratio; they can be found in various environments, from deep oceans to the human gut, showcasing their versatility and adaptability.

Phages were discovered by Frederick Twort and Felix d'Herelle separately. Twort was trying to find a vaccine in a virus-free environment, while d'Herelle discovered bacteriophages in a dysentery outbreak investigation during World War I. d'Herelle applied his findings to bacterial cultures and noted that turbid cultures became clear, demonstrating the lytic activity of bacteriophages against bacteria. His research on bacteriophages was compiled in a monograph titled "The Bacteriophage and Its Behavior," along with several other publications which established the field of "bacteriophagology," introducing new terminology to describe post-infection processes and providing detailed explanations of the purification and titration of bacteriophage cultures[23].

The structure of a bacteriophage is relatively simple, comprising genetic material, either DNA or RNA, in single or double-stranded configurations encased in a protein capsid (Fig. 3).

The primary structural forms of phages include a polyhedral capsid with a tail or a ploy hedral head without a tail, although the filamentous shaped phages are also available. Most of the phages have polyhedral capsid except filamentous phages. The structure of phage enables them to effectively locate suitable host bacterium where it attaches to exploit the cellular metabolic processes and produce numerous progeny that are robust enough to endure until they encounter a new host bacterium for infection. The capsid is attached to a tail which has fibers, used for attachments to receptors on bacterial cell surface. Bacteriophages infect bacterial cells and can reproduce through two different mechanisms: the lytic life cycle and the lysogenic life cycle, although two more mechanisms are known, namely pseudo-lysogenic and chronic. In the lytic life cycle, phages replicate within the host, ultimately leading to the destruction of the bacterial cell. Lysogenic cycle involves integration of phage DNA into host chromosome which keep replicating with the host for many generations[24]. Phage genes may reactivate under specific conditions to release fully assembled phage particles. (Fig. 4)

After its discovery in early 1900s, widespread use of bacteriophages in laboratory has revolutionized the field of molecular biology and biotechnology by solving numerous problems of molecular biology that were previously masked in mystery. By infecting and replicating within bacteria, bacteriophages provided researchers a window into the intricacies of molecular processes and genetics, unravelling the secrets of life at the most fundamental level.

The application of bacteriophages in genetic engineering and biotechnology enabled scientists to manipulate genetic material with unprecedented precision, fuelling advancements in fields such as recombinant DNA technology and gene editing.

It has been used as diagnostics, predominantly when the molecular tools were in its infancy. Diagnostic use of bacteriophages or the phage typing remained an integral part of the healthcare system and still now several laboratories use this system[25,26,27]. Classical value of the phage typing leads to the development of many new phage typing scheme, because it is relatively simple, reliable and don't need state of the are instrumentation.

As antibiotic resistance continues to escalate, phage therapy has once more emerged as a promising solution to address the issue of antimicrobial resistance (AMR). The lytic bacteriophages could revolutionize the treatment of bacterial infections, especially through their ability to target specific bacterial strains. This newfound understanding paved the way for the development of novel treatments for bacterial infections and antibiotic resistance, marking a significant leap forward in the dominion of medical science.

Evolution of Phage Therapy: A Historical Overview

Phage therapy has been around for 100 years (Fig. 5), with the first observations of phage activity made by Hankin in 1896. Early signal of phage therapy emerged 2 decades before the discovery of bacteriophage, when in 1896, British bacteriologist Ernest Hankin observed significant antibacterial activity in the "Holy" water of the Ganges and Jumna rivers in India. He conducted experiments with Ganges water, observed that unboiled water kills cholera germs in less than 3 hours and theorized that there was a "volatile agent" in Ganges water that could not survive heat and filtering[28,29]. Hankin recommend using river water instead of well water as a safer option to avoid cholera and other water-borne diseases.

The tobacco mosaic virus was recognized as the first virus in 1898, marking the beginning of virology studies[30]. The bacteriophage, an exciting virus that specifically

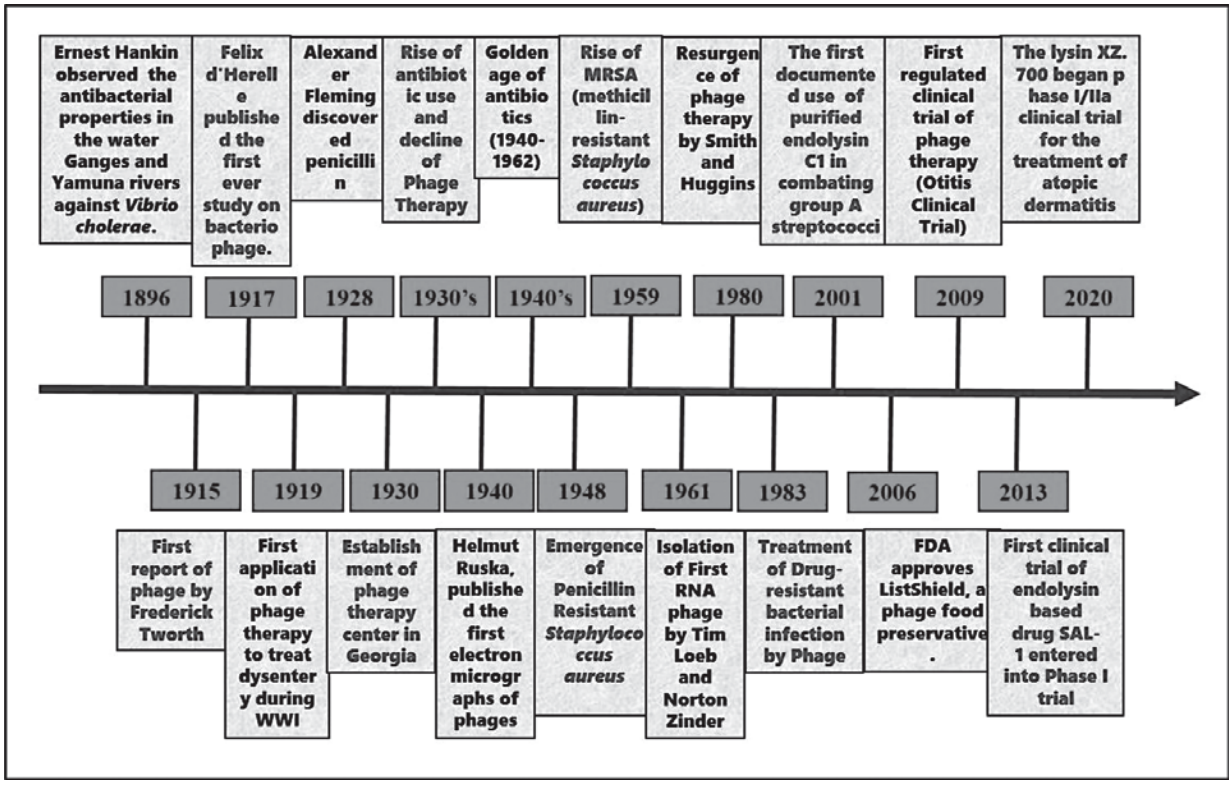

Fig. 5: Timeline of Phage Therapy from the discovery of bacteriophage to the present day

targets and infects bacteria, was independently discovered by two scientists in different geographical regions and scientific environments, who were conducting their own research in the early 20th century. Revolutionary findings by Frederick Twort in England in 1915 and Felix d'Hérelle in France in 1917 on phage discovary not only paved the way for new directions in microbiology but also established a foundation for future studies that would investigate the use of bacteriophages as a natural alternative to antibiotics, particularly in a time when antibiotic resistance has emerged as a critical global health challenge[31]. d'Hérelle was the pioneer in coining the term bacteriophage, which translates to bacteria-eater, to refer to phages as viruses and advocated for their potential application in therapeutic settings to treat bacterial infections[32]. This claim was only accepted after electron microscopy became available in the 1940s. Félix d'Herelle conducted the first therapeutic use of phages in 1919 at Paris's Hôpital des Enfants-Malades, resulting in successful treatments against various infections. d'Hérelle studied phage as treatment for bacterial infections in various countries and institutes[33]. He led the Bacteriophage Inquiry in India between 1927-1936, which was inconclusive due to technical difficulties, lack of knowledge about virus biology, and political turmoil[34]. The history of phage therapy dismissal during the 20th century was rich with politics, personal feuds, and unrecognised conflicts. The technology for studying viruses and knowledge about viruses was not developed enough at the time, and phages were not as efficient in clinical use as they were in laboratories[31]. Phage therapy was used worldwide with variable success until early 1940s. Following these key discoveries, interest in phage therapy grew, particularly in Eastern Europe and the Soviet Union, where research and clinical applications were actively pursued. During the 1920s and 1930s, phage therapy gained traction as a promising alternative to antibiotics, especially in the treatment of bacterial infections that were proving resistant to traditional treatments. Many hospitals and clinics began utilizing phages to treat patients suffering from a variety of infections, such as dysentery and cholera. In 1923, d'Herelle and his disciple George Eliava founded a phage-institute in Tbilisi, Georgia, which started producing phage propagates for diverse indications and became one of the leading centers for phage research and therapy, developing a range of phage preparations tailored for specific bacterial pathogens[35]. However, the advent of antibiotics in the 1940s led to a decline in interest and research in phage therapy in many parts of the world, particularly in Western nations. Antibiotics were widely adopted and hailed as miracle drugs, overshadowing the earlier enthusiasm for bacteriophages. This shift resulted in a significant decrease in funding and research efforts focused on phage therapy, causing it to remain largely confined to the Eastern European context. In recent decades, as antibiotic resistance has emerged as a pressing global health crisis, there has been a renewed interest in phage therapy. Researchers have started to explore the potential of bacteriophages as a viable treatment option in the face of resistant bacterial strains. Clinical trials and studies have been initiated to examine the efficacy and safety of phage therapy for various infections. This resurgence reflects a growing recognition of the limitations of antibiotics and the need for innovative approaches to tackle bacterial pathogens that have become resistant to multiple drugs.

Phage Therapy: A Promising Alternative to Antibiotics

Every problem presents an opportunity for solutions; it is likely that the creator of the universe engineered bacteriophages to manage the extensive populations of bacteria. We are now employing the lytic property of bacteriophage to confront the hurdle of AMR, which has led to the emergence of bacteriophage therapy as a promising alternative approach. Phage therapy is an innovative approach that utilizes bacteriophages, which are viruses that specifically infect bacteria, to treat bacterial infections[36]. Phage therapy operates through a different mechanism than antibiotics, utilizing bacteriophages which are highly specific to their bacterial hosts. The specificity and self-replicating nature of phages make them a promising treatment for multidrug-resistant infections. The power of bacteriophages to destroy the specific bacterial host, i.e, the lytic ability of bacteriophage is the fundamental concept of phage therapy. This principle is based on the unique ability of phages to target and destroy specific bacterial cells without harming the surrounding healthy tissue. Unlike traditional antibiotics, which can disrupt a wide range of microorganisms and lead to issues such as resistance and imbalances in the microbiome, phage therapy offers a more tailored method of treatment. When introduced into a patient's system, bacteriophages can identify and latch onto their specific bacterial targets. Once attached, they inject their genetic material into the bacterial cell, prompting the bacteria to produce new phage particles. This process ultimately leads to the destruction of the bacterial cell as it bursts, releasing more phages to continue the cycle of infection against the targeted bacteria. The application of phage therapy is particularly significant in an era of increasing antibiotic resistance, where conventional drugs are becoming less effective

against certain infections. This method not only offers a potential solution to combat resistant strains but also highlights the importance of personalized medicine, as phage therapy can be customized to match the specific bacterial infection a patient is facing[37].

Bacteria and phages engage in an ongoing evolutionary "arms race," with resistance mechanisms against antibiotics mirroring those against phages. As a result, phage engineering has emerged as a solution to enhance the effectiveness of phage therapy. Genetic engineering techniques like bacteriophage recombineering and electroporated DNA (BRED) enable the creation of engineered phages. The first successful treatment using engineered phages was repotred in 2019 in a patient with cystic fibrosis infected with antibiotic-resistant *Mycobacterium abscessus*[38]. This case used the bacteriophage recombineering and electroporated DNA (BRED) technique to engineer 2 phages as part of a 3-phage cocktail[39]. However, newer methods, such as incorporating CRISPR/Cas systems into phage products, show even greater potential. CRISPR/Cas9 systems enhance phage targeting of bacteria. Engineered bacteriophages can deliver CRISPR/Cas to efficiently kill bacteria by targeting virulence genes. Integrating CRISPR/Cas systems into phage engineering is crucial to enhancing the efficacy and consistency of phage therapy against resistant bacteria[40].

Sectors of health, such as hospitals, long-term care facilities, and the agricultural industry, are significantly threatened by the rise of multidrug-resistant (MDR) bacteria, resulting in challenges in treating infections effectively. These MDR bacteria pose a serious risk to public health by limiting the effectiveness of traditional antibiotic treatments, infection control protocols, and overall public health infrastructure.

Examples of phage therapy application in the health care system, with case reports illustrating its effectiveness, are becoming increasingly prominent as researchers and clinicians seek alternative treatments for antibiotic-resistant infections. In recent years, case reports have described compassionate use of phages for patients suffering from a severe and persistent MDR Mycobacterium abscessus infection. A patient with cystic fibrosis experienced a widespread infection from Mycobacterium abscessus after undergoing bilateral lung transplantation and receiving immunosuppressive therapy due to medication[41]. A 3-phage cocktail was utilized for the treatment, administered intravenously (IV) twice a day at a dosage of 10^9 plaque-forming units (PFUs) per administration for a duration of six months. The patient had clinical improvement in lung function, radiographic imaging, and clinical signs and symptoms, although without complete clearance of the infection. Within 6 months, the patient's condition improved significantly, demonstrating not only the therapeutic potential of phages but also highlighting the urgent need for further research and clinical trials to establish standardized protocols for their use.

The experimental approach to treating pneumonia or bacterial colonization of the airways in patients with cystic fibrosis (CF) remains largely confined to case reports. Another illustrative case involved a 5-year-old child who experienced multiple antibiotic treatment failures and chronic colonization by *Pseudomonas aeruginosa* was administered aerosolized bacteriophages from the Pyophage cocktail, sourced from the Eliava Institute in Georgia. A customized phage preparation was administered three times daily for periods of 6 to 10 days each month over three consecutive months, leading to a remarkable recovery and a decrease in bacterial load, an improvement in the child's clinical condition, including enhanced expectoration and weight gain. Following the third aerosol treatment, *P. aeruginosa* was no longer detected in the patient's sputum[42]. More recently, from 2007 to 2010, a study involving eight CF patients in Eliava Institute in Georgia, Tbilisi was conducted. These patients received aerosolized bacteriophages for 6 to 10 days in conjunction with standard antibiotic therapy, mucus-dissolving agents, and vitamins. The findings indicated a reduction in bacterial concentration in the patients' sputum and a decreased reliance on antibiotic treatments[43]. While these studies are promising and suggest that bacteriophages may effectively mitigate *P. aeruginosa* infections, the lack of standardized methodologies and the limited patient population make it challenging to draw definitive conclusions regarding the efficacy of bacteriophage therapy for this condition. Therefore, larger clinical trials, particularly those employing randomized designs, are essential to ascertain the effectiveness of bacteriophage-based treatments for pneumonia caused by *P. aeruginosa* in CF patients.

These cases underscore the promise of phage therapy as a viable option in the fight against drug-resistant bacterial infections, a growing concern in global health. As the medical community continues to explore and document such applications, it is clear that phage therapy may play an integral role in shaping the future of infectious disease treatment, offering hope to patients who face limited options in today's antibiotic landscape.

Another example is a 41-year-old male patient with Kartagener syndrome, who experienced a severe and life-threatening chronic infection caused by multidrug-resistant

Pseudomonas aeruginosa, showed a positive clinical response following treatment with iterative aerosolized phage therapies tailored to his specific bacterial isolate. The phage titers observed in successive sputum samples indicate active phage replication within the patient. The multidrug-resistant phenotype identified in these isolates is attributed to a combination of factors, primarily spontaneous chromosomal mutations. Notably, all isolates obtained post-phage treatment remain susceptible to phage. These findings underscore the potential for significant clinical improvement through personalized phage therapy, even when complete eradication of *P. aeruginosa* lung colonization is not achieved[44].

Phage therapy has shown success in treating chronic wounds and skin infections caused by Gram-negative bacteria like *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Acinetobacter baumannii* which are common in hospitals. Phage therapy has been effective in treating chronic wounds, targeting specific pathogens without harming beneficial skin bacteria. It is also researched for gastrointestinal disorders, respiratory infections (especially in patients with cystic fibrosis), and urinary tract infections. Combining antibiotics with phages has shown promise in fighting complex infections, illustrating phage therapy's potential as a complementary treatment to traditional antibiotics. Ongoing research in explores phages' applications in treating various infections, showing the versatility of phage therapy in revolutionizing infectious disease treatment.

Research into phage therapy has gained momentum, and clinical trials are underway to better understand its effectiveness and safety[45]. By utilizing the natural predatory behaviour of phages, this therapeutic option opens new pathways for treating bacterial infections, offering hope to patients who may have exhausted other treatment options. The principle of phage therapy represents a significant advancement in the fight against bacterial illnesses, reinforcing the continuous evolution of medical treatments in response to emerging health challenges.

Challenges in Phage Therapy

Although phage therapy is emerging as a promising alternative to traditional antibiotics in the fight against bacterial infections, it faces several challenges that must be addressed before it can be widely implemented in clinical practice. Phage therapy is intended to enhance, rather than substitute, the use of antibiotics by expanding the range of treatment alternatives and the understanding available to healthcare professionals concerning bacterial infections. However, the risks linked to phage therapy are amplified if healthcare providers choose to prescribe one or a limited number of synthetic phage products for minor infections, as this could lead to increased bacterial exposure to a single phage and promote the development of resistant mutations. Phage resistance is a significant concern in the application of phage therapy[46]. Like the bacterial strains can evolve mechanisms to withstand the effects of antibiotic treatments, bacteria can also adapt to resist specific bacteriophages. This phenomenon poses a challenge for the long-term effectiveness of phage therapies. While bacteriophages themselves have the ability to undergo evolutionary changes, the dynamic nature of bacterial resistance necessitates ongoing efforts in terms of adaptation and surveillance. Continuous monitoring of bacterial populations and their interaction with phages is essential to ensure that treatment remains effective and to modify therapeutic strategies accordingly[47]. The specificity of bacteriophages, which can target only particular strains of bacteria, also poses a significant hurdle, as it requires careful matching of the phage to the bacterial infection.

A critical problem arise from horizontal gene transfer in phage therapy applications, particularly as this process can facilitate the unintended acquisition of antibiotic resistance genes among bacterial populations[48]. Bacteriophages, which are well known to play a crucial and multifaceted role in horizontal gene transfer by injecting their own genetic material into bacterial cells that confer advantageous traits, such as antibiotic resistance or metabolic capabilities, thereby reshaping the genetic landscape of microbial communities[49]. This gene exchange not only undermines the efficacy of phage therapy, which aims to specifically target and eliminate harmful bacteria, but it also poses a significant risk of creating superbugs that are resistant to multiple forms of treatment. Antibiotic resistance in foodborne bacterial pathogens is a growing concern, recent studies highlight the role of phage-mediated transduction in spreading antibiotic resistance genes, particularly in pathogens like non-typhoidal *Salmonella* and Shiga toxin-producing *E. coli*[50]. Comprehending phage-bacteria interactions and AMR spread is crucial for effective phage therapy strategies. Progress in phage research and AMR monitoring is vital for responsible phage therapy use against escalating antibiotic resistance. Regulatory hurdles represent another critical challenge within the sphere of phage therapy. Unlike conventional antibiotics, which typically undergo a standardized approval process, phages exhibit a high degree of specificity, targeting only particular bacterial strains. This specificity complicates the regulatory approval process, as each phage preparation must be scrutinized through

rigorous testing protocols to confirm safety and efficacy. Regulatory agencies require comprehensive data to understand how phage therapy will perform in diverse patient populations and under various clinical conditions, adding an additional layer of complexity to the approval process[51]. The manufacturing and stability of phage preparations also present notable challenges. It is crucial that these preparations maintain their viability and effectiveness over time, regardless of storage and transportation conditions. The inherent instability of phages can be affected by factors such as temperature fluctuations and exposure to various environmental conditions. Ensuring that phages remain viable and potent is essential for their clinical application, requiring advanced manufacturing processes and stability testing to meet the demands of healthcare delivery.

Furthermore, the current landscape of clinical trials exploring phage therapy is limited[52]. While initial studies have indicated promising results in experimental models and specific compassionate-use cases, there is an urgent need for more extensive, large-scale, and controlled clinical trials. These trials are vital for validating the safety and efficacy of phage therapy across a broader range of clinical settings and patient populations. Robust clinical evidence is essential not only to support the integration of phage therapy into mainstream medical practice but also to facilitate its acceptance within the broader scientific and medical communities.

The Future Prospects of Phage Therapy

The future of phage therapy presents significant opportunities, particularly in the context of addressing the challenges posed by antibiotic-resistant bacteria, which are known for their resilience against conventional treatments. The advancement of phage therapy in clinical settings hinges on several critical areas of research and development. One crucial area is phage engineering, where the focus is on the genetic modification of bacteriophages to improve their efficacy against resistant bacterial strains[53]. By manipulating the genetic makeup of phages, researchers aim to enhance their targeting capabilities, allowing them to more effectively seek out and eliminate pathogenic bacteria. Additionally, these modifications can help phages evade the various defence mechanisms that bacteria have evolved, thus improving the overall success rate of phage therapy. Development of synergistic treatments that combine phages with antibiotics could provide a more comprehensive strategy for managing resistant infections. Establishing standardized protocols for these combinations would enable healthcare providers to optimize treatment regimens, enhancing the chances of clearing infections that are otherwise difficult to treat[54].

Very importantly, the creation of phage libraries and databases is essential for streamlining the clinical application of phage therapy. These repositories would facilitate rapid identification and selection of effective phages tailored to specific bacterial profiles. By analyzing the genetic characteristics of the infecting bacteria, clinicians could promptly access a database of phages, ensuring that the chosen treatment is the most effective for the given infection. This approach would significantly reduce the time required to initiate treatment, which is crucial in managing serious infections. Advancing phage delivery systems is vital for the success of phage therapy. Research should focus on improving the methods by which phages are delivered to the site of infection, including encapsulation technologies that ensure phages remain stable and bioavailable[55]. Enhanced delivery systems will promote targeted and sustained release of phages within the body, maximizing their therapeutic potential and improving patient outcomes[56].

Collectively, these areas of research represent the foundation upon which the future of phage therapy can be built, paving the way for its broader clinical application and effectiveness in combating some of the most challenging bacterial infections faced today.

Conclusion

Widespread antimicrobial resistance is slowly pushing us back to a pre-antibiotic era, we are witnessing a concerning resurgence of diseases that had been largely controlled. The implications of this shift are profound, without the ability to effectively treat infections, the advancements in modern medicine could be severely destabilised, leading to a public health crisis with the potential to destabilize modern medicine and lead to public health crises. The threat of reverting to a pre-antibiotic era, where common infections could lead to severe complications or even death due to the lack of effective treatments, prompting us urgent discussions about the future of medicine and public health. In response to this crisis, phage therapy emerges as a significant source of hope, offering a transformative approach that utilizes bacteriophages—viruses that specifically seek out and destroy bacterial pathogens. Phage therapy, an alternative to traditional antibiotics, offers a custom-made solution to combat bacterial infections resistant to conventional treatments by targeting specific bacteria while leaving beneficial microbiota untouched. The precision of

bacteriophages allows for personalized treatment, enhancing efficacy and minimizing side effects associated with broad-spectrum antibiotics[57]. Research and clinical trials are advancing understanding of phage-host interactions, leading to more effective formulations and treating complex infections. Scientists are exploring genetic and biochemical properties of various phages, which could lead to the development of phage cocktails, which target multiple bacterial species simultaneously[58]. This approach could be particularly beneficial in cases where polymicrobial infections are present, expanding the therapeutic potential of phage therapy[59]. Collaboration between the scientific community, healthcare providers, and policymakers is essential to address regulatory challenges and ensure equitable access to phage therapy for patients worldwide. Education and awareness campaigns can inform healthcare professionals and patients about the benefits and limitations of phage therapy, fostering informed choices regarding treatment options. Implementation of phage therapy symbolizes hope for those suffering from antibiotic-resistant infections and underscores the need for continuous innovation and adaptation in healthcare strategies. Maintaining open dialogue among researchers, clinicians, and policymakers is crucial to integrate phage therapy effectively and ethically, ultimately transforming the landscape of infectious disease treatment for generations to come.

Conflict of Interest

We have no conflicts of interest to disclose.

Acknowledgements

We are thankful to Indian Council of Medical Research, New Delhi for the support. We acknowledge help of Arghya Jyoti Paramanik, Sanjoy Biswas and Devendra Nath Tewari in preparation of the figures. ❐

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