Phage therapy – then and now

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Abstract: The "silent pandemic" – a WHO-coined phrase that relates to the rise and spread of antimicrobial resistance is a phenomenon so striking that in spite of living in the most prosperous times, we are threatened to return to the preantibiotic era when even the simplest of interventions and infections were possibly deadly. One of the possible solutions could be the application of natural predators of bacteria – bacteriophage therapy. Discovered over a century ago, phages have been studied extensively and have become irreplaceable tools in laboratory work. The knowledge they helped to generate is still enlarging, and was responsible, at least in part, for creating the whole scientific discipline of Molecular biology. Their application on the other hand has not been as continuous and fruitful. On the contrary, the history of phage therapy is one of rise and fall, enthusiasm and acceptance as well as rebuttal and oblivion, scientific misconceptions and dogmatism, bad corporate investments, politics and ideologies. As today we are equipped with far more knowledge on the biology of phages, bacteria and resistance than early phage enthusiasts, it is justified to attempt phage application once again. Here we elaborate on the early years and very first attempts of phage therapy and the troubles rising from those days as they serve as excellent cornerstones for future endeavors in the field. In parallel, the current state-of-the-art of phage therapy in different parts of the world is also presented.

Keywords: Bacteriophage; therapy; antimicrobial resistance

1. Introduction

Antimicrobial resistance (AMR) has emerged as a significant threat to global health and our ability to treat infectious diseases effectively. Over time, microorganisms have developed resistance to the drugs designed to kill them, rendering many commonly used antibiotics and antimicrobial agents less effective, or even ineffective. This phenomenon, fueled by factors such as overuse and misuse of antibiotics, inadequate infection prevention and control measures, and the lack of new antimicrobial discoveries, has led to a concerning rise in drug-resistant infections (Davies & Davies, 2010). The antibiotic resistance crisis is a complex issue that requires a multifaceted approach so there is an urgent need for a coordinated global response to preserve the effectiveness of our antimicrobial arsenal.

Bacteriophages, often referred to as phages, are viruses that specifically infect and replicate within bacteria. They are the most abundant biological entities on Earth and, as natural predators of bacteria, they play a crucial role in shaping microbial communities and maintaining their numbers. Phages have a unique ability to recognize and attach to specific bacterial strains, injecting their genetic material into the host cell. This genetic material then hijacks the bacterial machinery, leading to the production of new phages and ultimately causing the host cell to burst, releasing a new generation of phages (Kutter & Sulakvelidze, 2004).

Phage therapy, a promising field of medical research, holds the potential to advance and revolutionize the way we combat bacterial infections. Harnessing the power of bacteriophages to target and kill specific pathogen strains, and allying it together with traditional antibiotics, may be the key to addressing pressing challenges of healthcare (Gordillo Altamirano & Barr, 2019).

2. Advantages and disadvantages

Loc-Carrillo and Abedon (2011) have articulated the positive aspects of employing bacteriophages as antimicrobials, but also the possible weaknesses. Most advantages originate from the nature of phages, with replication and release of new virus particles resulting in the destruction of the bacterial host cell. Hence, obligately lytic phages are bactericidal agents, capable of increasing their numbers in the presence of the host, therefore self-adjusting the applied dose (Abedon, 2010). They are highly selective towards the host strain and pose no risk to the present beneficial members

of the microbiota (Mai *et al.* 2015). Although the interaction with mammalian cells has been recognized, phages do not infect them (Bodner *et al.* 2021). Phages are omnipresent, rather easily discovered, and have the tools, such as depolymerizing enzymes, to disrupt bacterial biofilms (Hughes *et al.* 1998). Furthermore, their implementation can be versatile, administered in different forms and in combination with other antimicrobials (Goodridge, 2010). Phages are already components of nature and therefore have a low environmental impact, consisting of mostly nucleic acids and proteins and being inactivated by external factors such as desiccation or sunlight (Iriarte *et al.* 2007). One of the shortfalls of bacteriophages as antimicrobials is that not all phages are suitable for therapeutic use. Phages that are temperate or that are released chronically do not result in host lysis, hence the bactericidal effect is absent. Also, some phages have low virulence due to a lack of efficiency in some stages of the infection process - poor adsorption or replication characteristics, or susceptibility to bacterial defences (Gill & Hyman, 2010). Furthermore, phages with narrow host range are often not enough to eradicate all strains present at the site of infection, especially in cases of polymicrobial infections, and the resistance to a phage can evolve rather fast (Castledine *et al.* 2021). However, by combining phages in cocktails or with other antimicrobials, phage products have an enhanced spectrum of activity rather than a single phage (Goodridge, 2010).

3. Historical perspectives

The idea of phage application is as old as the discovery of phages itself. Almost immediately upon their discovery at Pasteur Institute in 1917, Canadian-French scientist Felix d'Herelle applied phages, first in birds (1918) and then in human patients in 1919 in a children's hospital in Paris (Summers, 2001). It is worth noting the apparent degree of bravery and enthusiastic support from authorities, such as Emil Roux, the head of the Pasteur Institute at the time, and Victor-Henri Hutinel, the hospital's chief of pediatrics, for the application of this novel "drug", in times when the drug itself (i.e. bacteriophage) was not characterized in any way, apart from its antimicrobial activity. Nevertheless, this fast "translation" of laboratory results to patients gave much in return, as all of the treated patients, four boys with severe dysentery, fully recovered within a few days with symptoms ceasing after a single phage administration (Chanishvilli, 2012). Not to oversee, the "safety" of the preparation was investigated by ingestion from d'Herelle himself, alongside Dr Hutinel and several other hospital staff, one day prior to application to the patients. Similarly, d'Herelle was able to efficiently cure 4 individuals suffering from bubonic plague by injecting phages directly into patients' enlarged lymph nodes ("bubos"). The remarkableness of their recovery prompted D'Herelle to spotlight and secured him the invitation by the British government to go to India in 1927 to work on phage therapy for plague and cholera (Summers, 2001). This expedition opened the door for multiple trials of phage application in the community, in addition to the hospital environment, in several regions of India that suffered repeating cholera outbreaks. At one instance D'Herelle performed what could be called the first proto-clinical study in humans, as he divided over 200 individuals into treatment and control groups and observed not only the outcome (live/dead) but also the course of disease in phage administered vs. the control group (which was otherwise treated). The results were again very encouraging, as the mortality rate was ten-fold lower in the treatment group which, noteworthy, consisted exclusively of desperately ill patients (Dublanchet and Bourne, 2007). Others were reluctant to take the phage preparations mostly as a result of the Gandhian "non-cooperation movement", one of the many "extra-scientific" obstacles our predecessors had to deal with (Summers, 1993).

During his stay in India, d'Herelle had another visionary idea - to use phages as prophylactic agents. Upon realization that the wells used as the main water source in villages become the main source of infection during the cholera outbreak, he attempted to prevent this from happening by simply pouring phage preparations into the wells, thus saturating the drinking water with Vibrio phages (d'Herelle, 1929). The experiment was a total success and resulted in the establishment of "The Bacteriophage Inquiry" in India under the patronage of the Indian Research Fund Association, and closely monitored by the local governments. This project mainly studied the application of phage prophylaxis on a large scale, in several Indian provinces where cholera epidemics occurred regularly in association with religious festivals and pilgrimages. The project lasted for 6 years and yielded some sensational results, especially range-wise. One of the d'Herelle followers, Andre Raiga (1961) claimed that several districts, encompassing over a million people, were eradicated of cholera. However, the "Inquiry" ended field tests in 1937 due to many reasons including political unrest in India, funding constraints, as well as inconclusive data from the reports yielded thus far. Among others, statistical analyses were weak, and no definite conclusion could be drawn when addressing the main subject – which was not on the usability and efficacy of phages per se, but whether or not the phages were more effective than other strategies available, such as sanitation and vaccination. The design of the studies was flawed, controls were not adequate, strong

statistical evidence was missing, and desired comparisons could not be made. This indeed was the case, especially from today's perspective, as randomized, double-blind clinical studies with placebo controls are the gold standard of all modern epidemiological studies. It should be emphasized that the overall conclusions on phage application were not negative, but rather inconclusive or uncertain. The evidence was strong enough to indicate further research; however, this was not possible for other, mainly socio-political reasons. It should also be noted that in a clinical setting, performed at the same time in Calcutta Campbell Hospital, which was installed and funded by the same project, the results were much more scientifically sound, and better outcome regarding overall morbidity was clearly demonstrated for phage treated patients (Summers, 1993). It is noteworthy to mention that D'Herelle left India in 1928 to hold a position at Yale, USA, right before the "Inquiry" started, and others had been in charge of field and hospital tests.

A strikingly similar scenario regarding the evaluation of phage therapy occurred in the West, where firstly Eaton-Bayne-Jones report in 1934, followed by the Krueger-Scribner report 7 years later, extensively reviewed over 100 articles on phages in order to evaluate its effectiveness as therapeutics. Both reports agreed that the evidence is ambiguous but were clearly not in favor of phage therapy (Sulakvelidze *et al.* 2001). Although the authors, as we now know, made some completely false conclusions (e.g. that phages are enzymes or other high-molecular-weight proteins, rather than viruses), their words had a deciding role in abandoning phage therapy in the USA, mounting onto the penicillin discovery and application which occurred in parallel.

Several reasons are now clear to have made heavy blows on phage therapy acceptance in the West. Firstly, the methodology used in the research was not adequate to persuade many other scientists, as was already explained. Also, the irrefutable proof of the very nature of the phages was crucially lacking. Last but not least, several pharmaceutical companies that started production and marketing of phage preparations were not paying enough attention to the quality of their products and made products that simply "didn't work". Low titers of phages inside the preparations and their inadequate and narrow host range destroyed the reputation of the whole approach. We consider these as valuable lessons.

Although D'Herelle was an advocate of testing the activity of the phage against the specific bacterial strain in the laboratory before applying it to the patient and had openly criticized marketing of the phage preparations globally, he was also a target of much criticism from his peers, of whom many disputed the very essence of his discoveries, or that his results were biased. It seems that his moving from Yale, USA, to Tbilisi, USSR in the year 1934 had more severe consequences on the acceptance of the phage therapy in both East and West, as one would now fathom. An extensive review of the "Soviet Experience" that followed can be found in the excellent work of Chanishvili (2001).

4. Up-to-date phage therapy

Today, we witness the multiplication of reports on successful phage therapy cases. Although universal legislation for phage therapy still doesn't exist, research and medical institutions are building connections by which they would facilitate the administration of phage therapy through an alternative approach. It is important that these connections exist, as well as collections of characterized bacteriophages and well-defined protocols for quick production of high-quality phage preparations, since phage therapy is most often applied only as „compassionate" treatment of last resort, where rapid action is required. In a recent mini-review, Yang *et al.* (2023) sum up regulations of phage therapy across the world, in order to provide ideas and examples to other countries for tackling legislation challenges. Covered by national regulations, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Poland has treated over 1500 patients with bacteriophages in their Phage Therapy Unit up to year 2000, with a success rate of 85.9% reported (Weber-Dabrowska *et al.* 2000). In Belgium, permission was given for prescribing phages to individual patients as magistral preparations (Pirnay *et al.* 2018). One of the most well-known and media covered cases of successful phage therapy, accomplished by the joint effort of medical doctors and scientists, is the case of 68-year-old Thomas Patterson, infected with multi-drug resistant (MDR) Acinetobacter baumannii (Schooley *et al.* 2017). This triumphant feat was followed by the creation of the Center for Innovative Phage Applications and Therapeutics (IPATH) at the University of California, San Diego, in June 2018, for providing help to many other patients in need of an alternative treatment for infectious diseases. The results and lessons learned from the first 10 intravenous phage therapy cases at IPATH were described by Aslam *et al.* in 2020, as the safety, practicality of administration and efficiency of combination with antibiotics were demonstrated. However, two unsuccessful cases were noted. Taking into account that compassionate phage therapy is very often administered to patients with complex medical conditions that complicate the prognosis, futile cases are possible to happen. Nevertheless, healthcare institutions in other countries are resourcefully finding means to help and treat patients

with persistent MDR infections. Phage Australia was formed as a national alliance in order to systemize phage therapy and connect researchers and clinicians into a network across the country. Based on the Australian model, Lin *et al*. (2021) recently discussed the challenges of creating a sustainable phage biobank. Just recently, a victorious Italian phage therapy endeavour was published (Cesta *et al*. 2023), where a Pseudomonas aeruginosa chronic prosthetic joint infection was resolved by a combination of phage and meropenem, with no adverse events or relapse in a 2-year follow-up.

Besides conventionally applying only phages, increasing amount of research testifies how synergistic effects could be achieved by combining phages with antibiotics (Gomez *et al*. 2011; Torres-Barceló, 2014; Chaudhry *et al*. 2017). It has been shown that the evolutionary cost of developing phage resistance in bacteria could restore antibiotic sensitivity (Chan *et al*. 2016). In addition, phage-derived proteins demonstrate potent antimicrobial effects (Mushtaq *et al*. 2005; Born *et al*. 2014) which broadens the antimicrobial repertoire of phage application.

In the current age of bioengineering, the flaws of narrow host range or lysogeny of temperate phages can be overcome and transformed into effective or more potent antimicrobial agents. Lu & Collins (2007) upgraded a T7 phage with a biofilm-dispersing enzyme, which resulted in a significant reduction of biofilm and biofilm-embedded cell count. Mahichi *et al*. (2009) have modified the T2 phage creating a chimeric phage with an expanded host range. The first case of phage therapy including genetically engineered bacteriophages against disseminated *Mycobacterium* abscessus infection reported encouraging results and significant overall improvement in the treated patient (Dedrick *et al*. 2019)

Following the latest developments, the implementation of AI platforms could upgrade the perspective of personalized therapy by, for example, analyzing phage and bacterial genomes to predict and find the corresponding active phage without the extensive experimental testing of phage libraries. An interesting hypothesis of phage therapy in the year 2035 where ad hoc and on-site production of synthetic phages, designed by the AI and supported by crypto currency exchange between stakeholders, was presented by Jean-Paul Pirney in his visionary article (Pirnay, 2020).

5. Conclusion

It is now clear that large field studies undertaken in India from 1928-1936 were sort of biting off more than could be chewed. The enormous potential of such studies has been shattered by the flawed design and insufficient scientific rigour and scrutiny. Also, commercialization efforts of several pharmaceutical companies in the West trying to make "one size fits all" preparations and market them globally prior to understanding at least the most important aspects of phage biology, were painfully wrong. Even today, the clinical studies involving phage therapy that are underway have many different issues to address in terms of e.g. proper selection of patients as well as phages. Phages are now being collected in large biobanks and thoroughly studied in the laboratory with all the methods of modern science in order to select a phage suitable for application, as it is clear that only a portion of phages are such candidates. This step, as one of the crucial ones, needs much attention, and for example involves whole genome sequencing and extensive bioinformatic analysis, host range determination (which relies on extensive bacterial strain characterization), biosafety analyses etc. The early history of phage therapy teaches us that this is the proper way to pursue further investigations, as trust can be easily broken.

The field of phage therapy is constantly evolving, and the number of reported cases has increased. In conclusion, phage therapy presents a promising avenue for addressing the growing threat of antibiotic-resistant bacteria. With its ability to specifically target and eliminate harmful bacteria, phages offer a potential and perhaps more sustainable solution where traditional antibiotics have fallen short. The field of phage therapy has made significant strides in recent years, with numerous successful case studies and several ongoing clinical trials (Uyttebroek *et al*. 2022). However, obstacles remain. Regulatory hurdles and the need for personalized treatment approaches are among the challenges that need to be overcome. Further research and collaboration between scientists, clinicians, and regulatory bodies are crucial to harness the full potential of phage therapy in infectious disease treatment.

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