Bacteriophages as alternative to antibiotics: A review of therapeutic potential in the face of practical and regulatory challenges

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Abstract

Because of the ever-present issue of rising bacterial antibiotic resistance, bacteriophage (phage) therapy has garnered attention as a possible solution. This review covers the potential of phages as new antimicrobials. Previous research has demonstrated that phages are effective against bacteria, in animal trials and in human cases, and that phages have no negative effects on treated subjects. However, the lack of a generalised regulatory phage framework and well controlled clinical trials are a great hindrance to future clinical development and approval of phages. The aim of this paper is to show the current potential of phages and what future prospects phage treatment has.

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Introduction

Right now, the medical world is facing a big crisis, namely the rapid increase of antibiotic multidrug resistant bacteria worldwide (O'Neill 2014, Ventola 2015). Figure 1 shows the exponential increase of unique antibiotic resistance enzymes found (Li et al. 2019). This issue finds its origin in the extensive use of antibiotics. Ever since the discovery of penicillin in 1928 and its widespread use in the 1940s (HealthyChildren.org 2019), antibiotics have been the main way to treat bacterial diseases. However, due to the prevalent use of antibiotics, bacteria have evolved and become resistant to antibiotics. These drug resistant bacteria could cause the deaths of around 700,000 people every year (Górski et al. 2016). If nothing would be done, the death toll will even reach 10 million by 2050, which even exceeds the death toll of cancer. While this problem has been recognised as one of the biggest threats to modern medicine, there are still no real alternatives to antibiotics on the market. Though there is a bright side as some alternatives are being researched right now. This paper will delve into the prospects and challenges of bacteriophages (phage) as an alternative way to treat bacterial infections.

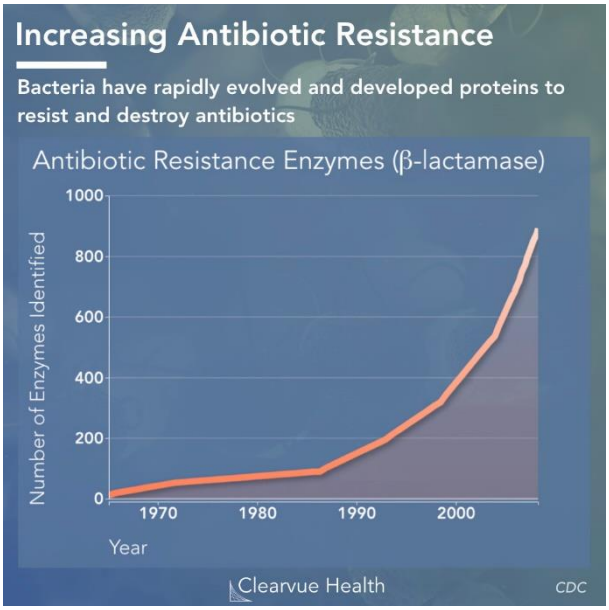

Figure 1, The Science of Antibiotic Resistance (Li, 2019). This graph shows the cumulative number of unique beta-lactamase enzymes identified. These enzymes can destroy the β-lactam ring in many antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems. Destruction of the β-lactam ring renders these antibiotics ineffective (Drawz and Bonomo 2010).

Bacteriophages

Bacteriophages (phages) are single or double stranded viruses with an RNA or DNA genome that infect bacteria. Their name “bacteriophage” actually means “bacteria eater” due to how they specifically infect bacterial cells and use them as hosts, taking over their metabolic functions, “eating” them from the inside. Phages are the most abundant biological beings on earth, estimated to be 10 times more numerous than bacteria (Batinovic et al. 2019). This huge number also implies immense genetic diversity within phages.

Phages live and evolve together with their bacterial hosts, playing a very important role in the diversity of microorganisms (Rodriguez-Valera 2009, Suttle 2005). Phages are simple organisms as they consist of only a protein capsid and a core. An essential part of phages is their life cycle. Based on their life cycle, phages can be separated into temperate phages and lytic phages. Temperate phages live in two life cycles. The first one is a lysogenic cycle where they are dormant as a prophage

inside of their host. In this cycle they are inactive and replicate together with the host until it receives an impulse, which causes it to enter the lytic phase. In the lytic phase the prophage creates new virions and lyses the host (Howard-Varona et al. 2017). Lytic phages, also known as virulent phages, do not have a lysogenic cycle. Instead, lytic phages upon entering their host take over the metabolic functions of their host to create virions and then lyse the host, releasing the virions (Casiens and Hendrix 2015). Temperate phages don't always lead to cell lysis and can play an important role in transduction, which leads to an easier spread of for example resistance. That is why lytic phages are the type of phages used in phage therapy,

But not all bacteria are infected by all phages. In fact, most phages have been found to only target a subset of bacterial species (Flores 2011). This might seem counterintuitive, as being able to infect a large range of bacteria would allow for more potential hosts and thus more reproduction. However, as mentioned, phages are generally very host specific in nature. Because phages can only replicate within their hosts and require the cell lysis of their host to spread to new hosts, they are considered obligate parasites (Koskella and Meaden 2013, Rodríguez-Rubio et al. 2020). If we take a look at aphid parasitoids for example, these parasites tend to specialise on specific hosts (Straub et al. 2011), even though the advantage of a broad host range seems appealing. The most accepted evolutionary argument for this comes down to the effect of "a jack of all trades is a master of none." as said by Britt Koskella (Koskella and Meaden 2013). Basically, when a being adapts to be a generalist, they need more general ways of infection, which does not allow for a specialised efficient pathway. Additionally, what might allow you to enter one host could completely block the phage from infecting another, which is called antagonistic pleiotropy. High specificity is an advantageous trait for clinical use, as using phages to kill off dangerous bacterial infections without causing much collateral damage to the human microflora is ideal. And since phages are specialised for bacterial hosts, they pose no direct threat to humans. In fact, phages can even be applied directly in human tissues without causing any danger to the patient (Weber-Dabrowska et al. 1987, Fabijan et al. 2020).

In summary, phages are biological beings that are parasitic and specifically target bacterial hosts. Phages are also generally specific to only a subset of bacterial species, and due to this specificity and how they aren't directly threatening to humans they have been selected as a potential anti-bacterial treatment option. In particular, lytic phages are the type of phages used in phage treatment due to their properties of only infecting and killing bacteria without a passive cycle where they duplicate with their hosts without killing them, like temperate phages do.

Phage treatment history

The idea of phage treatment is by no means a recent concept. Bacteriophages have been used as a way to treat infections in the past, with some of the earliest potentially successful treatments with phage therapy on pediatric dysentery, cholera, and bubonic plague (Caflisch et al. 2019). This sparked interest in phages as a potential treatment option in the west but also in the Soviet Union. However, researchers at the time were not able to create positive results, possibly due to an insufficient understanding of basic phage biology (Kutter et al. 2010, Summers 2012) as viruses in general were still very poorly understood at this time. Then when antibiotics were discovered, they quickly became widespread and became used as the main way of treating diseases, after which phage therapy was mostly abandoned in the west. On the contrary, eastern countries have used phage therapy till this day (Fruciano and Bourne 2007, Abedon et al. 2011, Abedon et al. 2017). This includes the countries Georgia and Russia, where products containing formulated therapeutic phages are directly available to the public, even without a prescript. However, a big issue is the lack of communication to the west, as even though the long and seeming positive history with phage treatment, very few has actually been publicised in English-language journals. This originates from multiple underlying issues, which

include the history of secrecy behind the Iron Curtain and language barriers. And even when considering the translated research there is still a lack of clinical trials that live up to the western standard (Abedon et al. 2011). Nowadays, with the rise of antibiotic resistant bacteria, phage therapy has been garnering more attention in the west once again.

Current applications of bacteriophages

Phage treatment is a process existing of multiple steps. Figure 2 shows the main steps of creating a phage treatment which is applicable in clinical settings (Vázquez et al. 2022). For phage treatment, first a phage needs to be isolated, then the phage needs to be grown and subsequently purified. This purified phage will then have to be made into a phage product that can be administered to patients, and some of the purified phage can be stored for future usage (Vázquez et al. 2022).

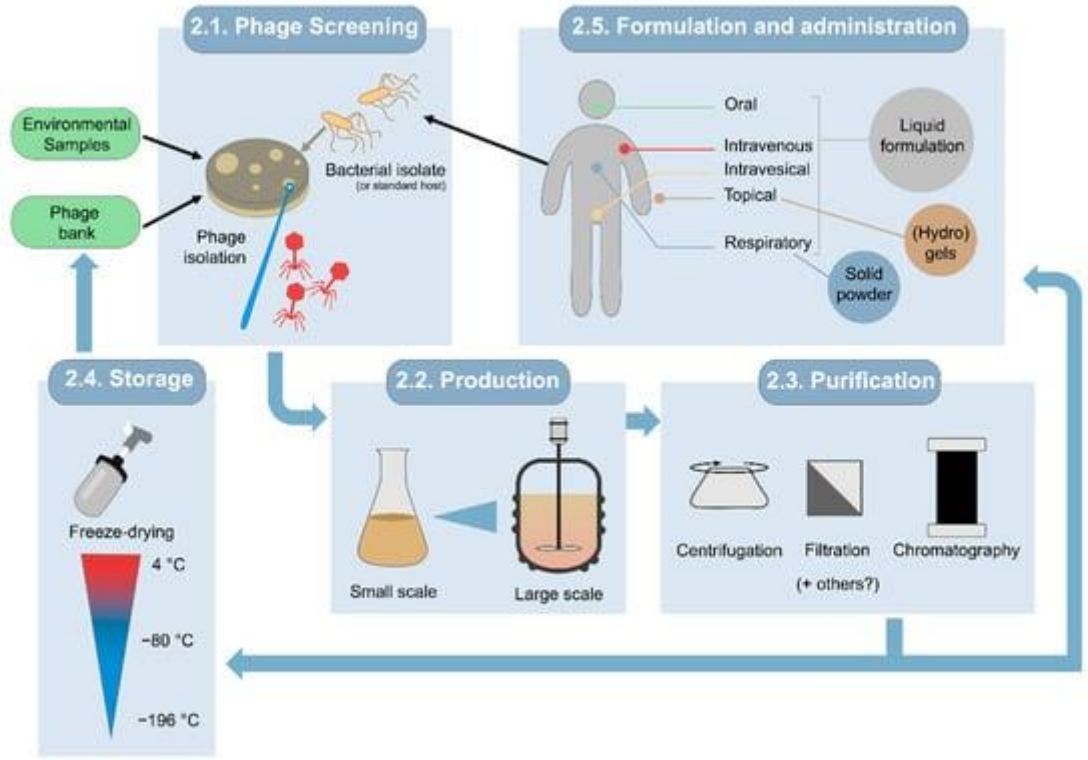

Figure 2, Steps in preparation of phage suspensions suitable for phage therapy, including screening, propagations, purification, storage, and formulation (Vázquez et al. 2022).

But have phages actually been proven to work as a way to treat bacterial diseases? Phages being able to efficiently kill bacteria in nature or in petri dishes does not mean that they would also be effective as a way to treat human diseases clinically. Luckily many studies do show positive therapeutic effects. Mice tests have been done where the mice have been infected with multiple drug-resistant *Klebsiella pneumoniae* bacteria where an 80% survival rate after phage treatment was found (Cao et al. 2015). The lungs were also in better condition after treatment with phages, only showing discrete lesions while most of the lung tissue was healthy. The mice in the control group all died and the majority of their alveolar space was completely destroyed by inflammatory exudate and also immune cell infiltrate, in combination with haemorrhage and consolidation. In other research with mice infected with *Pseudomonas aeruginosa*, which has a 100% lethality rate for mice after 24 hours, also showed interesting and promising results. Mice that received phage treatment up to 30 minutes after infection showed a 100% survival rate while this rate dropped down to 50% at 3 hours and 20% at 6 hours (Wang et al 2016). These results show the importance of a fast treatment response when using

phage therapy. Furthermore, mice research on open wounds infected with *Acinetobacter baumannii* showed positive effects like the wound only closing for phage treated during the study. Here phage therapy helped the mice recover from the infection. They discovered no significant changes in immune cell populations in circulation or secondary lymphoid organs after phage treatment (Rouse et al. 2020).

Phage treatment studies have also been done on non-rodents. In a study by Desiree et al. (Desiree et al. 2021) phage treatment in pigs has been examined, where phage treated pigs had significantly lower concentrations of the infecting bacteria when compared to untreated infected pigs. Because of this, these results support the use of phage treatment as an alternative to antibiotics. They also found that that treatment efficacy is expected to increase when applying phage multiple times during treatment. A study on sheep orally infected with *E. coli* and later orally administered phage therapy showed a substantial reduction of *E. coli* in the colon of the treated sheep (Raya et al. 2006).

There has been a plethora of other phage studies with positive findings on animals, but there are simply too many to be provided here.

As described by Abedon et al., the first human therapeutic phage trial has been recorded in France in 1919 (Abedon et al. 2011). There d'Hérelle treated children who were afflicted with severe *Shigella* dysentery using phages isolated at the Pasteur Institute from feces of soldiers. In the patients the symptoms ceased after only a single administration (Chanishvili 2012). But research was not immediately published as d'Hérelle was studying the general properties of phages in clinical context. Eventually, he published his findings and even opened his own phage laboratory called “Laboratoire du Bactériophage”. Here, the first commercial phage cocktails were produced, phage cocktails are a mix of different phages (Chan and Abedon 2012). However, as mentioned earlier, phage therapy was mostly abandoned in the west. Nonetheless, phage treatment did not completely disappear and humans have been treated with phage medications in other parts of the world, both in clinical trials as well as general treatment. The results of these studies and treatments have shown good prospects. Qin et al. found that a large majority of patients suffering from chronic wounds and ulcers that were treated with phage showed improvement (Qin et al. 2021). Phage therapy has been used and could be a great solution for typhoid fever, which is a fatal systemic infection caused by *Salmonella typhi* and *paratyphi* strains. Typhoid fever is the cause of 128,000 - 161,000 deaths globally each year. Phage therapy could also be used to combat cholera, shigellosis, food borne diseases and more (Khalid et al. 2020). In Poland thousands of patients have been treated with phages (Abedon et al. 2011) and phage therapy is still used but considered an experimental treatment (Żaczek et al. 2022). This is done mostly in association with the Hirszfeld Institute of Immunology and Experimental Therapy in Wrocńaw. Here phages are specifically selected for each patient from the large collection of phages they have available. The records of their 550 patients from 1981-1986 show cure rates for specific infection types ranging from 75% to 100% (Abedon et al. 2011). It is important that these are not clinical studies, and are instead often used as a last resort for chronic infections. This can result in unfavourable results due to the late start of the treatment and the general weakening of the patients due to the disease.

Łusiak-Szelachowska et al. specifically looked into elevated antibody levels after phage therapy and found that it depends per patient, as well as that it can take over a year for antibodies to disappear. Though, even if these patients have built up antibodies against phages used in therapy, it does not mean that the treatment will be ineffective (Łusiak-Szelachowska 2022).

To summarise, phage studies have been conducted with rodents and non-rodents, both showing promising results. There have also been human phage treatment cases. In these studies, phage

treatment likewise showed significant improvement in treated patients. These findings show how phage treatment does have the potential to work as a powerful anti-bacterial medication.

Limitations and concerns

Even though research results have shown potential of phage treatment, like other medicine, phage therapy also has limitations. These concerns about phage therapy should be discussed in order to see how relevant and critical these issues really are and or whether these concerns can be mitigated.

The main concern I would like to discuss first is the danger of phage resistance in bacteria and possible ways to avoid or reduce resistance development against phage therapy. After all, the rising interest in phages is largely due to the antibiotic resistance of bacteria. If those bacteria quickly develop resistance to phages, then phage therapy as a whole could be impractical. Because, just like with antibiotics, developing a resistance to any specific phage is inevitable (Dennehy 2012). Bacteria have a multitude of phage resistance systems; these consist of cell surface and extracellular modifications as well as intracellular modifications (Caflisch et al. 2019). However, there are multiple factors that do allow phage therapy to work around those bacterial resistance mechanisms. Firstly, unlike antibiotics, phages can evolve their own resistance against bacterial phage resistance like CRISPR and other phage resistance measures (Seed et al. 2013). Secondly, to avoid bacteria becoming resistant to one phage strain, it is possible to use phage cocktails consisting of multiple different phages that are virulent to the infecting bacteria, each targeting different surface receptors (Nilsson 2014). Thirdly, it is possible to use phages with fast adsorption rates and high number of virions upon bacterial cell lysis. That could allow a reduction of the bacterial population fast enough that there will not be many bacteria that can develop resistance and those cells can be killed by the immune system. (Levin and Bull 2004)

Another concern is the immunological response of the immune system to phages during treatment. Phages are all unique, with different proteins covering their surface. These peptides are not recognised by the human body and will therefore cause an immune response when detected. So, when phages are administered into wounds or in blood veins, the immune system will react to them. It has been found that ItG and ItM levels raised against the phage after multiple injections, but no anaphylactic reactions, deviations in core body temperature, or further negative effects were found after multiple injections of phage (Biswas et al. 2002). Other clinical and animal research also did not show severe immunologic reactions (Caflisch et al. 2019, Skurnik et al. 2007, Merril et al. 2006). Another concern that has to do with the immune response is that concentrations of phages after administration in veins decrease swiftly. This is largely due to innate immunity and phagocytosis in the blood and liver and only for a small part by the adaptive immune system (Sokoloff et al. 2000). Nonetheless, even if the body has an immune response and creates anti-phage antibodies, the phage therapy can still work as mentioned earlier (Łusiak-Szelachowska 2022). Optimisation of phage dose, that being the number of specific phage strains, as well as phage packaging can also help increase the effectiveness of phage therapy under the constraints of the immune response (Liang et al. 2023, Gembara and Dabrowska 2021).

The next challenge is phage storage. Phages are unique biological beings and as such, optimal storage for them can differ. Bacteriophages have evolved to survive unforgiving conditions, so there are robust phage strains which do not need any kind of specific storage. But not all phages are as stable, some phages need special handling (Ackermann et al. 2004). Here we discuss the main ways phages are stored, summarised based on the description by Skurnik et al. (Skurnik et al. 2007). Firstly, freeze drying. Freeze drying has allowed a large spectrum of phages to be stored for 20 years. However, freeze drying is dependent on the protectant used and infectivity quickly drops without any

protectant. Secondly, storage as a lysate at 20°C to 25°C. Phages have been known to survive for years as sterile lysates. Though no reports show a systematically studied therapy-friendly storage like this. Thirdly, storage at 2°C to 5°C. Much like storage at 20 to 25 degrees, not much has been studied about storage at these temperatures, but some unpublished results do show that the phage lysate used still holds a significant infectivity after 60 weeks. Finally, frozen storage. Freezing is done at -70°C to -80°C or with liquid nitrogen. Despite the fact that phages do survive freezing well, for phage therapy this method is not practical as phages should be able to be distributed to the client.

One last important detail is that we do not know everything yet. Lytic phage genomes with more than 50% of hypothetical genes that are either poorly understood or have no known function require in-depth research to make sure that these hypothetical genes cannot cause unforeseen effects later. And while phages cannot use eukaryotic cells as hosts, they can still have interactions with them (Putra and Lyrawati 2020). These interactions do not seem to be harmful to the hosts directly, but in humans phages could evolve to target symbiotic bacterial species within our gut, which would make them pathogenic.

So, while phage therapy has some challenges and concerns, they appear manageable but require more research. The biggest concern, the risk of phage resistance, exists. Luckily there are ways to combat resistance by for instance phage cocktails though the risk still remains.

Optimising phage therapy

In order for phages to be a good alternative to antibiotics, it is essential that phage therapy is optimised to be as effective as it can be for specific personalised and broad case usage. Some techniques are available for this.

Phage cocktails, also known as polyphage treatments, have already been used early on in phage therapy and they have proven to be a great way to enhance the effectiveness of treatment. To go a bit more in depth, phage cocktails consist of different phage strains. Cocktails can be designed in multiple ways. To start off with generalist cocktails, which goal is to target a large array of bacterial types, working more as a general cure. Such cocktails are used in Georgia, one of these being “Intestiphage”. This cocktail is not a static phage mixture but one that is updated every 6 months by additions of phages that target problem stains (Abedon et al. 2011). But a specialist phage cocktail can also be designed, which targets a bacterium or a bacterial strain more effectively, making it much harder for the bacteria to develop resistance. A cocktail like this would target a single species, with phages that can target different strains of the same bacterial species (Abedon et al. 2021). Having at least two phages that can infect a bacterial strain makes the cocktail more effective and make selection of resistance harder for bacteria (Abedon et al. 2021). Cocktails can be further optimised to target isolated bacterial strains from patients to have an even more successful result. Research by Pimay et al. showed that personalised phage cocktails, which are optimised for targeting the identified infecting bacteria, are critical to make the antibacterial effect of phage treatment the most effective (Pirnay and Kutter 2021). This specialised cocktail approach is used in Poland, where they use their library of phages to make a specialised cocktail for the patient (Abedon et al. 2011). When designing phage cocktails, it is additionally crucial to prevent that resistance to one phage would lead to resistance to all phages. To prevent this, avoidance of cross resistance is required. Cross resistance is when a bacterium has a mutation, which leads to resistance against a phage by for example deletion of the surface receptor used by the phage but also by other phages leading to general phage resistance (Pirnay and Kutter 2021).

Genetical engineering has already been used with phages and could end up playing an important role in phage treatment development in the future. With the ever-advancing techniques of genetical engineering, it is possible to genetically engineer phages for therapeutic goals. Phages can develop mutations that allow them to stay within the circulatory system for longer times (Merril et al. 1996), which would allow for a greater anti-bacterial effect. This is because the phage can evade the reticuloendothelial system (RES), which is an important part of mammalian host defence systems. RES organs remove phage particles from the circulatory system. The method these phages use to avoid the RES clearance could be generalised to also allow other phages to avoid the RES in the same way (Merril et al. 1996). Phages can also be modified to not cause lysis but still be lethal, which prevents an immune response from lysed bacterial components and toxins (Hagens and Blasi 2003). Combining these phage functions that both lower phage immunogenicity has however not yet been studied (Pires et al. 2016)0. It has also been suggested that phages can be designed to destroy bacterial biofilms (Pires et al. 2016). Using genetic engineering, phages that target specific DNA sequences have been developed (Citorik et al. 2014, Bikard et al. 2014). These phages target DNA sequences using a CRISPR-Cas based system, where RNA-guided nucleases on conjugative vectors or phagemids, induces a double strand break. This can lead to cell death or the loss of a plasmid. Bacteria without the target sequence are not affected by the phage, which allows for evolutionary pressure to lose the sequence. If the phage targets genes that cause antibiotic resistance, it is possible to selectively kill off resistant bacteria without harming commensal bacteria. The challenge is to develop an effective phage that can target the extensive range of, for example, bacterial antibiotic targets (Pires et al. 2016).

Phages can also be engineered to detect and identify bacteria (Pires et al. 2016). Creating phages that express fluorescence-encoding reporter genes can help with rapid and accurate diagnosis. This is due to how phage reporters are able to visualise low levels of bacteria, how they are very specific, avoiding off target hits which is important to prevent mislabelling bacteria, and they will only be expressed in living bacteria. This can allow for fast and accurate personalised phage treatment (Pires et al. 2016), which is an essential aspect for commercialised use.

Phage therapy is not necessarily a stand-alone therapy, antibiotics can still be of use in phage therapy. Phages can become even more effective when used in combination with antibiotics, this is referred to as phage-antibiotic synergy (PAS) (Diallo and Dublanchet 2022). PAS is explained by the fact that sub-lethal doses of antibiotics added to phage-infected bacteria result in significantly increased phage plaque sizes. The underlying mechanism is not very clear but it has been suggested that antibiotics could improve the efficiency of cell lysis due to bacterial elongation and corresponding cell wall weakness (Torres-Barceló et al. 2018). Early combined phage-antibiotic therapy, combining phages with Sulphonamides, showed success in rabbits (MacNeal et al. 1942) as well as humans (MacNeal et al. 1943). After the discovery of penicillin, phage-penicillin treatments were also studied. A study by Himmelweit et al. showed a significant decrease in complete lysis time for *Staphylococci* treated with both antibiotics and phages, as opposed to either alone (Himmelweit 1945). Further successful phage-penicillin therapy cases included endocarditis, bacteraemia, osteomyelitis and peritonitis (Diallo and Dublanchet 2022). In fact, the vast majority of phage-antibiotic combinations show positive results in the form of PAS, resulting in lower growth curves and higher kill curves (Tagliaferri et al. 2019). However, it is important to mention that there are also antagonistic phage-antibiotic combinations, where using either only the phage or the antibiotic gives better results (Diallo and Dublanchet 2022). While the mechanisms behind this are poorly understood, it is theorised that some antibiotics might damage phages directly while another option or complimentary cause is that antibiotics damage bacterial core functions (Torres-Barceló et al. 2018). The field of phage-antibiotic treatment still requires more research. Diallo et al. suggest that phage-antibiotic treatment can lead

to an increased and/or prolonged antimicrobial effect of classic antibiotics on resistant bacteria (Diallo and Dublanchet 2022). This is based on multiple findings. Evolutionary trade-off has been found, where evolution of phage resistance leads to a loss of antibiotic resistance. Phages and antibiotics also target bacteria in different ways, which has a similar effect like phage cocktails of reducing the risk of multi drug resistance by attacking multiple pathways. But for many phage-antibiotic combinations need to be tested whether they are synergistic or antagonistic. Furthermore, this field can make use of gene modification and other novel treatment techniques that have not yet been studied (Diallo and Dublanchet 2022).

Future phage testing

Even though phage research has been in the spotlight as a clinical tool, phage research is still in its infancy. The absolutely colossal diversity of phage types and structures that are still undiscovered need to be investigated in the future. Most naturally occurring phages have not even been produced in a lab (Pires et al. 2016). Besides the limited scope of the current knowledge on phages as a whole, phage therapy needs to be investigated according to rigorous scientific standards. Abedon has created a key list of considerations that are vital to be reported in phage studies. These considerations can offer assistance to create consistent guidelines that can guide researchers to include relevant details of phage research within publications (Abedon 2017). Relevant, consistent and detailed regulatory studies are essential for phage therapy to go from preclinical trials to phase 1,2 and 3 clinical trials, then via regulatory market approval to accepted usage in global medicine. Right now, there is no framework of regulatory rules specifically for phages in medicinal context in humans. But in Georgia where phage therapy is used on humans, such a framework is laid down in the healthcare system as standard application (Kutateladze 2015). Phage therapy as personalised treatment, where phage cocktails are made specifically for a patient's infections, will face regulatory issues. That is because personalised medicine breaks the groundwork of regulatory conventions as they are now (Furfaro et al. 2018). Phage is not a classical drug like antibiotics and the way of clinically accepting phage therapy needs to see a revision as none of the current regulations are suitable for phage therapy (Pelfrene et al. 2016). It is thus of essence that a dedicated phage therapy legal framework is developed so that phage therapy can smoothly be introduced into western medicine (Furfaro et al. 2018).

Currently, a lack of validated and detailed clinical trials is a big roadblock for the progress of phage therapy. Only a limited number of phage therapies have passed through stage 1 and 2 of clinical trials, but they did not have strict criteria and failed to show evidence of properly controlled clinical trials (Kutter et al. 2010). Caflisch et al. mentions that "the greatest impediment to standardised phagotherapy is well-controlled clinical trials" (Caflisch et al. 2019). What Caflisch means is that in current studies there is a lack of randomised control data and not enough independent studies that do not rely on data from compassionate use cases. In compassionate use cases, the main goal is to improve patients' health so these studies do not usually have the best conditions for therapeutic phage testing. That is because, besides phage therapy, other antimicrobials are applied and there is not a control group. The usage of other antimicrobials besides phages impedes the clarity of the effect phage treatment has had in these studies.

In the future, properly controlled, double-blind clinical trials are needed in order to progress phage therapy. Caflisch et al. suggest randomised controlled trials in well-defined infectious diseases, even if those diseases are not caused by antibiotic resistant bacteria (Caflisch et al. 2019). Anomaly et al. has a more extreme but interesting suggestion of compensating willing volunteers in exchange for infecting them with a pathogen and subsequently applying phage therapy (Romero-Calle et al. 2019).

Conclusion

The threat of emerging antibiotic resistant bacteria is becoming a bigger issue as antibiotic development has slowed down. Right now, the medical world needs a solution and phage therapy has once again come into the spotlight as a way to potentially prevent, regulate and cure bacterial infections. Phages have proven to be effective in treating bacterial infections in rodents, non-rodents and even humans (Khalid et al. 2020, Cao et al. 2015, Wang et al 2016, Rouse et al. 2020, Desiree et al. 2021, Raya et al. 2006, Qin et al. 2021).

Phage therapy also has some important concerns and limitations. But these concerns and limitations can be addressed by usage of phage cocktails, genetic engineering and more developed understanding of phages. Phage cocktails can for example help prevent bacterial resistance and help with creating a broader host range for treatment. Genetic engineering has many potential uses, (just to name a few options), ranging from better effectivity due to not being detected by RES clearance (Merril et al. 1996) to targeting specific gene sequences to guide bacterial evolution by selective pressure (Citorik et al. 2014, Bikard et al. 2014). It can even allow phages to make bacteria lose resistance to antibiotics and it can make phages destroy bacterial biofilms (Pires et al. 2016). However more research is needed in order to see how effective these techniques will eventually turn out to be.

One of the most important areas of phage treatment is phage cocktails. Phage cocktails will be essential in treating patients in the future. Therefore, it is vital that optimal phage mixes are studied to best treat diseases. While a generalist phage treatment plan for cocktails targeting the most common wild infectious bacteria is important, as it can be used as a general cure for many patients, it is also essential that a clinical guideline for personalised cocktails is formulated so that treatment can be designed for the individual needs of patients. Maybe even more essential is the genetic engineering of phages. Genetically engineered phages can be optimised in many useful ways like the ones mentioned above.

The greatest hurdle in the face of modernised phage treatment is a lack of a generalised clinical phage framework and well controlled clinical trials. So now it is just a matter of creating that framework, which can take the phage framework of Abedon (Abedon 2017) as inspiration. Though the existing guidelines in countries like Georgia can also be taken into consideration for this. From there, well controlled phage studies can be conducted, which could make use of compensated volunteer trials (Romero-Calle et al. 2019).

Discussion

Phage therapy was an innovative treatment idea that has finally come back into the public eye in the west with antibiotic resistant bacteria becoming more of an issue. While phages were largely abandoned due to multiple reasons, our increased understanding of phages and the development in genetic engineering allow us to view phages in a new light. Research shows that phages are an effective way to treat bacterial infections. Desiree et al. (Desiree et al. 2021) mentions that administering phages multiple times during treatment increases its effect. Finding optimal doses and regulatorily accepted treatment plans for humans should thus be investigated. Caflisch et al. suggest using phage therapy for not just dire cases with antibiotic resistant bacteria (Caflisch et al. 2019). I agree with this suggestion, as current research shows that phages can be an effective and personalised option for treatment. Using phages to treat general diseases will allow for targeted treatment without affecting the microbiome to a much greater extent than microbials ever could. For this, generalised broad cocktails would likely be the most efficient. Using phage treatment as the first line of medication against bacterial diseases will also help preserve the antibiotics that are still left, so that they can be used in cases where phage therapy is not an option. Those cases can include

bacteria for which no phage treatment is available due to it being a new or rare infection. Antibiotics can then be used to treat the infection or stall for time while a matching phage is found and cultured. And antibiotics in combination with phages can lead to an increased performance for both (Diallo and Dublanchet 2022) and certain antibiotics could be saved and used in conjunction with phages in dire situations. However, I do not mean to imply that personalised phage treatment is less important, as personalised treatment can lead to much better treatment results for individual patients (Pirnay and Kutter 2021).

I also believe that phage cocktail development is essential in creating broad range treatments and personalised cures against specific species and strains. Phage cocktails are also vital for the prevention of phage resistance development in bacteria. Even repetitive injections with phage cocktails show minimal innate and adaptive immune response in mice, which suggests if the same holds true in humans that systemic long term phage treatment would be possible (Weissfuss et al. 2023).

Genetic engineering has significantly advanced from when phages were largely abandoned in the west and it will play an essential role in the creation of phage medications as well as other clinical phage applications. Something that could also be useful to study is that phage resistance usually results in lower fitness for the bacteria. These fitness costs could be targeted and exploited against infecting bacteria as well (Romero-Calle et al. 2019). Phages could be genetically engineered to exploit this, and cocktails can use multiple phages which would lower the bacterial fitness if they become resistant, which can allow other phages, the immune system or other anti-bacterial medication to kill off the bacteria. Specifically, phages engineered to target antibiotic resistant bacteria used in combination with antibiotics could perhaps lead to great results, but this requires further research.

Right now, it is most important that more research is done for many aspects of phage therapy, and the hurdle of clinical approved standards and frameworks needs to be resolved. But the phage-based techniques could be of immense value in future medicine. And perhaps phage therapy could even end up being the solution to the antibiotic resistant bacterial crisis.

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