BACTERIOPHAGES: A SUITABLE ALTERNATIVE FOR ANTIBIOTICS?

Quentin Marsman[1]

[1] BSc Medicine, Radboud university medical center, Nijmegen, the Netherlands

Insights

Imagine you feel a little ill and you decide to call the doctor. The doctor suspects a common bacterial infection of the urinary tract. After a failed treatment with a broad-spectrum antibiotic, additional examinations reveal a multi-resistant bacterium in your body, resistant to most of the available antibiotics. You are worried about this result, as some of your loved ones were also infected by such a bacterium in the past and have not recovered from the infection. You receive further treatment after which you barely recover.

Even though this short story sounds dystopian, it could become a reality in the future. Due to our current antibiotic usage, both in medicine and the cattle industry, antibiotic resistance is on the rise. This could lead to infections that pose a therapeutic challenge and might even prove to be lethal. Therefore, an alternative is clearly needed. A possible alternative that is currently being researched is the treatment with bacteriophages. Could these bacteria-killing viruses be the solution we need?

In its first report about antimicrobial resistance, the World Health Organization has expressed the concern of an upcoming post-antibiotic era, where otherwise 'simple' infections may become a real threat again [1]. Currently, in the USA, it is estimated that more than 35,000 deaths each year are a result of infection by multi-resistant bacteria [2]. Globally, the numbers are increasing, with a possibility of 10 million deaths per year by 2050 [3, 4]. Furthermore, little is invested in the development of new antibacterial agents by pharmaceutical companies, as the antimicrobial agent market is less profitable than other markets, such as chemotherapy [5]. Overall, due to the limited number of treatment possibilities in bacterial infections, antibiotic resistance is a real threat to humanity in the present and future to come.

Alternatives to antibiotics are likely to be needed to prevent this post-antibiotic era from happening. Next to other alternatives such as antibodies, probiotics, lysins, and immune stimulation, bacteriophage and bacteriophage-based products have shown potential as an option to combat the antimicrobial resistance problem [6-8]. Every year, there is an increasing focus on bacteriophage therapy, which is shown by the growing amount of citations per year from 1100 around 2015 to 1400 in 2017 [9]. From 2017 on, the Dutch program 'Dokters van Morgen' made several episodes on bacteriophages and its application around the world, leading to more attention for phages among the public in the Netherlands [10, 11]. Despite the recognition, more investment is needed in these therapies as the current knowledge about bacteriophage therapy is lacking. Moreover, a review written in 2016 predicted only a nine per cent chance for bacteriophage therapy to be implemented for the infections caused by *C. difficile* and *P. aeruginosa* by 2025 [6].

In this article, we will address the question of whether bacteriophage therapy can become an alternative to antibiotic therapy. Starting with the biology of bacteriophages, the history and the therapy will be elaborated on, ending with a review of the opportunities and concerns regarding the therapy. Do you think bacteriophage therapy is the alternative we need to treat bacterial infections in the future?

History of phages

In 1915, Twort was the first to describe the possibility of a virus that could infect bacteria, but unfortunately, he could not confirm this hypothesis [12]. Two years later, the term bacteriophage was confidently coined by D'Herelle, who found the kind of viruses described by Twort [13]. Developing phage therapy was started with much enthusiasm, but the enthusiasm calmed down after the discovery of antibiotics and their use in the Second World War [14]. In the years after the war, in countries in Eastern Europe, specifically the Soviet Union and Poland, phage therapy was further used and developed. Most of the current knowledge and experience with the therapy is associated with The Hirszfeld Institute in Poland and the Eliava Institute in Georgia [15]. The Hirszfeld Institute is mainly focused on an individualised approach, specific for the bacterium in the patient, and reported cure rates of around 40 per cent [14, 15]. On the other hand, the focus of the Eliava Institute is more on the development and use of phage cocktails [14, 15]. In Georgia, the phage therapy was part of the standard care long before double-blinded clinical trials were developed; therefore, the experiences in these countries are not well documented [14].

Biology of bacteriophages

Bacteriophages are viruses that infect and kill bacteria [15]. In every natural environment where a bacterial host exists, the corresponding bacteriophage is also present. The phages play an essential role in many biological processes, after all, they are the most plentiful organism on the planet [16]. Bacteriophages act on specific bacteria or a specific strain of a bacterium [15]. Thus, when a bacterium has multiple strains, various bacteriophages can act on it. There are two types of bacteriophages that infect bacteria via two distinct pathways. The virulent phages have a lytic cycle in which the phage attaches itself to the bacterium, inserts its genome, multiplies, and lyses the cell, releasing the new viruses [17]. This lytic cycle can be compared to the way viruses infect humans. On the other hand, the temperate phages initiate a lysogenic cycle starting with the phage being dormant as a prophage, replicating along with its host, and sometimes beginning a lytic cycle through a specific trigger

[17]. Considering that these temperate phages can be beneficial to the bacteria by helping to encode genes for virulence factors, they should currently not be used in phage therapy [18-20].

How to set up bacteriophage therapy

A multi-step process should be followed to arrange a suitable treatment for a patient experiencing a multi-resistant bacterial infection (Figure 1). The first step in the treatment of a patient with phage therapy is the selection of the bacteriophage [15]. Without the proper selection of the right phage, failure of therapy is very likely [20]. The choice of phage can be quite simple in the case of a mono-bacterial infection, as only a phage that infects that specific bacterium needs to be selected [20]. For more complex situations, phage cocktails or banks may be needed. Phage cocktails are formulations with two or more phage species that each target a specific species of bacteria [20]. Phage banks are a collection of phages against specific bacteria that are isolated and can be used when needed [7]. The advantage of phage cocktails is its efficacy as you hit the bacteria with multiple phages, whereas the advantage of banks is the specificity of the therapy [20]. These banks and cocktails are especially useful when bacteria become phage-resistant during the treatment with a specific phage [20, 21]. But where do the bacteriophages in these banks and cocktails come from?

That is where the next three steps in the development of a proper treatment come into play, starting with the isolation of the phages [15]. For the isolation of the phages, an environmental sample that is known to contain the bacterial host, like water, soil, or cattle faeces, is needed [20]. For example, phages have been isolated in cattle feedlots that infect *E. coli* and *Salmonella* spp. [22]. In this process, strains of bacteria need to be isolated that are identical (or at least as similar as possible) to the strains of bacteria that will be encountered in the patient [20]. The next step, after isolation, is the characterisation of the phages, which is done to make sure that the phages are active against the bacterial strains present in the patient. Subsequently, the representative sample of the phages used is characterised to ensure that the treatment is active against the bacterial strains in the patient [20]. The last step is the preparation of the phages, which comprises amplification and purification [20]. This includes a titre of the phage preparation to ensure appropriate dosing and purity. When this step is completed, the patient can be treated with the phages (Figure 1).

Bumps in the road to implementation

Considering that much is known about the biology of bacteriophages and the way to set up treatment, bacteriophage therapy shows high potential. Nevertheless, implementation in the standard care worldwide seems far from easy. The first challenge is the lack of sufficient information retrieved from the currently performed controlled clinical trials [23]. In the last few years, two main clinical trials were performed [24, 25]. The first one reported on the safety of phage therapy for treating venous leg ulcers and found no adverse effects [24]. The second trial demonstrated the efficacy and safety of its use in chronic otitis [25]. Currently, several clinical trials are registered to take place in the next few years [23]. Compared to other drugs, controlled clinical trials for bacteriophage therapy come with unique considerations, such as the dosage, host spectrum activity, and phage resistance. The issues regarding broad bacterial infectivity and phage resistance are already tackled with effective methods like broad host range phages or cocktails [21]. As for safety during the trials, the purity of the preparation should be sufficient. This is a major challenge for widespread use, as viruses can mutate during the amplification for *in vivo* use, possibly troubling the purity of the virus. Quality parameters for the engineering process of phage preparation are already formulated [26].

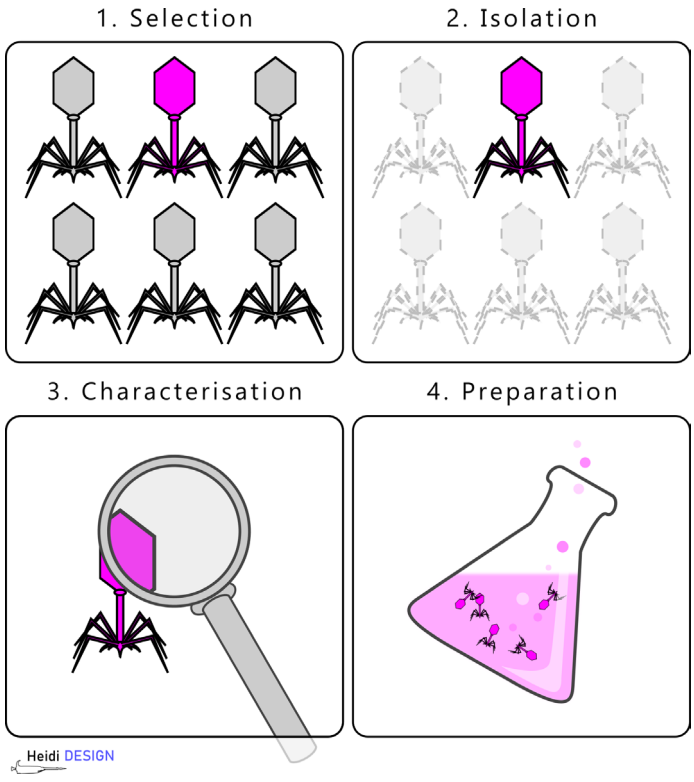

Figure 1: The development of bacteriophage therapy: a multi-step process
First the right bacteriophage has to be selected. After this selection, the phage should be isolated from an evironmental sample. Then, effectiveness against the bacterium is ensured and afterwards the phage preparation can be amplified and purified.

Outside of Georgia and Poland, no regulatory framework exists for the use of bacteriophages in the medical context [23]. In the literature, some researchers have suggested developing such a regulatory framework [27, 28]. Verbeken *et al.* analysed the stakeholder opinions and found that the stakeholders agreed about the need for a regulatory framework for the phage therapy; one for the properties and interactions of phages and one for the role of hospitals in the therapy [28]. Besides, a workshop with the European Medicines Agency acknowledged the need for a new regulatory framework as the current regulations are unsuitable for phages [29]. The magistral approach in Belgium is an example of a situation where political progression results in a framework for the use of phage therapy [30]. It centres on the preparation of tailored products that contain non-authorised ingredients (the phages) but have a certificate from a Belgian Approved Laboratory. These products can be used in Belgium to treat patients [31].

Although it has been approved in Belgium, the reaction of the general public on a possible widespread implementation of bacteriophage therapy is hard to predict. For example, vaccinations are not supported by everyone, often leading to discussion when new vaccinations enter the market. Injecting a living, functioning virus into the body of patients could lead to similar discussions. Would you inject yourself with a live virus?

Conclusion

Standing on the doorstep of the post-antibiotic era: does bacteriophage therapy bring light at the end of the tunnel? Indeed, bacteriophage therapy shows the potential to be one of the alternatives to antibiotics. These bacteria-

infecting viruses were discovered earlier than the antibiotics themselves, but the scientific Western world has slept on them for a long time. On the other hand, Eastern Europe, especially Poland, Georgia, and Russia, already has a lot of experience with treating patients with phage therapy. The engineering of phage therapy is known to be a multi-step process, comprising selection, isolation, characterisation, and preparation of the phages. However, the lack of clinical trials and a regulatory framework currently prevents bacteriophages from entering the drug market. Nevertheless, the magistral approach of Belgium shows the implementation of phage therapy is possible, giving us hope for a future with widespread bacteriophage use as a treatment for bacterial infections.

Acknowledgements

RAMS would like to thank Jelmer Raaijmakers, MSc, Department of Medical Microbiology, Radboudumc, Nijmegen, the Netherlands for providing the author of this article with feedback on this article.

References

1. Thomas, G. WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health. World Health Organization. (2014).
2. Cdc. Antibiotic resistance threats in the United States. U.S. Department of Health and Human Services, Atlanta. (2019).
3. Sugden, R., *et al.* Combatting antimicrobial resistance globally. *Nature microbiology* **1**, 16187 (2016).
4. O'neill, J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. (2014).
5. Projan, S.J. Why is big Pharma getting out of antibacterial drug discovery? *Current opinion in microbiology* **6**, 427-430 (2003).
6. Czaplewski, L., *et al.* Alternatives to antibiotics-a pipeline portfolio review. *The Lancet. Infectious diseases* **16**, 239-251 (2016).
7. Balogh, B., *et al.* Phage therapy for plant disease control. *Current pharmaceutical biotechnology* **11**, 48-57 (2010).
8. Kutter, E., *et al.* Phage therapy in clinical practice: treatment of human infections. *Current pharmaceutical biotechnology* **11**, 69-86 (2010).
9. Górski, A., *et al.* Phage Therapy: What Have We Learned? *Viruses* **10** (2018).
10. Dokters van Morgen. Bacteriofagen. (2020). Retrieved from: https://zorgnu.avrotros.nl/dossiers/item/bacteriofagen/#/ (Accessed: 15-10-2020)
11. Fagenbank. Bacteriofagen in de media. (2020). Retrieved from: https://www.fagenbank.nl/nederlands/in-de-media/ (Accessed: 15-10-2020)
12. Twort, F.W. AN INVESTIGATION ON THE NATURE OF ULTRA-MICROSCOPIC VIRUSES. *The Lancet* **186**, 1241-1243 (1915).
13. D'herelle, F. On an invisible microbe antagonistic toward dysenteric bacilli: brief note by Mr. F. D'Herelle, presented by Mr. Roux. 1917. *Research in microbiology* **158**, 553-554 (2007).
14. Abedon, S.T., *et al.* Phage treatment of human infections. *Bacteriophage* **1**, 66-85 (2011).
15. Kakasis, A. & Panitsa, G. Bacteriophage therapy as an alternative treatment for human infections. A comprehensive review. *International journal of antimicrobial agents* **53**, 16-21 (2019).
16. Keen, E.C. A century of phage research: bacteriophages and the shaping of modern biology. *BioEssays : news and reviews in molecular, cellular and developmental biology* **37**, 6-9 (2015).
17. Kutter, E.M. & Sulakvelidze, A. *Bacteriophages : biology and applications*, (Boca Raton (Fla.) : CRC Press, 2005).
18. Lin, D.M., *et al.* Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World journal of gastrointestinal pharmacology and therapeutics* **8**, 162-173 (2017).
19. Clark, J.R. Bacteriophage therapy: history and future prospects. *Future Virology* **10**, 449-461 (2015).
20. Gill, J.J. & Hyman, P. Phage choice, isolation, and preparation for phage therapy. *Current pharmaceutical biotechnology* **11**, 2-14 (2010).
21. Goodridge, L.D. Designing phage therapeutics. *Current pharmaceutical biotechnology* **11**, 15-27 (2010).
22. Johnson, R.P., *et al.* Bacteriophages for prophylaxis and therapy in cattle, poultry and pigs. *Animal health research reviews* **9**, 201-215 (2008).
23. Furfaro, L.L., *et al.* Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles. *Frontiers in cellular and infection microbiology* **8**, 376 (2018).
24. Rhoads, D.D., *et al.* Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. *Journal of wound care* **18**, 237-238, 240-233 (2009).
25. Wright, A., *et al.* A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. *Clinical otolaryngology : official journal of ENT-UK ; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery* **34**, 349-357 (2009).
26. Parracho, H.M., *et al.* The role of regulated clinical trials in the development of bacteriophage therapeutics. *Journal of molecular and genetic medicine : an international journal of biomedical research* **6**, 279-286 (2012).
27. Huys, I., *et al.* Paving a regulatory pathway for phage therapy. Europe should muster the resources to financially, technically and legally support the introduction of phage therapy. *EMBO reports* **14**, 951-954 (2013).
28. Verbeken, G., *et al.* Call for a dedicated European legal framework for bacteriophage therapy. *Archivum immunologiae et therapiae experimentalis* **62**, 117-129 (2014).
29. Pelfrene, E., *et al.* Bacteriophage therapy: a regulatory perspective. *The Journal of antimicrobial chemotherapy* **71**, 2071-2074 (2016).
30. Pirnay, J.P., *et al.* The Magistral Phage. *Viruses* **10** (2018).
31. Moelling, K., *et al.* A Wake-Up Call: We Need Phage Therapy Now. *Viruses* **10** (2018).