PHAGE THERAPY: COULD VIRUSES HELP RESOLVE THE WORLDWIDE ANTIBIOTIC CRISIS?

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Bacteriophages are bacterio-specific viruses. Involved in the origin of life and evolution, constituting a major part of the biosphere, they are promising as a sustainable, ecological and intrinsically cheap antibacterial. Félix d'Hérelle, one of the discoverers was the first to propose "phage therapy" in the early twentieth century. It was further developed at the Eliava Institute in Tbilisi, Georgia, and used in medical practice in all the previous Soviet Republics until now. The Western world, however, with the advent of antibiotics, forgot about phage therapy.

The antibiotic resistance crisis brought back phage therapy as a potential complementary or alternative treatment. The main problem is a lack of evidence-based studies using modern standards as well as the lack of an adapted regulatory framework. Attracting industrial partners and initiating studies in this context is difficult. Phage therapy is sporadically applied under certain conditions like the Helsinki Declaration or specific national regulations (for example, in Poland). This impedes scientific progress and clinical reimplementation.

Although several groups have set up animal and human studies, and bacteriophages are already used as antibacterials in the food industry, the clinical reimplementation is lacking while the antibiotic crisis is intensifying worldwide.

Antibiotic resistance (ABR), not a newly discovered biological phenomenon, is a fact and impacts negatively our global world society *(1-6)*. Fernando Baquero and colleagues described antimicrobial resistance as a "typical emerging characteristic of a dynamic, highly complex and self-organizing system evolving at the edge of chaos" *(7)*. The antibiotic crisis is obviously multifactorial and consequently not straightforward to resolve.

Although it seems not easy to cope with the global and complex ABR problem there is one approach, bacteriophage therapy, that could be an essential part in the process of resolving this antibiotic crisis (8–10). This is an antibacterial treatment approach that is scientifically proven, sustainable and timely, while intrinsically safe and cheaper than the development of a new antibiotic.

Bacteriophages

Bacteriophages were independently discovered and described almost a century ago by Dr Frederick Twort (1915), a UK microbiologist and Félix d'Hérelle (1917) a French-Canadian microbiologist *(11-12)*. It was d'Hérelle who coined the word bacteriophage and proposed to use these entities as antibacterial agents. An idea he quickly tried out in practice *(12)*.

Bacteriophages are bacterio-specific viruses that interact exclusively with bacterial cells (prokaryotes) *(13)*. The bacterial biochemical machinery that enables the interaction of bacteriophages and bacterial cells does not exisit in the cells that constitute our bodies (eukaryotic cells). This is why bacteriophages are bacterio-specific and non interactive with our body cells and in principle "safe" to use.

Factually bacterio-specific bacteriophages are composed of a nucleic acid genome packed in a protein capsid. In order to multiply itself the viral particle, called a virion, has to anchor itself on a specific bacterium. This happens through a specific bacterial outer membrane receptor that interacts with the virion's specific capsid coat molecular appendages such as the typical spikes. Once physico-chemically anchored on the bacterium's outer membrane, the bacteriophage injects its genomic material (composed of a nucleic acid) into the bacterial cell where it can be amplified and the capsid packed by the bacterial "hijacked" specific biochemical machinery.

Table 1: **The pros and cons of phages and antibiotics**

Phages	Antibiotics
Very specific (species or even strain specific and does not disturb the commensal flora)	Not specific (disturbs the commensal flora/ collateral damage)
Infecting bacteria need to be known (cocktails could solve this; use of rapid diagnostics)	Infecting bacteria don't need to be known (large spectrum antibiotics)
Development of new phage preparations: quick and cheap	Development of new AB: time consuming and costly
No side effects known so far	Multiple side effects
Complementary and synergistic	

This results in the production of tens of newly formed bacteriophages that will spread as virions after the infected bacterium is lysed. Indeed specific bacteriophage genome encoded and produced enzymes (holins) break open the bacterial cell wall after which the virions are released in the environment where they can look to infect again a specific (host) bacterium and restart the cycle through which the host bacterium is killed. It is this bacterial lysis process of natural lytic bacteriophages that is going to be used as a self-amplifying antibacterial in bacteriophage therapy for fighting specific bacterial pathogens. This bacterium killing process is independent of the bacterium's antibiotic resistance status. This means that, as well as a sensitive pathogen, a resistant pathogen will also be killed by the bacteriophage. Biofilms, the bacteria's main lifestyle modus especially in chronic infection, are known to inhibit antibiotic activity by a differential gene expression but not bacteriophage activity *(14–15)*. As such bacteria and antibiotics could even have a specific synergistic effect *(16)*. Here, specifically, research is needed in order to optimize both approaches: bacteriophages with or without antibiotics.

The advantage of phage therapy as a complimentary tool or substitute for antibiotics in the combat of bacterial infections is the existence of scientific evidence at several levels: theoretical, in vitro laboratory experiments and in vivo studies, in animal models and in humans.

The molecular biology that gave rise to the actual biotechnological industry was largely built thanks to the huge amounts of scientific experimental work carried out with the bacterial workhorse *Escherichia coli* and its bacteriophages. This gave a tremendous amount of experimental data enabling the development of a theoretical biological working framework. Furthermore, there exists a huge, and still increasing amount of laboratory experiments and in vivo studies using bacteriophages and their targeted bacterial host cells in animals and humans *(17–20)*.

Bacteriophage therapy

The human experience however is actually almost empirical and from the former Soviet Union republics with the Eliava Institute in Tbilisi (Georgia) as the main centre. In Poland, an actual EU country, phage therapy is in use under specific conditions. The Polish phage research centre (Ludwik Hirszfeld Institute of Immunology and experimental therapy in Wroclaw) is emerging as a very active scientific unit conducting and starting bacteriophage studies in order to help to re-implement phage therapy in routine medicine in accordance with actual scientific and medical care standards (19–20). In recent years, other countries have also conducted some human studies in accordance with modern biomedical standards and detailed clinical case reports are being published showing the potential of phage therapy *(20–26)*. Bacteriophages are in fact self-amplifying anti-bacterial agents that are eliminated naturally from our bodies by our reticulo-endothelial system, urine and faeces, after their targeted host cells disappear from the site of infection.

Safety issues

Are bacteriophages, bacterial viruses, safe for use in human beings? This is one of the first questions people will want to know if viruses are to be used on patients. In fact, we are living in an ocean of viruses which includes bacteriophages. Bacteriophages are apparently the most abundant biological entities in our biosphere *(27–29)*. Wherever there are bacteria there are bacteriophages. We eat, drink and carry in our bodies more bacteriophages than bacteria while the latter already exceed the number of cells in our body.

Bacteriophages co-evolve with their host bacteria and provide the earth's ecological equilibrium in several environmental or ecological niches. In fact, we have to think from the co-evolving couplet phage/bacterium that is continually co-adapting to each other and as such provides a long-term sustainable antibacterial approach.

Indeed for each existing or emerging pathogen there exists a bacteriophage, which makes bacteriophage therapy sustainable. This ecological point of view fits well in the emerging field of evolutionary/Darwinian medicine as well as the increasing societal and political interests in long-term

Figure 1: Large model of a phage. Courtesy, Perron, University Hospital of Geneva

sustainable and green approaches for managing our globalizing world *(30)*.

Bacteriophages, the other facet of a bacterium, have existed since the bacterial cells originated. Recent work from Raoult and Forterre brought viruses, including bacteriophages, into the tree of life and consider them as living biological entities, although a different kind than we are used *(31)*.

It is estimated that half of the bacterial biomass daily is lysed by lytic bacteriophages, while their total number in our biosphere approaches 10^{31}.

A recent study by the group of Rohwer showed that all epithelial mucus layers in multicellular organisms (metazoan), such as our gut mucus layer, is in fact a bacteriophage-based symbiotic defence system against potential invading bacteria *(32)*. This is one of the recent breakthrough studies showing the inherent and basic inoffensiveness and safety, of the use of bacteriophages as an antibacterial.

During the Seventies, the FDA had to set up a review of safety since bacteriophages were found in several vaccine preparations. The study review had to conclude that bacteriophages were not a safety issue and the vaccines containing phages were allowed to be used *(33)*.

Also researchers were allowed to apply phage phiX174 intravenously into HIV positive patients. The FDA concluded that bacteriophages are safe for humans. This knowledge in conjunction with the existing empirical clinical data as well as several animal and a handful of modern human applications show that, from the safety point of view, phage therapy concept is intrinsically safe *(33–35)*.

The advantages of phage therapy versus antibiotics

Phage therapy is bacteriospecific and has no or at least lower collateral damage then the use of antibiotics as far as is known. Even if small spectrum antibiotics are used more than the specifically targeted pathogen is influenced or hampered. The (side) effect of using antibiotics on our natural microbiota is so huge that it seems that a lot of actual diseases are associated with a microbiome disturbance caused by their use. This aspect is recently well-described in its global aspects by Blaser *(36)*.

By using phages we could prevent those side effects or in certain cases (*Clostridium difficile*), infections or other gastrointestinal diseases, could restore or re-equilibrate the situation.

Looking for a specific phage against a specific bacterium should always be possible and in a much shorter timeframe (days to weeks) than searching and developing a new antibiotic *(37–39)*. It should also be cheaper. This specifically makes phage therapy a relatively cheap "online" approach in the fight against specific bacterial infections.

Phage therapy in public health and in developing countries

The problem of antibiotic resistance not only affects individual patients in clinics, but exists in all types of health-care institutes, and affects whole communities. Remember the Enteroaggregative Hemorrhagic *Escherichia coli* (EAHEC) 0104:H4 outbreak in Berlin in 2011. Antibiotic use was even of questionable help *(39)*.

However, different research groups obtained potent lytic phages against this problematic enteroaggregative *E. coli*, either by the isolation of new phages from the environment or by selection and "improvement" of phages from existing collections, and this was often accomplished in a matter of days. As such, phages could probably have been used to help control the O104:H4 *E. coli* outbreak that caused the death of more than 50 patients. Unfortunately, authorized use of phages was not possible in this otherwise feasible phage therapy context, because under the existing medicinal product legislation such an anti-O104:H4 phage preparation would have taken years to develop, produce and approve. It is thus crucial to set up new therapeutic phage collections (including in low-income and emerging countries) and to maintain and continuously update existing phage collections, which can be

used to counter bacterial epidemics in a timely and cost-effective way.

In large parts of the world bacterial diarrhoeal diseases of all kinds are a big burden in public health. Diarrheal disease is one of the major problems in third world countries. Many common causative agents are multi-drug resistant (MDR) bacteria and considering the poor sanitation the control of those epidemics and pandemics such as cholera for example run quickly run out of control. Bacteriophages could play a significant role *(40)*.

The Mekalanos group showed some time ago the effective natural role of bacteriophages as agents at the origin of the natural collapse of the local endemic diarrheal epidemics in Bangladesh *(41–42)*. This observation brings us to the idea that further studying and optimization of this bacterial/phage interaction could bring phage therapy to public health authorities as a way of controlling or at least inhibiting diarrhoeal diseases, which are still a major issue, especially in developing countries.

Why is phage therapy not implemented?

Bacteriophages are natural biological entities that co-evolve with their bacterial host cell. This is in contrast with a chemical stable molecule like an antibiotic. We have to think of bacteriophages and bacteria as a couplet in continuous interaction. Since our actual pharmacoeconomic model, mainly based on stable static chemical molecules as a drug, and its associated highly sophisticated regulatory, quality and safety system it seems difficult in our modern Western countries to (re)introduce phage therapy which, in contrast to classic antibiotics, is based on a self-amplifying and co-evolving viral natural entity *(37–38; 43–44)*. Also intellectual property rights are a thorny issue since it is the basis for private/industrial incentives in the field. With respect to bacteriophages as natural biological entities, patents could be granted and exist, but how robust they are? They seem to be fragile and thus not very attractive for the classical big pharmaceutical companies in the context of today.

However, seeing the impact of ABR on our societies, we think that the phage therapy concept should be taken in consideration as a valuable instrument to resolve our actual antibiotic crisis worldwide.

The current hurdles that SMEs need to overcome to put phage therapeutics on the European Union or United States markets are throttling, and largely undetermined. Therefore, some phage companies decided to circumvent some regulatory obstacles by seeking first to market phage products for agricultural and food applications, where regulations are less stringent. For example, in 2006 the FDA approved a bacteriophage preparation on ready-to-eat meat and poultry products as an antimicrobial agent against *Listeria monocytogenes*.

Conclusions and perspective

Conclusively we can state that the use of bacteriophages as antibacterial agents makes sense, scientifically and empirically. Bacteriophages, bacteriophage therapy, as a tool for resolving the antibiotics resistance crisis, should be re-introduced in regular medicine all over the globe where it could be optimized for specific treatments. It will not only help to treat individual patients at hospitals or in other health-care settings, but it could be a real beneficial tool from the standpoint of public health for inhibiting and/or controlling emerging bacterial epidemics especially gastrointestinal infections or chronic bacterial-related diseases. Especially in developing countries where bacterial diarrheal disease, amongst others, is a major part of the infectious burden and phage therapy could bring a solution.

Phage therapy can also be applied in conjunction with or without antibiotics, depending on the situation, while in general a more judicious use of antibiotics should be promoted.

Further it seems that all future strategies should be integrated and involve different fields: human and veterinary medicine, the agro-bio and food industry.

It would be important today to be open to a more sustainable ecological approach enabling the development, optimization and implementation of phage therapy as a recognized scientifically meaningful bacterial treatment approach. •

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