Evolutionary biology and development model of medicines: a necessary "*pas de deux*" for future successful bacteriophage therapy

Review article

Abstract

The increase in frequency of multidrug resistant bacteria worldwide is largely the result of the massive use of antibiotics in the second half of the 20th century. These relatively recent changes in human societies revealed the great evolutionary capacities of bacteria towards drug resistance. In this article, we hypothesize that the success of future antibacterial strategies lies in taking into account both these evolutionary processes and the way human activities influence them. Faced with the increasing prevalence of multidrug resistant bacteria and the scarcity of new antibacterial chemical molecules, the use of bacteriophages is considered as a complementary and/or alternative therapy. After presenting the evolutionary capacities of bacteriophages and bacteria, we show how the development model currently envisaged (based on the classification of bacteriophages as medicinal products similar to antibacterial chemical molecules) ignores the evolutionary processes inherent in bacteriophage therapy. This categorization imposes to bacteriophage therapy a specific conception of what a treatment and a therapeutic scheme should be as well as its mode of production and prescription. We argue that a new development model is needed that would allow the use of therapeutic bacteriophages fully adapted (after *in vitro* "bacteriophage training") to the etiologic bacteria and/or aimed at rendering bacteria either avirulent or antibiotic-susceptible ("bacteriophage steering"). To not repeat the mistakes made with antibiotics, we must now think about and learn from the ways in which the materialities of microbes (*e.g.* evolutionary capacities of both bacteriophages and bacteria) are intertwined with those of societies.

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The advent of microbiology, notably around the figures of Pasteur and Koch at the end of the 19th century, until the advent of metagenomics at the beginning of the 21st century, produced an unprecedented wealth of knowledge and data on microbes. Medical microbiology has greatly contributed to the increase in the life expectancy of humans and their domestic animals, partly due to the discovery and use of antibacterial chemical molecules (hereafter referred as antibiotics) as early as the first half of the 20th century. The discovery of antibiotics, their massive production from the 1940s onwards, and their widespread use in the second half of the 20th century even led, for a time, to the hope of a world in which infectious diseases would be but a distant memory (O'Neill, 2014).

However, our societies are currently under the influence of a new threat, whose similarities with the infections that humans have experienced so far should not make us lose sight of what is new and the conclusions we may be able to draw. The increase in the frequency of bacteria that are resistant to several currently available antibiotics (Davies & Davies, 2010; Jee *et al.*, 2018) suggests a present and future increase in mortality due to these bacteria (O'Neill, 2014).

This is by no means a return to a "pre-antibiotic" era. The multidrug-resistant (MDR) bacteria that are currently emerging have little to do with the bacteria that patients and health services were confronted with only a few decades ago (Landecker, 2015). The emerging bacteria are adapted to the different medical, agricultural and industrial contexts in which they have been placed. It is these adaptive skills of bacteria and more generally of organisms that we wish to focus on in this article, in order to discuss the increase in antimicrobial resistance (AMR) and determine the answers we wish to provide. In human health, it is usually a question of doing everything possible to counter the evolutionary skills of microbes (e.g. tri-therapy against HIV which aims to reduce the probability of the appearance of a resistant viral variant). But what could a treatment for infectious bacterial diseases look like if, far from merely fighting it, it actively accompanied the evolutionary processes of the different actors (i.e. bacteria and antibacterial agents, as well as host responses)?

The field of Science and Technology Studies (STS) has shown that the production of knowledge about living things, what we do with this knowledge, and the consequences of its applications are the result of both the state of knowledge at a given moment and the intellectual, political and material context in which this knowledge is used (Keller, 1983, 1995; Latour, 1988, 1993; Jasanoff *et al.*, 1995). For these reasons, we found it necessary to develop a strong

interdisciplinary approach, mixing Life Sciences, Biomedical Sciences and Human and Social Sciences. What brings us together here, an evolutionary virologist and an anthropologist of science and health, is a similar awareness of the materiality (*sensus* properties and physical reality of an object or, here, of an organism) of the living (human or non-human): their characteristics, skills and modes of existence. In this sense, it is also important to pay attention to the materiality of our societies, their infrastructures, what they produce and induce.

In this article, we will first draw up an inventory of the complexity and entanglement of the factors (scientific, social, political) suspected to be involved in the increase in the frequency of AMR. While AMR is a good illustration of evolutionary mechanisms, there is a great risk that these will only be perceived in a negative way, as a set of capabilities that should be countered or prevented at all costs. This is why we then present bacteriophage therapy, the use of bacteriophages (i.e. bacterial viruses) to treat bacterial infections, focusing in particular on the co-evolutionary capacities of bacteriophages and bacteria, and showing how these can be one response, among others, to the problem of AMR.

However, we will also illustrate in a third and final part of the article the difficulty in compatibility of bacteriophage therapy with current infrastructures, and the need to think and work on the creation of new development models likely to take into account the evolutionary capacities of living organisms, both for curative (treating bacterial infections) and preventative purposes (avoiding the emergence of biological entities that are pathogenic for humans and resistant to antibiotics insofar as is possible).

The rise of bacterial antibiotic resistance

Antimicrobial resistance is a natural phenomenon, resulting from a set of molecular mechanisms that certain microorganisms such as bacteria harbor to protect themselves against other biological entities. What seems to have changed in recent decades, however, is not so much the existence of antibiotic resistance genes but the increase in the frequency of acquisition and the accumulation of these genes in the bacterial populations living in human-shaped ecosystems. That is to say in ecosystems where antimicrobial selective pressure is strong, both spatially and temporally.

The increase in the prevalence of bacteria resistant to multiple antibiotics is a phenomenon that cannot be fully understood and apprehended if we consider evolutionary processes and socio-

economic changes separately. To start with, technical innovations, but also the reorganization of certain industries, led to the mass production of penicillin in the early 1940s. This was soon followed by other antibiotics. Although some scientists warned very early on against the massive and unreasonable use of antibiotics, due in particular to the rapid emergence of resistance in certain bacterial species (Holmes *et al.*, 2016), antibiotics were used first for preventative and curative reasons in livestock and fish farms, before being used, in sub-therapeutic doses, but systematically and widely, as growth promoters (Bud, 2005). Antibiotics have become what anthropologists Clare Chandler and Laurie Denyer Willis have called a 'quick-fix', molecules used widely throughout the globe to rapidly alleviate problems of care, productivity, hygiene and inequality, underlining the structural and systemic dimension of the AMR problem (Denyer Willis & Chandler, 2019).

This increase in prevalence can thus be analyzed within a context of the conjunction between bacterial evolutionary abilities and structural and infrastructural changes specific to capitalism, particularly the acceleration that the latter underwent after the Second World War (Landecker, 2015). More precisely, AMR can be understood by the increase of all the basic evolutionary forces over time: (i) creation of heritable variations , (ii) strong selection pressures due to the use of high concentrations of antibiotics (Holmes *et al.*, 2016) and rapid response (Davies & Davies, 2010; Diaz Högberg *et al.*, 2010) to selection, (iii) large population size due to large use of antibiotics throughout the world in different ecosystems (e.g. same antibiotics in human and animal health, particularly on the 28 billion farm animals on the planet; Moulin *et al.*, 2008; Tang *et al.*, 2017), and (iv) high migration rate between hosts, thanks to intensification of trade (especially in the agro-food sector) and human exchanges (tourism, business travel) (Holmes *et al.*, 2016). Thus, a mutant that once appeared locally and disappeared locally by random effect, or because of a too high selective cost (in the absence of antibiotics) induced by its resistance molecular mechanism compared to the resident bacteria, can now be transmitted worldwide. Finally, (v) bacteria show a great ability to recover genetic information from horizontal gene transfers after recombination events and/or integration of different genetic mobile elements, both intra- and interspecifically, and even between genera. A large number of these resistance genes is concentrated and accumulates on plasmids, which led the microbial evolutionary biologist Michael Gillings to refer to "xenogenetic pollutants": if antibiotics pollute the soil, the plasmids not only pollute but also duplicate and spread more and more (Gillings, 2013; Gillings & Paulsen, 2014; Gillings *et al.*,

2018). For instance, this is the case for the transfer of the plasmid allowing the expression of the alarming metallo-β-lactamase NDM-1 in different *Echerishia coli* genotypes but also in *Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis* and *Salmonella enterica* (Johnson & Woodford, 2013), or the transfer of efflux pumps known to allow resistance to different families of antibiotics (Nolivos *et al.*, 2019).

This profound intertwining of the various factors and actors involved in the increase in the frequency of antibiotic-resistant bacteria has led to the production of a scientific and institutional framework that partly makes it possible to account for it. The concept of One Health, used jointly by the World Health Organization, the FAO (Food and Agriculture Organization) and the OIE (World Organisation for Animal Health) in the early 2000s highlights the highly interspecific dimension of human, animal and environmental health. Although it has made it possible to develop interdisciplinary research programs and thus to foster dialogue, discussions and collaborations between people who did not necessarily work together before, this concept nevertheless has its limitations, in particular by failing to take into account local idiosyncrasies, inequalities in access to healthcare and consumer goods, or the different modes of agriculture and animal husbandry (Hinchliffe, 2015). One Health can thus lead to a desire to seek 'universal' solutions, even though mechanisms of evolution and the way in which they are applied are profoundly situated and embedded in specific socio-geo-historical contexts.

Interdisciplinary works are central to disentangling parameters involved in AMR, since understanding the problem of antibiotic resistance requires thinking together about local practices, regulations, pharmaceutical companies, factories, production chains and biological entities. Chemical antibiotic molecules have been used in different ways (with varying consequences) as growth promoters on farms, for example, or as 'magic bullets' to ensure the health of workers, and, in the first phase of antibiotic development (i.e. during the 1944's), to support the survival of injured soldiers in conflict zones (Figure 1).

In this story, the materiality and the modes of existence of microbes, and especially their enormous adaptive and evolutionary abilities, have been forgotten. Indeed, the development of capitalism is based on extractivism. That is to say on the massive exploitation of natural resources, which are reified and commodified to meet different objectives. If nature is thought to be controllable, manageable, and exploitable, then there is no room to consider about the reactions and evolution of non-human living beings. The irruption of antibiotic resistance as a public health

problem thus forces us to think about what has hitherto remained in the shadows: the materiality of (microbial) living and its evolutionary capacities. Whether new antibiotics, complementary or alternative therapies and treatments are involved, we now have to take into account the materiality of the different biological entities and the material infrastructure of modern societies.

Multiple new categories of antibiotic agents are now under investigation: immunity modulating agents (vaccines and immunostimulants; Lipsitch & Siber, 2016; Jansen *et al.*, 2018), antimicrobial peptides (Brogden *et al.*, 2005; Cotter *et al.*, 2013), pro- and/or prebiotics, plant extracts such as polyphenols (Daglia, 2012), inhibitors targeting pathogenicity (such as inhibitors of bacterial quorum sensing, biofilm growth or virulence factors; Cheng *et al.*, 2014) , as well as purified bacteriophage lytic enzymes (Kim *et al.*, 2019) or bacteriophages per se (Gordillo Altamirano & Barr, 2019; Kortright *et al.*, 2019). In this context, bacteriophages appear to be particularly promising for rapid development at different levels since they are relatively simple to isolate and produce, but also because of their co-evolution with their hosts, which has been inherent to their persistence on Earth since first they appeared. Note that if the regulatory framework was different, bacteriophage therapy could allow for the principles of co-evolution in infectious processes to be taken into account. It thus requires actors concerned with controlling infections (doctors, veterinarians, agronomists, etc.) to think and act with evolution.

Bacteriophage *versus* antibiotic potentialities

The existence of bacteriophages was highlighted at the beginning of the 20th century (Twort, D'Hérelle) and bacteriophage therapy was then conceptualized and researched by Félix d'Hérelle in late 1910s (D'Hérelle, 1917). This therapy was almost abandoned from the 1940s onwards in Western Europe and North America, partly due to the development of antibiotics. However, it continued to be practiced occasionally in these countries from the 1950s onwards, to deal with antibiotic-resistant bacterial infections. Meanwhile, bacteriophages have enabled the development of molecular biology thanks to fundamental discoveries such as operons and regulator genes involved in lysogeny of temperate phage systems (Lwoff, 1953; Jacob & Monod, 1961), the development of the basis of genetics and molecular evolution by demonstrating the phenomena of random mutations over time (Luria & Delbrück, 1943), or even more recently the renewed

development of gene editing through the discovery of the CRISPR system (Barrangou *et al.*, 2007; Brouns *et al.*, 2008) which recently led to the award of the Nobel Prize in Chemistry to E. Charpentier and J. Doudna.

Several types of biological cycles can be observed in bacteriophages, of which the two most frequent are the lytic and lysogenic cycles. In the first case, the so-called "virulent" bacteriophages only use this lytic cycle and can be compared to "predators" of the bacteria. In the second case, the genome of so-called "temperate" bacteriophages is integrated into that of the bacteria and no bacterial lysis happens during the lysogenic stage. This integration allows vertical transmission from mother to daughter cells. Following an environmental stress (e.g. antibiotics) or other unknown mechanisms, the bacteriophage DNA is excised and the lytic cycle takes place. Temperate bacteriophages can not be used in bacteriophage therapy due to several factors : (i) such types of bacteriophage might lysogenize the bacterial host that would then become homoimmune to further infection (Gandon, 2016), (ii) there is a risk of horizontal transfer of effectors or toxins (Boyd, 2012) or antibiotic resistance genes (Schuch & Fischetti, 2006; Shousha *et al.*, 2015; Calero-Cáceres *et al.*, 2019) as well as specialized and generalized transduction. Only 'virulent' bacteriophages will thus be considered in this article. Although bacteriophages and antibiotics are not incompatible and can even act synergistically (Tagliaferri *et al.*, 2019), it is nevertheless interesting to consider what distinguishes them.

1 – Host Range

Antibiotics target fundamental molecular mechanisms present in large classes of bacteria, such as the inhibition of cell wall synthesis in the case of molecules of the β-lactam family (e.g. penicillin) or the inhibition of DNA or RNA synthesis (quinolones targeting topo-isomerases) or proteins (macrolides or aminoglycosides targeting certain subunits of the ribosomes). These antibiotics thus present a relatively broad spectrum of action since they target molecular sites common across different classes of bacteria. New antibiotics under development rely on targeting more specific features of a bacterial species (Wright & Sutherland, 2007), but the range of chemical antibiotic molecules remains relatively broad.

The interactions between bacteriophages and their hosts are much more specific and diverse. This specificity is thanks to several steps of the biological cycle of bacteriophages: (i) recognition of a receptor located on the bacterial outer surface (LPS, pilus, capsule, porin or lipo-teichoic acid), (ii) adsorption and the capacity of the bacteriophage to deliver its genetic material to the host cytoplasm, (iii) replication, (iv) expression and appropriate assembly of proteins, and (v) lysis (Chevallereau *et al.*, 2016). Single bacterial genotypes can thus be the target of several bacteriophages using different infection mechanisms (Allen *et al.*, 2017). For the recognition stage, for example, some *Salmonella enterica* bacteriophages adsorb via LPS, others via membrane proteins (OmpF, BtuB, TolC), others via flagellar proteins (Chaturongakul & Ounjai, 2014). To persist within the cytoplasm, bacteriophage genomes must avoid recognition, modification and degradation by restriction enzymes or the CRISPR system (van Houte *et al.*, 2016). The reproductive capacity of bacteriophages therefore depends on their adaptation level to the bacteria with which they are in contact. Consequently, bacteriophage capacities to fulfill each step of the biological cycle determine the qualitative (i.e. host range) and quantitative capacities for infection (i.e. the number of phage offspring resulting from infection) (Hyman & Abedon, 2010). Depending on the molecular mechanisms of infection, the host range of a bacteriophage can therefore be specific to a single genotype within a bacterial species, while other bacteriophages can infect bacteria of different genera (Koskella & Meaden, 2013; Meaden & Koskella, 2013; Cazares *et al.*, 2021). From the point of view of the bacteriophage, the hosts are seen in terms of the presence/absence of multiple molecular mechanisms (e.g. proteins, DNA targets and states, etc) to which it must be pre-adapted and/or adapt.

These differences between bacteriophages (biological entities) and antibiotics (chemical molecules) in modes of action and host range lead to differences in (i) the mechanisms and possibilities of bacterial resistance and (ii) the effects of bacteriophage and antibiotic administration on human health.

2 – Resistances and Evolution

Although we may observe an evolution of bacteria towards resistance to antibiotics as well as to bacteriophages, the mechanisms of resistance are different. On the one hand, bacteria become resistant to antibiotics through several major mechanisms: (i) modification or degradation of the

toxic molecules themselves, (ii) modification, protection or overproduction of the targets of the toxic molecules, or (iii) reduction of the internalization or increased externalization of the toxic molecules via efflux pumps. The processes by which these resistance mechanisms appear are different (single point mutations or acquisition of mobile genetic elements), and they strongly influence the frequency of emergence of resistant bacteria (Peterson & Kaur, 2018). For example, resistance to quinolones can be acquired via a low number of single mutations whose frequency of appearance depends on the antibiotic concentration (Harmand *et al.*, 2017, 2018), or via plasmid acquisition. While single mutations can be involved in the resistance to a specific drug and are then generally vertically transmitted, the horizontal transfer of mobile genetic elements (integron, plasmid, transposon) allows the acquisition of different types of resistance mechanisms which can be specific to a family of antibiotic molecules (e.g. against quinolones, see Li *et al.*, 2019), or resistance mechanisms to different antibiotic families (e.g. efflux pumps; Fernández & Hancock, 2012; Lin *et al.*, 2018). Unfortunately, such horizontal transfers of mobile elements are frequent within as well as between species (Johnson & Woodford, 2013; Nolivos *et al.*, 2019).

Antibiotic resistance is often associated with reduced bacterial fitness in environments where antibiotics aren't in use (Davison *et al.*, 2000). Depending on species, population structure, molecular mechanisms targeted, genetic background and environment, fitness costs range from several percent up to as much as 400%. Moreover, fitness costs associated with resistance can be decreased rapidly and efficiently after reversion (rare) or accumulation of compensatory mutations without a loss of resistance (and even sometimes with a gain in fitness). Compensation can occur by acquisition at other loci of new (epistatic) mutations, but also by gene conversion or gene duplications. According to this rapid processes, antibiotic resistant bacteria persist over time within ecosystems (Björkman & Andersson, 2000; Andersson & Hughes, 2010; Hall *et al.*, 2015).

On the other hand, the bacterial mechanisms of resistance to bacteriophages can be of different types (Oechslin, 2018; Torres-Barceló, 2018). Thus, mutations or complete deletions of genes allow the modification of bacteriophage receptors (van Houte *et al.*, 2016; Gordillo Altamirano & Barr, 2019) but bacteria can also resist bacteriophage infection through "constitutive" systems of degradation (via restriction enzymes and/or CRISPR systems with chromosomal genes) or modification (methylation) of bacteriophage DNA and/or RNA, with some of these mechanisms having only been recently discovered (Barrangou & Horvath, 2017; Bernheim & Sorek, 2019). While few studies are available concerning *in vivo* bacterial fitness costs associated to resistance,

initial studies show that bacterial genotypes resistant to bacteriophages are rare. For instance, two *Pseudomonas aeruginosa* resistant genotypes isolated *in vitro* presented great physiological costs and a very low infectivity rate *in vivo*. Interestingly, none of these variants emerged *in vivo*, most likely because resistant mutations affected bacterial surface determinants important for infectivity (pilus motility and LPS formation), inducing a high selective cost and therefore preventing the emergence of such resistant bacteria (Oechslin *et al.*, 2017).

One possible explanation for such absence of development of resistant bacterial genotypes might be that bacteriophages target main bacterial structures participating in the biological cycles of the bacteria. In fact, several studies reveal that resistant bacteria exhibit lower virulence (a phenotype that often results from host cell bacterial infection), motility and growth rates (Le *et al.*, 2014; León & Bastías, 2015) [see our conclusion paragraph for further exploitation of these results]. Another explanation may be that the host immune system eliminates the few remaining bacteria, thus preventing any appearance of resistant ones. This hypothesis is supported by an *in vivo* study in which variants of *P. aeruginosa* resistant to bacteriophages only emerged within immuno-compromised mice, while none were observed in wild-type mice (Roach *et al.*, 2017).

In view of these abilities of bacteria to resist antibiotic agents (chemical or bacteriophage), it is important to consider the processes of discovery and synthesis of new molecules that make it possible to kill resistant bacteria. For chemical molecules, these processes are human-driven and thus relatively slow and extremely costly (DiMasi *et al.*, 2016), as shown by the sharp decline over the last few decades of the discovery and marketing of new antibiotic molecules (Ventola, 2015), with pharmaceutical companies focusing on the development of molecules for people with chronic conditions.

By comparison, the discovery of new bacteriophages can be rapid (anything from a few days to a few weeks), given the number and diversity of bacteriophages on our planet (Dion *et al.*, 2020), illustrated by the increase in publications of new bacteriophages (Adriaenssens & Brister, 2017) and particularly in environments where the targeted bacteria are found, such as feces and waste water (Weber-Dąbrowska *et al.*, 2016).

3 – Side effects

Because of both their nature and their modes of action, bacteriophages and antibiotics are also distinguished by the side effects expected when administered to humans, domestic animals and plants. As antibiotics are catabolized by the body (kidneys, liver, immune system), they must be administered regularly, sometimes several times a day (depending on pharmacodynamics and pharmacokinetics). Moreover, these processes of degradation of chemical molecules can be toxic for the organs involved, thus limiting the concentration and the time during which they can be applied without causing too much damage to the organism. Even if they are partially degraded by the immune system, bacteriophages benefit from their amplification by the very bacteria they target. Interestingly, phase I and II clinical trials have not reported any toxic effects of the application of bacteriophages (Sarker *et al.*, 2016; Furfaro *et al.*, 2018; Jault *et al.*, 2019; Leitner *et al.*, 2021).

Finally, due to the use of bacteriophages with a narrow host-range, their therapeutic use should have only a moderate impact on resident non-targeted bacterial communities and thus cause minimal disruption to ecosystems (Wang *et al.*, 2019). However, *in vitro* and *in vivo* experiments (in animal models) are still necessary to measure the impact of bacteriophage therapy on non-targeted bacterial communities. The principle of using bacteriophages with a narrow host-range also means that treatment with bacteriophages has a potentially limited impact on the selection of resistant genotypes in the non-targeted bacteria. At a global scale (i.e. Earth), population sizes of emerging resistant bacteria should then be very limited. On the contrary, antibiotics have more long-term impacts on diverse ecosystems (Jernberg *et al.*, 2010) such as, when ingested orally, damage on the whole commensal gut bacterial community and selection of resistance among all the susceptible bacteria (e.g. all Gram positive when using Vancomycin), which might in turn be passed on to later pathogen species by horizontal transfer.

4 – Conception of care

Because bacteriophages and antibiotics harbor different evolutionary abilities (the former can co-evolve *in situ* with bacteria while the latter can not), some practitioners of bacteriophage therapy are openly questioning the notion of eradication of the etiologic bacteria as a results of their therapeutic treatment. They thus further promote approaches to chronic bacterial infections that allow the maintenance of an infection at a low noise level, at a tolerable threshold for the patient, which would allow them to live in acceptable conditions (Brives and Pourraz, 2020). While in practice this second option is often applied, it is usually by default. It is thus proposed that a therapeutic treatment to accompany the infection be made a principle practice, to render it viable for patients, instead of fighting against the etiologic bacteria by any possible means.

In the case of acute bacterial infections, the rapid decrease in concentration of the pathogen by antibiotics remains by far the best solution. However, the use of therapeutic bacteriophages as complement could provide both a means of treating multidrug-resistant bacterial infections as well as of preventing the emergence of new antibiotic resistance (by not using increasingly valuable antibiotics), while preserving the other bacterial species present in the microbiotas. Because they make it possible to be more precise (some bacteriophages can target one genotype only; Allen *et al.*, 2017), therapeutic treatments using bacteriophages have the advantage of not destroying commensal microbial communities, the roles of which we are currently hardly aware of, for humans as well as for other non-human living beings. Now a days, bacteriophage therapy allows personalized therapy to treat infectious diseases, which, in the near future, could even be more efficient, thanks to instant synthetic bacteriophages, Artificial Intelligence and Distributed Ledger Technology (Pirnay, 2020). Our hypothesis therefore is that what may emerge (notably with bacteriophage therapy) is a new way of conceiving medicine, which actively takes into account evolutionary and ecological processes (sometimes called "evolutionary medicine"; Shanks & Pyles, 2007; Corbellini, 2008; Nesse & Stearns, 2008).

It is at this point, however, that we need to think about both the materiality of living organisms and material infrastructures, particularly concerning the way in which bacteriophages can become entities that can be used in human therapy. It is therefore towards regulation that we must turn.

The materiality of the context: regulation, production and uses of medicines

Although bacteriophages and antibiotics present significant differences, both in terms of their nature and their mode of action, they are nevertheless subject to the same regulations for their production and their uses in human health. Today, bacteriophages are considered by both the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) as "industrial medicinal products", a decision that was adopted by the EMA in 2011 and renewed in 2015. This categorization has several major consequences: (i) medicines must be produced according to Good Manufacturing Practices (GMP); (ii) their composition must be defined and stable over time, both in terms of the active molecule and the excipients, (iii) they must demonstrate their safety and efficacy in Randomized Controlled Trials (RCTs); (iv) they must be subject to a marketing authorization (MA). The production of bacteriophages has thus rapidly found itself placed in the context of an industry-led drug economy (Brives and Pourraz, 2020) in which drug-related regulations impose a fixist conception of bacteriophages that must remain "stable" over time.

Moreover, since 2019, this categorization has been accompanied by guidelines produced by the EMA, which require bacteriophages to be developed as closely as possible to the standards and norms set up for antibiotics, imposing a specific conception of what a treatment and a therapeutic scheme should be.

1 – Production

Good Manufacturing Practices (GMPs) require the construction of dedicated production lines. GMPs are part of the quality assurance system set up by the pharmaceutical industry to guarantee the production of medicines in a systematic, standardized and controlled manner according to the quality standards appropriate to their use. Firstly, while the pharmaceutical industry develops facilities in which different production lines are located side by side, the high risk of bacteriophage contamination of bacteria requires dedicated sites with clean rooms for the production of bacteriophages. In addition, all the norms and standards specific to bacteriophage production and

to the administration of proof of their effectiveness have to be built in. This is a financial, material and time-consuming investment, which only start-ups accept to make for the moment, constituting a first obstacle in the development of bacteriophage therapy (Fauconnier *et al.*, 2020).

In addition, two other problems arise with regard to GMPs. The first concerns the bacterial strains necessary for the production of bacteriophages. The qualification of bacteriophages as medicinal products imposes their production on avirulent and known bacterial strains available in collections (Pirnay *et al.*, 2015). However, the isolation and selection of the bacteriophage(s) active against an etiological agent is most often carried out using the bacteria responsible for a patient's infection. For therapeutic use, these bacteriophages must then be produced in large numbers in aqueous solutions. However, it is generally not possible to produce bacteriophages from these pathogenic strains insofar as (i) the characteristics of these strains are generally unknown, as this requires time and financial resources; (ii) when they are known, these strains sometimes harbor plasmids, resistance genes, toxins, or even prophages, and their mutation/recombination rates may also be more or less large (e.g. RecA+, MutS+). The high specificity of bacteriophages and bacteria further implies that the bacteria used in production will most often be different for each search for bacteriophage isolation.

The second problem concerns the issue of viral variation during production. Indeed, insofar as the active elements are viruses, their production inevitably suffers from genetic variations linked to mutations occurring during replication (Duffy *et al.*, 2008; Gago *et al.*, 2009). To give an (under)estimation, for a hypothetical bacteriophage of the Caudivirales type (Myo- Podo- or Siphoviridae), with 100 kb double-stranded DNA and a mutation rate of 10^{-8}/site/cycle, the production of 100mL of therapeutic solution (10^8PFU/mL) produces at least 50.10^6 mutants, i.e. a proportion of about 5 mutants per 1000 bacteriophages, without taking into account the diversity present in the stock solution. This is a far cry from the traditional standards imposed on antibiotics. In other words, the key advantage of bacteriophages which is their ability to co-evolve against evolved resistance, is a hindrance to their standardized production.

2 – Formulation

To this first series of problems, which stem from a discrepancy between regulatory expectations and the evolutionary abilities of bacteriophages, we can add a second series of problems lies in

how bacteriophage therapy has been envisioned historically. The preferred approach was initially the implementation of bacteriophage cocktails, in order to mimic the "broad spectrum" effects of antibiotics. This strategy has long been favored in former USSR countries. In Russian pharmacies, for example, one can find solutions of bacteriophages in which the leaflet indicates the targeted bacteria, generally between 5 and 10 different bacterial species. Retro-engineering studies have revealed the presence of 29 bacteriophages in the "Pyophages" cocktail and around 30 in the "intesti" cocktail (Zschach *et al.*, 2015; Villarroel *et al.*, 2017; McCallin *et al.*, 2018).

It is precisely in this regulatory context that the "Phagoburn" clinical trial (NCT02116010) was set up, the conclusions of which were particularly disappointing in view of the financial investment involved, for several reasons. (i) Given the inclusion criteria - burns caused by mono-infections by *E. coli* or *Pseudomonas aeruginosa*, when patients suffer most often from burns due to poly-microbial infections (Brogden *et al.*, 2005) - the number of inclusions was particularly low. (ii) The cocktails of bacteriophages targeting a large range of bacterial genetic diversity showed very low concentrations of active bacteriophages after a few months due to unknown inhibition of the bacteriophages while stored together. (iii) In a post-hoc study, etiologic bacteria isolated from patients showed, that they were *a priori* resistant to the bacteriophages present in the applied cocktail (Jault *et al.*, 2019). Typically, such results suggest that a cocktail of bacteriophages adapted to the etiologic bacteria would have been more appropriate. Similar conclusions could also been drawn after a clinical trial for treating urinary tract infections (NCT03140085) (Leitner *et al.*, 2021; Pirnay & Kutter, 2021).

3 – Prescription: "prêt-à-porter" or "sur-mesure"?

Such failure of bacteriophage therapy based on predetermined cocktails, initially recommended by the European Medicines Agency does not prevent this strategy from always being favored over more adapted cocktails. This further reveals the weight antibiotics carry as an infrastructure (Chandler, 2019), and more specifically as an onto-epistemological infrastructure, enforcing how phages must be conceptualized and used (as chemical molecules), and how evidence of their efficacy must be produced (Brives & Pourraz, 2020).

This infrastructure is partly, but only partly, based on the regulatory framework in which phage therapy is currently embedded, as it is also based on modes of production and consumption that developed in the second half of the 20th century (for more details, notably on the socio-anthropological aspects of phage therapy and its relationship to antibiotic therapy, see Brives & Froissart, submitted). And this regulatory framework is increasingly contested, openly or not, by the people involved in bacteriophage therapy, because of its inability to support the specificities of bacteriophages, whether in terms of production or application. The first occurrence of this type of reflection is found in an article published in 2011, in which the authors oppose two strategies: the use of predetermined cocktails or a "*prêt-à-porter*" strategy, and the use of bacteriophages which are active on the etiological bacteria only, or a "*sur-mesure*" strategy (Pirnay *et al.*, 2011). The latter strategy is based on realization of phagograms that evaluate the ability of infection of the isolated etiological bacteria by various characterized bacteriophages from a collection, in order to select the one(s) which harbor the highest efficiency of plating (EOP).

As we mentioned however, the actual framework based on the development of antibiotics prevents, or at least makes it difficult to implement and routinize the "*sur-mesure*" strategy. For this reason, in 2016 a scientific and medical team from the Queen Astrid Military Hospital (Brussel, Belgium) assisted by lawyers, proposed in 2016 an effective change of the regulatory framework in Belgium, detailed in a major article: "The Magistral Phage" (Pirnay *et al.*, 2018). Thus, in belgian regulatory policies, bacteriophages are no longer considered as 'medicinal products', but as 'active pharmaceutical ingredients' (APIs) used in magistral preparations (compounded prescription drugs in the United States). This re-qualification allows for the use of bacteriophages produced according to a fixed and public monograph, the biological quality of which must be certified by an independent laboratory. This is a first and crucial step towards developing a new model of medicine based on the evolutionary abilities of bacteriophages and bacteria to control bacterial diseases.

Conclusion

The worldwide increase in frequency of multidrug resistant bacteria is not only the result of the capacities of microorganisms, but also of changes in societal structures and infrastructures. In particular, the massification of the production and consumption of meat, fish and vegetables, as well as the increase in the frequency and speed of exchanges in a globalized world. This is why it

seems fundamental to us to consider the constraints imposed by these infrastructures on the development of this therapy, in order to avoid making the same mistakes as with antibiotics. The development of any new antibacterial therapy should indeed be designed to limit the creation and propagation of resistant genotypes.

In the previous sections, we have shown how the regulatory frameworks and the dominant drug development model are particularly unsuitable for bacteriophage therapy. The categorization of bacteriophages as 'medicinal products' implies a lengthy and costly production process (i.e. GMP-compliant production, stable product definition, time-consuming and expensive randomized controlled trials and marketing authorization). Such a categorization risks favoring the commercialization of a small number of bacteriophage solutions that will either be very expensive and therefore reserved for high-income countries (Nagel *et al.*, 2016; Fauconnier *et al.*, 2020), or will have a large spectrum of action ("*prêt-à-porter*") in order to allow economic returns on investment (Brives & Pourraz, 2020) with a non-negligible probability of the appearance of resistance. However, the few recent clinical trials using "*prêt-à-porter*" bacteriophage cocktails mostly failed to show any effect of treatment using bacteriophages (Sarker *et al.*, 2016; Jault *et al.*, 2019; Leitner *et al.*, 2021) while case reports using the "*sur-mesure*" cocktails tend to demonstrate the relevance and effectiveness of this approach (Jennes *et al.*, 2017; Schooley *et al.*, 2017).

All these data lead us to believe that a new development model is needed, which can support the evolutionary capacities of bacteriophages and bacteria. The change in regulation made in Belgium is a first step, in that it has facilitated production of a greater number of bacteriophages, which is necessary in order to take into account the high specificity of their interactions with bacteria. But the changes needed must be of greater magnitude if we are to cope with the increase in infections caused by multidrug resistant bacteria. They imply, among other things, taking into account the material infrastructures of production and distribution, encouraging dialogue between experts, mutualizing existing competences, but also supporting the training of both scientists and health professionals on innovative therapies based on the evolutionary capacities of bacteriophages and bacteria. These requirements are far more extensive than the current framework allows. For example, the ability of bacteriophages to adapt to their host target in the laboratory could be exploited to increase the effectiveness of therapy. The relatively high mutation and recombination rates of phages (Duffy *et al.*, 2008) as well as their large population size in the laboratory (cultures can easily be grown in flasks containing milliliters to liters) could allow for the rapid selection of

new variants of a bacteriophage against which the target bacterium has acquired resistance. Such bacteriophages, which bypass bacterial resistance, can indeed be produced through 'evolutionary training', performed *in vivo* (Morello *et al.*, 2011; Friman *et al.*, 2016; Burrowes *et al.*, 2019). This process, traditionally carried out in the laboratory in evolutionary experiments, involves serial passage of bacteriophages over polymorphic (susceptible and resistant) populations of target bacteria so that natural selection favors bacteriophage variants with the best reproduction rate and a wider host range. More interestingly, 'trained' bacteriophages tend to better prevent the emergence of resistant bacteria, through adaptive mutations as well as recombination (Borin *et al.*, 2021).

On the other hand, from a bacteria point of view, the focus should no longer be on eradicating etiological agents but rather on targeting virulence factors (e.g. LPS or flagellum), as has already been proposed for chemical molecules (Vale *et al.*, 2014). For example (for a recent review see Mangalea & Duerkop, 2020), the process of flagellum loss (virulence factor) has been observed in natural populations of *Vibrio cholerae* in which the frequencies of virulent genotypes decrease in the presence of bacteriophages in favor of avirulent genotypes (Seed *et al.*, 2014). Another process known as bacteriophage steering has also recently been implemented (Gurney *et al.*, 2020). For instance, transmembrane proteins of the efflux pump type allowing antibiotic resistance of *Pseudomonas aeruginosa* are targeted by a bacteriophage (Chan *et al.*, 2016, 2018). Bacteria that have mutated this gene will thus be favored, and the mutant genotypes (no longer having functional efflux pumps) will then become sensitive to antibiotics (Gurney & Brown, 2021). Such process of “bacterial resensitation” has also been observed in other bacteriophage-bacteria interactions such as *Acinetobacter baumannii* (Gordillo Altamirano *et al.*, 2021) This last strategy thus makes it possible to envisage bacteriophage therapy as a complement to or as a way to "potentiate" antibiotics (Torres-Barceló & Hochberg, 2016).

To conclude, this is in no way a question of renouncing the biological quality criteria for health products. Following the arguments presented in the present article, we believe that it is necessary to adapt the current regulations, or at least to envisage a second regulatory framework in parallel with the first. This new framework applied to bacteriophage therapy should take into account the advantages of the evolutionary nature of bacteriophages for therapeutic use to treat pathogenic bacteria, which will anyhow continue to evolve in reaction to bactericidal molecules. But we also believe that it is necessary, and even unavoidable, to rethink the existing links between applied

researches and development models, and to build real dialogues concerning the use of bacteriophages not only in human health, but also in animal health and biocontrol (for further socio-anthropological considerations, see Brives & Froissart, submitted). We can no longer deny the evolutionary abilities of microbes, and we cannot repeat the mistakes made with antibiotics. Instead, we must think about and learn from the ways in which the materialities of microbes are intertwined with those of societies.

References

Adriaenssens, E. & Brister, J.R. 2017. How to name and classify your Phage: an informal guide. *Viruses* **9**: 70.

Allen, R.C., Pfrunder-Cardozo, K.R., Meinel, D., Egli, A. & Hall, A.R. 2017. Associations among Antibiotic and Phage Resistance Phenotypes in Natural and Clinical Escherichia coli Isolates. *mBio* **8**: e01341-17.

Andersson, D.I. & Hughes, D. 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat Rev Microbiol* **8**: 260–271.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., *et al.* 2007. CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes. *Science* **315**: 1709–1712.

Barrangou, R. & Horvath, P. 2017. A decade of discovery: CRISPR functions and applications. *Nat Microbiol* **2**: 17092.

Bernheim, A. & Sorek, R. 2019. The pan-immune system of bacteria: antiviral defence as a community resource. *Nat Rev Microbiol* 1–7.

Björkman, J. & Andersson, D.I. 2000. The cost of antibiotic resistance from a bacterial perspective. *Drug Resistance Updates* **3**: 237–245.

Borin, J.M., Avrani, S., Barrick, J.E., Petrie, K.L. & Meyer, J.R. 2021. Coevolutionary phage training leads to greater bacterial suppression and delays the evolution of phage resistance. *Proc Natl Acad Sci U S A* **118**.

Boyd, E.F. 2012. Bacteriophage-encoded bacterial virulence factors and phage-pathogenicity island interactions. *Adv Virus Res* **82**: 91–118.

Brives, C. & Pourraz, J. 2020. Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures. *Palgrave Comm* **6**: 1–11.

Brogden, K.A., Guthmiller, J.M. & Taylor, C.E. 2005. Human polymicrobial infections. *The Lancet* **365**: 253–255.

Brouns, S.J.J., Jore, M.M., Lundgren, M., Westra, E.R., Slijkhuis, R.J.H., Snijders, A.P.L., *et al.* 2008. Small CRISPR RNAs Guide Antiviral Defense in Prokaryotes. *Science* **321**: 960–964.

Bud, R. 2005. Antibiotics, big business, and consumers. The context of government investigations into the postwar American drug industry. *Technol Cult* **46**: 329–349.

Burrowes, B.H., Molineux, I.J. & Fralick, J.A. 2019. Directed in Vitro Evolution of Therapeutic Bacteriophages: The Appelmans Protocol. *Viruses* **11**: 241.

Calero-Cáceres, W., Ye, M. & Balcázar, J.L. 2019. Bacteriophages as environmental reservoirs of antibiotic resistance. *Trends Microbiol* **27**: 570–577.

Cazares, D., Cazares, A., Figueroa, W., Guarneros, G., Edwards, R.A. & Vinuesa, P. 2021. A novel group of promiscuous Podophages infecting diverse Gammaproteobacteria from river communities exhibits dynamic intergenus host adaptation. *mSystems* **6**.

Chan, B.K., Sistrom, M., Wertz, J.E., Kortright, K.E., Narayan, D. & Turner, P.E. 2016. Phage selection restores antibiotic sensitivity in MDR Pseudomonas aeruginosa. *Scientific Reports* **6**: 26717.

Chan, B.K., Turner, P.E., Kim, S., Mojibian, H.R., Elefteriades, J.A. & Narayan, D. 2018. Phage treatment of an aortic graft infected with Pseudomonas aeruginosa. *Evol Med Public Health* **2018**: 60–66.

Chaturongakul, S. & Ounjai, P. 2014. Phage–host interplay: examples from tailed phages and Gram-negative bacterial pathogens. *Front Microbiol* **5**.

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., *et al.* 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Front Microbiol* **5**.

Chevallereau, A., Blasdel, B.G., Smet, J.D., Monot, M., Zimmermann, M., Kogadeeva, M., *et al.* 2016. Next-generation "-omics" approaches reveal a massive alteration of host RNA

metabolism during bacteriophage infection of Pseudomonas aeruginosa. *PLoS Genet* **12**: e1006134.

Corbellini, G. 2008. Evolution Based Medicine (EBM) an epistemological framework for thinking of, and dealing with the so called "crisis of medicine." *Medicina nei Secoli* **20**: 115–139.

Cotter, P.D., Ross, R.P. & Hill, C. 2013. Bacteriocins — a viable alternative to antibiotics? *Nat Rev Microbiol* **11**: 95–105.

Daglia, M. 2012. Polyphenols as antimicrobial agents. *Curr Op Biotechnol* **23**: 174–181.

Davies, J. & Davies, D. 2010. Origins and Evolution of Antibiotic Resistance. *Microbiol Mol Biol Rev* **74**: 417–433.

Davison, H.C., Woolhouse, M.E.J. & Low, J.C. 2000. What is antibiotic resistance and how can we measure it? *Trends Microbiol* **8**: 554–559.

Denyer Willis, L. & Chandler, C. 2019. Quick fix for care, productivity, hygiene and inequality: reframing the entrenched problem of antibiotic overuse. *BMJ Global Health* **4**: e001590.

D'Hérelle, M.F. 1917. Sur un microbe invisible antagoniste des bacilles dysenteriques. *C. R. Acad. Sc.* 373–375.

Diaz Högberg, L.D., Heddini, A. & Cars, O. 2010. The global need for effective antibiotics: challenges and recent advances. *Trends Pharmacol Sci* **31**: 509–515.

DiMasi, J.A., Grabowski, H.G. & Hansen, R.W. 2016. Innovation in the pharmaceutical industry: New estimates of R&D costs. *J Health Econ* **47**: 20–33.

Dion, M.B., Oechslin, F. & Moineau, S. 2020. Phage diversity, genomics and phylogeny. *Nat Rev Microbiol* 1–14.

Duffy, S., Shackelton, L.A. & Holmes, E.C. 2008. Rates of evolutionary change in viruses: patterns and determinants. *Nat Rev Genet* **9**: 267–276.

Fauconnier, A., Nagel, T.E., Fauconnier, C., Verbeken, G., De Vos, D., Merabishvili, M., *et al.* 2020. The unique role that WHO could play in implementing phage therapy to combat the global antibiotic resistance crisis. *Front Microbiol* **11**.

Fernández, L. & Hancock, R.E.W. 2012. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin Microbiol Rev* **25**: 661–681.

Friman, V.-P., Soanes-Brown, D., Sierocinski, P., Molin, S., Johansen, H.K., Merabishvili, M., *et al.* 2016. Pre-adapting parasitic phages to a pathogen leads to increased pathogen clearance and lowered resistance evolution with Pseudomonas aeruginosa cystic fibrosis bacterial isolates. *J Evol Biol* **29**: 188–198.

Furfaro, L.L., Payne, M.S. & Chang, B.J. 2018. Bacteriophage therapy: clinical trials and regulatory hurdles. *Front Cell Infect Microbiol* **8**.

Gago, S., Elena, S.F., Flores, R. & Sanjuan, R. 2009. Extremely high mutation rate of a hammerhead viroid. *Science* **323**: 1308.

Gandon, S. 2016. Why be temperate: lessons from bacteriophage λ. *Trends Microbiol* **24**: 356–365.

Gillings, M. & Paulsen, I.T. 2014. Microbiology of the Anthropocene. *Anthropocene* **5**: 1–8.

Gillings, M., Westoby, M. & Ghaly, T.M. 2018. Pollutants That Replicate: Xenogenetic DNAs. *Trends Microbiol* **26**: 975–977.

Gillings, M.R. 2013. Evolutionary consequences of antibiotic use for the resistome, mobilome and microbial pangenome. *Front. Microbiol.* **4**.

Gordillo Altamirano, F.L. & Barr, J.J. 2019. Phage therapy in the postantibiotic era. *Clin Microbiol Rev* **32**: e00066-18.

Gordillo Altamirano, F.L., Forsyth, J.H., Patwa, R., Kostoulias, X., Trim, M., Subedi, D., *et al.* 2021. Bacteriophage-resistant Acinetobacter baumannii are resensitized to antimicrobials. *Nat Microbiol* **6**: 157–161.

Gurney, J. & Brown, S.P. 2021. Building reusable phage and antibiotic treatments via exploitation of bacteria-phage coevolutionary dynamics. *bioRxiv* 2021.03.31.437900.

Gurney, J., Brown, S.P., Kaltz, O. & Hochberg, M.E. 2020. Steering Phages to Combat Bacterial Pathogens. *Trends Microbiol* **28**: 85–94.

Hall, A.R., Angst, D.C., Schiessl, K.T. & Ackermann, M. 2015. Costs of antibiotic resistance – separating trait effects and selective effects. *Evol Appl* **8**: 261–272.

Harmand, N., Gallet, R., Jabbour-Zahab, R., Martin, G. & Lenormand, T. 2017. Fisher's geometrical model and the mutational patterns of antibiotic resistance across dose gradients. *Evolution* **71**: 23–37.

Harmand, N., Gallet, R., Martin, G. & Lenormand, T. 2018. Evolution of bacteria specialization along an antibiotic dose gradient. *Evol Lett* **2**: 221–232.

Hinchliffe, S. 2015. More than one world, more than one health: Re-configuring interspecies health. *Social Sci & Med* **129**: 28–35.

Holmes, A.H., Moore, L.S.P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., *et al.* 2016. Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet* **387**: 176–187.

Hyman, P. & Abedon, S.T. 2010. Chapter 7 - Bacteriophage host range and bacterial resistance. *Adv App Microbiol* **70**: 217–248.

Jacob, F. & Monod, J. 1961. Genetic regulatory mechanisms in the synthesis of proteins. *J Mol Biol* **3**: 318–356.

Jansen, K.U., Knirsch, C. & Anderson, A.S. 2018. The role of vaccines in preventing bacterial antimicrobial resistance. *Nature Medicine* **24**: 10–19.

Jasanoff, S., Markle, G.E., Peterson, J.C. & Pinch, T. 1995. *Handbook of Science and Technology Studies*. SAGE Publications.

Jault, P., Leclerc, T., Jennes, S., Pirnay, J.P., Que, Y.-A., Resch, G., *et al.* 2019. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *The Lancet Inf Dis* **19**: 35–45.

Jee, Y., Carlson, J., Rafai, E., Musonda, K., Huong, T.T.G., Daza, P., *et al.* 2018. Antimicrobial resistance: a threat to global health. *The Lancet Inf Dis* **18**: 939–940.

Jennes, S., Merabishvili, M., Soentjens, P., Pang, K.W., Rose, T., Keersebilck, E., *et al.* 2017. Use of bacteriophages in the treatment of colistin-only-sensitive Pseudomonas aeruginosa septicaemia in a patient with acute kidney injury—a case report. *Critical Care* **21**: 129.

Jernberg, C., Löfmark, S., Edlund, C. & Jansson, J.K.Y. 2010. 2010. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* **156**: 3216–3223.

Johnson, A.P. & Woodford, N. 2013. Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. J Med Microbiol **62**: 499–513. Microbiology Society,.

Keller, E.F. 1983. *A feeling for the organism : the life and work of Barbara McClintock*, New York : W.H. Freeman&Co Ltd.

Keller, E.F. 1995. *Refiguring Life: Metaphors of Twentieth-century Biology*. Columbia University Press.

Kim, B., Kim, E.S., Yoo, Y.-J., Bae, H.-W., Chung, I.-Y. & Cho, Y.-H. 2019. Phage-Derived Antibacterials: Harnessing the Simplicity, Plasticity, and Diversity of Phages. *Viruses* **11**: 268.

Kortright, K.E., Chan, B.K., Koff, J.L. & Turner, P.E. 2019. Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria. *Cell Host & Microbe* **25**: 219–232.

Koskella, B. & Meaden, S. 2013. Understanding Bacteriophage Specificity in Natural Microbial Communities. *Viruses* **5**: 806–823.

Landecker, H. 2015. Antibiotic resistance and the biology of history. *Body & Society* **22**: 19–52.

Latour, B. 1988. *Science in Action*, Harvard University Press.

Latour, B. 1993. *The Pasteurization of France*, Harvard University Press.

Le, S., Yao, X., Lu, S., Tan, Y., Rao, X., Li, M., *et al.* 2014. Chromosomal DNA deletion confers phage resistance to Pseudomonas aeruginosa. *Sci Rep* **4**: 1–8.

Leitner, L., Ujmajuridze, A., Chanishvili, N., Goderdzishvili, M., Chkonia, I., Rigvava, S., *et al.* 2021. Intravesical bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomised, placebo-controlled, double-blind clinical trial. *Lancet Infect Dis* **21**: 427–436.

León, M. & Bastías, R. 2015. Virulence reduction in bacteriophage resistant bacteria. *Front Microbiol* **6**.

Li, X., Zhang, Y., Zhou, X., Hu, X., Zhou, Y., Liu, D., *et al.* 2019. The plasmid-borne quinolone resistance protein QnrB, a novel DnaA-binding protein, increases the bacterial mutation rate by triggering DNA replication stress. *Mol Microbiol* **111**: 1529–1543.

Lin, J., Zhou, D., Steitz, T.A., Polikanov, Y.S. & Gagnon, M.G. 2018. Ribosome-targeting antibiotics: modes of action, mechanisms of resistance, and implications for drug design. *Annu Rev Biochem* **87**: 451–478.

Lipsitch, M. & Siber, G.R. 2016. How Can Vaccines Contribute to Solving the Antimicrobial Resistance Problem? *mBio* **7**: e00428-16.

Luria, S.E. & Delbrück, M. 1943. Mutations of Bacteria from Virus Sensitivity to Virus Resistance. *Genetics* **28**: 491–511.

Lwoff, A. 1953. Lysogeny. *Microb Mol Biol Rev* **17**: 269–337.

Mangalea, M.R. & Duerkop, B.A. 2020. Fitness Trade-Offs Resulting from Bacteriophage Resistance Potentiate Synergistic Antibacterial Strategies. *Infect Immun* **88**.

McCallin, S., Sarker, S.A., Sultana, S., Oechslin, F. & Brüssow, H. 2018. Metagenome analysis of Russian and Georgian Pyophage cocktails and a placebo-controlled safety trial of single

phage versus phage cocktail in healthy Staphylococcus aureus carriers. *Environmental Microbiology* **20**: 3278–3293.

Meaden, S. & Koskella, B. 2013. Exploring the risks of phage application in the environment. *Front Microbiol* **4**.

Morello, E., Saussereau, E., Maura, D., Huerre, M., Touqui, L. & Debarbieux, L. 2011. Pulmonary Bacteriophage Therapy on Pseudomonas aeruginosa Cystic Fibrosis Strains: First Steps Towards Treatment and Prevention. *PLoS ONE* **6**: e16963.

Moulin, G., Cavalié, P., Pellanne, I., Chevance, A., Laval, A., Millemann, Y., *et al.* 2008. A comparison of antimicrobial usage in human and veterinary medicine in France from 1999 to 2005. *J Antimicrob Chemother* **62**: 617–625.

Nagel, T.E., Chan, B.K., De Vos, D., El-Shibiny, A., Kang'ethe, E.K., Makumi, A., *et al.* 2016. The developing world urgently needs phages to combat pathogenic bacteria. *Front Microbiol* **7**: 882.

Nesse, R.M. & Stearns, S.C. 2008. The great opportunity: Evolutionary applications to medicine and public health. *Evol Appl* **1**: 28–48.

Nolivos, S., Cayron, J., Dedieu, A., Page, A., Delolme, F. & Lesterlin, C. 2019. Role of AcrAB-TolC multidrug efflux pump in drug-resistance acquisition by plasmid transfer. *Science* **364**: 778–782.

Oechslin, F. 2018. Resistance development to bacteriophages occurring during bacteriophage therapy. *Viruses* **10**: 351.

Oechslin, F., Piccardi, P., Mancini, S., Gabard, J., Moreillon, P., Entenza, J.M., *et al.* 2017. Synergistic interaction between phage therapy and antibiotics clears Pseudomonas aeruginosa infection in endocarditis and reduces virulence. *J Infect Dis* **215**: 703–712.

O'Neill, J. 2014. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. *Review on antimicrobial resistance*.

Peterson, E. & Kaur, P. 2018. Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. *Front Microbiol* **9**: 2928.

Pirnay, J.-P. 2020. Phage Therapy in the Year 2035. *Front Microbiol* **11**: 1171.

Pirnay, J.-P., Blasdel, B.G., Bretaudeau, L., Buckling, A., Chanishvili, N., Clark, J.R., *et al.* 2015. Quality and Safety Requirements for Sustainable Phage Therapy Products. *Pharm Res* **32**: 2173–2179.

Pirnay, J.-P., De Vos, D., Verbeken, G., Merabishvili, M., Chanishvili, N., Vaneechoutte, M., *et al.* 2011. The Phage Therapy Paradigm: Prêt-à-Porter or Sur-mesure? *Pharm Res* **28**: 934–937.

Pirnay, J.-P. & Kutter, E. 2021. Bacteriophages: it's a medicine, Jim, but not as we know it. *Lancet Infect Dis* **21**: 309–311.

Pirnay, J.-P., Verbeken, G., Ceyssens, P.-J., Huys, I., De Vos, D., Ameloot, C., *et al.* 2018. The magistral phage. *Viruses* **10**: 64.

Roach, D.R., Leung, C.Y., Henry, M., Morello, E., Singh, D., Santo, J.P.D., *et al.* 2017. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host & Microbe* **22**: 38–47.

Sarker, S.A., Sultana, S., Reuteler, G., Moine, D., Descombes, P., Charton, F., *et al.* 2016. Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children from Bangladesh. *EBioMedicine* **4**: 124–137.

Schooley, R.T., Biswas, B., Gill, J.J., Hernandez-Morales, A., Lancaster, J., Lessor, L., *et al.* 2017. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. *Antimicrob Agents Chemother* **61**: e00954-17.

Schuch, R. & Fischetti, V.A. 2006. Detailed Genomic Analysis of the Wβ and γ Phages Infecting Bacillus anthracis: Implications for Evolution of Environmental Fitness and Antibiotic Resistance. *J Bacteriol* **188**: 3037–3051.

Seed, K.D., Yen, M., Shapiro, B.J., Hilaire, I.J., Charles, R.C., Teng, J.E., *et al.* 2014. Evolutionary consequences of intra-patient phage predation on microbial populations. *eLife* **3**: e03497.

Shanks, N. & Pyles, R.A. 2007. Evolution and medicine: the long reach of "Dr. Darwin." *Philosophy, Ethics, and Humanities in Medicine* **2**: 4.

Shousha, A., Awaiwanont, N., Sofka, D., Smulders, F.J.M., Paulsen, P., Szostak, M.P., *et al.* 2015. Bacteriophages isolated from chicken meat and the horizontal transfer of antimicrobial resistance genes. *Appl Environ Microbiol* **81**: 4600–4606.

Tagliaferri, T.L., Jansen, M. & Horz, H.-P. 2019. Fighting pathogenic bacteria on two fronts: Phages and antibiotics as combined strategy. *Front Cell Infect Microbiol* **9**: 22.

Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., *et al.* 2017. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *Lancet Planetary Health* **1**: e316–e327. Elsevier.

Torres-Barceló, C. 2018. Phage therapy faces evolutionary challenges. *Viruses* **10**: 323.

Torres-Barceló, C. & Hochberg, M.E. 2016. Evolutionary Rationale for Phages as Complements of Antibiotics. *Trends Microbiol* **24**: 249–256.

Vale, P.F., Fenton, A. & Brown, S.P. 2014. Limiting Damage during Infection: Lessons from Infection Tolerance for Novel Therapeutics. *PLOS Biology* **12**: e1001769. Public Library of Science.

van Houte, S. van, Buckling, A. & Westra, E.R. 2016. Evolutionary ecology of prokaryotic immune mechanisms. *Microbiol Mol Biol Rev* **80**: 745–763.

Ventola, C.L. 2015. The antibiotic resistance crisis. *Pharm Therapeutics* **40**: 277–283.

Villarroel, J., Larsen, M.V., Kilstrup, M. & Nielsen, M. 2017. Metagenomic Analysis of Therapeutic PYO Phage Cocktails from 1997 to 2014. *Viruses* **9**: 328.

Wang, X., Wei, Z., Yang, K., Wang, J., Jousset, A., Xu, Y., *et al.* 2019. Phage combination therapies for bacterial wilt disease in tomato. *Nat Biotechnol* **37**: 1513–1520.

Weber-Dąbrowska, B., Jończyk-Matysiak, E., Żaczek, M., Łobocka, M., Łusiak-Szelachowska, M. & Górski, A. 2016. Bacteriophage procurement for therapeutic purposes. *Front Microbiol* **7**.

Wright, G.D. & Sutherland, A.D. 2007. New strategies for combating multidrug-resistant bacteria. *Trends Mol Med* **13**: 260–267.

Zschach, H., Joensen, K.G., Lindhard, B., Lund, O., Goderdzishvili, M., Chkonia, I., *et al.* 2015. What Can We Learn from a Metagenomic Analysis of a Georgian Bacteriophage Cocktail? *Viruses* **7**: 6570–6589.

List of tables

Table 1. Comparisons of evolutionary potentials of two anti-bacterial agents

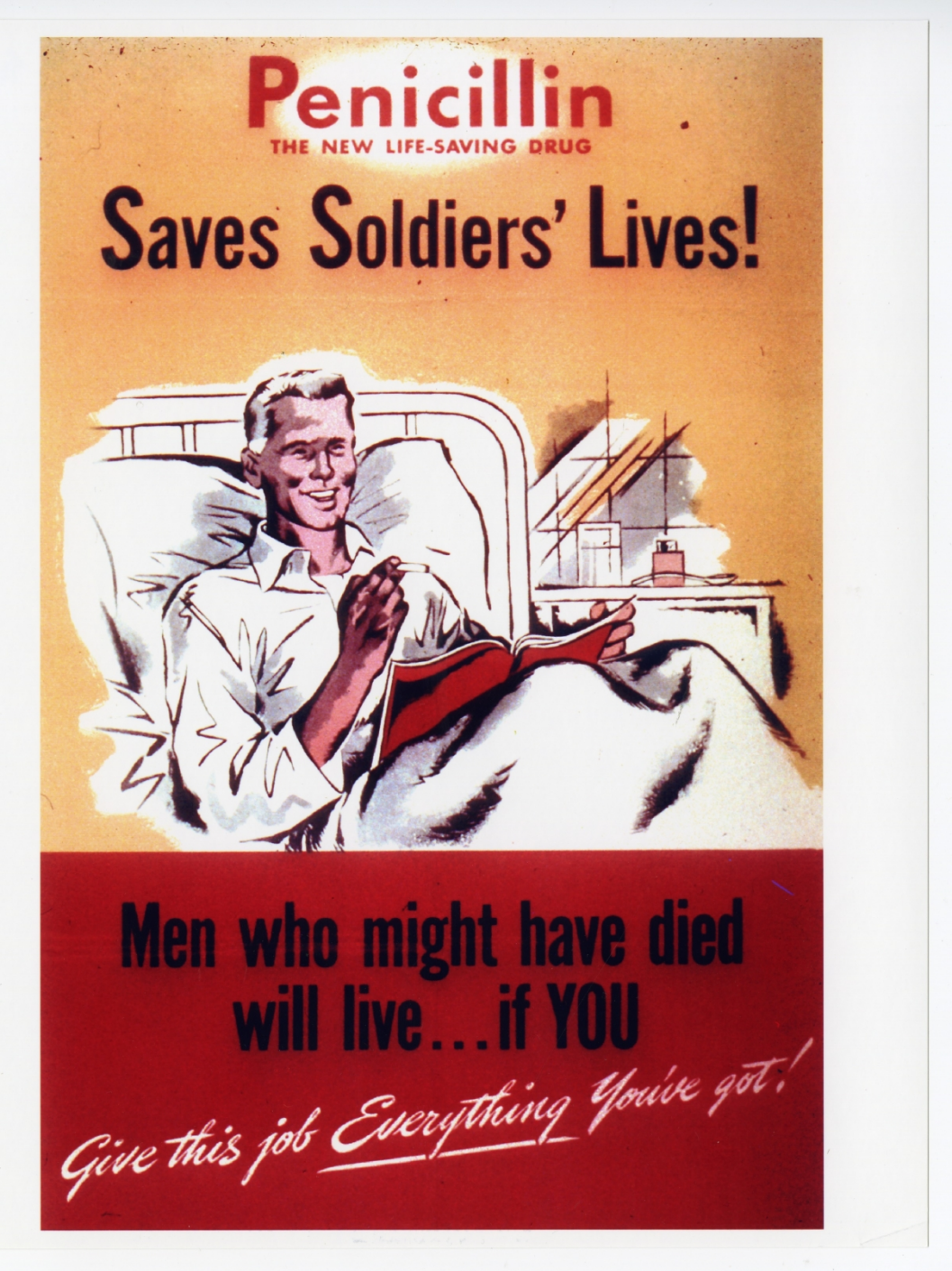
	Antibiotics	**Bacteriophages**
Diversity of types available	Limited / dereasing	Large
Host Range	Large	Variable, can be specific to one bacterial genotype
Bacterial resistance evolution	yes	yes
Evolution / resistance-bypassing	no	yes (*ex vivo* & *in situ*)
***In situ* auto-amplification**	no	yes
Toxicity	yes	no

List of figures

Figure 1. United States Federal Government World War II poster, for Penicillin. Credit: Science History Institute. (https://commons.wikimedia.org/wiki/File:Penicillin_poster_5.40.tif)

Graphical abstracts

Current regulation framework and development models consider therapeutic bacteriophages as "medical products" similar to chemical antibacterial molecules. Such classification impede bacteriophage therapy from taking advantage of the evolutionary capacities of both bacteriophages (high diversity, fast *in vitro* adaptation and increase in efficiency against the etiologic bacteria) and bacteria (mutations towards bacteriophage resistance render bacteria avirulent or antibiotic sensitive). Pictograms from the Noun Project (https://thenounproject.com)



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