Universidade de Lisboa

Faculdade de Farmácia

Opportunities and Regulatory Challenges of Phage Therapy

Joana Rodrigues Ribeiro

Dissertation supervised by Professora Madalena Maria Vilela Pimentel and
co-supervised by Professor Bruno Miguel Nogueira Sepodes

Master in Regulation and Evaluation of Medicines and Health Products

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Abstract

In recent years, a global health crisis has emerged, mainly driven by the ineffectiveness in fighting pathogenic bacteria causing deathly infections. Bacteria have evolved to become resistant to antibiotics at an exceedingly fast rate which resulted in multi-drug resistant bacteria for some of which no available antibiotic is effective. The urgency in finding a new therapy against pathogenic bacteria combined with the possibly-beneficial antibacterial features of bacteriophages resulted in the re-emergence of phage therapy.

Phage therapy is the study and application of bacteriophages to be therapeutically used against bacteria. Even though this therapy was developed many years ago, it was forgotten following the introduction of antibiotics, in the Western world. In the East, phage therapy was kept going and introduced in clinical practice as a common medicine. The re-emergence of phage therapy in the West has been taking a slow pace since not enough non-clinical and clinical data has been gathered to ensure safety and efficacy of phage products. However, the main obstacle for phage therapy implementation is the current pharmaceutical legislation and its inflexibility regarding personalized medicines.

Europe and USA frameworks are designed for industrially-made pharmaceuticals to be largely distributed. Even though phage therapy can be developed following this path, a more sustainable option following a custom-made preparation for a specific patient that could better explore the unique characteristics of bacteriophages is not compatible with the current regulatory framework of medicinal products. Each national authority has adapted this new concept to its own regulation to nationally implement phage therapy. International organizations like EMA and FDA were pushed to intervene and are following the initiatives launched at national level to try and implement a phage therapy-inclusive regulatory framework.

Keywords: phage therapy, antimicrobial resistance, regulatory framework, personalized medicine, EMA

Resumo

O aparecimento da resistência aos antibióticos por parte das bactérias, apesar de incorretamente considerado um novo problema devido ao alarmismo criado pelos mídia, esteve sempre presente, mesmo antes da introdução da terapêutica antibiótica e é hoje abordada como uma crise global. Esta crise hoje vivida é assim considerada devido à ineficácia no combate às infeções bacterianas causadas por bactérias multirresistentes.

Através da expressão de genes de resistência, as bactérias tomam partido de mecanismos de resistência e alterações bioquímicas e estruturais, neutralizando a eficácia dos antibióticos, e promovendo a própria infecciosidade e sobrevivência bacteriana. A resistência aos antibióticos pode ser intrínseca e está naturalmente presente no organismo, relacionada com a fisiologia da bactéria, ou pode ser extrínseca sendo adquirida ou adaptada. A acumulação da resistência antimicrobiana acontece principalmente devido à aquisição de genes de resistência, através da transferência horizontal de genes, mas pode também acontecer através de mutações nas bactérias. Quando expostas a uma pressão externa, como a exposição aos antibióticos, as bactérias que adquiriram mecanismos de resistência terão vantagem em relação às outras bactérias suscetíveis, sobrevivendo e replicando-se.

O uso incorreto e abusivo dos antibióticos na agricultura, produção animal e medicina levou a uma das grandes emergências de saúde pública global. De facto, foi estimado que, anualmente, as infeções causadas por bactérias resistentes aos antibióticos causaram cerca de 700,000 mortes globais e que, se a situação não for controlada, pode chegar a 10 milhões de mortes globais, em 2050. É necessária uma reforma no que diz respeito à disponibilização e utilização dos antibióticos por parte do ser humano de forma a controlar o uso despropositado e desmedido destes produtos antimicrobianos que deveriam apenas ser usados para tratar doenças causadas por bactérias patogénicas. Por exemplo, nos Estados Unidos, 80% dos antibióticos disponibilizados no mercado são utilizados para a produção alimentar. Este tipo de comportamentos precisa de ser alterado começando pela disponibilização desmedida dos antibióticos, à desinformação relativamente ao assunto na população geral, mas principalmente nos profissionais de saúde, ao diagnóstico empírico e incorretas prescrições, à incorreta dosagem ou incumprimento do regime terapêutico por parte dos pacientes, à falta de controlo sanitário para a correta eliminação de resíduos antibióticos, etc. No entanto, mesmo que estes comportamentos fossem agora alterados, as bactérias já apresentam mecanismos de multirresistência aos antibióticos existentes, o desenvolvimento de novos antibióticos é cada vez mais lento e desinteressante para as indústrias farmacêuticas e são escassas as alternativas antimicrobianas.

Os bacteriófagos ou fagos, são vírus que infetam, exclusivamente, bactérias com a possível finalidade de as eliminar por lise celular. Esta alta especificidade bacteriana dos fagos sugeriu a inúmeros cientistas a possibilidade de criar uma terapia antimicrobiana baseada nos bacteriófagos: a terapia fágica. Felix d'Herelle foi o responsável pela descoberta oficial dos fagos mas foi também o impulsionador da aplicação terapêutica destes vírus para combater infeções bacterianas, desenvolvendo vários estudos clínicos. Em parceria com o bacteriologista George Eliava, d'Herelle possibilitou a produção de preparações de fagos que foi replicada por todo o mundo a nível comercial. Diversas implicações históricas e políticas atrasaram o desenvolvimento da terapia fágica, mas o maior inimigo à evolução desta terapia inovadora foi a introdução dos antibióticos na prática clínica no início dos anos 40. A terapia fágica continuou a ser desenvolvida em alguns países de leste, pertencentes à ex-União Soviética, mas a Europa ocidental rejeitou este novo conceito, apoiando-se nos antibióticos. No entanto, dada a crescente crise global, dos últimos anos, relativa à multirresistência das bactérias aos antibióticos, à ineficácia dos antibióticos existentes e à escassez de alternativas, a terapia fágica (re)surge como um excelente candidato.

Comparada com os antibióticos a terapia fágica apresenta diversas vantagens como a alta especificidade para um determinado hospedeiro bacteriano, evitando o contacto com bactérias comensais benéficas para o organismo, e por consequente, apresenta menos efeitos secundários quando administrado; por ser o organismo mais diverso do mundo o seu isolamento seria mais fácil do que a dependência dos antibióticos em processos laboratoriais para a sua disponibilização; os fagos têm a capacidade de auto-replicação e como tal as dosagens e regimes terapêuticos seriam mais curtos do que os antibióticos, etc. Algumas desvantagens da terapia fágica como o espetro reduzido, a falta de eficácia, a possibilidade da emergência de resistência bacteriana aos fagos e de transdução de genes de resistência antibiótica podem ser ultrapassadas através da combinação de diferentes fagos que infetam a mesma bactéria, ou de diferentes fagos que infetam diferentes bactérias, numa mistura denominada cocktail. No entanto, comparada com os antibióticos que representam uma terapêutica bem estabelecida com décadas de experiência clínica e com métodos de produção industrial padronizados, a terapia fágica carece de evidências clínicas e pré-clínicas assim como de métodos de produção viáveis e sustentáveis, e que, por necessitar de uma adaptação geral da medicina moderna e por mostrar incompatibilidade com os diferentes esquemas regulamentares globais, tem visto a sua introdução no mercado farmacêutico algo dificultada.

A terapia fágica pode ser desenvolvida seguindo duas abordagens diferentes: como uma preparação de composição fixa, preparada industrialmente para ser amplamente distribuída pelo mercado farmacêutico (*prêt-à-porter*) ou pela preparação de uma mistura de fagos previamente isolados e guardados em bancos específicos, e posteriormente selecionados seguindo uma prescrição adaptada ao paciente, o que resulta num produto final de composição variada (*sur mesure*). Esta última abordagem foi desenvolvida para que as características únicas dos bacteriófagos possam ser exploradas ao nível terapêutico desenvolvendo uma abordagem mais sustentável e viável.

Tanto na Europa como nos Estados Unidos, a terapia fágica foi considerada um medicamento biológico, apesar de as jurisdições pertencerem a oficinas diferentes, na Europa pertencente aos medicamentos biológicos nos Estados Unidos o *Office of Vaccines Research and Review* (OVRR) pertencente à oficina dos biológicos. Devido à sua natureza biológica e ao facto de ser considerado um medicamento anti-infeccioso, os produtos à base de fagos requerem a atribuição de uma autorização de introdução no mercado através do processo centralizado. O processo centralizado obriga a seguir padrões clínicos, de produção e de distribuição incompatíveis com o desenvolvimento de preparações de fagos personalizadas e as organizações e empresas interessadas em desenvolver estes produtos ainda não conseguiram introduzir estes produtos no mercado. De forma a contornar este problema, diferentes países europeus e os Estados Unidos têm vindo a desenvolver a terapia fágica seguindo o uso compassivo de fagos para pacientes com infeções bacterianas resistentes a necessitar de alternativas terapêuticas. No entanto, esta abordagem apenas permite o uso esporádico e urgente de fagos terapêuticos, sem conseguir explorar o verdadeiro potencial da terapia fágica.

A implementação desta terapia dentro dos esquemas regulamentares existentes só é possível se estes forem alterados, adicionando novas categorias e/ou modificando as definições existentes. No entanto, a terapia fágica pode servir-se da experiência de outros medicamentos que, tal como esta terapia, se apresentam complexos ou particulares e que, também estes, viram a sua introdução regulamentar dificultada. De forma a contornar essas dificuldades, diversas exceções e novos conceitos foram introduzidos e estão hoje implementados. Com base na experiência destes medicamentos e seguindo a abordagem implementada na Bélgica: a fórmula magistral de fagos, a terapia fágica poderia ser implementada e introduzida nos esquemas regulamentares seguindo um processo centralizado. Desta forma seria possível implementar a terapia fágica ao nível do mercado farmacêutico europeu, americano e internacional e possivelmente ver resolvida a crise de saúde pública global corrente.

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Acronyms and Abbreviations

AMR	Antimicrobial Resistance
ANSM	Agence Nationale de Sécurité du Medicament et les Produits de Santé as National Agency for Medicines and Health Products Safety
API	Active Pharmaceutical Ingredient
ARGs	Antimicrobial Resistance Genes
ASMF	Active substance master file
ASP	Antimicrobial Stewardship Program
BMP	Biological Medicinal Product
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CF	Cystic fibrosis
CFR	Code of Federal Regulations
CTD	Common Technical Document
CORDIS	Community Research and Development Information Service
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded deoxyribonucleic acid
dsRNA	Double-stranded ribonucleic acid
E. coli	Escherichia coli
EMA	European Medicines Agency
FAMHP	Federal Agency for Medicines and Health Products
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HGT	Horizontal Gene Transfer
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IND	Investigational New Drug Application
IPATH	Innovative Phage Applications and Therapeutics
MA	Marketing Authorization

MAA	Marketing Authorization Application
MDR	Multidrug Resistance
OVRR	Office of Vaccines Research and Review
P. aeruginosa	Pseudomonas aeruginosa
PTMP	Phage therapy medicinal product
QA	Quality Assurance
QAMH	Queen Astrid military hospital
QC	Quality Control
QP	Qualified Person
RNA	Ribonucleic acid
RES	Reticuloendothelial system
SmPC	Summary of Product Characteristics
ssDNA	Single-stranded deoxyribonucleic acid
ssRNA	Single-stranded ribonucleic acid
USA/US	United States of America
UV	Ultraviolet
VAMF	Vaccine Antigen Master File
WHO	World Health Organization

1. Introduction

Modern medicine as we know and benefit from in our current days is only possible after it suffered a great revolution with the introduction of antibiotics in clinical practice (1). For many decades, antibiotics have saved millions of lives while being used in the treatment of severe infectious diseases, possibly preventing a broad spread of a pathogenic organism that could be the cause of a global epidemic (2). In our current days, antibiotics are essential in different fields of clinical practice including surgical interventions, transplants, prophylactically, and severe and chronical infections (3).

However, as it was expected, the overuse and misuse of antibiotics by humans combined with a natural predisposition of bacteria to develop evolutionary changes against a threat, allowed bacteria to develop multiple mechanisms of antibiotic resistance (2). Antimicrobial resistance (AMR) is a global health threat based on the capacity of a pathogenic organism to adapt over time in such a way that the corresponding medicines can no longer trigger a therapeutic effect. The fast spread of multi-drug resistant bacteria around the world is specially alarming since the current antibiotics are not efficiently eliminating these "super bugs" (4). We are entering a post-antibiotic era where minor common infections can be deadly since the available antibiotics can no longer fight the pathogenic bacteria and the pharmaceutical industries show no interest in developing new ones that will rapidly loose its efficiency (5). It is urgently necessary to develop effective antibacterial products to stop this global crisis (2). But, instead of trying to replace ineffective antibiotics with new ones, perhaps we should try to explore a different strategy easily adapted to the natural behaviour of bacteria.

One of the most promising candidates as an antibacterial compound are the once forgotten bacteriophages. As the name suggest, these are viruses that infect and kill bacteria following a natural high specificity at the strain level without being able to infect mammalian cells (6). Compared with antibiotics, bacteriophages show many differences regarding mode of action, pharmacokinetics and pharmacodynamics properties which could be advantageous or limitative (1). Since its discovery, the therapeutic use of phages, called phage therapy, has been successfully used in the Eastern Europe. Only recently the Western Countries started to acknowledge bacteriophages as a solution for the antibiotic global crisis (6).

Even though the introduction of bacteriophages in clinical practice may bring a new set of opportunities for modern medicine, it also brought many worries, especially regarding regulatory concerns (2). Classifying phage therapy has resulted in controversial discussions since phage therapy does not seem to clearly fit in any

medicinal product category (7). Then, phage therapy has been developed as an over-the-counter product, available in countries like Russia and Georgia. However, the unique characteristics of phages like its narrow therapeutic spectrum can be more suitably and profitably applied to a personalized therapy (8). From this, phage therapy can be developed as a formulation of fixed composition based on a *prêt-à-porter* approach or with a tailored composition specifically adapted for a certain patient on a *sur-mesure* approach. Even though the latter approach is presented as a more advantageous option, it is not compatible with the current licensing processes (9). Phage therapy production shows a set of challenges regarding manufacturing and formulation which will reflect in the safety, quality and efficacy of the finished product (10).

Regulatory agencies of both Europe and US (and around the globe) are already discussing on how to address the licensing and market introduction of phage products: either by adapting phages to other medicinal products frameworks or by trying to create a new framework based on the experience of similar medicinal products (11). The process of phage therapy introduction in our current framework still needs a lot of work and assessment, but in the long term, a promising future may be expectable for bacteriophages in modern medicine.

2. Bacteriophages

Bacteriophages or phages, as more commonly named, are viruses that infect bacteria and replicate within it, by a host depending access (12,13). Among the most abundant and widely distributed organisms on earth, phages can kill a specific bacterial cell while they are unable to infect mammalian cells. This targeted infection allowed the possibility of a therapy phage based, especially as an antimicrobial (1,13).

2.1 History of Bacteriophages and Phage Therapy

To understand the discovery and evolution of phages at the hands of humans, we need to go back over a century, when the occurrence of bacterial parasites in the environment were first reported, in 1896, by an English bacteriologist, Ernest Hanbury Hankin (14,15). This took place in India, in the Ganges and Jamuna rivers, where the bacteriologist noticed some kind of self-purification, despite the unsanitary conditions of the waters with which the inhabitants had to live (15,16). He performed a study of these waters, microscopic based, compared to the waters of European rivers resulting on the identification of a "biologic organism" that was able to cross a filter membrane with milipores and provoke an antibacterial activity (1,15). Another bacteriologist, Nikolay Gamaleya, observed a similar event, two years later, but instead of *Vibrio cholerae*, he worked with *Bacillus subtilis* (17).

Despite these previous findings, it was only in 1915 that Frederick Twort proposed the idea of an ultramicroscopic and "transparent" virus capable of trapping and transform bacteria, producing an antimicrobial effect (18,19). He was able to filter cultures of *Staphylococcus* and identify zones of transmissible lysis, however, he wasn't able to explain it nor present definite conclusions, and due to financial difficulties, he could only provide a description of the event (16,19). This was important, however, to the official discover of phages, since this article was read by Felix d'Herelle, a French-Canadian microbiologist from the Pasteur Institute in Paris (18). A few years previous to Twort article, from 1906 to 1909, d'Herelle's attention was attracted to a severe epidemic in locust plagues, in Mexico, and tried to fight it using *Enterobacter aerogenes*. Over the course of the experiment, d'Herelle noticed transparent areas in bacterial preparations, presumably caused by a virus that was mistakenly identified as the reason of locust infection. He isolated this virus against locust but no effect was observed, and d'Herelle ignored this strange finding and kept his initial plan (16). But, in 1917, by combining Twort article and his own findings in Mexico, Felix d'Herelle made the official discover and first communication to the Academy of Sciences, about these bactericidal viruses (18,20). Following an outbreak of severe dysentery hemorrhage in French soldiers, he developed

a treatment, phage based, against *Shigella* strains, where he noticed, again, the transparent spots on the preparations, similar to the ones of his Mexico study (16,20). In that same year, he presented his findings in the Academy of Science meeting and published it, using for the first time the term he invented: *bacteriophage* (20,21). Unlike Hankin and Twort, and several other scientists, many awarded with Nobel prizes, d'Herelle had no doubts about the nature and biology of his findings, and bacteriophages as we know them today, still follow d'Herelle's conception of phage as a virus (16,18).

Even though d'Herelle's findings had a major impact by raising awareness of phages, his greatest merit came when he started to apply his new knowledge to human and animal antibacterial therapy. Two years after his discover, in 1919, d'Herelle conducted a study to treat dysentery with antidysenteric phages, in Paris, at the Hôpital des Enfants-Malades (16). He started with safety testing by administering the phage preparation to the clinical supervisor, also head of hospital's pediatrics; to hospital interns, and to d'Herelle himself. With safety parameters assured, the preparation was administered to a sick 12 years old boy with dysentery, who, after only one dose of the preparation, showed a significant improvement, symptoms free (16,18). The same happened to three other children with bacterial dysentery and the first document reporting a successful phage based therapy was published. From here, several other studies were conducted, by d'Herelle and other concerned scientists, including the first attempts to treat cholera and/or plague in many Asiatic and African countries, with a successful and significant reduction of mortality by cholera, in India. D'Herelle assembled this and many other studies in his book "Bacteriophage and the Phenomenon of Recovery", published in 1935 and written in Russian (1,16).

Despite d'Herelles best efforts, the existing knowledge and all the successful studies regarding phage therapy wouldn't have happened without George Eliava, a bacteriologist from Georgia responsible for founding the Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV), in 1923 (1,16). Together, d'Herelle and Eliava built what would become the primary institution for developing and producing phage based preparations. After successful achievements with d'Herelle's first experiments with phage preparations, this therapeutic method became popular and so, its commercial production started (1,21). There were produced five phage preparations at d'Herelle's laboratory in Paris, and brought to the market by the French company called Laboratoire du Bacteriophage, known today as L'Oreal. The preparations were named *Bacté-coli-phage*, *Bacté-rhino-phage*, *bacté-intesti-phage*, *Bacté-pyo-phage* and *Bacté-staphy-phage*. The Americas also had phages produced, for exemple, in the south, the Oswaldo Cruz Institute in Brazil produced, in a year, 10,000 doses of

antidysenteric phages preparations to help the Latin American countries, in 1924. For the north, the United States (US) had the Eli Lily Company, in Indianopolis, producing, in 1940, seven phage preparations for human use to treat infections. Some of these infections included abscesses, wounds, vaginitis, upper respiratory tract and mastoid infections, caused by *Escherichia coli (E. coli)*, *Staphylococcus* sp., *Streptococcus* sp. and other pathogens. These products were produced in two different forms: one as a bacteriologically sterile phage lysate, such as Staphylo-lysate, Ento-lysate, Neiso-lysate and Colo-lysate; the other as a gel preparation soluble in water, such as Colo-jel, Ento-jel and Staphylo-jel (16,21).

At this point, various historical and political subjects had an impact on the outcome of bacteriophages future, starting with World War II. The first time a bacteriophage was actually seen by the man and its first pictures were taken was in 1939, by Dr.Helmut Ruska, in Berlin, Germany, with and electron microscope (18,21). This new information would be shared with the world had it not been for the start of the war jeopardizing possible friendly exchanges between the European countries. At this time, d'Herelle's conclusions about phage's biological nature were still being questioned by the scientific community and these first microscopic images would have helped clarify any still existing doubts. So, the countries' struggle to cooperate with each other delayed the disclosure and consequent evolution of the available knowledge about phages. Adding to that, some doubts started to rise concerning the efficacy and validity of phages, whether used therapeutically or prophylactically. The development of a therapy, back then, was based on a weak assessment with short non-blind trials to a specific patient, random reports, control groups from other studies and no required standards. Instead of preparing the phages separately and then mix them in a polyvalent product, it was easier to mix the phages first and let them grow together, selecting only the most virulent and rapidly replicating phages presented on the final product. This resulted in low viable preparations with low titre and narrow strain range phages and with too high concentrations of preservative in its constitution inactivating phage's chemical activity (16,18). Worse still, the literature available, at the time, including the scientist's findings, laboratory and clinical trials, general and safety recommendations; was written in Russian creating a new barrier against the development of a phage based therapy (1). There were also concerns regarding administration and preservation of this therapy in general clinics and in similar health-provider facilities. Compared to other medicines, phage therapy required facilities and storage conditions too complicated and specific. This had also a strong impact on how physicians and health professionals handled this new and complex

therapy, given how accustomed they were with simple medications and of easiest administration, storage and production (18).

However, the greatest enemy to phage therapy evolution was discovered in 1928: the antibiotics, with a peak in the 1940s. Of easiest production and administration, a more stable preparation and more effective, antimicrobials represented the new promising therapy against bacterial infections. Even though phages were proved safe and efficient, the western world saw this therapy as uncertain and with contradictory results that didn´t comply with any standards, and so, took them off the market to replace them with the new antibiotics. Of course the advent of the War also brought some consequences on this matter since phage therapy was developed originally in the Soviet Union and used by the German army and, at the time, the western world was rejecting any scientific discover or study original from the Union of Soviet Socialist Republics (USSR). Despite the lack of interest by the western world, phage therapy continued to be used and developed in the former Soviet Union, Georgia and Poland, especially since antibiotics were only available to the Allies, even if in small quantities (16,18,21).

So, on the whole, phage therapy was no longer an option and was rapidly forgotten, but not for too long (21,22). The widespread use of antibiotics resulted in the emergence of the first bacterial strains resistant to penicillin. The fastest response to this problem was based on the development of new classes of antibiotics and modification of the old ones, to maintain the efficacy of antimicrobials (18,22). However, the over-use and improper use of antibiotics fastened the adaptation of bacteria and resulted in the emerge of the first multidrug-resistant (MDR) bacteria (22). So, antimicrobials were not (and still aren't) matching the need to fight a bacterial disease and started to grow the need for alternatives, being phage therapy one promissing candidate (1,21).

2.2 Phage Abundance, Biology and Classification
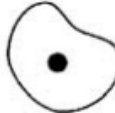
Phages are estimated to be the most numerous, diverse and ubiquitous organisms existing on Earth (22,23). It is estimated a range from 10^{30} to 10^{32} for the total amount of phage particles in the biosphere, outnumbering bacteria by a ratio of 10:1 (6,24). They can be isolated from soil, aquatic surfaces, human and animal feces and sewage, but some types of phages can also easily be grown in a laboratory (1,22). Representing a great role in the ecosystem, phages are responsible for maintaining and regulating the bacterial balance by reducing and transforming bacteria and their genetic material (6,25). And so, even though it is not certain, phages can have a great impact in bacteria diversification with possible genetic exchanges resulting in a co-evolution between both

(25). In order to cope with this diversity, phages are also greatly diverse since they have a narrow host range and infect only a limited number of strains or serotypes of bacteria (23,24). To understand the weight of their existence, it is known that phages are responsible for diminishing 20%-40% of bacteria on aquatic surfaces, everyday (1). All these actions regarding bacteria come about due to phage's fast replication, the ability to strongly survive despite adverse conditions and their high specificity (6,22).

Bacteriophages are obligate intracellular parasitic viruses which means that they cannot finish their life cycle without taking advantage of a host, in this case, bacteria. This natural high specificity to a particular bacterial species or subgroup of species is possible through a variety of surface receptors in bacteria. They differ according to bacteria classification: gram-positive bacteria can have teichoic acids, cell wall proteins (CWPs) and peptidoglycan components; while gram-negative bacteria can have lipopolysaccharides (LPS), flagella and pili (6,10). Regarding phage's constitution, all phages contain proteins and one type of nucleic acid which, depending upon the phage, can be either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), single or double stranded. This genetic material is enclosed within a protein capsid which can be polyhedral, filamentous, pleomorphic or connected to a tail which can be short or long with the latter being contractile or non-contractile. The existence of a tail permits to the tailed phage the injection of its DNA into the host (26). The order *Caudovirales* groups the tailed phages with icosahedral capsids containing a dsDNA genome, and represent more than 96% of the population of bacteriophages (10). Until recently bacteriophage classification was based on morphological features and type of nucleic acid. In 2018 the International Committee on Taxonomy of Viruses (ICTV) classified phages in 12 families, 5 of which belonging to the tailed phages (26) as described in Table 1.

Shape	Family	Characteristics	Morphotype	Example
Tailed (*Caudovirales*)	*Myoviridae*	Linear dsDNA, long contractile tail		T4
	Podoviridae	Linear dsDNA, short non-contractile tail		T7
	Siphoviridae	Linear dsDNA, long and non-contractile tail		λ
	Ackermannviridae	Linear dsDNA, long contractile tale Star-like structures		AG3
	Herelleviridae	Linear dsDNA, long contractile tail		SPO1
Polyhedral	*Microviridae*	Circular ssDNA, icosahedral capsid		phiX174
	Corticoviridae	Circular dsDNA, complex capsid		PM2
	Tectiviridae	Linear dsDNA, icosahedral capsid, inner lipidic membrane		PRD1, AP50
	Leviviridae	Linear ssRNA, icosahedral and spherical capsid		MS2, R17

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	Cystoviridae	Linear dsRNA, segmented, spherical capsid		phi6
Filamentous	*Inoviridae*	Circular ssDNA, long filaments		fd
Pleomorphic	*Plasmaviridae*	Circular dsDNA, no capsid, lipidic envelope		MVL2

Advances in sequencing technology, genomics and bioinformatics allowed a better understanding of phages' genomic diversity which opened the door for the proposal of new families sharing a set of core genes, following a genome-based classification (29). In 2020 the virus taxonomy suffered a rearrangement in which all viruses, including prokaryotic and eukaryotic, are classified into 15 hierarchical ranks. Phages are now distributed into 4 realms, equivalent to the domain rank used for the classification of cellular organisms. The dsDNA tailed phages are unified in a class called *Caudoviricetes* (29). Figure 1 presents a schematic division following the new genome-based classification.

Realm	Kingdom	Phylum	Class	Order	Family
Duplodnaviria	Heunggongvirae	Uroviricota	Caudoviricetes	Caudovirales	Myo/Podo/Sipho Ackermannviridae Autographiviridae Chaseviridae Demerecviridae Drexlerviridae Guelinviridae Herelleviridae Rountreeviridae Salasmaviridae Schitoviridae Zobellviridae
Monodnaviria	Loebvirae	Hofneiviricota	Faserviricetes	Tubulavirales	Inoviridae Plectroviridae Paulinoviridae
	Sangervirae	Phixviricota	Malgrandaviricetes	Petitvirales	Microviridae
Varidnaviria	Bamfordvirae	Preplasmiviricota	Tectiliviricetes	Kalamavirales	Tectiviridae Corticoviridae Autolykiviridae
				Vinivirales	
	Helvetiavirae	Dividoviricota	Laserviricetes	Halopanivirales	Matsushitaviridae
Riboviria	Orthonavirae	Lenarviricota	Leviviricetes	Norzivirales	Atkinsviridae Suinviridae Fiersviridae Solspiviridae Blumeviridae Steitzviridae
				Timlovirales	
		Vidaverviricetes	Vidaverviricetes	Mindivirales	Cystoviridae Picobirnaviridae
			Duplopiviricetes	Durnavirales	

Figure 1 - Virus ranks containing bacteriophages from the Master Species List of ICTV.
The order Caudovirales is highlighted since it will eventually be deleted. The families Finnlakeviridae and Plasmaviridae are not represented since they are still unranked. Adapted from (28)

Bacteriophages interact differently with their host and so, according to their mode of infection, they can be either classified as virulent or temperate phages, and can then be characterized according to their life cycle: virulent phages undergo lytic life cycle only, and temperate phages can choose to follow through lytic or lysogenic cycle (1,6,30). To the moment, there have been identified and studied four phage life cycles: lytic, lysogenic, pseudo-lysogenic and chronic cycles (30). As mentioned above, bacteriophages are highly specific, infecting only bacteria by binding through receptors present exclusively at the bacteria surface (1,24). This is the first step of infection: the adsorption of the phage to the bacterial cell, which is mediated by fibers of the tail or of some analogous structure of phages with no tail (1,23). This fibers are specific to a bacterial receptor that was not designated to bind to phages, representing an evolution and adaptation that permitted the assignment for each phage their respective bacteria (6,12). Then, after an irreversible attachment through phage's base plate (or some analogous structure) to the bacteria, there is the need to bring the tail closer to the bacterial cell wall, in the case of tailed phages, in order to inject the genetic material. In

contractile tails the sheath contracts, shortening the distance of the tail to the cell wall, and a small incision enables the genome to pass through the hollow tail to the interior of the bacteria (6,23). The differences between each life cycle start from here (1).

In virulent (or strictly lytic) phages, the genome inserted in the cytoplasm of the bacteria triggers a set of fast changes and takes over the host metabolism and biosynthetic machinery (1,23). This allows the production of phage´s mRNA and some proteins needed, not only for the synthesis of phage's DNA, but also to block the bacterial DNA, RNA and biomachinery production (1,22). The phage DNA is then used to make the structural parts of the phage such as capsids and tails, but to also produce lysis proteins that compromise the bacterial cell envelope (10,30). When the synthesis is completed, copies of the invasive phage are available within the bacterial cell (1,6) and their lytic proteins trigger the bacterial cell lysis, releasing the virions to the extracellular environment (1,23). From this point, the new phages can infect another bacteria and start a new lytic cycle (6,23).

So, virulent phages only undergo a lytic cycle, induce cell lysis and contribute to progeny synthesis and release. Temperate phages can follow the same path, but can also follow a lysogenic life cycle as it is represented in Figure 2 (10,31). In this case, when the genome is injected in to the bacterial cytoplasm it can integrate in the host genome in the form of a prophage (or independently as a plasmid) replicating with the bacteria genome on a vertical transmission (23,30). The daughter bacterial cells inherit this prophage, making them a lysogen, that is not affected by the invader, maintaining its normal metabolism (1,6). When subjected to a stressful environment, like ultraviolet (UV) radiation, the prophage can leave the quiescent state and start a lytic cycle (6,10). Phages adopting a temperate life cycle instead of a strictly lytic one can be transducers of bacterial genetic material. Working as a vehicle for these genes, temperate phages incorporate parts of the host genome and transduce it to other bacterium on a horizontal transmission base (23). These genes may encode specific features like bacterial virulence, antibiotic resistance or biofilm formation which will enhance the infectivity and survival of bacteria (22). Because of that, part of the evolution of bacteria relies on phage transduction since it is responsible for balancing the bacterial population (30). And so, temperate phages are not recommended and highly avoided for phage therapy (10).
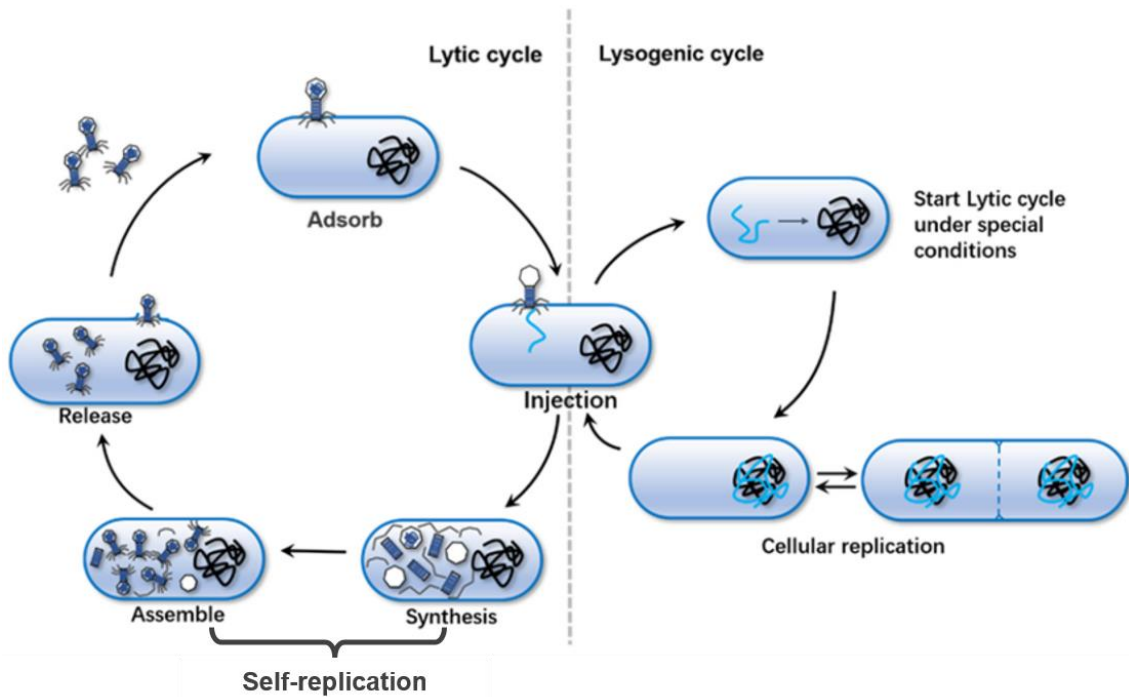
Figure 2 - Lytic and lysogenic cycle of phages. Adapted from (32)

Phages can undergo switches between their mode of infection, from lytic to lysogenic or the other way around. But the first hasn't been very well studied and when there is a switch referred for a phage usually is the switch from lysogenic to lytic mode. The ability to "decide" which mode of infection to follow is mediated through cellular and genetic mechanisms and depends on physical and chemical conditions. Depending on such conditions, the switch from lysogenic to lytic mode happens with a process called induction (30). This process is basically an internal or external stress, like temperature, change in pH, bacterial number and growth, nutrition, UV radiation, or a chemical action, that damages the DNA and induce a SOS response to try and repair it. The prophage is induced to leave the quiescent state and to excise the chromosome, letting the genetic switch happen. This switch is a gene expression process characterized by a linkage between regulatory proteins, promoters and operators. So, lysogeny appears as an anti-lytic gene cluster that maintains its activity if nothing disrupts the normal cell conditions (30,33).

Besides these two main life cycles, there are two other modes of infection less studied. Pseudo-lysogeny, as the name suggests, neither induces cell lysis and progeny synthesis like a lytic cycle, nor integrates the host genome and transmit it to bacterial daughter cells like in lysogeny (30). A pseudo-lysogenic phage is characterized as an episome that suspended its replication process when there was no cellular energy available. This intermediate state is ideal when growth conditions are adverse, and the

phage progeny wouldn't prosper (25,30). Once there is a more stable environment, this phage can either follow a lytic or a lysogenic cycle. Pseudo-lysogeny may affect both lytic and temperate phages. All three types of replication cycle described can become virulent at some point, but not for filamentous phages that undergo a chronic life cycle. The phage becomes a parasite that do not disrupt the host membrane and won't consequently cause cell lysis. Instead, it establishes a relationship with the bacteria supporting its growth and possible biofilm development (30).

So, by gathering the morphological and biological features about phages it was possible to establish a preference for virulent phages from the *Caudovirales* order to be used in phage therapy since it showed a better performance as an antimicrobial compared to other non-*Caudovirales* phages (1,27).

3. Antimicrobial resistance and phage therapy re-emergence

As crucial as the discover of antibiotics might have been for modern medicine and public health, it has also brought several concerns with the emergence of antimicrobial resistance (AMR). Confronted with this problem, scientists had to come up with possible solutions and/or alternatives. Hence, bacteriophages are being considered by the scientific community as a possible alternative therapy to help overcome this public health emergency (3,34).

3.1 What is AMR

Before the discover of antibiotics, infectious diseases were the main causes of death, specially caused by bacteria (1). With the discover of penicillin by Alexander Fleming, as the first of many antibiotics, came the revolution of modern medicine and parameters like life expectancy were improved (1,35). This new therapy allowed a more robust combat against several human infectious diseases preventing many possible global epidemics, as the years passed (1). It also played an important role on World War II by helping manage and control infections amongst soldiers (5). Nowadays, antimicrobials are a fundamental tool in clinical practice including surgeries, treatments of chronic diseases, sepsis, organ transplant and dialysis (3). However, and as Fleming stated while accepting his Nobel Prize in 1945, "The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug, make them resistant" (36). So, the difficulties brought by AMR were predicted even before they started to appear. What people don't know and easily misjudge is that AMR is not a recent problem, it existed way before the discover and synthesis of antimicrobials (37).

To understand how old AMR is, some bacteria isolated from glacial waters and permafrost with thousands of years were studied and showed resistance against some antimicrobials like ampicillin and vancomycin. It was actually a natural process, just like penicillin was created from a type of mold that naturally defends itself against bacteria, some strains of staphylococcus were naturally resistant to penicillin, with no human influence (37). And so, AMR only became a problem with the misuse and overuse of antimicrobials by humans that lead to the selection of resistant bacterial strains (35). Chronologically, the first strains of *Staphylococcus* resistant to penicillin were identified in 1940, previous to the first penicillin clinical use. But, instead of exploring a new polyvalent strategy that fits each specific situation of antimicrobial resistance, the scientific community tried to replace the "weak" antimicrobial by developing a new one

(5,35). In 1959, methicillin was already introduced as a substitute to penicillin but, no longer than a year later, a strain of *Staphylococcus* resistant to methicillin was reported. In 1972, was introduced vancomycin to fight methicillin resistant strains but in 1979, 1989 and 1997, there were reported strains of *Staphylococcus*, *S. aureus* and *Enterococcus*, respectively, resistant to vancomycin. The same happened with other antimicrobials like tetracycline and levofloxacin, Figure 3. So, the introduction of a new antimicrobial would eventually be followed by a report with a new resistant bacteria strain. Adding to that, after the 1980s, the development of new classes of antimicrobials started to slow down (5). To have an idea, the Food and Drug Administrations (FDA) approved sixteen new antimicrobials, between 1983 and 1987, whereas between 2010 and 2016, only six new antimicrobials were reported (1). With time, it became clear how the development and introduction of a new antimicrobial cannot keep up with how fast bacteria can evolve (37).

ANTIBIOTIC RESISTENCE IDENTIFIED | ANTIBIOTIC INTRODUCED

penincillin-R *Staphylococcus* 1940

1943 penincillin

1950 tetracycline
1953 erythromycin

tetracycline-R *Shigella* 1959
(MRCA) methicillin-R *Staphylococcus* 1960

1959 methicillin

penincillin-R *Pneumococcus* 1965
erythromycin-R *Streptococcus* 1968

1967 gentomicin

1972 vancomycin

gentomicin-R *Enterococcus*
vancomycin-R *Staphylococcus* 1979

1985 ceftazidime imipenen

ceftazidime-R *Enterobacteriaceae* 1987

levofloxacin-R *Pneumococcus* 1996
vancomycin-R *Enterococcus* 1997
imipenen-R *Enterobacteriaceae* 1998
linezolid-R *Staphylococcus* 2001
vancomycin-R *Staphylococcus* 2002

1996 levofloxacin
2000 linezolid
2003 daptomycin

ceftaroline-R *Staphylococcus* 2011

2010 ceftaroline

Figure 3 - Chronological comparison between the introduction of an antibiotic and the emergence of the correspondent resistance. Adapted from (37)

So, what is antimicrobial resistance (AMR)? It is the capacity of bacteria, viruses, fungi, and parasites to withstand the mechanisms used by antibiotics. As explained before, it is a naturally selected feature that can be gene manipulated to evolve. As a result, infections caused by these resistant pathogens become difficult or even impossible to treat (5,36). Bacterial resistance can be characterized as intrinsic, acquired or adaptive. Intrinsic resistance is a natural process possible due to inherent features of bacteria, that were not originated trough mutations or gene acquisition. So, it is independent of any antimicrobial action, universally found in the genome of the bacteria and is not coded by any particular gene (5,37). Despite the innate nature of this resistance mechanism, intrinsic resistance is developing and spreading within pathogenic and non-pathogenic bacteria, which means that the mechanisms developed by environmental bacteria to interact with other microbes and to defend themselves are being identified in the pathogenic bacteria seen in clinical practice. This phenomena is possible through a concept called (environmental) resistome, which is a reservoir that encompasses all the antimicrobial resistance genes in bacteria with or without a pathogenic activity, developed through the years as a survival mechanism (37). When this resistance elements are passed or expressed to pathogenic bacteria it can develop human diseases with clinically relevant antimicrobial resistance (5). Examples of intrinsic resistance include the resistance to ampicillin by *Klebsiella pneumoniae*, the cephalosporin resistance by *Enterococcus faecium* and *faecalis*, the vancomycin resistance by gram-negative *bacilli*, and the glycopeptide resistant gram-negative bacteria (5,37).

The real problems are posed with the two other types of antimicrobial resistance. Acquired antimicrobial resistance, as the name suggests, happens when a former sensitive bacterium acquires some resistance against antimicrobials (5). The development of acquired AMR is mediated by several factors but the misuse and overuse of antimicrobials is certainly the principal responsible. There are two ways for bacteria to acquire resistance, either by gene mutation of their DNA during replication or through horizontal gene transfer (HGT). While rapidly replicating, some bacteria can develop random genetic mutations. Some of these mutations may turn bacteria resistant to antibiotics and, consequently, enhance its chance of survival against this therapeutic method. Compared with other non-antibiotic-resistant bacteria, these mutated bacteria have a survival advantage and are selectively selected to continually multiply (37).

In the case of horizontal gene transfer, it happens when bacteria acquire new genetic material from an external source. Is divided in three main processes: transformation,

transduction and conjugation. Transformation consists in the uptake of DNA fragments from lysed bacterial cells to incorporate the bacterium chromosome. Not all bacteria can pass through this process. Transduction involves the exchange of genetic material from a bacterium to another through a bacterial virus, named bacteriophage. Conjugation consists in transferring the genetic material directly from one bacterial cell to another through physical contact. It can involve a plasmid which is an extrachromosomal circular DNA that can independently replicate within a cell, in this case, a bacterium. A single plasmid may present more than one resistance genes which allows the transference of multidrug resistance in a single exchange. In fact, the accumulation of AMR is mainly due to HGT aided by plasmids and mobile genetic elements (5,37).

Mutation

RESISTANT BACTERIA

SUSCEPTIBLE BACTERIA

ANTIBIOTICS

Through the process of cell reapplication, some bacteria develop mutations that make them resistant to antibiotics.

Bacteria with the resistant mutation have a better chance of survival against antibiotics.

Resistant bacteria continue to multiply, even when exposed to antibiotics.

Horizontal gene transfer

ANTIBIOTIC-RESISTANCE GENE

Antibiotic-resistant genetic material is transferred between different bacteria cells. This can happen in three different ways: transformation, transduction and conjugation.

Figure 4 – Acquired resistance: mutation and HGT. Adapted from (38)

Adaptive resistance is the other type of AMR that brings clinical problems by making antimicrobials ineffective. In contrast to the genetic changes of acquired resistance, this type of resistance results in modulations of genes after an environmental alteration. So, instead of developing irreversible gene expressions, it results in epigenetic changes that pass on to progeny as heterogenic gene expression profiles. This type of resistance can occur after changes in concentration gradients or after exposure to antimicrobials, either way, resulting in a reversible or irreversible phenotype of heterogeneity (5,39).

3.2 AMR: a public health emergence. What can be done?

To visualize, more clearly, the damage AMR has caused, several entities captured these consequences by studying and collecting specific statistics. Lord Jim O'Neill, a renowned British economist conducted a review that estimated a total of 700,000 deaths, caused by antimicrobial resistant bacterial infections, annually, that can go up to 10 million by the year of 2050 (40), taking over today's serious conditions like cancer, diabetes and heart disease. In USA, in 2019, these resistant infections affected over 2.8 million people, with many hospitalizations, part of them resulting in 35,000 deaths, annually. Annually, the US healthcare system spends between 28 to 45 billion dollars, annually (37) representing the possible damages to health and to the economy, caused by AMR (41). For this reason and as explored more ahead, the World Health Organization (WHO) considered AMR one of the greatest public health emergencies (1).

As discussed before, AMR and its evolution is a natural process, that can also be spread as a consequence of human errors like the overuse and misuse of antimicrobials, unsanitary conditions, the non-therapeutic usage of antimicrobials in agriculture, aquaculture, food production and industry, the release of antimicrobial residues into the environment, the inadequate prevention of infections and the misinformation when using antimicrobials (5,42). As the common public already knows, the legitimate use of antimicrobials as therapeutic reasons for humans and animals is already causing problems regarding AMR. Very often, when prescribed, antimicrobials are indiscriminately used either because the administered dosage is not correct, or because the treatment regime is not completed, either way, this behaviour contributes for the development of AMR and its spread. But is not the therapeutic usage of antimicrobials that brought antimicrobials to this urgent situation (35,43).

After the World War II, the need for a higher food production stimulated the search for supplements that could improve animal nutrition. Antibiotics were introduced as growth promoters to increase animal weight gain. Some studies presumed that antibiotics alter and reduce the animal gut microflora resulting in higher nutrition intake levels when feeding. Adding to that, antibiotics are also used in food production as preventive treatments, where animals are exposed collectively to the same dose, to avoid a collective infection that would invalidate any product derived from those infected animals (35,43). So, antibiotics as growth promoters and/or prophylactics brought an economical advantage, to both food producer and consumer, great enough that, for example in the USA, 80% of the marketed antibiotics are used in food production (35). As a result, animals have higher circulating concentrations of antibiotics, that were not

necessary. And, as the economy arises, more animal food is consumed and consequently, higher is the consumption of antibiotics through food. It was estimated that antibiotic usage in food animals may go from 63,151 tons in 2010 to 105,597 tons by 2030 (35). With time, it was identified the risk of developing AMR in enteric bacteria of animals that were transmissible to the consumer. When animals are in herds enclosed in a small place, these conditions benefit the pathogenic transmission. This may result in the identification of an animal that, even though it was never exposed to the antibiotic, its bacteria are resistant to it and will pass on these resistant genes to the consumer (35,43).

To fight this uncontrolled and unnecessary use of antibiotics, many measures were taken and are still in force. Starting by trying to encourage the search for alternatives to improve the well-being and performance of animals in food production. With our modern times, there can also be found solutions by researching the genetic and immunology of resistance in animals as well the environment and how it can be improved. Other measures with a stronger impact are based in only allowing the use of antibiotics with minor or no use in human treatment for food production. For example, tetracycline, penicillin and sulfa drugs were considered unavailable as growth promoters. In fact, in the most developed countries, a legislation is available in order to control and forbid the use of antibiotics as growth promoters, but the producers found ways to overcome the law, either by under reporting the use, or misreporting it as a therapeutic use (43).

After we consume and metabolize the antibiotic, its residues need to be released into the environment. For humans and animals, it is known that between 30% to 90% of consumed antibiotics are released through urine and excrements, and so, a higher consume of antibiotic leads to a higher release of residues in human and animal waste and sewage. This discharge of residues also occurs through hospitals, private residences, farms, industry, agriculture and waste landfills. Unfortunately, when unused or expired, antibiotics are simply left in the garbage, which won't lead to degradation, but can possible allow for resistance genes to be transferred to the environmental bacteria from soils and water, making them resistant. These resistance genes are then transferred to humans and other biospheres through food industry and waste residues, when in contact with pathogenic or non-pathogenic bacteria. As the years passed, the selected AMR genes consumed and exposed to humans started to affect our microbiome, making our commensal bacteria resistant to antibiotics (35). The possible solutions in order to prevent the antibiotic residues waste start when taking measures in health and food production, since less use of antibiotics results in less antibiotic residues

waste. Then, a proper scheme should be set to properly manage manure: manure storage to help decompose it, digestion through fermentation or aerobic processes (depending on the type of antibiotic) and biological treatment of antibiotic genes in manure. Regarding wastewater, it is essential to develop a sanitation and sewage treatment for the undeveloped countries that could prevent many infections and thus reducing the need for antibiotics. Then, our worldwide water treatment implemented needs to be updated since it is not designed to eliminate antibiotics or ARGs. Also, with the implementation of reutilized wastewaters as an ecological step it was identified the need to update and manage this treatment system to eliminate residues of several medicines and to use it as critical control point for the spread of antibiotics (44). The Figure 5 schematizes the process.
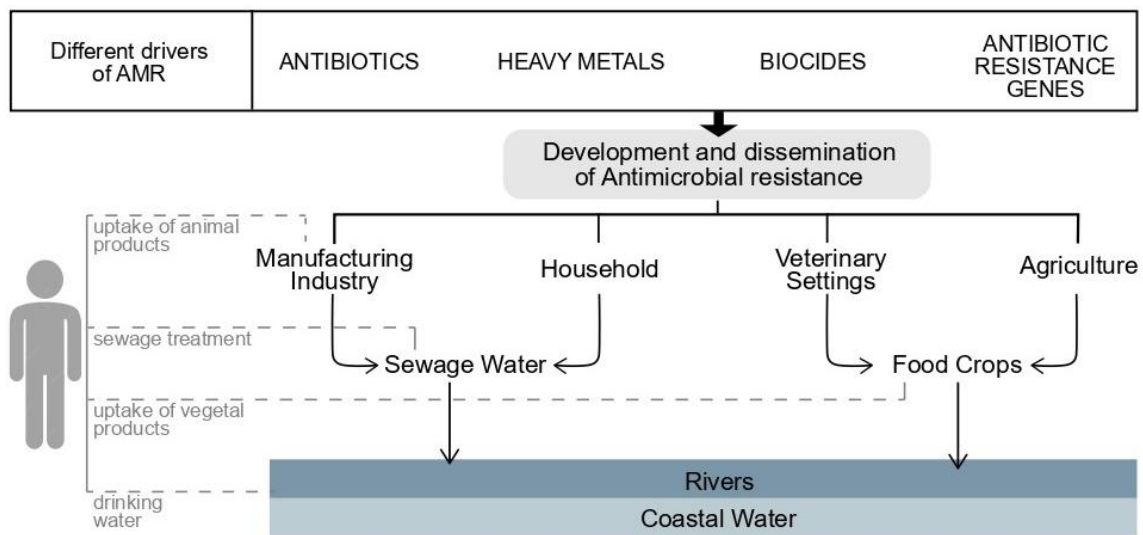
Figure 5 - Generalized scheme of different AMR drivers and their possible route of transmission to humans. Adapted from (35)

The use and misuse of antibiotics is still out of control in several countries "outside" Europe and USA. Pharmacies dispense antibiotics without prescription. It was estimated that more than half of the worldwide antibiotic prescriptions weren't necessary (35,36). So, the problem isn't only caused by the general public, but also by health professionals. Nowadays, it is possible to find many prescribers lacking crucial knowledge and information about antibiotics and AMR (36,42). So, to reduce these unnecessary prescriptions and antimicrobial misuse there should be an investment in schools and health professionals training, especially by following the One Health Approach: Starting with students, it is important to establish an awareness about these worldwide health problems since their first academic years. And although they can recognize the AMR emergence, they also admit lacking information and knowledge which doesn't give them much confidence when prescribing antimicrobials. And so, it was created the

Antimicrobial Stewardship Programs (ASPs), an institutional initiative focused in monitoring and guiding health professionals through protocols and guidelines in how to prescribe and use antimicrobials (36,45). The core elements for implementing the hospital ASPs, defined by CDC (Centers for Disease Control and Prevention), represented in Table 2, were updated in 2019 and can be applied to all hospitals. And so, as health professionals improve their knowledge and efficiency when dealing with antimicrobials, they can influence their current and future colleagues so that a great impact can be seen within the general public (42,46).

Table 2 - Core Elements of Hospital ASPs (2019) by CDC. Adapted from (46)

Core Elements of Hospital Antibiotic Stewardship Programs	
Hospital Leadership Commitment	Dedicate necessary human, financial, and information technology resources
Accountability	Appoint a leader or co-leaders, such as a physician and pharmacist, responsible for program management and outcomes
Pharmacy Expertise (previously "Drug Expertise")	Appoint a pharmacist, ideally as the co-leader of the stewardship program, to help lead implementations efforts to improve antibiotic use
Action	Implement interventions, such as prospective audit and feedback or preauthorization, to improve antibiotic use
Tracking	Monitor antibiotic prescribing, impact of interventions, and other important outcomes, like *C. difficile* infections and resistance patterns
Reporting	Regularly report information on antibiotic use and resistance to prescribers, pharmacist, nurses, and hospital leadership
Education	Educate prescribers, pharmacists, nurses, and patients about adverse reactions from antibiotics, antibiotic resistance, and optimal prescribing

In 2017, the WHO shared a list of the 12 bacterial agents most threatful to public health regarding antibiotic resistance (47) listed in Table 3. The division in critical, high or medium priority categories was based on how urgent it is to develop a new antibiotic against the bacteria in question (48). The list is based in the following criteria: 1) how deadly the infection is, 2) if its treatment requires hospitalization and for how long, 3) the frequency of resistance when infecting a community, 4) how it is spread amongst animals, between animals and humans or between two persons 5) how easily they can be prevented (if measures like good hygiene or vaccination are enough), 6) how many

alternative options are available (if there is any) and 7) if there are new antibiotics being developed to target them (47).

Table 3 - WHO list of antibiotic-resistant priority pathogens. Adapted from (47)

Group	Family	Important features
Critical	*Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing	Great threat in hospitals, nursing homes Patients in ventilators and blood catheters Causes bloodstream infections and pneumonia
High	*Enterococcus faecium*, vancomycin-resistant *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant *Helicobacter pylori*, clarithromycin-resistant *Campylobacter* spp., fluoroquinolone-resistant *Salmonellae*, fluoroquinolone-resistant *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant	More common diseases such as gonorrhea and food poisoning caused by *salmonella*
Medium	*Streptococcus pneumoniae*, penicillin-non-susceptible *Haemophilus influenzae*, ampicillin-resistant *Shigella* spp., fluoroquinolone-resistant	There are still effective antibiotics available

A vast list of risk behaviours and natural phenomena are identified every year when it comes to the evolution of pathogenic bacteria against antibiotics, and the scientific community works hard to overcome these difficulties. However, if there isn't an obvious

and urgent reason to develop a new therapy, like we have seen with the vaccine against COVID-19, and many other historical events like wars and plagues, the only incentive is profit or economic benefit. And in the specific case of antibiotics, the pharmaceutical industry shows no interest in investing in a saturated market with poor prospective (43). So, the industry focused in developing new strategies to overcome the antimicrobial resistance urgency, either by enhancing the antibiotics' effectiveness, by combining therapies, including antibiotics, or by developing new therapies (3,5). Regarding antibiotics, their effect can be increased when enhancing the antibiotic availability that results in a better drug delivery, or by increasing its therapeutic concentration inside the bacterium (5). Antibiotics can also be combined with other antibiotics, with another drugs or therapy, or with adjuvants, creating a synergetic effect (49). Modern technologies allowed the scientific community to branch out and propose alternatives like antimicrobial peptides, photodynamic therapy, silver nanoparticles, monoclonal antibodies, phytochemicals, and, as mentioned above, the use of bacteriophages: phage therapy (5,49).

4. Phage Therapy: Current Status and Applications

4.1 Bacteriophages Applications and Phage Therapy

Even though bacteriophages are most likely associated to phage therapy, there are other applications for these viruses in different areas. Phages can be used as detectors for bacteria, as environmental indicators, as plague control in plants, as gene transfer and deliver, in food production and preservation and, for the medical area, as biomaterials for tissue regeneration, as drug vehicles or as phage vaccines (6,50). Phages as a vaccine product are still being developed, and even though it's not yet used in practice, it may help prevent and treat complicate and chronic infections by several pathogens, including resistant bacteria, viruses and parasites or as a trigger of the immune response against cancer, for example. There are being developed three types of phage-based vaccines: phage DNA vaccines that will encode an antigen and activate the cellular and immune response of the host; phage-displayed vaccines that express the antigen in the phage surface to start an immune response against this antigen; and hybrid vaccines that is a combination of both vaccine types previously mentioned (21,51). These vaccines show stability, reduced costs when largely produced, simple storage and transportation needs and the possibility of an induced immune response that can overcome some of the limitations of our common vaccines (21).

The prophylactic use of bacteriophages, even though not yet recommended, could have a crucial impact by replacing preventive antibiotics that are dispensable and contribute to the spread of AMR. Phages can also help regulate the human and animal microbiota and contribute for the prevention of several diseases. Also, by preventing diseases in animals, especially in food production, an indirect prevention of infections in humans that are only transmittable through food consumption could be achieved (52). However, the most promising application of bacteriophages still relies on the therapeutic use as an antimicrobial to fight bacterial infections: Phage Therapy.

With time, phage therapy has shown different levels of popularity, and even though it was forgotten in the western countries, it kept growing in the east allowing its development and study. And nowadays, the need for a therapy against pathogenic bacteria that could overcome AMR and replace antibiotics led the scientific community to consider other alternatives, including phage therapy (21). At first sight, bacteriophages looked like the perfect candidate with its high specificity to bacteria and harmlessness to any other organism, but its uncertainty regarding eukaryotic cells infection, the possible emergence of immunogenicity and bacterial resistance, and several other regulatory hurdles brought some fears when evaluating this alternative (53).

4.2 Advantages and Limitations of Phage Therapy

The renewed interest in bacteriophages as a therapeutic alternative to fight pathogenic bacteria and as a possible replacement for antibiotics relies on many advantageous factors, particularly when compared with antibiotics (10). However, phage therapy presents also several limitations, especially when compared to antibiotics, as the Table 4 resumes.

Table 4 - Advantages and disadvantages of bacteriophages and antibiotics. Adapted from (54)

	Advantages	Disadvantages
Bacteriophages	1. High host-specificity 2. Safe, little side effects 3. Independent from antibiogram 4. Wide availability in biosphere 5. Antibiofilm properties 6. "Self-replicating", self-limiting	1. Narrow spectrum 2. No empiric treatment (phagogram) 3. Less predictable pharmacology 4. Little efficacy data 5. Vague regulatory framework
Antibiotics	1. Broad spectrum 2. Empiric treatment 3. Well-studied pharmacology 4. Qualitative RCT's * 5. Easy availability	1. Low host-specificity 2. AB-related side effects 3. Depending on antibiogram 4. Challenging development 5. No antibiofilm properties 6. Small therapeutic window (toxicity levels)

*RCT- Randomized Clinical Trial

4.2.1 Advantages of therapeutic bacteriophages

High host specificity

One of the most attractive features of bacteriophages against bacteria is its high host specificity allowing a targeted action against a specific pathogen by binding to its membrane receptors (1). Antibiotics have a broad host range targeting both good and bad bacteria, killing the pathogens but also our probiotics that help with digestion, nutrient production and pathogenic protection which may result in associated side effects like nausea, bloating, diarrhoea and yeast infections (55). Phages can only infect and lyse the one pathogen they can recognize and have no influence in the human or animal microbiome (34) resulting in little to no side effects, which is a clear advantage over antibiotics (49).

Safety and tolerability

The minor side effects identified above result from an indirect effect of antibiotics by undesirably killing the commensal microbiota (56). However, antibiotics are also associated with more severe effects like neurotoxicity, cardiotoxicity, hepatotoxicity, nephrotoxicity and allergic reactions. Antibiotics may interact with other drugs and are

commonly associated with intolerability by the organism. Even though the non-clinical and clinical studies on phages are not sufficient to consider phage therapy safe, the many years of co-existence with humans and animals may lead to an empirical deduction that phages have a higher safety and tolerability, especially compared with antibiotics (1).

Wide availability and versatility

Antibiotics are industrially produced through fermentation under controlled and necessary conditions which represents a challenging and slow development process (54). As the most numerous and diverse entities on Earth, phages can be easily isolated from the environment, which represents a rich and unlimited source of bacteriophages (56). Phages are then more easily available than antibiotics which may result in a faster therapeutic development. In fact, it is faster and cheaper to find a phage targeting a specific host than trying to develop new classes or molecules of antibiotics against the specific pathogen.

Biofilm penetration

Sometimes, the bacterial infection can be presented as a biofilm, an ecosystem of bacteria grouped within a self-produced matrix that help improve survival and persistence in hostile environments (2). Amongst other properties, biofilms work against the elimination of bacteria by demining its effectivity and by blocking the passage or adsorption of antibiotics. Because of this, antibiotics have a very limited effect on these complex ecosystems which could be a dangerous situation in need of a fast solution (1).

Bacteriophages have shown promising results on the eradication of biofilms (1). Phages can encode enzymes, named depolymerases that help bacteriophages to penetrate the host envelope and inject the DNA at the beginning of an infection cycle. Many of these exopolysaccharide depolymerases are also able to degrade the biofilm structure and therefore help the bacteriophage to reach the bacterial membrane receptors and start the viral infection (2).

Co-evolution between phages and bacteria

Phages show one of the best advantages, if not the most important, compared with antibiotics: the ability to mutate alongside bacteria. Phages can mutate faster or as fast as bacteria when it evolves to resist a phage infection, by mimicking those mutations and develop new infectivity. As it will be addressed more ahead, the emergence of resistant bacteria against phages can be a future possibility. If bacteria evolve to avoid a phage infection, phages can also change to keep up with these updates and be used in therapy (1).

Dosage and administration

Bacteriophage's mode of action involves the lysis of a bacterium and replication of phages that can only happen in the presence of bacteria. So, general rules of pharmacology are not applicable to self-replicating phages that are also self-limiting since the absence of bacteria would cease the phage activity (54). Some advantages can be reached from these characteristics. The self-replication of phages allows for a treatment based on a single dose to maintain the pharmacological effect, contrary to the multiple administrations required to ensure the efficacy of antibiotics (1). However, the unique pharmacodynamic and pharmacokinetic properties of phages can be limiting as will be addressed more ahead.

4.2.2   Disadvantages/limitations of therapeutic bacteriophages

Narrow spectrum

Even though the high specificity of phages is presented as one of the most promising characteristics of phages, it is also responsible for its narrow spectrum and consequent limited range of applicability (32). Usually, bacterial infections are polymicrobial, which means that more than one species of bacteria is causing the infection (1). The narrow spectrum of phages does not permit an effective action against these polymicrobial infections (32). Also, when the pathogenic bacteria are not identified or when there is not enough time to identify the pathogenic bacteria like it happens in acute infections, the treatment is usually based on an empirical approach using broad spectrum antibiotics (54). With bacteriophages this is not possible.

To overcome this problem, it can be prepared a cocktail of different phages that target different bacteria (54). However, for this approach to work it would be necessary to identify the pathogenic bacteria and to test its sensitivity to different phages to select the efficient ones (1). Compared to antibiotics, this is a clear disadvantage that consumes time and resources.

Development of phage-resistant bacteria

To prevent another global crisis regarding resistant bacteria, the scientific community is addressing the possibility of resistance developing against phages by bacteria (1). Since not enough clinical trials have been conducted to study the emergence of phage-resistance in bacteria no certain conclusions can be settled (54). However, investigators are concluding that it is an almost certain and inevitable event (51).

Just like it happens with antibiotic-resistant bacteria, several mechanisms can be developed or possessed by bacteria to overcome an infection by phages. However, these mechanisms are different than antibiotic-resistant bacteria (1). Bacteria prevent

viral infections through mutation and selection, surface-receptor loss or modification, chemicals secretion to avoid phage adhesion to the bacterial surface, blockage of the phage DNA injection, inhibition of phage replication and release, inhibition of phage DNA integration to the clustered regularly interspaced palindromic repeats/CRISPR associated system (CRISPR/Cas) (25).

The broad-spectrum activity of antibiotics allows for the treatment of a wide variety of bacterial pathogens, which also means that all these pathogens will be exposed to the antibiotic. If not correctly eliminated, these bacteria will survive and may develop resistance mechanisms against the antibiotic and pass them through HGT. The high host specificity of phages allows for an infection where only the targeting bacteria is exposed to the phage (57). And if the bacteriophage treatment can successfully eliminate all the pathogenic bacteria, at a faster rate than they can self-replicate, the risk of developing phage-resistant bacteria is lower, mainly when compared with antibiotic-resistant bacteria (34).

Contribution to AMR development in bacteria

Not only there is the possibility of bacteria to develop resistance against phages, but phage therapy can also work as a vehicle for AMR in bacteria. As mentioned before, when identifying the most adequate phage for a treatment, there should exist an obvious preference for strictly lytic phages, since temperate phages can follow a lysogenic cycle and contribute for the horizontal gene transfer of ARGs through the mechanism of transduction (1). And so, a temperate phage in contact with a resistant bacterium may encapsidate the host ARGs and transduce it to another bacterium where it can recombine with into the bacterial chromosome or replicate as a plasmid (10). This new genetic information can have major effects on the recipient bacteria, contributing to new capabilities and mechanisms of resistance that make bacteria more virulent (32).

Pharmacokinetic properties

Phages have been clinically used without properly assessing the complex dynamics resulting from the interactions between the human organism, phages and bacteria. In fact, the unpredictable or poor results of conducted clinical trials of phage therapy are commonly a result of pharmacokinetic properties of phages combined with the pharmacodynamics between phages and bacteria (58).

In order to reach a clinically significant elimination of pathogenic bacteria, the number of phages that must reach and infect bacteria must be high enough without causing side effects. For this to work, the titre of both phages and bacteria must be high at the site of infection since the self-replicating mechanism of phages depends on the existence of a

host bacterium. However, the rate at which phage self-replicates in one or more cycles may not be enough and a multiple-dose regimen may be best (58). And so, the advantageous single dose of a phage preparation compared to the multiple doses of antibiotic (1) may not be possible. Adding to that, phage particles are much bigger than antibiotic molecules which limits the administration dose, lowers the rates of uptake and transportation and may result in lower circulating phages (58).

Then, since phages are mainly composed of proteins and genetic material, they can be easily degraded when in contact with human metabolism, like in the stomach or liver (32). The mode of administration can also have an impact in pharmacokinetics of phages (31) with some advantages and disadvantages depending on the final objective, like the Table 5 shows.

Table 5 - Routes of administration for phage therapy. Adapted from (31)

Delivery Route	Advantages	Disadvantages
Intraperitoneal	Higher dosage volumes possible Diffusion to other sites	Extent of diffusion to other sites may be overestimated in humans (most data from small animals)
Intramuscular	Phages delivered at infection site	Slower diffusion of phages (possibly) Lower dosage volumes
Subcutaneous	Localized and systemic diffusion	Lower dosage volumes
Intravenous	Rapid Systemic diffusion	Rapid clearing of phages by the immune system
Topical	High dose of phages delivered at infection site	Run-off from target site if phages suspended in liquid
Intrarectal	Slow, stable release of phages over long time	Limited applications/sites Risk of insufficient dosing Technically challenging to manufacture
Oral	Ease of delivery: Higher dosage volumes possible	Stomach acid reduces phage titer Non-specific adherence of phages to stomach contents and other microflora
Aerosol	Relative ease of delivery Can reach poorly perfused regions of infected lungs	High proportion of phages lost Delivery can be impaired by mucus and biofilms

When using phage therapy against Gram-negative bacteria, especially in high doses of phages, the lysis of several bacteria may release endotoxins in dangerous quantities

and provoke an endotoxic shock (25) and potentially cause undesired inflammatory reactions (56).

Impact on immune system

The success of phage effectivity highly depends on the influence of the innate and adaptive immune response (31). Phages can potentially trigger the immune system and induce responses towards the elimination or inactivation of the bacterial viruses (25,34).

Phages can be recognized by pattern recognition receptors (PRRs) and activate an innate immune response recruiting phagocytes to the infection site (31). The reticuloendothelial system (RES) is responsible for a fast clearance of phages¸ mainly in the liver and spleen. The effectiveness of phage clearance depends on structural features of the viral capsid where a minor change on the composition of the phage protein coat could affect its duration while blood circulating or its immunogenicity (25).

Also, the therapeutic success and efficacy of phages can be hampered with the induction of phage-neutralizing antibodies. This is a highly variable situation that can increase its activity after multiple administrations of the treatment (31). Very few trials have been finished and conducted, and no clear correlation has been defined between anti-phage antibodies and phage therapy (54). Conclusions regarding immunogenicity of phages are controversial and should be considered during phage screening (31).

On the other side, the induction of immune cells will also result in the elimination of the pathogenic bacteria. From here, genetically altered phages could be developed to create an infection against bacteria, without triggering the immune system against phages but to trigger the immune system against pathogenic bacteria (50).

It is possible to encounter many other limitations of phage therapy as we approach it from different applicable alternatives but the principal barrier for phage therapy to evolve, at this time, are the many regulatory hurdles and clinical evaluation difficulties (57). A descriptive critical reflection will be presented more ahead.

4.2.3 Approaches and options to overcome phage therapy limitations

Most of the disadvantages or limitations described above are overcome by combining different phages forming a phage mixture or cocktail (49). These cocktails can be a combination of different targeting phages when facing a polymicrobial infection, or, it can be a combination of different phages targeting the same host to prevent the spread and development of resistant bacteria against phages (32,49).

Phage therapy may also benefit from a meticulous screening of phages and further establishment of phage library. This way, if the pathogenic bacteria is identified it would only be necessary to consult previously assessed data and match the correspondent infectous phage, available in a phage library, previously isolated (32). To ensure the efficacy of the phage preparation, a phagogram must be perfomed. A phagogram is an analogous procedure of an antibiogram to previously test the sensibility of bacteria to phages and correctly select the efficient phage (59).

Phage engineering technologies are also being developed to overcome some of phages limitations. Some examples include expanding the phage host range through modification of the ligand protein, alteration of the viral capsid to improve phage stability in the blood circulation (51), or even the enginering of temperate phages to became strictly lytic (60).

Phage therapy can also be combined with antibiotics to reach a synergistic effect and efficiently eliminate bacteria (25). In fact, since phages can penetrate into biofilms, a combinatory use with antibiotics would ensure a successful approach and elimination of pathogenic bacteria (10).

4.3 Phage selection and preparation

In order to select a bacteriophage, it is firstly necessary to isolate it. As already mentioned, phages are ubiquitous, and so, they can be isolated from everywhere in the environment. Adding to this, it has been shown that phages can outnumber their host even while evolving, and so, nature can provide an endless source of phages. From this natural limitless source, it is important to select the most probable environments for a phage reservoir. The easiest way to find these spots rests in finding the respective host, in this case, bacteria. d´Hérelle suggested this approach and suggested the isolation of phages from recovered or in recovery patients from bacterial infections. And since these infected patients were gathered in hospitals, it was clear that another obvious reservoir would be the hospital wastewater, that is the current starting point for phage isolation. Other isolation sources can be selected if the phage strain needed colonizes different ecologic environments like rivers, lakes or known contaminated drinking waters (61).

After isolation, the bacteriophage needs to be genetically and phenotypically characterized by having its genome sequenced, its morphology assessed by electron microscopy and its growth parameters determined (62). As already discussed, not all phages are suitable for therapy and so, a set of relevant features must be gathered. First, the selected phage must be strictly lytic, show efficacy and specificity against the pathogen, not affecting the microbiota (62). It is important to determine the host range,

i.e. determine the bacterial genera, species and strains the phage can lyse. Its interaction with the infected organism must have a positive impact either by activating the immune system to help eliminate the pathogen or by having no immune interaction at all (62,63).

As previously discussed, phages should be formulated in a cocktail of different host-specific phages to ensure a higher host range and avoid the selection of phage resistant bacteria (1). Noteworthy when designing a cocktail, it is important to evaluate the behaviour of each phage in the cocktail, in order to guaranty they are not inactivated or lose title. As it happens with other medical products, every formulation should be prepared according to good manufacturing practices (GMP) to ensure adequate sterilization and purification, and quality assurance (QA) standards (62,63). As a biological product, the production of a phage preparation must follow a set of orientations divided into upstream and downstream processes. The first stage is the upstream processing that includes isolation of host cells and phages to create cell banks, the establishment of a cell culture and infection. Then, the downstream processing includes the process of purification until the bulk product is made. Each selected phage must be produced and purified individually. And finally, each purified phage is mixed into the cocktail and formulated to meet the final preparation (6). The Figure 6 resumes the process.
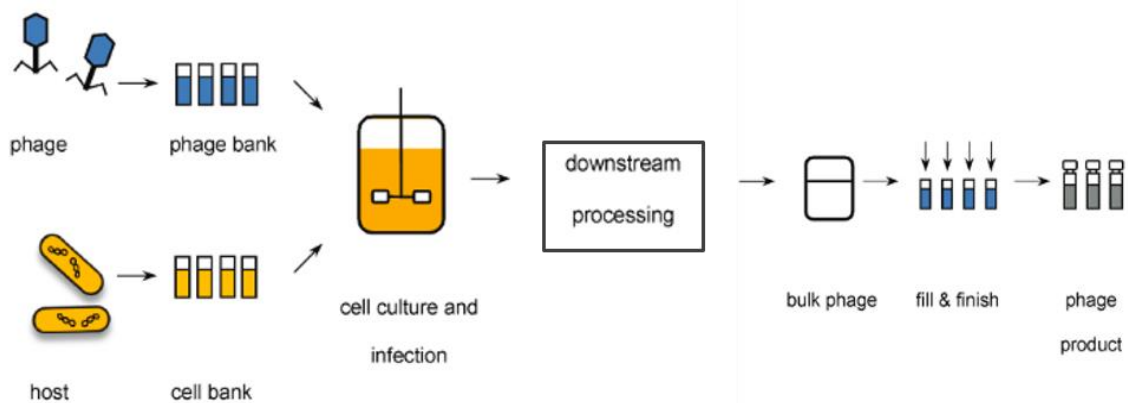
Figure 6 - Overview of phage manufacturing. Adapted from (6)

The specifications for the formulation differ according to the route of administration. It can be through oral, topical, inhalation, intravenous, intramuscular, intraperitoneal or intrasubcutaneous administration.(6) The ideal pure phage formulation presents no impurities or contaminants like bacterial endotoxins, genetic material and proteins (other than the ones in the formulated phage) (6,63). It is important to notice that if the formulation will be used in systemic administration, the purification should be stricter. As for the number of phages needed in the preparation to result in the elimination of the

bacteria it is estimated that for one bacterium there should be 10 or more virions, which results in a concentration of 10^8-10^{10} bacteriophages/ml (62,63).

To maintain the viability of phage preparations it is essential a particular attention to the storage conditions. Regarding temperature, there are preparations that can maintain their stability at room or body temperature, others at 4ºC and others require lower temperatures that can go down to -80ºC. In terms of the format for storing the phage, it can be more stable when stored in a liquid form, in a dry powder, or combined in both forms with some stabilizing agents.

These formulations need also to keep its stability after administration until it reaches its target, and as mentioned before, it varies according to the route of administration. For instance, the body temperature must have no negative impact on the phage (61). Also when administered orally the phage must not be affected by the acidic pH of the stomach (31) or if topically administered it must not be excessively sensitive to UV light exposure so that it is inactivated before reaching the pathogenic bacteria (61). In order to reach favourable conditions, the phages may be encapsulated for oral administration, but when there is an interest in eliciting the immune response of the patient, an intravenous administration is more suitable (61).

4.4 Phage Therapy Current Worldwide Status and Clinical Trials

As already discussed, phage therapy has a long history that dates back to 1919 when the first study with therapeutic bacteriophages was performed (16). Its fame continued in the western Europe, especially in Poland, Georgia and Russia where this therapy was integrated in their health system until today (62,64). However, the studies conducted in the Eastern countries were lacking validity and consistence that resulted in several mistakes, resulting in phage therapy lose its credibility (1,64). The few clinical trials on phage therapy available were either written in Russian or lacked scientific basis due to scientific limitations of the time. The overall knowledge of biology and life cycle of phages was short, the manufacture and preparation of the formulations was rudimental, the preparations showed impurities and weak effectivity since the selection of the bacteriophage was not as accurate as today and the final result was not sufficient to fit be accepted following other countries' standards (1,31). Nevertheless, given the recent need for new therapies against bacterial infections, phage therapy has risen but more clinical trials need to be designed in accordance with the rigorous standards designed by the Western world (64).

4.4.1 Available bacteriophages sources and therapy methods

Phage production was much greater before the break off of the Soviet Union, great enough to satisfy the needs of almost all Soviet countries. Nowadays, the Eliava Institute of Bacteriophage, Microbiology and Virology (EIMBV) company called Eliava BioPreparations, Ltd. produces phage preparations enough to cover the market in Georgia and to export to few countries (16) In the past, various phage preparations were produced to target multiple strains of bacterial infections with such successful results that some of these preparations are still used in the present. For example, the Pyophage is used against *Staphylococcus aureus*, *P. aeruginosa (Pseudomonas aeruginosa)*, *Streptococcus proteus* and *E. coli* for skin wounds, urinary or digestive tract infections. The Intesti Bacteriophage is another example of a phage preparation against *Shigella*, *Salmonella*, *E.coli*, *Proteus vulgaris*, *P. mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* for prophylactic use or treatment of gastrointestinal infections (1,65). Adding to these preparations, the EIMBV has also available other over the counter preparations like Enko Bacteriophage, Fersisi Bacteriophage, SES Bacteriophage, Staphylococcal Bacteriophage, for enteric and/or pyo-inflamatory infections (65,66). These preparations consist in cocktails of different phages that can target different bacteria and be sold in pharmacies as an over-the-counter product following the so called *prêt-à-porter* approach This approach is not in accordance with the regulatory standards of the European Medicines Agency (EMA) and FDA for medical application in humans and is not available for application in the Western world (65).

On the contrary, in Poland, Wroclaw, where exists the only phage therapy unit of central Europe, the Hirszfeld Institute with two branches in Cracow and Czestochowa, it has been developed an approach based on the *sur-mesure* concept. It consists on a personalized treatment adapted to the needs of a specific patient. It can be developed as an experimental program. The phage preparation is considered, by the national authorities, an Unproven Intervention in Clinical Practice inserted in the Declaration of Helsinki, Article 37 which is the use of an intervention that is not justified on the basis of available evidence. Some European countries have followed the steps of Poland and implemented phage therapy following the *sur-mesure* approach (65,67).

Since 2007, the Queen Astrid military hospital (QAMH) in Brussels, Belgium, has been developing phage treatments under the Declaration of Helsinki, Article 37, in clinical practice. With access to a vast phage bank from different centres in Canada, USA, Georgia, UK, Israel, Switzerland, Japan, Korea and Poland, available at the https://www.bacteriophage.news/phage-banks-collections/ website. The QAMH worked

with treatment requests mainly within the hospital or the wound burn centre, however, since 2017, and after extensive publicity and public information spreading, the number of request went up and from around the world, presented in Figure 7. The QAMH received 151 requests in 2017, particularly after the broadcast of two documentaries in the Netherlands, one on 21st March entitled: Bacteriophages: an alternative to antibiotics? and the other on 24th October entitled: Doctors of tomorrow (68). Between April 2013 and April 2018 the centre received 260 requests to apply a phage based treatment, but only 15 presented the eligible criteria and were submitted to phage treatment, 12 of those in the QAMH (21,68). Figure 7 shows the international transfers of phages from and to the QAMH to clinical practice between 2015 and 2020 (69). The QAMH had also impact in clinical trials, especially in its participation in the PhagoBurn trial, discussed more ahead (53). In 2018, the Belgian government introduced a legislation for phage production and clinical applicability of phage therapy (21). More recently, in Belgium, it was introduced the Magistral Phage, a magistral preparation defined in Europe by "any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient" in Article 3 of Directive 2001/83 and Article 6 quater, § 3 of the Law of 25 March 1964, phage based (53,70).
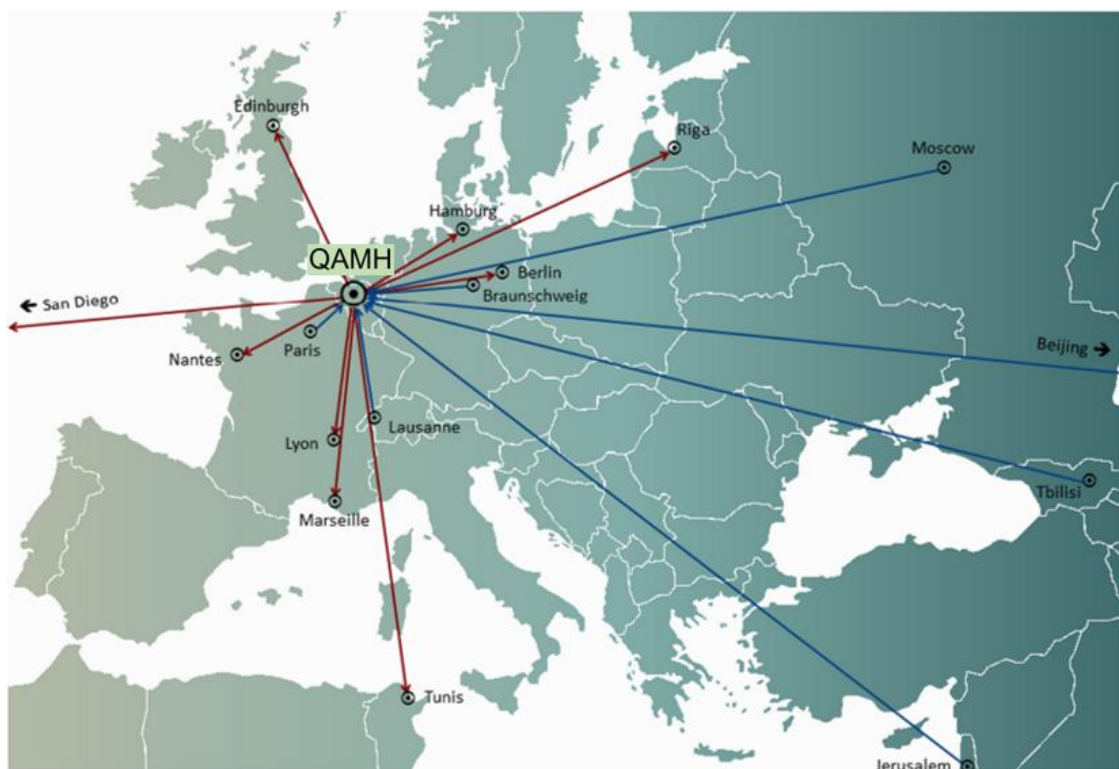

Figure 7 - Transfers of phages between the Queen Astrid military hospital (QAMH) in Brussels and the rest of the world for clinical application between 2015 and 2020. The red arrows represent transfers from the QAMH to other countries and the blue arrows represent the international transfers for QAMH. Adapted from (69)

In France, since 2006 until today, the Pherecydes Pharma company produces several phage preparations either to use in clinical trials or for therapeutic purposes following the *sur-mesure* approach (70). The company follows the indications of WHO against the priority bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli* to develop the suspensions for the compassionate use (71). A topic that will be developed more ahead. Pherecydes Pharma was also a promoter for the PhagoBurn trial (70).

In the US, in June of 2018, the first Center for Innovative Phage Applications and Therapeutics (IPATH) of the North America was launched by the University of California San Diego School of Medicine after six cases of urgent bacterial infection were successfully treated with phage cocktails. These preparations were approved by the FDA as emergency investigational new drugs (72). Also, located in California, the Armata pharmaceuticals is responsible for developing phage preparations against key resistant bacteria, especially those in the WHO priority pathogens list. With two phage cocktails against *Pseudomonas aeruginosa*, one for cystic fibrosis (CF) or non-CF bronchiectasis patients and the other against pneumonia; and a novel biologic product against Staphylococcus aureus causing bacteraemia and bone joint infection (73).

With the increased interest in phage therapy comes an increase in the demand and request for these preparations that are dependable on the availability of characterized phages. And so, it is important to establish an international network of phage banks that is constantly growing and updating. For example, in North America, the US has its phage bank in the state of Virginia called the American Type Culture Collection (ATCC Bacteriophage Collection, and in Canada, Québec, there is the Felix d'Hérelle Reference Center of Bacterial Viruses ate the University of Laval (2). In Europe, as part of the Leibniz Institute, the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) in Braunschweig, Germany, is a collection centre of yeasts, bacteria, fungi and bacteriophages that can be ordered (2,70). There is also the Fabenbank in Delft, in the Netherlands and the National Collection of Types Cultures (NTCT) Bacteriophage Collection in Salisbury, UK. In Asia, the Bacteriophage Bank of Korea is based in Yongin, South Korea (2).

4.4.2 Phage Clinical Trials

Phage therapy clinical trials have been developed since the discover of bacteriophages in the eastern Europe, especially in the IMBV in Tbilisi, Georgia and in the Ludwig Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw,

Poland (1). But not long after the beginning of these trials, especially during the 1930s, some concerns started to rise regarding safety and efficacy of phage preparations (2). The clinical information available is based in a rationale of pharmacodynamic efficacy and in the empirical use of phages, securing its safety data on the over the years-coexistence with animals and humans (70). Additional clinical information must be gathered through adequate controlled clinical trials with specific care towards the trial design. The trial design is similar to a standard drug clinical trial but with some particularities adapted to phage preparations such as the dosage, route of administration and bioavailability (64). And so, the clinical trial should be randomized and controlled and must gather specific data on characterization and selection of the phage, patient and host. Then, information on the efficacy including dosage, formulations, route of administration and antibiotic compatibility must be added to the previous pharmacologic tests and gathered in a detailed report for future references and possible study replication (2,22).

Since phage therapy has been suggested as an alternative to antimicrobials, over the last few years, the number of clinical trials has increased, but only a small percentage are completed (2). Through different web platforms it is possible to freely access a worldwide database of clinical trial records. Either with Global Clinical Trial data (GCT) (https://www.globalclinicaltrialsdata.com/) or ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home). The keyword "phage" was introduced on both databases search, resulting in 81 and 79 studies, respectively. Not all the results showed correspond to a study based on a treatment where a bacteriophage is applied or administered. When introduced the key word "phage therapy" the results were narrowed to 50 (74) and 34 (75), respectively. However, it is clear that most clinical trials regarding age therapy registered in both platforms take place in the US or Europe, as the Figure 8 shows.
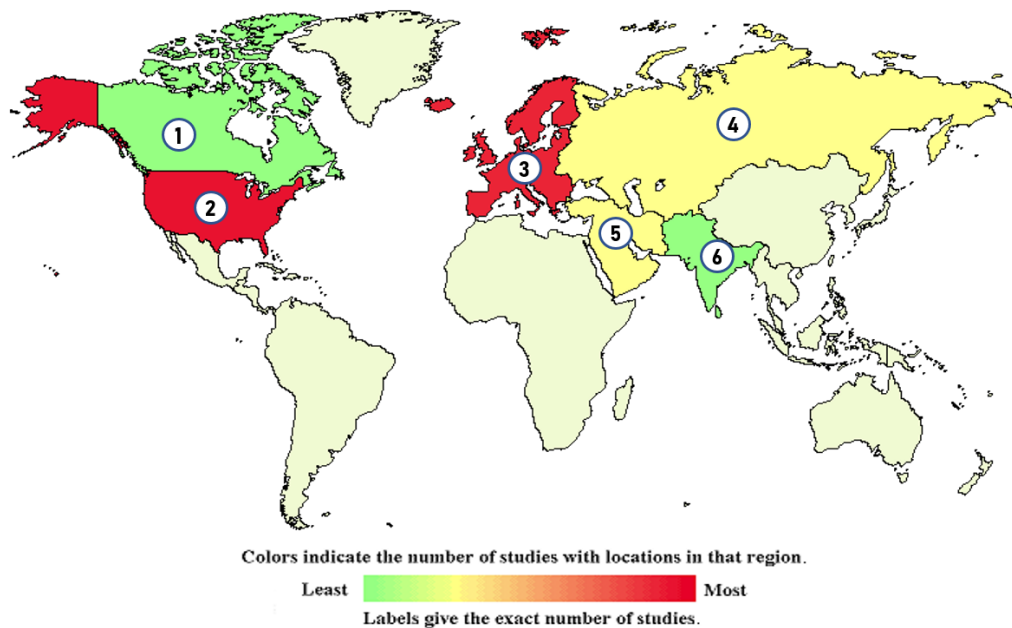
Figure 8 - Number of clinical trials in each world region. 1) 1 in Canada in green 2) 10 in USA in red 3) 9 in Europe in red 4) 2 in North Asia in yellow 5) 2 in Middle East in yellow and 6) 1 in South Asia in green. Adapted from ClinicalTrial.gov (75)

Since phage therapy has little literature and study reports available from when it was developed in the eastern Europe, the new trials developed in the West cannot benefit from previous clinical experience of the East. Adding to that, the process of preclinical and clinical trials is extensive and thorough in order to be approved for marketing authorization. For example, in the US, a new drug takes approximately 12 years to gain regulatory approval for market introduction since the start of a preclinical trial. And so, the first study reports on phage therapy in the west were important as breaking points for the study of phage therapy in Europe and the US, even though only a few were conducted and would likely fail (31). The first clinical trial on phage therapy in the US was conducted in the Southweast Regional Wound Care Center in Lubbock, Texas, to evaluate the safety of a phage preparation to treat venous leg ulcers. This randomized, double-blind controlled trial started in September 2006, with two groups, the patients treated with a phage cocktail targeting *Staphylococcus aureus*, *E. coli* and *P. aeruginosa*, and the control group treated with a saline solution. The results from this Phase I clinical trial showed no adverse reactions to the phage cocktail, however, the final results were similar between the treatment with phage and the control group. But this was expected since the efficacy was not being tested and the bacteriophages were not chosen for its infectivity (2,75).

A randomized double-blind, placebo-controlled phase I/II clinical trial was also conducted by Nestlé, in Switzerland in collaboration with the Dhaka Hospital of the International Centre for Diarrhea Disease Research in Bangladesh, between 2009 and

2011, to test the safety and efficacy of a phage cocktail orally administered to hospitalized children with acute diarrhea caused by *E. coli*. Although efficacy was not achieved due to mistakes made, mainly because the target of the phage cocktail was not the main cause of the acute diarrhea, no significant adverse effects were reported (2).

Both US and Switzerland studies showed the same mistake and can be used for future reference as how important it is to identify the etiologic agent(s) of the bacterial infection and how susceptible to the phage cocktail it is (2).

In 2013 was launched the largest multicentered, open label, European clinical trial on phage therapy called PhagoBurn. This randomized single-blind controlled phase I/II clinical trial was conducted in 27 patients from 5 centres: the QAMH and the Hospital Center Sart-Tilman in Belgium, the *Hôpital s'instruction des armées* Percy and the *Centre hospitalier* ST Joseph et St Luc in France, and the *Centre Hospitalier Universitaire Vaudois* in Switzerland (2,75). The purpose of this trial was to assess the efficacy of one cocktail with 12 strictly lytic phages, topically applied, for the treatment of infected wound burns by MDR bacteria *E. coli* and *P. aeruginosa* with a control group treated with a standard emulsion cream with 1% silver sulfadiazine, in a total of 27 patients (21). This prospective trial was the first conducted under GMP and Good Clinical Practices (GCP) that showed great significance for the future of phage studies/ clinical trials design (2). The trial ended in February 2017 with a detailed final report summarized in the Community Research and Development Information Service (CORDIS) website of the European Commission (https://cordis.europa.eu/en). Although a reduction in bacterial burden was observed in patients treated with the phage preparation, efficacy was limited since the outcome was slower than in the control group. One of the problems encountered regarding efficacy was because the dosage administered was much lower (10-100 PFU/mL) than the planned (1×10^6 PFU/mL) due to a drop in phage titre as a consequence of the GMP manufacturing (2,21). Adding to this, a great part of the budget was spent producing one batch of the investigational product and took 20 months to be finished (53). Worse, wound bacteria were not tested for susceptibility to the phage preparation, and bacteria resistant to a low phage dose were recovered from patients with treatment failure (2,21). Despite all these problems, the phage cocktail was safer than the standard treatment which presented adverse effects like pneumonia, bronchitis and septic shock (21). Since the PhagoBurn was the first trial to follow European standards with GMP and GCP, it gave a real perspective about safe phages production

and how important it is to develop detailed regulatory requirements for phage therapy (53).

Although only a few finished clinical trials are available, it is important to notice how long a trial can take until it is finished and so, a great number of clinical trials are currently being developed targeting different infections through different routes of administration. The most common complications studied on trials are septicemia or venous leg ulcers, heart and pulmonary diseases, gastrointestinal disorders, skin and soft tissue infection like burn wounds infection, bone or joint infection, urinary tract infection, chronic otitis media and biofilm infection (31,76). The routes of administration studied in clinical trials are oral, intraoperative, intravenous, topical, inhalation and intrarectal (22).

As it will be discussed more ahead, one of the principle hurdles regarding phage therapy implementation concerns ensuring efficacy, safety and quality of the product. In fact, the current European regulatory provides specific Regulations and Guidelines so that a harmonized non-clinical and clinical assessment can comply with the Human Medicinal Product Directive 2001/83/EC (77). The same happens in the US following the Code of Federal Regulations in Title 21 (78). However, phage products are not yet introduced on the market and consequently cannot benefit from the experience of a previous licensed phage product. As an investigational medicinal product some exemptions can be considered for the clinical development of a phage-based product, so that a smoother and stronger progress can be achieved.

5. Regulating Phage Therapy

With the new found interest in phage therapy within the last years came also a search for useful applications of bacteriophages against pathogenic bacteria as well as new pre-clinical and clinical evidence and worldwide studies. In fact, when submitting the word "bacteriophage" in the PubMed platform, it gives access to 76,062 results from 1921 until today (79), but when submitting the words "phage therapy" the platform gives access to 5,688 results from 1946 until today, with the majority of these results updated to the platform between 2010 and today, with a total of 4,256 results (79). However, phage therapy rapidly showed some limitations that would hold back its thriving within the scientific community mainly due to scarce availability of valid clinical data and the incompatibility with our current pharmaceutical legislation (8).

5.1 Current Phage Therapy Approaches Worldwide

As mentioned above, two approaches can be used for application of phage products to treat a bacterial infection, either as a single or multiphage preparation. One approach is the application of a ready-to-use product available on a pharmacy, or the personalized concept where each preparation is tailor made for a specific patient (69). As it will be discussed below, each of these approaches bring specific regulatory hurdles which have delayed the implementation and development of phage therapy, especially in the western world.

5.1.1 Eastern World

Bacteriophages have been used and produced in Georgia, Russia and Poland for prophylaxis and treatment of bacterial infections for over a century (16,52). The production numbers went down with the break of the Soviet Union and, nowadays, the Eliava BioPreparations, Ltd. is responsible for phage products production and distribution in Georgia. In Russia and in Georgia cocktails of phage lysates for topical or oral administration, either targeting one or more bacterial species, are available in local pharmacies for treatment of several types of infections (ex: intestinal, urinary, wound infections). However, the manufacturing of these preparations doesn't follow any specific guidelines or national requirements which may result in contaminated preparations that cannot assure safety and efficacy (80).

In Poland, for nearly 50 years, personalized phage lysates preparations were supplied to the hospitals as an act of good faith when facing a patient with a serious condition. Although phage therapy was not approved in Poland, this special treatment was still conducted, under medical supervision following the standard procedures at that time. When Poland joined the European Union as a Member State, the Hirszfeld Institute

of Immunology and Experimental Therapy created its own Phage Therapy Unit (PTU) and adapted the regulatory provisions in accordance with EU standards (67,81). In Poland, as in other EU countries, phage therapy is considered an experimental treatment and used in accord with the Declaration of Helsinki and under Polish regulations. The guidelines determined by the Declaration of Helsinki for experimental treatment in Poland have been deployed in the Medical and Dental Professions Act of 5 December 1996, chapter 4. Together, these two documents plus the Constitution of Poland and the ethical code of the Polish Medical Association conduct the basis for the application of phage therapy through the compassionate use concept. Since the PTU was the first center for phage therapy that was ethically approved in Europe it became a national and international role model when structuring phage therapy, specially following the compassionate use (81).

Regarding phage availability in Poland, contrary to what happens in Russia or Georgia, phage therapy is not a standard practice and so, bacteriophage preparations are not available on the national market in pharmacies or online and are limited only to the phage therapy candidate patients (81).

5.1.2 Western World

Although phage therapy poses many opinion differences throughout the globe, the western world was able to reach a consensus when classifying the therapeutic use of phages. As a biological medicinal product (also called biological drug in the US), phage therapy concept is addressed differently in each Member State or in the US (80). However, no framework is currently including phage therapy as a medicinal product and many efforts are being done to meet a satisfactory conclusion (76). Either facing safety, stability or regulatory difficulties, phage therapy is not well fitted in the current legal standards for its production and marketing introduction. And so, several stakeholders, either private companies or national institutions are studying and suggesting new approaches to turn the situation around (80).

Following the example of Poland, other EU countries and the US adopted the compassionate use approach under the Declaration of Helsinki, approved by the national regulation in Poland, through a temporary use authorization (ATU) in France, by the FDA under emergency investigational new drug (eIND) in the USA and, in Australia, through special access schemes by the Therapeutic Goods Administration (TGA) (82). In France, the phage preparations used for a specific patient treatment are not produced under GMP standards, but follow similar quality norms when manufactured in Pherecydes Pharma or QAMH. Also, in 2021, it was created the program PHAGE*in*LYON to develop

novel phage applications in therapy. Similar to this program, the San Diego IPATH was created with the same objective suggesting a model for phage therapy implementation that could be fitted in other countries. It required a network for trading experiences and results of clinical trials from each European academic reference center mediated by the respective national health authorities (80).

Belgium was one of the first countries to adopt the compassionate phage therapy use in Europe and its gathered experience while adopting this concept allowed for the development of the concept of magistral phage (80). This concept uses phages as the active pharmaceutical ingredient (API) available on a pharmacy and are mixed by or under the supervision of the pharmacist following a prescription from a physician for a specific patient (53). The concept of preparing a specific mixed drug was also adopted in the US but instead of magistral phage it is known as compounded prescription drugs (82).

5.2 Regulatory Status of Phage Therapy

With the renewed interest in phage therapy reaching different parts of the globe as well as new suggestions for its applicability, came also the need to apply the corresponding current regulatory framework (83). However, when implementing a new therapy in Europe or in the US, a set of required steps are mandatory to grant a final quality product (80). Starting with defining the status of phage therapy, which has been the subject of discussion between regulatory authorities of different countries or Member States (80,83). In fact, the nature of bacteriophages and its mode of action differs from conventional therapies in a way that the current regulatory frameworks may not be well fitted (84).

5.2.1 Medicinal Product/Drug Status

Since phage therapy is simply the use of bacteria eating viruses so that a therapeutic effect can be observed when used against a bacterial infection, it can fall under the European definition of a medicinal product (7) as *"any substance or combination of substances presented as having properties for treating or preventing disease in human beings or (…) which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis"*, Article 1(2) of Directive 2001/83/EC (77). The same happens in the US, but instead it is defined as a drug in the section 201(g) of the Federal Food Drug and Cosmetic Act (FD&C Act) that states *"articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals"* and *"articles (other than*

food) intended to affect the structure or any function of the body of man or other animals." (85). So, the medicinal product based on the application of bacteriophages for therapeutic reasons is called phage therapy medicinal product (PTMP). The terminology of phage therapy medicinal product (PTMP) is not consensual between Europe and US. Europe does not commonly use the term, but for this work it will be adopted since there is some difficulty defining phage therapy inside medicinal products.

As a medicinal product, a set of requirements from the health agencies must be established including obtaining a Marketing Authorization (MA) that can only be obtained if non-clinical and clinical data is provided that demonstrates the quality, safety and efficacy of the product (77,86). But since no phage product for human treatment has been currently placed on the market and only few clinical trials have been concluded, there is no clinical and regulatory sufficient evidence to be followed by a new phage product (87). And so, further clinical conclusions will help to establish more evidence for a more robust regulatory scheme for such a new medicinal product (8). In Europe, a phage product must be manufactured as an investigational medicinal product (IMP) defined as *"a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial (…) or when used for an unauthorized indication"* in the Directive 2001/20/EC, article 2(d) (88). To ensure a quality and safe final product, the manufacture process must be in compliance with the GMP rules including a rigorous quality control (QC) scheme, non-clinical and Phase I, II and III clinical trials and then a MA (7,89). Adding to that, the medicinal product must also have each of its constituents evaluated in quality and quantity, and also, in the case of a PTMP, a set of criteria like the absence of prophages and antibiotic resistance in the bacteria used to produce phages, impurities control, the use of strictly lytic phages with high host specificity that show potency and purity must be met (2). In the US, this new medicinal product is considered an investigational new drug subjected to the Code of Federal Regulations title 21, chapter 312 and an IND (investigational new drug application) must be approved (78).

5.2.2 Biological Medicinal Product Status

On 8 June 2015, the EMA organized a conference entitled *"Workshop on the therapeutic use of bacteriophages"* that brought together the different stakeholders of industry, academia, legislators and regulatory authorities to discuss and reflect about the therapeutic use of bacteriophages (90,91). With the purpose of helping the development of phage therapy, this workshop focused in reviewing the regulatory framework, the quality aspects and its current clinical evidence (90). From this exchange of experience

resulted a consensus opinion that phage therapy falls under the European regulatory scheme on biological medicinal products (BMP) (86). In the same way as Europe, the FDA organized a public workshop on 10 and 11 July 2017, entitled *"Bacteriophage Therapy: Scientific and Regulatory Issues"*, So that the experience from the clinical and scientific community regarding regulatory and scientific considerations could be shared. In this workshop, phage therapy was clearly considered as a biological drug (2) that should be developed under the specifications of an Investigational New Drug and regulated by the Office of Vaccines Research and Review (OVRR) within the Center for Biologics Evaluation and Research (CBER) (85).

According to EMA, a biological medicinal product is defined as a product *"the active substance of which is a biological substance"*, as specified in Part I of Annex I of Directive 2001/83/EC (77,86). Phages are biological entities that naturally lyse bacteria, and so, when used in therapy, the bacteriophage is responsible for exerting the pharmacological or immunological action (77), which means that is working as the active substance. Phages alone can easily be considered a biological product, but they are also considered to be produced by a biological source like a bacterium, and are also considered to be extracted from a biological source like an infected wound or the wastewater of an hospital (92). This information is in line with the definition of biological substances defined in the Directive as *"a substance that is produced by or extracted from a biological source and that needs a combination of physico-chemical-biological testing together with the production process and its control for its characterization and the determination of its quality"* (77). And since phages are biological substances and also the active substance in a phage therapy preparation, phage preparations can fall under the definition of a biological medicinal product.

5.2.3 Natural phages in PTMP status

The following information will be focused on PTMPs based on natural phages since it has been the most common approach when developing therapeutic bacteriophages products (7). Information on altered or modified phages will be presented more ahead.

Once settled that phages are a biological medicinal product, the logical next step is to try and associate PTMP to one of the classes that subcategorize a BMP. This poses one of the greatest challenges regarding phages: should bacteriophages be classified as an advanced therapy medicinal product (ATMP)? The Regulation (EC) No 1394/2007 of the European Parliament and of the council of 13 November 2007 defines ATMPs as *"complex therapeutic products"* that include gene therapy, somatic cells therapy and tissue engineering (93). Bacteriophages present a set of features that resemble the

complexity of ATMPs: instability when administered, uncontrolled replication, possibility of developing mutations, unique pharmacokinetics, incomplete understanding of the function and therapeutic, etc. (92). However, natural phages are not genetically modified, and cannot be considered a gene, somatic cells nor engineered tissues medicinal product, and consequently, do not fall under the definition of an ATMP (83).

The nature and co-existence with humans, even though complex, does not necessarily means a requirement for a legal definition, as it happens with other complex medicinal products. The complexity referred to some ATMPs is different than the complexity of PTMPs, and bacteriophages could better benefit from a more experienced and used strategy like probiotics with the finality of improving microbial ecology (92).

As mentioned before, the co-existence with bacteria results in a constant natural change and adaptation of bacteriophages, which would require a constant updating of phage cocktails. Not only to keep up with the evolution and diversification of bacteria, but especially when a bacterial resistance is developed (92). Therefore, on a regulatory basis, bacteriophages share a great similarity with other BMP, the human and veterinary vaccines, for example, the flu vaccine that is updated every year (92). And so, phage therapy as a medicinal product can have its development and manufacture processes following the experience of human and veterinary vaccines implementation (7). It requires both bacteria cell banks and phage banks with controlled identity, purity and potency with the finality of producing PTMP batches compliant with GMPs (7). Some companies are producing phage preparations following this regulatory scheme within the vaccines unit of EMA. But it is important to mention that phages are not vaccines in its whole, their scope is to produce a therapeutic effect and can be used prophylactic and to trigger an immune response. The opposite happens with vaccines that can produce a therapeutic effect, but it is not its main scope. PTMP can be seen as *"therapeutic vaccines"* (92).

5.2.4 Genetically Modified phages (GMO phages) status

As mentioned before, most developed or in development PTMPs use natural phages. However, natural phages show some limitations in isolation, in stability due to phage neutralization in a cocktail, in resistance development to phage infection by bacteria that can be enhanced with phage enginery. The final scope is to reach a higher infectivity from bacteriophages by modifying what can be improved. Compared to natural phages, altered phages need to have some additional regulatory requirements, depending on the type of engineering (7).

In Europe, the Directive 2001/18/EC on the Deliberate Release into the Environment of Genetically Modified Organisms (GMO) must be followed and an environmental risk assessment (ERA) must be carried (94) defined in the Article 2(8) as *"the evaluation of risks to human health and the environment, whether direct or indirect, immediate or delayed, which the deliberate release or the placing on the market of CMOs may pose"* (94). To perform a risk assessment *"it is necessary to establish a common methodology (…) based on independent scientific advice"* and be carried out before its release (94), resulting on a complete assay of the phage GMO (7).

Depending on its engineering type, the GMO phage can be considered an ATMP by EMA and benefit from a centralized authorization procedure (93). However, some countries do not accept GMOs which can bring a difference of opinion on whether or not it should be placed on the market and difficult the implementation of these products. Something that should be projected to better be prepared for these regulatory hurdles and technicalities, for GMOs in general and for phage GMOs (7).

In the US, a natural phage is supervised by the OVRR and a GMO is supervised by the Office of Tissues and Advanced Therapies (OTAT), and since they are both considered a biologic product, they fall under the supervision of the CBER. Both offices must work together to evaluate and develop a phage GMO (7). In fact, the FDA does not face natural and engineered phages with too much difference, the existing difference is between common medicinal products and phages in general. With that in mind, a PTMP of engineered phages follows a similar path of natural phages, but its IND must contain an environmental assessment with manufacture background information (95).

Temperate phages can be seen as an obstacle when developing a therapeutic phage since it can lead to lysogeny that consequently may lead to the transduction of virulence or antibiotic resistance genes to bacteria (22). The possibility of engineering temperate phages to present only a lytic form by reducing or removing the risk of transduction of bacterial genes permits an increase on the number of available therapeutic phages. For example, no virulent phages have been discovered to fight infections caused by *Clostridium difficile* and *Mycobacterium abscessus* and could benefit from a therapy based on engineered temperate phages (22). Also, temperate phages can be engineered to become a vehicle for synthetic genes with advantageous properties. For example, they may code antibiotic-sensitive genes as an adjuvant to antibiotics. Even though this approach poses many improvements for phages' efficacy, the inherent ethical issues associated with GMOs have been posed to engineered phages which are not readily accepted, and consequently, are being less explored (2).

5.3 Two Different Approaches

Phages nature and intrinsic characteristics differ from the average active substance of a medicinal product (8) and so, instead of basing the therapy technique in this information, the experts proposed two approaches based in the applicability, manufacturing and delivering strategies of phage therapy: ready-to-use and personalized approaches (2). It is important to notice that the implementation of one does not mean the end of the other, and these approaches can complement each other (92). However, the regulatory implementation cannot be the same.

In fact, a phage preparation *"either prepared industrially or manufactured by a method involving an industrial process"* (77) is a medicinal product subjected to a MA, according to the Directive 2001/83/EC. This current pharmaceutical framework would easily fit to phage therapy if it included only the preparations of fixed composition (2). However, phage therapy brought a great interest when developed as a tailor made concept that is based on a preparation with a variable composition for the need of a specific patient, which does not fit well with the current regulatory framework and hampers the process of establishing an uniform one (96).

5.3.1 Ready-to-use or prêt-à-porter approach

Also called over-the-counter, this model is focused on a predefined polyvalent phage cocktail with a fixed composition against a specific infection or pathogenic target that is manufactured at an industrial scale (2,96). These phage cocktails can be used against an identified bacteria and so the preparation must have multiple phages that target a single bacterium, but when the infectious agent is not identified, the cocktail must have multiple phage strains targeting multiple species of bacteria (22). This concept is designed for a regular use against a common infection and can be found in Russian or Georgian markets and pharmacies since phage therapy is not yet approved in other countries (16).

A medicinal product of fixed composition shows more tolerability, quality and consistency and the pharmaceutical industrial stakeholders are more interested in this approach where a *"one-size-fits-all"* product can better fit with the conventional economical set up (22,53). This approach also matches the current regulatory framework.

In order to reach a relevant efficacy with this approach, a large number and variety of phages must be present in the cocktail. However, the number of available phage strains for medical practice is low and the process of isolating the pathogenic bacteria and selecting the more suitable phage normally results in few or none phage-bacteria

correspondence and is a difficult and expensive one (53,96). Then, there is the problem of uncertainty of phages since its nature is associated with variability in host targeting and triggering phage resistance in bacteria (22,69). And since new phages can be isolated from nature every day, the phage product would need to be frequently updated which would mean that this ready-to-use preparation would be time-limited, which is incompatible with the fixed composition concept (9). The framework of FDA and EMA states that a finished medicinal product that goes through registration and approval cannot be modified, and if the product is altered, a new approval must be granted (96). Worsening the situation, it is not sure if the need for a high quantity of phages in preparations can contribute to the growing and spreading of phage resistance in bacteria, just like it happened with antibiotics (69).

5.3.2 Personalized or *sur mesure* approach

Also called tailor-made or custom-made approach, it is based on a phage preparation designed for a certain patient with a specific bacterial infection, highly depending on the host specificity of phages (83,96). First, the infecting bacteria must be isolated from the patient to be tested for sensitivity or resistance to a collection of phages, in a phagogram (83). Then, one or more phages are selected from a phage bank or from the environment and be formulated in and *ad-hoc* polyphage cocktail to successfully lyse the bacteria (84,96). Phage banks, which are a collection of purified phages, are a prerequisite of this tailored approach, and require a structured phage selection criteria and an updated phage library (22), discussed more ahead. A cocktail specifically prepared for a patient in need can be used as a last resort approach and fall under the Declaration of Helsinki (83), like it happens in Poland; or it can be prescribed by a physician and prepared by a pharmacist, like it happens in Belgium following the magistral phage concept (53).

As mentioned before, phages are continuously co-evolving with bacteria and so, new phages can be isolated from the environment every year, and be added to the phage banks, making sure that phage preparations are updated and optimized.(69,84) This led to the beneficial association of a personalized PTMP against chronic infections, for example (84).

This personalized approach can also be linked to intrinsic sustainability since the phage selection is based on its ability to lyse only the identified bacterium, reducing the possibility of selective pressure regarding phage resistance (69). However, this model can be time consuming if a new specific phage is needed and its manufacture includes isolation, identification and experimental testing that can take days to weeks (9,22). Adding to that, the identification of a pathogenic bacterium causing a more complex

infection in more than one organ may need a more thorough analysis including a biopsy, which delays and complicates the process (96). Then, these customized polyphage preparations are adapted to a specific bacterial infection ad have a variable composition, which compared to conventional medicines results in impracticability of testing a finished product since it is required that the final product must be tested including a quantification of each active ingredient. On a product of variable composition for a unique finished product, the quantification of each active ingredient is an unreasonable requirement. The same thing happens with stability testing and non-clinical studies, which is, again, almost unfeasible for a finished product (11).

Even though this approach is the most promising one for phage therapy, it has numerous limitations regarding regulatory specifications (9). First, since phages are the active ingredient in the preparation, they must follow the industrial standards for its manufacture, and be in accordance with GMP requirements, following a QC scheme, and be attested by a Qualified Person (QP). However, the phage formulation does not have a fixed composition and cannot follow these required standards (11). The regulatory definition of PTMPs can be placed between industrially prepared medicinal products and magistral formulas, but there is no established procedure for this in-between scheme (83).

The Figure 9 schematizes the two complimentary paradigms of phage therapy for clinical application of phage products, from prescription to administration (84).
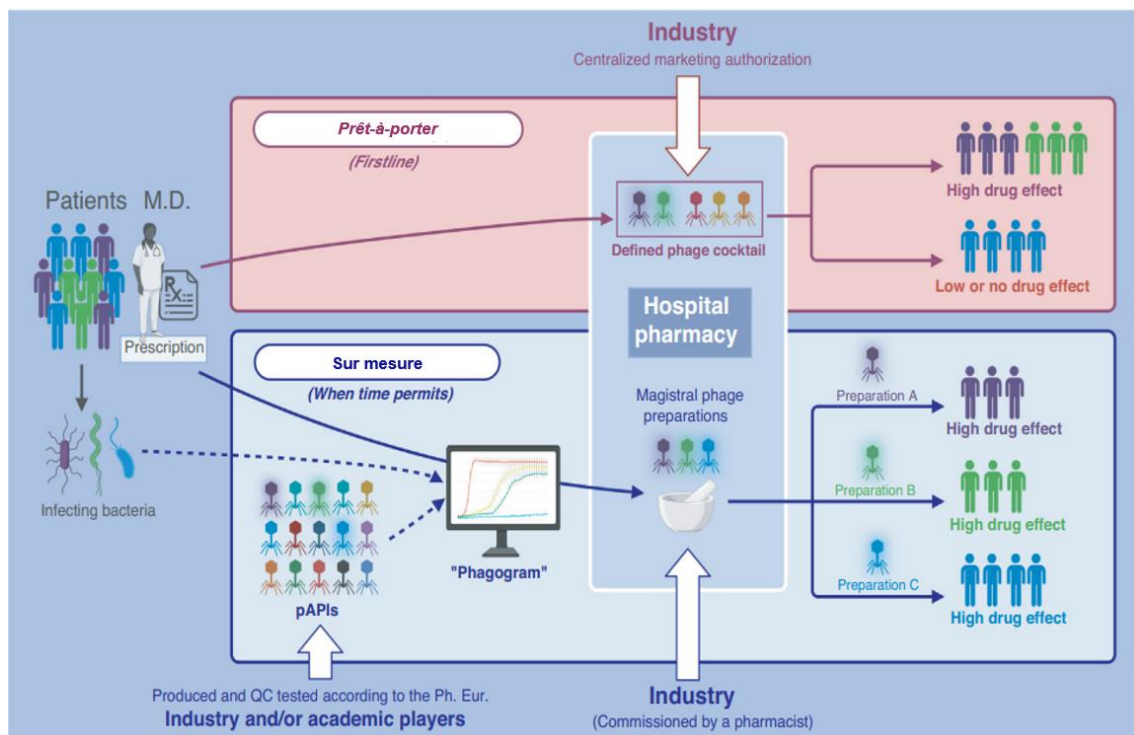

Figure 9 – Representation of the two approaches for phage therapy clinical application. Adapted from (84)

5.4 Regulatory frameworks of Phage Therapy

5.4.1 Learning from the PhagoBurn trial

With the main objectives of testing the safety, efficacy and tolerability of a cocktail preparation with 12 natural phages against bacterial infected burn wounds (59) while following a GMP and GCP manufacturing process, the PhagoBurn clinical trial had the final outcome of boosting the recognition of phage therapy as a therapeutic option to be implemented (86). Whatever the final outcomes, this trial would serve to learn how to better develop a PTMP in clinical trials and to trigger interest among regulatory authorities.

There is no specific guidance for phage preparation or implementation and the pharmaceutical industry and regulatory agencies are trying to apply the existing guidelines for the manufacture and testing of phages (97). For example, this trial followed the GMP manufacturing and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline Q2(R1) for validation and analytical procedures (98). However, the manufacturing of a phage cocktail in accordance with GMPs was challenging. The manufacturing process took twice as much time, from the initial 12 months to actual 25 months (59), since the phage cocktail had 12 and 13 active ingredients (each cocktail) compared to the single active ingredient of an antibiotic and the QC has to be applied to each active ingredient (86). Adding to that, when trying to enlarge the volume of phages through scale-up it was shown that some phages, due to its nature, are difficult to be largely produced, actually resulting in the removal of 2 phages from each cocktail (86,98). In fact, up scalling the production of phages is not recommended, even though phages are natural entities, they are not prepared to being largely produced and it could result in genetic changes and unexpected alterations in phages (92).

After that, it was identified a decrease on the concentration of phages resulting from interactions within the cocktail that altered the phage titer.(59) In fact, using a mixture with more than ten phages is challenging and can affect the stability of the cocktail. Consequently, it was very difficult to establish the shelf-life of the product. It was concluded that the titration method should follow QC validation and the phage cocktail must have a limited number of phages, not more than ten (86,98). This stability hurdles resulted in lower doses of active phages administered, a consequent lower rate of multiplicity of infection that resulted in a slow propagation of phages and a consequent slower bacteria reduction (59). However, despite the lower doses of phages, the lytic cycle still happened, resulting in the lysis of bacteria. This suggests that if the initial

cocktail with the right dose of phages was applied, the elimination of the pathogenic bacteria would have happened much faster (98).

In order to simplify the protocol and to avoid any delay on administration to patients there is a temptation in skipping the susceptibility testing to phages, like a phagogram.(59) However, the PhagoBurn trial demonstrated the importance of testing bacterial susceptibility to the phage cocktail by successfully achieving the primary endpoint of bacteria burden reduction and determining the efficacy of the cocktail before it was administered and along the course of the clinical trial (59,98). Regarding safety, in the PhagoBurn trial phages were applied topically which raises less concerns with serious adverse events than a systemic administration. Indeed, there was an absence of major adverse events which was in accordance with the safety profiles of the used phages (59). Despite these hopeful results, it was not possible to definitely conclude that these preparations were safe and efficacious since the selected population suffered a reduction enough to lose credibility (59).

The main objective of this trial was to test two phage cocktails to prove its ability to perform a therapeutic action against E. coli and P. aeruginosa infections. Other endpoints were also tested like GMP production of the product, the application of testing and QC procedures to phage preparations, and the clinical testing according to GCP to prove the efficacy and safety of these preparations. These achievements represent a great progress regarding phage therapy development and a future reference for market implementation (98).

5.4.2 Phage Banks

An important issue when implementing phage therapy is based on storage and availability of phages. The nature of phages makes its synthetically produce a very difficult option, which means they need to be extracted from a biological source and be used directly or stored. The first option is based on taking advantage of the infected patient and its biologic material from whom the bacteriophage could be isolated, without needing long-term storage. However, this would require on-site facilities for identifying the phages and test the susceptibility of bacteria, on every place where phage therapy could be administered. Also, this option can only be available when a customized treatment is being conducted (92). The second option, already being developed, is by establishing phage repositories following GMP standards that are carefully monitored, allowing the availability of vast a collection of phages to be used in treatment. These phage repositories are called phage banks (63).

An established phage bank system cannot work without a corresponding bacteria bank system, since the preparation of a phage suspension requires a phage seed obtained from a bacterial cell substrate (11). The international guideline ICH Q5D *Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products* (99) should be followed for the preparation of the phage suspension. Following the guideline the banked phages and bacteria must be characterized for phenotypic and genotypic markers to ensure indentity, viability, potency and purity (90). The FDA also provides requirements for industry in the production of viral vaccines on *Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications* (100) that could be applied to phages. In here, a set of recommendations for characterizing and qualifying cell substrates, viral seeds and other biologics, could be applied to phages. Following this document, it is recommended to have both a master cell bank with viral seeds of uniform composition and a working cell bank that uses vials from the master bank to expand the available viral seeds which represents a useful method when producing large number of batches, as it may happen with a PTMP of fixed formulation, industrially prepared (100). However, on a customized PMTP, a master cell lot without a working lot could be sufficient. Both phages and bacteria should undergo genotypic and phenotypic characterization, viability, potency, purity (90) and additionally, bacteria must be tested for identity, absence of prophages, ARGs and virulence factors (11).

Not only a customized approach of phage therapy would highly depend on these phage banks, but the phage preparations of fixed composition would also benefit from available collections of well-characterized and stored phages to be used in preclinical and clinical environments (101). It is then crucial to develop a license scheme for phage libraries to facilitate the availability of therapeutic phages, especially against the most pathogenic bacteria, while ensuring safety and quality. These phages are ready to be combined on a custom-made formulation, and in order to be selected, they are tested with a bacterial isolate from the patient to test for sensitivity (90). Not only the authorities would develop this licensed phage collections, but also academic and other non-profitable organizations and of course, private companies (96). However, this licensing would require a major shift in what is considered the "normal" regulatory processing (11).

Since no MA has been granted to a PTMP, the first step is to understand which information the applicant should gather to submit a MAA (MA application) in Europe, also called Biologics License Application (BLA) in the US (11). These documents must follow the Common Technical Document (CTD) format, an international set of guidelines for

registering medicines from the ICH. In Europe, the CTD format is mandatory when submitting an MAA, whereas in the US it is optional, but strongly recommended when submitting an new drug application (102).

5.4.3 Adapting the current regulatory framework to overcome phage therapy limitations

The difficulty in presenting enough clinical evidence and the lack of guidance and guidelines on how to implement phage therapy resulted in a delayed development of these phage-based products with no PTMP introduced in the market since no MA has been granted to these products (11). However, phage therapy can benefit from the work and investigation done with other *"new"* or *"undefined"* therapies to fit in the pharmaceutical market as it is established (11). Plus, if this therapy is considered the solution for an urgent or unmet medical need of an persistent infection, it can be used as an experimental treatment without finished clinical testing and without a MA (87).

The multi-strain dossier

This concept is currently used in the veterinary field for the regulation of inactivated vaccines against avian influenza, blue tongue and foot-and-mouth disease, that require fast and/or frequent changes in the strains of the final product and which (100), consequently, do not fit well with the regulatory scheme of vaccines (11).

The *Guideline on data requirements for multi-strain dossiers for inactivated veterinary vaccines* (100) defines the multi-strain concept as *"a single dossier containing the relevant data for a unique and thorough scientific assessment of the different options of strains/combinations of strains permitting the authorization of inactivated vaccines against antigenically variable viruses (…)"*, meaning, that the applicant or authority needs only one dossier for the different strains of the same vaccine (100). Which won't require a separate authorization for each vaccine strain or a new authorization for a new combination of vaccine strains. Instead, the terms of the MA would suffer a variation covered on the Regulation (EC) No 1234/2008 *concerning the examination of variations to the terms of marketing authorisations for medicinal products for human use and veterinary medicinal products* (103). This Variations Regulation stipulates the time needed for efficacy and safety assurance depending on the type of variations or extension applied to the MA existing: minor variation type IA or IB, major variation type II and extension of AM which is the longest assessed. The multi-strain dossier is considered a type II variation since it is a modification to the strain or combination of strains (100).

If applied to phage therapy, this concept could solve some of the uncertainties at regulatory and scientific levels. Regarding phage preparations production, the multi-strains dossier guidelines (section 7, IIIb.2.A1) states that a maximum number of strains should be established for the final product (100). Something that should be considered when applied to phage strains as was also concluded in the PhagoBurn trial. Another benefit from this concept if applied to phage therapy is based on the suggestion of clinical testing, methods validation and specifications establishing on a finished product of a single strain to further be extrapolated to a strains-combined finished product (section 7, Section IIIb.4 Part 4) (100). To phage therapy, the stability or/and efficacy testing could be made using a single phage product and that information could be used for a polyphage finished product combining those pre-tested phages (11).

Regarding regulatory concerns, the big problem of phage therapy regarding approval of a finished product with a variable composition could be solved (11). In fact, the guideline (100) states that an MA for a multi-strain dossier should *"specify the strains that may be included in the final product as well as the maximum amount and number of strains"* but *"the number and type of strains included in the final product should be adapted to the current epidemiological situation at the time of formulation of the final product"* (100). Which means that a PTMP could be approved with a stipulated composition and be released with an updated and/or customized composition, as long as it is correctly labelled (86). This approach could be used for PMTPs of fixed composition and industrially prepared.

However, besides being a concept for veterinary use only, the guidelines clearly stipulates that the submission of a multi-strain dossier for emergency use is also not appropriate (100), which could be the case of a tailor-made PTMP. But phage therapy can still take advantage of the Variations Regulation. Any modification in the composition of a phage cocktail would result in the replacement, addition or elimination of a phage strain which would be considered as a new product that would require a new MA (77). Nonetheless, besides the multi-strain dossier, another exceptional case, included in human medicinal products, suffered a change of active substance and did not need a new MA, and was additionally assessed as a type II variation: the human influenza vaccine, which, in the US is named FluMist (103). Replacing a phage strain with another could follow the process of the influenza vaccine that was accelerated, with a shorter assessment justified by a seasonal disease. It would still be a complex process that needed to ensure efficacy and safety despite the finished product being modified (90).

The homologous group

The *Guideline on allergen products: production and quality issues* states *"Due to the high number of allergens in an allergen extract or in an allergen extract mixture and the cross-reactivity of the individual components, it is impossible to determine all relevant parameters for the allergens within a given extract or a defined allergen extract mixture"* (91). To tackle this situation, an *"extrapolation of stability data among members of taxonomic families were (…) used by applicants"* (91) in the previous *Note for Guidance on "Allergen Products"* (CPMP/BWP/243/96). However, this guidance had poor limits and the homologous group concept came to replace the taxonomic families' concept (91).

The homologous group concept allows for the same data extrapolation but instead of basing its division groups by taxonomy, these groups are *"defined and justified by scientific criteria, restricts extrapolation to a few parameters while at the same time it retains the flexibility needed"* (91). A member of each homologous group is "selected as the representative species" and then *"to a limited extent, data on quality, safety and efficacy can be extrapolated from the representative source to other members of the homologous group"* (91). Of course the applicability of this concept could face many challenges, mainly when decisions making on which characteristics should the phages groups be based. It this concept could be considered for phage therapy, a lot of work and study would be needed.

As already mentioned, the great diversity of bacteriophages must require some flexibility when developing a phage product and the homologous group concept may be the solution (11). The representative phage would provide the required data, for example, on stability, on safety for nonclinical studies, an environmental assessment risk in genetic altered phages, but the other phages of the group would not have to present such extensive data. This would also facilitate the introduction of new phage strains and/or mutants to the phage libraries (11).

This concept also brings an important detail about the correct characterisation when isolating phages. Following the example of allergens, the taxonomic group was replaced with the homologous group which suggests that a group based only on taxonomic criteria is not limiting and strong enough. And so, when isolating phages, its characterisation should include genetic sequencing accompanied by a phenotypic characterization (11).

The hospital exemption

This concept was brought as an exemption applied to some ATMPs. The Regulation (EC) No 1394/2007 on ATMPs (93) requires that all these products must obtain a MA

through a centralized procedure and demonstrate equivalent standards compared to other medicines (104). However, some ATMPs were not produced with the intention of market introduction, even though some manufacturing processes were the same, the finished product was adapted for a specific patient, without the purpose of profiting. And so, the concept of a marketing authorization ceased to be applicable (8).

The Regulation (EC) No 1394/2007 in Article 28 (2'7) amends the Directive 2011/83/EC stating that any ATMP *"prepared on a non-routine basis according to specific quality standards, and used within the same Member State in a hospital under the exclusive professional responsibility of a medical practitioner, in order to comply with an individual medical prescription for a custom-made product for an individual patient"* (93) is exempted from following the Directive and from mandatorily getting a MA (77). These ATMPs are subjected to a national legislation and authorization by the competent authority of the Member State and *"shall ensure that national traceability and pharmacovigilance requirements as well as the specific quality standards (…) are equivalent to those provided for at Community level"* (93).

As already discussed above, PTMP cannot be considered an ATMP, and consequently, cannot benefit from the exemptions applied to it (92). However, the similarities between both therapies, specially the concepts of a *"non-routine manufacturing"* of a preparation for an *"individual patient"* leave open the possibility of a tailor-made approach of a PTMP based on these ATMPs (8). In order for PTMPs to benefit from this exemption, either are introduced in the ATMPs as a new category, at the same level of gene, somatic-cells and tissue engineered medicinal products, or, and probably the best option, by applying a specific hospital exemption to phage therapy (92).

Even though these ATMPs are exempted from a MA, the national rules still need to match the quality standards of the centralized marketed products, specially the GMP standards, which should be similar (92). This topic may be difficult for hospitals implementing phage therapy, since complying with GMP standards is not easy.

This Regulation also prevents the possible unbalanced competition between ATMPs following a centralized MA and ATMPs following the hospital exemption stating in Article 19(1) that *"the fee for marketing authorization shall be reduced by 50 % if the applicant is a hospital or a small or medium-sized enterprise and can prove that there is a particular public health interest in the Community in the advanced therapy medicinal product concerned"* (93). This problem can also be addressed, in the future, when developing both ready-to-use and tailor-made phage preparations.

5.4.4 <u>Possible regulatory framework based on current approaches</u>

The autogenous (or autologous) vaccines

This concept is applied to veterinary vaccines defined in the Directive 2001/ 82/EC on the Community code relating to veterinary medicinal products as "inactivated immunological veterinary medicinal products which are manufactured from pathogens and antigens obtained from an animal or animals from a holding and used for the treatment of that animal or the animals of that holding in the same locality", Article 3(b) (105). The definition is actually given as an exemption to the Directive 2001/ 82/EC, with national authorities responsible for accepting these products on the correspondent Member State (11). For this reason, there have been different approaches for implementing the autogenous vaccine concept.

In France, a well-developed regulation for national implementation of this concept states that the preparation of veterinary autogenous vaccines should be undertaken by a qualified person or company or organization employing a qualified person that acquired a license by the French Food Safety Agency.(106) So the authorization is granted to the QP and not to the vaccine (11). These vaccines follow the Good Preparation Practices (GPP) ensured by the authorities that conduct inspections. The pharmacovigilance information on safety and efficacy should be reported by the prescribing veterinarian (11,86).

Even though these vaccines' guidelines tend to be based on those for industrially prepared products, they can actually be tailored and follow the rules of magistral formulas. This ambiguous definition situation is similar to phage therapy, and could help model a regulatory framework for phage based products just like Belgium did with the so called magistral phage (11).

However, besides being a veterinary product, vaccines in general have also a different fundamental action as PTMPs. Vaccines principal objective is immunological triggering, instead of the therapeutic effect of PTMPs. Also, these vaccines are made of inactivated pathogens, and PTMPs is based in living viruses (11).

Compassionate use

The Regulation (EC) No 726/2004 of the European Parliament and of the Council *laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use,* Article 83 (107) present a legal framework for the therapeutic application of unauthorized medicinal products, also called compassionate use (87). As stated, the compassionate use has two principal requirements: it can only be available for a group of patients *"with a chronically or seriously debilitating disease or whose disease is considered to be life- threatening, and*

who cannot be treated satisfactorily by an authorised medicinal product" (107) and the *"medicinal product concerned must either be the subject of an application for a (centralized) marketing authorisation or must be undergoing clinical trials"* (107). EMA provides a *Guideline on compassionate use of medicinal products, pursuant to article 83 of regulation (EC) no 726/2004* on how to implement the compassionate use, but each Member State is in charge of implementing and coordinating this concept according to the correspondent national rules (96).

This concept could be applied to the tailor-made version of phage therapy, however, the regulation clearly states that the compassionate use is intended for a specific group of patients (107), and not a highly specified PTMP to treat a specific patient with a bacterial infection.(83) And so, phage therapy cannot follow this approach, but can benefit from and adapted compassionate use to a named-patient basis (83).

In the US, a similar approach was adopted by the FDA, called expanded access on the Code of Federal Regulations (CFR), title 21, part 312.3 (subpart I) (78) where an unauthorized medicinal product is applied to *"serious diseases or conditions when there is no comparable or satisfactory alternative therapy to diagnose, monitor, or treat the patient's disease or condition"* (78) but in this case, it can only be used outside clinical trials (87).

The "specials" scheme/ named-patient exemption

When consulting the Directive 2001/83/EC, the Article 5 presents a set of possible exemptions from its requirements, including for *"medicinal products supplied in response to a bona fide unsolicited order, formulated in accordance with the specifications of an authorised health-care professional and for use by an individual patient under his direct personal responsibility"* (77). This exemption allows for a compassionate use of a product but on a named-patient basis, and not for a group of patients.(83) Which means that an unauthorized medicinal product could be used for an individual patient without any better therapeutic options that are ineffective or non-existent (97).

This definition matches the paragraph 37 of the Declaration of Helsinki of the World Medical Association (108) on *Unproven Interventions in Clinical Practice* that states *"In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering"* (108). Which means that the need of a patient must be correctly justified so that this exemption can be applied. Phage therapy could

be introduced as an alternative to ineffective antibiotics and MDR bacteria for which no other solution exists.

Even though the compassionate use definition is the one referred above, nowadays, when mentioning the term "compassionate use" it is both a definition and a regulatory pathway for a special access to a specific patient (82). From now on, the term "compassionate use" will be addressed following this definition.

In the US, a similar approach was adopted by the FDA, called expanded access (or more commonly known as compassionate use) on the Code of Federal Regulations, title 21, part 312.3 (subpart I) (78) where an unauthorized medicinal product is applied to *"serious diseases or conditions when there is no comparable or satisfactory alternative therapy to diagnose, monitor, or treat the patient's disease or condition"* (78). In the US, the compassionate use is regulated by the regulatory authority, the FDA, and in Europe the EMA provides directives on how to process the compassionate use, but each Member State manages independently these directives accordingly to the correspondent national regulation (82).

The compassionate phage therapy has been approved in Poland by the national regulation that established a legislation for phage therapy without a MA (82). But in the US and other EU countries, the compassionate use of phages has to be done on a special access. The QAMH, in Belgium, has occasionally treated patients with phages under the paragraph 37 of the Declaration of Helsinki, requested from patients of neighbouring countries that do not have this concept implemented and available (80).

In France, the French National Agency for Medicines and Health Products Safety (ANSM) approved the compassionate use under a temporary use authorization (82), following and assessing each specific case (80). Since phage therapy is not an approved product, they do not need to follow standardized industrial processes (70,80). The Pherecydes Pharma and QAMH produce bacteriophages that do not follow GMP standards to be formulated, but present a similar quality. The Temporary Specialized Scientific Committee (CSST) was created to evaluate and discuss the applicability of phage therapy and send expertise recommendations to ANSM (96). In fact, in 2019, the committee concluded that *"all of the issues raised by phage therapy have led to a plea for the setting up of a national platform for the orientation and validation of the use of phages in order to manage this use in France and which could eventually work towards the implementation of academic production of phages for clinical use from a phage library. In view of the critical issues at stake, it is expected that this platform will be set up at a ministerial level with the authorities involved in the organization of care"* (80). This

plea was followed by the development of a technical report to ensure the safe use of phages in France (80). The request for the compassionate use of phages must be done by a prescribing physician and be produced under its entire responsibility and the hospital pharmacist (80).

In UK, the Medicines and Healthcare Products Regulatory Agency (MHRA) released guidance documents on the supply and manufacturing of unlicensed medicinal products (109). The medicinal product must be *"specially manufactured or imported to the order of a doctor, dentist, nurse independent prescriber, pharmacist independent prescriber or supplementary prescriber"* (109) which means that after a prescription, the health professional contacts directly the supplier that manufactured the product (11). The supplier must hold a Manufacturer's "Specials" license for its manufacturing facility that should comply with GMPs, and the batch must be released by a quality controller (11). The "Specials" do not need to be released by a QP and do not follow the pharmacovigilance requirements, since they are not authorized products (11).

This named-patient approach has been proposed as a regulatory model for personalized medicines which can include the tailor-made PTMP (11). Even though it would be a great option for phage therapy application on a named-patient basis, it could not be used for a broad group of infected patients. If phage therapy is to be prospectively considered as an alternative to antibiotics, then a much correspondent broader approach must be considered (11). This concept is poorly detailed in the Community Code, and when applied, it is differently licensed within Member States, and so, it is mostly used for the reformulation of licensed medicines (83).

In this regard, the Article 5 of Directive 2001/83/EC presents another option for the use of an unauthorized medicinal product stating that a *"Member States may temporarily authorise the distribution (…) in response to the suspected or confirmed spread of pathogenic agents, toxins, chemical agents or nuclear radiation any of which could cause harm"* (77). It has not been possible for phage therapy to benefit from this exemption yet, but an outbreak of MDR pathogenic bacteria may require such measure.

The magistral formula

Sometimes, the pharmaceutical industry cannot meet the specific needs of a patient through the medicines available on the market and so, a customized preparation could be the solution. This practice is called pharmaceutical compounding on which the doctor can have access to personalized treatments for a specific patient that are not available (53) and do not require regulatory approve, being regulated by the national authorities in Europe or by state boards in the US (110). The differences in definition, applicable

standards and compounding settings of this practice between Europe and US result from lacking of an harmonized international regulatory framework (110).

In the EU, the compounding practice is defined in the Directive 2001/83/EC, Article 3 in two categories: the paragraph 1 refers to *"any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient"* (77) known as magistral formula, and paragraph 2 refers to the officinal formula , which is not relevant in this case. The physician must prescribe the magistral formulation for a given patient, which is prepared by a pharmacist or under the supervision of a pharmacist following specific standards (53). The regulation of the magistral formula is mainly assured by the national authority since an harmonized framework hasn't been reached (110).

In the US, the compounding concept hasn't also been a harmonized concept, but the Compounding Quality Act in Title I of the Drug Quality and Security Act exempts these compounded preparations from several requirements and ads new requirements to ensure quality and safety production on the compounding facilities (110). These facilities can be pharmacies (or outsourcing facilities, but are no relevant in this case) that prepare the prescribed compounded medicines for a certain patient and are assessed by the State Boards of Pharmacy, also called compounding pharmacy (110). The compounding process is done by a pharmacist or a licensed physician that mix, combines or alters the ingredients to form a personalized drug for the need of a specific patient (111). The compounding pharmacy in the US matches the definition of the magistral formula in the EU (96).

The magistral phage

In January 2018, the Belgian Federal Government in cooperation with the Federal Agency for Medicines and Health Products (FAMHP, the competent authority for medicines in Belgium) were able to develop a regulatory strategy for phage therapy based on the magistral formulation: the magistral phage (87). The active ingredients, in this case phages, must meet the requirements of either the European Pharmacopeia, the Belgian Pharmacopeia or an official Pharmacopeia. In the absence of one of these documents, the Minister of Public Health under the advice of the national Pharmacopeia Commission can be responsible for authorising these active ingredients (53).

If these ingredients are not authorized they can still be compounded in a preparation if a certificate of analysis is licensed by a Belgian Approved Laboratory, a quality control laboratory accredited by the Belgian regulatory authorities (53). The certificate should present data on identity and quality control concluded through current standards methods (11). Through this accreditation, these laboratories are in charge of testing the

batch release of the medicinal product, which is equivalent to the GMP certification (53). In the case of phage therapy, this allowance for unauthorized active substances in magistral formulation was maintained since it would be impossible to license every individual phage as an active ingredient to be used on magistral formulation (11).

The magistral phage is considered an enhanced approach of the magistral formula (11). Besides involving the medical doctor, the patient, the manufacturer, the approved laboratory and the pharmacist required for a standard licensing of unauthorized active substances, the magistral formula elaboration also requires the involvement of the FAMHP. And so, the FAMHP offers the possibility of scientific and/or technical information exchange between the stakeholders trough the existing concept of the Scientific-Technical Advice (STA) procedure (53). This is a voluntary procedure, with no legal obligations, which should be based on a reliable and honest communication between each party. By gathering these enhanced features, the uncommon and innovative nature of phage therapy could be overcome (11).

The Biological Master File

The Master File concept was introduced in the US for an applicant developing a medical device. Commonly, the developer of a device needs access to the product or the manufacturing facilities of another party, that consequently does not want to disclosure its secret or confidential information. The master file concept allows the preservation of information from the medical device industry while allowing its harmonized exchange of data. This concept was adapted for other products, including biological products, named Biologics Master Files submitted to CBER (112).

Even though a regulation for the Biologics Master File concept is still being processed, the applicants can submit it to CBER as a voluntary submission of information to provide confidential information to the FDA about the facilities, processes and utilities used in manufacture, processing, packaging, or storing of a licensed product (113). Other applicants or drug developers, may be authorized by the Master File holder, to reference information from the Master File to support a submission to FDA without disclosing confidential information (113).

In the EU, it was developed the concept of Active substance master file (ASMF) procedure. However, the *Guideline on Active Substance Master File Procedure* in Annex 5 does not allow the biological active substances to follow the ASMF procedure, since a biological must be approved as a whole and not from an active substance to another (114). But then again, vaccines are also excluded for the applicability of this concept, and some have been certified with a vaccine antigen master file (VAMF) certification.

According to the Guideline for VAMF certification, a VAMF *"contains all relevant information of biological, pharmaceutical and chemical nature for one given vaccine antigen, which is common to several vaccines from the same MA applicant or MA holder"* (115). This certification allows *"reducing the number of dossier submissions and data evaluations carried out for the same vaccine antigen"* and *"harmonising the data for a given antigen present in several vaccines"* (115) which ensures consistency throughout the European Commission. It is impossible not to notice the similarity with phage therapy.

This VAMF concept could be adapted to phage therapy as a Biological Master File (BMF) that would undertake a centralized assessment to be valid throughout the European Commission, through a Certificate of Compliance. Instead of following the master file for an antigen, in the case of phage therapy, it would be applied to the active substance: phages. The use of a master file is optional, however, if the applicant decides to opt for a BMF procedure, it must be submitted for all the active substances of the final preparation, where one BMF is submitted per phage (115). The diagram of the Figure 10 resumes the process.
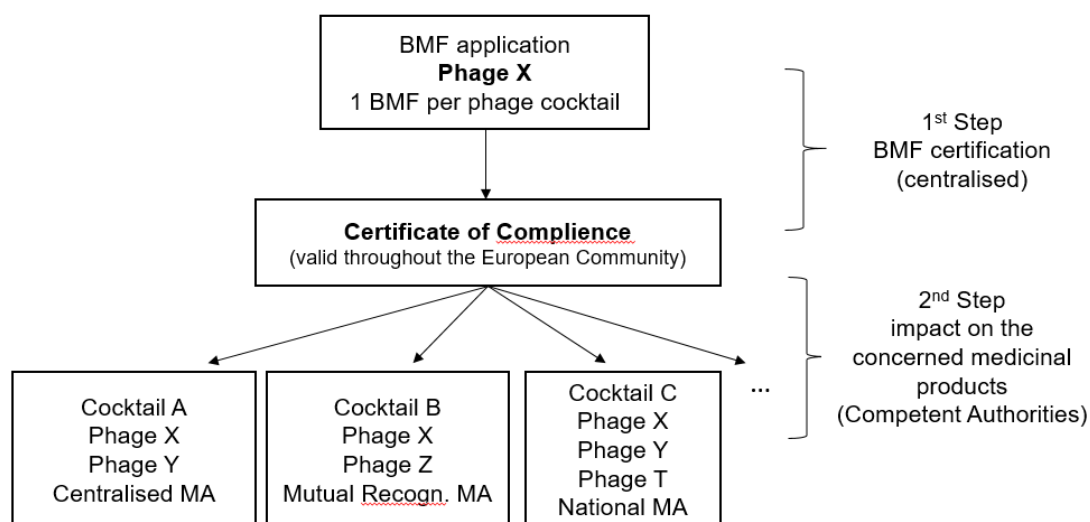
Figure 10 - General principles of a Biological Master File (BMF) for a phage product, based on the (vaccine antigen master file (VAMF) procedure. Adapted from (115)

A BMF applied to a tailor-made PTMP could solve some industrial and safety problems that are delaying its regulatory implementation (53). The submission of a BMF for each individual phage or to homologous groups of phages would cover the manufacturing of each phage, which would allow for the production of phages to be subjected to licensing procedure, the need to submit a quality module for approval, compliance with GMP manufacturing and batch release by a QP. Also, the safety profile would be performed on each active substance following the BMF, since it is impossible to submit the complete non-clinical data of a personalized polyphage preparation (83).

In this way, PTMPs could be licensed at the active substance level and not at the finished product level, to be used in the magistral phage.

Orphan Designation

In order to support and facilitate the development, evaluation and authorization of new therapeutics for a rare disease the regulatory authorities are allowed to grant the orphan designation to a medicinal product targeting these diseases. A rare disease is defined as any disease affecting only a small percentage of the population, between 6-7% of the population, however, this definition can vary depending on each jurisdiction (116).

In the EU, the purpose of the Regulation (EC) No 141/2000 on orphan medicinal products on Article 1 is to *"lay down a Community procedure for the designation of medicinal products as orphan medicinal products and to provide incentives for the research, development and placing on the market of designated orphan medicinal products"* (117) and is responsibility of the Orphan Regulation also established the Committee for Orphan Medicinal Products (COMP) (116). In Europe, a rare disease is a *"life-threatening or chronically debilitating condition affecting not more than five in 10 thousand persons"* (117). The orphan designation is granted by the European Commission and offers the following incentives: protocol assistance of scientific advice, access to a centralized market authorization without having to present complete non-clinical and clinical data, ten years of market exclusivity, in other words, ten years of protection from market competition, fee reduction and other specifications (118). The orphan designation is removed if the above criteria ceases to exist at the end of the 10 years exclusivity (117).

In the US, the orphan designation is granted to a drug if the *"number of people affected by the disease or condition for which the drug is to be developed is fewer than 200,000 persons"* (78) as stated on the 21 CFR 316.21(1). The Office of Orphan Products Development (OOPD) is responsible for evaluating the requests for orphan drug designation, assessing the development of products for the diagnosis and/or treatment and for organizing a database of orphan drugs (116). FDA also provides some incentives to the orphan drug's sponsor: tax credits for qualified clinical trials, exemption from user fees and potential seven years of market exclusivity after approval (85). Once the FDA grants an orphan designation it can only be removed if false data or omitted information are detected (116).

If an MDR bacterial infection is causing a disease with potential to be considered a rare disease, a PTMP could be granted with the orphan designation and be administered

under the compassionate use (118). The pharmaceutical company named Adaptive Phage Therapeutics (APT) has received the Orphan Drug Designation for the PhageBank as an indication against prosthetic bone and joint infections caused by MDR bacterial infections, approved by the FDA (95,119). Meaning that this approach can easily be applied to phage therapy, specially under the compassionate use or magistral formula.

6. Discussion

As previously discussed, no MA has been granted to a PTMP following a set of regulatory difficulties, which in turn results from the difficulties to provide relevant safety and efficiency proof in clinical studies. And in order for the clinical testing to grow the pharmaceutical industry and regulatory authorities need to make an investment in its development, which, in turn, it's not appealing without a scientific and regulatory basis (8). This loop needs to be broken. In fact, the application of phages with other purposes may help establish a regulatory approval (61).

As a matter of fact, in 2006, the FDA has approved a food additive containing phages called ListShield (24). As the name suggests, a phage cocktail against Listeria was used in a disinfectant spray for deli meats ready to consume. This phage-based food additive was recognized as *"generally regarded as safe"* (GRAS), being the first phage product granted with such designation. Which means that the possible implications associated with the approval of phage products could be more easily managed (24,61).

To this day, the application of phage therapy has been based on the adaptation of the current approaches and regulatory frameworks to try and gather useful data and experience while taking advantage of eventual approval schemes that may fit. And so, the relevant authorities must develop a dual pathway capable of ensuring a well-structured framework that at the same time allows and encourages the participation and investing of specialized companies (61).

In order to correctly fit phage therapy within a regulatory approach it is first important to define it within the current framework. As already discussed, a phage product is considered a medicinal product or drug, which requires isolation, characterization and propagation according to GMP standards with the final purpose of obtaining a MA (7). However, developing a medicinal product in compliance with GMP standards is a real challenge and depends on extensive financial resources. As a medicinal product in development, some features like impurity and stipulated dose are not defined, since no clinical trial was concluded to present this information. And so, the clinical trials on this in-developing product can help solve some of the uncertainties associated with phage production (7). However, the risk is high with no guarantee of positive results. Bearing in mind that the main organizations currently developing phage therapy are non-profit, or hospitals, academia or small or medium companies, which means only having access to a tight budget, they can't afford to invest on a risky and expensive therapy (8), which represents an obstacle for the development of phage therapy. From this point, an interested investor will try to develop its phage product out of the scope of the Directive

2001/83/EC and Regulation 1394/2007 in the EU or from the Federal Food, Drug, and Cosmetic Act (92).

Since phage therapy has not been included in any category inside a medicinal product, exemptions from other regulated medicinal products could be used (92). As already discussed, a therapy based on altered and engineered phages with the purpose of enhancing its infectivity may belong to the class of ATMPs, if developed with flexible technical requirements and regulatory schemes, considering the phage's unique features (7). As so, they may benefit from a centralized procedure if produced with a fixed composition, or benefit from the hospital exemption if developed for a customized preparation, and follow a national procedure. In the case of natural phages, the ATMP status is not suitable but could be adapted (92). In fact, PTMPs are easily compared with other medicinal products integrated within ATMPs. For example, according to the Directive 2001/83/EC in Annex I part IV, a therapy based on somatic cells substantially manipulated or used in a non-homologous way integrates the ATMP class (77). And since phages can be cultivated, which, according to the Directive, is considered a manipulation action, they could fit in the class of ATMPs (92). Also, the autologous ATMPs englobing somatic cell therapy and tissue engineered medicinal products share with phages the need for a customized preparation since its composition changes from one patient to another. These products are process-driven basing its quality, non-clinical and clinical data on a risk-based approach, which is suggested for a customized PTMP. Adding to that, the hospital exemption can be applicable to an ATMP that fulfils a number of criteria, as already discussed, which is the same criteria gathered by some customized PTMPs (8). The similarities suggest that PTMPs should either be integrated as a new viral category within the ATMP framework, or, if integrated in the biologics or in a new framework, a new hospital exemption could be designated for PTMPs (92).

If, in contrast, a phage product is considered a biological medicinal product, the PTMP must follow the Directive 2001/83/EC. A biological PTMP of fixed composition could be produced as a phage cocktail targeting MDR bacteria following the experience of vaccines, beneficiating from its development and manufacturing process (7). However, bacteriophages are associated with an evolutionary instability resulting from the co-existence with bacteria. As bacteria evolve, bacteriophages match the evolutionary update resulting in a new strain of phages which, if considered for therapy, needs to undergo clinical, regulatory and ethical approval and additional time and costs for the producer. And for this new strain to be added to the phage cocktail, according to the FDA and EMA regulations, it is considered a new product and thus requires a new approval (24). Also, if for some reason, a large-scale resistance to the phage preparation

is developed, the product ceases to have a purpose and must be taken of the market. Which can happen very early in the lifecycle of the product and consequently represents a major risk for the MA holder. The best way to tackle this situation would be based on a yearly update of the phage preparation, following the example of the flu vaccine (92).

Without entering in too much detail, the European variations also called post-approval changes in the US could considerate a phage strain modification in a cocktail as a type II/PAS variation (120). Table 6 can help understanding the similar concepts in both Europe and the US. A phage cocktail would require the addition, removal or replacement of a phage strain which is categorized as a change needing an MA extension, which means that it must be granted as a new MA or must be included to its related MA (103). However, in Annex I of the Regulation 1234/2008 is stated that the extensions of MA are an exception for the replacement or addition of a strain or a combination of strains and for "changes to the active substance of a seasonal, pre-pandemic or pandemic vaccine against human influenza" (103). A well-defined exception like this could be developed for PTMP variations. However, some concerns may be raised when comparing both viruses. First, phages are expected to be more unstable compared to the better-studied and more predictable flu viruses. And so, there is no data regarding altering a phage formulation that ideally can have up to 10 phages that, compared to the 2022/2023 quadrivalent flu vaccine (121), is a much riskier change. The homologous group concept could be introduced to assure safety and stability based in phenotypic similarities instead of powerless taxonomic comparisons (11). And so, the concept of a yearly updated phage product could benefit from the experience of the manufacturing methods of the multi-strain dossier but with its own exception when performing an alteration to its formulation as a type II variation, with the safety and stability of phages having a solid testing basis on phenotypic criteria (103).

Table 6 - Summary of variations and anticipated implementation dates in Europe and US. Adapted from (120)

Europe				USA		
Variation	Type	Anticipated implementation time	Guidelines approval timeline	Type	Anticipated implementation time	Guideline approval timeline
Admin	Type IA$_{IN}$	14 days before submission	N/A	AR	Up to 1 year before submission	N/A
	Type IA	Up to 1 year before submission	N/A			
Minor	Type IB	Up to 3 months after submission	30 days	CBE-0	One receipt of submission by FDA	N/A
				CBE-30	30 days after receipt of submission	6 months
Major	Type II	Up to 6 months after submission	60 days	PAS	Up to 6 months after submission	4 months

Notes: AR- Annual Report, CBE- Changes Being Effected, PAS – Prior Approval Supplements

A completely different scheme must be followed to approach a customized biological PTMP. First, if concluded that phage therapy can't indeed fall under the ATMP category, then, and as already concluded by several regulatory authorities, it must fall under the biological medicinal product definition. But, as so, it cannot benefit from a hospital exemption exclusive for the benefit of ATMPs (104). Besides all the obvious advantages brought by hospital exemption, PTMPs could also beneficiate from the experience gathered through the national procedure. In other words, the development process of phage therapy in each Member State could gather enough experience to develop a more structured framework (8). An adapted hospital exemption could then be applied to a customized PTMP inside the biologics. However, this may not be the more suitable option for customized PTMPs.

As already mentioned, a biological PTMP developer will try and fall out of the scope of the Directive 2001/83/EC, for which the Community code presents Articles 3 and 5 (77). Article 3 is referring to the magistral formula and Article 5 is applied to the named-patient scheme, with some similarities and differences between them. Both Articles define the set of criteria that should be gathered so that a product can be exempted from its Directive (77). Either clearly stated or indirectly referred, both Articles require a

particular need of a patient in order to justify the exemption applicability. This point is also important when trying to differentiate these two approaches with the hospital exemption. Even though a great similarity can be found between hospital exemption and the named-patient scheme if both would to be applied to phage therapy, the hospital exemption would lack the patient special need criteria (77).

The hospital exemption already gives space for some worrying when used in ATMPs, which could be passed on to phage therapy. A nationally approved hospital exempted ATMP may lead to a decrease in prescriptions for centralized approved products by the physicians. This problem should be addressed in regulatory guidelines stating that an ATMP should not follow the hospital exemption if an existing equivalent centrally approved product can be used or if, in the meantime, an equivalent one becomes available (104). The United Kingdom addresses this concern in its legal framework for the named-patient/ "specials" scheme stating *"an unlicensed medicinal product may only be supplied in order to meet the special needs of an individual patient. An unlicensed medicinal product should not be supplied where an equivalent licensed medicinal product can meet the special needs of the patient"* (109). The hospital exemption is not clearly limited like the "specials" scheme and, for this reason, the regulators and manufacturers show preference for the latter. Indeed, the regulatory authorities are asking for better defining boundaries to harmonize the situation, but it is predictable that an unclear and open-to-mistakes concept should not be used by phage therapy (104).

So, what would be the more suitable approach for a customized development of phage therapy? As already stated, the producers and investors are not interested in following the Directive for time and money consuming reasons which results in the application of exemptions only possible through the national procedure. Some authors have raised the following question: should a tailor-made PTMP be included in the Human Medicinal Product Directive? In fact, according to Article 2, the Directive *"shall apply to medicinal products for human use intended to be placed on the market in Member States and either prepared industrially or manufactured by a method involving an industrial process"* (77) which, in the case of customized phage products, only works if falling under the scope of an exemption. In fact, besides not being industrially prepared, the current hospitals developing customized phage formulations for in-house use are not interested in placing these preparations in the market (92). This definition matches the named-patient scheme which, even though highly important for the evolution of a regulatory basis for phage therapy by gathering medical experience, while helping the patients in need for an antibiotic therapy; would not be a considerable option if trying to implement a customized phage therapy broadly available. Besides limiting the access of phage

therapy to emergency or special cases, the named-patient scheme also gives the impression that it should only be used as last resort. Altogether, these features cause pharmaceutical industries to lose interest in investing nor helps the general acceptance of the public, which delays the progress of a customized phage therapy (63).

A current optimal solution would be based on the magistral phage concept of Belgium. This enhanced model is based on the magistral formula concept and adapted following the experience of other medicinal products (11) so that is flexible enough to keep up with evolving bacteria by having updated active ingredients, while prioritizing the patient safety (96). First, this approach presents less restrictive regulatory obligations like GMP compliance for its formulation since it is considered a magistral formula (77). Then, a set of additional or adjusted requirements could be applied based on the autologous vaccines regulatory model, that do not need to file a full application on quality, security and efficacy dossier but must comply with GMP production of the active ingredient. This duality could exist through the licensed phage banks englobing biological master files for each phage strain (11). In this way, the industrial aspects of the active ingredient's manufacturing process are covered by the master file including mainly GMP, QP and other quality issues, but also some safety issues (83). As and addition, the biological master file of phages would need to be included in the EU legislation, since it is already included in the FDA (11). Also, following the example of the VAMF procedure, the biological master file would need to be of unrestricted access to information in production control and processes for the producer of the finished product since the quality of a biological active substance depends on the complete information (83).

It is possible to find some weaknesses associated with unlicensed products following a National Procedure that, for being exempted from some of the requirements of the licensed products may result on a poor quality, safety and efficacy data (11). For example, some legal requirements need to be part of the MA like a Summary of Product Characteristics (SmPC) or the package leaflet. Both have the purpose of providing more information, for the healthcare professionals or to the patients, respectively. The SmPC is subject of approval by the competent authorities after being completed by the applicant with information on how to use the product safely. By following the indications on this document, the prescriber of this product is protected if any unpredicted effect occurs, as it is legal responsibility of the manufacturer. However, an unlicensed product does not require a SmPC, which by law, means that if a problem with the product is identified, then the prescriber takes full responsibility (11).

It is possible, however, to tackle this problem by benefitting, again, from the experience of medical devices, in this case, when they are custom-made. The Regulation 2017/745 on medical devices already presents the information on how to license custom-made devices, and, to prevent a similar situation as custom-made phages, the prescriber has the legal responsibility of the specific design characteristics but anything else can be responsibility of the manufacturer. This situation can only be possible through an informative statement that must indicate "which general safety and performance requirements have not been fully met" (122). A similar statement could be adapted to customized PTMPs representing a SmPC but with general information of the homologous group of PTMPs. Then, the specific and variable from one product to another information could be included in a multi-strain dossier which in turn would be part of the statement of the manufacturer. Any post-market information gathered by the manufacturer should be added to update the multi-strain dossier.

The magistral phage concept starts with a phage seed correctly characterized and following the system of master and working cell bank. From the phage lot it is produced the API that will be externally assessed on its quality and properties by the national approved organization, like a laboratory. Both private and public organizations can produce these phage APIs that need to be accompanied by its corresponding batch record protocol with details on batch process production. From this controlled environment, the phage APIs are transferred to the magistral pharmacy in phage libraries. Upon a medical prescription, a phagogram is performed (when possible) to test the sensitivity of bacteria to phages to select the correct API. Gathered the more suitable phages, the personalized formula can be prepared and administered, always followed by the manufacturer statement including the multi-strain dossier (53).

The magistral phage model can be followed by different Member States, which will consequently apply its own Regulations. It is important to, in the future, try and establish a harmonized framework for the magistral formula so that phage therapy can be broadly used at a higher scale. The US can also follow the magistral phage concept, which has been approached in the context of clinical trials (95).

The current regulatory framework is not adapted for the introduction of tailor-made patient specific phage products and even though each country or Member State has and is developing their adapted regulatory structure at national level (8), in the future it will be necessary to try and implement phage therapy following the Centralised Procedure, in Europe (123). Natural or engineered phages included in phage therapy work as anti-infection treatment with a new mechanism of action, which means they are an innovative

therapy. As an innovative therapy of biological origin it must follow a centralised procedure in order to be granted a MA. The main objective of the Centralised Procedure is to ensure that the approach to the regulation of medicines is consistent and harmonised across the whole EU. A set of product information to healthcare professionals and patients is generated including public assessment reports, summaries of products characteristics (SmPC) and information leaflets available in all EU languages. In fact, the disadvantages previously mentioned regarding the National Procedure are identified in comparison with the Centralised Procedure. So, not only the Centralised Procedure would help the harmonization of phage therapy in the EU but is actually mandatory following the phage therapy implementation concepts (123).

Pharmaceutical companies show also some disinterest in developing phage products since there could exist some fears regarding intellectual property protection of phage products (9,96). Around the world, the patent-attributing courts generally decline the requested patents for products based on any form of life or in genetic material such DNA and RNA. If patents for biological organisms were easily allowed, every time a searcher used a panted organism he would be obligate to give money to the patent holder (96). Phage-based formulations are biological entities composed of genetic material and proteins which can pose the above described difficulties for intellectual protection of phage products (9,96). This represents another disadvantage compared with antibiotics since the latter are chemical compounds industrially made and can be more easily patentable. However, several phage products have been patented in the US and Europe, which can further contribute to the introduction of a phage based therapy against bacterial infections (96).

7. Conclusion

If the critical current scenario based on the unavailability of effective therapeutic options against bacterial infections continues, phage therapy may regain its status as an established antibacterial (61). In fact, bacteriophages full potential is not totally explored and the ongoing clinical trials may prospectively help the development of phage therapy. After many scientific advances on better understanding the interactions between phages and bacteria, the conditions needed for phage therapy implementation ensuring safety and efficiency are gathered (2). However, beyond the clinical context, a set of additional challenges can arise: the need for efficient phage screening methods, stability assurance during storage and transportation, the need to enlarge phage collections and create specific phage banks and the need to adapt regulatory frameworks to personalized phage products while following European and US standards.

Phage therapy can be better used and exploited as a personalized medicine. However, there are no specific guidance for personalized phage products, especially in the EU framework (11). Member States of the EU are currently nationally developing phage products for the specific need of a patient unable to fight a bacterial infection. The European and American legal frameworks provide some exceptions regarding atypical or under developed medicinal products to facilitate and motivate the development of new treatments. Even though the described exceptions of other particular medicines like the flu vaccine cannot be applicable to phage products, the previous experience gathered by the applications of these concepts can be beneficially used to design a suitable regulatory framework for phage therapy. In fact, the compassionate use of bacteriophages is already being developed and carried out in the US and in some European countries (90) which is, for now, a good way for the introduction of phages in the medical field, while enlarging the available clinical and biological data of phages.

However, phage therapy cannot fulfil its full potential as a personalized therapeutic for exceptional and emergency use only. The magistral phage concept implemented in Belgium represents a sustainable and feasible option to allow the access for patients to personalized phage products (84). Further efforts are needed to implement such a robust concept that not only allows to raise interest in possible investors since this concept could be easily adapted to a more profitable pharmaceutical market, but also contributes to the centralisation of phage products.

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