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Phage therapy: An overview of the features, challenges, and possibilities as an alternative to antibiotics

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Abstract
Antibiotic resistance is a public health concern that jeopardizes the efficacy of the therapies with these drugs and raises the possibility of epidemic events involving the emergence and re-emergence of infectious diseases whose treatments can be delicate or ineffective. As a result, this public health issue necessitates the search for feasible alternatives, such as phage therapy, which employs bacterial viruses to treat bacterial infections. In this way, the goal of this research is to look at the qualities of phage therapy as well as potential clinical applications and challenges.

Keywords: Bacteriophages, phage therapy, bacterial resistance, infectious diseases

Introduction
Infectious infections were pronounced death sentences until the discovery of penicillin, a chemical isolated for the first time from the fungus *Penicillium notatum* and efficient against a variety of bacterial pathogens. It's worth noting that the discovery of penicillin also marks the start of the antibiotics golden age, during which several scientists from around the world collaborated to discover most of the drugs currently used in clinical practice, resulting in a slew of medical breakthroughs that improved the quality of life and longevity of the world's population [1].

However, the inappropriate use of antibiotics has increased the incidence of difficult-to-treat infections [2], as well as the epidemiologic risks of transmission and maintenance of resistant pathogens within human populations, jeopardizing all of medicine's advances, quality of life, and longevity achieved since the discovery of antibiotics [3, 4].

In this context, phage therapy is a procedure that uses bacterial viruses to treat bacterial infections (figure 1), representing a natural strategy to combat bacterial resistance with several advantages over chemical antibiotics, including specificity against specific bacterial strains, the ability to self-replicate, presence in abundance at the infection site as long as bacteria are available for replication, and the ability to follow bacterial evolution [5].

Highlighting Frederick Twort explored the biological activity of phages for the first time in 1915, and Felix d'Hérelle established the efficiency of these viruses by effectively treating numerous infants hospitalized with dysentery in 1919. Phage therapy, which had been dormant for almost a century due to the discovery of antibiotics, was resurrected in the last few years due to an alarming surge in bacterial strains resistant to multiple antibiotics [6, 7]. In this sense, the purpose of this study is to look at the pharmacological features of phage therapy as well as prospective therapeutic uses as an alternative to antibiotics.

Phage therapy's history and current use around the world
After the success in treating bacterial dysentery in France in 1919, the microbiologist Felix d'Hérelle had a significant influence on European countries such as Russia, Poland, and the former Soviet Union. He also influenced the conduct of phage therapy research in the United States, where promising results were achieved for treating various types of bacterial infections [8, 9].

However, after WWII, and with the discovery of antibiotics, the use of bacteriophages in therapeutics faded into obscurity for a long period. This was aggravated by linguistic variations in the languages of countries like Russia and its neighbors, who continued to explore phage therapy but with limited international dissemination. In addition, the focus on these viruses switched to their use as a model organism in molecular biology, leading to key advancements including restriction enzymes, gene cloning, the CRISPR-Cas gene editing system, and phage display technology [10-12].

Then, many years later, due to the worldwide exponential development in antibiotic-resistant and multidrug-resistant bacterial strains, interest in phage therapy was reignited, though it is currently limited as a health service to a few European nations with varying regulatory statuses [13].

The Eliava Institute in Georgia provides phage therapy as a standard healthcare practice; in Belgium, the marketing of phages in generic pharmaceutical formulations is legal; and in other countries, a few institutions, such as the Center for Innovative Phage Application and Therapeutics in the United States and the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Poland, conduct phage therapy under an experimental regime [13, 14].

The Bacteriophages' Pharmacological Properties

In general, pharmacological therapy with any drug should consider pharmacokinetic aspects such as 1) absorption, which is dependent on permeability to biological barriers, physical-chemical properties such as molecular size, lipophilicity, and degree of ionization, 2) Distribution profile based on the degree of blood perfusion, the affinity for body tissues, as well as the affinity for plasma proteins 3) metabolism involving many enzymatic processes, such as those catalyzed by the CYP450 enzymatic complex, which tends to make xenobiotics more polar, allowing them to be 4) excreted mostly through the kidney [15-17].

Highlighting the antimicrobial must reach an appropriate concentration at the site of action and engage with a molecular target to provide the desired pharmacodynamic effect while taking into account the likelihood of drug interactions and adverse events [2,18].

In this perspective, considering the aforementioned processes, phage therapy has a different pharmacological profile than chemical medications.

Bacteriophage absorption and distribution are negligible since these viruses have a high degree of penetration over biological barriers due to their nanoscale size, allowing them to reach anatomical areas such as the central nervous system and difficult-to-reach organs like bones [11]. Furthermore, because bacteriophages are not xenobiotics, metabolism does not affect them, therefore their clearance is determined by bacterial density to enable viral multiplication, immune system activity, and excretion in urine (which can be beneficial in situations of urinary tract infection) and feces [19-21].

In this context, phage therapy is classified as an active strategy in terms of dosing schedules since it can be given in a single dose and does not require dosing plans to keep serum concentrations within a range where the pharmacological effect can be observed due to the ability for viral reproduction [19]. However, the immune system and physical-chemical properties found in specific anatomical areas (such as the acidic pH in the stomach) can rapidly lower phage quantities in the body after a single dose, which may be comfortable for the patient but may limit therapeutic efficacy [22-24].

However, pharmaceutical formulations that protect phages can overcome this constraint, allowing larger viral densities to reach the bacterial pathogen [25]. This point will be addressed later.

Furthermore, phage therapy's pharmacodynamics are linked to a series of events that alter the expression of bacterial structural and functional genes, resulting in the death of the target bacteria, where genomic and proteomic differences play a role in the phage-bacterial cell interaction [26]. In this context, it's well known that phages that infect gram-negative bacteria engage with protein or bacterial appendices such as the capsule, flagella, and pile, whereas phages that infect gram-positive bacteria contact with saccharides, or teichoic acid moieties [27, 28]. This selectivity (figure 2) is a promising benefit since it eliminates the likelihood of interactions of the phages with the normal microbiota, thus reducing the risks of dysbiosis [5].

Selectivity, on the other hand, can be a disadvantage due to the high genetic diversity found in bacteria, which can result in differentiated expression of molecules used by phages in host recognition, causing variability in phage lytic activity across strains of bacteria from the same genus or species [29].

In this sense, the ideal bacteriophage for phage therapy is a highly virulent lytic virus capable of infecting a wide range of bacteria species, producing a large progeny after infecting a single cell and maintaining high viral densities at infection sites. Besides this, the ideal phage should be completely devoid of genes encoding for antibiotic resistance, toxin, and virulence factors, and be able to withstand the technical processes of formulation production [30–32].

Additionally, pharmaceuticals containing phages as therapeutic actives should have a high degree of purity and specified quality criteria. To avoid the development of resistance in the target bacteria against the phages, and to ensure the successful treatment of infections when the identification of the bacterial pathogen is not available, phage cocktails containing several different viruses are preferable rather than monotherapy with just one type of phage [8, 33].

Bacteriophages as an alternative to antibiotics

Antibiotics became more widely used after their discovery, and bacterial resistance to antibiotics became a public health issue, which has been documented since the 1950s [34]. This has encouraged the creation of various research lines aimed at extending the useful life of existing antibiotics as well as developing novel medications and drug delivery methods. In this context, bacteriophages have reemerged as a therapeutic resource [35, 36], but in a different way from the 1910's due to significant scientific and technological advances in the fields of bacteriology, virology, molecular biology, and medical-pharmaceutical sciences [12, 37, 38], which have enabled a wider understanding of bacteriophage’s biology and their biotechnological applications in health.

As a result, given the current state of knowledge about the pharmacological aspects of phage therapy, several possibilities emerge, particularly in cases where antibiotics have a short safety interval, such as among patients who use any medication in a continuous regimen [20], as those receiving psychiatric treatment with antidepressants; patients with diabetes type 2 receiving antidiabetic drugs; and polypharmacy patients, such as those with heart disease who may need to take multiple medications, and are prone to experience drug interactions or pharmacological treatment deregulation, which can lead to increased morbidity due to the underlying disease when antibiotics are required [39-42].

Besides, the literature highlights a significant number of drug interactions with antibiotics, such as the use of amoxicillin with venlafaxine that can cause serotonergic syndrome, furosemide with gentamicin can increase the diuretic's ototoxicity, and penicillin with warfarin can increase the risk of hemorrhage, as well as antibiotic interactions with food [43-46].

In this context, phage therapy is a safe alternative to

antibiotics for patients with chronic diseases who are receiving pharmacological treatment with two or more drugs, because the pharmacology of phages differs from that of chemical drugs, as previously discussed, and does not interfere with the pharmacokinetics or pharmacodynamics of chemical drugs [20, 47, 48]. Noting that antibiotic allergy is another important concern in the treatment of bacterial infections that can be overcome with phage therapy [9, 49], and these viruses may also represent a potential therapeutic resource for allergic disorders, as they have modulatory properties on interleukin secretion 10, which suppresses inflammatory response pathways involving eosinophils and mast cells [50].

Furthermore, we propose that phage therapy be used as a treatment and prophylaxis for chronic diseases in which bacterial agents play a role as an infectious determinant, such as *Helicobacter pylori* in the development of intestinal gastritis and peptic ulcers, *Campylobacter pylori* in cases of Guillain-Barré syndrome, and *Borrelia burgdorferi* in cases of Lyme arthritis [51].

And, as Kingwell [30] points out, the technological potential of bacteriophages represents an open field of applications, with a wide range of possibilities reported in the literature, including ways to prevent contamination of hospital environments, soft tissue dressings to prevent infections [52], decontamination of patients and health professionals [53], biofilm elimination [54], and foodborne disease prevention [55]. Noting that bacteriophages also are an important topic in bacterial control for industrial interests, particularly in the food and livestock industries [56–58], where antibiotics are used in large quantities and contribute to the emergence and spread of bacterial resistance around the world, affecting human health outside of the hospital environments [59].

Phage therapy safety and efficacy

Phage therapy is safe from an ecological standpoint, because the human beings have been exposed to a vast number and diversity of phages in nature since the beginning of human species [60]. Even molecular evidence shows that the human virome has a great number and diversity of phages, which plays a vital role in maintaining homeostasis and participates in processes that promote disease in cases where the microbiota function is disrupted [61-64].

The clinical use of bacteriophages, on the other hand, necessitates the delivery of a virus or set of viruses at high titers at the site of infection, usually through routes that these organisms do not ordinarily have access to in the human body [24, 53, 65]. As a result, clinical trials are required to ensure the safety and efficacy of phages for treating bacterial illnesses, in order to provide evidence to help doctors choose and employ phage therapy appropriately [14, 30].

In this session, studies with low strength of evidence, such as case reports present positive outcomes reported in the literature, implying that phage therapy is effective; however, the variables determining the success of the phage therapy intervention are not fully understood and are insufficient to ensure generalizations to standardize the outcomes.

Khawaldeh, *et al.* [66], for example, report on the efficacy of bacteriophages in the topical treatment of refractory urinary tract infection caused by *Pseudomonas aeruginosa* in combination with antibiotics. Jennes, *et al.* [67] also report efficacy in a complicated case involving a 61-year-old male who contracted septicemia from the same pathogen, had several clinical complications, and was treated with a phage-specific against *Pseudomonas aeruginosa* administered intravenously, resulting in a successful recovery from sepsis but unfortunately, the patient died of a *Klebsiella pneumoniae*-related bacterial infection.

Furthermore, the results of investigations with high strength of evidence, such as group control studies and double-blind randomized clinical trials, are inconclusive.

In this context, Bruttin *et al.* [68] compared a group of volunteers who received T4 phages in titers of 10^5 PFU x mL^{-1} by oral route with a placebo group, reporting no adverse effects or differences between the groups; and in a double-blind clinical trial phase I, Rhoads *et al.* [69] showed safety but equivocal efficacy in 42 patients who were treated with phages for leg ulcers by topical route.

Similarly, Sarker *et al.* [70] compared the outcomes of a test group that received oral doses of a Russian coliphage with a placebo group in a randomized study, finding no adverse reactions or efficacy, but differing from Wright *et al.* [71], who reported the effectiveness in the treatment of 24 patients with chronic otitis caused by *Pseudomonas aeruginosa* multi-resistant to antibiotics. Furthermore, McCallin *et al.* [13] report in a review the clinical outcomes of 29 studies of patients who received phage therapy to treat various bacterial infections, finding that 22 patients had success, while 7 had partial improvement but no complete resolution of the infection, similar to Jault *et al.* [72], who report a reduction in bacterial load of wounds in burned patients but without infection cure. Adding to the conclusion that clinical investigations on the efficacy of phage therapy show conflicting results, implying that further studies are needed to provide a predictable therapeutic response [13].

Challenges to overcome in phage therapy

The high specificity of bacteriophages for their bacterial hosts, as well as the vulnerability of many of these viruses to environmental conditions inherent in formulation preparation and administration routes (figure 3), are the primary limiting constraints in bacteriophage pharmaceutical application, besides, detecting genes that express antibiotic resistance and virulence factors are also challenges that must be considered in the methodological design of research to obtain candidates for phage therapy [27, 33, 54].

In this review we will focus on the challenges concerned to host specificity and resistance to environmental factors.

In this context, bacteriophages' specificity for bacterial hosts is mediated by molecular recognition structures involved in pathogen-host interaction [73], posing a challenge for clinical practice when the etiological agent and its molecular characteristics are unknown, necessitating an assessment of the range of hosts and determinants for host-pathogen interaction [60]. This can be time-consuming; however, using formulations containing multiple phages increases the likelihood of success, as well in preventing bacterial resistance to phages through mechanisms such as adsorption inhibition, restriction/modification system, abortive infection, reduced infection vigor, and interference with viral dissemination [74].

Thus, the ability of phages to infect and lyse different bacterial hosts can be evaluated by two methods: 1) the host range, in which the ability of phages to infect and lyse different bacterial hosts is compared by their titers, where high values are desired; and 2) the efficiency of plating, which is a comparison of bacterial titers taking into account the reason present in the titers of hosts different from the one

the phage was obtained (E.O.P. = test host/obtaining host) [31, 75].

These procedures must be pursued in tandem in order to obtain more information about the phages' profile of infectivity to various bacterial hosts [75, 76]. Furthermore, it is strongly recommended that the hosts utilized in the experiments be clinical isolates, as this will give the data more confidence concerning human phage therapy [77].

In this regard, Chang *et al.* [78] demonstrate experimentally the importance of knowing the profile of susceptibility and resistance of target bacteria to phages in their study, in which they isolated the Phage PVE20 from sewage samples, preserved it in phosphate buffer at a titer of 10^{10} PFU/mL, and tested it against seven strains of antibiotic-resistant *Pseudomonas aeruginosa*, finding that five of the seven were susceptible, one was completely resistant, and another was partially resistant.

Moreover, the resistance of bacteriophages to environmental factors and characteristics of the routes of administration, such as temperature, pH, ionic strength, divalent ion concentration, and immune system activity, can influence the densities of phages that reach the infection site, compromising the efficacy of phage therapy [79, 80].

To protect phages from the aforementioned factors, the resistance and susceptibility of viruses intended for phage therapy should be experimentally evaluated, contributing to a broader characterization, as well as the design of storage conditions and the preparation of specific pharmaceutical formulations for phage therapy, which can improve phage pharmacokinetics by increasing their bioavailability at the infection site [27, 54, 81, 82]. In this context, several methods for developing pharmaceutical formulations for phage therapy are described in the literature, such as spray freeze drying [83], encapsulation, incorporation of phages in matrix systems containing biopolymers such as proteins, saccharides, lipids [84, 85], or semi-solid colloidal systems and solid formulations [86], in addition to formulations aimed at treating infections in specific organic systems [24, 87, 88].

In this context, Chang *et al.* [89] used lactose and leucine as excipients to protect the PEV20 phage and then prepared powders using the spray drying method, resulting in inhalable phage powders that were biologically and physically stable over long-term storage (12 months) at room temperature. They also demonstrated that the phage powders were non-toxic to lung alveolar macrophage and epithelia, moreover, the formulation was tested in rodent model for treating *Pseudomonas aeruginosa* respiratory infection, confirming the viability of phage PEV20 dry-powder formulation for treating antibiotic-resistant *P. aeruginosa* lung infection. Highlighting the study presented statistical significance when compared to the controls [90].

Figures

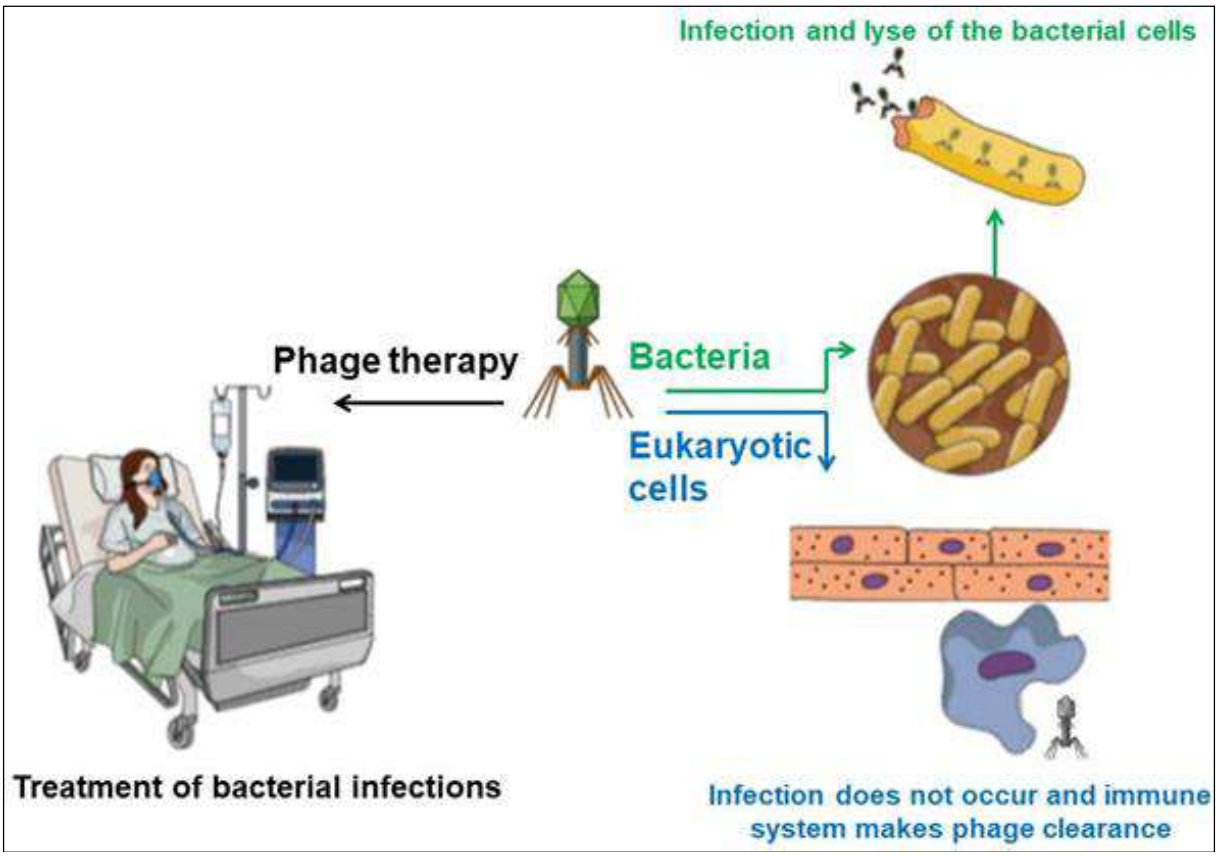

Fig 1: Phage therapy concept

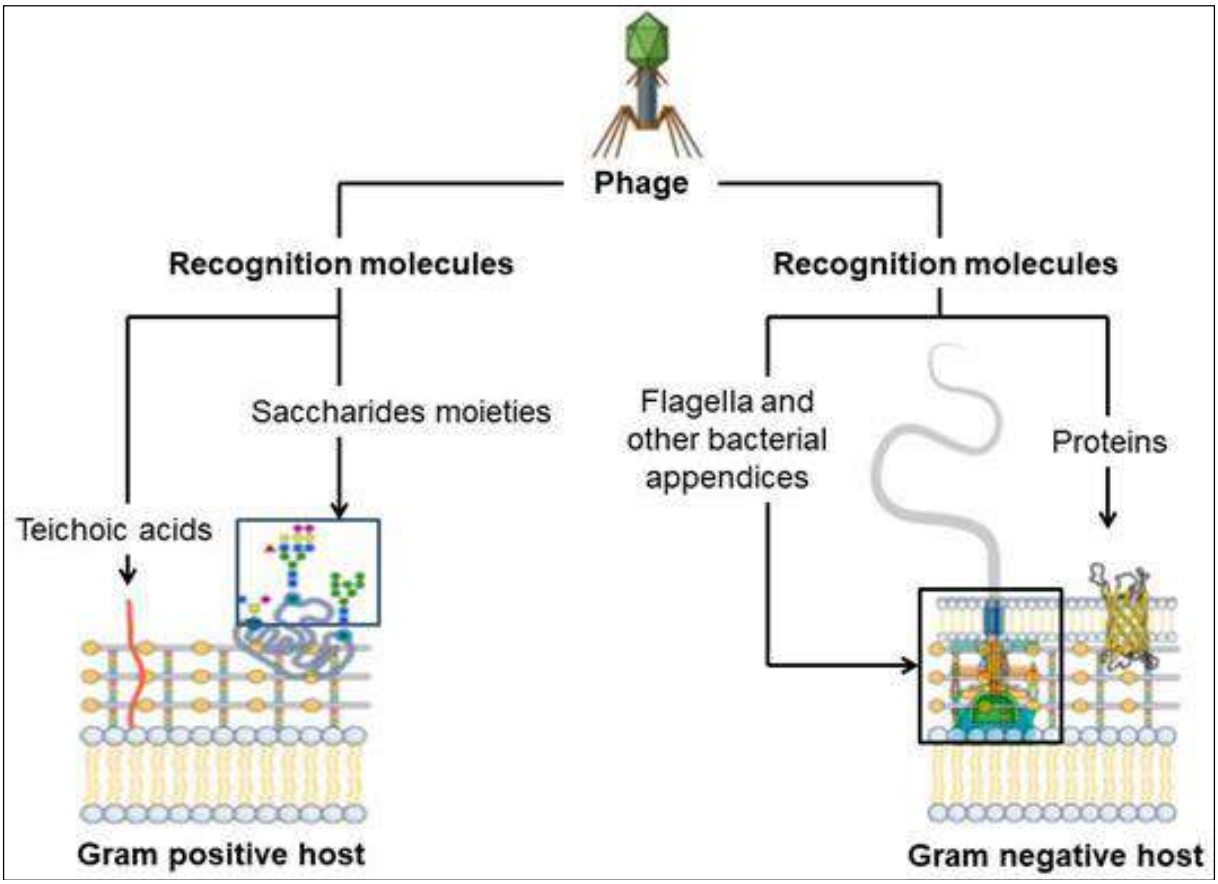

Fig 2: The selectivity of bacteriophages for hosts depends on biochemical moieties in the bacterial surface

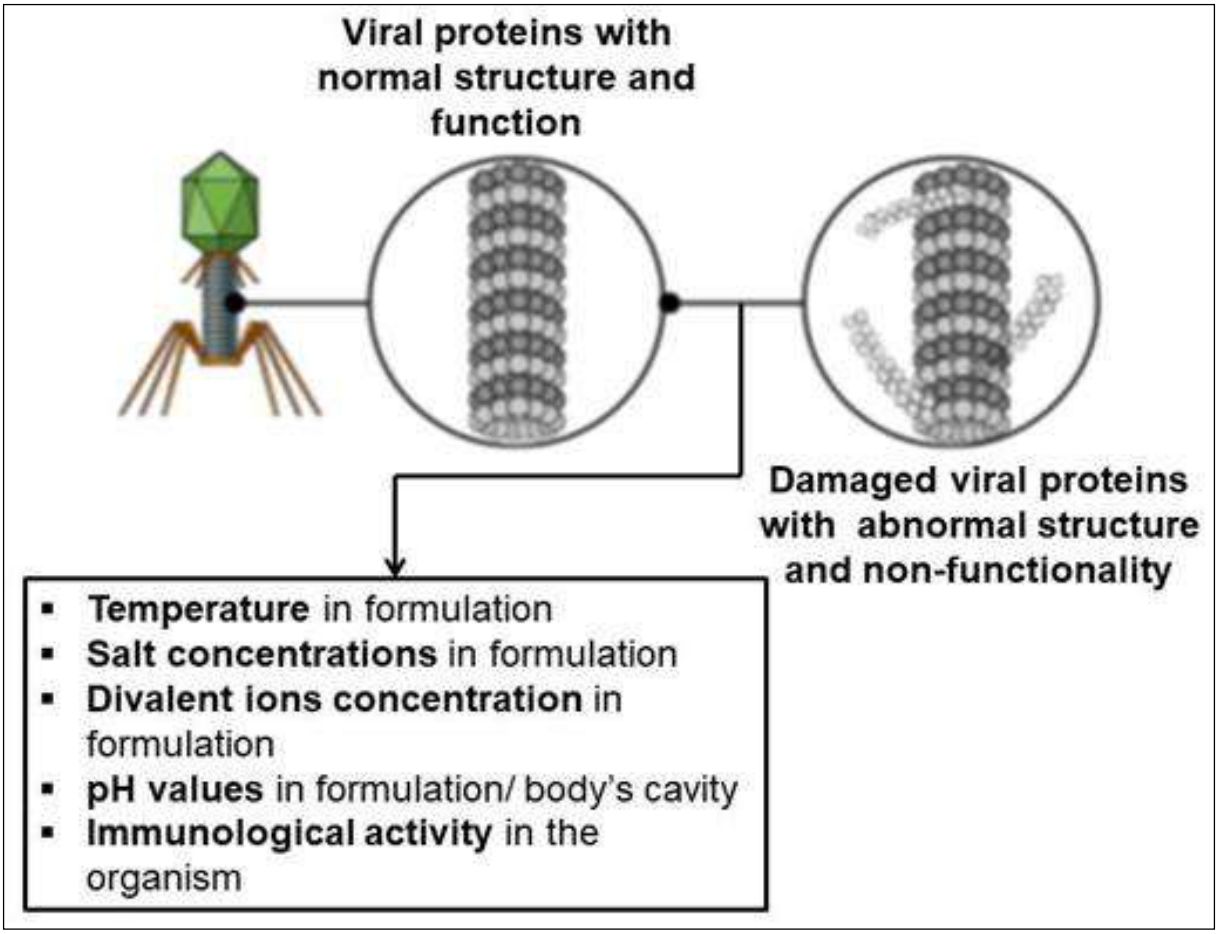

Fig 3: Resistance to environmental conditions, as well as pharmaceutical formulation development and application route

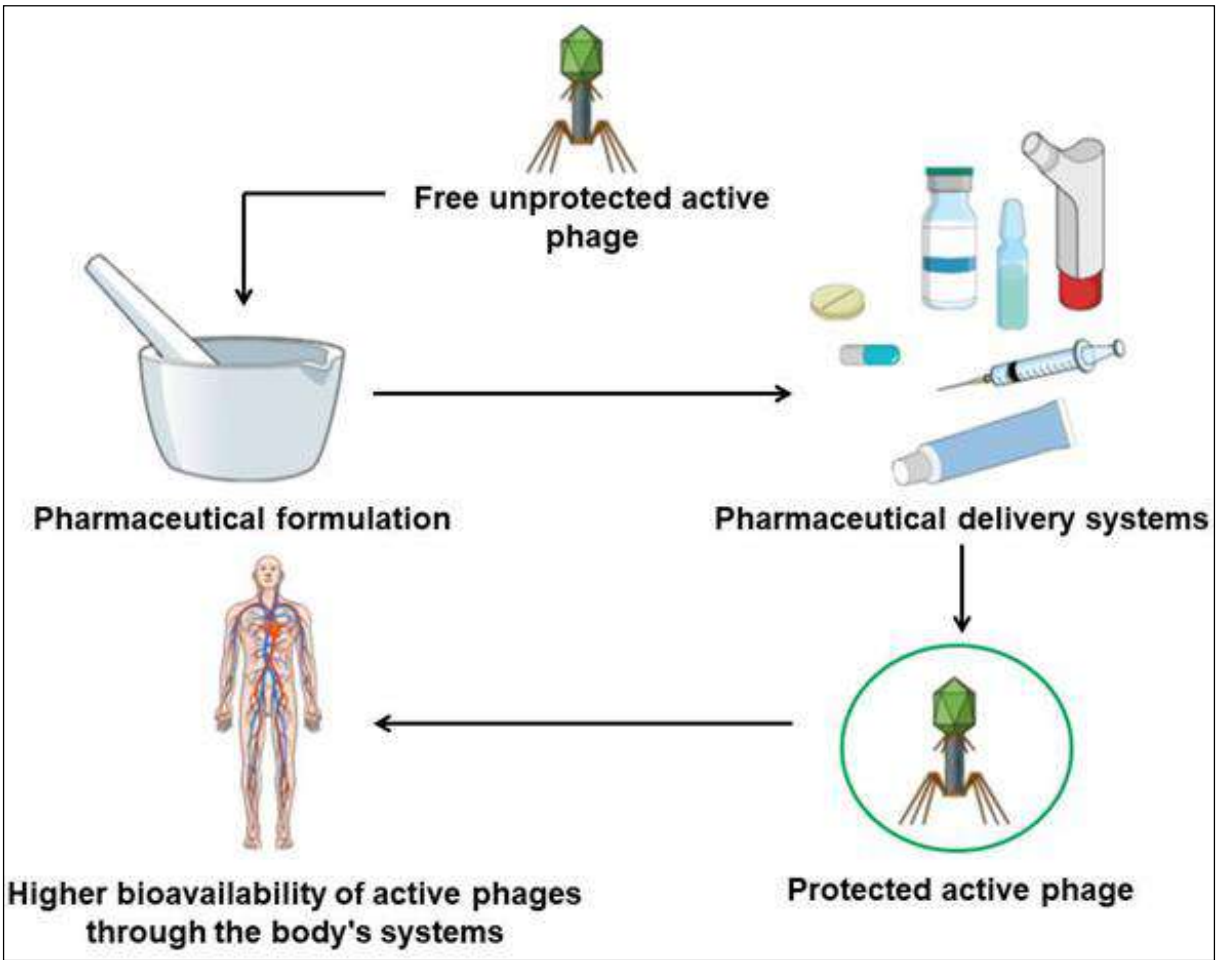

Fig 4: Pharmaceutical formulations may increase the availability of phages in the infection site and improve the outcome of the patient. Phage cocktails are more suitable than monotherapy to treat infections without laboratorial diagnose of the bacterial pathogen

Conclusion

Because bacteriophages are obligate cellular parasites of bacterial cells, therefore they represent a natural way to control specific bacteria populations. However, because these viruses are biologically diverse, formulations must be tailored to the unique characteristics of each phage isolated, and their effects on the target bacterial species.

In this perspective, this review suggests that phage therapy's variable results could be due to 1) phage non-survival in delivery systems, as well as the natural process of clearance in the human body; or 2) a failure to consider elements intrinsic to the host range of the phages studied in the reviewed studies.

As a result, we propose at the conclusion of this paper that, in addition to extensive characterization, bacteriophages should be evaluated against collections of clinical bacterial isolates as well as different environmental conditions in order to generate subsidies regarding the physicochemical conditions required to keep phages viable in formulations that enable satisfactory bioavailability to human infection sites.

Conflict of Interest

Not available

Financial Support

Not available

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