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Review

Exploring Bacteriophage Applications in Medicine and Beyond

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Abstract: Bacteriophages, or phages, are microscopic viruses that specifically infect and replicate within bacterial hosts. Their unique ability to target and control bacterial populations makes them valuable tools with applications ranging from human medicine and agriculture and environmental management to biotechnology. In this comprehensive review, we explore the diverse and promising medical and non-medical applications of bacteriophages, highlighting their pivotal role across various niches. From safeguarding food production through pathogen control to their innovative utilization in wastewater treatment, bacteriophages prove to be versatile agents. To achieve applications of phages on a larger scale, it is necessary to make the legal framework more suitable and flexible, create special approval programs (e.g., for novel antimicrobial drugs), and promote targeted research and development activities on phages. Additionally, a more intensive exchange between academia, industry, regulatory authorities, and stakeholders in the health system should be pursued.

Keywords: bacteriophages; non-medical applications; food; agriculture; biotechnology

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1. Bacteriophage Applications in Medicine

Bacteriophages or "phages" are viruses that kill bacteria and are usually used in the treatment of bacterial infections (Figure 1). Phages are a potent, promising, possible solution to the antibiotic-resistant epidemic (half a million people die every year from multidrug-resistant (MDR) bacteria, and 10 million are expected by 2050). In 1896, Ernest Hanbury Hankin, a microbiologist from England working as a Chemical Inspector and Bacteriologist for the Government of the United Provinces and the Central Provinces of India, found an idiopathic agent that possessed a high killing activity against *Vibrio cholera* and decreased its spread [1]. In 1900, Gamaleya confirmed this observation against *Bacillus subtilis*. In 1915, the British microbiologist Frederick Twort observed a plaque determined by lysis in the culture of *Staphylococcus* that appeared many times with different strains. The first article published confirming the presence of phage was by Twort in The Lancet, raising the hypothesis that it is a virus agent [2]. In 1917, two years later, the French-Canadian bacteriologist Felix d'Herelle, who investigated the outbreak of hemorrhagic dysentery in France, observed a plaque similar to that described by Twort, which he named lysis plates [3,4]. For the first time, he studied the characterization of this unidentified agent and confirmed that it is an obligate parasite that has the ability to invade bacteria, thus naming it bacteriophages [5]. Since then, many phages have been identified against harmful bacteria such as *Escherichia coli*, *Salmonella typhi*, *Vibrio cholera*, *Pasteurella multocida*, *Yersinia pestis*, *Streptococcus species*, *Neisseria meningitides* and *Pseudomonas aeruginosa* [6]. In 1923, The International Bacteriophage Institute was established in Tbilisi, Georgia. During World War II, the Soviet Union and Eastern Europe had restricted stock of antibiotics and, accordingly, enhanced the development of phage therapy. The practice of phage therapy in the Soviet Union has been well advised and is still widely used in Russia and Eastern European countries for more than 80 years [7]. During the 1920s and 1930s, Soviet scientists made significant contributions to the understanding and application of phages in therapeutic settings. Félix d'Hérelle played a crucial role in advancing phage therapy and collaborated with Soviet scientists during this period. D'Hérelle conducted research in the Soviet Union and established close ties with Soviet scientists, including renowned microbiologist Giorgi Eliava [8]. Together, they recognized the potential of bacteriophages as a tool to combat bacterial infections. Soviet scientists have conducted extensive studies on phages and their applications. They have recognized the diverse nature of bacteriophages, with each type being specific to certain bacteria, and this specificity became a key aspect of phage therapy. Researchers isolated and characterized numerous bacteriophages, building a foundation of knowledge on their behavior and effectiveness against various bacterial strains. In 1950 and after, bacteriophages were tools in molecular biology, and some institutions developed programs for the treatment of patients with phages and supportive therapy [9]. In addition to laboratory studies, in 1980, Soviet researchers conducted clinical trials to evaluate the therapeutic potential of phage therapy. They treated patients with bacterial infections using bacteriophage preparations and observed encouraging results. The success of these early trials further fueled the interest in phage therapy and spurred its widespread use within the Soviet Union. [10]. After the dissolution of the Soviet Union in 1991, the history of phage therapy in the newly independent states took a different trajectory. The early 1990s marked a period of transition and uncertainty in the newly independent states. The dissolution of the Soviet Union led to economic challenges, including funding shortages and a decline in scientific infrastructure. Consequently, phage therapy research and development faced significant obstacles during this time. From 2000 to 2005, phage therapy research regained force, with a focus on evaluating its effectiveness in clinical trials and exploring potential applications. Collaborations with international researchers and institutions helped sustain and advance phage therapy research. The recognition of the global threat posed by antibiotic resistance led to increased international interest in phage therapy, prompting collaborations and knowledge sharing between researchers worldwide [11].

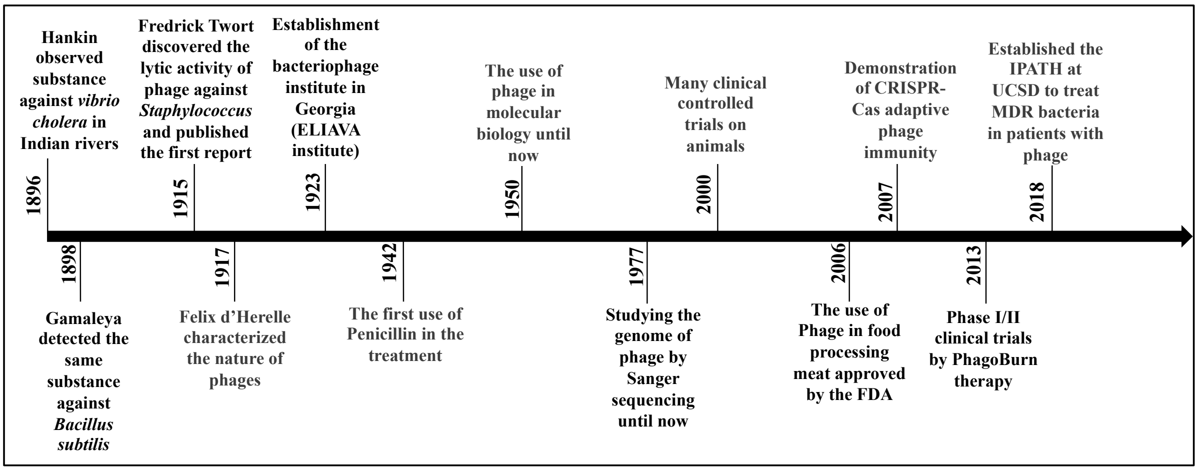

Figure 1. History of bacteriophages.

The use of phages as antimicrobial agents is one of the key areas of interest in phage applications, especially given the global issue of antibiotic resistance affecting human, animal, and environmental health [12–14]. Since the early 2000s, bacteriophages have garnered renewed attention from researchers, physicians, patients, and emerging biotechnology companies for treating infections that poorly respond to antibiotics. Bacterial infections are often caused by multiple pathogens or strains in a patient-specific composition. Therefore, to address potential bacterial phage resistance, effective phage preparations must contain mixtures of different phages (phage cocktails) or be individually tailored for each patient. Hence, phage therapy comprises two basic approaches: (1) off-the-shelf treatments, which use pre-defined phage preparations available for specific indications; and (2) personalized therapies, which involve phage preparations specifically tailored to patients or their pathogen strains, using phages from specialized phage collections (phage biobanks) or newly isolated phages.

Theoretically, phage therapy offers many potential advantages over traditional antibiotics. Unlike bacteriostatic antibiotics, which permit the development of antibiotic resistance, lytic phages are bactericidal and completely lyse bacterial cells as part of their life cycle [15]. Phages attack crucial cellular processes such as DNA transcription and translation, making it difficult for bacteria to develop resistance [16]. Furthermore, phages co-evolve with their bacterial hosts, enabling them to infect even phage-resistant bacteria, unlike traditional antibiotics. Phages also maintain the normal microbiome balance, unlike broad-spectrum antibiotics that can disrupt the body's protective normal flora. Phages' specificity to particular bacterial species or strains makes them ideal for targeting pathogens selectively [17]. Additionally, the discovery of new antibiotics has slowed, whereas the identification of new bacteriophages has accelerated due to their vast biodiversity in various niches. Despite these benefits, some limitations of phage therapy compared to traditional antibiotics need to be addressed before its widespread acceptance in clinical use (Figure 2).

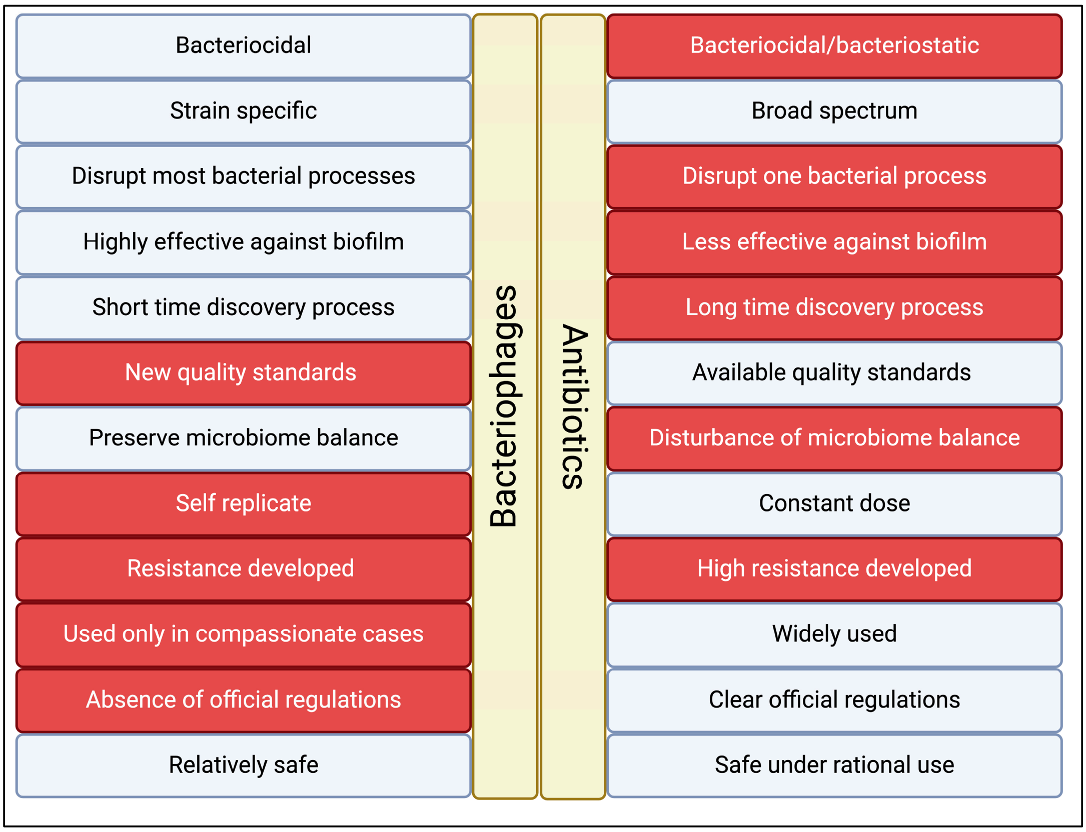

Figure 2. The advantages (blue) and disadvantages (red) of bacteriophages versus traditional antibiotics.

In fact, “the enemy of my enemy is my friend” actually describes the bacteriophage lytic cycle. Phages have been used in medicine since 1919 to treat *Shigella dysenteriae*, ten years before the discovery of the first antibiotic, “Penicillin” [8,18]. Despite more than a century of diverse studies on phage applications, our understanding of phages and their derived agents remains incomplete. Despite their potential, the widespread acceptance of phages within both modern medical and non-medical approaches has yet to emerge. Notable exceptions can be observed in Georgia, Poland, and Russia, where phage therapy has been employed for an extended period [19]. Given the extensive exploration of therapeutic phage applications, in the following sections, our review aims to provide a comprehensive overview of the recent literature focusing on applications of bacteriophages beyond medicine (Figure 3).

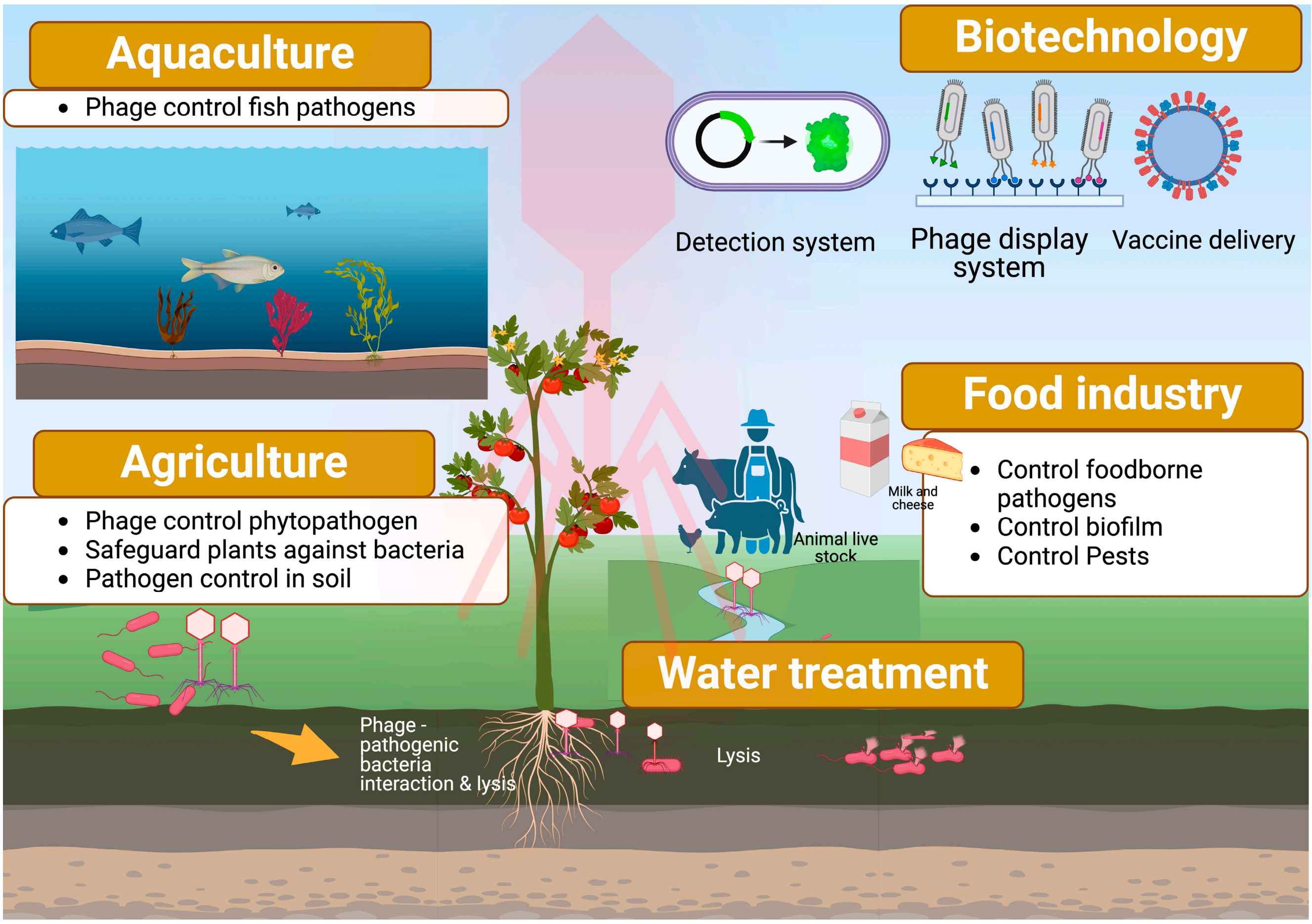

Figure 3. Applications of bacteriophages in non-medical areas.

2. Bacteriophage Applications in Food Industry

Phages have proven effective in combating bacterial threats throughout the entire food production chain. Beyond their role in preventing and treating diseases in animals and plants, phages play a crucial role in detecting and impeding the growth of foodborne pathogens during and after food processing. One notable application involves the engineering of reporter phages, enabling the bioluminescence-based detection of the foodborne pathogen *Listeria monocytogenes* in items like contaminated milk, cold cuts, and lettuce. To enhance food safety, phage solutions can be applied by dipping or spraying, designed to eliminate common pathogens that may contaminate various food products. This method of phage biocontrol has achieved significant commercial success in the food industry. Numerous products are now available on the market specifically formulated to combat bacteria such as *Salmonella*, *E. coli*, *L. monocytogenes*, and others. Moreover, phages find application in sterilizing surfaces within food processing facilities. Areas prone to bacterial contamination, such as conveyor belts and food transport racks, can be effectively sanitized using phage-based methods, contributing to the overall hygiene and safety of food production processes.

Various studies have demonstrated that the direct application of lytic phages to ready-to-use food can significantly decrease the presence of potentially harmful foodborne bacteria. For instance, when lytic phages were administered to chicken skin previously contaminated with *Campylobacter jejuni* or *Salmonella enterica* serovar Enteritidis, there was a notable increase in phage titers, resulting in a substantial reduction in the bacterial load of these pathogens by less than 1–2 $\log^{10}$ unit [20]. In a different study, a cocktail of virulent phages targeting *Salmonella flexneri*, *Salmonella dysenteriae*, and *Salmonella sonnei* effectively minimized the risk of *Shigella* spp. contamination in ready-to-eat spiced chicken products [21]. The impactful results from these studies have led to the approval of various phages for the decontamination of food products. Notably, the U.S. Food and Drug Administration (FDA) has approved a blend of six phages, known as ListShield™ -LMP-102^{TM} and developed by Intralytix Inc. in Columbia, IN, USA, as a food additive. This approval specifically targets ready-to-eat poultry and meat products, aiming to control contaminations caused by *Listeria monocytogenes* [22]. Several other comparable products are currently available, further highlighting the expanding use of phages in the food industry. EcoShield comprises three lytic phages specifically targeting *E. coli* O157:H7. Additionally, SALMONELEX, created by Micreos Food Safety in Wageningen, Netherlands, is designed to target *Salmonella* spp. in food products. Another product, Listex P100, also from Micreos Food Safety, Wageningen, Netherlands, features a single phage targeting *L. monocytogenes* [23].

The formation of microbiological biofilms on equipment surfaces poses a significant challenge in food production. Bacterial biofilms are characterized by the aggregation of cells surrounded by a self-produced matrix of extracellular polymeric substances, adhering to both living and non-living surfaces. Cells within biofilms exhibit high resistance to adverse environmental conditions, antibiotics, and disinfectants. In the fresh produce sector, pathogens like *L. monocytogenes*, *Salmonella*, *E. coli*, and *Yersinia* can adhere to plant tissues, forming robust biofilms [24,25]. The intrinsic structure of vegetables hinders the effectiveness of sanitizers against these microorganisms, necessitating the development of solutions harmless to humans yet capable of eliminating biofilms on plant tissues. Bacteriophages offer promising potential for the creation of safe sanitizers. For instance, *C. jejuni*, a pathogenic bacterium known to form biofilms on commonly used industrial materials like polyvinyl chloride and stainless steel, was targeted using lytic bacteriophages CP8 and CP30. Isolated from poultry excreta, these phages were applied to prevent the formation of *C. jejuni* biofilms on glass Petri plates, resulting in a significant reduction of 1.0–3.0 log CFU/cm^2 in the viable count just 24 h after infection [26]. *L. monocytogenes* demonstrates the ability to form biofilms on various surfaces within food production settings, including conveyor belts, floor drains, stainless steel equipment, and product transportation racks. In a previous study, ListShield$^{\mathrm{TM}}$, a mixture of six lytic phages targeting *L. monocytogenes*, effectively removed 72 h biofilms formed on stainless steel surfaces by most of the tested strains after four hours of treatment at 12 °C [27]. Additionally, Soni et al. demonstrated ListShield$^{\mathrm{TM}}$'s efficacy on fresh channel catfish fillets, achieving a reduction in *L. monocytogenes* of between 1.4 and 2.0 log CFU/g at temperatures of 4 °C, 10 °C, and 22 °C [28].

The regulatory status of phage uses in food safety lacks global standardization, and several factors contribute to the varying regulatory landscapes across different countries. One significant influence is the presence of Novel Food Regulations in many authorities. These regulations control the approval and use of novel food ingredients, potentially encompassing phages. The primary goal is to ensure the safety and accurate labeling of new or unconventional foods. Consequently, phages employed in food applications may undergo a regulatory approval process to establish their safety and efficacy before gaining permission for use. Another critical factor is the emphasis on Risk Assessment and Safety Evaluation. Regulatory authorities typically demand a thorough evaluation of novel food ingredients, including phages. This assessment entails scrutinizing potential adverse effects on human health, exploring the likelihood of gene transfer or antibiotic resistance development, and evaluating the stability and persistence of bacteriophages in the food environment. Additionally, regulatory authorities often require transparent and precise labeling of foods treated with phages. This includes information on the presence of phages, their specific targets, and any necessary handling or storage instructions. Moreover, the regulatory landscape is significantly influenced by country-specific regulations. Each nation maintains its regulatory framework for food safety, incorporating guidelines for novel ingredients like phages [22,29–31].

3. Bacteriophage Applications in Controlling Phytopathogens

Phytopathogens pose a significant threat to plants, encompassing various parasitic plants, fungi, viruses, nematodes, and bacteria [32]. These pathogens exhibit high virulence, possess adaptability to changing environments, and are challenging to manage effectively. Given their impact on plant health, efficient disease control management is crucial to maintaining a stable and reliable food supply for consumers. In recent times, the prohibition of certain control agents, such as pesticides and antibiotics, in Western countries due to their undesirable toxic characteristics has necessitated the exploration of alternative approaches [33]. A notable and growing interest in the field involves the use of bacteriophages as biocontrol agents to address phytopathogens. The initial successful field trial of bacteriophages dates back to 1935 when Stewart's wilt disease caused by *Pantoea stewartia* was effectively treated. In this trial, bacteriophages were introduced to combat a highly virulent strain of *Agrobacterium tumefaciens*, leading to the complete inhibition of bacterial activity [34,35].

Bacteriophages, with their diverse biocontrol capabilities, find extensive applications in agriculture for safeguarding plants against various bacterial diseases. In a demonstration of their efficacy as biocontrol agents, a phage cocktail consisting of approximately six isolated bacteriophages targeting *Pseudomonas syringae*, the causative agent of bacterial canker disease on kiwifruits, has been evaluated. The outcomes of this trial revealed promising results, suggesting that the phage cocktail PHB09 holds significant potential as a therapeutic cure for bacterial canker disease [36]. Additionally, Liu et al. isolated and characterized two novel bacteriophages targeting *Xanthomonas oryzae*, the causative agent of bacterial leaf blight disease in rice [37]. Bacteriophages employed for biocontrol in agriculture must exhibit environmental stability, demonstrating resilience against factors such as UV radiation, temperature variations, and chemical agents. Additionally, they must possess lytic characteristics [38]. Agriphage, a phage-based product developed by the approved U.S. company Omnilytics, serves as an effective tool in controlling bacterial spot diseases affecting peppers and tomatoes [39]. Several bacteriophage enzymes, including ΦXo411 and Lys411, have been isolated due to their demonstrated lytic activity against the pathogenic bacterium *Xanthomonas* [40]. The experimental trial showcased remarkable results, with fruits treated with the phage suspension displaying a 92% survival rate and no signs of disease development. RSL1, a phage targeting *Ralstonia solanacearum*, has displayed remarkable resilience to high temperatures (37–50 °C). In practical applications, tomato plants infected with *R. solanacearum* exhibited wilting symptoms within four days of infection. However, when exposed to phage φRSL1, these plants showed no signs of wilting. The phage effectively prevented the characteristic wilting pattern by limiting the growth of *R. solanacearum* cells [41]. Phages have found application in field conditions and greenhouses for effective disease control. In the case of *R. solanacearum*, phages were directly applied to the rhizosphere through soil drenching, demonstrating efficacy in suppressing the development of wilting in tomato plants. For soil-borne pathogens such as *Xanthomonas euvesicatoria*, *X. campestris* pv. *campestris*, and *Pectobacterium carotovorum* subsp. *carotovorum*, a foliar spraying method was employed to mitigate disease incidence in plants caused by these pathogens [42].

To enhance the host range and overcome limitations associated with a single bacteriophage, formulations of phages are often prepared into a bacteriophage cocktail combining multiple phages [43]. This strategy proves effective in compensating for host range constraints and significantly reduces the likelihood of the development of phage-resistant bacteria, such as phage control against pathogens like *P. syringae* [44]. While bacteriophage cocktails demonstrate promise, there remains a need for further research in the agricultural domain to fully realize and implement the role of bacteriophages on a global scale. In the propagation of crops, the stability of phage cocktails is dependent on the phages' resistance to adverse environmental factors, highlighting the importance of continued investigation and refinement in this field.

4. Bacteriophage Applications for Pest Control

The application of bacteriophages to combat bacterial diseases in agriculture is gaining potential as a safe and effective alternative to traditional antibiotics. Beyond their conventional role in targeting bacteria harmful to crops, the application of phages becomes broader in weakening or eradicating certain insect pests that rely on their microbiome for essential physiological functions. Insects like aphids could be vulnerable to this approach. Similarly, symbiotic relationships in pests like termites, flies, mosquitoes, and roaches open new possibilities. Importantly, recent research showcased the potential of phages to remove a significant portion of *Pseudomonas aeruginosa* in the gut of *Musca domestica*, leading to profound changes in the flies' gut microbiome and disrupting their normal development [45]. The adult mosquito microbiota primarily stems from the larval microbiota, connected to the water habitat's microbial composition. Disturbing the microbiota of mosquito larvae holds promise for influencing mosquito biology. Recent experimentation demonstrated the modulation of Anopheles larvae microbiota using bacteriophages in their water habitat. Gnotobiotic *Anopheles* larvae, hosting *Enterobacter*, *Pseudomonas*, *Serratia*, and *Asaia*, experienced reduced survival and larval development when phages targeting Enterobacter and Pseudomonas were introduced into the larval water. Moreover, a synergistic effect was observed when both phages were simultaneously applied [46].

5. Bacteriophage Applications for Water Treatment

Bacteria are pivotal in wastewater treatment plants, playing a crucial role in the biological conversion of nutrients [47]. While essential, certain bacterial species can become problematic, instigating competition with organisms responsible for conversion and removal processes and thereby detrimentally impacting the overall treatment process. Bacteriophages have emerged as a potential solution to addressing environmental challenges associated with wastewater treatment. Their applications include mitigating issues like foam formation induced by microorganisms and enhancing the dewaterability and digestibility of post-aerobic processes, thereby increasing substrate availability for subsequent anaerobic treatment [48–50]. Phages also contribute to combating pathogenic bacteria and regulate competition between undesirable bacterial species and functionally important microbial populations in wastewater systems [47,49].

In a study, four bacteriophages isolated from an activated sludge system were combined to create a cocktail. This cocktail significantly reduced the abundance of the genus *Gordonia*, improving the wastewater treatment process tenfold compared to untreated reactors [51]. Additionally, another phage, GTE7, isolated from an Australian wastewater plant demonstrated polyvalent characteristics by causing the lysis of three species of *Gordonia* and two species of *Nocardia* among 65 strains of mycolic acid-producing bacteria [52]. The bacteriophage SnaR1, sourced from a wastewater treatment plant, was isolated to control *Sphaerotilus natans*, a filamentous bacterium known for its detrimental effects. With different multiplicities of infection of SnaR1, including an MOI of 10, there was a notable 83% reduction in the growth of *S. natans* DSM 6575 after 24 h of infection [53].

Certain bacteria, including *Vibrio*, *E. coli*, *Shigella*, and *Salmonella*, pose significant risks to human health, making their control a top priority. A study has highlighted the potential of polyvalent bacteriophages introduced into an activated sludge system to thrive and effectively suppress multidrug-resistant *E. coli* NDM-1-producing strains [54]. Importantly, this intervention did not disturb the overall population of heterotrophic bacteria. Additionally, bacteriophage AS1 isolated from sewage wastewater exhibited lytic activity against *E. coli* S2 [55]. Three other distinct bacteriophages—sww65, sww275, and sww297—isolated from wastewater demonstrated promising results in reducing *Salmonella* and other members of the *Enterobacteriaceae* family, both in vitro and within wastewater systems [56]. While the study and utilization of bacteriophages in wastewater treatment systems have become increasingly prominent, challenges persist in accurately determining bacteriophage infections outside laboratory conditions. The complexity of microbiota within biological treatment systems encompassing numerous potential hosts presents difficulties, as it may not precisely mirror the in vitro environment reproduced in a controlled setting. This complexity hinders the straightforward application and assessment of bacteriophages in real-world wastewater treatment scenarios.

6. Bacteriophage Applications in Aquaculture

Aquaculture and fish food production constitute a rapidly expanding sector in the global food industry. As reported by the Food and Agriculture Organization (FAO), global fish production surged to 179 million tons in 2018, with a remarkable 500% increase in fish food production over the last 30 years [57]. This substantial growth underscores the significant role of aquaculture in meeting the demand for fish and seafood products worldwide. Diseases originating from bacteria represent a primary cause of elevated fish mortality and substantial economic losses in aquaculture. Traditionally, antibiotics have been the primary choice for therapeutic and prophylactic purposes in aquaculture [58]. However, bacteriophages emerge as a promising alternative for controlling pathogenic bacteria in this setting. The effectiveness of phages as biocontrol agents in aquaculture has been demonstrated through various application methods, including direct introduction into water, oral administration through food, and injection. The utilization of phages instead of antibiotics has been extensively reported for eliminating pathogenic bacteria like *Vibrio*, *Pseudomonas*, *Aeromonas*, and *Flavobacterium*. In recent times, numerous successful instances of phage therapy for both preventive and therapeutic purposes in aquaculture have been documented. Notably, Phage VPp1 and A3S, along with Vpms1, effectively managed *V. parahaemolyticus* in oysters and shrimp [59]. Additionally, studies have showcased the efficacy of phage PLgY-16 in mitigating lactococcosis in yellowtail (*Seriola quinqueradiata*) infected with *L. garvieae*, administered either orally or intraperitoneally. Another example is the use of phage PPpW-4, delivered through fish feed, to combat bacterial hemorrhagic ascites disease in ayu fish (Plecoglossus altivelis) caused by *P. plecoglossicida* [60]. In the context of *Flavobacterium psychrophilum*, the Gram-negative bacterium responsible for bacterial cold-water disease in salmonid species, studies have demonstrated the effectiveness of phages such as PSV-D22 in treating infections in live fish eggs [61]. Additionally, infectious diseases in aquaculture caused by *Vibrio* genus bacteria have exhibited susceptibility to phage biocontrol. The application of phages in treating *Penaeus monodon larvae* infected with *V. harveyi* resulted in a noteworthy 85% survival rate, surpassing the 65–68% survival observed following antibiotic treatment [60]. Phages targeting specific bacterial strains such as *Vibrio* sp. Va-F3 [62], *Vibrio coralliilyticus* [63], *Vibrio alginolyticus* [64], *P. aeruginosa* [65], *Aeromonas hydrophila* [66], and *Flavobacterium columnare* [67], along with other fish pathogens, have been applied.

Despite significant progress in laboratory research, the practical application of phage therapy in aquaculture faces several challenges that need to be addressed. The complexity of application scenarios necessitates customized approaches for key parameters in phage therapy, including phage selection, dose, and administration method. However, comprehensive systematic reviews and analyses of these aspects are currently lacking. Additionally, limited attention has been given to exploring the implications of phage therapy in aquaculture on environmental health, food safety, and techno-economic practicability in the existing literature. These gaps highlight the need for further research and comprehensive assessments to bridge the knowledge and implementation gaps in the practical application of phage therapy in aquaculture.

7. Bacteriophage Applications as Environmental Sanitizers

Hospital-acquired infections pose a significant threat to patient well-being and contribute to increased morbidity and mortality within healthcare facilities, with a close link to the escalating challenge of antimicrobial resistance [68]. Surfaces in hospitals consistently harbor various bacteria, creating a serious risk of transmission and patient colonization and further causing hospital-acquired infections. Among these microorganisms are methicillin-resistant *Staphylococcus aureus*, *P. aeruginosa,* and *E. coli* strains [69,70]. Bacteriophages are suggested as a category of bio-sanitizer owing to several advantageous characteristics. Notably, they exclusively target bacteria and can maintain viability for extended periods, acting as a preventive measure against bacterial recontamination. Phages have low toxicity, are environmentally friendly and non-corrosive, and do not alter food properties or emit harmful or unpleasant odors. Therefore, they are considered safe for human consumption, with no associated safety concerns related to oral ingestion [71].

The scientific literature offers numerous examples showcasing the beneficial impact of phages on target bacteria present on surfaces relevant to food and health settings. These studies provide evidence supporting the potential adoption of phage-based bio-sanitizers. However, a closer examination reveals certain drawbacks when comparing phage-based solutions to traditional chemical sanitizers. Notably, phage-based bio-sanitizers exhibit a limited host range, exclusively affecting bacteria and lacking the broad-spectrum activity necessary for inactivating nonbacterial microorganisms like fungi and viruses. Additionally, concerns about the development of resistance are not unique to chemical sanitizers. Despite proposed solutions to addressing resistance, their practical viability remains untested. Furthermore, the majority of studies assessing phage-based bio-sanitizers have occurred in controlled laboratory settings, underscoring the imperative for real-world testing to ascertain their robustness and efficacy in practical sites.

8. Bacteriophage-Based Biotechnological Applications

Phage-based biotechnology applications encompass various innovative strategies, contributing to advancements in diagnostics, molecular biology, and nanotechnology. The specificity of phages, determined by receptor binding proteins (RBPs), plays a pivotal role in targeting bacterial pathogens. A study elucidated the interaction between RBPs and *P. aeruginosa* [72], paving the way for phage RBP-based detection systems, as demonstrated in a *Shigella* detection [73]. Endolysins with cell-binding domains (CBDs) targeting Gram-positive bacteria offer high specificity and are leveraged for bacterial diagnosis. Another study exploited these CBDs in combination with phages to enhance sensitivity in detecting viable *Listeria* cells [74]. Another application involves using phages to eliminate common sample contaminations, thus improving target bacteria recovery and enhancing detection accuracy, as seen in the removal of *Enterococcus faecalis* from vaginal samples [75].

Phage display, a potent technique enabling the display of foreign peptides/proteins on phage surfaces, facilitates the identification of diagnostic biomarkers. A phage display system was utilized to identify specific markers for tuberculosis detection, showcasing high sensitivity and specificity [76]. Advancements in structural phage biology and display technology give rise to landscape phages, contributing to the development of nanomaterials with applications in bioscience, medicine, material science, and engineering. Phages also serve as molecular biology tools, with newly developed phage–bacterium systems functioning as molecular switches to study protein–DNA and protein–protein interactions in living cells [77]. In the realm of nanotechnology, phages and their proteins prove valuable in constructing highly ordered and self-assembling nanostructures, opening avenues for diverse applications in the nanobiosciences [78].

Bacteriophage-based vaccines are emerging as a potent alternative to traditional vaccines, addressing limitations such as stability and immunogenicity. These vaccines exploit the inherent properties of bacteriophages to improve antigen presentation and immune response. There are three main types of phage-based vaccines: (1) Phage display vaccines, where bacteriophages display antigens on their surface. Phages are genetically engineered to express foreign antigens on their coat proteins. The displayed antigens can then stimulate the immune system more effectively than free antigens. (2) Bacteriophage DNA vaccines: In this strategy, bacteriophages are used as vectors to deliver DNA encoding the antigen into host cells. Once inside the host cells, the DNA is expressed, and the produced antigen stimulates an immune response. This method combines the benefits of DNA vaccines, such as the ability to induce both humoral and cellular immunity, with the stability and efficiency of phage delivery systems. (3) Hybrid phage vaccines, in which a combination of phage display and DNA vaccines are used. These phages carry both the displayed antigens and the genetic material encoding additional antigens. The use of phages as antigen carriers offers several advantages, such as increased antigen stability and preventing its degradation, ensuring a longer half-life in the bloodstream as well as enhanced immune activation via the activation of T-helper cells. Overall, phage-based vaccines represent a versatile and innovative approach to vaccination, potentially offering more effective and durable protection against a wide range of diseases. With the emergence of cutting-edge bioengineering tools and the vast reservoir of undiscovered phages and phage proteins awaiting exploration, the future holds promise for an array of captivating phage-based biotechnological applications.

9. Conclusions

In this review, we have explored the history of lytic phage therapy and its therapeutic effects. Phages show significant potential for controlling bacteria-related infections. Despite promising applications, challenges remain in translating phage therapy to clinical practice. Continued research and development are essential to overcome these obstacles and fully realize the potential of phage therapy in medicine. Additionally, the non-medical applications of bacteriophages reveal a promising landscape across agriculture, environmental management, and biotechnology. From ensuring food safety to enhancing wastewater treatment and contributing to advanced nanostructure construction, bacteriophages exhibit remarkable adaptability. The future of phages holds exciting prospects for innovative and sustainable solutions in diverse non-medical domains. As bioengineering tools continue to advance, we anticipate more breakthroughs that will establish bacteriophages as essential contributors to our efficient solutions.

Author Contributions: A.E., Conceptualization, supervision, Investigation; writing–original draft; data curation; writing–review and editing. R.F.R., data curation; investigation; analysis; writing–original draft. M.A.A.S., visualization; project administration; writing–original draft. M.R.E.; A.F., data curation; investigation, writing–review and editing. A.H.A.E.-A., data curation; investigation; writing–original draft. H.O.K., Conceptualization, supervision; writing–original draft; data curation; writing–review and editing. All authors have read and agreed to the published version of the manuscript.

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