

Bacteriophages as Sustainable Biocontrol Agents in Animal and Agricultural Systems

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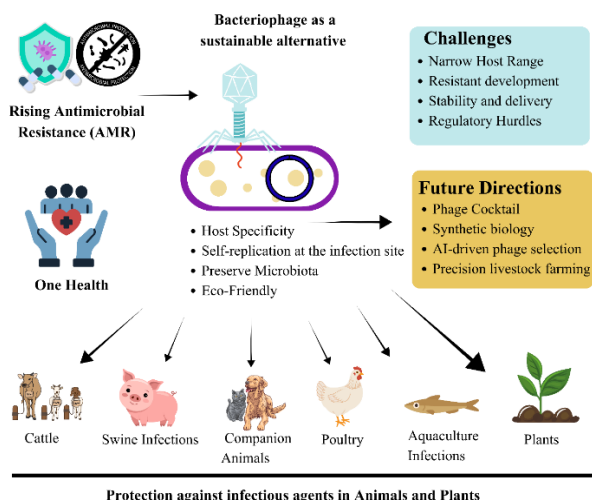
Abstract

Bacteriophages can be a green solution to address bacterial resistance and control bacteria in animals. These viruses are useful in the treatment of poultry, aquaculture, livestock, and other related fields because they target only harmful bacteria, leaving the host unaffected. Bacteriophages invade pathogenic bacteria at the infection site and replicate within the infected region. Many studies have demonstrated that phages can control diseases in animals, including mastitis in dairy cattle, infections in swine, Salmonella and Campylobacter infection in poultry, and infections associated with wounds in pet animals. The development of engineering phages has widened their spectrum and increased their effectiveness to give a more targeted solution to complex infections. Conversely, there are other challenges currently in progress, such as the variety of host phages that can be targeted, how bacteria can develop resistance to phages, stability and transportation issues, uniform regulations, and establishing manufacturing requirements. The development of new delivery forms, including the use of microencapsulation, is currently underway so that these phage applications can be used in a variety of settings in a convenient form. Various guidelines are being established to ensure phage therapy is safe in animals, both in veterinary practices and in food production. The review explains recent advances in phage research, shows where phage treatments are already being used in animals, and describes how phage therapy may be employed in veterinary medicine down the road. In this review, the authors point out the possibilities of bacteriophages to change the system of managing animal health and decrease the use of antibiotics, and make agriculture sustainable. Moreover, bacteriophages possess not only evolutionary interest in the replication process of plant organellar DNA but also has applied importance in controlling plant pathogens, further demonstrating their worth beyond animal health.

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Graphical Abstract



Introduction

The misuse and overuse of antibiotics in farm animals leads to antimicrobial resistance (AMR), which threatens all humans and animals to a considerable extent (Bava et al., 2024). Unless preventive action is taken, bacterial AMR is likely to surpass malaria, as well as HIV/AIDS, as one of the leading causes of death by 2050. This could lead to the death of more than 10 million people every year, especially in developing countries (O'Neill et al., 2016; Usman et al., 2023). The concept of One Health acknowledges the connectedness of human, animal and environmental health, since people and animals experience similar infections and therapies, including antibiotics. The AMR and the introduction of new methods may be effectively tackled by bacteriophage therapy. Bacteriophages, which are viruses that target and destroy bacteria, present a possible treatment option for AMR infections to address this new issue (Kortright et al., 2019; Strathdee et al., 2023). Phage therapy is better than antibiotics because it targets the specific host, does little harm to the body's microbes, and multiplies where necessary to reduce the need for frequent treatments (Abeldon et al., 2011). Because of rising AMR and strict rules limiting antibiotic use in animal farming, researchers are now revisiting phages as a safe alternative (Jiang et al., 2024).

Before the discovery and widespread use of antibiotics, bacteriophage therapy was created. Canadian microbiologist Felix d'Hérelle proposed using syringes containing bacteriophages from patients with dysentery to treat *Shigella* infections at the beginning of the 20th century (Nikolich & Filippov, 2020). In 1931, Tsulukidze was the first surgeon to apply phages in surgery. In 1938, another famous Soviet

surgeon, Burdenko, proposed the use of phages against purulent disease by the *Proteus* species, *Staphylococci*, *Streptococci*, and *Escherichia coli* (Metsemakers et al., 2023). According to Mannapperuma and Santos (2004), bacteriophages had been used in veterinary medicine prior to the presence of antibiotics, primarily in Eastern Europe and the Soviet Union. However, Western nations began to overlook phage therapy with the discovery of penicillin and several other antibiotics (Jiang et al., 2024). However, Western researchers lost interest in phage therapy in the 1940s as penicillin and other broad-spectrum antibiotics became more widely available. Antibiotics successfully cured a wide range of pathogenic organisms and were easier to produce, standardize, and administer. As a result, phage development was postponed in many regions, except the Soviet Union and the Eastern Block, where research institutions were unable to stop their work due to challenges in obtaining antibiotics (Fortier & Sekulovic, 2013).

AMR threats and rising regulations against antibiotics use in farming sparked new interest in phage therapy in the late 20th century and early 21st century (Castanon et al., 2007). Some studies have demonstrated that treating livestock and poultry infections with phages can work against *Salmonella*, *Campylobacter*, *E. coli*, and *Staphylococcus aureus*. Many of these pathogens lead to severe economic problems by causing diseases in animals and humans (Chapman et al., 2016). Phage therapy was initially used in animals to treat infections caused by *S. aureus* in bovine mastitis, specifically in dairy cows. One study established that the injection of the mammary gland with a specific phage in large numbers minimized bacterial counts and clinical symptoms, which underlines its suitability in the management of dairy cows (Wernicki et al., 2017). Recent investigations in swine have determined that oral supplementation of phages against *E. coli* can greatly lower diarrhea during the post-weaning period (Yu et al., 2017). The recent research studies have shown that phage therapy has been effective in decreasing the occurrence of *Salmonella*, *Campylobacter* and *E. coli* in poultry and swine. Ahmadi et al. (2016) showed that pre-exposure of phages reduced *S. enteritidis* in quail and that encapsulation of the phages reduced *Salmonella* contamination in broiler chickens. The evolution of molecular biology has increased phage specificity and lytic efficiency, and research is being conducted to explore their application to control gut microbiota and to improve animal health (Heselpoth et al., 2019). Along with these innovations, there are still issues with uncontrollable phage-bacteria interactions, bacterial resistance, regulation, and protocols (Zampara et al., 2020). In addition, recent achievements in the field of molecular biology have allowed producing phages, which are more efficient and capable of working with a broad range of

hosts. CRISPR-Cas edits and synthetic biology have enabled the capability to alter phages that have the potential to breach the defense of a bacterium, which can assist veterinary medicine (Chaudhry et al., 2017). This review aims to emphasize the potential of bacteriophages in changing the way, animal health is being managed, lessening the use of antibiotics, and ensuring long-term agriculture sustainability. This review will explain the functionality of the phages, its use in different animals, challenges and advantages of using the phages in contemporary agriculture.

Understanding of Bacteriophage Antimicrobial Functions

Bacteriophages (phages) are viruses that attack and replicate within bacteria. They are practically present in every habitat where bacteria are predominant (soil, water, and the gastrointestinal tracts (GIT) of organisms) (Jäckel et al., 2019). The selection of the microbial community plays an essential role, where phage performs its lytic activity. The ability to selectively attack certain strains of a bacterial host enables its use as a potential candidate in veterinary medicine and animal health care, especially as an alternative to antibiotics (Jiang et al., 2024). However, phages may affect the pathogenicity of the bacteria directly by attacking the pathogenic bacteria, regulating the expression of virulence proteins in the bacteria, or through resistance to antibiotics (Lopez et al., 2012).

Classification and Lifecycle of Bacteriophage

Phages are classified morphologically into three main families, *Myoviridae* (with contractile tails), *Podoviridae* (with short, non-contractile tails), and *Siphoviridae* (with long, flexible tails). These structural differences influence the mechanisms by which phages attach and infect their bacterial hosts (International Committee on Taxonomy of Viruses Executive Committee, 2020). There are a vast amount of morphological and genomic phenotypes of phages which are mainly discussed in terms of their physical appearance and nucleic acid composition. Although by far the largest proportion of known phages are dsDNA phages, phages whose genomes are comprised of ssDNA, dsRNA, and ssRNA have been reported. The International Committee on Taxonomy of Viruses (ICTV) has defined more than 50 viral families of the double-stranded (ds) DNA bacteriophages, but there is a known family of no less than seven species of the Cystoviridae family of a single type of the bacteriophage, the family of Cystoviridae, and only one family of the dsRNA bacteriophages is known (Mäntynen et al., 2023). Classified dsRNA phage isolates have a comparatively limited host range compared to dsDNA

phages that have been isolated from a broad variety of bacterial hosts. But recent discoveries of new dsRNA phages and metatranscriptome analysis of viral sequences from various settings reveal that dsRNA phages are more common in nature than previously thought (Chen et al., 2022; Li et al., 2022). Bacteriophages have two main life cycles; namely lytic and lysogenic cycles. Lytic phages replicate themselves at the expense of the bacterial host, and upon release of their offspring, they cause the death of bacteria. Lysogenic phages can form prophages, which can be integrated into the bacterial genome (Garvey et al., 2020), constituting nearly 20% of the bacterial genome, and recent studies have discovered that the average number of prophages in the bacterial genomes of the human gut is only 5% (Gauthier et al., 2022; Inglis et al., 2024). One type of prophage is plasmid phages, which replicate together with bacterial genome, and is in the form of an extrachromosomal element. Oxidative stress, temperature, pH, or chemicals are the environmental conditions that activate lysogenic phages and cause a shift to the lytic cycle (Henrot & Petit, 2022). Apart from these two typical lifestyles, certain phages continue to exist inside bacteria as pseudo-lysogens, which means they remain a non-replicating, non-integrating plasmid. This state appears to be temporary and is triggered by unfavorable environmental factors for the bacteria, such as bacterial malnutrition (Cenens et al., 2013; Łoś & Węgrzyn, 2012). When the environment changes, pseudo-lysogenic phages can transition between a lytic and a lysogenic cycle. *In vitro*, pseudo-lysogeny has been demonstrated for certain *P. aeruginosa* and *E. coli* phages (Nabergoj et al., 2018). The specificity of this attack comes from connections between phage receptor-binding proteins and lipopolysaccharides, teichoic acids, flagella, and pili on the surface of bacteria (Schmelcher et al., 2012).

Advances in Phage Research and Therapy

Phage research has recently resurfaced as an alternative and innovative means of addressing the rising risk of AMR. Phage-therapy has been used successfully in Eastern Europe for approximately 90 years without endangering patients' health (Tang et al., 2019). This treatment has been shown to be effective in the treatment of the AMR diseases in Poland (Selle et al., 2020). It offers a feasible alternative to current treatment methods, in particular, the treatment of diseases with significant fatality rates, i.e., *Clostridium difficile* infection (CDI). Despite the challenges yet to be eliminated, bacteriophage treatment has provided an opportunity to combat infectious diseases and AMR (Amjad et al., 2024). With the recent achievements in molecular biology and sequencing, the discovery of new phages with high lytic activity, which can infect several different hosts has become

much easier. Metagenomics and synthetic biology are now applied by researchers to improve the reliability of phages, which solve some problems of naturally occurring phages (Chaudhry et al., 2017). Building on this, several have proposed that combining various phages against different bacterial receptors can make the treatment more effective and minimize the chances of resistance forming (Abbas et al., 2022).

Bacteriophage-Derived Mechanisms in Plant Biology and Disease Control

In addition to their potential utilization as bio tools to combat bacterial diseases in the field, bacteriophages are closely related in evolutionary terms to plant molecular machinery. Research shows that major components of plant organelle DNA replication machinery are structurally and functionally homologous to enzymes encoded by bacteriophages. As an example, the protein AtTwinkle of the *Arabidopsis thaliana* (AtTwinkle) is the two-targeted primase-helicase that supports the mitochondrial and plastid DNA replications and appears architecturally and functionally close to the T7 bacteriophage primase-T7-helicase (gp4), forming an active replisome with the organellar DNA polymerases *PolIA* and *PolIB* (Peralta-Castro et al., 2023). In this evolutionary context, plant organellar replication systems suggest that they are more homogeneous with animal mechanisms in terms of replication systems, rather than resembling bacterial phage. What is more, bacteriophages are under active investigation as renewable substitutes to antibiotics in crop defense (Hasan & Ahn, 2022). Success in pepper (or tomato) plants has already been achieved in controlling *Xanthomonas euvesicatoria* pv. *Euvesicatoria*, the causal agent of the bacterial spot disease, uses phage-based treatments, overcoming the problem of copper-resistant strains (Eski et al., 2022). Accordingly, bacteriophages can indeed be seen as direct biocontrol in the field of plant pathology, and as having an echo in the actual architecture of plant organellar genome maintenance systems.

Mechanism of Action of Bacteriophage in the GIT

After infecting bacteria, phages multiply within them and proliferate rapidly, using the bacterium's genome for protein synthesis and energy production to support rapid growth. Bacteriophages are employed in antimicrobial phage therapy due to their ability to identify, adsorb, grow, and lyse bacteria exclusively within the cells they inhabit (Burrowes et al., 2011). Phage therapy, in the simplest sense, is the employment of viruses specific to infectious bacteria to stop the growth of harmful bacteria (Carrillo et

al., 2005). Bacteriophages can typically be successful against antibiotic-resistant Gram-positive and Gram-negative bacteria (Chhibber et al., 2013). It is demonstrated to be safe and comparatively side-effect-free (Jończyk-Matysiak et al., 2015), to use in the treatment of human systemic and local illnesses (Rose et al., 2014). However, there are two methods of bacterial reproduction: lysis and lysogenesis. When the virus is released, each bacterial cell is lysed in the case of lytic phages (Newell et al., 2010). Phage replication occurs in two key life pathways: the lytic cycle and the lysogenic cycle, which have distinct mechanisms and consequences related to bacterial physiology and antibiotic treatment.

Lytic cycle (Virulent Phage)

The lytic cycle starts with the specific attachment of a bacteriophage to the cell surface of bacteria through the receptor-binding proteins (Manyi-Loh et al., 2018). After attaching, the phage transfers its genetic material to the cytoplasm of the host. The viral genome is generally kept free in this cycle and does not get incorporated into the host chromosome. Rather, it quickly takes over the metabolic machinery of the host to replicate its nucleic acids and produce viral proteins (Reganold & Wachter, 2016). Messenger RNA molecules, which regulate the host's ribosomes, are directly copied by DNA viruses. Later, during the translation process, the newly translated proteins come together to form the phage's capsid and tail, which then escape from the host cell. The newly generated fragments then proceed to infect and replicate additional host cells. As an example of horizontal gene transfer (HGT), the host chromosome is occasionally packed into the capsid during phage replication, rather than incorporated into the phage genome (Jajere et al., 2019). Treating antibiotic-resistant pathogenic bacteria with their respective lytic cycles is one direct use of lytic-cycle-only phages.

Lysogenic cycle (Temperate Phage)

While temperate bacteriophages can also follow a lytic growth cycle, they have evolved a different method of multiplication that does not kill the bacterial host. A lysogenic cycle in which the viral genetic material is incorporated into the genome of bacteria, called a prophage, and continues to replicate without causing death to the host (Chen et al., 2020). According to Cieřlik et al. (2021), each bacterial daughter cell that obtains an integrated bacteriophage genome at this stage is called a prophage. However, viral genetic material infection can change bacterial phenotypes because the virus integrates its genome into the host. Based on this change, it is clear that most typical strains of bacteria are dangerous. Hydrogen

peroxide can also be used to prevent this kind of lysogenic conversion by creating glutathione, reactive oxygen species and overexpressing transcriptional repressors (Keen & microbiology, 2012). The lysis of bacterial cells is the crucial step that separates the lytic cycle from the lysogenic one (Fortier & Sekulovic, 2013). The phage-encoded endolysin (lysin) can enter and hydrolyze the peptidoglycan layer because of the protein holin, which creates holes in the cytoplasmic membrane. Cell lysis and the release of daughter phages that can infect further microbial cells are the outcomes of this (Fortier & Sekulovic, 2013) and the entire cycle may last from 20 minutes to two hours (Abd-El Wahab et al., 2023), as shown in Figure 1.

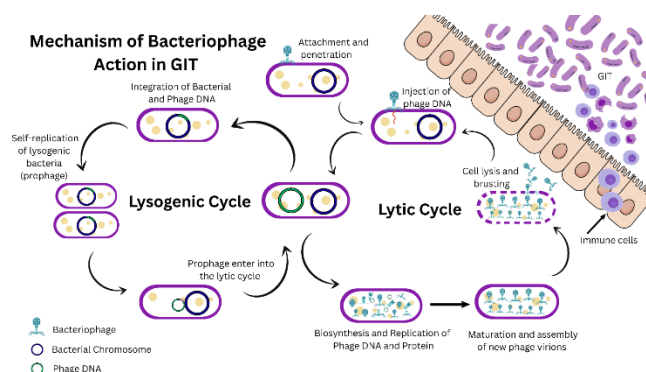


Figure 1: Bacteriophage Action in GIT. Illustration of the mode of action of a bacteriophage in the gastrointestinal tract of an animal, showing the specificity of the pathogenic bacteria with conservation of beneficial bacteria in the GIT.

Advantages of Bacteriophages Over Antibiotics

High Host Specificity

Specificity of action is the most critical feature of bacteriophages that is the possibility to kill only certain types of targeted bacteria and not other microorganisms (Domingo et al., 2018). Broad spectrum antibiotics kill all the bacteria present in the body whereas phages only kill those bacteria that they are designed to kill. The main advantage of this solution is that it protects the probiotic and commensal bacteria that help to maintain the microbial balance within the gut and other mucous membranes. The phages will not cause other bacteria to develop resistance as they infect a few hosts. This feature is particularly relevant to animals producing food, as the immunological and digestive system of the animal is based on the stable gut microbiome (Jäckel et al., 2019). Phages can prevent or lessen infections in animals by selectively destroying

pathogens, thereby maintaining the animal's healthy microbiome.

Bacteriophages are believed to be much safer and more palatable because they exclusively proliferate in the particular bacterial cells they are intended to infect (Kakasis & Panitsa, 2019). The main issue directly linked to the use of antibiotics is that they do alter the entire microbiome by eradicating potentially helpful bacteria and encouraging the development of secondary illnesses (Domingo et al., 2018). The most effective way of using phages in therapy is by using phages as cocktails to enhance their ability to kill, increase their target coverage, or prevent the development of phage resistance (Chan et al., 2013). The efficacy of some phage mixtures that are developed to combat *Salmonella* have been evaluated by use of challenge tests in pigs and poultry (Martinez et al., 2019).

While bacteriophages are useful in treating diseases caused by a single bacterium, infections caused by multiple harmful bacteria are common in clinical cases (Abd-El Wahab et al., 2023). Other risks associated with using bacteriophages include the difficulty of preparing bacteriophages for therapeutic use and the fact that not all issues related to the physiology of these viruses have been resolved (Lin et al., 2022). Additionally, the lysogenic cycle's specificity may increase the likelihood that harmful genes could proliferate throughout the environment. Because bacteriophages in the lysogenic condition can also spread genes and toxins that render bacteria resistant to antibiotics, they pose a major risk to public health (Abd-El Wahab et al., 2023). Since 2011, the phages have been classified as medical items in the EU and therapies in the US (Guo et al., 2020).

Self-Replicating Nature

Bacteriophages are excellent candidates for use in the identification and control of pathogens at every stage of the food production process, from farm to fork, because of their innate capacity to specifically infect and eradicate their target bacteria, as well as the fact that they are prevalent in the environment and safe for both humans and animals (Vikram et al., 2021). Their capacity to multiply at the site of infection means that a small dose can be applied to further combat germs over a long duration. A phage multiplies upon infecting a colony forming several young phages to infect other bacteria. The positive feedback from the mechanism increases treatment effectiveness, particularly in areas with high bacterial populations (Abedon et al., 2011). One dose of phages may stop the growth of bacteria that require repeated antibiotic treatment (Abbas et al., 2022). The two-in-one formulation of the drug is also useful in the veterinary facility in cases, where a system of giving several doses of the drug would be difficult. One major benefit of phages is that they can be fed

to animals to protect them against *Salmonella* and *Campylobacter* for extended periods of time, meaning antibiotics can be used less frequently (Ahmadi et al., 2016). In a similar fashion, intramammary phage is very effective in the treatment of mastitis in dairy cattle, and does not need to be reapplied frequently (Wernicki et al., 2017).

Low Toxicity and its Environmental Impact

As phages do not interact with eukaryotic cells, they are regarded as safe for animal use. While some antibiotics can harm the stomach, cause allergic reactions, and be toxic to the liver and kidneys but phages do not affect normal mammalian health (Heselpoth et al., 2019). Many safe studies of phage therapy have been carried out in labs and the field, and no severe side effects have ever been seen in the treated animals (Zampara et al., 2020). Besides being host selective and the enzymes that they encode, the resistance of bacteriophages to environmental conditions highlights their capabilities to be used as a biocontrol agent in various aquatic environments or biotechnological processes throughout the consumer water cycle (Reyneke et al., 2024). Bacteriophage biocontrol can also provide the chance to aid the bioremediation of natural water sources and to improve a number of industrial or treatment processes due to a specific elimination of microbial contaminants and pathogens that can prove hard to be eliminated with the help of conventional microbial control methods. This is due to the presence of bacteria in the consumer's water cycle, including bacteria that develop biofilms during food processing, wastewater, and water distribution systems, as well as bacteria that are resistant to antibiotics and drugs (Bisen et al., 2024; Cristobal et al., 2021). In aquaculture, researchers are considering phage therapy instead of antibiotics because it helps to remove *Aeromonas hydrophila* and *Vibrio anguillarum*, without endangering helpful aquatic microbiota (Chaudhry et al., 2017). Phage grazing livestock in agriculture has demonstrated the possibility of reducing antibiotic drainage and preventing the emergence of resistance genes in any given environment (Fortier & Sekulovic, 2013).

Applications for Phage Therapy in Livestock

In animal husbandry, bacteriophage-based antimicrobial therapy has been seen as an ecologically friendly substitute to antibiotics in the treatment of bacterial diseases. Viruses can be used in fish, poultry, swine, and cattle production since they only infect one host, multiply, and do minimal harm to the microbiota. The application of phages in different areas has been discussed by many researchers and proven to improve the health status of animals, reduce the number of infections, and decrease the possibility of animals spreading diseases to humans (Jiang et al., 2024).

Cattle and Swine

Mastitis is a pathogenic disease of dairy and beef cattle, and it is commonly caused by *S. aureus*, *E. coli*, and *S. uberis* (Wernicki et al., 2017). The pathogens developing resistance to the traditional antibiotics through the establishment of a protective biofilm cause a failure in treatment of the pathogen. Research studies have proven that using phage therapy can decrease infections within a cow's udder. Specifically, giving lytic phages to *S. aureus*-infected cows into the affected udder reduced the somatic cell count and clinical symptoms (Wernicki et al., 2017). Moreover, a type of phage cocktail has been produced to help control multiple types of mastitis, making them more effective and reducing the odds of resistance (Zampara et al., 2020).

Pigs are vulnerable to bacterial infections including *Salmonella*, *E. coli* and *Brachyspira hyodysenteriae* which may cause huge losses. Enterotoxigenic *E. coli* causes diarrhea following weaning in pig farming and this is normally managed by prophylactic antibiotics. Nevertheless, in many locations, antibiotic growth promoters are more restricted, so the application of phages has received significant attention. Giving oral phages that target Enterotoxigenic *E. Coli* to weaned pigs has been shown to decrease diarrhea and improve the conversion of food into growth (Yu et al., 2017). Phage cocktails which are meant to attack various *E. coli* virulence factors have become more effective than by themselves. Phage therapy has also been employed to help control *Salmonella Typhimurium* in pigs, and results thus far indicate that phages reduce intestinal bacteria and fecal contamination (Abbas et al., 2022). Swine dysentery is a disease caused by *Brachyspira hyodysenteriae* and is being experimentally tested as a treatment using phages. Farmers need alternative management methods since bacteria have developed resistance to tiamulin and valnemulin. Targeting phages can be beneficial, according to applied research, but further research is needed to ensure that the technique is reliable and efficient for wider application (Fortier & Sekulovic, 2013).

Poultry and Aquaculture

Several researchers have found that phages can reduce the amount of *Salmonella Enteritidis* and *Salmonella Typhimurium* in broiler chickens. Administration of phages by adding them to drinking water or feed is proven to cut back on pathogens in the cecum and fecal matter, making it less likely that contaminations occur during processing, as shown in Summary Table 1 (Ahmadi et al., 2016). Besides, microcapsules containing phages are being used to help them release at the correct point in the digestive tract by

Table 1: Summary of Bacteriophage Applications in Different Animal Species

Animal Species	Target Pathogen	Phage Application Method	Outcome / Efficacy	Citations
Broiler chickens	<i>Salmonella Enteritidis</i>	Oral administration	Reduced cecal colonization, improved gut health	(Grabowski et al., 2022; Sarrami et al., 2022)
Laying hens	<i>Salmonella Typhimurium</i>	Dietary supplementation	Lowered pathogen load and enhanced immunity	(Lee et al., 2021)
Swine	<i>Escherichia coli</i>	Feed or water supplementation	Reduced post-weaning diarrhea and improved weight gain	(Ahmadi et al., 2016)
Dairy cattle	<i>Staphylococcus aureus</i>	Intramammary infusion	Decreased somatic cell count, reduced mastitis symptoms	(Wernicki et al., 2017)
Fish	<i>Aeromonas hydrophila</i>	Water treatment or feed additive	Reduced mortality, controlled infection spread	(Ture et al., 2022)
Companion animals	<i>Pseudomonas aeruginosa</i>	Topical application	Effective wound healing, reduced inflammation	(Wood et al., 2023)
Equines	<i>Brachyspira hyodysenteriae</i>	Oral or intravenous	Improved hoof health, reduced laminitis-associated infection	(Archambault & Rubin, 2018)

making them steadier, thus supporting better treatment effectiveness (Abbas et al., 2022). *Campylobacter jejuni* is a deadly threat and a common cause of gastroenteritis in humans in poultry. Sequential use of group II and III phages appears to control *Campylobacter* in broilers, without creating fast resistance, as reported by Hammerl et al. (2014). In addition, it has been studied that phages can be used to wash or spray corpses in order to minimize contamination during slaughter and processing (Olson et al., 2022). Phages are also being investigated by researchers to enhance the immunological response of poultry by modifying the bacteria in their gastrointestinal tract. As a few studies suggest, phages can enhance the microbial diversity of the gut, which might benefit digestion, enhance nutrient absorption, and increase resistance to disease (Jäckel et al., 2019).

Fish bacterial infections are being successfully treated using phage therapy, which mostly keeps fish from contracting vibriosis and edwardsiellosis. Recent studies have demonstrated that phages administered by injection, oral, or immersion decrease the number of bacteria and mortality in infected aquaculture populations (Heselpoth et al., 2019). The application of Edwardsiaceae phages to treat Edwardsiaceae in tilapia enhanced the survival rate and decreased the symptoms of infections. In place of conventional antibiotics, phage mixtures are also being used to treat *Aeromonas* infections in carp (Jamal et al., 2015).

Considering the above, a global perspective of bacteriophages is shown in Figure 2.

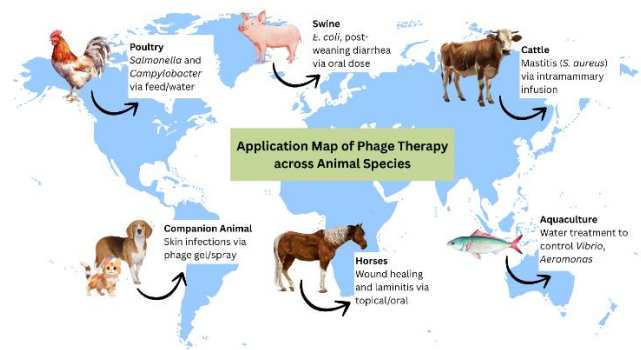


Figure 2: A Global perspective of bacteriophages used in various animal species, with a focus on targeted pathogens and delivery systems in the production of livestock/poultry, aquaculture, and companion animals.

Phage-Based Products and Delivery System

Over the recent years, the bacteriophage products have received more focus considering that farmers are increasingly adopting alternative ways of treating diseases. To eliminate certain bacterial pathogens such as *Salmonella*,

E. coli, *Listeria monocytogenes*, and *Campylobacter* found in poultry, livestock, and aquaculture, these formulations are developed.

Phage formulations are created using various combinations, delivery systems, and application manners, including liquids, powders, microencapsulated versions, and spray or

feed additives (Hussain et al., 2017). The way phages are delivered aims at ensuring that they stay active in the adverse environment including low pH, high temperatures, and enzyme digestion in order to reach the target site safe. Poultry and swine are usually given medicine in their water or feed, but for dairy cattle, topical and intramammary administration is preferred, as shown in Table 2 (Jiang et

Table 2: Overview of Phage-Based Formulations and Application Methods

Animal Type	Formulation Type	Delivery Method	Target Pathogen(s)	Application Context	Citations
Poultry	Liquid suspension	Drinking water	<i>Salmonella</i> , <i>E. coli</i>	Pre-harvest control	(Zhao et al., 2022)
Poultry	Lyophilized powder	Feed supplementation	<i>Salmonella Gallinarum</i>	Prevention of fowl typhoid	(Żbikowska et al., 2020)
Swine	Microencapsulated phages	Oral	<i>Escherichia coli</i>	Control of post-weaning diarrhea	(Loh et al., 2021; Thanki et al., 2022)
Dairy Cattle	Intramammary solution	Infusion into the udder	<i>Staphylococcus aureus</i>	Treatment of mastitis	(Wernicki et al., 2017)
Aquaculture	Water-soluble formulation	Water treatment	<i>Aeromonas hydrophila</i> , <i>Vibrio spp.</i>	Disease prevention in fish farms	(Zampara et al., 2020)
Companion animals	Topical gel/spray	Direct application to the skin/wounds	<i>Pseudomonas aeruginosa</i> , <i>S. pseudintermedius</i>	Treatment of dermatitis and otitis	(Wood et al., 2023)
Broilers	Spray formulation	Surface application on the carcass	<i>Campylobacter</i> , <i>Salmonella</i>	Post-harvest decontamination	(Goode et al., 2003)
Ruminants	Encapsulated feed additive	Oral via feed	<i>Escherichia coli</i> O157:H7	Reduction of pathogen shedding	(Stanford et al., 2010)
Food processing	Phage cocktail in wash/spray	Sprayed on meat surfaces	<i>Listeria monocytogenes</i>	Decontamination of ready-to-eat meats	(Perera et al., 2015)
Livestock	Combination therapy	Oral + probiotic/prebiotic synergy	Multi-pathogen control	Integrated disease management	(Abbas et al., 2022; Naureen et al., 2020)

al., 2024). Using phages together with probiotics, prebiotics, or organic acids helps make them work better and less likely to cause resistance (Pelyuntha & Vongkamjan, 2022).

Restrictions and Problems of Phage Therapy to Animals

Narrow Host Range

Phages have a major advantage in their ability to infect only specific hosts, but this trait prevents them from being widely used in practice. While many broad-spectrum antibiotics treat various bacteria at once, most phages are created for a specific strain or species (Abd-El Wahab et al., 2023). Since phages should be aligned with the pathogen, pathogen testing is needed prior to initiation of therapy. This can at times be troublesome in field conditions, where no quick diagnostics is involved.

In order to overcome this issue, researchers have embarked on using phage cocktails that target various regions of the same bacteria or different bacterial species linked to a particular disease (Jäckel et al., 2019). While cocktails made, infections and lysis is more likely, they also cause difficulties during vaccine formulation, standardizing them, and getting approval. The effect of phages can vary between different ground conditions and different bacteria, so the treatment to use in each area may require adjustment based on the types of bacteria present in that area (Zampara et al., 2020).

Development of Bacterial Resistance to Phages

Phages and bacteria are evolving ways to avoid and resist each other. Strategies that bacteria often use consist of receptor modifications, CRISPR-Cas offers a backup protection, restriction-modification systems, plus special infections to foil the virus (L.-C. et al., 2013). The phage binding can be blocked by mutations in outer surface proteins or LPS, whereas the phages targeting the bacterium with CRISPR-Cas immunity can be identified and destroyed (Xiong et al., 2020). The speed of evolution of the phages and bacteria is an indication that phage libraries need to be updated and monitored after time to maintain treatment efficiency. Genetically altered microorganisms called engineered phages are a way to introduce medicine that circumvents bacterial resistance (Chaudhry et al., 2017).

Delivery Issues and Regulatory Hurdles

The presence of enzymes, temperature, pH, and UV light can all kill phages and reduce their utility during production and use. The phages typically do not withstand the acidic environment of the stomach and enzymes of the GIT, but

they are often delivered orally to cattle and poultry due to the convenience of this method (Abbas et al., 2022). To solve these difficulties, many approaches are used, such as microencapsulation, lyophilization, and putative drugs in alginate or chitosan (Ahmadi et al., 2016). They make sure that the phage is not damaged until it gets to the digestive tract where it is released and only at the infected site. Researchers are also investigating controlled-release formulations and bio-adhesive gels to ensure phages remain active and give better treatment results when bacteria are targeted on the skin or within the mammary glands (Wernicki et al., 2017). Animal phage therapy regulations are still being developed gradually and are not yet integrated. Various legal frameworks treat the phage-based goods differently causing different standards of assessment and entry into the market. The Center for Veterinary Medicine at the FDA regulates phages prepared for veterinary use in the United States, while the European Medicines Agency oversees those prepared in the EU (Naureen et al., 2020). Phage therapy must show that the preparations are safe, pure, powerful, and produce the same results all the time. Production of phages can hardly be a consistent process across multiple batches due to the nature of phages as biological organisms and the variations in the process of phages propagation. Standardized procedures of phage testing, property description, and quality maintenance are needed to be approved by the regulatory authorities and used in many applications (Zampara et al., 2020).

Immune Response and Quality Control Challenges

While phages are usually harmless to eukaryotes, their interaction with a patient's immune system could still affect the results of treatment. Certain studies indicate that phages may change the level of inflammation, when they interact with toll-like receptors, which creates new cytokines and activates immune defense (Miernikiewicz & Dąbrowska, 2022). The release of endotoxins by bacterial cells during phage therapy of Gram-negative bacteria raises the risk of complications linked to sepsis. Lipopolysaccharides (LPS) can be released when a great number of bacteria die, resulting in inflammation and septic shock (Heselpoth et al., 2019). Due to the reduction of released endotoxin, it is possible to use endolysin derivatives which inhibit Gram-negative bacteria but do not kill them, in addition to using anti-inflammatory drugs, or simply timing their doses (Zheng & Zhang, 2024).

Specialized facilities and reliable methods are needed to guarantee that phages are produced in large quantities in a safe manner and of consistently the same quality. The process generally involves researchers growing host bacteria and proceeding with filtration, centrifugation and chromatography used to clean the phages. It's crucial to

control contamination risks, process cell fragments, and produce antibiotics to meet pharmaceutical requirements (Callaway et al., 2004). The quality control should check the stability of the genetics since phages can be changed during replication, which can affect the nature of the hosts, or the activity of the phage. Thorough testing is required to ensure that final chemicals maintain their beneficial qualities and are incapable of horizontally transmitting or lysogenizing genes (Fortier & Sekulovic, 2013).

Regulatory Landscape and Approval Pathways

Phages can be the byproducts of the food safety or biocontrol industry, or they can be regarded as biological agents in certain countries and fall under the jurisdiction of something similar to probiotics or vaccine regulations (Jiang et al., 2024). The list of regulations regarding bacteriophages in veterinary medicine is country-specific and depends on the purpose of the product, but it is constantly changing. Because there is no uniform process for certifying phages, unlike antibiotics, it can be very challenging to find a consistent approach and path to market (Naureen et al., 2020). Understanding these distinctions is crucial to maintaining products' efficacy, safety, and consistency wherever they are used.

Global Regulatory Frameworks

EMA regulates veterinary medicines in the EU using the Committee on Medicinal Products to Veterinary Use (CVMP). Phage therapies used for treatment must adhere to Directive 2001/82/EC, which explains the necessities for ensuring quality, safety, and efficacy. Guidance on immunological veterinary medicinal products is issued by the EMA according to which the Categorization 1-4 (grounded on microbial characterization and batch stability) can be reasonable in the case of phage formulations (Jordá et al., 2023). Phage therapy is not covered by a special set of rules, which makes it difficult to get products through registration. United States FDA categorizes phage products into two major guidelines. CVM takes care of animal therapeutic treatments, whereas the Center of Food Safety and Applied Nutrition (CFSAN) is concerned with phage uses in food processing and biocontrol. Animal health phage products must be thoroughly tested in the same manner as conventional antimicrobials including tests of how the products are absorbed and utilized by animals, safety and controlled trials in animals. Currently, most phages are approved by the FDA for food safety, especially to control *Listeria monocytogenes* in prepared meats (Perera et al., 2015). Various locations have reacted by choosing a more flexible path. In Georgia and Poland, where phage therapy has been used for a long time, regulatory authorities refer to

common regulations but highlight testing through research rather than using strict medical antibiotic rules (Fortier & Sekulovic, 2013). The agricultural and veterinary chemical legislation in Australia enables registration of phage-based biocontrol products provided the products are safe and their use is warranted.

Labeling and Quality Standards

Standardization of phage-based products is important and therefore, it is necessary to have uniform protocols to ensure consistency and reproducibility in isolation, purification and formulation of the products. The problem with a significant impact is that phages are biological materials and their propagation and stability may be inconsistent across different host strains, growth environments, and storage conditions (Zampara et al., 2020). Contrary to the synthetic antibiotics, phages should be grown in live bacterial hosts, which is a concern regarding risks of contamination, endotoxins, and long-term stability of gene structure. There is also a significant difference in the labeling standards of various jurisdictions. Some countries insist on specifications of the host range, titer concentration (e.g. the number of plaque-forming units per mL), and usage guidelines, whereas others have less strict labeling protocols, especially regarding phage products that are applied to agricultural sources (Naureen et al., 2020). Balancing these criteria will be important in easing international trade and consumer trust in interventions based on phage. To be reliable and manufactured on constant, screening, purification, and formulation processes have to be standardized in case of phage-based products. Because phages are living organisms, the approach to making each batch consistent is complicated due to host effects, growth, and storage changes (Zampara et al., 2020). Because phages rely on bacterial hosts to multiply, a question of the need to maintain purity, overcome toxins, and constant genetic composition might be raised. All regions have various labeling standards. Some nations require a lot of information on the host range, titer concentration, and use of the phage, whereas others follow less strict guidelines for products mainly meant for agriculture (Naureen et al., 2020). Through standardization of the methodologies, the nations can facilitate the international trade and assure consumers to use phages medically.

Concerns of Biosafety and Genetically Modified Organisms

Phage therapy regulations are concerned with the possibility of genetically modified organisms being used to change the ability of phages to kill bacteria or to affect more hosts. While engineering phages with CRISPR-Cas or synthetic

biology creates hopeful options for their use, it also creates questions about possible unplanned interactions with other organisms (Chaudhry et al., 2017). Only a limited number of products using phages have come out of laboratory research due to the challenging nature of getting them approved (Jäckel et al., 2019). However there are still debates amongst researchers, industry players and regulators to make sure that the new methods are safe and innovative.

Future Prospects of Phage Therapy

With AMR on the increase and the urgent necessity to find a sustainable way of replacing antibiotics, there are increased interest in the application of bacteriophages to combat infections in animal bodies. Even though much has been discovered about phages, their host specificity, and medicinal benefits, there is still a lot to study to make full use of them. Further development of the search of phages, their optimal preparation, control of regulations, and the integration of phage treatment in agricultural activities and One Health is a subject of study in the future.

Phages Discovery and Characterization

The application of metagenomic and bioinformatic techniques to increase phage libraries is one of the key areas of future research. Researchers have discovered certain phages with distinct hosts and methods for eliminating those hosts because of improved sequencing technology. In metagenomic studies, it was discovered that phages may inhabit very diverse places, such as farmlands, water, and animal intestines (Jiang et al., 2024). These results can be used to choose phages that particularly infect *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, and *E. coli*. Comparative genomics and scan tools for prototypical CRISPR sequences, like CRISPR Finder, can find the genes that cause host identification, explosive lysis, and strategies to get around resistance mechanisms (Grissa et al., 2007). The realization of these relationships will inform the selection of the most appropriate phages to use in therapy and help to develop effective phage combinations that would not develop resistance (Zampara et al., 2020).

Phage Engineering and Synthetic Biology

Synthetic biology provides us with new means of making phages more useful to veterinary medicine. Phages can be modified with genetic engineering to infect a greater variety of bacteria, work better, or cause less reaction in the host. As an example, Gram-positive genes that encode endolysins have been connected to modified vectors, which have enhanced Gram-negative bacteria treatment capabilities

(Heselpoth et al., 2019). Moreover, CRISPR-Cas tools are currently being employed to manipulate phage-host interactions and target antibiotic-resistant isolates (Khalid et al., 2021). Besides, advances in this field now make it possible to adjust receptor-binding proteins in phages, so they can either identify new types of receptors or evade bacterial immune systems, like restriction-modification and CRISPR, that try to stop them (Chaudhry et al., 2017). Even so, these inventions could generate special phages better able to fight diseases, but such valuable phages would need to be carefully made and watched.

Artificial Intelligence and Integration with Precision Livestock Farming

The use of artificial intelligence and machine learning is now being applied to make inferences on phages and hosts relationships more accurate and utilize it to enhance phage therapy. Through research on large datasets on phage DNA sequences, types of infections bacteria cause, and environmental aspects, AI can select the best phage combinations for a given infection (De Jonge et al., 2019). With predictive modeling, it is possible to estimate bacterial resistance and shape phage mixes that continue to work effectively for a long period. Such simulations may also be used before human trials by predicting phage responses in the stomach due to pH shifts or when interacting with the immune system. Such tools can save both time and money in conducting experiments, which can speed up the movement of phage-based treatments into the clinic (Torres-Acosta et al., 2021).

Precision farming can make use of phage therapy because it supports efficient approaches to preventing and treating diseases in animals. Personalized phage therapies created using real-time analysis of the microbiome and detection of pathogens can make treatments more effective and reduce the exposure of people to extra antibiotics (Tartor et al., 2024). Animals could be treated fast and efficiently in the poultry and swine farm with the quick diagnosis and phages generated at the farm. Moreover, thanks to wearable sensors and digital systems, it's possible to catch infection outbreaks early and give phages to animals at the most advantageous time (Abbas et al., 2022). Combining these practices is equivalent to the objectives of precision agriculture and enhances sustainability and efficiency of animal farming.

The AMR Gene Transfer and Long-Term Ecological Impact

Even though phages are usually thought to be harmless and environmentally friendly, further studies should be done to assess their long-term effects, mainly on the behavior of

microbial communities and the sharing of genetic information. When some temperate phages carry genes that cause problems, such as making bacteria resistant to antibiotics, using them may cause unexpected problems (Fortier & Sekulovic, 2013). Consequently, every phage preparation should be thoroughly screened for lysogenic and mobile genetic elements before being used in animals that produce food products (Hammerl et al., 2014). Consecutive studies into the mechanisms of phage therapy to changes the composition and the functioning of the gut microbiota are significant as well. Despite bacteria often being very selective toward phages, phages may affect microorganisms in the body by influencing nutrient levels, immune responses, and general health (Callaway et al., 2004). The monitoring of these changes as time passes in treated and untreated animals will provide us with helpful information regarding the safety of phage treatments and their sustainability.

Harmonization and Standardization of Regulations

The variations in the regulation of phage therapies in the world serve as a significant impediment to its adoption. While routine antibiotics are approved similarly everywhere, the outcome for phage therapy is often different according to jurisdiction, making it hard to control quality, labels, and market access (Naureen et al., 2020). The quality and reliability of the resulting products is guaranteed by international standards of phages isolation, research, and testing. Working together, researchers, industry experts, and regulators will be important for coming up with adaptable ways to supervise phages and assess both their effectiveness and how safe they are (Jordá et al., 2023). Phage products can be introduced into the market sooner through public-private cooperation and persuade farmers, veterinarians, and customers to accept them.

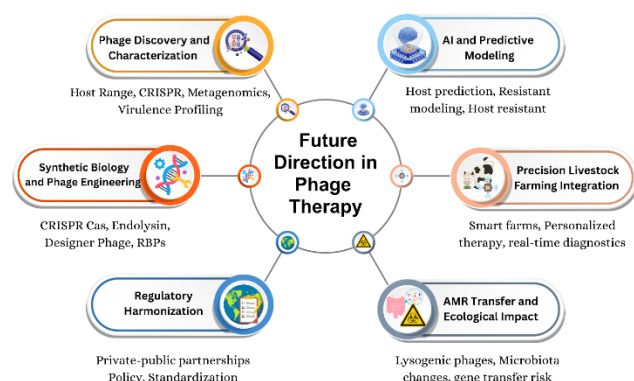


Figure 3: Future directions in phage therapy

The six main factors that will determine the future of bacteriophage therapy in veterinary and animal health are shown in radial infographic (Figure 3). These are (1) Phage Discovery and Characterization where metagenomics and CRISPR profiling is used; (2) Synthetic Biology and Phage Engineering where designer phages and enhanced host specificity will be determined; (3) Artificial Intelligence and Predictive Modeling is given, which aims at optimizing phage-host selection and resistance prediction; (4) Integration with Precision Livestock Farming will be provided and this aims at real-time and personalized treatments; (5) Ecological Impact and AMR Transfer will be considered where issues of safety, gut microbiota. Taken collectively, all these themes would be a multidisciplinary road map to the successful and sustainable application of phage therapy in animal agriculture.

Conclusion

The application of bacteriophages is an effective, powerful, and eco-friendly method of battling bacterial diseases in animals. Since they only attack the specific ones and keep the good bacteria of the microbiome intact, these types of drugs can be very useful as a substitute for antibiotics, which are causing the latter to become more resistant and areas around them, to cause some sort of negative effects environmentally. Phage treatment has been found to be beneficial in livestock, poultry, aquaculture, and pet animals demonstrating that it can be applied to help control infections and increase productivity. Scalability of phage therapy on the platform of recent high-throughput screening and production technologies allows solving the problem of mass spread in the different agricultural environments. To a macro level, phage therapy is limited by issues like dissimilar phage reactions to hosts, disadvantages in the production and administration of the therapy, and the absence of coherent regulations. The phage-based therapy could be improved even more and simplified using precision-agriculture technologies, such as in-time monitoring of pathogens and automated distribution/in-store delivery. It is hoped that research will address the obstacles to date to find means of surmounting them through the development of novel means of executing engineered phages, data tools, and more effective delivery equipment. Additionally, the incorporation of phage therapy into One Health can reduce the issue of drug resistance because all health issues, including human, animal, and environmental health, are taken care of. Phage therapy can become the standard veterinary procedure with the help of international collaboration to regulate the environment. Phage-based approaches will reduce the extent to which we are employing antibiotics in animals and make farming more viable as more science and regulations are backing it. The

potential of contributing to global health, such as the zoonotic diseases, also throws additional light on their contribution as the basis of animal health plans of the future. With the rise in the popularity of natural and environmentally sound methods, bacteriophages have become an adequate solution to take care of animal health and guarantee the safety level of the population, health, and the environment. Among the findings is the fact that the functional and evolutionary connections between bacteriophages and plant systems, which run the gamut (including the phage-like structure of the plant organellar replisome to the effective use of phages to control phytopathogens), indicate that bacteriophage research informs both animal and plant health and thus a comprehensive, One Health-focused approach to antimicrobial sustainability.

Author Contributions

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Quadrat Ullah & Waqas Haider: visualization, validation, writing – review and editing.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Abbas, R. Z., Alsayeqh, A. F., & Aqib, A. I. (2022). Role of bacteriophages for optimized health and production of poultry. *Animals*, 12(23), 3378.

- Abd-El Wahab, A., Basiouni, S., El-Seedi, H. R., Ahmed, M. F., Bielke, L. R., Hargis, B., Tellez-Isaias, G., Eisenreich, W., Lehnher, H., & Kittler, S. (2023). An overview of the use of bacteriophages in the poultry industry: Successes, challenges, and possibilities for overcoming breakdowns. *Frontiers in microbiology*, 14, 1136638.
- Abedon, S. T., Kuhl, S. J., Blasdel, B. G., & Kutter, E. M. (2011). Phage treatment of human infections. *Bacteriophage*, 1(2), 66-85.
- Ahmadi, M., Karimi Torshizi, M. A., Rahimi, S., & Dennehy, J. J. (2016). Prophylactic bacteriophage administration more effective than post-infection administration in reducing *Salmonella enterica* serovar Enteritidis shedding in quail. *Frontiers in microbiology*, 7, 1253.
- Amjad, N., Naseer, M. S., Imran, A., Menon, S. V., Sharma, A., Islam, F., Tahir, S., & Shah, M. A. (2024). A mini-review on the role of bacteriophages in food safety. *CYTA-Journal of Food*, 22(1), 2357192.
- Archambault, M., & Rubin, J. E. (2018). Antimicrobial resistance in *Clostridium* and *Brachyspira* spp. and other anaerobes. *Antimicrobial Resistance in Bacteria from Livestock and Companion Animals*, 447-470.
- Bava, R., Castagna, F., Lupia, C., Poerio, G., Liguori, G., Lombardi, R., Naturale, M. D., Mercuri, C., Bulotta, R. M., & Britti, D. (2024). Antimicrobial resistance in livestock: A serious threat to public health. *Antibiotics*, 13(6), 551.
- Bisen, M., Kharga, K., Mehta, S., Jabi, N., & Kumar, L. (2024). Bacteriophages in nature: Recent advances in research tools and diverse environmental and biotechnological applications. *Environmental Science and Pollution Research*, 31(15), 22199-22242.
- Burrowes, B., Harper, D. R., Anderson, J., McConville, M., & Enright, M. C. (2011). Bacteriophage therapy: potential uses in the control of antibiotic-resistant pathogens. *Expert review of anti-infective therapy*, 9(9), 775-785.
- Callaway, T., Anderson, R., Edrington, T., Genovese, K., Harvey, R., Poole, T., & Nisbet, D. (2004). Recent pre-harvest supplementation strategies to reduce carriage and shedding of zoonotic enteric bacterial pathogens in food animals. *Animal Health Research Reviews*, 5(1), 35-47.
- Castanon, J. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry science*, 86(11), 2466-2471.
- Cenens, W., Makumi, A., Mebrhatu, M. T., Lavigne, R., & Aertsens, A. J. B. (2013). Phage–host interactions

- during pseudolysogeny: Lessons from the Pid/dgo interaction. *Bacteriophages*, 3(1), e1003269.
- Chan, B. K., Abedon, S. T., & Loc-Carrillo, C. J. F. m. (2013). Phage cocktails and the future of phage therapy. *Future Microbiology*, 8(6), 769-783.
- Chapman, B., Otten, A., Fazil, A., Ernst, N., & Smith, B. A. (2016). A review of quantitative microbial risk assessment and consumer process models for *Campylobacter* in broiler chickens. *Microbial Risk Analysis*, 2, 3-15.
- Chaudhry, W. N., Concepcion-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., & Levin, B. R. (2017). Synergy and order effects of antibiotics and phages in killing *Pseudomonas aeruginosa* biofilms. *PLoS One*, 12(1), e0168615.
- Chen, Y.-M., Sadiq, S., Tian, J.-H., Chen, X., Lin, X.-D., Shen, J.-J., Chen, H., Hao, Z.-Y., Wille, M., & Zhou, Z.-C. (2022). RNA viromes from terrestrial sites across China expand environmental viral diversity. *Nature Microbiology*, 7(8), 1312-1323.
- Chen, Y., Yang, L., Yang, D., Song, J., Wang, C., Sun, E., Gu, C., Chen, H., Tong, Y., & Tao, P. (2020). Specific integration of temperate phage decreases the pathogenicity of host bacteria. *Frontiers in Cellular and Infection Microbiology*, 10, 14.
- Chhibber, S., Kaur, T., & Kaur, S. J. P. o. (2013). Co-therapy using lytic bacteriophage and linezolid: effective treatment in eliminating methicillin resistant *Staphylococcus aureus* (MRSA) from diabetic foot infections. *PLoS One*, 8(2), e56022.
- Cieřlik, M., Bagińska, N., Jończyk-Matysiak, E., Węgrzyn, A., Węgrzyn, G., & Górski, A. J. V. (2021). Temperate bacteriophages—The powerful indirect modulators of eukaryotic cells and immune functions. *Viruses*, 13(6), 1013.
- Cristobal-Cueto, P., García-Quintanilla, A., Esteban, J., & García-Quintanilla, M. J. A. (2021). Phages in food industry biocontrol and bioremediation. *Antibiotics*, 10(7), 786.
- De Jonge, P. A., Nobrega, F. L., Brouns, S. J., & Dutilh, B. E. (2019). Molecular and evolutionary determinants of bacteriophage host range. *Trends in microbiology*, 27(1), 51-63.
- Domingo-Calap, P., & Delgado-Martínez, J. J. A. (2018). Bacteriophages: protagonists of a post-antibiotic era. *Antibiotics*, 7(3), 66.
- Eski, D. B., Eski, A., & Darcan, C. (2022). The future of phage-mediated biocontrol of tomato bacterial diseases. *Journal of Agricultural Biotechnology*, 3(1), 11-24.
- Fortier, L.-C., & Sekulovic, O. (2013). Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence*, 4(5), 354-365.
- Garvey, M. J. A. (2020). Bacteriophages and the one health approach to combat multidrug resistance: is this the way?. *Antibiotics*, 9(7), 414.
- Gauthier, C. H., Abad, L., Venbakkam, A. K., Malnak, J., Russell, D. A., & Hatfull, G. F. (2022). DEPhT: a novel approach for efficient prophage discovery and precise extraction. *Nucleic acids research*, 50(13), e75-e75.
- Goode, D., Allen, V., & Barrow, P. (2003). Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Applied and environmental microbiology*, 69(8), 5032-5036.
- Grabowski, Ł., Węgrzyn, G., Węgrzyn, A., & Podlacha, M. (2022). Highly different effects of phage therapy and antibiotic therapy on immunological responses of chickens infected with *Salmonella enterica* serovar Typhimurium. *Frontiers in immunology*, 13, 956833.
- Grissa, I., Vergnaud, G., & Pourcel, C. (2007). CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic acids research*, 35(suppl_2), W52-W57.
- Guo, Z., Lin, H., Ji, X., Yan, G., Lei, L., Han, W., Gu, J., & Huang, J. (2020). Therapeutic applications of lytic phages in human medicine. *Microbial pathogenesis*, 142, 104048.
- Hammerl, J. A., Jäckel, C., Alter, T., Janzyk, P., Stingl, K., Knüver, M. T., & Hertwig, S. (2014). Reduction of *Campylobacter jejuni* in broiler chicken by successive application of group II and group III phages. *PLoS One*, 9(12), e114785.
- Hasan, M., & Ahn, J. (2022). Evolutionary dynamics between phages and bacteria as a possible approach for designing effective phage therapies against antibiotic-resistant bacteria. *Antibiotics*, 11(7), 915.
- Henrot, C., & Petit, M. A. J. M. m. (2022). Signals triggering prophage induction in the gut microbiota. *Molecular Microbiology*, 118(5), 494-502.
- Heselpoth, R. D., Euler, C. W., Schuch, R., & Fischetti, V. A. (2019). Lysocins: bioengineered antimicrobials that deliver lysins across the outer membrane of Gram-negative bacteria. *Antimicrobial Agents and Chemotherapy*, 63(6), 10.1128/aac.00342-00319.
- Hussain, M. A., Liu, H., Wang, Q., Zhong, F., Guo, Q., & Balamurugan, S. (2017). Use of encapsulated bacteriophages to enhance farm to fork food safety. *Critical reviews in food science and nutrition*, 57(13), 2801-2810.
- Inglis, L. K., Roach, M. J., & Edwards, R. A. (2024). Prophages: an integral but understudied component

- of the human microbiome. *Microbial genomics*, 10(1), 001166.
- Jäckel, C., Hammerl, J. A., & Hertwig, S. (2019). *Campylobacter* phage isolation and characterization: what we have learned so far. *Methods and protocols*, 2(1), 18.
- Jajere, S. M. (2019). A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Veterinary world*, 12(4), 504.
- Jamal, M., Chaudhry, W. N., Hussain, T., Das, C. R., & Andleeb, S. (2015). Characterization of new Myoviridae bacteriophage WZ1 against multi-drug resistant (MDR) *Shigella dysenteriae*. *Journal of Basic Microbiology*, 55(4), 420-431.
- Jiang, A., Liu, Z., Lv, X., Zhou, C., Ran, T., & Tan, Z. (2024). Prospects and challenges of bacteriophage substitution for antibiotics in livestock and poultry production. *Biology*, 13(1), 28.
- Jończyk-Matysiak, E., Łusiak-Szelachowska, M., Kłak, M., Bubak, B., Międzybrodzki, R., Weber-Dąbrowska, B., Żaczek, M., Fortuna, W., Rogóż, P., & Letkiewicz, S. (2015). The effect of bacteriophage preparations on intracellular killing of bacteria by phagocytes. *Journal of Immunology Research*, 2015(1), 482863.
- Jordá, J., Lorenzo-Rebenaque, L., Montoro-Dasi, L., Marco-Fuertes, A., Vega, S., & Marin, C. (2023). Phage-Based biosanitation strategies for minimizing persistent *Salmonella* and *Campylobacter* bacteria in poultry. *Animals*, 13(24), 3826.
- Kakasis, A., & Panitsa, G. (2019). Bacteriophage therapy as an alternative treatment for human infections. A comprehensive review. *International journal of antimicrobial agents*, 53(1), 16-21.
- Keen, E. C. (2012). Paradigms of pathogenesis: targeting the mobile genetic elements of disease. In (Vol. 2, pp. 161): Frontiers Media SA..
- Khalid, A., Lin, R. C., & Iredell, J. R. (2021). A phage therapy guide for clinicians and basic scientists: background and highlighting applications for developing countries. *Frontiers in microbiology*, 11, 599906.
- Kortright, K. E., Chan, B. K., Koff, J. L., Turner, P. E. J. C. h., & microbe. (2019). Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. *Cell Host and Microbes*, 25(2), 219-232.
- Lee, S. H., Kim, Y. B., Kim, D.-H., Lee, D.-W., Lee, H.-G., Jha, R., & Lee, K.-W. (2021). Dietary soluble flaxseed oils as a source of omega-3 polyunsaturated fatty acids for laying hens. *Poultry science*, 100(8), 101276.
- Li, D., Li, Y., Li, P., Han, Q., Zhang, T., Yang, B., Wu, W., & Yang, H. (2022). Phage phiZ98: A novel tri-segmented dsRNA cystovirus for controlling *Pseudomonas* strains with defective lipopolysaccharides in foods. *Food Research International*, 162, 112197.
- Lin, J., Du, F., Long, M., & Li, P. J. M. (2022). Limitations of phage therapy and corresponding optimization strategies: a review. *Molecules*, 27(6), 1857.
- Loc Carrillo, C., Atterbury, R. J., El-Shibiny, A., Connerton, P. L., Dillon, E., Scott, A., & Connerton, I. F. (2005). Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Applied and Environmental Microbiology*, 71(11), 6554-6563.
- Loh, B., Gondil, V. S., Manohar, P., Khan, F. M., Yang, H., & Leptihn, S. (2021). Encapsulation and delivery of therapeutic phages. *Applied and environmental microbiology*, 87(5), e01979-01920.
- Lopez, C. A., Winter, S. E., Rivera-Chávez, F., Xavier, M. N., Poon, V., Nuccio, S.-P., Tsolis, R. M., & Bäumler, A. J. (2012). Phage-mediated acquisition of a type III secreted effector protein boosts growth of *Salmonella* by nitrate respiration. *mBio*, 3(3), 10.1128/mbio.00143-00112.
- Mannapperuma, J. D., & Santos, M. R. (2004). Reconditioning of poultry chiller overflow by ultrafiltration. *Journal of Food Process Engineering*, 27(6), 497-516.
- Mäntynen, S., Salomaa, M. M., & Poranen, M. M. J. V. (2023). Diversity and current classification of dsRNA bacteriophages. *Viruses*, 15(11), 2154.
- Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. J. M. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*, 23(4), 795.
- Martinez, M. N., Watts, J. L., & Gilbert, J. M. (2019). Questions associated with the development of novel drugs intended for the treatment of bacterial infections in veterinary species. *The Veterinary Journal*, 248, 79-85.
- Metsemakers, W.-J., Onsea, J., Moriarty, T. F., Pruidze, N., Nadareishvili, L., Dadiani, M., & Kutateladze, M. (2023). Bacteriophage therapy for human musculoskeletal and skin/soft tissue infections. *Clinical microbiology and infection*, 29(6), 695-701.
- Miernikiewicz, P., & Dąbrowska, K. (2022). Endocytosis of bacteriophages. *Current Opinion in Virology*, 52, 229-235.
- Nabergoj, D., Modic, P., & Podgornik, A. J. M. (2018). Effect of bacterial growth rate on bacteriophage

- population growth rate. *MicrobiologyOpen*, 7(2), e00558.
- Naureen, Z., Malacarne, D., Anpilogov, K., Dautaj, A., Camilleri, G., Cecchin, S., Bressan, S., Casadei, A., Albion, E., & Sorrentino, E. (2020). Comparison between American and European legislation in the therapeutical and alimentary bacteriophage usage. *Acta Bio Medica: Atenei Parmensis*, 91(Suppl 13), e2020023.
- The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks. *Nature Microbiology*, (2020). 5(5), 668-674.
- Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh, M., Langelaar, M., Threlfall, J., & Scheutz, F. (2010). Food-borne diseases—the challenges of 20 years ago still persist while new ones continue to emerge. *International journal of food microbiology*, 139, S3-S15.
- Nikolich, M. P., & Filippov, A. A. J. A. (2020). Bacteriophage therapy: developments and directions. *Antibiotics*, 9(3), 135.
- O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations.
- Olson, E. G., Micciche, A. C., Rothrock Jr, M. J., Yang, Y., & Rieke, S. C. (2022). Application of bacteriophages to limit *Campylobacter* in poultry production. *Frontiers in microbiology*, 12, 458721.
- Pelyuntha, W., & Vongkamjan, K. (2022). Combined effects of *Salmonella* phage cocktail and organic acid for controlling *Salmonella* Enteritidis in chicken meat. *Food Control*, 133, 108653.
- Perera, M. N., Abuladze, T., Li, M., Woolston, J., & Sulakvelidze, A. (2015). Bacteriophage cocktail significantly reduces or eliminates *Listeria monocytogenes* contamination on lettuce, apples, cheese, smoked salmon and frozen foods. *Food microbiology*, 52, 42-48.
- Peralta-Castro, A., Cordoba-Andrade, F., Díaz-Quezada, C., Sotelo-Mundo, R., Winkler, R., & Briebe, L. G. (2023). The plant organellar primase-helicase directs template recognition and primosome assembly via its zinc finger domain. *BMC Plant Biology*, 23(1), 467.
- Reganold, J. P., & Wachter, J. M. (2016). Organic agriculture in the twenty-first century. *Nature plants*, 2(2), 1-8.
- Reyneke, B., Havenga, B., Waso-Reyneke, M., Khan, S., & Khan, W. J. M. (2024). Benefits and challenges of applying bacteriophage biocontrol in the consumer water cycle. *Microorganisms*, 12(6), 1163.
- Rose, T., Verbeken, G., De Vos, D., Merabishvili, M., Vanechoutte, M., Lavigne, R., Jennes, S., Zizi, M., & Pirnay, J.-P. (2014). Experimental phage therapy of burn wound infection: difficult first steps. *International journal of burns and trauma*, 4(2), 66.
- Sarrami, Z., Sedghi, M., Mohammadi, I., Kim, W. K., & Mahdavi, A. H. (2022). Effects of bacteriophage supplement on the growth performance, microbial population, and PGC-1 α and TLR4 gene expressions of broiler chickens. *Scientific Reports*, 12(1), 14391.
- Schmelcher, M., Donovan, D. M., & Loessner, M. J. (2012). Bacteriophage endolysins as novel antimicrobials. *Future microbiology*, 7(10), 1147-1171.
- Selle, K., Fletcher, J. R., Tuson, H., Schmitt, D. S., McMillan, L., Vridhambal, G. S., Rivera, A. J., Montgomery, S. A., Fortier, L.-C., & Barrangou, R. (2020). In vivo targeting of *Clostridioides difficile* using phage-delivered CRISPR-Cas3 antimicrobials. *mBio*, 11(2), 10.1128/mbio.00019-00020.
- Stanford, K., McAllister, T., Niu, Y., Stephens, T., Mazzocco, A., Waddell, T., & Johnson, R. (2010). Oral delivery systems for encapsulated bacteriophages targeted at *Escherichia coli* O157: H7 in feedlot cattle. *Journal of Food Protection*, 73(7), 1304-1312.
- Strathdee, S. A., Hatfull, G. F., Mutalik, V. K., & Schooley, R. T. J. C. (2023). Phage therapy: From biological mechanisms to future directions. *Cell*, 186(1), 17-31.
- Tang, S.-S., Biswas, S. K., Tan, W. S., Saha, A. K., & Leo, B.-F. J. P. (2019). Efficacy and potential of phage therapy against multidrug resistant *Shigella* spp. *PeerJ*, 7, e6225.
- Tartor, Y. H., Ammar, A. M., & AbdelKhalek, A. (2024). Integrating Alternative Therapies to Combat Multidrug-Resistant Bacteria Causing Infections in Equine. *Zagazig Veterinary Journal*, 52(3), 315-335.
- Thanki, A. M., Clavijo, V., Healy, K., Wilkinson, R. C., Sicheritz-Pontén, T., Millard, A. D., & Clokie, M. R. (2022). Development of a phage cocktail to target *Salmonella* strains associated with swine. *Pharmaceuticals*, 15(1), 58.
- Torres-Acosta, M. A., Castaneda-Aponte, H. M., Mora-Galvez, L. M., Gil-Garzon, M. R., Banda-Magaña, M. P., Marcellin, E., Mayolo-Deloisa, K., & Licona-Cassani, C. (2021). Comparative economic analysis between endogenous and recombinant production of hyaluronic acid. *Frontiers in Bioengineering and Biotechnology*, 9, 680278.

- Ture, M., Cebeci, A., Altinok, I., Aygur, E., & Caliskan, N. (2022). Isolation and characterization of *Aeromonas hydrophila*-specific lytic bacteriophages. *Aquaculture*, 558, 738371.
- Usman, S. S., Uba, A. I., & Christina, E. (2023). Bacteriophage genome engineering for phage therapy to combat bacterial antimicrobial resistance as an alternative to antibiotics. *Molecular Biology Reports*, 50(8), 7055-7067.
- Vikram, A., Woolston, J., & Sulakvelidze, A. (2021). Phage biocontrol applications in food production and processing. *Current issues in molecular biology*, 40(1), 267-302.
- Wernicki, A., Nowaczek, A., & Urban-hmiel, R. (2017). Bacteriophage therapy to combat bacterial infections in poultry. *Virology journal*, 14, 1-13.
- Wood, S. J., Kuzel, T. M., & Shafikhani, S. H. (2023). *Pseudomonas aeruginosa*: infections, animal modeling, and therapeutics. *Cells*, 12(1), 199.
- Xiong, D., Dai, W., Gong, J., Li, G., Liu, N., Wu, W., Pan, J., Chen, C., Jiao, Y., & Deng, H. (2020). Rapid detection of SARS-CoV-2 with CRISPR-Cas12a. *PLoS biology*, 18(12), e3000978.
- Yu, H., Ding, X., Li, N., Zhang, X., Zeng, X., Wang, S., Liu, H., Wang, Y., Jia, H., & Qiao, S. (2017). Dietary supplemented antimicrobial peptide microcin J25 improves the growth performance, apparent total tract digestibility, fecal microbiota, and intestinal barrier function of weaned pigs. *Journal of animal science*, 95(11), 5064-5076.
- Zampara, A., Sørensen, M. C. H., Grimon, D., Antenucci, F., Vitt, A. R., Bortolaia, V., Briers, Y., & Brøndsted, L. (2020). Exploiting phage receptor binding proteins to enable endolysins to kill Gram-negative bacteria. *Scientific reports*, 10(1), 12087.
- Żbikowska, K., Michalczuk, M., & Dolka, B. (2020). The use of bacteriophages in the poultry industry. *Animals*, 10(5), 872.
- Zhao, H., Li, Y., Lv, P., Huang, J., Tai, R., Jin, X., Wang, J., & Wang, X. (2022). *Salmonella* phages affect the intestinal barrier in chicks by altering the composition of early intestinal flora: Association with time of phage use. *Frontiers in microbiology*, 13, 947640.
- Zheng, T., & Zhang, C. J. M. b. (2024). Engineering strategies and challenges of endolysin as an antibacterial agent against Gram-negative bacteria. *Microbial Biotechnology*, 17(4), e14465.

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