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# Bacteriophages as an Emerging Alternative and Supplement to Antibiotics, including Prophylaxis to Limit Antibiotic Use

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# Abstract

The rise of antimicrobial resistance (AMR) has created a critical need for alternatives to traditional antibiotics. Bacteriophages, viruses that specifically target bacteria, are emerging as a potential solution. This literature review examines the use of bacteriophages, both as alternatives and supplements to antibiotics, highlighting their applications, advantages, and challenges. The concept of Phage-Antibiotic Synergy (PAS) is explored, demonstrating how phages can enhance the efficacy of antibiotics under certain conditions. This study discusses the current use of bacteriophages in medical, agricultural, and food safety contexts, underscoring their potential to address the global threat of AMR. Despite promising findings, limited research underscores the need for further investigation to fully understand the capabilities and limitations of bacteriophage therapy.

# 1 Introduction

Antibiotics are a class of drugs used to treat infections caused by bacteria. They work by either killing bacteria (bactericidal) or inhibiting their growth and reproduction (bacteriostatic). They are providing treatments effective against several and various bacterial diseases that once may have been fatal, and in this way, they have revolutionized medicine. Already back in ancient times, the use of natural substances with antibacterial properties started. But the modern era of antibiotics began in 1928, with Alexander Fleming's discovery of penicillin – which became the first widely used antibiotic. [1]

During a Nobel Lecture in 1945 Alexander Fleming said "But I would like to sound one note of warning. Penicillin is to all intents and purposes non-poisonous so there is no need to worry about giving an overdose and poisoning the patient. There may be a danger, though, in underdosage. It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body. The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant." [2]

And as Alexander Fleming feared – we have reached the point where an overuse of antibiotics has led to a spread of antibiotic resistance in a number of bacteria. [3]
In this literature study I want to look into the use of bacteriophages as a possible supplement, or even alternative to antibiotics. I will look into when it can be used, what it can be used for, how it is used today and what challenges we are facing in the use of the bacteriophages.

## 1.1 What is antimicrobial resistance?

Antimicrobial resistance occurs when pathogens develop resistance to the effects of drugs meant to eliminate them. As a result, they survive and continue to multiply. This makes resistant infections extremely challenging, and in some cases, impossible to treat.

Antimicrobial resistance (AMR) represents a severe threat to both animal and human health worldwide, causing the deaths of at least 1.27 million people globally yearly. According to a study done by the Global Research on AMR, it is expected that drug-resistant infections will cause the death of more than 39 million people from now to 2050, if further action is not taken. [4] The resistant microorganisms spread easily between countries and even continents, though humans, animals, food and the environment. [5] This makes AMR a global challenge requiring international collaboration and needs to be solved in a "one health" perspective. The global antibiotic use needs to be reduced.

Antimicrobial resistance (AMR) threatens many of the advancements in modern medicine. It complicates the treatment of infections and increases the risks associated with various medical procedures and treatments, including surgeries. [6] The world is facing an antibiotic pipeline where there is a lack of new antibiotics being developed, while bacteria develop resistance to existing ones. This is why alternatives to antibiotics are essential. This could decrease the antibiotic resistance, ensure better treatments of complex infections, and support the global public health.

The Norwegian National Antibiotic Strategy (2015–2020) states that "The threat of antibiotic resistance cannot be eliminated, but we must implement measures to reduce the development of resistance and at the same time adapt to minimize the consequences for humans and animals. This requires renewed efforts across multiple sectors." [7]

# 2 Bacteriophages

## 2.1 What are bacteriophages?

Viruses are often specific to an exact host, and even to specific cells within that particular host. Almost every living organism has its unique set of viruses that can attempt to infect its cells, showing the large diversity of the types of viruses that exist. Bacteriophages, also known as phages, are viruses that specifically target and infect bacteria. [8]

Over billions of years, bacteriophages have progressed to specialize in infecting and killing bacteria, making them crucial in keeping bacterial populations under control. We can find them everywhere – in our bodies, in the food we eat, in our homes, and in nature. It's estimated that there are 100 nonillion ($10^{31}$) phages on Earth, making them the most abundant organisms on the planet. [9]

Viruses are usually linked to disease, but bacteriophages are different types of viruses that do not cause a threat. As an alternative, they play an important role in the fight against an actual threat: antibiotic resistance. Some bacteriophages can be used as a possible alternative to antibiotics, and some can be used in conjunction with them. Contrasting antibiotics, bacteriophages target and kill bacteria without causing the same side effects or problems. This makes bacteriophages a very efficient, natural, and environmentally friendly alternative to the traditional antibiotics. Bacteriophages have a wide range of applications, particularly within human medicine, veterinary medicine, aquaculture, agriculture, and the food industry. [10]

## 2.2 History

The history of bacteriophages dates back to 1915. Frederick Twort, a British scientist was working on a vaccine for smallpox and kept getting his plate contaminated with staphylococci. He observed that between all the bacterial growth, there were some clear spots where bacteria did not grow or was killed. Although his research on the smallpox vaccine was unsuccessful, Twort published his observations on these mysterious clear spots but did not pursue further investigation. [11, 12]

At the same time, Félix D'Herelle made similar observations as Twort, but in dysentery patients. He isolated the clear spots from the plates, incubated them with the dysentery bacteria, and found that the bacteria were completely eradicated. D'Herelle spent the rest of

his career attempting to develop bacteriophages as therapeutic agents. He published his findings in 1917 and continued his work, treating dysentery patients with phage therapy. [13]

A lot of research and multiple experiments on the use of bacteriophages were done during this time, especially in the Soviet Union. There was a major challenge during this time – the fact that the bacteriophage therapy requires accurate identification of the bacterial strain for effective treatment. [14] In the 1920s, they lacked the necessary knowledge and equipment, leading to multiple unsuccessful attempts at phage therapy.

Another significant challenge was the limited understanding of phage structure and composition. Before the discovery of the electron microscope, there was considerable debate within the scientific community about whether phages were viruses, enzymes, or something else entirely. [15] This lack of understanding hampered the production and storage of bacteriophages.

Consequently, further research into bacteriophages halted when Alexander Fleming discovered penicillin. [14] Antibiotics target different types of bacteria, and some target only a few (narrow-spectrum antibiotics), but comparing antibiotics to bacteriophages, they all have a broad specter. Therefor antibiotics became a lot easier to use and outcompeted the phages completely in the west.

In some countries they continued the experiments and use of bacteriophages, and they are still used frequently in countries like Poland, Russia, and Georgia.

Today, however, with the rise of antibiotic resistance posing an extreme threat to public health and the environment, interest in bacteriophages is resurging. With our increased knowledge and advanced technology, bacteriophages are being reconsidered as a critical tool in the ongoing battle against bacterial infections. [16]

## 2.3 Work of bacteriophages

Viruses are simple organisms that cannot reproduce on their own. They need a host cell to do so. In nature, bacteriophages work as bio-controllers. If a bacterial species becomes too dominant, they will be set back by bacteriophages, ensuring the wide microbial diversity that the ecosystems need. Bacteriophages are the most abundant organisms on the planet. They have developed together with their host bacteria, resulting in each bacteria species having their own bacteriophages.

There are both pros and cons to the fact that bacteriophages are very specific. It is great that they only attack their target, without causing damage to the other bacteria. For example, antibiotics that are applied per os, a normal side effect will be diarrhea. With bacteriophages we will not have those side effects, as they leave the healthy microflora unaffected. On the other hand, it also means that we need to have exact information about the bacteria to be able to give the correct treatment

There are many types of bacteriophages, each suited for different applications. Understanding which bacteriophage is appropriate for a given use is crucial. Some are better suited than others. Bacteriophages come in various genetic forms, such as DNA and RNA, either single- or double-stranded. [17]

The most commonly known and studied group is the Caudovirales [18] a double-stranded DNA virus with a head and tail. [19] The Caudovirales-order is the one most used in medical purposes. At the end of their tail, they have receptor-binding structures recognizing and binding specific bacteria. Once the bacteriophage has bound the bacteria, their tail works as a syringe injecting their DNA into the host bacteria, taking control of the host for replication. [20]

## 2.4 Lytic and lysogenic life cycle

Bacteriophages multiplicate in different ways. They can have a lytic, or a lysogenic cycle (figure 1).

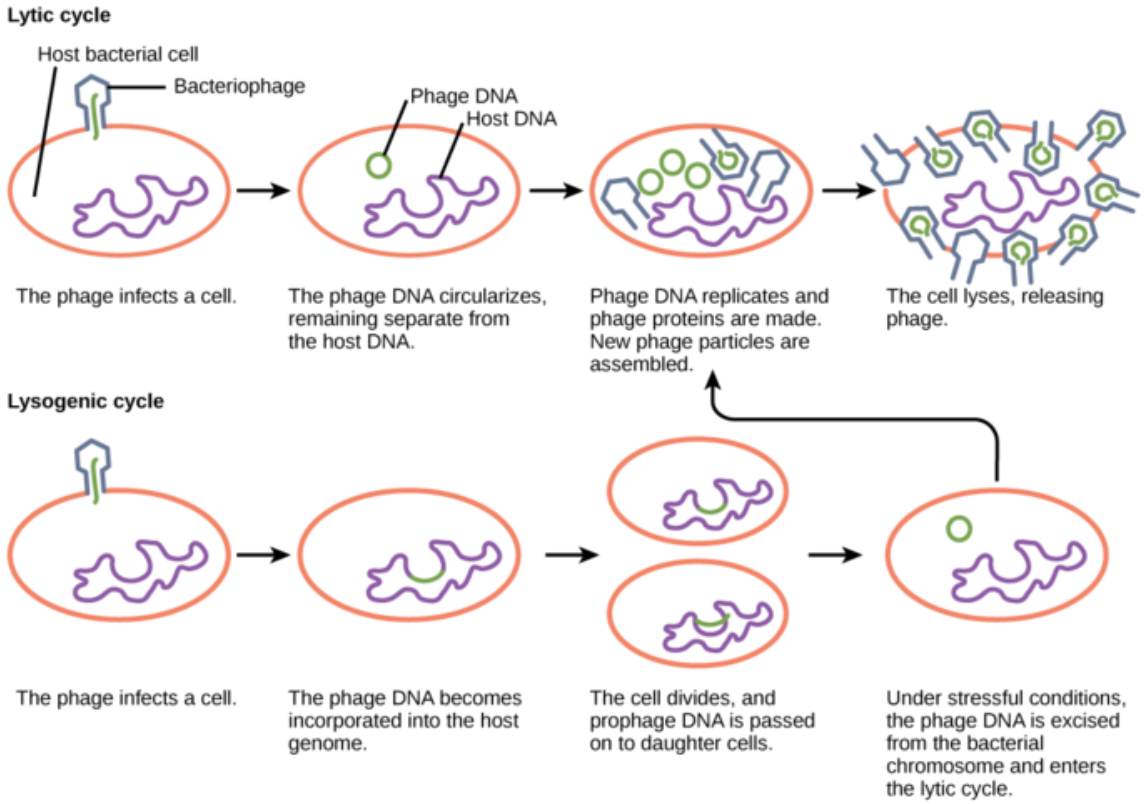


Figure 1 The lytic and lysogenic cycles of bacteriophages [8]

*The lytic cycle* starts with the specific recognition of and binding to the bacterial host. Thereafter the bacteriophage injects the viral genome into the host and takes control of it. The physiological activities of the bacteria are taken over and are used for the reproduction of viruses, manufacturing more viruses. The viruses then burst out of the cell by lysing the host cell. As soon as they are released, the bacteriophage progeny can find new hosts to infect. Lytic bacteriophages are the most suitable for medical use. All the viruses of the order Caudovirales can multiply in a lytic cycle.

*The lysogenic* cycle involves the incorporation of the viral genome into the host cell genome and passing it on to subsequent generations. This type of multiplication cycle allows the host cell to survive and reproduce, and as the viral genome is incorporated into the bacteria DNA, the virus will be reproduced in all cell offsprings. [21] The lysogenic bacteriophages increase their own multiplication by increasing the multiplication of the bacteria. That is why they

often carry genes that help the bacterial hosts' multiplication capacity. These genes can be hazardous, increasing antibiotic resistance. These types of bacteriophages can in other words reshape bacteria and make them more dangerous. [22]

In addition to the lytic cycle, some of the Caudovirales bacteriophages can also reproduce in a lysogenic cycle. [8] Lysogenic bacteriophages can lead to bacteriophage resistance and an unwanted gene transmission between bacteria. It is therefore important to do a quality control of the host bacteria that is used for the production of bacteriophages, to ensure that lysogenic bacteriophages do not enter the final product.

# 3 Bacteriophage therapy

## 3.1 Production of Bacteriophages and Quality Controls

The biological nature of bacteriophages means they are entirely dependent on host cells for reproduction. To produce bacteriophages at scale, the first step involves cultivating a high concentration of the specific host bacteria under optimal conditions. This environment is designed to maximize bacterial growth, ensuring a sufficient host population for phage infection. [23] Once the host bacteria reach the desired concentration, the specific bacteriophage is introduced into the culture.

### 3.1.1 Propagation and Cleansing

Once injected with bacteriophages, the host bacteria become production centers for viral replication. The bacteriophage infects the bacteria, using the host's machinery to reproduce. Over time, as the phages replicate within the bacterial cells, they cause the bacteria to lyse (burst), releasing new bacteriophages into the solution. [24, 25] This process continues until the bacterial culture is effectively consumed by the bacteriophages.

When all the host bacteria have been lysed, the resulting solution contains both the desired bacteriophages and remnants of the bacterial debris. At this point, purification processes are necessary to remove the bacterial remnants and isolate a clean solution of bacteriophages. [26] Quality control measures dictate the level of purification required, ensuring that the final product is safe for the intended use.

### 3.1.2 Selecting Non-Virulent Hosts

In bacteriophage production, it is critical to choose non-virulent strains of bacteria as the host organisms. Virulent, drug-resistant, or especially multi-resistant bacterial strains should be avoided due to safety risks. Using virulent strains not only increases the potential danger during the production process but also complicates the purification and containment of the phages, which may lead to contamination with harmful pathogens. [25]

For industrial-scale production, where large quantities of host bacteria are required, the selection of safe, non-pathogenic strains becomes even more critical. Industrial facilities must ensure that they are not accidentally cultivating dangerous or drug-resistant bacteria during phage production, which could pose serious risks to human and animal health, or the environment. [27]

### 3.1.3 Quality Control and Regulatory Oversight

In addition to selecting the appropriate bacterial host, the quality of the final bacteriophage product depends heavily on quality control protocols. These protocols cover every step of the process, from bacterial cultivation to phage propagation and purification. Thorough strategies must be in place to control the level of cleaning and purification, guaranteeing that all bacterial debris is correctly removed from the solution. [28]

Regulatory bodies often set detailed standards for bacteriophage production, especially if the phages are meant for use in medical or food safety applications. How strictly they control the purification can vary depending on the intended use of the bacteriophages. For example, bacteriophages used in food production may require less stringent purification than those used in pharmaceutical products. Regardless of the exact application, following these guidelines is necessary to ensure the safety, efficacy, and quality of the bacteriophage preparations. [29]

In addition to the above mentioned, quality control measures are important to ensure the correct use of bacteriophages. As bacteriophages are specific viruses that target specific bacteria, their biological effectiveness relies on selecting the correct bacteriophage-host bacteria pair.

Proper quality control measures must ensure that the bacteriophages possess the appropriate primary structure and reproductive cycle, and that the production system maintains a high

standard. This helps minimize the risk of producing bacteriophages or genes that are unwanted, ensuring the safety and efficacy of bacteriophage therapy.

## 3.2 Challenges

### 3.2.1 Preventing Resistance Development

Bacteria have developed complex mechanisms to avoid bacteriophages to recognize and bind to them, contributing to increase phage resistance. However, bacteriophages have evolved strategies to oppose these bacterial defenses, establishing a continuous evolutionary battle that has lasted billions of years. [30] To improve bacteriophage therapy, it's important to implement strategies that maximize phage efficacy and minimize bacterial resistance.

Some key strategies for controlling bacterial resistance in phage therapy:

1. **Cocktail Strategy**: Combining different bacteriophages targeting the same bacteria is beneficial. By using this cocktail strategy, the chances of the bacteria creating a resistance to all the bacteriophages at once are significantly reduced. This method can compare to antibiotic combination therapies, which also work to outdo bacterial defenses by attacking from multiple fronts. [31]

2. **High Doses, Short Intervals**: Another approach highly efficient in avoiding the development of bacterial resistance involves using high concentrations of bacteriophages over a short period of time. This concentrated treatment gives bacteria less opportunity to adapt or develop resistance mechanisms towards the phages. However, careful monitoring and dosing is critical as we must ensure that high doses do not cause unwanted side effects in patients.

3. **Avoiding Physiological Changes in Bacteria**: In some cases, bacteriophages can initiate harmful physiological changes in their bacterial hosts. For instance, in patients with cystic fibrosis who are infected with *Pseudomonas aeruginosa*, it has been observed that some bacteriophages can make the bacteria form biofilms. Biofilms are protective layers that screen bacteria from being attacked, making them harder to eradicate. It is therefore important to do thorough research and document the effects that given phages have on exact bacteria, to avoid worsening infections rather than curing them. [32]

### 3.2.2 Immune System Considerations

In addition to bacterial resistance, the immune system of both humans and animals can also pose challenges to bacteriophage therapy. [33] Over time, the body can develop immune responses against phages, especially in the case of repeated exposure to the same bacteriophage. This immune recognition can reduce the effectiveness of phage therapy, as the body's defenses can neutralize the phages before they can even act on the bacterial infection. [34]

This highlights the importance of selecting bacteriophages carefully, not only for their effectiveness against bacteria but also for their capability to escape or moderate the hosts immune response. In some cases, alternating between different phage types or developing phages with immune-evasive properties may help extend the efficacy of treatment.

### 3.2.3 Comprehensive monitoring

While phage-based therapies are developing, they are facing challenges that must be controlled to make them safe and effective for different use. This demands careful monitoring and strict quality checks. Currently, rules for phage therapy differ around the world, leading to practices not being consistent. Quality control focuses on keeping bacteriophages free from contamination and ensuring they remain genetically stable so they only target harmful bacteria. [35]

There are ongoing efforts to develop consistent global standards, which would make it easier to both develop and approve phage therapies in different regions. [35] Research highlights the need for specific production methods to meet high safety standards, including testing to avoid resistance and harmful genetic changes. [36] These steps are vital for ensuring that bacteriophage therapy can be used safely and effectively worldwide.

### 3.2.4 Knowledge about the target bacteria

Luckily, with today’s modern diagnostic methods, we are able to detect exactly what bacteria is causing disease in an individual or in an outbreak. As we already know, bacteriophages are extremely specific in what bacteria they can infect and kill. Each bacteriophage has its own target. Knowledge about the bacteria causing disease is therefore highly necessary. This means that the use of bacteriophages requires that the treatment of individuals or outbreaks

has time to wait until it is decided what bacteriophages shall be used, and if they are available.

Since bacteriophages, in contrast to many antibiotics, have a narrow spectrum, we need to combine a large number of phages to get a wider spectrum. This is an advantage if we are not able to get a diagnosis that is as specific as needed in the use of one type of phage.

### 3.2.5 Endotoxin effect

One of the huge advantages of bacteriophages is that they are generally safe to use. Through a lot of research, it is proven that phages have few adverse side effects in humans and animals. [37, 38] This is especially an advantage in immunocompromised patients, like those with cancer or different chronic diseases.

However, since effective phages can lyse bacteria within a few minutes [39], phage therapy may induce a quick and significant release of endotoxins. A few studies have reported transient adverse side effects during treatments using phages, which include inflammation, hypotension, and fever. [39–41] This is likely due to allergic reactions or in some cases an endotoxin shock in reaction to the endotoxins released from the bacteria. Side effects can also occur if the cleansing of the phages is not sufficient. Therefore, quality control in the production of the bacteriophages is extremely important to avoid or reduce side effects.

## 3.3 Use of bacteriophages today

Bacteriophages are already being utilized in various sectors today. In clinical settings, bacteriophages are used to treat bacterial infections, especially those resistant to antibiotics. They specifically target pathogenic bacteria without disturbing beneficial microflora, offering an alternative to traditional antibiotics. Some applications include treating chronic wounds and gastrointestinal infections. [35]

In agriculture and food safety, phages are used as biocontrol agents. This involves applying bacteriophages to disinfect nonliving surfaces, equipment, or environments. [42] They also play a significant role in reducing bacterial contamination in food production, from pre-harvest to processing. Phage-based solutions help control pathogens like *E. coli* and *Salmonella* in livestock and crops, ensuring safer food supply chains.

In the food industry, bacteriophages are increasingly used to prevent foodborne diseases. Phage formulations are applied to raw and minimally processed foods, such as meat and fresh produce, to lower bacterial loads without using chemicals that could alter the product's quality. This targeted approach maintains food safety standards while supporting environmentally friendly practices. [43]

The growing use of bacteriophages highlights their potential to address antibiotic resistance, promote sustainable agriculture, and ensure food safety through natural and targeted bacterial control.

In this section, I will highlight several examples of how bacteriophages are used today.

### 3.3.1 CUSTUS®YRS

**Yersinia Ruckeri**

Enteric Redmouth disease (ERM) is primarily a freshwater disease of salmonids, but seawater cases are occasionally reported following the transfer of Atlantic salmon to sea. According to an article on The Fish site (2018), the number of such cases has increased in regions where the pathogen is present. [44]

ERM, also known as Yersiniosis, has spread to salmonids worldwide, affecting both Atlantic salmon (*Salmo salar*) and rainbow trout (*Onchorhynchus mykiss*). The disease is caused by the pathogen *Yersinia ruckeri.* ERM disease is an important acute or chronic disease, primarily affecting salmonids. It is characterized as hemorrhagic septicemia where we see massive destruction of tissues, especially lymphoid and hematopoietic tissues in the spleen and liver. [45]

Salmonids seem to be the most sensitive to Yersiniosis, compared to freshwater fish species like European eels and carp. They are prone to suffer disease outbreaks, especially in young life stages, and in worse cases leading to significant mortalities and economic losses for the industry.

Treatments usually rely on in-feed oral use of antibiotics, including amoxicillin, oxolinic acid, and florfenicol. In vitro testing has shown rapid resistance development against several antibiotics, and there is a growing drug resistance problem. [44] Also, a study carried out in China, doing a surveillance of ERM from April 2016 to August 2018, found that antibiotic resistance profiles and genes of *Y. ruckeri* were found. *Y. ruckeri* had appeared resistance to

14 of the 20 antibiotics, and 39 antibiotic resistance genes were detected positively from 95 specific primers. [46]

As the rise of antibiotic resistance poses a major threat to human health, and the use of antibiotics in food production is partly responsible, the company STIM made headlines when gaining approval for a phage product that effectively eliminates harmful bacteria, providing an alternative solution for treating a disease that currently relies only on antibiotics. [47]

**The first bacteriophage product in fish farming in Norway**

In 2018 STIM produced CUSTUS®yrs, as the first bacteriophage product used for Norwegian fish farming. They are working to control the balance between the tolerance and the infection pressure of the fish. The bacteriophages are specialized to infect and kill *Yersinia Ruckeri*. The bacteriophages in CUSTUS®yrs offer a straightforward and effective defense against harmful bacteria levels in the fish's environment. It is primarily used during operations that carry a high risk of yersiniosis outbreaks. [48]

Disease outbreaks are often seen shortly after the fish is released into a natural body of water in the sea, after mechanical delousing or other similar handling. These disease outbreaks are likely to be due to stress-related activation of a subclinical infection. [49]

CUSTUS®yrs is used in the biofilters in connection with washing down the pens, sedation tank in connection with vaccination, and in the well-boat in connection with delousing. In other words, bacteriophages are used to control the disease pressure during stressful operations where the fish is the most susceptible to disease.

## Results

Some data on the use of CYSTUS, and the results:

### 1: Vaccination

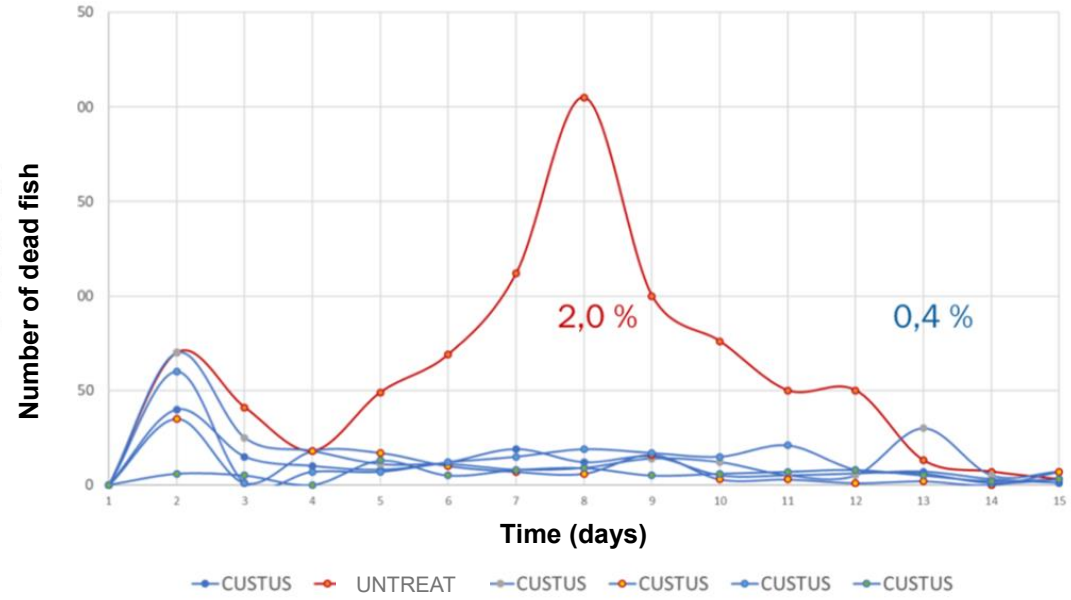


Figure 2 Vaccinated vs unvaccinated fish in smolt facility *[50]*

This graph (figure 2) shows the mortality rate after vaccination in the smolt facility. Norwegian smolt are constantly exposed to pathogen pressure, which they can usually withstand. However, as previously mentioned, disease outbreaks tend to occur when the fish are subjected to stressful procedures, such as vaccination. As shown in the graph, by adding CUSTUS®YRS (bacteriophages) to the sedation pool, the levels of Yersinia bacteria are kept low, preventing disease outbreaks during vaccination.

**2: Transportation**

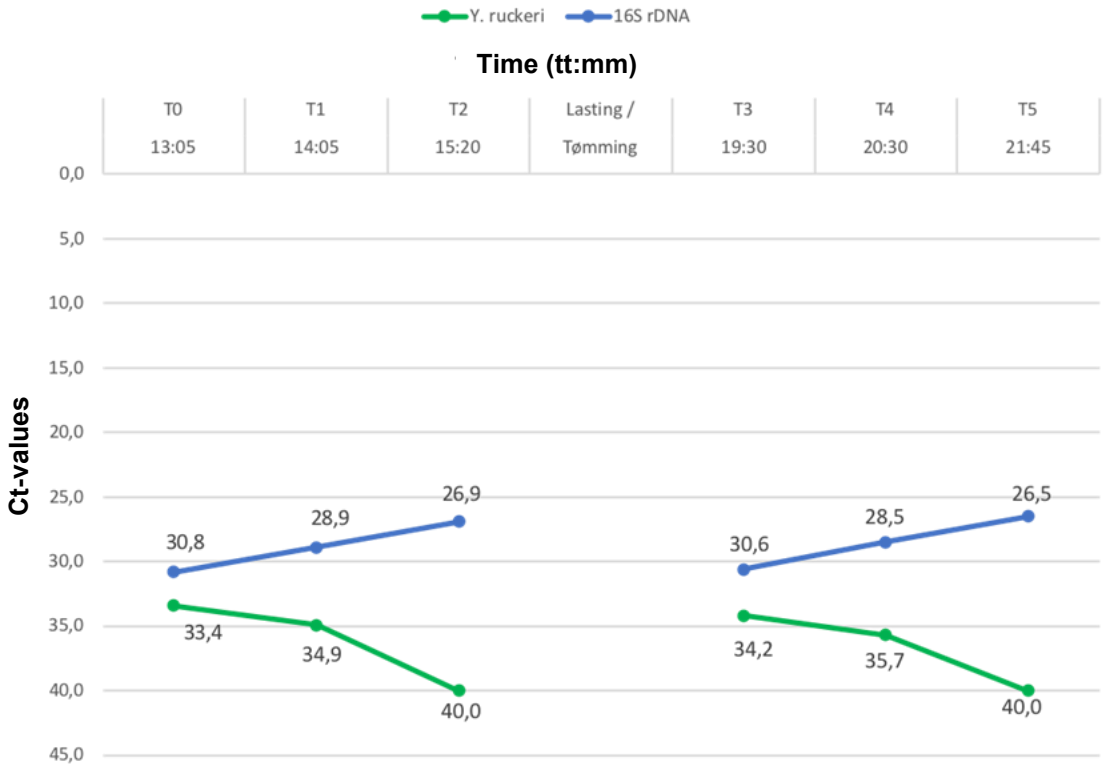


Figure 3 *Y. ruckeri* compared to other bacterial levels [50]

This graph (figure 3) illustrates that the overall bacterial levels (blue) increase the longer the fish are kept in the well boat, while the level of *Yersinia ruckeri* (green) decreases due to the use of CUSTUS®YRS. During transport in a well boat, the fish release significant amounts of bacteria. As a result, if the fish carry *Y. ruckeri*, the transport can lead to major outbreaks and the spread of the bacteria.

**3: Mechanical delousing**

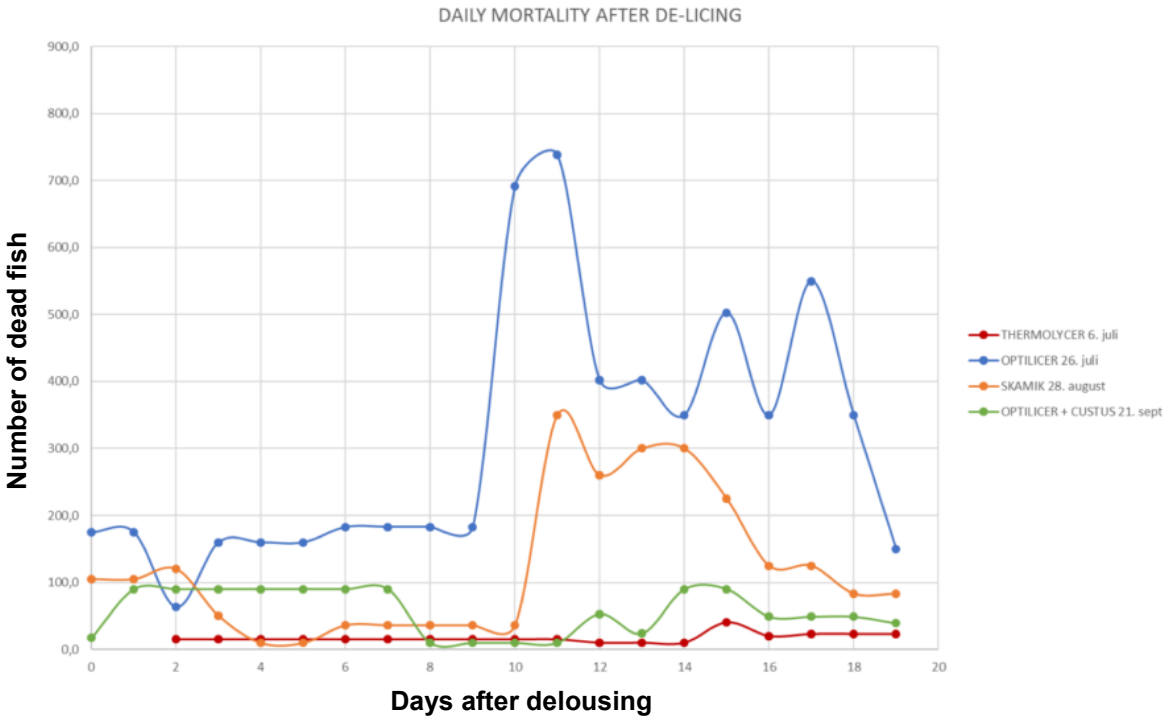


Figure 4 CUSTUS®YRS in delousing [50]

This graph (figure 4) demonstrates the use of CUSTUS®YRS during the fourth delousing process for a fish farming company that previously experienced high mortality rates during the last two delousing. During mechanical delousing in a well boat, the fish are subjected to significant stress and exposed to high infection pressure. The results show low mortality rates in this case, thanks to the use of CUSTUS®YRS, which effectively controls the *Yersinia ruckeri* infection pressure. [50]

CUSTUS®YRS is composed only of two bacteriophages, but still covers the diversity of the target bacteria Y. ruckeri that the Norwegian salmon is affected by. However, it is not efficient against Norwegian variants of Y. ruckeri that attack rainbow trout.

### 3.3.2 Food safety

Food safety refers to handling, preparation, and storage of food in ways that reduce the risk of individuals becoming sick from foodborne illness. [51]

There are four main principles in food safety; clean, separate, cook, and chill. These are applied in different stages of the food production chain. It is very important to avoid cross-

contamination. For example, lettuce crops should not be farmed in the same place as beef cattle graze. The same lettuce should not be cut with the same knife as the one you use for your chicken.

Despite significant efforts from producers and government to ensure food safety for consumers and more awareness of these principles – foodborne diseases continue to be the main reason for hospitalization and other fatalities. Each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 420,000 deaths. Of these disease cases, a large number is due to *Escherichia coli* (*E. coli*). [52]

*Escherichia coli* (*E. coli*) is a bacterium that is found in the gut of all humans and mammals. Most *E. coli* strains are harmless. However, some strains can cause severe foodborne illness. The consumption of raw or undercooked meat, raw milk, and contaminated raw vegetables is the primary way of transmission of these *E. coli* strains to humans. These will be in focus for the rest of his chapter. [53]

There are several variants of pathogenic *E. coli*. Both intestinal and extraintestinal. In this thesis I will focus on three of the intestinal ones;

- Enterotoxigenic *E. coli* (ETEC): the *E. coli* type responsible for the most deaths
- Enteropathogenic *E. coli* (EPEC): one of the most common causes of bacterial gastroenteritis
- Enterohemorrhagic *E. coli* (EHEC): the most virulent type of *E. coli*, not treatable with antibiotics

The main source of food poisoning of *E. coli* is fecal contamination of food and water. The contamination of ETEC and EPEC is more commonly from the human gastrointestinal tract, while for EHEC the major cause of contamination is fecal matter from animals. This tells us that for ETEC and EPEC the contamination is often due to human handling and food hygiene failure.

Symptoms of ETEC and EPEC are most commonly watery diarrhea and stomach pain, and occasionally also vomiting. They pose a huge risk of dehydration, especially if vomiting. This makes it hard to rehydrate orally, and in developing countries this poses an especially high risk, as hospitals are not available to everyone.

EHEC usually gives hemorrhagic diarrhea and severe cramps. This strain also produces Shiga-toxin, which in severe cases can damage organs, such as the kidneys, and in the worst case lead to death. [54]

As these pathogens pose a huge threat to humans and animals, we need ways to decrease their occurrence.

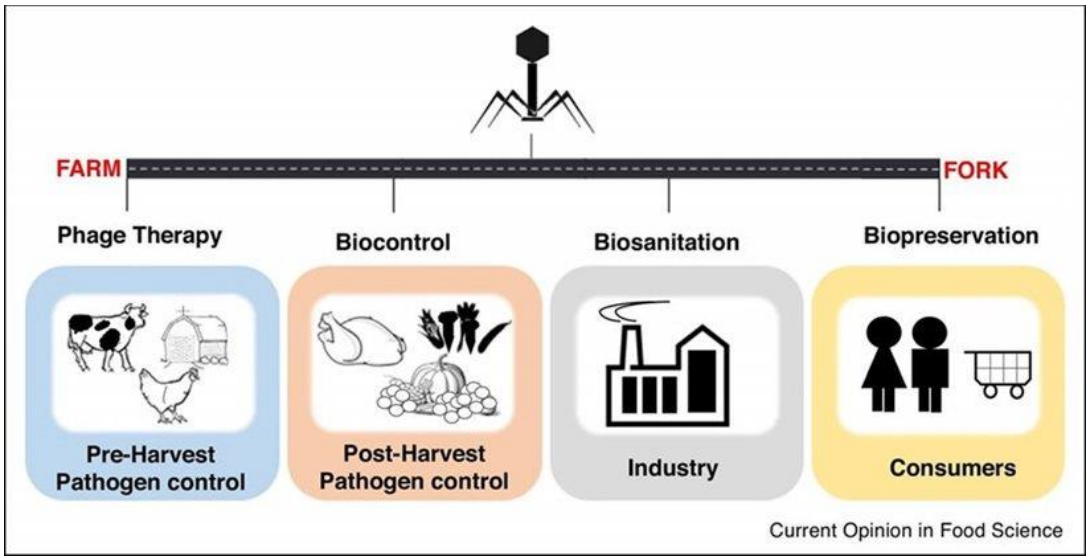


Figure 5 From Pre-Harvest to Consumers [55]

Bacteriophages are used for controlling bacterial pathogens throughout the whole food production process, from the farm to post-harvest stages (figure 5). On farms, phage therapy can be applied directly to livestock or to plants to reduce bacterial contamination. Bacteriophages are able to target specific bacterial threats and therefore are offering an alternative or supplement to conventionally used antibacterial techniques. [56, 57]

Today, pasteurization, pressure preparation, radioactivity, and antiseptics are conventional antibacterial techniques used to decrease bacterial activity in nutrition. Unfortunately, these techniques have many downsides like malfunctions due to corrosiveness, their nutritional significance, and the fact that these cleansing methods eliminate all contaminants – including the beneficial bacteria naturally occurring in the food. Luckily, the quantity of viable bacteriophage treatments licensed for health and safety purposes has expanded in the last few years. Due to their selectivity and stability, the bacteriophages have a lot fewer side effects than the previously mentioned antibacterial techniques. [58]

**Bacteriophage to control *E. coli* contamination**

Enterohemorrhagic *E. coli* strains produce Shiga toxin. One of the common serotypes is the *Escherichia coli* O157: H7. This is as previously mentioned, an important food and waterborne pathogen causing hemorrhagic diarrhea, and in severe cases also hemolytic-uremic syndrome (HUS) in humans. [59]

Recent research shows that vegetables, fresh milk, and UHT-preserved milk polluted with *E. coli* have been effectively treated with phage formulations specific to *E. coli*. *E. coli* type 0157:H7 was dramatically decreased on different types of vegetables, due to one of the phages. Research has shown that a mix of three phage types, the so-called "cocktail strategy", was beneficial for the decrease in the re-growth of *E. coli* at different temperatures. [60]

Also in ongoing research on meat systems, researchers have done thorough inspections of bacteriophage use in the treatment of the Shiga-toxin-producing *E. coli*. Eighth commodities including beef mince, cooked chicken, chicken breast, salmon, cheese, etc., were investigated and they found that the bacteriophages were highly effective in reducing pathogen levels of the analyzed foods. The amount of *E. coli* 0157:H7 was reduced significantly. [61]

Some examples of specific products that are already on the market for the control of EHEC 0157:H7 in pre- and post-harvest are:

- Finalyse®
    - A pre-harvest hide wash, that has been proven to significantly reduce *E. coli* O157:H7 at an exposure time of 5 minutes. This means that the levels of the *E. coli* O157:H7 are decreased before the beef enters the packing plant. Finalyse® is great as a first step in a long food process chain. [62–64]
- EcoShield™
    - Approved for being blended into ground beef, as an environmentally friendly alternative to the previously tried efforts to remove *E. coli* O157:H7.
    - Three lytic bacteriophages are being used as biocontrol agents
    - Science has shown that EcoShield™ significantly reduced the levels of the bacterium in experimentally contaminated beef by ≥ 94% and in lettuce by 87% after a 5-minute contact time.

- However, the effect after a one-time application of EcoShield™ did not include the protection of the foods from recontamination with *E. coli* O157:H7. Their results exhibit that EcoShield™ is effective in drastically reducing contamination of beef and lettuce with *E. coli* O157:H7, but does not protect against potential later contamination, for example, due to unsanitary handling of the foods post-processing. [65]

Previously, efforts to reduce the *E. coli* O157:H7 contamination have mainly focused on washing, chemical application, and gamma-irradiation – each of which has huge environmental and nutritional impacts. Today's use of bacteriophages seems to only be the beginning of the change to a more environmentally friendly food production market. The products mentioned are only examples of how bacteriophages are used to control food safety today, and as already mentioned the number of bacteriophage-treatments qualified for health and safety purposes are increasing rapidly.

## 3.3 BAS: Bacteriophage-antibiotic synergy

A common method of using bacteriophages involves combining them with antibiotics to enhance their effectiveness. Research has shown that phages can reduce the Minimum Inhibitory Concentration (MIC) of antibiotics, even for bacteria that are resistant. However, interactions between phages and antibiotics can vary, sometimes showing antagonistic effects. This interaction often depends on the antibiotic class, with variations within the same class impacting the outcome. [66]

This relationship is described by the term Phage-Antibiotic Synergy (PAS), which describes how sublethal doses of antibiotics can increase phage production. [67] This is not limited to antibiotics – it also applies to other environmental stressors. The PAS effect is an example of phages adapting to challenging environments by increasing their replication when the host bacteria are under stress. Antibiotics, alongside other stressors, threaten bacterial survival and, as a result, also increase phage reproduction. As a response, phages optimize their production in the presence of proper bacterial hosts.

A study done in 2018 explored various combinations of bacteria, phages, antibiotics, and other stressors. It proved that infections could be managed more efficiently with a combination of low-dose antibiotics and phages due to the PAS effect, which could also help limit the development of antibiotic-resistant bacteria. [68]

However, not many studies have been done on the synergistic relationship between phages and antibiotics. Despite the limited research, current findings suggest that this method may become valuable for using bacteriophages in the future.

## 3.4 Strengths and weaknesses of bacteriophages

### 3.4.1 Strengths

**Self-limiting doses**

New phages will be produced as long as there are bacteria to kill. During the process of killing bacteria, the phages are able to increase in number specifically where the hosts are located. There are some limitations, such as dependence on a relatively high density of bacteria. Since the phages themselves contribute to establishing their own dose, this process is called "auto-dosing". [69]

**Their specificity**

The phages are not harmful to other organisms as they do not kill other bacteria including our microflora, like antibiotics or sanitation chemicals can do[70]. They do not kill human or animal cells either [71], as for example some sanitary chemicals [72] and antibiotics do. [73]

**Environmentally friendly**:

Bacteriophages are eco-friendly compared to conventional plant protection products in the way they fight against pathogenic bacteria. Due to their specificity to their target bacteria, they are not expected to have a negative impact on the bacterial biodiversity and serve as a green and sustainable solution in crop production and protection. [74, 75]

**Reduction of antibiotic use**

Phages can reduce antibiotic resistance in two ways:

- in a less direct way; if phage therapy is used when possible, fewer antibiotics will be used and therefore less resistance will be developed. [76]
- In a direct way; phages can be used to directly kill antibiotic-resistant bacteria and can be used in therapy or to prevent the resistant microorganisms from spreading. [77, 78]

**Natural origin**

Public resistance to laboratory-synthesized drugs and genetically modified organisms (GMOs) is growing [69], with more consumers prioritizing natural and organic foods and therapeutic options. The fact that bacteriophages are naturally occurring, and non-GMO presents a significant advantage. It aligns with consumer demand for more natural, eco-friendly solutions, making phages an appealing option for biocontrol in food safety and other applications.

Phages are suitable for food safety, especially in the context of raw foods, like raw milk, beef tartare, and other minimally processed foods. Phages can reduce the amount of harmful bacteria in these products, enhancing safety without the use of synthetic chemicals that may be considered less healthy. [79]

However, the idea of adding viruses to food and pharmaceuticals may be misinterpreted by the general public, and concerns of the concept may occur. Despite this, the specific and targeted nature of phages, combined with their natural origin, offers biocontrol in food safety that is unique as it does not alter the organic status of food.

These factors make bacteriophages promising in the ongoing efforts to balance natural food production with effective pathogen control.

Not only are the bacteriophages suitable for raw foods, but they have been tested successfully on a wide variety of food products, including raw and cooked meats, vegetables, and ready-to-eat products, making them useful across different food industries. [57]

### 3.4.2 Weaknesses

**Narrow spectrum**

Due to the bacteriophage's high specificity, their host spectrum is extremely narrow. Bacteriophages can manage diseases caused by a single, known bacterial species, but most bacteriophages are so specific that they cannot even target all pathogenic strains of a single bacterial species, some only act on certain species of a specific bacterial genera. Unfortunately, infections in actual clinical cases are usually caused by multiple pathogenic bacteria. Therefore, the desired therapeutic effect is difficult to reach with single bacteriophages. [80]

Phages will have huge limitations on presumptive treatment (treatment beginning prior to the identification of the pathogens causing the disease or problem). However, in "phage-cocktails", where multiple phages are combined, the lytic spectrum of phage products can be much broader than the one of an individual phage. But even the phage solution we call "broadly acting phages" has more selective specter than the "narrow-spectrum" antibiotics. [69]

**Phage resistance**

Bacteria can develop resistance against phages, as they can against antibiotics. But as they have an advantage with mutating and overcoming resistance, this is less of an issue compared to antibiotics. The resistance development in phage therapy can often be countered by having multiple different phages in a phage cocktail solution so that if the bacteria develop resistance to one phage, the rest can still kill it and prevent the bacterium from passing the resistance on to the next generation. [81, 82]

**Potential for horizontal gene transfer**

Lysogenic phages can be the source of horizontal gene transfer and contribute to antibiotic resistance genes (ARGs) spread. Theoretically, transduction may result in the emergence of new microbes or even more resistant genes in bacteria. [76, 83] Yet, the exact role of phages in the spread of ARGs remains unknown.

**Storage and environmental conditions**

Commercial phage products often claim that they are robust towards various environmental conditions. However, research gives a different perspective. Phages are highly sensitive to multiple external factors, such as pH, temperature, and ion concentration. These factors can significantly affect their stability and effectiveness. Given the differences in products with varying temperatures, textures, and acidity levels, it seems challenging to maintain a consistent phage solution with reliable qualities across all environments. Based on this, it is clear that biocontrol using phages requires careful consideration of these limitations.

- Temperature: they are sensitive to extreme temperatures, as high temperatures can denature the phage proteins, while lower temperatures may decrease their viability.
- pH: Most phages are stable in the pH range of 5-9, and outside of this range phages may lose their infectivity.

- Ions and salinity: phages can be inactivated by high concentrations of salts or sugars, particularly NaCl and sucrose. [84]

In other words, maintaining optimal conditions for temperature, pH, and ion concentration is crucial for their stability and application, especially in food safety and agriculture.

# 4 Discussion, conclusion

There are still many unknown aspects of bacteriophage therapy, but new and ongoing research continues to broaden our understanding of how phage therapy can be optimized.

Antibiotics target a broad range of bacteria, often harming beneficial microbiota and contributing to antibiotic resistance. In contrast, bacteriophages are highly specific, attacking only certain bacterial strains without affecting the surrounding beneficial bacteria. This specificity makes phages a promising alternative in the therapy of infections, especially in the context of rising antibiotic resistance. However, phage therapy faces multiple challenges, including regulatory issues and the need for personalized treatment approaches. [85]

## 4.1 What challenges should bacteriophage therapy help address?

### 4.1.1 Antimicrobial Resistance (AMR)

AMR poses a huge global health threat, where many bacterial infections are no longer treatable with traditional antibiotics. Bacteriophages offer a promising solution as they specifically target bacteria and can therefore target antibiotic-resistant bacteria. This could help reduce the reliance on antibiotics and slow the development of resistance. The specificity of phages also minimizes the interference with beneficial bacteria, unlike broad-spectrum antibiotics.

### 4.1.2 Food Safety and Production

Phage therapy has already been applied within the food production industry to control bacterial contamination in livestock, fish, and crops. Bacteriophages can be used from pre-harvest to post-harvest stages, helping to prevent foodborne illnesses caused by bacteria like *E. coli* and *Salmonella*. Since phages are natural and can be used in organic or minimally processed foods, they offer an attractive alternative to chemical treatments.

### 4.1.3 Control of Infections in Immunocompromised Patients

Immunocompromised individuals, such as cancer patients or patients going through complex treatments, are highly vulnerable to bacterial infections. Phage therapy's specificity and low risk of side effects make it a safer option for these patients, as it avoids the broad-spectrum harm that antibiotics can cause.

### 4.1.4 Environmental Sustainability

The overuse of antibiotics in agriculture, aquaculture, and medicine has led to environmental contamination, which accelerates the number of resistance genes in bacteria. Phages, as natural biocontrol agents, offer a more environmentally sustainable option. They specifically target harmful bacteria without affecting the surrounding ecosystem, unlike chemical disinfectants or antibiotics, which can harm non-target species.

These aspects highlight how bacteriophage therapy can improve the control of infection across medical, agricultural, aquacultural, and environmental sectors.

## 4.2 Strengths and weaknesses of this study

This thesis has a strong theoretical framework and practical relevance.

Today, the focus on bacteriophages as alternatives or supplements to antibiotics is highly relevant to the current challenges. With antimicrobial resistance being one of the biggest global health issues we have, this research addresses an important area that has a major impact on public health, food safety, and environmental sustainability. I am highlighting both the potential of bacteriophages, and the limitations of their use by discussing the challenges related to environmental stability, resistance, and specificity of bacteriophages. This gives a detailed and realistic view. I am using some practical applications and case studies, like the use of CUSTUS®YRS in Norwegian fish farming and EcoShield™ in livestock, to connect theoretical knowledge to real-world applications.

There are several weaknesses in this study. Firstly, bacteriophage therapy is still a relatively unexplored topic, which limits the number of available case reports and studies. Furthermore, the methods of bacteriophage therapy vary significantly, reducing the comparability between them. My thesis is based on secondary data and literature reviews, and not original experimental work. There is also a limited focus on long-term clinical data, as there are few long-term clinical trials available for reference. By including some specific

case studies, while valuable, it might limit the applicability of my conclusions. The field of bacteriophage research is rapidly changing, and some information might become outdated soon, as new studies and technological advances develop. Keeping the thesis up to date with the latest findings could be a challenge, especially for such a dynamic area.

## 4.3 The future

Due to the specificity of bacteriophages, it is unlikely that phage therapy can replace broad-spectrum antibiotics. In acute situations requiring immediate antimicrobial treatment, there is no time for sensitivity testing for bacteriophages. However, it is crucial to preserve the effectiveness of broad-spectrum antimicrobials by using them only when absolutely necessary and for shorter periods. It is still uncertain what role bacteriophages may eventually play in clinical practice, however, there are several other potential applications. They have numerous properties that can make them compelling alternatives to chemical antibiotics. Many of the concerns associated with phage therapy can be manageable through a combination of proper selection of phages, effective formulations, and a greater understanding of their application.

With the rise of antibiotic-resistant bacterial infections, phages offer many advantages with relatively few drawbacks. Advances in our understanding of phage biology, combined with stricter medical and food safety standards compared to the early days of phage therapy [86], may give bacteriophages a second opportunity to demonstrate their full potential in Western medicine.

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