Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

Review

Bacteriophages as Biocontrol Agents in Food Microbiology

Logan Gildea[1*], Joseph Atia Ayariga[2*] and Boakai K. Roberson[1]

[1] The Microbiology Program, College of Science, Technology, Engineering, and Mathematics (C-STEM), Alabama State University, Montgomery, AL, 36106
[2] The Biomedical Engineering Program, College of Science, Technology, Engineering, and Mathematics (C-STEM), Alabama State University, 1627 Hall Street, Montgomery, AL, 36104
*Corresponding author: lgildea5810@myasu.alasu.edu; ayarigajosephatia@yahoo.co.uk

Abstract: Bacteriophages have been recognized as potential biocontrol agents in the food industry. Bacteriophages have been proposed for a variety of applications within this industry including bio preservation, pathogen detection, and as an alternative treatment method to antibiotics in animal health. The potential applications of bacteriophages are widespread throughout the entire food production process and serve to enhance food quality, prevent foodborne illnesses, and enhance the efficiency of production. The ability of bacteriophages to lyse bacterial targets with high specificity and pose no threat to mammalian cells or natural microbiota is unique and relevant in terms of suitability for food safety. This review will outline potential and current applications of bacteriophages and their respective impacts on the field.

Keywords: bacteriophages; biocontrol; food production; bacterial resistance

1. Introduction

An integral part of human life is the consumption of food products. Our everyday life revolves around the preparation and consumption of food. While it is essential to consume food products it also provides a pathway to infection caused by foodborne pathogens such as *Salmonella* spp.,**[1,2]** *Escherichia coli, Campylobacter* spp., and *Clostridium* spp. Annually foodborne pathogens account for around 179 million illnesses, 486,000 hospitalizations, and 6,000 deaths in the United States alone **[3]**. Even with enhanced technology, thorough quality control, and advanced sterilization methods these pathogens persist within the environment. The ever-evolving nature of food production, due to consumer demand and economic considerations, presents challenges in maintaining food quality and safety. The most efficient antimicrobial methods in food production are good hygiene and the utilization of antimicrobial agents such as biocides and disinfectants. These practices must be applied in every aspect of the food production process to ensure safety and quality. Regardless of the many preventative measures in place, bacterial communities persist through development of resistance, biofilm formation, and other mechanisms **[4-6]**. This bacterial persistence is why development of new novel biocontrol agents, such as bacteriophages, are crucial for this constantly evolving field. This review will overview relevant literature and commercial products that utilize bacteriophages as agents of biocontrol in the food industry.

2. What is Biocontrol?

Biocontrol refers to the use of one or more organisms to inhibit or control other organisms. Agents of biocontrol can be divided into two categories: living organisms, such as phages or indirect actions or byproducts of an organism, such as the production of bacteriocins. In general biocontrol encompasses the use of lactic acid bacteria, bacteriocins, endolysins, bacteriophages, and 'protective' cultures. Biocontrol relies on the ability of agents to carry out microbial interference. Microbial interference refers to the general non-specific inhibition or destruction of one microorganism by other members of the same environment.

© 2022 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

A major benefit of biocontrol is, unlike other microbial control methods, it is derived from natural and green sources. Biocontrol products are typically less harsh and are more widely publicly accepted in the current market **[7,8]**. Biocontrol agents are characterized as nontoxic and naturally produced agents that can withstand physiological conditions and maintain a narrow spectrum of antimicrobial activity. While bacteriophages represent an up-and-coming biocontrol technique, some biocontrol techniques are currently already widely used. The bacteriocin nisin was first introduced commercially nearly 60 years ago and has been utilized in numerous food and beverage products **[9]**. Nisin is a bacteriocin produced by some strains of *Lactococcus lactis* and is a lantibiotic that has been used in the control of *Clostridium* spp. and *Enterococcus* spp. **[10]**. Another common group of biocontrol agents are endolysins. Endolysins are proteins, typically derived from bacteriophages, that function to exogenously lyse bacteria. Endolysins are typically paired with *holins,* a protein that perforates the peptidoglycan layer of gram-positive bacteria to allow for the entry into the cytoplasm of the cell. Endolysins have been employed as agents of biocontrol against *Clostridium perfringens*, *Clostridium fallax*, and *Listeria monocytogenes* **[11-13]**. The final agents of biocontrol and primary focus of this review are bacteriophages. Bacteriophages are abundantly present in the natural world and phage typing has been utilized to identify phages and their associated bacterial hosts. Lytic bacteriophages have been studied since the early 20th century but have more recently have gained attention as potential agents against antibiotic resistant bacteria.

The Role of Bacteriophages in Food Microbiology

Prokaryotic viruses, otherwise known as bacteriophages or phages, are viruses that infect bacterial species with high specificity **[14-23].** This high specificity allows bacteriophages to target pathogenic foodborne bacteria while not affecting the natural microbiota of food products or the humans who consume them **[24]**. Phages are found in every environment suitable for bacterial growth and are already present in all the foods we consume. Research on phages have identified bacteriophages associated with almost every known pathogenic bacterium **[25]**. While phages are one of the most abundant organisms on earth it is important to differentiate which phages are appropriate for use in food microbiology. The broadest classification method of phages is based on their lifestyle which can be either lytic or lysogenic. Lytic phages function to kill bacteria through lyses while lysogenic phages allow the bacteria to persist while infected with the virus (Figure 1). In terms of therapeutics and food microbiology, it is highly inadvisable to utilize lysogenic phages for bacterial control due to the persistence of the bacteria **[25]**. Lytic phages are preferred for the treatment of bacteria in that it does not allow the bacteria to persist which is the desired goal in food microbiology.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

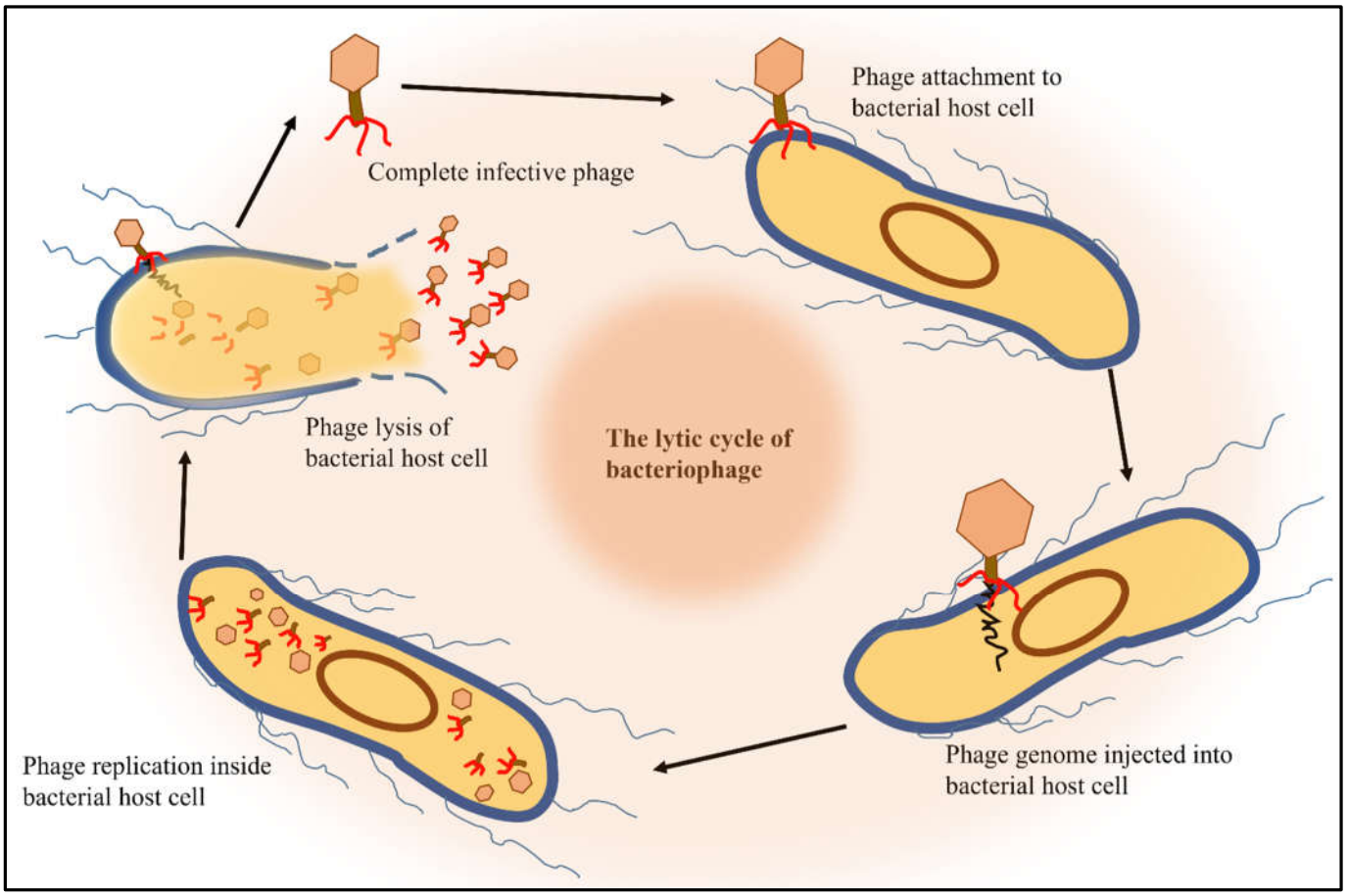

Figure 1. Lytic cycle of bacteriophages. The lytic cycle is one of the two cycles of bacteriophage replication that leads to the lysis and destruction of the infected cell. These bacteriophages that employ the lytic cycle have been shown to have significant importance in biocontrol of bacteria in livestock infections, these virulent phages are specific to their host. In the lytic cycle, the bacteriophage genome do not integrate with the host bacterial cell genome, however, the DNA are found in the cytoplasm of the host cell as separate and free-floating molecule, also it replicates separately from the host bacterial DNA.

Phage biocontrol is progressing towards acceptance as a novel and green technology that could eventually replace some standard methods of intervention such as chemical antimicrobials **[27-29]**. Unlike common chemical antimicrobial interventions such as alcohols, chloride compounds, and bisphenols; bacteriophages are naturally occurring organisms that are safe for consumption and can be readily used at multiple points in food processing without sacrificing the quality or safety of the product **[30,31]**. Bacteriophage preparations are either distinguished as mono-phage treatments or phage cocktails. Mono-phage treatments consist of one singular phage type and is used to target a singular known pathogen present in the food product. Whereas phage cocktail preparations are a mixture of multiple phage types and can be utilized for treatment of food products with pathogens that readily develop resistance or are diverse. Phage cocktails are designed primarily as a preventative treatment that can be utilized to ensure that common pathogens are unable to colonize a food product. The acceptance of phage treatment has consistently grown since the FDA granted clearance to ListShield™ a *Listeria monocytogenes*-specific phage cocktail used as a food additive in 2006 **[32]**. Since this major steppingstone, multiple phage treatments for foodborne pathogens including *Salmonella spp*., *E. coli*, and various other common foodborne bacteria have been accepted by the FDA as safe additives to food products. A major step in the progression of phages as a commonly accepted food additive was the FSIS directive 7120.1. This directive stated that addition of phages to livestock prior to slaughter and food is permitted, which promoted the production of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

multiple lytic phage additives such as *E. coli* O157:H7-targeted phages, used for the reduction of aerosol contamination from the hides of cattle and P22-*Salmonella*-targeted phages in the use of poultry and fresh meats **[33,34]**.

One of the beauties of bacteriophages is their ability to be incorporated in multiple aspects of food processing including animal therapy, sanitation, biocontrol, and preservation. The versatility of phages promotes them as a sensible option as novel methods are developed to enhance and preserve the safety and quality of food products. Adaption of phage treatment methods would provide not only ease of use but also decrease economic loss associated with spoilage and product contamination **[35,36]**.

3. Phages as Agents of Animal Therapy

An important aspect of food production is the ability to maintain healthy livestock. While many focus on the harvesting and preparation aspect of food production, animal health is a major contributor to the prevalence of foodborne pathogens **[37]**. Several pathogens can infect farm animals asymptomatically, which can lead to contamination and public health hazards. Many of these pathogen stem from the increased prevalence of antibiotic-resistance within farm animals that can be contributed to the mass and sometimes inappropriate use of antibiotics **[38]**. While these pathogens are antibiotic resistant, they are still susceptible to phages **[39]**. This contributing factor is a major reason why the prospect of phage therapy has gained so much interest with the post-antibiotic era quickly approaching.

The most common bacterial pathogens of concern associated with poultry are *Salmonella* spp., *E. coli, Campylobacter* spp., and *Clostridium* spp. **[40]**. These bacterial pathogens have shown to be increasingly resistant to antibiotic treatments as well as antimicrobial compounds **[41]**. The European Food Safety Authority (EFSA) reported that roughly 28.6% of *Salmonella* spp. and 34.9% of *E. coli* found in food sources were multidrug resistant **[42]**. Considering this data, it is crucial to incorporate new and novel methods for the treatment of these pathogens. Literature has examined the efficacy of various phage treatments against several common foodborne pathogens such as *Salmonella* spp., *E. coli, Campylobacter* spp., and *Clostridium* spp. outlined in the following subsections.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

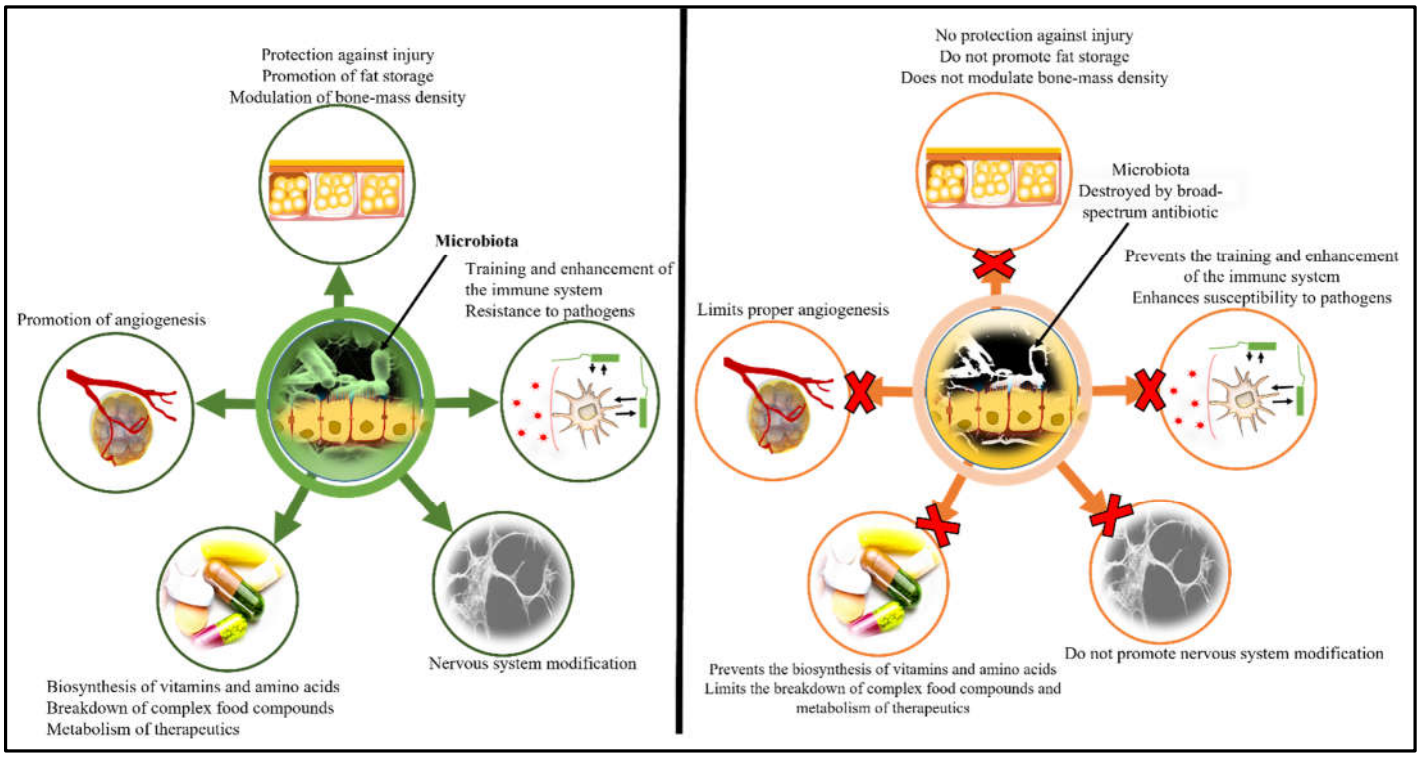

Figure 2. Potential side effects of antibiotic use in control of bacterial in livestock. The left shows the main functions microbiome in most livestock/humans. It has been demonstrated that microbiota provides special benefits to the livestock and human hosts alike. These bacteria are involved in biosynthesis of vitamins and amino acids, breakdown of complex food compounds and metabolism of therapeutics, development, and training of the bacterial host's immune system **[43-45].** Microbiota has also been demonstrated to inhibit the colonization of the gut of their animal host with pathogenic bacteria **[46,47]**. It has been revealed also that microbiota modifies the nervous system, encourages fat storage, promote angiogenesis, provide protection for epithelial tissues of its host **[48-52].** The use of broad-spectrum antibiotics usually leads to the destruction of the microbiota of the animal or the human host **[53]**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

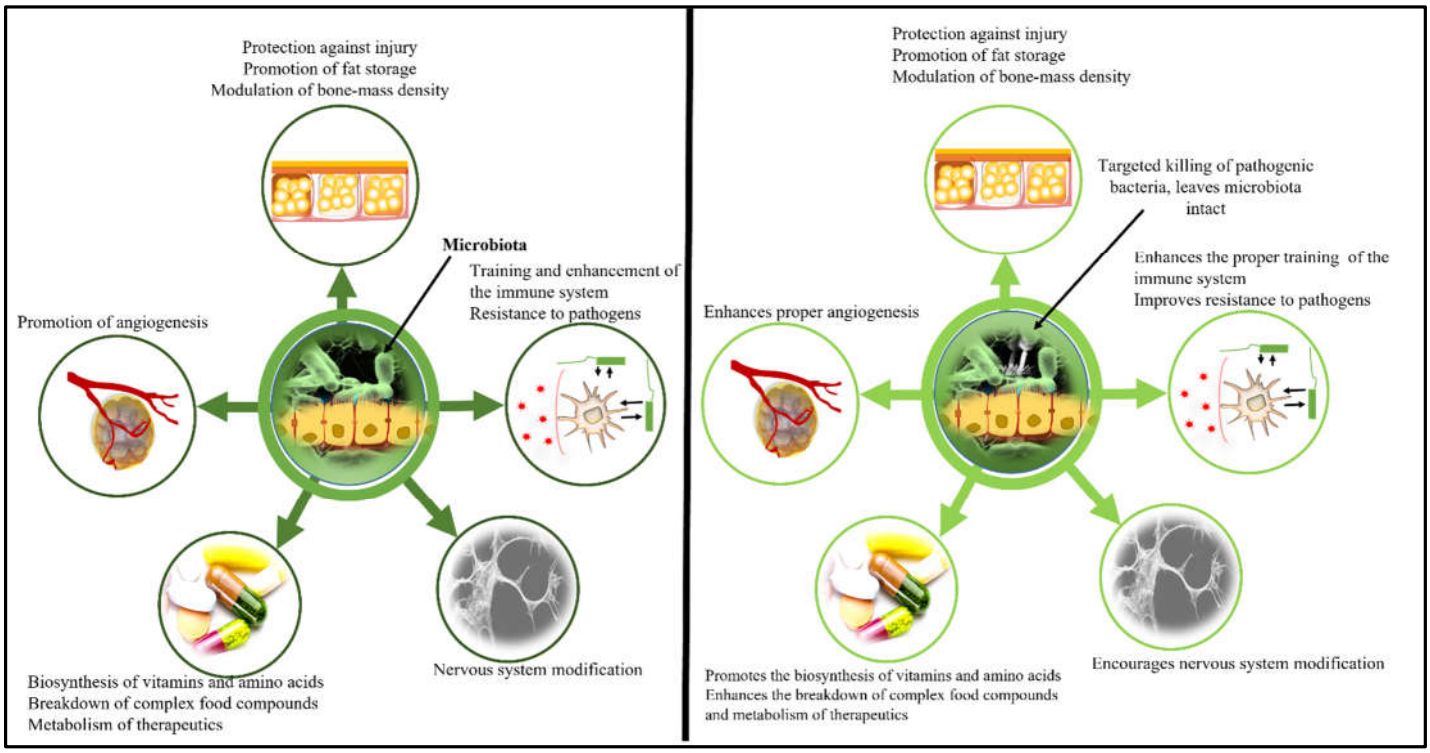

Figure 3. Advantages of bacteriophage application in the control of bacterial infection in livestock.

Similar to Figure 2, the left panel in Figure 3 shows the main functions of microbiome in most livestock/humans and these benefits are either enhanced, promoted, or not inhibited when phages are used in the bacterial control of pathogenic bacterial (Figure 3, right panel) in the host instead of the microbiota destroying broad-spectrum antibiotics **[54]**.

3.1. Salmonellosis

Salmonella spp. are prevalent pathogens in the realm of foodborne disease, causing disease in a variety of endothermic animals, humans, and leading to significant production losses in livestock **[55,56]**. *Salmonella spp*. can be divided into host-restricted and non-host-restricted classifications. Host-restricted *Salmonella spp.* are characterized as bacterium that produced severe, typhoid-like illness in a single host. Non-host-restricted *Salmonella* serotypes, such as *S. enteritidis* and *S. typhimurium*, typically result in moderate gastrointestinal infections across a broad range of species and account for most foodborne bacterial infections **[57]**. Phage therapy and biocontrol has primarily focused on non-host-restricted *Salmonella spp*. due to their prevalence and relation to most foodborne *Salmonella* outbreaks.

Phage therapy has been extensively studied in the poultry industry for the control of *Salmonella*. Poultry products account for most of the *Salmonella* outbreaks reported the United States every year **[56]** so the development of specific biocontrol methods such as phage therapy are essential for the prevention of *Salmonella*-related illness. Phage treatments in poultry can potentially address several issues of *Salmonella* infections such as gut colonization, horizontal transmission, and prevalence of multi-drug resistant infections **[33,55,57]**. Several studies have outlined the success of various phage treatments aimed to reduce *Salmonella* colonization within poultry prior to harvest. The results of these studies are outlined within Table 1.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

Table 1. Phage biocontrol studies of Salmonella spp. in poultry.

Phage Treatment	Target	Results	Reference
Lytic Phage Cocktail	*S. enteritidis, S. hadar, S. typhimurium*	Salmonella colonization of the ceca was reduced approximately 2.19-2.53 $\log_{10}$ CFU/g.	**[55]**
Mono-phage Treatment as a feed additive	Prevention of Horizontal infection by *S. enteritidis*	Significant reduction ($P<0.05$) of intestinal colonization by up to 1 $\log_{10}$ CFU/g after 21 days and prevention of S. enteritidis infection through horizontal infection.	**[58]**
Salmonella Specific Phage Cocktail incorporated with water supply	Reduction of *Salmonella* colonization in the intestine	Reduction of mean Salmonella colonization of the Intestinal tract by up to 1.6 $\log_{10}$ CFU/mL.	**[59]**
Prophylactic Mono-phage Treatment (PSE)	*S. enteritidis* within the ceca	Prophylactic Treatment reduced the detection of S. enteritidis to 20% 7 days after administration.	**[60]**

The studies referenced in Table *1* outline several methods in which phages can be utilized for the prevention and control of *Salmonella* within poultry food sources **[55,58-60]**. The versatility of phage therapy as a preventative treatment as well as a therapeutic is a benefit not possessed by typical chemical intervention methods. Research has even determined that in the case of phage treatment against *Salmonella* infections, the prevalence and persistence of bacteriophage-insensitive mutants (BIMs) can be reduced through responsible treatment methods **[55]**. Phage therapy's ability to lyse antibiotic-resistant *Salmonella* spp. is also a major upside in terms of phage incorporation into food production.

3.2. Colibacillosis

One of the most prominent pathogens responsible for food contamination and foodborne disease is *Escherichia coli*. A gram negative, anaerobic, rod-shaped, coliform bacterium, *E. coli* is responsible for the majority of reported foodborne illnesses and several significant outbreaks in the United States **[61]**. In terms of food production, *E. coli* is also responsible for considerably high mortality in poultry **[62]**. This can be attributed to the occurrence of avian respiratory tract infections causing Colibacillosis. Development of alternative preventative methods such as phage treatments is crucial to the reduction of economic loss and potential hazards caused by *E. coli* infections in poultry.

Numerous studies have attempted to progressively introduce and develop phage treatments for the treatment and prevention of *E. coli* infections within poultry. The efficacy of phages as a preventative method was confirmed by Huff et al in 2002 through use of a high phage titer phage cocktail administered through aerosol spray as a therapeutic measure. Phage treatment of *E. coli* challenged chickens reduced the mortality from 50% in the control group to 20% in the treated group **[63].** Other studies have utilized aerosol phage treatments as therapeutics for poultry and confirm the reduction of mortality due to phage treatment **[64,65]**. One of the notable drawbacks of the aerosol treatment of livestock is that it must be administered immediately as studies suggest that efficacy is significantly lowered after 24hrs of *E. coli* challenge **[66]**. To address this efficacy issue, Huff et al., also administered a combination of two phages, DAF6 and SPR02, through direct intermuscular injection into *E. coli* challenged chickens. This treatment method resulted in significantly lower mortality whether the combination was injected immediately or up to 48 hours after bacterial challenge **[66]**. This study alone illustrates the importance of phage administration methods to the successful prevention of foodborne pathogens.

Another potential threat of colibacillosis, other than respiratory infections, is the development of meningitis or septicemia. Unlike respiratory infections, in which aerosols can be utilized, these infections require intramuscular or intracranial administration for phage treatment. Research has shown that intramuscular or intracranial administration of phage K into *E. coli* challenged chickens resulted in a complete elimination of mortality

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

related to these infections when injected immediately. Even administration following development of clinical signs of infection resulted in a 70% survival rate in phage treated chickens **[67]**. The ability of phages such as phage K to multiply in blood and penetrate the blood-brain barrier supports the idea that phages could serve as viable treatment methods for acute infections.

3.3. Campylobacteriosis

In the United States and European Union (EU) *Campylobacter* is a significant contributor to acute bacterial foodborne disease with 95% of reported diseases being the result of the species *C. jejuni* **[42]**. *Campylobacter* has been responsible for six separate outbreaks since 2010 in the United States alone. *C. jejuni* infections are incredibly prevalent and Campylobacteriosis has been linked to Guillian-Barré Syndrome and reactive arthritis **[68]**. A major obstacle in the prevention of *Campylobacter* contamination in food products is its high adaptability and relatively low infectious dose **[69]**. Both characteristics significantly lower the efficacy of standard prevention methods such as antibiotics and other antibacterial agents. The UK Food Standards Agency reported increased occurrence of antibiotic resistance in *Campylobacter spp.* against ciprofloxacin, tetracycline, nalidixic acid, streptomycin, and erythromycin **[70]**. This prevalence stresses the need for new and effective novel prevention and therapeutic agents.

Phage therapy has been studied rather extensively in the treatment of Campylobacteriosis. Due to the high adaptability of *Campylobacter spp.* multiple phage therapy or phage cocktails have been suggested as more efficacious treatment methods. *Campylobacter* phages are arranged into three groups based upon their structure, genome size, and host receptor **[71]**. Literature suggests that group I and II *Campylobacter* phages utilize multiple receptors for the infection of host cells making them ideal for treatment of *Campylobacter* infections **[72-74]**. The efficacy of group II and III phages CP14 (III) and CP68 (II) in combination therapy was observed in Vrolix chickens by Hammerl et al. in 2014. Phage cocktail treatment of chickens inoculated with a 10^9 CFU of *C. jejuni* resulted in a 3 $\log_{10}$ CFU/g reduction of *C. jejuni* colonies within the caeca **[75]**. It was also concluded that phage cocktail treatment was more efficacious than phage monotherapy using CP14 or CP68. Additionally, the prevalence of resistant *Campylobacter* was significantly reduced through the application of phage cocktail treatment **[75]**. *Campylobacter* colonization of the caeca in birds does not appear to be very invasive allowing for oral phage administration to be a preferred delivery method. Phage cocktails administered through gavage and incorporated with feed reduced *C. jejuni* and *C. coli* colonization by 2 $\log_{10}$ CFU/g in broiler chickens **[76]**. The results presented suggest that phage treatments could be a valuable tool for the control of *Campylobacter* spp. in food production.

3.4. Clostridiosis

Clostridium spp. are considered a pathogen with significant impact due to its association with enteric disease in poultry. These enteric diseases caused by *Clostridium* species, primarily *C. perfringens*, are difficult to diagnose due to clostridial species being present in the gut as a natural microbe **[77]**. *C. perfringens* is characterized as a gram-positive, rod shaped, anaerobic, spore-forming bacterium that is one of the leading causes of foodborne disease. Typical diagnostic methods such as alpha-toxin detection and isolation cultures cannot be used for confirmation of infection since they are present within the gut naturally **[77]**. The most common enteric disease associated with *C. perfringens* in poultry is necrotic enteritis. The poultry industry annually loses 5 to 6 billion per year due to the loss caused by necrotic enteritis **[78]**. This disease is prevalent in broiler chickens but has also been diagnosed in a variety of avian species such as turkeys, waterfowl, and ostriches. The prevention of *C. perfringens* infections in poultry would not only reduce economic loss associated with food production but also reduce the prevalence of related foodborne disease in humans.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

Phage therapy has been studied as a potential therapeutic in chickens for the reduction of symptoms and disease progression. Initial study of *C. perfringens* phages revealed that phage-encoded endolysins target and hydrolyze the peptidoglycan mesh of *C. perfringens* with high efficacy leading to bacterial lysis **[79]**. The use of purified phage endolysins show promise as a phage-protein therapy to reduce *C. perfringens* colonization **[11,12,80,81]**. Miller et al, in 2010 determined a five-phage cocktail, termed INT-401, administered orally reduced *C. perfringens* associated mortality by 92% **[82]**. These results concluded that phage treatments could be a viable agent of biocontrol of *C. perfringens* in poultry. Further study to standardize phage treatments of *Clostridium* spp. is a key to the advancement in treatment of this pathogen.

4. Challenges of Bacteriophage Biocontrol

Research on bacteriophages as biocontrol agents for targeting pathogenic bacteria with high specificity has become increasingly prevalent throughout literature as described in the previous sections. However, the advancement of phage biocontrol faces two major challenges: firstly, general consumer acceptance and secondly resolution of technical constraints involved with development and standardization. Before phage preparations can be introduced as a replacement to standard treatment methods these two challenges must be addressed and resolved.

The most significant technical challenge of phage biocontrol is consistent efficacy. While *in vitro* and *in vivo* studies have illustrated success, the efficacy of phage treatments in commercial usage is highly dependent on the phages that are utilized within the treatment due to the associated specificity of phages. A common phenomenon observed with phage treatments is the initial decrease of bacterial colonization, followed by a restoration of bacterial growth **[83,84]**. The result of this is a drastic decrease of bacteria within the food source initially but not a complete elimination of the pathogen. Phage treatments must also be targeted to the location of colonization within the organism, leading some broad treatment methods to be ineffective in complete eradication. Therefore, oral administration of phages is typically successful in the treatment of intestinal tract infections but non-efficacious to secondary infections such as Colibacillosis related meningitis infections **[67]**. Dosage determination is another technical challenge of phage treatments. Unlike antibiotics, phages are living organisms that can actively replicate within the body while lysing target bacteria. The plethora of phages as well as the conditions they are subject to within the body leads to variation in the rate of replication and production of viral progeny. This variation makes determination of exact dosage requirements challenging and requires further study. Theoretically phage concentration should increase exponentially as lysis of the target bacteria occurs, however recent studies have suggested that phages do not increase exponentially after application to food products **[64,85,86]**. This lack of exponential growth is related to the environmental conditions that typically have low water activity (a_w) levels for preservation purposes. This low a_w directly correlates to the reduction in phage mobility within the environment **[34]**. This obstacle could be avoided through increased phage concentrations which would increase the probability of phage particles meeting the pathogen in food products with lower a_w levels **[55,87-90]**. Another potential methodology to increase phage therapy efficacy is the use of high titer aerosol treatments. This method would be utilized following the harvesting of animals for food products and applied directly to the entire surface of the food product. This treatment would drastically reduce the potential contamination of food products, especially in cases such as *Campylobacter* contamination, where infective doses are relatively low. Standardization of proper application will ultimately resolve the challenge of phage efficacy in food product biocontrol.

A challenge that addresses both the efficacy and public acceptance of phage biocontrol is the quantitative bacterial reduction associated with this treatment method. As outlined in previous sections, phage treatments typically reduce bacterial colonization of food products by approximately 1-3 $\log_{10}$ CFU **[75]**. This reduction is considerably lower

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

than some hasher intervention methods such as chemical agents and irradiation, that reduce bacteria by approximately 5 $\log_{10}$ CFU **[75]**. This differentiation in CFU reduction may lead consumers and industry to shy away from phage therapy methods, however, there still is value in the reduction potential of this method. While the bacteria may not be fully eliminated from the food product it is still drastically reduced rendering it safer for consumption. Additionally, food products exhibiting $\log_{10}$ CFU levels greater than 3 are typically the result of other contamination issues introduced throughout the production process **[91]**. A study that exhibits the significance of even a 1-3 $\log_{10}$ CFU reduction is a risk assessment conducted jointly by the FDA and USDA's FSIS that quantified the effect of contaminated deli meats on the morality rate of the elderly **[92]**. According to the analysis in this study, even a 10-fold (1 $\log_{10}$) and 100-fold (2 $\log_{10}$) reduction of bacteria in pre-retail contamination would reduce the mortality rate by approximately 50% and 74% respectively **[92]**. This data suggests that even though phage biocontrol methods do not always fully eradicate bacterial contamination they still may yield significant potential in applications of food safety and public health.

Another significant challenge for the introduction of phage biocontrol methods is public acceptance of the method. Recent consumer trends in terms of food appear to be aligning to provide entry of phage treated products into the market. Trends indicate that consumers are more likely to avoid chemically and antibiotically treated products in comparison to organic and locally produced 'green' products **[7,8]**. This trend is promising to phage biocontrol in that phage treatments are a natural, organic, and targeted approach to provide safe food products. While this is promising there is still a natural apprehension of consumers to the concept of using viruses on the food that they consume. This is mostly due to unfamiliarity with the method and the generally low scientific literacy of the common public. Before widespread utilization of this technology the public must be educated on the safety, efficacy, and ubiquity of bacteriophages in their food products.

5. Approved Phage Biocontrol Products

While the widespread acceptance of phages as biocontrol agents is still pending, some phage biocontrol products are currently approved and have begun being utilized in the food production industry. Most of these products are utilized in the Eastern World, where phage applications have been more exhaustively studied and are more widely accepted **[93]**. These commercial phage products are viewed as natural and green technologies. These phage products contain naturally occurring, GMO free phages and are typically suspended in water-based solutions that are free of harsh chemicals typically associated with biocontrol. Most importantly these phage products, compared to other safety intervention methods that typically cost 10-30 cents per pound of treated food, only cost 1-4 cents per pound of treated food **[94]**. This drastic financial difference is another major advantage of phage biocontrol products in comparison to traditional intervention. It is important to note however that this is the cost associated with a product designed for very specific treatment of a singular pathogen. Incorporation of multiple products does increase the overall cost of phage biocontrol. Overall, the biological properties of lytic phages used in commercial phage biocontrol products make them a very attractive modality for further improvement of our food products. An increasing number of companies involved in the production and commercialization of phage biocontrol methods will further advance the relevance of this modality in the food production industry **(**Table 2**)**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

Table 2. Commercial Phage Biocontrol Products. Adapted and modified from Moye et al., 2018. A brief list of relevant commercial phage biocontrol products.

Company	Phage Product	Target Organism(s)	Regulatory	References
FINK TEC GmbH (Hamm, Germany)	Secure Shield E1	*E. coli*	FDA, GRN	**[88,95-100]**
	Ecolicide® (EcolicidePX™)	*E. coli O157:H7*	USDA, FSIS	
	EcoShield™	*E. coli O157:H7*	FDA, FCN 1018; Israel Ministry of Health; Health Canada	
Intralytix, Inc. (Baltimore, MD, USA)	SalmoFresh™	*Salmonella spp.*	FDA, GRN 435, USDA, FSIS Directive 7120.1; Israel Ministry of Health; Health Canada	**[101,102]**
Micreos Food Safety (Wageningen, Netherlands)	PhageGuard S™	*Salmonella spp.*	FDA, GRN 468; FSANZ; Swiss BAG; Israel Ministry of Health; Health Canada	**[103,104]**
		E.coli O157:H7	FDA, GRN 757(Pending)	
Passport Food Safety Solutions (West Des Moines, IA, USA)	Finalyse®	*E. coli O157:H7*	USDA, FSIS Directive 7120.1	**[105]**
PhageLux (Shanghai, China)	SalmoPro®	*Salmonella spp.*	FDA, GRN 603	**[106]**

6. Conclusion

The emergence of antibiotic resistant pathogens is a major threat to public health, especially when these pathogens are present in our food sources. The lack of antibiotic development and the continual rise of antibiotic resistant pathogens makes development of alternative applications essential to the preservation of public health and the prevention of foodborne outbreaks. While phage therapy is highly variable dependent on target pathogen, location of infection, and complexity of the infection, recent studies outlined in this review demonstrate how phages can significantly reduce these pathogens. The reduction of dangerous pathogens such as *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp., and *Clostridium* spp. has a beneficial effect on both livestock and human health. Mitigation of these infections in livestock directly correlates to the safety of the food products that we consume **[3]**. Overall inclusion and further development of phage products not only would increase the safety of food products but also decrease the associated economic burden of foodborne pathogens on the food industry. As expressed throughout this review, poultry production is the industry in which we could first see widespread application of phage biocontrol **[33,55,57-60]**. Unlike some food production processes, the poultry industry utilizes highly integrated production systems that are more amenable to phage therapy. Poultry production systems allows for the flexibility to introduce phages at multiple points in production including feed, water, aerosol sprays, and modified packaging **[55-60]**. Before these treatments are utilized it is key for the installation of a regulatory framework that would allow for the incorporation of phage treatments. Organizations such as the USDA in the United States or the EU in Europe are responsible for this framework.

A potential drawback of phage products is the ability of bacterial species to develop resistance mechanisms against phages **[106,107]**. While resistance development is an

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

obstacle of any treatment method it is important to note that resistance to one phage does not confer resistance to all phages. For this reason, phage cocktails appear to be an ideal administration method for biocontrol **[55,59,63-65]**. Literature also suggests there is a fitness cost associated with the development of phage resistance in bacteria resulting decreased growth of bacteria even without the presence of phage **[108]**. Another aspect of phage therapy that must be considered is the specificity of phages and what this means in terms of intervention design. When considering the host specificity of phage, it may be beneficial to target bacterial species that are genetically homologous. Genetically homologous pathogens are more attractive targets for phage therapy in that there is not a wide variation phenotypical and genotypical factors that must be taken into consideration. Bacterial species such as *E. coli* pose difficulties for phage treatments due to their wide genetic diversity. Consideration of factors such as this will be important in the development of phage intervention protocols as well as regulatory framework. It is important that phage intervention is used responsibly to prolong the efficacy of this method and avoid the issues that we now face with modern day antibiotic intervention methods. Potential methods that could circumvent issues associated with antibiotic treatments would be regulatory rotation or reformulation of phages used in intervention methods to retain efficacy and avoid resistance. Overall, the future of phages in the food production industry is promising and quickly approaching.

References

1. Gildea, L., Ayariga, J.A., Ajayi, O.S., Xu, J., Villafane, R. and Samuel-Foo, M., 2022. Cannabis sativa CBD Extract Shows Promising Antibacterial Activity against Salmonella typhimurium and S. newington. Molecules, 27(9), p.2669.
2. [1] Ayariga, J.A., Abugri, D., Amrutha, B. and Villafane, R., 2022. Capsaicin potently blocks Salmonella typhimurium invasion of Vero cells. bioRxiv.
3. Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M., Roy, S. L., Griffin, P. M. (2011). Foodborne Illness Acquired in the United States—*Major Pathogens. Emerging Infectious Diseases*, 17(1), 7-15. https://doi.org/10.3201/eid1701.p11101.
4. Møretrø, T., & Langsrud, S. (2004). Listeria monocytogenes: Biofilm formation and persistence in food-processing environments. *Biofilms*, 1(2), 107-121. https://doi.org/10.1017/S1479050504001322
5. Bridier, A., Sanchez-Vizuete, P., Guilbaud, M., et al. (2015). Biofilm-associated persistence of food-borne pathogens. *Food Microbiology*, 45(B), 167-178. https://doi.org/10.1016/j.fm.2014.04.015
6. Newell, D.G., Koopmans, M., Verhoef, L., et al. (2010). Food-borne diseases — The challenges of 20years ago still persist while new ones continue to emerge, *International Journal of Food Microbiology*, Volume 139, Supplement, 2010, Pages S3-S15, https://doi.org/10.1016/j.ijfoodmicro.2010.01.021
7. Reganold, J.P.; Wachter, J.M. Organic agriculture in the twenty-first century. *Nat. Plants* 2016, 2, 15221. http://dx.doi.org/10.1038/nplants.2015.221
8. Woods, T.; Ernst, M.; Tropp, D. Community Supported Agriculture–New Models for Changing Markets; U.S. Department of Agriculture, Agricultural Marketing Service: Washington, DC, USA, 2017. https://www.ams.usda.gov/sites/default/files/media/CSANewModelsforChangingMarketsb.pdf
9. Delves-Broughton, J., Blackburn, P., Evans, R.J. et al. Applications of the bacteriocin, nisin. *Antonie van Leeuwenhoek* 69, 193–202 (1996). https://doi.org/10.1007/BF00399424
10. Hurst, A. 1981. Nisin. *Adv. Appl. Microbiol.* 27:85-123. https://doi.org/10.1016/S0065-2164(08)70342-3
11. Zimmer M, Scherer S, Loessner MJ. Genomic analysis of *Clostridium perfringens* bacteriophage phi3626, which integrates into guaA and possibly affects sporulation. J Bacteriol. 2002;184(16):4359–68. http://www.ncbi.nlm.nih.gov/pubmed/12142405
12. Zimmer M, Vukov N, Scherer S, Loessner MJ. The murein hydrolase of the bacteriophage phi3626 dual lysis system is active against all tested *Clostridium perfringens* strains. Appl Environ Microbiol. 2002;68(11):5311–7. http://www.ncbi.nlm.nih.gov/pubmed/12406719
13. Gaeng, S., S. Scherer, H. Neve, and M.J. Loessner. 2002. Gene cloning and expression and secretion of *Listeria monocytogenes* bacteriophage-lytic enzymes in *Lactococcus lactis*. *Appl. Environ. Microbiol.* 66:2951-2958. https://doi.org/10.1128/aem.66.7.2951-2958.2000
14. Maura, D. and Debarbieux, L., 2011. Bacteriophages as twenty-first century antibacterial tools for food and medicine. Applied microbiology and biotechnology, 90(3), pp.851-859
15. Ayariga, J.A.; Gildea, L.; Villafane, R. Ɛ34 phage tailspike protein is resistant to trypsin and inhibits Salmonella biofilm formation. Enliven Microb. Microb. Tech. 2022, 9, 002.
16. Ayariga, J.A.; Gildea, L.; Wu, H.; Villafane, R. The Ɛ34 phage tailspike protein: An in vitro characterization, structure prediction, potential interaction with S. newington LPS and cytotoxicity assessment to animal cell line. bioRxiv 2021 https://doi.org/10.1101/2021.09.20.461090.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

17. Gildea, L.; Ayariga, J.A.; Robertson, B.K.; Villafane, R. P22 phage shows promising antibacterial activity under pathophysiological conditions. Arch. Microbiol. Immunol. 2022, 6, 81–100.
18. Joseph A. Ayariga, Karthikeya Venkatesan, Robert Ward, Hongzuan Wu, Doba Jackson, and Robert Villafane (2018) Initiation of P22 Infection at the Phage Centennial, Frontiers in Science, Technology, Engineering and Mathematics, Volume 2, Issue 2, 64-81
19. Gildea, L.; Ayariga, J.; Abugri, J.; Villafane, R. Phage Therapy: A Potential Novel Therapeutic Treatment of Methicillin-Resistant Staphylococcus aureus. Preprints 2021, 2021110397 (doi: 10.20944/preprints202111.0397.v1)
20. Williams, J., Venkatesan, K., Ayariga, J.A., Jackson, D., Wu, H. and Villafane, R., 2018. A genetic analysis of an important hydrophobic interaction at the P22 N-terminal domain. Archives of virology, 163(6), pp.1623-1633.
21. Ayariga, J.A. and Villafane, R., 2021. Single Amino Acid Change Mutation in the Hydrophobic Core of the N-terminal Domain of P22 TSP affects the Proteins Stability. bioRxiv.
22. Ayariga JA, Gildea L, Villafane R. Extended ε34 Phage TSP Renatures after Urea-Acid Unfolding. Enliven: Microb Microbial Tech. 2022; 9(1): 001.
23. Atia, A.J., Azumah, A.D., Deepa, B. and Dean, D., 2021. Tuning Phage for Cartilage Regeneration. In Bacteriophages. IntechOpen.
24. Divya Ganeshan, S.; Hosseinidoust, Z. Phage Therapy with a Focus on the Human Microbiota. *Antibiotics* **2019**, *8*, 131. https://doi.org/10.3390/antibiotics8030131
25. Clokie, M. R., Millard, A. D., Letarov, A. V., & Heaphy, S. (2011). Phages in nature. *Bacteriophage*, *1*(1), 31–45. https://doi.org/10.4161/bact.1.1.14942
26. Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World journal of gastrointestinal pharmacology and therapeutics*, *8*(3), 162–173. https://doi.org/10.4292/wjgpt.v8.i3.162
27. Cristobal-Cueto, P.; García-Quintanilla, A.; Esteban, J.; García-Quintanilla, M. Phages in Food Industry Biocontrol and Bioremediation. *Antibiotics* **2021**, *10*, 786. https://doi.org/10.3390/antibiotics10070786
28. Lawrence D. Goodridge & Bledar Bisha (2011) Phage-based biocontrol strategies to reduce foodborne pathogens in foods, *Bacteriophage*, 1:3, 130-137, DOI: 10.4161/bact.1.3.17629
29. Vikram, A.; Woolston, J.; Sulakvelidze, A. Phage Biocontrol Applications in Food Production and Processing. *Curr. Issues Mol. Biol.* **2021**, *40*, 267-302. https://doi.org/10.21775/cimb.040.267
30. Sillankorva, S.M., Oliveira, H., Azeredo, J. (2012). Bacteriophages and Their Role in Food Safety. International Journal of Microbiology. https://doi.org/10.1155/2012/863945
31. Coffey, B., Mills, S., Coffey, A., McAuliffe, O., Ross, R.P. (2010). Phage and Their Lysins as Biocontrol Agents for Food Safety Applications. *Annual Review of Food Science and Technology*, 1:1, 449-468. https://doi.org/10.1146/annurev.food.102308.124046
32. Yang, S., Sadekuzzaman, M., Ha, S.D. (2017). Reduction of Listeria monocytogenes on chicken breasts by combined treatment with UV-C light and bacteriophage ListShield, *LWT*, Volume 86, Pages 193-200, https://doi.org/10.1016/j.lwt.2017.07.060.
33. Waseh S, Hanifi-Moghaddam P, Coleman R, Masotti M, Ryan S, et al. (2010) Orally Administered P22 Phage Tailspike Protein Reduces *Salmonella* Colonization in Chickens: Prospects of a Novel Therapy against Bacterial Infections. PLOS ONE 5(11): e13904. https://doi.org/10.1371/journal.pone.0013904
34. Hudson, J.A., Billington, C., Cornelius, A.J., Wilson, T., On, S.L.W., Premaratne, A., King, N.J. (2013) Use of a bacteriophage to inactivate Escherichia coli O157:H7 on beef. *Food Microbiology*, Volume 36, Issue 1, Pages 14-21, https://doi.org/10.1016/j.fm.2013.03.006
35. Pulido, R.P., Burgos, M.J.G., Gálvez, A., López, R.L. (2016) Application of bacteriophages in post-harvest control of human pathogenic and food spoiling bacteria, Critical Reviews in Biotechnology, 36:5, 851-861, https://doi.org/10.3109/07388551.2015.1049935
36. Kahn, L.H., Bergeron, G., Bourassa, M.W., De Vegt, B., Gill, J., Gomes, F., Malouin, F., Opengart, K., Ritter, G.D., Singer, R.S., Storrs, C. and Topp, E. (2019), From farm management to bacteriophage therapy: strategies to reduce antibiotic use in animal agriculture. Ann. N.Y. Acad. Sci., 1441: 31-39. https://doi.org/10.1111/nyas.14034
37. González Garcia EA. Animal health and foodborne pathogens: enterohaemorrhagic O157:H7 strains and other pathogenic Escherichia coli virotypes (EPEC, ETEC, EIEC, EHEC). Pol J Vet Sci. 2002;5(2):103-15. PMID: 12189946.
38. Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules (Basel, Switzerland)*, *23*(4), 795. https://doi.org/10.3390/molecules23040795
39. Yosef, I., Manor, M., Kiro, R., Qimron, U. (2015). Temperate and Lytic bacteriophages programmed to sensitize and kill antibiotic-resistance bacteria. *PNAS*. 112 (23) 7267-7272. https://doi.org/10.1073/pnas.1500107112
40. Acheson, D., Allos, B.M. (2001). Campylobacter jejuni Infections: Update on Emerging Issues and Trends, *Clinical Infectious Diseases*, Volume 32, Issue 8, 15 April 2001, Pages 1201–1206, https://doi.org/10.1086/319760
41. Andersson, D. I. (2003). Persistence of antibiotic resistant bacteria. *Current Opinion in Microbiology*, *6*(5), 452–456. doi:10.1016/j.mib.2003.09.001.
42. European Food Safety Authority (EFSA). The European Union Summary Report on Trends and Sources of Zoonoses, Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2017. 2018. [Internet]. https://www.ecdc.europa.eu/sites/portal/files/documents/zoonoese-food-borne-outbreaks-surveillance-2017-updated.pdf
43. Hill, D.A. and Artis, D., 2009. Intestinal bacteria and the regulation of immune cell homeostasis. Annual review of immunology, 28, pp.623-667.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

44. Renz, H., Brandtzaeg, P. and Hornef, M., 2012. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nature Reviews Immunology, 12(1), pp.9-23.
45. Sonnenberg, G.F. and Artis, D., 2012. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity, 37(4), pp.601-610.
46. Kamada, N., Chen, G.Y., Inohara, N. and Núñez, G., 2013. Control of pathogens and pathobionts by the gut microbiota. Nature immunology, 14(7), pp.685-690.
47. Lawley, T.D. and Walker, A.W., 2013. Intestinal colonization resistance. Immunology, 138(1), pp.1-11.
48. Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. and Medzhitov, R., 2004. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell, 118(2), pp.229-241.
49. Stappenbeck, T.S., Hooper, L.V. and Gordon, J.I., 2002. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proceedings of the National Academy of Sciences, 99(24), pp.15451-15455.
50. Reinhardt, C., Bergentall, M., Greiner, T.U., Schaffner, F., Östergren-Lundén, G., Petersen, L.C., Ruf, W. and Bäckhed, F., 2012. Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling. Nature, 483(7391), pp.627-631.
51. Bäckhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F. and Gordon, J.I., 2004. The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the national academy of sciences, 101(44), pp.15718-15723.
52. Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F. and Patterson, P.H., 2013. The microbiota modulates gut physiology and behavioral abnormalities associated with autism. Cell, 155(7), p.1451.
53. Winek, K., Engel, O., Koduah, P., Heimesaat, M.M., Fischer, A., Bereswill, S., Dames, C., Kershaw, O., Gruber, A.D., Curato, C. and Oyama, N., 2016. Depletion of cultivatable gut microbiota by broad-spectrum antibiotic pretreatment worsens outcome after murine stroke. Stroke, 47(5), pp.1354-1363.
54. Winek, K., Engel, O., Koduah, P., Heimesaat, M.M., Fischer, A., Bereswill, S., Dames, C., Kershaw, O., Gruber, A.D., Curato, C. and Oyama, N., 2016. Depletion of cultivatable gut microbiota by broad-spectrum antibiotic pretreatment worsens outcome after murine stroke. Stroke, 47(5), pp.1354-1363.
55. Atterbury, R.J.; Connerton, P.L.; Dodd, C.E.; Rees, C.E.; Connerton, I.F. Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of Campylobacter jejuni. Appl. Environ. Microbiol. 2003, 69, 6302–6306. http://dx.doi.org/10.1128/AEM.69.10.6302-6306.2003
56. CDC. "Salmonella Homepage." Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 18 Nov. 2021, https://www.cdc.gov/salmonella/index.html
57. 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), 504–521. https://doi.org/10.14202/vetworld.2019.504-521
58. Lim, T., Kim, M., Lee, D., Lee, Y., Park, J., Youn, H., Lee, H.J., Yang, S., Cho, Y., Lee, J., Park, S., Choi, I., Song, C. (2012). Use of bacteriophage for biological control of Salmonella Enteritidis infection in chicken, *Research in Veterinary Science*, Volume 93, Issue 3, Pages 1173-1178, https://doi.org/10.1016/j.rvsc.2012.06.004.
59. C. Borie, I. Albala, P. Sànchez, M. L. Sánchez, S. Ramírez, C. Navarro, M. A. Morales, J. Retamales, J. Robeson; Bacteriophage Treatment Reduces *Salmonella* Colonization of Infected Chickens. *Avian Dis* 1 March 2008; 52 (1): 64–67. doi: https://doi.org/10.1637/8091-082007-Reg
60. Ahmadi M., Torshizi M.A.K., 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, Volume 7, https://doi.org/10.3389/fmicb.2016.01253
61. CDC. "Reports of Selected E. Coli Outbreak Investigations." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 15 Nov. 2021, https://www.cdc.gov/ecoli/outbreaks.html
62. Janßen, T., Schwarz, C., Preikschat, P., Voss, M., Philipp, H.C., Wieler, L.H. (2001). Virulence-associated genes in avian pathogenic Escherichia coli (APEC) isolated from internal organs of poultry having died from colibacillosis, *International Journal of Medical Microbiology*, Volume 291, Issue 5, Pages 371-378, ISSN 1438-4221, https://doi.org/10.1078/1438-4221-00143
63. Huff WE, Huff GR, Rath NC, Balog JM, Xie H, Moore PA, et al. Prevention of *Escherichia coli* respiratory infection in broiler chickens with bacteriophage (SPR02). Poult Sci. 2002;81(4):437-41. http://www.ncbi.nlm.nih.gov/pubmed/11998827
64. Oliveira A, Sereno R, Azeredo J. *In vivo* efficiency evaluation of a phage cocktail in controlling severe colibacillosis in confined conditions and experimental poultry houses. Vet Microbiol. 2010;146(3-4):303–8. http://www.ncbi.nlm.nih.gov/pubmed/20605377
65. El-Gohary FA, Huff WE, Huff GR, Rath NC, Zhou ZY, Donoghue AM. Environmental augmentation with bacteriophage prevents colibacillosis in broiler chickens1. Poult Sci. 2014;93(11):2788–92. http://www.ncbi.nlm.nih.gov/pubmed/25214555
66. Huff W, Huff G, Rath N, Balog J, Donoghue A. Evaluation of aerosol spray and intramuscular injection of bacteriophage to treat an *Escherichia coli* respiratory infection. Poult Sci. 2003;82(7):1108–12. http://www.ncbi.nlm.nih.gov/pubmed/12872966
67. Barrow P, Lovell M, Berchieri A. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. Clin Diagn Lab Immunol. 1998;5(3):294–8. http://www.ncbi.nlm.nih.gov/pubmed/9605979
68. Altekruse, S. F., Stern, N. J., Fields, P. I., & Swerdlow, D. L. (1999). Campylobacter jejuni—An Emerging Foodborne Pathogen. *Emerging Infectious Diseases*, *5*(1), 28-35. https://doi.org/10.3201/eid0501.990104

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

69. Black RE, Levine MM, Clements ML, Hughes TP, Blaser MJ, Black RE. Experimental *Campylobacter jejuni* infection in humans. J Infect Dis. 1988;157(3):472-9. https://doi.org/10.1093/infdis/157.3.472.
70. Food Standards Agency (FSA). Antimicrobial resistance in Campylobacter jejuni and Campylobacter coli from retail chilled chicken in the UK (Year 3: 2016–17) [Internet]. 2018. https://www.food.gov.uk/sites/default/files/media/document/antimicrobial-resistance-in-campylobacter-jejuni-and-campylobacter-coli-from-retail-chilled-chicken-in-the-uk-year-3-2016-17.pdf.
71. Jäckel C, Hammerl J, Hertwig S. *Campylobacter* phage isolation and characterization: what we have learned so far. Methods Protoc. 2019;2(1):18. http://www.mdpi.com/2409-9279/2/1/18.
72. Coward C, Grant AJ, Swift C, Philp J, Towler R, Heydarian M, et al. Phase-variable surface structures are required for infection of Campylobacter jejuni by bacteriophages. *Appl Environ Microbiol*. 2006;72(7):4638–47. https://doi.org/10.1128/AEM.00184-06
73. Sørensen MCH, van Alphen LB, Harboe A, Li J, Christensen BB, Szymanski CM, et al. Bacteriophage F336 recognizes the capsular phosphoramidate modification of *Campylobacter jejuni* NCTC11168. J Bacteriol. 2011;193(23):6742–9. https://doi.org/10.1128/JB.05276-11
74. Sørensen MCH, van Alphen LB, Fodor C, Crowley SM, Christensen BB, Szymanski CM, et al. Phase Variable Expression of Capsular Polysaccharide Modifications Allows *Campylobacter jejuni* to Avoid Bacteriophage Infection in Chickens. Front Cell Infect Microbiol. 2:11 https://doi.org/10.3389/fcimb.2012.00011
75. Hammerl JA, Jäckel C, Alter T, Janzcyk P, Stingl K, Knüver MT, et al. Reduction of *Campylobacter jejuni* in Broiler Chicken by Successive Application of Group II and Group III Phages. PLoS One. 2014;9(12):e114785. https://dx.plos.org/10.1371/journal.pone.0114785
76. Carvalho CM, Gannon BW, Halfhide DE, Santos SB, Hayes CM, Roe JM, et al. The *in vivo* efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens. *BMC Microbiol*. 10(1): 232 http://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-10-232
77. Cooper KK, Songer JG, Uzal FA. Diagnosing clostridial enteric disease in poultry. J Vet Diagn Invest. 2013 May;25(3):314-27. doi: 10.1177/1040638713483468. Epub 2013 Apr 9. PMID: 23572451
78. Skinner, J.T., Bauer, S., Young, V., Pauling, G., Wilson, J. (2010). An Economic Analysis of the Impact of Subclinical (Mild) Necrotic Enteritis in Broiler Chickens. *Avian Dis* 1 December 2010; 54 (4): 1237–1240. doi: https://doi.org/10.1637/9399-052110-Reg.1
79. Fischetti VA. Bacteriophage endolysins: A novel anti-infective to control Gram-positive pathogens. Int J Med Microbiol. 2010; 300:357–62. https://doi.org/10.1016/j.ijmm.2010.04.002
80. Wernicki A, Nowaczek A, Urban-Chmiel R. Bacteriophage therapy to combat bacterial infections in poultry. Virol J. 2017;14(1):179. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28915819
81. Gervasi T, Horn N, Wegmann U, Dugo G, Narbad A, Mayer MJ. Expression and delivery of an endolysin to combat *Clostridium perfringens*. Appl Microbiol Biotechnol. 2014;98(6):2495–505. http://www.ncbi.nlm.nih.gov/pubmed/23942878
82. Miller, R.W., Skinner, J., Sulakvelidze, A., Mathis, G.F., Hofacre, C.L. (2010). Bacteriophage Therapy for Control of Necrotic Enteritis of Broiler Chickens Experimentally Infected with *Clostridium perfringens*. *Avian Dis* 1 March 2010; 54 (1): 33–40. doi: https://doi.org/10.1637/8953-060509-Reg.1
83. Guenther, S.; Herzig, O.; Fieseler, L.; Klumpp, J.; Loessner, M.J. Biocontrol of Salmonella Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. Int. J. Food Microbiol. 2012, 154, 66–72. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.12.023
84. Kang, H.W.; Kim, J.W.; Jung, T.S.; Woo, G.J. wksl3, a new biocontrol agent for Salmonella enterica serovars Enteritidis and Typhimurium in foods: Characterization, application, sequence analysis, and oral acute toxicity study. Appl. Environ. Microbiol. 2013, 79, 1956–1968. http://dx.doi.org/10.1128/AEM.02793-12
85. Soni, K.A.; Desai, M.; Oladunjoye, A.; Skrobot, F.; Nannapaneni, R. Reduction of Listeria monocytogenes in queso fresco cheese by a combination of listericidal and listeriostatic GRAS olivhttp://dx.doi.org/10.1016/j.ijfoodmicro.2012.01.010
86. Chibeu, A.; Agius, L.; Gao, A.; Sabour, P.M.; Kropinski, A.M.; Balamurugan, S. Efficacy of bacteriophage LISTEXTM P100 combined with chemical antimicrobials in reducing Listeria monocytogenes in cooked turkey and roast beef. Int. J. Food Microbiol. 2013, 167, 208–214. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.08.018
87. Oliveira, M.; Viñas, I.; Colàs, P.; Anguera, M.; Usall, J.; Abadias, M. Effectiveness of a bacteriophage in reducing Listeria monocytogenes on fresh-cut fruits and fruit juices. Food Microbiol. 2014, 38, 137–142. http://dx.doi.org/10.1016/j.fm.2013.08.018
88. Abuladze, T.; Li, M.; Menetrez, M.Y.; Dean, T.; Senecal, A.; Sulakvelidze, A. Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by Escherichia coli O157:H7. Appl. Environ. Microbiol. 2008, 74, 6230–6238. http://dx.doi.org/10.1128/AEM.01465-08
89. Leverentz, B.; Conway, W.S.; Janisiewicz, W.; Camp, M.J. Optimizing concentration and timing of a phage spray application to reduce Listeria monocytogenes on honeydew melon tissue. J. Food Prot. 2004, 67, 1682–1686. http://dx.doi.org/10.4315/0362-028X-67.8.1682
90. Soffer, N.; Woolston, J.; Li, M.; Das, C.; Sulakvelidze, A. Bacteriophage preparation lytic for Shigella significantly reduces Shigella sonnei contamination in various foods. PLoS ONE 2017, 12, e0175256. http://dx.doi.org/10.1371/journal.pone.0175256
91. Álvarez-Astorga, M., Capita, R., Alonso-Calleja, C., Moreno, B., Del, M., García-Fernández, C. (2002) Microbiological quality of retail chicken by-products in Spain, Meat Science, Volume 62, Issue 1, Pages 45-50, https://doi.org/10.1016/S0309-1740(01)00225-X
92. US Food and Drug Administration Center for Food Safety and Applied Nutrition (FDA). Quantitative Assessment of Relative Risk to Public Health from Foodborne Listeria Monocytogenes Among Selected Categories of Ready-to-Eat Foods; US Food and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 April 2022 doi:10.20944/preprints202204.0309.v1

Drug Administration Center for Food Safety and Applied Nutrition: College Park, MD, USA, 2003. https://www.fda.gov/downloads/food/scienceresearch/researchareas/riskassessmentsafetyassessment/ucm197330.pdf
93. Pirnay, JP., De Vos, D., Verbeken, G. et al. The Phage Therapy Paradigm. *Pharm Res* 28, 934–937 (2011). https://doi.org/10.1007/s11095-010-0313-5
94. Viator C.L., Muth M.K., Brophy J.E. Costs of Food Safety Investments. RTI International; Research Triangle Park, NC, USA: 2015. [(accessed on 19 March 2018)]. Report. https://www.fsis.usda.gov/wps/wcm/connect/0cdc568e-f6b1-45dc-88f1-45f343ed0bcd/Food-Safety-Costs.pdf?MOD=AJPERES
95. Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage Applications for Food Production and Processing. Viruses, 10(4), 205. https://doi.org/10.3390/v10040205
96. Sharma M., Patel J.R., Conway W.S., Ferguson S., Sulakvelidze A. Effectiveness of bacteriophages in reducing Escherichia coli O157:H7 on fresh-cut cantaloupes and lettuce. J. Food Prot. 2009;72:1481–1485. doi: 10.4315/0362-028X-72.7.1481
97. Carter C.D., Parks A., Abuladze T., Li M., Woolston J., Magnone J., Senecal A., Kropinski A.M., Sulakvelidze A. Bacteriophage cocktail significantly reduces Escherichia coli O157:H7 contamination of lettuce and beef, but does not protect against recontamination. Bacteriophage. 2012;2:178–185. doi: 10.4161/bact.22825
98. Boyacioglu, O., Sharma, M., Sulakvelidze, A., Goktepe, I. Biocontrol of Escherichia coli O157:H7 on fresh-cut leafy greens. Bacteriophage. 2013;3:e24620. doi: 10.4161/bact.24620
99. Ferguson S., Roberts C., Handy E., Sharma M. Lytic bacteriophages reduce Escherichia coli O157:H7 on fresh cut lettuce introduced through cross-contamination. Bacteriophage. 2013;3:e24323. doi: 10.4161/bact.24323
100. Magnone J.P., Marek P.J., Sulakvelidze A., Senecal A.G. Additive approach for inactivation of Escherichia coli O157:H7, Salmonella, and Shigella spp. on contaminated fresh fruits and vegetables using bacteriophage cocktail and produce wash. J. Food Prot. 2013; 76:1336–1341. doi: 10.4315/0362-028X.JFP-12-517
101. Sukumaran A.T., Nannapaneni R., Kiess A., Sharma C.S. Reduction of Salmonella on chicken meat and chicken skin by combined or sequential application of lytic bacteriophage with chemical antimicrobials. Int. J. Food Microbiol. 2015;207:8–15. doi: 10.1016/j.ijfoodmicro.2015.04.025.
102. Sukumaran A.T., Nannapaneni R., Kiess A., Sharma C.S. Reduction of Salmonella on chicken breast fillets stored under aerobic or modified atmosphere packaging by the application of lytic bacteriophage preparation SalmoFreshTM. Poult. Sci. 2016;95:668–675. doi: 10.3382/ps/pev332
103. Grant A., Parveen S., Schwarz J., Hashem F., Vimini B. Reduction of Salmonella in ground chicken using a bacteriophage. Poult. Sci. 2017;96:2845–2852. doi: 10.3382/ps/pex062
104. Yeh Y., de Moura F.H., Van Den Broek K., de Mello A.S. Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground beef. Meat Sci. 2018;139:44–48. doi: 10.1016/j.meatsci.2018.01.007
105. Arthur, T.M., Kalchayanand K., Norasak, A., Getahun, E., Tommy L., and Koohmaraie, M. (2017) Foodborne Pathogens and Disease. Jan 2017.17-22.http://doi.org/10.1089/fpd.2016.2189
106. Thanki, A.M., Hooton, S., Gigante, A.M., Atterbury, R.J., Clokie, M.R.J. Potential Roles for Bacteriophages in Reducing *Salmonella* from Poultry and Swine. InTech Open. March 2021. DOI: 10.5772/intechopen.96984
107. Anni-Maria Örmälä & Matti Jalasvuori (2013) Phage therapy, Bacteriophage, 3:1, DOI: 10.4161/bact.24219
108. Mihnea, M.R., Duerkop, B.A. (2020). Fitness trade-offs resulting from bacteriophage resistance potentiate synergistic antibacterial strategies. Infection and Immunity. 88 (7). https://doi.org/10.1128/IAI.00926-19