Applications of *Salmonella* bacteriophages in the food production chain

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Salmonella outbreaks have been linked to various food products such as dairy, eggs, poultry, and fresh produce. Food contamination with *Salmonella* can occur at any stage along the food production chain. Phage applications have gained considerable interest for use as biocontrol agents against foodborne pathogens, including *Salmonella*. This review provides information to highlight recent advances and intervention studies on using *Salmonella* phages for controlling *Salmonella* in various stages of food production. Phages can be potential means to eliminate and control *Salmonella* starting from primary production, in postharvest foods (as antimicrobial food additives), and on food processing facilities (as biosanitizing agents). This control measure, if effectively and suitably implemented, will contribute to reduction of *Salmonella* contamination in the food production chain resulting in lower incidence of *Salmonella* outbreaks worldwide.

Keywords: food production chain, phage-based applications; biocontrols; *Salmonella* phage; *Salmonella*

1. Introduction

The World Health Organization (WHO) asserts that food- and water-borne diarrhoeal illnesses lead to 2.2 million deaths globally each year [1]. Diseases linked to consumption of contaminated food with pathogens thus are of importance for development of effective control measures to reduce the incidence of foodborne illnesses and outbreaks. The US Centers for Disease Control and Prevention (CDC) reported estimates of foodborne illness in the US, which indicated 48 million cases, resulting in 127,839 cases of hospitalization and 3,037 deaths each year [2]. Among these cases, *Salmonella* is the leading cause of hospitalization (19,336) and is the top pathogen responsible for the most foodborne deaths (378). *Salmonella* can cause salmonellosis which is common and widely distributed foodborne diseases in humans. In addition, the gastrointestinal tract of animals is well-known as the major reservoir of *Salmonella* spp., and most *Salmonella* contamination in foods can be linked to those of animal origin [3]. Illnesses and outbreaks involving *Salmonella* have commonly been linked to various sources, including animal- and plant-based raw materials and food products [4, 5]. Common sources include poultry, beef, eggs, fresh-cut produce, and sprouts [5, 6]. According to the Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA), 75% of the annual cases of human salmonellosis is due to the consumption of contaminated poultry, beef, and egg products [7]. *Salmonella* can be transmitted into foods at different stages of the food production chain (from farms to consumers). For example, *Salmonella* can be shed with animal feces involved in primary production, which can lead to contamination in various foods, including animal meat and fresh produce at this stage and/or other steps of production. *Salmonella*-contaminated feces can spread in the farm or field environments and/or water sources, which can then transmit to foods at any point such as crop, farm, livestock feed, food manufacturing, processing, and retailing [8].

Currently, a number of control strategies have been established to reduce *Salmonella*, starting at bio-security to manage farm hygiene, feed, water, and rodents [9]. The EU has extensively focused on the reduction of *Salmonella* levels in poultry by, for example, carcass treatment to reduce *Salmonella* contamination [9, 10]. Effective strategies for controlling and reducing *Salmonella* are considerably important for food safety. Applications of bacteriophages have gained interest and become an alternative approach for preventing or treating bacterial diseases [11, 12], monitoring and detecting bacterial pathogens [13–15], and improving food safety [16–18]. Phages are host-specific as indicated by their ability to infect and kill specific genus or species of a bacterial pathogen. Phages have mechanisms to kill bacterial host cells via the insertion of the phage DNA and multiplication of the phage progeny inside the host. Each bacterial cell will burst after the population of phages comes out from the host cell. Therefore, phages act as a special and natural antimicrobial agent against bacterial pathogens while representing harmless entities to humans and animals [19].

Phage-based biocontrol agents have emerged as a promising tool for controlling a wide range of pathogens in a variety of food safety applications, including food products and food processing environments [10, 20, 21]. A number of commercial phage products (cocktails or individual phages) have been previously granted GRAS status (Generally Recognized As Safe), and are currently available for use against several foodborne pathogens [22]. In 2006, a phage preparation ListShield™ (LMP-102™), manufactured by Intralytix Inc., received approval for use as an antimicrobial food additive to control *Listeria monocytogenes* in ready-to-eat meat and poultry products [22]. In 2007, the FDA (U.S. Food and Drug Administration) approved the use of phage-based preparation (manufactured by OmniLytics Inc.) against *E. coli* and *Salmonella*. Another commercial phage preparation called SalmoFresh™, manufactured by Intralytix Inc., recently received regulatory approval in 2013 for use in eliminating *Salmonella* in poultry products and other foods. This product claims to have the ability to control particular strains of common *Salmonella enterica* serovars, including Typhimurium, Enteritidis, Heidelberg, Newport, Hadar, Kentucky, Thompson,

Georgia, Agona, Grampian, Senftenberg, Alachua, Infantis, Reading, and Schwarzengrund [23]. The USDA also issued a no objection letter for the use of phage preparation, Armament (manufactured by OmniLytics Inc.) for use to control *Salmonella* on poultry [21]. In addition, Biotector®, developed by CJ CheilJedang (http://www.cj.co.kr/cj-kr/, Seoul, South Korea), is the first phage-based product to replace antibiotics in animal feed. This phage preparation is targeting *Salmonella* spp. that can cause fowl typhoid and pullorum disease. This review highlights recent advances and intervention studies in using phage-based biocontrol agents for controlling *Salmonella* in various stages of the food production chain. Phage-based strategies can be applied to reduce *Salmonella* contamination, starting from primary production (preharvest), by decontamination of livestock (food-producing animals) or produce seeds, to postharvest or processing. Phage treatments in the postharvest or processing steps include the use of phages as antimicrobial food additives and sanitizing agents for various surfaces in food processing facilities.

2. Applications of a phage-based biocontrol agent against *Salmonella*

2.1 Control of *Salmonella* in primary production (preharvest)

Farms associated with food-producing animals, e.g., poultry, swine, cattle, and sheep, are common reservoirs of *Salmonella*. Major serovars of *Salmonella* associated with animal farms include Enteritidis, Typhimurium, Kentucky, and 4,5,12:i:– [24]. For primary production, controlling *Salmonella* loads at this initial stage can minimize the risk of *Salmonella* contamination in the later stages of food production. The potential uses of bacteriophages for controlling *Salmonella* at the farm level have been studied by several approaches. For example, use of phages to prevent or reduce colonization of domesticated livestock with *Salmonella* was investigated using poultry and swine *in-vivo* models [25-28]. Models involved sprout seeds or fresh produce were also studied for the potential benefit of *Salmonella* phages [29, 30]. In addition, phages have been evaluated for effectiveness against *Salmonella* in the application to decontaminate surfaces that are involved in food processing [31].

For the studies of *Salmonella* phages using poultry *in-vivo* models, Atterbury et al. (2007) [32] showed the phage efficacy against *Salmonella* in broilers. Three phages representing broad host range were individually used to treat broilers that were colonized with specific *Salmonella* strains at 38 days of age. Phage Φ151 reduced more than 4.2 log CFU of *Salmonella* Enteritidis within 24 h compared with the control. Phage Φ10 reduced more than 2.19 log CFU of *Salmonella* Typhimurium. However, phage Φ25 was ineffective at reducing *Salmonella* Hadar colonization. Selection of appropriate phages, optimization of timing and delivery methods are important for effective phage-based control strategies. Another study [33] reported the ability of phages in reducing *Salmonella* Enteritidis in infected chicks (3×10^3 CFU/bird) after treatment cloacally with 1×10^9 PFU/bird (phage WT45Φ). A phage mixture, consisting of 1×10^8 PFU (phage CB4Φ) and 1.2×10^8 PFU (phage WT45Φ) was given via oral gavage to these chicks. All treatments showed that *Salmonella* Enteritidis levels were significantly reduced from cecal tonsils at 24 h as compared with the untreated controls. Borie et al. (2008) [34] tested a *Salmonella* phage cocktail (combination of three phages, BP1, BP2, and BP3), in a form of aerosol spray or drinking water, with 10-day-old chickens infected with *Salmonella* Enteritidis. Chickens were treated with the phage cocktail, at the MOI (multiplicity of infection) of 10^3, at 24 h prior to challenging with *Salmonella* Enteritidis (9.6×10^5 CFU/ml). The phage cocktail could reduce the incidence of *Salmonella* infection in chickens over a 20-day period to 72.7% compared to the control group (100%). In addition, both phage delivery methods could reduce the intestinal colonization of *Salmonella* Enteritidis. This study suggests that phage treatment, either by aerosol spray or drinking water, may be a potential alternative to antibiotics for the reduction of *Salmonella* infection in poultry.

Previous reports have shown that the overuse of antibiotics in farm animals can further generate a global issue associated with the increasing emergence of antibiotic-resistant foodborne pathogens. Use of phage-based biocontrol-agent as an alternative has recently gained more interest. To treat infected chickens, Lim et al. (2012) [27] used bacteriophage (ΦCJ07), isolated from sewage effluent, mixed with feed as a feed additive at three concentrations (10^5, 10^7 and 10^9 PFU/g). Different concentrations of the feed additive were given to one-day-old chicks infected with 5×10^7 CFU/bird of *Salmonella* Enteritidis for a course of 21 days. After 1, 2, and 3 weeks of treatment, *Salmonella* was reduced by phage treatments at 10^7 and 10^9 PFU/g as compared with untreated controls. As *Salmonella* was reduced in these infected chickens, the horizontal transmission of *Salmonella* to other contact animals or environments could then be prevented from occurring. To test phages as a therapeutic agent for reducing *Salmonella*, a mixture of three *Salmonella* phages, comprising of 10^{11} PFU of each phage, was orally administered to broilers in a study by Fiorentin et al. (2005) [35]. A reduction of *Salmonella* Enteritidis PT4 by 3.5 log units was observed in caeca of broilers after 5 days of treatment. In addition, samples of caecal content collected at 10, 15, 20, and 25 days after treatment showed that broilers still had lower levels of *Salmonella* Enteritidis PT4. Another study by Gonçalves et al. (2014) [28] also orally treated broiler chickens that received 10^7 CFU/ml of *Salmonella* Enteritidis with a phage cocktail. A reduction of *Salmonella* Enteritidis counts was observed at 3 h posttreatment as indicated by the presence of 10^3 CFU/g in cecal suspension. This study suggests a promising approach in using phage therapy to reduce preslaughter loads of *Salmonella*. The reduced loads can allow for a lower likelihood of *Salmonella* contamination in chicken carcasses, poultry, and poultry products.

Phage applications have been studied in swine. Callaway et al. (2011) [25] tested a phage cocktail against *Salmonella* Typhimurium that was inoculated to growing swine by oral gavage at 2×10^{10} CFU/pig. Pigs were treated with a phage cocktail (3×10^{9} PFU) at 24 h and 48 h. Reduction of *Salmonella* was monitored in fecal samples every 24 h for 96-h period. To evaluate the efficiency of the phage suspension in the intestine and rectum of the pigs, pigs were euthanized after 96 h. A reduction in *Salmonella* Typhimurium levels in both cecal and rectal contents was observed, but a greater reduction ($p < 0.05$) was observed in the rectal contents. Phage applications have also been studied on older pigs that were ready for the markets. In a study by Wall et al. (2010) [36], a phage cocktail (15 ml; 10^{9} PFU/ml) was given via oral gavage to pigs (250 lbs) that had previously received *Salmonella* Typhimurium at 5×10^{9} CFU/pig. Treatment with a phage cocktail reduced *Salmonella* levels in both contents, 95% in cecal content ($p < 0.05$) and 90% ileal content ($p = 0.06$). Overall, a phage mixture could effectively reduce *Salmonella* levels in ileal, cecal and tonsil.

In addition, phages were applied to treatments not only as a liquid suspension form, but also as other alternative forms, e.g., microencapsulated phages. Several studies have optimized the conditions for microencapsulated form of phages, including *Salmonella* phages [37, 38]. Microencapsulated phages were developed to prevent phages from harsh conditions such as acid in order to increase the efficacy against *Salmonella* [26, 37]. A study by Saez et al. (2011) [26] developed a microencapsulated phage mixture to control *Salmonella* Typhimurium. This study compared the efficacy of microencapsulated phages between the two delivery methods, mixing with the feed before administration and giving directly via oral gavage. Results showed that pigs fed with the microencapsulated phages directly for 5 days shed less *Salmonella* Typhimurium compared to the gavage and control groups. After 6 h posttreatment, *Salmonella* Typhimurium counts in ileal (2 log CFU/ml) and cecal (2.7 log CFU/ml) were lower in the feed pig group than ileal (3 log CFU/ml) and cecal (3.7 log CFU/ml) in the control group. Microencapsulation is another technique that can deliver effective phages for the reduction of *Salmonella* colonization and shedding in pigs. Minimizing the colonization and shedding of *Salmonella* in pigs by phage applications is promising approach which can further reduce the incidence of *Salmonella* contamination in primary production and other food-associated environments throughout the food chain.

Previous reports have shown a frequent occurrence of *Salmonella* contamination in sprouts and some leafy greens [4, 5]. Outbreaks linked to *Salmonella* contamination in fruits and vegetables, e.g., tomatoes, alfalfa sprouts, and cantaloupe, have been reported [6]. Control of *Salmonella* is thus crucial for the global fruit and vegetable industry. In primary production of fruits and vegetables, phages exhibit a promising alternative to eliminate *Salmonella* contamination in seeds and field environments. Several studies have evaluated use of *Salmonella* phages in controlling *Salmonella* in seeds to reduce the incidence of contamination in sprouts after seedling [29, 39]. Ye et al., 2010 [29] used a phage cocktail (combination of six *Salmonella* phages, F01, P01, P102, P700, P800, and FL41) together with the *Enterobacter asburiae* JX1 to reduce multiple *Salmonella* serovars in mung beans and alfalfa seeds. Beans and seeds were first soaked in a suspension of *Enterobacter asburiae* JX1 (10^{6} CFU/ml) and in a phage cocktail (10^{6} PFU/ml) for 20 min. Combined treatments could inhibit growth of *Salmonella* on mung bean sprouts and alfalfa sprouting seeds. A *Salmonella* phage cocktail in this study could reduce *Salmonella* populations by 6.72 log CFU/g on sprouting mung beans and alfalfa sprouts after 4 days at room temperature. Results suggest an alternative chemical-free approach for controlling *Salmonella* contamination on sprouting seeds. In other types of plant seeds, Pao et al. (2004) [40] evaluated two *Salmonella* phages for controlling *Salmonella* Typhimurium, Enteritidis, and Montevideo in inoculated mustard and broccoli seeds. *Salmonella* counts were increased in all inoculated seeds during soaking. However, mustard seeds showed greater growth of the inoculated *Salmonella* than broccoli seeds. Among the phages tested, phage-A could reduce *Salmonella* counts by 1.37 log units on mustard seeds, while the mixture of phage-A and phage-B could reduce *Salmonella* counts by 1.50 log units in the soaking water of broccoli seeds. However, in another study by Kocharunchitt et al. (2009) [39], two *Salmonella* phages (SSP5 and SSP6) were tested for the ability to control *Salmonella* Oranienburg artificially contaminated in alfalfa seeds (10^{7} CFU/ml, for 1 h). After treatment with a phage solution at MOI of 70 (12 h at 25°C), seeds were sprouting within 5 days. A reduction of only 1 log unit was observed. Overall, the two phages could not effectively reduce the *Salmonella* populations, suggesting a phage-resistance phenomenon in this study as high levels of background microflora in the seeds may offer alternative phage attachment sites. The efficacy of phage treatments may be varied depending on several factors, e.g., types of foods, phage concentration applied, and levels of *Salmonella* populations present in the foods.

In primary production, manure compost is widely used in produce fields. Contaminated manure compost can lead to pathogen contamination of the fields, fresh produce or runoff, and can then transfer to nearby fields or water sources. Good quality of manure compost is thus essentially required according to the Good Agricultural Practices (GAPs). Phages can be used to control *Salmonella* in manure compost. For example, Heringa et al. (2010) [41] used a mixture of five phages at MOIs of 1, 10, and 50 to treat *Salmonella* Typhimurium on dairy manure compost at different moisture contents (30, 40, 45, and 50%). The results showed a reduction of more than 2 log units within 4 h at all moisture levels compared to the control. This study also reported that reductions in autoclaved compost were less than those observed in non-autoclaved compost. This could be due to the reduction of competitive microorganism in the autoclaved compost.

Phage applications can be used in livestock or food animals to reduce preslaughter loads of *Salmonella* in the animals' intestines that might be shed into the environment as summarized in Table 1. In addition, waste or unwanted materials inside animals' intestines may favor multiplication of *Salmonella*. Upon withdrawal, *Salmonella* can still grow on these materials, leading to a subsequent contamination of carcasses in food processing facilities. Minimizing the shedding of *Salmonella* can thus be crucial for the reduction of *Salmonella* contamination in post-slaughter carcasses, meat or skin during slaughter and carcass processing.

2.2 Control of *Salmonella* in postharvest or processing

Several strategies applying phage-based biocontrol agents have been investigated for controlling *Salmonella* in postharvest or processing. Most studies have shown promising results on the reduction of *Salmonella* in postharvest foods as well as those observed after phage application in primary production. Postharvest foods that have been previously studied to evaluate the efficacy of phages against *Salmonella* include meat carcasses or outer skin, vegetables, and fresh fruit, while processed foods include a variety of ready-to-eat foods as summarized in Table 1.

Table 1 Summary of phage-based biocontrol applications for controlling *Salmonella* at different stages of food production.

Stage of food production – sample	Target *Salmonella* serovar [reference]	*Salmonella* phages applied and application method[a]	Result	Limitation
Primary production (preharvest)				
Livestock (chicken)	*S.* Enteritidis [27]	Phage ΦCJ07 mixed with feed and was given to one-day-old chicks infected with 5×10^7 CFU/bird for 21 days at 3 MOIs* (0.01, 1, and 100).	*Salmonella* counts were not detected in 70% of contact chickens treated with phage at MOI 100 at 3 weeks after treatment.	
Livestock (broilers)	*S.* Enteritidis [35]	A phage mixture of phage CB4Φ (1×10^8 PFU) and phage WT45Φ (1.2×10^8 PFU) was orally administered to chicks.	In all treatments, *S* Enteritidis levels were significantly reduced from cecal tonsils at 24 h compared to the untreated controls.	• Some phages can sporadically survive in the digestive tract. • Some phages are not inactivated or successfully replicated in the digestive tract.
	S. Enteritidis [28]	A phage cocktail (11 phages, 10 ml at 10^9 PFU/ml) was orally treated to broiler chickens that received 10^7 CFU/ml of *S.* Enteritidis	A reduction of *S.* Enteritidis counts was observed at 3 h posttreatment in cecal suspension and crop suspension.	
Livestock (swine)	*S.* Typhimurium [36]	A phage cocktail (15 phages, 15 ml at 10^9 PFU/ml) was given via oral gavage to pigs (250 lbs) that received 5×10^9 CFU/pig of *S.* Typhimurium.	Treatment with a phage cocktail reduced *Salmonella* levels by 95% in cecal content and 90% ileal content.	
	S. Typhimurium [25]	A phage cocktail (3×10^9 PFU) was orally treated to growing swine that received 2×10^{10} CFU/pig of *S.* Enteritidis	A reduction in *S.* Typhimurium levels in both cecal and rectal contents was observed, but a greater reduction ($p < 0.05$) was observed in the rectal contents.	
Sprout seeds (alfalfa seeds)	*S.* Oranienburg [39]	Two *Salmonella* phages (SSP5 and SSP6) were used to treat alfalfa seeds (10^7 CFU/ml) at MOI* 70 (12 h at 25°C).	Only a 1-log CFU/g reduction of *Salmonella* was observed after seeds were sprouting within 5 days.	• Some phages show incomplete lysis against *Salmonella*. • Physiological and/or genetic changes of surviving *Salmonella* cells may prevent phage lysis.
Sprout seeds (mung beans and alfalfa seeds)	Multiple *Salmonella* serovars [42]	A phage cocktail (six phages) was used with *E. asburiae* JX1. Beans and seeds were soaked in suspension of JX1 (10^6 CFU/ml) and in a phage cocktail (10^6 PFU/ml) for 20 min.	A reduction of 6.72 log CFU/g was observed on sprouting mung beans and alfalfa sprouts after 4 days at room temperature.	

Stage of food production – sample	Target *Salmonella* serovar [reference]	*Salmonella* phages applied and application method[a]	Result	Limitation
Manure compost	*S.* Typhimurium [41]	A phage cocktail (five phages) was treated against *S.* Typhimurium at MOIs 1, 10, and 50 on dairy manure compost at different moisture contents (30, 40, 45, and 50%).	A reduction of more than 2 log units within 4 h was observed at all moisture levels compared to the control.	
Postharvest				
Chicken skin	*S.* Enteritidis [43]	A mixture of phages with chemical agents was used on chicken skin with *S.* Enteritidis (10^5 CFU/cm^2).	A reduction of 1 log CFU/cm^2 was observed.	• Several factors, such as phage/host/food interactions, and effects of processing can vary efficacy of phages treatments as biosanitizing agents.
Pig skin	*S.* Typhimurium [44]	A phage cocktail (four phages) was applied on pig skin at MOI 10 or higher at 4°C over 96 h.	At the MOI of at least 10, counts of multidrug-resistant *S.* Typhimurium U288 were reduced to undetectable level.	
	S. Enteritidis and *S.* Typhimurium [30]	A phage cocktail (three phages) was applied on the pig skin by a spraying method at MOI of 4.4×10^4 and left at 33°C for 6 h.	A reduction of greater than 4 and 2 log CFU/cm^2 was observed for *S.* Typhimurium and *S.* Enteritidis, respectively.	
Poultry (chicken breast)	*S.* Enteritidis and *S.* Typhimurium [30]	A phage cocktail (three phages) was used to test with chicken breast by a dipping method at MOI of 10^3.	The chicken breast dipped with the phage cocktail and kept at 4°C for 7 days showed a reduction of 2.2 and 0.9 log CFU/g for *S.* Typhimurium and *S.* Enteritidis, respectively.	
Meat (raw and cooked beef)	*S.* Typhimurium [45]	Phages at MOI of 10^1 or 10^4 were used on raw and cooked beef. Treated meat was stored at 5°C and 24°C.	A reduction of 2 to 3 log CFU/cm^2 at 5°C and more than 5.9 log CFU/cm^2 at 24°C was observed.	
Fresh-cut produce (honeydew melon and apple slices)	*S.* Enteritidis [46]	A phage mixture (four phages) was tested on fresh-cut honeydew melon and apple slices.	A reduction of 3.5 log units when stored at 5°C and 10°C, and 2.5 log units when stored at 20°C was observed on fresh-cut honeydew melon slices. No significant decrease in *Salmonella* counts was observed on apple slices.	• Intrinsic factors such as pH of apple slices (pH 4.2) may have influence on efficacy of the phage mixture against *Salmonella* as phages may be inactivated.
Processing				
Surfaces in food processing facilities	*S.* Kentucky and *S.* Brandenburg [31]	A phage cocktail (six lytic phages) was tested on stainless steel and glass surfaces.	A reduction of 2.1 to 4.3 log units of *Salmonella* counts was observed.	
Processed foods – various ready-to-eat foods and dairy products (hot dogs, sliced turkey breast, seafood, chocolate milk)	*S.* Typhimurium [47]	Phage (FO1-E2) was used to treat foods that were inoculated with 10^3 CFU of *Salmonella* cells and then treated with 3×10^8 PFU/g (MOI* 10^5) for 6 days at 8°C or 15°C.	All phage-treated *Salmonella* cells were completely eliminated in foods at 8°C. At 15°C, a reduction of 5 log units on turkey deli meat, and in chocolate milk was observed. A reduction of 3 log units was observed on hot dogs and in seafood at 15°C.	• Structure and chemical compositions of different food matrices may have influence on efficacy of the phage-based intervention against *Salmonella*.
Processed foods (cheese)	*S.* Enteritidis [48]	Phage SJ2 was applied to pasteurized- and raw-milk cheese.	*Salmonella* was not detected after 89 days of storage at 8°C in pasteurized milk cheese. *Salmonella* counts were 50 CFU/g after 99 days of storage at 8°C in raw milk cheese.	

Stage of food production – sample	Target *Salmonella* serovar [reference]	*Salmonella* phages applied and application method[a]	Result	Limitation
Processed foods – various foods (energy drinks, whole and skimmed milk and apple juice)	*S.* Typhimurium [49]	Phage P22 at 10^8 PFU/ml was used to treat liquid food samples that were spiked with *Salmonella* (initial level of 10^4 CFU/ml) at 4°C.	A reduction of 2 to 4 log units was observed after 48 h at 4°C.	
Processed foods (packaged lettuce)	*S.* Enteritidis and *S.* Typhimurium [30]	A phage cocktail (three phages) was used to test with lettuce by a dipping method at MOI of 10^4 at room temperature.	A reduction of 3.9 log CFU/g for *S.* Typhimurium and 2.2 log CFU/g for *S.* Enteritidis was observed.	

[a] Approximate MOI (multiplicity of infection; ratio of phages to bacterial cells) of phages applied in the study is indicated with (*).

2.2.1 Applications of *Salmonella* phages in postharvest foods

Previous studies have evaluated phage-based biocontrol against *Salmonella* on meat carcasses or outer skin as *Salmonella* can be shed from animals, passed to environment via animals' feces and could possibly cause contamination in the meat during slaughtering and carcass processing. Hungaro et al. (2013) [43] combined a mixture of phages with chemical agents (i.e., sodium dichloroisocyanurate, peracetic acid, lactic acid) to reduce *Salmonella* Enteritidis on chicken skin with initial levels of 10^5 CFU/cm^2. Independent treatments with a phage cocktail or chemical agents showed similar results as indicated by a reduction of 1 log CFU/cm^2 in *Salmonella* Enteritidis counts. This study suggests that a phage cocktail can be employed as an alternative biocontrol agent to reduce *Salmonella* Enteritidis contamination on poultry carcasses in an industrial setting during carcass processing. Similarly, a study by Goode et al. (2003) [50] reported a reduction of *Salmonella* populations by up to 2 log units on chicken skin over 48 h after the application of *Salmonella* phages at the MOIs of 10^2 and 10^3. While development of phage-resistance may occur, this study showed that application of phages at higher MOI, up to 10^7, exhibited a potential capability to eliminate other *Salmonella* strains that showed high levels of resistance. To reduce *Salmonella* contamination on post-slaughter pig skin, Hooton et al. (2011) [44] tested a phage mixture against multidrug-resistant *Salmonella* Typhimurium U288 by applying this phage mixture to pig skin at MOI of 10 or higher. Results showed that the phage cocktails could effectively control *Salmonella* Typhimurium as indicated by a reduction of cell numbers to the undetectable levels. A study by Spricigo et al. (2013) [30] also evaluated the effectiveness of a phage cocktail (combination of three phages) on pig skin. A phage cocktail was applied on the pig skin by a spraying method and left at 33°C for 6 h. A reduction of greater than 4 CFU/cm^2 and 2 log CFU/cm^2 were observed for *Salmonella* Typhimurium and *Salmonella* Enteritidis, respectively.

For meat upon slaughtering and carcass processing, such as fresh chicken breast and raw beef, phage applications have been evaluated for the ability to reduce *Salmonella* contamination. A study by Spricigo et al. (2013) [30] evaluated the effectiveness of a phage cocktail (combination of three phages) on chicken breast by a dipping method. The chicken breast dipped with the phage cocktail and kept at 4°C for 7 days showed a reduction of 2.2 log CFU/g and 0.9 log CFU/g for *Salmonella* Typhimurium and *Salmonella* Enteritidis, respectively. Bigwood et al. (2008) [45] used the phages at MOIs of 10 or 10^4 to reduce *Salmonella* Typhimurium PT160 on raw and cooked beef. Treated meat was stored at 5°C and 24°C to simulate the conditions of refrigerated and room temperature storage. Phage treatment on raw and cooked beef in this study showed a reduction of 2 to 3 log CFU/cm^2 at 5°C and more than 5.9 log CFU/cm^2 at 24°C. *Salmonella* phages applied onto fresh meat upon slaughtering and carcass processing showed a marked ability in reducing *Salmonella* populations during storage at refrigeration or room temperatures. Overall, this suggests an alternative strategy for controlling *Salmonella* during distribution to processing facilities, processing, or even at the retail operation level before reaching the consumers' kitchens.

Phage treatments have been evaluated on fresh-cut produce, fresh leafy greens, and packaged produce. Various studies have reported the efficacy of phages in reducing *Salmonella* levels on these food commodities. Leverentz et al. (2001) [46] evaluated the effects of a phage mixture on the reduction of *Salmonella* in fresh-cut produce. The phage mixture could effectively reduce *Salmonella* counts on fresh-cut honeydew melon slices by 3.5 log units when stored at 5°C and 10°C, and by 2.5 log units when stored at 20°C. The study also compared phages to chemical sanitizers on honeydew melon slices. Results showed that the phages mediated a greater reduction of *Salmonella* than chemicals. However, the phages were not effective against *Salmonella* on apple slices as indicated by no significant decrease in *Salmonella* counts, while a reduction in phage titer was observed. Some explanations for the unsatisfactory results include the acidic pH of the apples which may have inactivated the phages. Overall, this study suggests effects of food types on the efficacy of phage treatments against *Salmonella* populations. Red tomatoes are another type of fresh produce that showed a similar unsatisfactory result upon phage treatment against *Salmonella*. Ye et al. (2009) [42] studied the use of a phage cocktail (combination of five lytic phages) on red tomatoes. The phage cocktail showed unsatisfactory result on the reduction of *Salmonella* Javiana populations. Another study by Spricigo et al. (2013) [30] tested a mixture of three phages as a cocktail (10^9 PFU/ml) on romaine lettuce. A reduction

in numbers of *Salmonella* Enteritidis of more than 2 log CFU/g and *Salmonella* Typhimurium of more than 3 log CFU/g was observed upon phage treatment at room temperature.

Processing surfaces require appropriate cleaning and sanitizing procedures to reduce contamination of *Salmonella*. *Salmonella* contamination of processing areas or surfaces can link to cross-contamination in food products. Phage applications have been studied for use as sanitizing agents on various surfaces in food processing facilities. For example, Woolston et al., 2013 [31] tested a cocktail of six lytic *Salmonella* phages against *Salmonella* Kentucky and Brandenburg on stainless steel and glass surfaces. Phage treatments showed 2.1 to 4.3 log units of *Salmonella* counts. This study also replaced two phages that could lyse *Salmonella* Paratyphi B in the phage cocktail. Treatment of this phage cocktail showed 2.1 to 4.3 log units of *Salmonella* Paratyphi B counts. Results suggested that a phage cocktail could be a promising tool for effectively reducing *Salmonella* levels on hard surfaces.

2.2.2 Applications of *Salmonella* phages in processed foods

Various types of processed foods, including ready-to-eat foods have been linked to *Salmonella* contamination and outbreaks [4, 51]. Several studies have evaluated direct applications of *Salmonella* phages to processed foods. In addition, previous studies have reported high efficacy of phages for controlling *Salmonella* contamination in different food matrices. Guenther et al. (2012) [47] evaluated the efficacy of phage FO1-E2 in reducing *Salmonella* Typhimurium in a variety of ready-to-eat foods and dairy product (i.e., hot dogs, sliced turkey breast, seafood, chocolate milk). Foods were inoculated with 10^3 CFU of *Salmonella* cells and then treated with 3×10^8 PFU/g for six days. All phage-treated foods incubated at 8°C resulted in complete eradication of *Salmonella*. At 15°C, phage treatments could significantly reduce *Salmonella* levels by 5 log units on turkey deli meat, and in chocolate milk. However, a reduction of 3 log units was observed after phage treatment on hot dogs and in seafood at this temperature. Phage Felix-O1 was also evaluated against *Salmonella* Typhimurium DT104 on chicken frankfurters. Results showed a reduction of 1.8 to 2.1 log units of *Salmonella* counts after phage treatment [52]. Another study by Zinno et al. (2014) [49] tested phage P22 against *Salmonella* Typhimurium in various foods (liquid eggs, energy drinks, whole and skimmed milk, apple juice, chicken breast, and chicken mince). All liquid food samples (energy drinks, whole and skimmed milk and apple juice) were spiked with *Salmonella* (initial level of 10^4 CFU/ml) and treated with phage P22 at 10^8 PFU/ml at 4°C. A reduction of 2 to 4 log units was observed after 48 h at 4°C. Phage treatment has been evaluated for controlling *Salmonella* contamination during the manufacture, ripening, and storage of cheddar cheese made from raw and pasteurized milk [48]. This study demonstrated that a single phage, SJ2 had the ability to control *Salmonella* Enteritidis after 89 days of storage at 8°C in pasteurized milk cheese as *Salmonella* was not detected. However, the raw milk cheese contained *Salmonella* counts of 50 CFU/g after 99 days of storage at 8°C. In addition, a reduction in *Salmonella* counts was observed in comparison to the control where growth of *Salmonella* Enteritidis increased to a final concentration of 10^3 CFU/g. Overall, the effectiveness of phages has been shown in various types of postharvest foods. However, several factors may contribute to the efficacy of phages against *Salmonella* populations present on foods. Differences in food matrices and food processing conditions can result in varied results from phage applications.

3. Conclusions

Phage applications have gained much interest for use as biocontrol agents against foodborne pathogens, including *Salmonella*. Effective control measures of *Salmonella* at any stages of food production are thus essential to reduce foodborne outbreaks worldwide. Several studies have shown that phages can be a potential means to eliminate and control *Salmonella* starting from primary production, in postharvest foods (as antimicrobial food additive), and on food processing facilities (as biosanitizing agents). Although advantages of phage applications have been markedly demonstrated, some limitations have been encountered. Both intrinsic and extrinsic factors can have influence on phage/host/food interactions which can lead to variations in the efficacy of phage treatments. Current phage applications still need improvement with regards to further evaluation of the suitability and appropriate use of phages. Further research in this area also needs to be implemented to minimize the incidence of food contamination and foodborne disease outbreaks.

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