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Mini Review

A Review on the Applications of Listex™ P100 Bacteriophage

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Abstract

Listeria monocytogenes is a Gram-positive, small rod-shaped bacterium that causes listeriosis in animals and humans from eating contaminated food. It can mostly affect susceptible individuals with defective immune systems. Also, the mortality rates, as a result of listeriosis, can be varied between 30 to 75%. *Listeria* had been reported to be more allergic to antibiotics than Gram-positive bacteria. However, according to reports, *listeria* has recently been allergic to the resistance to these antibiotics. Such an increase in antibiotic resistance in *listeria* is in line with the worldwide pattern of the increasing prevalence of antibiotic resistance, including multiple antibiotic resistance in many bacterial groups. Therefore, one of the ways to reduce the incidence of this disease in animals and humans is the biological control of this bacterium by bacteriophage. Bacteriophages have been shown to be effective in controlling *L. monocytogenes* in food. In 2006, the US Food and Drug Administration approved the preparation of two bacteriophages (Listex™ P100 and LMP-102) for use in certain foods to control *L. monocytogenes*. The prevalence of foodborne pathogens is the use of phages as biocontrol agents in food. Also, the activation of Phage Listex™ P100 against multiple *L. monocytogenes* serovars allows it to kill *L. monocytogenes* and its immunogenicity in food and clinical products. The prevalence of foodborne pathogens is the use of phages as biocontrol agents in food. Also, the activation of Phage Listex™ P100 against multiple *L. monocytogenes* serovars allows it to kill *L. monocytogenes* and its immunogenicity in food and clinical products.

Keywords: *Listeria* monocytogenes, Bacteriophage, Listex™ P100, Bacteriophage Safety

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Introduction

Listeria monocytogenes

Listeria monocytogenes (*L. monocytogenes*) is a small Gram-positive bacterium that causes listeriosis in animals and humans from eating contaminated food. The prevalence of this bacterium in nature is so much so that contamination of food is likely.[1,2,3] The ability of this bacterium to grow in a wide range of temperatures (0.4 °C to 45 °C) and pH values (4 to 6.9), allows it to stay in food processing environments for a long time. Listeriosis has been reported with the consumption of undercooked meat, seafood, cantaloupe, contaminated dairy products, unwashed raw vegetables and chicken.[4,5] Listeriosis is a serious disease transmitted to humans by contaminated food, which can be sporadic or prevalent. It can mostly affect susceptible individuals with defective immune systems. Also, the mortality rates, as a result of listeriosis, can be varied between 30 to 75%.[6] This disease is extremely dangerous in pregnant women, adults, infants, and people with immunodeficiency. It can cause various symptoms in humans, including abortion in pregnant women, neonatal sepsis, intrauterine infection in the form of granulomatosis, encephalitis, meningitis, meningitis, meningitis, myocarditis, hepatic necrosis, and skin and gastrointestinal complications.[7] The clinical signs of invasive listeriosis are usually severe and emerge as meningoencephalitis.[8] The incubation period of this disease in susceptible adults is estimated at three to 70 days and an average of three weeks. In infected infants, it takes several days to several weeks for symptoms to appear.[9] Notwithstanding the reality that fewer than 2,300 instances were reported in the European Union (EU)/European Economic Area (EEA) between 2008 and 2015, *Listeria* which have caused considerable concern. This concern is mainly due to the overall annual mortality rate of 12-20%, the involvement of at-risk groups, and the increment in the verificated instances between 2008 and 2015.[1]

Listeria Antibiotic Resistance

Listeria has been reported to be more allergic to antibiotics than gram-positive bacteria, and this allergy to *Listeria* has recently been reported to be resistant to these antibiotics. Such an increase in antibiotic resistance in Listeria is in line with the global template of increasing prevalence of antibiotic resistance. There are recurrent reports of pathogens that are resistant to almost all extant antibiotics. In the past, people with listeriosis were usually treated with penicillin or ampicillin with aminoglycoside, although chloramphenicol, erythromycin, or tetracycline have been used alone or in combination. The current treatment for all types of listeriosis is a combination of ampicillin and gentamicin. Antibiotic

resistance in bacteria is associated with the overuse of antibiotics in animals and humans. It is clear that antibiotic resistance is widely reported not only in pathogens but also in all bacteria. Also, the presence of antibiotic resistance in individuals poses major risks to human health.[10]

The growing number of multidrug-resistant bacteria and the complete ban on the use of antibiotics in animal feed in the European Union, as well as the partial ban in the United States, have led to growing research into the use of bacteriophages to fight bacterial infections in animals and humans. Therefore, one of the ways to reduce the incidence of this disease in animals and humans is the biological control of this bacterium by bacteriophage.[11]

Bacteriophages

Bacteriophages (or viruses) are widespread in nature, and their life cycle is closely linked to that of bacterial cells. These bacteriophages are known as bacterial parasites because they lack the cell structure and enzymatic system needed to synthesize proteins, absorb nutrients, or make new particles, and as defective organisms can only reproduce in living cells.[11,12,13] These bacteriophage viruses can bind and penetrate specifically to their specific bacteria, and today, with increasing antibiotic resistance, they can be a proper alternative for the treatment of bacterial infections and pathogens that are transmitted through food items. They can also be used as alternatives to complex molecular methods to identify species and subspecies of bacteria. Concerns about the use of various antibiotics as an antimicrobial agent and growth factor in diets and ultimately reducing the quality of food have brought the need to use phages as natural biological control agents.[14,15] Unlike antibiotics, which may kill beneficial bacteria in addition to harmful bacteria during treatment, phages have an enzyme in their tail filaments that acts only on a specific molecule on the surface of the bacterium and destroys it after entering a specific bacterium. Also, phages are so self-limited that they are destroyed after the destruction of the desired bacteria.[16,17] Bacteriophages have long been shown to be potent as a biotherapeutic agent. Recent approvals for the use of Phage *L. monocytogenes* for food safety have increased the motivation to research phages in other foods. At present, phage-based products focus on the microbial species *L. monocytogenes*, *Campylobacter*, *Salmonella*, and *Escherichia coli*.[18,19] The antibacterial effect of phages has also been confirmed in the fight against infections caused by *L. monocytogenes*, such a *Salmonella* and *Campylobacter* spp. It is one of the common diseases between animals and humans that causes food poisoning with a mortality rate of more than 30% in humans.[11] Bacteriophages are effective in controlling *L. monocytogens* on food. In 2006, the FDA approved the preparation of two bacteriophages (Listex™ P100 and LMP-102) for use in certain foods to control *L. monocytogenes*. The Australian/New Zealand Food Standards (FSANZ) also approved Listex™ P100 as a processing aid to lessen *L. monocytogenes* contamination in a variety of foods, some of which are collected in Table 1.

Table 1. Commercial Phage Preparations Approved for Use in Food Production

Company	Phage preparation	Targeted bacteria	Range of products	Year of implementation	Approved by	References
Intralytix Inc, Baltimore, MD, USA	LMP-102™	*L. monocytogenes*	Meat, poultry	2007	FDA	20
EBI Food Safety, Wegeningen, Netherlands	LISTEX™ P100	*L. monocytogenes*	All food products	2007	FDA, USDA	21
OmnilyticsT Salt Lake City, UT, USA	BacWash™	*Salmonella*	Hides of livestock	2007	USDA FSIS	22
OmnilyticsT Salt Lake City, UT, USA	AgriPhage™	*Xanthomanas capestris pv. Vesicataria P. syringae pv tomato*	Tomato, pepper	2006	FDA, USDA FSIS	23

*In 2006 approved for cheese

Immunity of Bacteriophages

Non-toxicity of Phage

Phages are very specific and can only infect a very limited range of host bacteria. All available evidence shows that their oral consumption (even in large quantities) is completely harmless to humans. Safety studies have been performed, for example, with *Listeria* P100 phage, in which mice were fed with high doses of phage without measurable effects compared with controls.[14] Preference in the clinical and non-clinical use of phages and their antimicrobial enzymes have been stimulated by the need for new types of antibacterial drugs to address the ongoing problem of antibiotic resistance. Although phages are often compared to antibiotics, it is well established that they also have advantages and disadvantages as therapeutic drugs. It is generally accepted that antibiotic therapies cause higher levels of damage to existing microflora due to non-specific activities, while phage treatment minimizes this damage. Total phage-based therapy has traditionally been used in Eastern Europe to treat a variety of infections, from diabetic foot ulcers to stomach complaints. This type of treatment uses the lytic proliferation cycle of bacteriophages.[24]

WHO data confirm the decline of the effectiveness of antibiotic therapy. The spread of bacteria resistant to several groups of antibiotics makes it more difficult to treat various diseases, especially in children.[25]

Bacteriophages have long been used in children for therapeutic and prophylactic purposes .Since phage therapy has been superior to the logical methods used in contemporary controlled studies, many people who dealt with phages described successful treatments but did not conduct comparative studies. However, phage administration seems safe even in children after intravenous injection. It can be concluded that the results of therapeutic application and prevention of phages against multidrug-resistant pathogens are very encouraging.[26]

Listex™ P100 Bacteriophage (Phage P100)

Bacteriophage P100 belongs to the family *of Myoviridae* Caudovirales, respectively. This family of phages has the general feature of having a contracted tail and head. The tail, made of a central core surrounded by a contractile helical sheath, is separated from the head by a collar. In general, these phage families have larger heads, more DNA, and higher molecular weights than other phage families. These phages are usually sensitive to the processes of freezing and thawing and causing osmotic shock. Different genera of the *Myoviridae* family differ in genetic organization, DNA replication mechanism, and the presence of unusual bases or DNA polymerases.[27] Listex™ P100 bacteriophage (Phage P100) has been obtained from the U.S. Food and Drug Administration (USFDA) and the United States Department of Agriculture (USDA) (Wageningen, The Netherlands). Phage P100 is active against multiple *L. monocytogenes* serovars.[28] Following the request of the European Commission, the EFSA Panel on Biological Hazards (BIOHAZ) provided scientific confirmation of the application file submitted by Micreos BV (Netherlands) to approve of Listex™ P100 for treatment or use and reduce surface contamination of raw fish with *L. monocytogenes*. In this study, the European Food Safety Authority (EFSA) study concluded that the substance did not cause toxic problems for humans because bacteriophage P100, which is used as an active ingredient, had no effect on consumers and organisms except for *Listeria* and may endanger their safety.[29] Other products such as ListShield, EcoShield and SalmFresh bacteriophages are also used commercially by Intralytix to reduce the number of *L. monocytogenes*, *Escherichia coli O157* and *Salmonella enterica*. Gum and toothpaste are also made by Micreos, which contains Listex™ P100, which is effective against *L. monocytogenes*, and New Horizons Diagnostic. It contains a bacteriophage mixture and is effective against *streptococcus* species for oral health use. The FSANZ and Switzerland also approved the use of Listex™ in cheese and other foods in 2012.[30,19,31,32] P100, similar to A511, has a wider range in the genus *Listeria*, although P100 is wider than A511 and more than 95% of the 250 the tested strains of *Listeria* are susceptible to this phage.[27]

Chibeu et al. studied the effect of bacteriophage Listex™ P100 with chemical antimicrobials in reducing *L. monocytogenes* in the presence or absence of chemical antimicrobials Potassium Lactate (PL) and Sodium Diacetate (SD) in cooked turkeys and grilled beef and in the absence of antimicrobials reduce *L. monocytogenes*, respectively: 2.1 $\log_{10}$ CFU/cm^2 and 1.7 $\log_{10}$ CFU/cm^2 were observed in cooked turkey and grilled beef. In this study, they found that Listex™ P100 reduces *L. monocytogenes* and can act as a barrier to increase meat immunity in combination with chemical antimicrobials.[4]

In a similar study in 2017, Cláudia et al. examined the effect of Listex™ P100 bacteriophage on pork ham and concluded that Listex™ P100 bacteriophage had the most effective reduction in *L. monocytogenes.*[33]

Conclusion

Although there are many obstacles to using bacteriophage to overcome pathogens, the current results of the use of bacteriophage treatment and prevention methods are encouraging and there is a clear need for phage therapy in children. In addition, findings show that an effective phage therapy can be much cheaper than antibiotic therapy. Yet, there is still lack of well-controlled large-scale formal clinical studies on its safety and efficacy. Bradley et al., believe that the lack of new antibiotics requires re-evaluation and re-administration of bacteriophages. The prospect of bacteria as a valuable tool in biotechnology forces us to emphasize contemporary medicine and use the unlimited possibilities offered by bacteriophages for common interests.[26]

The resurgence of research into phage biology and treatment is partly due to the growing need for new agents to treat drug-resistant infections. Despite a long clinical history in Eastern Europe and early success in the food industry, commercialized phage products have not yet entered other sectors. This relative failure is partly due to the inherent biological limitations of all phages.

Natural antimicrobial compounds have become increasingly popular due to changes in consumer positions relative to the use of chemical preservatives in food processing surfaces. Bacteriophages belong to the class of natural antibiotics, and their effectiveness in controlling bacterial pathogens in the food industry has led to the production of various phage products that have already been approved by the USFDA and USDA. Most of these products are used in farm animals or animal products such as meat, as well as in agricultural and horticultural products.

Treatment with specific phages in the food industry can prevent crop rot and the spread of bacterial diseases, and ultimately promote safe environments in the production, processing, and use of plant foods for animals and plants. Also, the activation of phage P100 Listex™ against multiple *L. monocytogenes* serovars allows it to kill *L. monocytogenes* and its immunogenicity in food and clinical products.

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Authors' Contributions

All authors have equal contribution in this study.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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