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Bacteriophages: Potential antagonistic agents against medically and agriculturally harmful bacteria

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Abstract

Bacteriophages are viruses that have evolved to be natural predators of prokaryotes, infecting and killing bacteria without harming the environment or human health. Antibiotic-resistant bacteria are significant threat to humanity; millions of yearly suffer from these pathogens. Antibiotic resistance in disease-causing bacterial species is growing very commonly and rapidly both in developed and developing countries. Discovery and synthesis of new drugs are costly; moreover, low income-nations cannot afford the cost of manufacturing, research and development. The exploitation of diverse bacteriophages as antibacterial agents has much potential, especially against drug-resistant bacteria. However, phage biologists are grappling consistently to meet the regulatory standards for correct and safe strategies to introduce phage therapy in the routine health care system. Due to this hitch, fewer human trials have been evaluated where phage is used as a therapy against bacterial infections. On the other hand, the importance of bacteriophages in the agri-food business has recently received researchers' attention. Bacteriophages are used as preservatives for food storage. However, since bacteriophages have been licensed recently as food additives, interest in "edible viruses" has grown. This review focuses on several vital aspects of bacteriophages, such as their isolation and identification, mode of action against their hosts, successful pieces of evidence where phages are used as a therapy against drug-resistant bacteria, and their use in food safety in the field of agriculture.

Keywords: Bacteriophages, Drug resistance, Bacteria, Antibiotics, Agriculture, Biotechnology

1. Introduction

In the present scenario, antibiotic-resistant bacteria are one of the immense hurdles before treating the infections caused by them. The situation is more worsens in the case of immuno-suppressed and immunocompromised patients. According to one report published (November 13, 2019; https://www.healio.com) by the Centers for Disease Control and Prevention (CDC) that “One death observed after every 15 minutes in the USA due to severe infections caused by antibiotic-resistant bacteria”. Further it is estimated that ~700,000 individual’s die yearly from antimicrobial resistance and 10 million people will die by 2050 [1]. Antibiotic resistance in microbes is widely neglected research area in a highly populous country like India. Moreover, no appropriate data can reveal the exact cause of bacterial resistance. To overcome this problem of increasing antibiotic resistance among the bacteria, many thorough, intensive research and development inputs are needed to synthesize the new target-specific antibiotics. However, developing successful and large-spectrum antibacterial drugs requires excellent infrastructure, time, and cost. But, there is no guarantee about how many years the newly developed drug will work effectively against perilous bacterial infections [2, 3]. So, many opportunities exist

beyond antibiotic approaches to curb severe bacterial diseases. Implementing prokaryotic predators called bacteriophages shows fantastic antagonistic results against the bacteria that can be excellent options to use as therapeutic agents [4]. Bacteriophages were initially isolated and identified by Fredrick Twort [5] and Felix d Herelle [6]. Soon it was realized that bacterial infections could be prevented or cured by administering bacteriophages as they impose antibacterial properties and self-replicating mechanisms. Bacteriophages can successfully infect the bacteria by binding on their surfaces at specific receptor sites, injecting their genetic materials into the prokaryotic cells, and finally lysing them.

It has been observed the availability and affordability of antibiotics is tough in low income countries especially in many African and Asian countries. These nations cannot afford the expenditure on infrastructure that is prerequisite to start research on discovering of novel antibiotics and their proper trials. In these countries budget on health care system is far less as recommended by the world health organization (WHO). Moreover most of the countries struggling with the numerous monetary challenges such as rapid inflation, nutrition insecurity, costly borrowing, and mounting debt [7]. In these circumstances phage therapy may work as an alternate without expending too much cost. In current article, we critically reviewed the various studies and trials of bacteriophage therapy against multi-drug resistant bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli, Mycobacterium tuberculosis, Acne vulgaris*, and other miscellaneous prokaryotes.

2. Phage-inspired anti-prokaryotic approaches and mechanisms

In the Figure 1, illustrates how the phages can successfully adsorb on the surface of pathogenic bacteria, inhibit them from further colonizing, and mitigate the biofilm formation process. Phages can also kill the bacteria by using enzymes and introducing their drug-sensitizing genes in the host body.

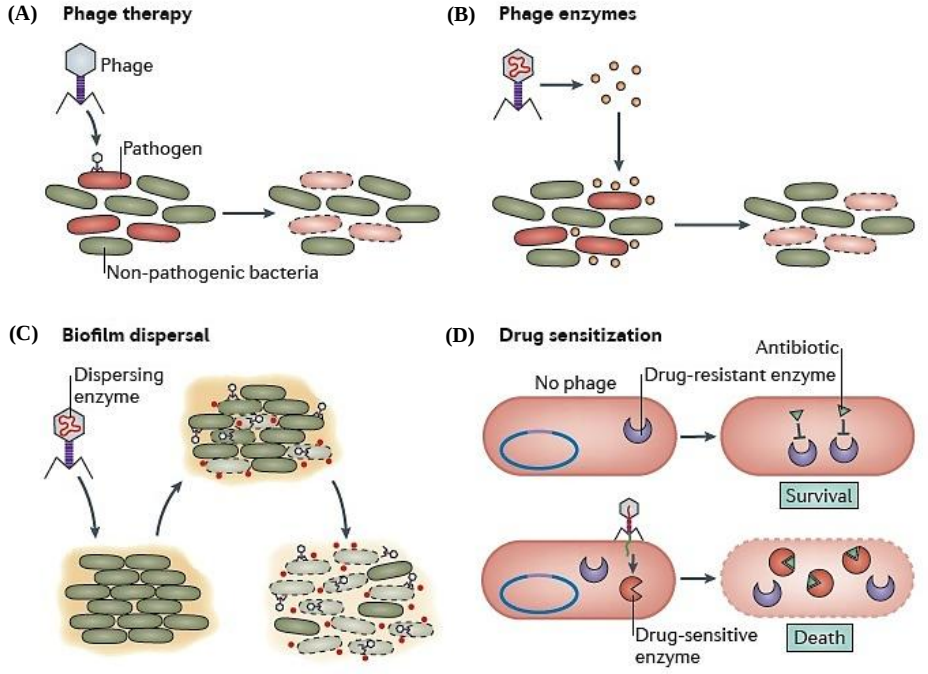

Figure 1 A) The specificity of phages can be explored for phage therapy, by which phages target particular bacterial pathogens. B) Phage products, such as enzymes, can be used to target specific bacteria, including pathogens. C) Phages can be used to disrupt biofilms, by targeting bacteria embedded in these structures, and can be engineered to release specific enzymes that degrade the biofilm matrix. D) Phages can be used to sensitize antibiotic-resistant bacteria. For example, phages can introduce antibiotic-sensitive genes into drug-resistant hosts, and this strategy can be combined with antibiotic treatment [8].

It has been observed some bacteria showed the resistance to the phage attacks by using diverse strategies such as DNA restriction-modifications, mutations, through blocking of receptors used by phages, release of extracellular materials that prevent the phage DNA entry in to the bacterial cytoplasm and restriction on the assembly of the phages inside the bacterial host. The phage resistance that emerged in the bacteria could be an obstacle before the success of phage therapy. It has been also observed; the phages have an ability to develop the mechanisms that may work against the phage resistant prokaryotes. On the other hand it's also true; the phage resistance is considered as a minor hurdle for the practical use of phages in clinic, through use of multi phages in

cocktail, further also the blend of phages and antibiotics, proposed as an approach for reducing the rise of phage resistant bacteria [9].

3. Insights on the antibiotic resistance mechanisms followed by the bacteria

With time, antibiotic resistance has increased, and several pathogenic bacteria used their developed mechanisms to escape from the lethal effect of antibiotics. Figure 2 exhibits the release of some enzymes by bacteria that degrade the functioning vital rings of antibiotic structures. Some bacteria apply efflux pumps in their cell wall to push back antibiotics that enter their cell. Many bacteria received resistant genes to work against the anti-bacterial drugs.

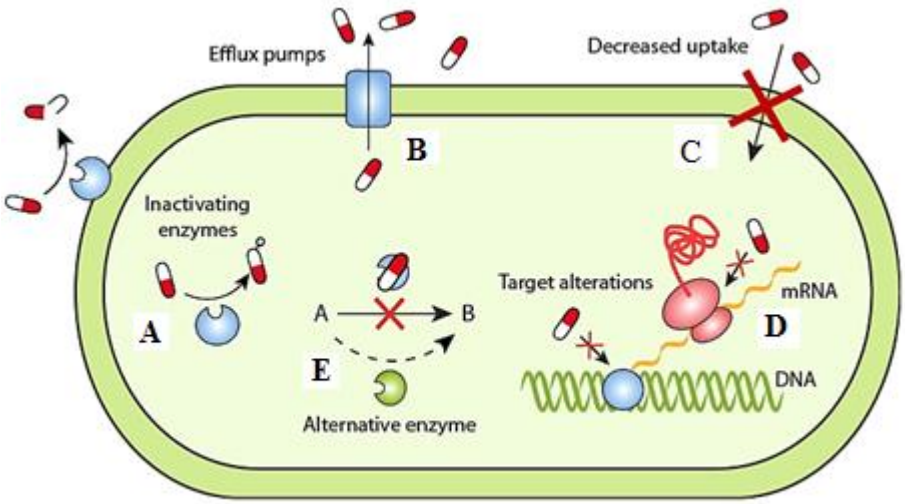

Figure 2 Bacterial pathogens have developed many strategies to neutralize the anti-prokaryotic drugs: A. Bacteria can utilize the cleaving biocatalysts like β-lactamases that breaks the β-lactam rings. B. Origin of efflux pumps to through antibiotics out of the bacterial cell. C. Prokaryotes can also alter their cell wall to lessen the antibiotic influx. D. Bacteria can alter the genetic targets of many antibiotics. E. Replacement of enzyme targets of antibiotics with an alternative enzymes that carry out the similar function [10].

Generally prokaryotes (especially Gram negative bacteria) produced the enzymes such as *B*-Lactamase and macrolide esterases that can denature the β-lactams and macrolides. Moreover the antibiotic resistance genes are often located on plasmids or transposons and can be transferred from cell to cell through conjugation, transformation, or transduction. The gene exchange among the bacteria is responsible for the rapidly spreading of resistance in prokaryotes [11].

4. Isolation and identification of bacteriophages

Bacteriophages differ from other antibacterial strategies because they selectively infect pathogenic bacteria, including multidrug-resistant bacteria. Bacteriophages are environmentally safe and effective in small amounts and have no adverse effects on the human body or agricultural stored products. The isolation of bacteriophages can be done from sewage water specimens and soil samples (collected from various places). Isolation of bacteriophages can be done by plating techniques, Figure 3. This technique is used to detect and count specific phages from the enriched sample. At the same time, plaque assay involves seeding a lawn of host bacteria with a small phage sample. In Japan, 49 *S. aureus* isolates were obtained from the milk of mastitic cows for the isolation and identification of a *staphylococcus* strain bacteriophage. As a result, 15 isolates were obtained that were positive for coagulase and hemolysin, and two of them were chosen for further analysis. As a result, SA039 had the broadest host range, producing clear plaques on 13 of the 15 isolates, while SA012 made clear plaques on eight isolates and was the only phage capable of producing a clear plaque on a non-mastitic *S. aureus* strain [12]. In Uttar Pradesh (India), *S. aureus* strains were identified from human clinical samples. Each sample was streaked over mannitol salt agar and then tested biochemically. Following the isolation and identification of *S. aureus* phages, researchers determined that the polyvalent lytic phage P-27/HP was effective against a broad spectrum of multi-drug resistant (MDR) *S. aureus*-caused human illnesses. Phage P-27/HP revealed high lytic efficiency for eliminating the *S. aureus* [13]. A descriptive cross-sectional study was conducted in Nepal (Kathmandu) to analyze the phages in water samples collected from various rivers and their lytic effect on pathogenic bacterial strains. They isolated the few lytic phages against MDR bacteria [14]. Another study determined in which pure

phage strain was isolated from untreated sewage water. Following host range analysis and stability testing at varying temperatures and pH, a plaque assay was performed to determine the phage titer against MDR *E. Coli*. It was determined that sewage water contained various bacteriophages. Bacteriophages were found to be highly specific against tested *E. coli* strains and could not lyse strains from other species after repeated plating [15]. Anti-Arthrobacter bacteriophage species were isolated from soil using an optimized enrichment technique, yielding dozens of distinct phages from various soil types. In addition, samples of urban sewage were collected for bacteriophage isolation and identification. In this study, two strategies were used; i) Two sewage samples were obtained and processed by several steps of filtration and purification. ii) 10 ml of urban sewage mixed with the culture of a bacterial strain to obtain lysates of bacteriophages. *P. aeruginosa*, *Salmonella enterica*, *Staphylococcus sciuri*, and *Enterococcus faecalis* were all killed by isolated phages [16].

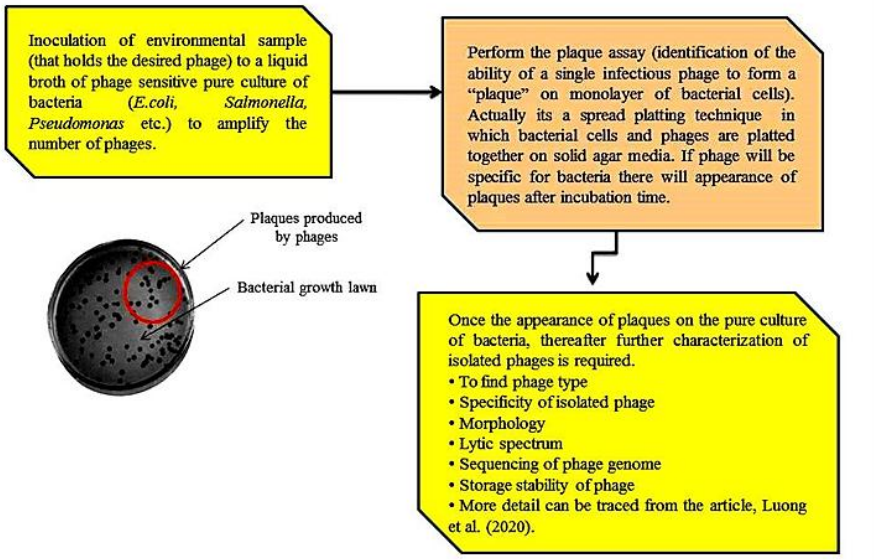

Figure 3 Steps involved in the isolation, identification and characterization of bacteriophages.

5. Phage selection criteria for therapeutic purposes (Phage Properties)

Still the methodology is almost same as adapted by Felix d'Herelle (pioneer in phage isolation) for isolation and selection of desirable phages. Isolated phage simply mixed through with the susceptible bacteria and incubated at optimum temperature and later monitored for the lysis of bacterial cells [17]. Affinity, or specificity, is the most crucial consideration when selecting phages for therapeutic application [18]. A cocktail of different phages may broaden their lytic spectrum, allowing them to confirm that the targeted bacterium is sensitive to the chosen phage before using it [19]. Because phage cocktails have a broader lytic effect against bacteria than single phages, phage cocktails are recommended for dealing with the problem of phage specificity [20]. Because of several characteristics, including the efficacy of phage therapy and the possibility of preventing adverse effects, phages can be considered as beneficial for therapeutic purposes [21]. First, it is advised to utilize lytic phages exclusively. Observable turbid plaques are typically produced by temperate phages [22]. Ackermann (2005) [23] estimates that about half of the phages isolated from the environment are temperate. It is critical to remember that most therapeutic phages are discovered in environmental samples. If prophages are present, a bacterial strain may undergo lysogenic conversion, reducing the therapeutic benefit of phages. As a result, it is not recommended to use phagesholding genes for lytic phage repressors, integrases, or transposases for medicinal purposes [24], [25], [26]. For safety reasons, every page that can be used therapeutically should be sequenced.

6. How the phages identify their specific bacterial host

Bacteriophages identify their specific bacterial host with the help of receptors present on their surfaces. Bacteriophage attaches to the surface of bacteria and releases their nucleic material into the bacteria. Specific bacteriophage attaches to the particular bacteria only. When bacteriophage enters bacterial cells, it initiates its life cycle. Life cycles can be classified into three types: lytic, lysogenic, and chronic infection [27]. Bacteriophages may replicate inside the bacterial cells and lyse the bacterial cell to release new copies of bacteriophages into the surrounding environment. This lysis of cells causes plaque formation. Phages attach to the bacterial cell with the help of specific surface receptors that may be outer membrane proteins (protein receptors) like (OmpA and OmpC), lipopolysaccharides, or other components of bacterial cells such as bacterial capsules, pilli, and flagella.

These receptors allow phages to target specific bacterial cells, invade, replicate, and ultimately destroy them. When the interaction between phages and bacterial cell occurs, sheath contraction and invasion of nucleic acid into the cell. Once a phage enters the bacterial cell, it replicates by lytic or lysogenic life cycle [28]. Bacteriophages cannot infect eukaryotic cells because eukaryotic cells do not have such types of receptors.

7. Successful evaluation and efficacy of bacteriophages as therapeutic agents

The emergence and spread of antimicrobial resistance have been widely documented over the last 30 years. However, the search and development of novel antibiotic classes have yet to begin to satisfy the demand, despite the progress in modern biotechnology [29]. Since the level of antibacterial resistance increased considerably from the 1980s to 1990s, the search for alternative treatments progressed. However, with few results, different scientists and companies turned to phage isolation for treatment. Recently, phage-related activities have also increased in western countries, resulting in a significant surge in bacteriophage-related scientific papers [30].

7.1 Use of bacteriophage therapy against Pseudomonas aeruginosa

P. aeruginosa is a multidrug-resistant gram-negative bacterium that causes nosocomial, acute, and chronic infections. It has binding projections like flagella, pili, and biofilms, allowing it to survive on surfaces and medical devices. Some chromosomal effects and some drug efflux mechanisms make it MDR. The bacteriophages can degrade the bacterium's envelope and inhibit the biofilm by producing certain specific enzymes. Bacteriophage PAK-P3 was isolated from the sewage and used as pulmonary bacteriophage therapy to treat multidrug-resistant lung infections like cystic fibrosis Bacteriophage therapy may be effective in preventing skin graft disease [31]. The *P. aeruginosa* 3719 (6.0×10^5 CFU) was successfully inhibited to cause the infection on the skin grafted on laboratory burned guinea-pigs by use of lytic phage BS24 (1.2×10^7, Plaque forming units (PFU)). The BS24 was isolated from the sewage. This study supports the criteria to apply the effective phages on burned patient's skin that may protect it from destruction of grafts caused by antibiotic resistant *P. aeruginosa* [32]. The *P. aeruginosa* causes the severe infection in chronic rhinosinusitis (CRS) patients. Fong et al. [33] prepare the cocktail of four phages that reduced the growth of *P. aeruginosa* isolated from the CRS patients within 48h up to 76% reduction in growth by applying the phage titre 10^8 PFU/mL was optimum. Another study found that PELP20 could be used as bacteriophage therapy in mice models of chronic lung infection [34]. A cocktail of six bacteriophages (PYO2, DEV, E215, E217, PAK-P1, and PAK-P4) effectively reduced respiratory infection in a mouse model and bacteremia in wax moth larvae in [35]. Polyethylene glycol (PEG) ointment loaded with bacteriophage was prepared for treating burn wounds against *P. aeruginosa*, resulting in histopathological improvement in burn wounds with no allergic reactions [36].

7.2 Use of bacteriophage therapy against Escherichia coli

In general, *E. coli* is a common causative agent of diarrhoea and urinary tract infections (UTIs). It is a Gram-negative bacterium that is part of the normal intestinal flora. Among the strains are enteropathogenic *E. coli*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli* and entero toxigenic *E. coli* are well known to adherent to human intestinal tissues. Most of the *E. coli* strains can be infected by the *Escherichia* virus T4 bacteriophage [37]. The bacterial outer membrane contains receptors such as outer membrane porin C (OmpC trimer protein) and lipopolysaccharide, which the bacteriophage recognizes via its long tail fibers (LTFs). Stone and colleagues [28] discovered that LM33P1 infects the *E. coli* strain O25b. This strain was resistant to fluoroquinolones and ß-lactamase. Tsui, [38] studied the effectiveness of five adherent-invasive *E. coli* (AIEC) phages against adherent and non-adherent *E. coli*. The most common enterohemorrhagic *E. coli* (EHEC) was *E. coli* O157:H7, which is caused by infection by ingestion of contaminated food or water and causes diarrhoea and UTI [37].

7.3. Use of bacteriophage therapy against Acne vulgaris

Acne is the most common dermatological infection in the world, and the *Propionibacterium acne* bacteria cause it. It is a significant challenge for both patients and doctors. It can sometimes leave scars on the skin. Approximately 50 million people in the United States are currently infected with this skin infection, with 85% of those affected being between the ages of 12 and 25 [27]. The *P. acnes* cause inflammation in the sebaceous glands [39]. The *P. acne* are non-motile, microaerophilic, and opportunistic Gram-positive bacteria. It is very frequent on the skin of healthy persons. The *P. acnes* secretes enzymes that breakdown the skin components and chemotactic proteins, leading keratinocytes and inflammatory cells to generate pro-inflammatory cytokines such IL-8, IL-12, IL-1IL1, tumour necrosis factor-alpha, and reactive oxygen species [27]. Acne treatment needs topical and systemic antibiotics for an extended period, which contributes to resistant *P. acnes* strains. Antibiotic resistance in *P. acne*s has increased by nearly 40% globally [40]. According to reports from the United States,

Europe, and Asia, the most common resistance pattern appears to be erythromycin/clindamycin-resistant *P. acnes*. Resistance levels, on the other hand, vary with geography. Europe, Singapore, and Hong Kong have high rates of erythromycin/clindamycin-resistant *P. acnes* (45-91%), as do countries that use antibiotics sparingly, such as Japan and Korea, which have significantly lower resistance rates (2-4%) [27]. Since, 1964 it is recognized may viruses especially bacteriophages existed on human skin as non pathogenic microbes [41]. Marinelli et al. [42] observed in phage genome regions that encode phage endolysin, that is conserved in all tested *P. acnes* phages. These enzymes probably bind to essential elements of the *P. acnes* cell wall and may kill the several strains of *P. acne* [42]. Some formulations, including oil base cream, water-oil nanoemulsion, and paraffin oil-based lotion, successfully deliver bacteriophages to affected skin. The *P. acnes* biofilm development is inhibited by bacteriophage. Rimon et al. [43] reported through his experiments on mouse model and concluded the topical phage application on effected skin may reduce the acne vulgaris infection to spread. Further they noticed the phages might be used as an additional therapeutic tool along with the use of traditional antibiotics ingestion.

7.4. Use of bacteriophages against the methicillin-resistant Staphylococcus aureus

MDR bacteria are methicillin-resistant *S. aureus* (MRSA). It is a major therapeutic problem for human health. The *S. aureus* is a normal human commensal, but it can cause opportunistic infections such as pneumonia, osteomyelitis, food poisoning, and toxic shock syndrome. The phages can reduce the adherence, invasion, and cytotoxicity of MRSA. It was discovered that Phage SaGR51Φ1can be used to cure chronic PJI (prosthetic joint infection caused by MRSA). Phage MH-1 was acted against MRSA in another study. A hospital sewage sample was collected, and six bacteriophages effective against MRSA strains from burn patients were isolated. Only one bacteriophage, the MH-1 phage, demonstrated broad lytic activity against the tested MRSA and MSSA strains from burn patients. For the binding process, phage MH-1 can detect bacterial cell surfaces components such as lipopolysaccharides, peptidoglycan, thioacids, oligosaccharides, capsules, outer membrane proteins, and fimbria [44]. Because of their broad host range, other bacteriophages, such as *Staphylococcal* phage GH15, can be used as a therapeutic agent against MRSA infection. The *Staphylococcal* phage GH15 has a high ability to lyse MRSA strains [45]. In another study, 100 staphylococci isolates were obtained from Al-Sadar hospital, Al-Barsa General hospital, Ibn Ghazwan Hospital, and daycare centers in Barsa. Bacteriophages were also obtained from sewage samples obtained from Al-Sadar hospital. A total of 20 bacteriophages were isolated, and eight phages were chosen. These eight bacteriophages were tested for their host range. But only three bacteriophages (ΦSA1, ΦSA2, and ΦSA3) were further selected to check their lytic ability against MRSA isolates. It was concluded that all three bacteriophages produce clear large to medium-sized plaques. These three bacteriophages can be used as therapeutic agents against MRSA strains. Microscopically these three bacteriophages belong to the *Siphoviridae* family [46]. In another study, the French National Agency for Medicines and Health Products Safety (ANSM) created various phages (PP1493, PP1815, and PP1957) in an appropriate environment to target MDR *S. aureus*. These phages were isolated from multiple backgrounds. They are members of the *Silviavirus* and *Rosenblumvirus* genera [47].

7.5. Use of bacteriophages against the Mycobacterium tuberculosis

The mycobacterial infections like tuberculosis and leprosy are primitive and still big challenges to many developing countries. Initially mycobacteriophages were isolated during, 1940. Phages against the mycobacteria may helpful to combat the newly emerging extensively drug resistance tuberculosis bacteria [48]. Recently, Hashemi et al. [49] reviewed many phages have been isolated and characterized against the different strains of mycobacterium.

7.6. Evaluation of bacteriophages against Listeria monocytogenes

One of the important bacteria is *Listeria monocytogenes*. The source of this infection is contaminated products such as meat, fruits, vegetables, and various milk products. Two *Listeria* species have been identified as pathogenic bacteria capable of causing disease in humans and animals, and these are *L. monocytogenes* and *L. vanovii*. The *L. monocytogenes* is responsible for causing infections mainly in the United States especially in neonates, pregnant women, elderly patients, and immunocompromised (impaired cell-mediated immunity). It is rarely reported in non-pregnant adults in India. The *L. monocytogenes* is a tiny rod-shaped, facultative anaerobic microorganism that causes listeriosis. *L. Monocytogenes* can survive and reproduce in various environments, including low temperatures, pH, and salt concentration, making them multidrug-resistant bacteria. A mostly *L. monocytogenes* bacterium shows the resistant to cefotaxime, cefepime, fosfomycin, oxacillin, and licosamides. Bacteriophages in food products can be used to control *L. monocytogenes.* Bacteriophages can be used in food products against *L. monocytogenes* in advanced countries such as the United States, Canada, and Switzerland. In Europe, a specific bacteriophage called IZSAM-1 was isolated from a floor drain in the cheese salting area of an

Italian blue cheese dairy factory. It was further characterized and demonstrated broad host range activity against 21 strains of *L. monocytogenes* [50]. Another study isolated two bacteriophages against *L. monocytogenes*, LMP1 and LMP7, from chicken feces. These two bacteriophages were lytic and inhibited the growth of *L. monocytogenes* ATCC 7644, 15313, 19114, and 19115. The lytic activity of LMP1 and LMP7 in milk under refrigerated conditions was evaluated as a biocontrol agent in refrigerated foods. These two phages' cocktails can be used as a biocontrol agent in various dairy products [51]. In another study,110 sewage samples were collected and processed in Kerala, India, to isolate and identify the bacteriophages specific to the *L. monocytogenes*. Out of 110 models, 18 bacteriophages could clear the entire bacterial load [52].

8. Biocontrol action of bacteriophages against the bacteria for the food preservation

Bacteriophages become a new hope for the food industry for providing the safe food without chemical based preservatives. It has been observed, when the phages used as safety weapons doesn't change the properties of the food. Bacteriophage kills the pathogenic bacteria of poultry such as *Salmonella enterica, Shigella* species, *Campylobacter jejuni*, *L. monocytogenes*, and *E. coli* 0157:H7. With the help of bacteriophages we can preserve all kind of food such as dairy products, vegetables, sea food and many other food products. Bacteriophages could substantially reduce the risk of food contamination. Bacteriophage-based biocontrol activities have a long evaluation history because it is considered a safe and potent antibacterial strategy. Employing phages to combat bacterial contamination and infections is entirely possible but needs further dedicated efforts. Further, the phages also can be used as natural preservatives for increasing the shelf life of high-protein foods like varieties of meats (Table 1).

Table 1 Effective phages against the food spoiling and pat hogenic bacteria, modified from Sanna et al. [56].

Name of the sensitive bacteria	Name of the effective phage	Mode of the action of phage
Campylobacter jejuni	29C	Applied on chicken skin for the inhibition of *Campylobacter* infection
E. coli O157:H7	KH1/SH1	Administered rectally and orally to sheep and cattle
E. coli O157:H7	PPO1, e11/2 and e4/1c	Application on the surface of meat
Listeria monocytogenes	LMP-102 andLM -103	Phages mixed with nisin and applied on Honeydew melons to reduce the bacterial count
Campylobacter jejuni	CP8 and CP34	Protection of chickens
Listeria monocytogenes	LMP-102	Used as a food additive, mixture of 6 *Listeria* specific phages
E. coli O157:H7	ECP-100	Application to foods having hard surfaces
Salmonella	SP6	Applied on chickens to fight against the *Salmonella* infection
Salmonella	Felix O1	Microencapsulation technique use to ensure phage protection from external environment before giving antagonistic action to *Salmonella*
Listeria monocytogenes	P100andA511	Applied to ready-to-eat foods
Salmonella	P7	Application is made to *Salmonella*-treated meat

9. Challenges of the phage applications before their implementation

It's tough to predict the exact phage type that will combat successfully with the bacterial infection so it's always to be preferred the phage cocktail for treating the disease. Further time prediction is also a considerable issue, how much time phages will take to eradicate the bacteria after inserted in vivo applications. Phage mutations are also one of the major issues for their long term stability. Phage shelf life is the big concern for the successful treatment of bacterial diseases. Different techniques are developed for the storage of the phages such as freeze drying, extrusion dripping method that needs skills and experience. There are some organizations such as Phages for Global Health should raise awareness for the phage therapy in laboratories and healthcare staff [53].

The next major obstacle is culturing of phages at a large scale because their production procedures could be more convenient and need skilled scientific human resources. Modifying the wild strains of the phages before bringing in therapy or food preservation is challenging at genetic and molecular levels. It is also possible that most phages will not show suitable antibacterial activities at a large scale because most studies on phages occurred only at laboratory scales. Limiting factors like microbial load vary according to the situation, which can provide non-specific phage binding sites and act as a mechanical barrier. Other factors like temperature, pH, and inhibiting substances are significant before the phage action. Other restrictions include reduced diffusion rates that lessen the possibility of host-phage collisions. The effectiveness of bacteriophages in food should be assessed case-by-case, as with all food bio preservatives. Phages from the same niche or habitat should be maintained apart to preserve proper phage performance since they are likely better suited to thrive and multiply there. The fitness and physiological state of the bacteria influence the rate of phage infection. So the phages should need a wide range of physiological tolerances and infrequently adherence capacity to their host [54].

10. Conclusion and futuristic prospects

The present review highlighted the various aspects on phage research such as their isolation, characterization and applications against the diverse pathogenic bacteria. Moreover the phages act as antagonistic in natural (human body) and artificial (wastewater treatment plants) environments against the MDR bacteria, without harming any ecosystem. Unlike antibiotics, bacteriophages chose different mechanism to neutralize the bacteria. Single dose of phage can destroy large amount of multi-drug resistant pathogenic bacteria. Phages infect their target bacteria only and does not infect or harm the normal flora of human body [55]. Since the last decade, MDR bacteria have been considered a significant threat, especially to humans and their livestock. It becomes necessary to evolve new antibacterial agents to control the disease caused by MDR bacteria. Bacteriophages not only kill the bacteria but also restrict them from developing any resistant variants. Further, the phages did not affect the body's normal flora or aggravate dysbiotic disturbances. Indeed phages were discovered 82 years ago, but their implementation could be more varied in the medical and agro-food industries. So more dedicated efforts are required and collaborations from the laboratory to the field.

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