

BACTERIOPHAGE: A NOVEL TOOL FOR THE ERADICATION OF BIOFILM

Abstract

Biofilms are communities of organisms associated with biotic or abiotic surfaces and encapsulated in extracellular polymers. They form on biotic and abiotic surfaces, including human tissues and medical devices, posing a serious threat to chronic diseases. In addition, current antibiotics and disinfectants have limited ability to completely remove biofilms. In this review, the authors provide an overview of bacterial biofilm formation and its properties, burden, and phage evolution. In addition, recently, phages and phage-derived enzymes used to attack bacteria in the biofilm process have been described. According to the results, it can be concluded that although phages and phage-derived products have been used successfully to break down biofilms, they are often not sufficient to destroy all bacteria. However, combining phage and/or phage-derived products with other antibiotics, including antibiotics, nanoparticles, and antibiotic peptides, would be an effective way to remove biofilms from medical devices and treat their infectious diseases.

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I. INTRODUCTION

1. **Bacteriophage:** In 1915, Twort discovered that phages do not recognize molecules that inhibit bacterial growth. In 1917, D'Herelle first isolated and discovered phages and developed a phage against some typhoid fevers caused by *Salmonella gallinarum* [1]. With advances in research on the use of phages in the treatment of humans and animals, it is clear that great results have been achieved in the use of phages in the fight against infectious diseases [2]. Phages are the most abundant bacterial group in the world. Life cycles are strictly related to brain cells. Phages are also called parasites or viruses because they do not have the cell structure and enzymes necessary for food, protein synthesis or the production of new products, and harmful bacteria can recycle in cells. The ubiquity of phages is important in enhancing their penetration and ability to fight disease. Phages have been isolated from natural environments, including human and animal waste, sewage, water bodies, soil, food, and other organisms [3]. The genetic material in these organisms is encapsulated in a protein coat in the form of DNA or RNA [4]. According to the DNA structure, phages can be divided into three groups: phages with double-stranded DNA, phages with single-stranded DNA, and phages with RNA. Most phages are known to have a genome composed of double-stranded DNA. Based on capsid symmetry, two types of phages can be distinguished: isometric (polyhedral) and spiral phages [5].

- **Phage Replication Cycle:** The replication of phages and eukaryotic viruses is similar in many ways. Both are required for the adsorption, penetration, replication of nucleic acids, formation of virus particles and their release from host cells. Phages bind specifically to specific bacteria and have potent bactericidal activity against Gram-positive and Gram-negative bacteria. Some phages show affinity for a single organism type, while others have a broad spectrum of activity. The specificity of phages and the diversity of their activities depend on the presence of bacterial cell surface receptors, from which we can distinguish LPS fragments, pili and other surface proteins [6].

Most phages, except filamentous phages, have polyhedral capsids. The capsid attaches to a tail containing fibers used to attach to the receptor or to the bacterial cell surface [7]. Phages become infected and multiply in two ways; lytic life cycle and lysogenic cycle.

- **Lytic Cycle:** The lytic cycle is characteristic of phages and consists of bacterial cell-associated adsorption and binding of phage proteins to pre-recognized receptors of bacterial cells such as Gram-positive or Teichoic acid and lipoteichoic acid. Lipopolysaccharide (LPS) for Gram-negative bacteria [8].
- **The Penetration Phase:** This phase consists of the rupture of the cell wall of the bacteria by the bacteriophage enzymes and insertion of the genetic material into the host cells.
- **Eclipse Phase:** Next phase is the eclipse phase, which involves the replication of nucleic acid and proteins comprising the structural part of the capsid, while replication of the bacterial DNA is subdued. This is accompanied by the formation and maturation of the bacteriophage, lysis of the bacterial cell and the liberation of daughter phages. These daughter phages are then capable of infecting other cells [9].

T1 and T4 are the examples of bacteriophages that undergo lytic cycle.

- **Lysogenic Life Cycle:** This cycle involves the integration of the genetic material of the phage into the bacterial chromosome and its replication as part of the bacterial DNA, resulting in the emergence of a prophage [6]. When a phage resides on a chromosome, it is called a prophage and replicates with the bacterial genome during cellular replication. Often prophages encode virulence genes that can be transferred horizontally from one pathogen to another via a process called transduction [10]. Phages with the lysogenic cycle include λ *E. coli*; Mu against *E. coli*, *Salmonella*, *Citrobacter* and *Erwinia*; MM1S. lung inflammation; and ϕ 11 *Staphylococcus aureus* [11].

There are many different routes of phage infection, including chronic infection, pseudo lysogenic infection, and abortion, depending on the environment and the type of infected cell. Not all cause cell death and phage particle replication.

In general, the production of daughter virions does not involve lysis of the infected cell, so virions are not released extracellularly [9]. If severe conditions such as ultraviolet (UV) radiation occur, the prophages will disappear due to bacterial lysis. Phages are known to be ubiquitously important, infecting more than 140 genera, and can be considered the most biologically diverse with about 10^{31} phages in the world [12].

2. **Biofilm- Formation, Dispersal and the Risk of Dissemination:** Biofilm formation consists of several stages such as reversible attachment, irreversible attachment, colonization, maturation and dispersal. Organisms living in biofilms have unique mechanisms that ensure attachment to the origin site, colony formation and growth of the ecosystem, and the continuous degradation of biofilms. The adhesion of bacteria to surfaces can be accelerated by factors such as strong shear strength, bacterial mobility, and electrostatic interactions between the bacteria and the surface. In the case of "reversibly attached", there are equal amounts of attached bacteria and free-floating bacteria. However, many types of microbial cells readily attach to the surface, including flagella, pili, pili, and glycocalyx. In terms of microbial attachment to medical devices, cell-cell adhesion of bacteria to biomaterials and biomaterial-surface interactions are explained. For example, staphylococcal bacteria express cell surface proteins, particularly staphylococcal surface proteins-1 and -2 (SSP-1 and SSP-2), are limited to pilus-like polymers on the cell surface and are associated with *S. epidermidis* adhesion. relating to polystyrene [14]. In addition, capsular polysaccharide/adhesin plays a role in optimizing coagulase-negative staphylococcal isolates for biomaterials [15]. Also, *S. epidermidis* affects the adhesion of these bacteria to the polymeric surface; This protein not only adheres to the polystyrene surface, but can also bind to vitronectin, suggesting that bacterial adhesion to this role will be at an initial level. Adhesion to plasma protein coated polymer surfaces.

- **Molecular Signalling of Biofilm:** As the density of cells in old biofilms changes, gene expression of cells in the biofilm is controlled by a process called nucleation detection (QS). Thanks to this system, bacteria secrete chemical signals called auto inducers, which are constantly produced and increase as the biofilm density increases.

Some of the important factors required for bacterial survival in biofilms are prepared by physiological processes such as motility, sporulation and release of harmful substances; all change when the concentration of auto inducers that continue to cause gene mutations reaches a critical level. Gram-negative bacteria and Gram-positive bacteria secrete acyl homoserine lactone molecules and oligopeptide molecules, respectively [16]. It has been reported that *Pseudomonas aeruginosa* biofilms are associated with N-(3-oxo-dodecanoyl)-L-homoserine QS molecules. It was also investigated that N-(3-oxo-lauroyl)-L-homoserine QS molecule plays a role in suppressing the host's immune system and increasing the virulence of *Pseudomonas aeruginosa* biofilms [17].

- **Dispersal or Detachment of Bacteria from a Biofilm and the Risk of Dissemination:** When the site of the biofilm bursts and can form new areas, this drastic decision affects the host; this is a process called biofilm dispersal, which further promotes distribution in the host (Donelli, 2006). Biofilm propagation is a process that occurs at the end of the biofilm life cycle, where cells in the biofilm, which are part of a complex, static, slow-growing microbial population, are dispersed. In contrast, most infectious diseases are highly contagious [18]. Interestingly, blast cells are truly specialized cells, unlike biofilm-derived bacteria, and in addition, blast cells have the ability to attach to a new location and initiate biofilm growth.

The intracellular molecule cyclic di-GMP (c-di-GMP) has been reported to regulate the transition from biofilm to planktonic phenotype. It has also been investigated that the decrease in intracellular c-di-GMP may lead to the deterioration of some diseases. This intracellular molecule acts as a second messenger molecule in the bio membrane and is responsible for the intracellular diffusion mechanism [19]. Biofilm dispersal affects not only motile organisms but also non-motile organisms. Let's take *S. aureus* as an example. In *S. aureus*, not only has the challenge of agr-related QS regulatory genes been shown to be involved in biofilm formation, but its activation has also been reported to promote the release of *S. aureus* cells from biofilms. Various conditions, such as changes in nutrients, temperature, and oxygen, trigger the diffusion process. Sometimes bacteria in biofilms can be associated with infection with the help of chemical markers such as acyl homoserine lactones, diffusible fatty acids and peptides.

II. FACTORS INFLUENCING BIOFILM FORMATION

Biofilms and Health Care Associated Infections: Today, home medical devices such as urinary catheters, central venous catheters, endotracheal tubes (ETTs), endotracheal devices, heart valves, peritoneal nephrostomy medical devices and other household devices have become important tools in the treatment of hospitalized patients. These home medical devices are mostly used in hospital patients and are suitable for more than 25% of hospital patients. Risks associated with microbial infection and biofilm formation increase morbidity and mortality in hospitalized patients. The problem is directly related to the time the medical equipment is implanted in the hospital, the longer the time, the greater the risk of biofilm in the patient [13].

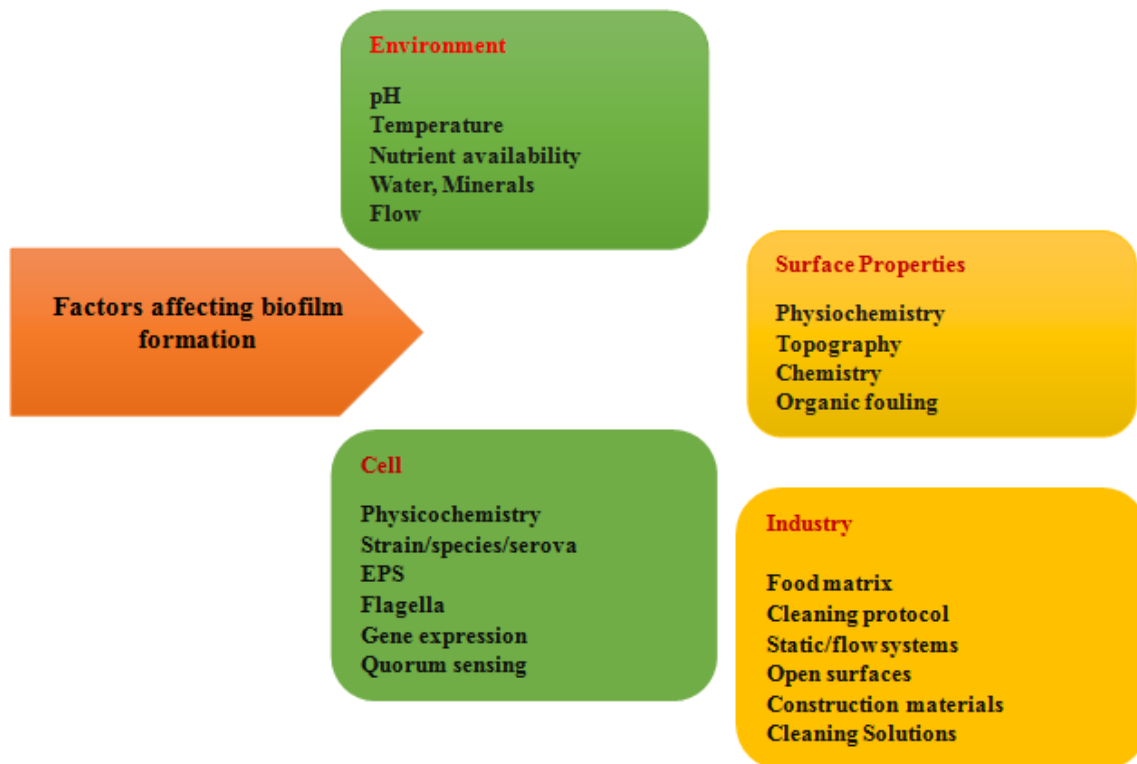


Figure 1

Nosocomial infections (HCAIs) can be caused by a variety of risk factors, including prolonged hospitalization, immunocompromised patients (post-chemotherapy, transplant patients, and patients with genetic diseases), invasive procedures, and home wound care (HPA, 2012a). The largest cause of HCAI is the nosocomial "supergerm" methicillin-resistant *Staphylococcus aureus* (MRSA), a common cause of sepsis or bacteremia in the healthcare setting. Although many organisms, including fungi, bacteria, viruses and prions, can form biofilms on medical devices, bacteria are the most common HCAIs (HPA, 2012b). Bacteria can spread to medical equipment through the skin of patients or medical personnel, contaminated water, or other external environmental sources. Too few bacteria can contaminate medical equipment and can extend to equipment problems. Although many diseases have been investigated in the treatment of infectious diseases, the most common bacteria associated with biofilm formation on medical devices and widely considered to be the main cause of HCAI are *Staphylococcus epidermidis* and *Staphylococcus aureus* [14].

Micro-organisms such as *A. baumannii*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* have been recognized as multidrug -resistant Gram-negative bacteria and are becoming more widespread in long-term care facilities and acute care hospitals. In actuality, these species are frequently the reason of biofilm-based HCAIs, including catheter-associated urinary tract infection (CAUTI) [21].

III. PHAGE THERAPY

In 1919, phages were first used as a therapeutic agent in human, that was the time when they were just discovered [22]. Phage therapy started back in 1896, when the existence

of antibacterial activity against *Vibrio Cholera* the causative agent of cholera was first reported by Ernest Hankin, cholera was considered one of the deadliest peril humans had faced. Frederick Twort, in 1915 hypothesized that antibacterial activity could be due to the phage, but he did not follow his discovery therefore in 1917, bacteriophages were discovered by Fe'lix d'He'relle. In 1925, d'He'relle drew attention towards phage therapy by reporting the treatment of plague (four types) by anti-plague phages. The Eliava institute in Tbilisi Georgia is considered the pioneer in this regard where phage therapy is extensively studied and applied [23].

1. Phages as Antibacterial Agents: Phages are prominent candidates for exploitation as antibacterial agents as they are the natural killers of bacteria. Phages have multiple intrinsic characteristics which make them delightful candidate for such applications. Phages cannot incorporate their DNA into the genome of eukaryotic cells or can replicate in such cells. They are highly specific in their bactericidal potential. Some phages are strain specific and they normally target a single bacterial species, which means that there is little or no effect on the natural microbiota of the patient/animal. This is a powerful advantage over the many broad range anti-microbials (including antibiotics) which are commonly used now a days. Besides, there have been no reports of harmful effects on eukaryotic cells by purified phages. Phages are non-toxic in nature because humans are exposed to phages in the natural environment every day without any adverse side effects. Phages multiply in the presence of the target bacteria and are not able to multiply in its absence and thus ultimately get eliminated from the human/animal which indicates that the phages are considered to be self-dosing. Phages also have the aptness to lyse bacteria present in a biofilm, mucous membrane and suppurative wounds. These are bacterial niches, which are unsuitable for treatment with antibiotics [24].

2. Other Potential Applications of Bacteriophages

- **Phage Display:** In 1985, Smith first proposed phage display. Phage display is probably the first phage used as a tool in modern biotechnology. Phage display is a unique molecular technique for combining peptides with new products. In this process, the protein-coding DNA is fused to the phage coat protein gene and the desired protein is expressed on the surface of the phage fragment or phage. The most widely used phage in phage display technology is M13, the filamentous phage of *Escherichia coli*. In addition, phages such as lambda phage and T7 phage are widely used in phage display systems [4].

Phage libraries can be used for analysis and isolation of peptides, these peptide molecules are specific and have a good relationship with the target protein. These unique peptides can be used as reagents in drug development to validate molecular recognition as well as reduce receptor limitations. These peptides can also be used as therapeutic agents by inhibiting receptor-ligand interactions or acting as agonists [25].

Other than this, phage display can be used for various applications which include mapping of epitopes, in vaccine design, in study of interaction between protein-protein, in determination of specificity of enzymes and inhibitors, screening for anticancer peptide and protein and in the screening for receptors [26].

- **Phage Typing:** Phages can infect and destroy only one or a few types of organisms, making them unique to their hosts. The specificity of these phages is very useful and widely used in bacterial infections, making them useful for typing viruses, a process called phage typing and detection of viruses [27]. In phage typing, specific phages use sensitive models to identify microbial pathogens. The sensitivity of detection can be increased if phages that bind to bacteria are detected by specific bacteria [28]. To identify unknown bacteria, many different phages are placed in the lawn of unknown bacteria, plaques (open areas) will form on the lawn when the phages start to infect and degrade a certain type of bacteria, which is easier for Research to identify the disease. specific diseases. In addition, there are other methods commonly used to identify pathogenic bacteria; such as the use of phages that specifically provide genes (such as Lux) or the use of green fluorescent protein expressed after infection. [4].
- **Phages used as Vehicle for the Delivery of Vaccines:** Phages are widely used as vaccine delivery vehicles. Phages can be used in two different ways. First, phages can be used directly, they carry antigens to their surface. Second, in DNA vaccines, the genes responsible for the synthesis of antigens are first integrated into the phage genome, which then blocks the DNA, which will act as a vehicle for vaccine delivery. Phage can be generated using phage display technology to display antibody peptides on its surface [4]. All antigenic peptides expressed in phage have been used as vaccines in animal models in various studies.
- **Phages used in Targeted Gene Delivery:** Phages have been used as special delivery vehicles and are widely used [29]. The phages used for gene delivery are similar to those used for DNA vaccine delivery, in which the inner DNA is protected by the phage's coating. The phage coating protects the DNA from degradation after injection. The ability of phages to display foreign proteins on their surface allows them to target specific cells necessary for effective gene therapy. Phage display and covalent conjugation are widely used to release targets and form molecules on the phage surface [30].
- **Phages Therapy in Plants:** For the elimination of plant pathogens, bacteriophages have been widely used as a bio-control. Phage mediated bio-controls of plant pathogens such as *Xanthomonas pruni* associated with the infection of cabbage, peppers and peaches, *Ralstonia solanacearum* cause infection in tobacco. They have been successfully used against *Xanthomonas campestris* cause spots on tomatoes and against *Pseudomonas talaasi* which cause blotch of mushrooms [31].

The use of phage therapy in plants is still in its infancy. New phages are continually being discovered and used in many ways for the use of phage therapy. Treatment of infected crops with the aid of phage therapy has been successful, including the treatment of some diseases associated with soybean blight [32]. Many phages have been approved by the US Food and Drug Administration (FDA) for use in products intended for human consumption. Many companies and organizations are focusing on the discovery, isolation, and commercialization of phage products to control disease, healthcare, and food processing in the environment, and in hopes of reducing product losses and production costs [33].

- **Phage Therapy in Animals:** Bacteriophages are widely used for the treatment and elimination of infection in chickens, calves, pigs and lambs, infections caused by *Escherichia coli* and *Salmonella*. In 1983, experiment performed by Smith and Huggins proves that, a single dose of phage R is very effective in preventing death in mice due to septicemia caused by *E. coli*, this infection cannot be treated with multiple doses of antibiotics. They eventually used bacteriophages for the treatment of infections caused by *E. coli* (entero toxigenic) in neonatal lambs, calves and pigs [34]. Animal studies have shown that phage therapy works in animal system. However, many experiments with animals, recommended that the therapeutic application of phages in animals have some advantages over antibiotics.
- **Phages in Food Industry:** Foodborne diseases are the contamination of food that are caused by many pathogenic bacteria. Currently, food borne pathogens like *Listeria* and *Salmonella*, followed by *E. coli* and *Campylobacter jejuni* are the major cause of death. Bacteriophages can be used effectively and safely to eradicate the food born pathogenic bacteria that contaminate the food. For example, *Listeria* phage P100 (under the commercial name Listex P100) was developed to eradicate biofilms present in processed meat products and on factory working surfaces, and has already been authorized in the United States by the Department of Agriculture (Fister *et al.*, 2016). Other commercial bacteriophages products have been targeted for other pathogenic species as for example *S. enterica* or *E. coli* (SalmofreshTM and ScoShieldTM, respectively) [35].
- **Eradication of Biofilm by Phages:** Formation of biofilm is an important strategy that is adopted by the bacteria for the survival purpose. During this strategy, microorganisms secrete huge amount of extracellular polymer. Microorganisms' aggregates to form biofilm either on living or non-living surfaces. Normally, environmental conditions stimulate most of the bacteria to form biofilm. In humans, formation of biofilms causes severe diseases and the biofilm is resistant to antibiotics and host immune system. An alternative therapy must be needed to control biofilm-associated diseases, since biofilms are resistance to anti-microbial drugs [36].

But outside of the capsid, phages are equipped with enzymes (such as EPS deloplimerase) that break down extracellular polymers and infect bacterial biofilms, allowing the phage to enter bacteria embedded in the EPS matrix. Once the lytic cycle is complete, the phage offspring are released, removing the biofilm embedded in the bacterial layers, increasing biofilm dispersal. In order to prevent biofilms, high doses of vaccines are often needed to observe the effects of biofilms with minimal biohit.

In one of the studies, Gabisoniya found that the implementation of phages on *in-vitro* colonies of the pathogen *P. aeruginosa* not only prevented additional biofilm formation but it also degraded existing biofilm. Biofilm formed on the surface of the medical devices by *L. monocytogenes*, *P. aeruginosa*, and *Staphylococcus epidermidis* have been eliminated via phage treatment [38].

These discoveries are highly admissible to the problem of persistent infections caused by implanted medical devices such as catheters, lenses, and prostheses where biofilm formation is common.

IV. ADVANTAGES OF PHAGE THERAPY

Phages are natural, latent and special agents with many therapeutic advantages over conventional antibiotics. Phages can control bacterial cells by inducing bacterial lysis. It is known that phages are active against both Gram-positive and Gram-negative bacteria and are also active against bacteria that are highly resistant to antibiotics in the environment [39].

Bacteriophages have some of the most desirable properties and important advantages that make them enthralling applicant for tackling antibiotic resistance in bacteria. Some of the most important advantages are given as follows:

- Bacteriophages are environmentally friendly.
- Isolation and identification of suitable bacteriophage for the phage therapy is rapid, relatively easy and cost effective.
- Bacterial resistance against phage develops about ten times slower than the resistance to that of antibiotic [40].
- Under very harsh environmental conditions, phages tend to replicate continuously and they remain infective until the host bacterial population density has been significantly reduced [41].
- Once the bacteria get lysed by the lytic phage it will not be able to regain its viability; by contrast antibiotic therapy, in which the targeted bacteria might not get killed, which facilitate the development of antibiotic resistance [42].

Apart from these generalized advantages there are some specific advantages that the phage therapy have, they are as follows:

- Phages are considered to self-dosing or auto-dosing, because phages are capable of increasing in number in the presence of host bacteria (depending on the host population density) and are not able to multiply in its absence and thus ultimately gets eliminated from the human/animal.
- Bacteriophages are highly specific for their host cell is another advantage of phage therapy over antibiotics. They target specific bacterial strain without affecting the normal beneficial bacterial micro flora of the body, due to this property the chance of secondary infections is reduced.
- Low inherent toxicity, phages are inherently non-toxic, since phages consist mostly of nucleic acids and proteins. Most of the phages exhibit relatively narrow host range which limits the number of bacterial types with which selection for specific phage-resistance mechanism can occur. Thus, the chances of resistance associated with phage therapy is low. Because when phages infect and kill bacteria, the mechanism they use is different from those of antibiotics, specific antibiotic resistance mechanism do not translate into mechanism of phage resistance.
- Bacteriophages are non-toxic to humans, thus there are no severe side effects and is suitable for use in humans, since phage do not infect eukaryotic cells [40].

REFERENCE

- [1] Atterbury, R.J. (2006). The age of phage. *Poult Int.*;45:18–22.
- [2] Summers. W. (2005). Bacteriophage research: early history. In: Kutter E, Sulakvelidze A, editors. *Bacteriophages biology and applications*. Boca Raton: Crc Press;. p. 5–27.
- [3] Leverentz, B., Conway, W.S., Janisiewicz, W., Camp, M.J. (2004). Optimizing concentration and timing of a phage spray application to reduce *Listeria monocytogenes* on honeydew melon tissue. *J Food Protect.*;67:1682–6.
- [4] Clark, J.R. and March, J.B. (2006). Bacteriophages and biotechnology: vaccines, gene therapy and antibacterials. *Trends in Biotechnol* ; 24: 212-18.
- [5] Brüßow, H., Kutter E. (2005). Phage ecology. In: Kutter E, Sulakvelidze A, editors. *Bacteriophages biology and applications*. Boca Raton: Crc Press; p. 128–63.
- [6] Domingo-Calap, P., Georgel, P., Bahram, S., (2016). Back to the future: bacteriophages as promising therapeutic tools. *HLA.*;87:133–40.
- [7] Ackerman, H.W. (1998). Tailed bacteriophages: the Caudovirales. *Adv Virus Res* , 51:135-201.
- [8] Rakhuba, D.V., Kolomiets, E.I., Szwajcer Dey, E., Novik, G.I., (2010). Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol J Microbiol.*;59:145–55.
- [9] Weinbauer, MG. (2004). Ecology of prokaryotic viruses. *FEMS Microbiol Rev.*;28:127–81.
- [10] Boyd, E.F., Brussow, H., (2002). Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. *Trends Microbiol* 10: 521–529.
- [11] Guttman, B., Raya, R., Kutter, E. (2005). Basic phage biology. In: Kutter E, Sulakvelidze A, editors. *Bacteriophages biology and applications*. Boca Raton: Crc Press;. p. 29–66.
- [12] Bergh, O., Borsheim, K.Y., Bratbak, G., Heldal, M. (1989). High abundance of viruses found in aquatic environments. *Nature*340: 467–468.
- [13] Donlan, R. M. (2001). Biofilms and device-associated infections. *Emerg Infect Dis* 7, 277–281.
- [14] Von Eiff, C., Heilmann, C., Peters G. (1999). New aspects in the molecular basis of polymer-associated infections due to staphylococci. *Eur J Clin Microbiol Infect Dis* 18, 843–846.
- [15] Muller, E., Hußner, J., Gutierrez, N., Takeda, S., Goldmann, D. A., Pier, G. B. (1993). Isolation and characterization of transposonmutants of *Staphylococcus epidermidis* deficient in capsular polysaccharide/adhesin and slime. *Infect Immun* 61, 551–558.
- [16] Lindsay, D. and von Holy, A. (2006). Bacterial biofilms within the clinical setting: what healthcare professionals should know. *J Hosp Infect* 64, 313–325.
- [17] Driscoll, JA., Brody, SL. & Kollef, MH. (2007). The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* 67, 351–368.
- [18] McDougald, D., Rice, S. A., Barraud, N., Steinberg, P. D. & Kjelleberg, S. (2012). Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. *Nat Rev Microbiol* 10, 39–50.
- [19] Karatan, E. and Watnick, P. (2009). Signals, regulatory networks, and materials that build and break bacterial biofilms. *Microbiol Mol Biol Rev* 73, 310–347.
- [20] Kaplan, S.L., (2010). editors. *Textbook of pediatric infectious diseases*. 6th ed. PA, USA: Saunders Elsevier; pp. 725–42.
- [21] Niveditha, S., Pramodhini, S., Umadevi, S., Kumar, S. & Stephen, S. (2012). The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). *J Clin Diagn Res* 6, 1478–1482
- [22] Summers, W.C. (1999). *Bacteriophage discovered. Felix d’Herelle and the Origins of Molecular Biology* Yale University Press, 47-59.
- [23] Sulakvelidze, A., Kutter E. (2005). Bacteriophage therapy in humans. In: Kutter E, Sulakvelidze A, editors. *Bacteriophages: Biology and Application*. Boca Raton, FL: CRC Press;. pp. 381–436.
- [24] Azeredo, J. and Sutherland, I., (2008). The use of phages for the removal of infectious biofilms. *Current pharmaceutical biotechnology*;9(4):261.
- [25] Sidhu, S.S. (2000). Phage display in pharmaceutical biotechnology. *Curr Opin Biotechnol*, 11(6):610-616.
- [26] Krylov, V.N., Tolmachova, T.O., Akhverdyan, V.Z. (1993). DNA homology in species of bacteriophages active on *Pseudomonas Aeruginosa*. *Arch Virol.*;131:141–51.
- [27] Gill, J., Abedon, S.T. (2012). Bacteriophage ecology and plants. *Virology J*; 9: 9.
- [28] Watson, B.B. and Eveland, W.C. (1965). The application of the phage fluorescent antiphage staining system in the specific identification of *Listeria monocytogenes*. I. Species specificity and immunofluorescent sensitivity of *Listeria monocytogenes* phage observed in smear preparations. *J Infect Dis*, 115(4):363-369.

- [29] Barry, M.A., Dower, W.J., Johnston, S.A. (1996). Toward cell-targeting gene therapy vectors: selection of cell-binding peptides from random peptide presenting phage libraries. *Nat Med*, 2(3):299-305.
- [30] Larocca, D., Kassner, P.D., Witte, A., Ladner, R.C., Pierce, G.F., Baird, A. (1999). Gene transfer to mammalian cells using genetically targeted filamentous bacteriophage. *FASEB J*, 13(6):727-734.
- [31] Hertwig, S., Hammerl, J.A., Appel, B., Alter, T. (2013). Post-harvest application of lytic bacteriophages for biocontrol of foodborne pathogens and spoilage bacteria. *Berl Munch Tierarztl Wochenschr* ; 126(9-10): 357-369.
- [32] Susianto, G., Farid, M.M., Dhany, N.R., Addy, H.S. (2014). Host range for bacteriophages that infect bacterial blight pathogen on soybean. *Procedia Environ. Sci.*, 20, 760–766.
- [33] Meaden, S. and Koskella, B. (2013). Exploring the risks of phage application in the environment. *Front. Microbiol.*, 4, 358.
- [34] Smith, H.W. and Huggins, M.B. (1983). Effectiveness of phages in treating experimental *Escherichia coli* diarrhea in calves, piglets and lambs. *J Gen Microbiol*; 129: 2659–2675.
- [35] Gutiérrez, D., Rodríguez-Rubio, L., Martínez, B., Rodríguez, A., and García, P. (2016). Bacteriophages as weapons against bacterial biofilms in the food industry. *Front. Microbiol.*7:825. doi: 10.3389/fmicb.2016.00825.
- [36] Azeredo, J. and Sutherland, I., (2008). The use of phages for the removal of infectious biofilms. *Current pharmaceutical biotechnology*;9(4):261.
- [37] Anwar, H., Strap, J.L., Chen, K., Costerton, J.W. (1992). Dynamic interactions of biofilms of mucoid *Pseudomonas aeruginosa* with tobramycin and piperacillin. *Antimicrob Agents Chemother* ;36: 1208-1214
- [38] Motlagh, A.M., Bhattacharjee, A.S., Goel, R. (2016). Biofilm control with natural and genetically-modified phages. *World J Microbiol Biotechnol*; 32: 67.
- [39] Wittebole, X., Rock, S.D., Opal, S.M. (2014). A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence* 5, 226–235. doi:10.4161/viru.25991.
- [40] Parasion, S., Kwiatek, M., Gryko, R., Mizak, L., Malm, A. (2014). Bacteriophages as an alternative strategy for fighting biofilm development. *Pol J Microbiol*; 63:137-145.
- [41] Schmelcher, M. and Loessner, M.J. (2014). Application of bacteriophages for detection of foodborne pathogens. *Bacteriophage*, 4, e28137.
- [42] Stratton, C.W. (2003). Dead bugs don't mutate :susceptibility issues in the emergence of bacterial resistance. *Emerg. Infect. Dis.* 9, 10–16. doi: 10.3201/eid0901.020172.

