The Pharma Innovation Journal 2021; 10(5): 389-394

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(5): 389-394

www.thepharmajournal.com
Received: 05-03-2021
Accepted: 14-04-2021

Dr. Satyabrat Dutta
M.V.Sc. Scholar, Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, India

Dr. Richa Sarkar
M.V.Sc. Scholar, Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, India

Dr. Kaushik Poran Bordoloi
M.V.Sc. Scholar, Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, India

Dr. Champak Deka
M.V.Sc. Scholar, Department of Veterinary Medicine, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, India

Dr. Palash Jyoti Sonowal
M.V.Sc. Scholar, Department of Veterinary Surgery and Radiology, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, India

Corresponding Author:
Dr. Satyabrat Dutta
M.V.Sc. Scholar, Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, India

Bacteriophage therapy to combat antibiotic resistance: A brief review

Dr. Satyabrat Dutta, Dr. Richa Sarkar, Dr. Kaushik Poran Bordoloi, Dr. Champak Deka and Dr. Palash Jyoti Sonowal

Abstract
Antimicrobials or antibiotics are one of the most fruitful forms of therapy against pathogenic microbial infection however their broad and frequently indiscriminate use resulted in a widespread antimicrobial resistance that has caused a significant threat to public health and environmental security. Therefore, implementation of a substitute but potent effective ways to kill pathogenic bacteria and reduce the environmental risks are urgently needed. As a unique century-old natural remedies, bacteriophage (phage) therapy has a high efficiency in detecting and killing pathogenic microorganism in numerous environmental or ecological systems. Phages are the viruses which usually target and cause lysis of bacteria naturally without any antagonistic effect on human or animal bodies. For this reason, many scientists across the world have proposed that they can be used, alone or together with typical antibiotics, to treat pathological conditions. The increasing risk of antimicrobial resistance has revived the interest on Phage therapy. The main motive of this article is to provide a brief but comprehensive review of all aspects of natural phage therapy to counter the antibiotic resistance on a global scale.

Keywords: Antimicrobial, antibiotics, antimicrobial resistance, bacteriophage, phage therapy

Introduction
Antimicrobial resistance (AMR) is a global, growing and major public health problem which threating to life of both human and animals. With the increasing of the world economy in the last 2-3 decades, a cumulative amount of antibiotics has been unconstrained into the environment along with the waste from households, industries, agricultures, animal husbandry sectors and medical activities such as hospitals or pharmaceutics etc. (Chen *et al*., 2016; Fang *et al*., 2018; Garner *et al*., 2019; Jiao *et al*., 2018) [12, 23, 26]. This has resulted in the selection and spread of antibiotic-resistance genes/bacteria in the surrounding environment, which threatens the efficacy and potency of antibiotic in battling bacterial infections (Burch *et al*., 2017; Chen *et al*., 2018; Couch *et al*., 2019; Liu *et al*., 2019) [9, 11, 16, 41]. The World Health Organization, on its 1st global report on resistance of antibiotics, has highlighted the threat of an approaching post anti-microbial era, in which many common infections will no longer have a cure (Cohen, 1992) [15]. The Centre for Disease Control (CDC) estimates that at least 23000 casualties per anum in the United States are an outcome of infection by drug resistant bacteria (Jernigan *et al*., 2020) [32]. On the other hand, pharmaceutical companies are showing a less interest of developing and manufacturing novel antimicrobial agents, the reason behind is antibacterial market being less gainful than other pharmaceutical products (Projan, 2003) [53]. Considering the condition, the possibility of developing chemo-therapeutic agents that are substitute to antimicrobials possibly will be of great aid in the fight against resistance to antibiotics. The complications in the treatment of many lethal bacterial infections have led scientists to review phages as therapeutic agent. Bacteriophages and bacteriophage-based products have the potential to become one of the most successful replacements to combat antibiotic resistance (Czaplewski *et al*., 2016) [17]. Phages are the viruses that attack only a single bacterial strain or species/strains.This specificity together with the killing capacity makes them the natural enemies of bacteria without any negative effect on human or animal cells (Dewangan *et al*., 2017; Lyon, 2017) [18, 42]. For this cause, it is supposed that phage can be used, alone or in together with antibiotics, to treat pathogenic bacterial infections (Domingo-Calap *et al*., 2016) [20]. Several retrospective studies on use of Bacteriophages *in vitro*, in laboratory animals as well as in humans have been conducted in both the USA and Europe.

The novel interest in phages is exemplified by their wide use as medicinal and biological control purposes in agriculture and related fields of food production and animal husbandry etc. in many European countries.

Bacteriophage

Bacteriophages are very small viruses of about 20-200 nm in size, are probably the most ancient and ubiquitous existing viruses on biosphere (keen, 2015) [36]. As they are omnipresent in the environment including soil, water, air, and the intestines of humans and other animals, (Simmonds and Aiewsakun, 2018; Yu *et al*., 2016) [3, 56] and can be isolated for therapeutic purposes from any environmental source, in which the target pathogen is likely to be presented, with sewage probably being the richest source of bacteriophage (Weber-Dabrowska *et al*., 2016) [64]. They mostly infect their host bacterium in a species or even strain specific manner. The structures of bacteriophages are relatively simple and composed of mostly proteins (60%) that encapsulate a specific genome (40%) which is either DNA or RNA but never both (Williamson *et al*., 2017) [66]. They have been categorized according to their morphological characters, nucleic acid content, the site of predilection, and the specificity of bacterial species they attack. On the basis of their morphology and genomic nucleic acid phages can be classified into Siphoviridae (with long, non-contractile tails), Podoviridae (short, stubbed tails), Myoviridae (with long, contractile tails), and Filamentous phages (International Committee on Taxonomy of Viruses, ICTV). As depicted in Fig. 1, a typical phage structure is consisting of a capsid(head), filled with DNA, long tails with a collar or neck, a base plate with short spikes and tail fibres (Adriaenssens and Brister, 2017; Tolstoy *et al*., 2018) [2, 58]. Most of the phages genome have double-stranded DNA (dsDNA) while a small proportion has single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), or single-stranded RNA (ssRNA) (Aiewsakun *et al*., 2018) [3, 56]. On the basis of mode of replication whether or not their DNA genome is integrated into the bacterial genome, phages can also be classified into lytic or virulent phages and lysogenic or temperate phages (Bao *et al*., 2018; Hobbs and Abedon, 2016) [5, 28]. Their replication cycle implies adhesion to and invasion of the bacterial surface. However, to initiate attachment, structures of phage have to match with the receptor present in strain-specific variants of bacteria. Once the Phage enters the bacterial, the host cell synthetic machinery is redirected to the production of new phage genome and viral proteins. Finally, assembly and packing of virus particle occurs, and host are lysed with the release of new phage virions that can infect another host cell bacterium (Young, 2013) [68]. Most of the virulent phages use two kinds of proteins to lyse their host bacterial cell, the holins and the endolysins. The holins are utilized for the puncture of the bacterial cell membrane and work as a synergistic tool for the endolysins, which are accountable for the lysis of the bacterial cell wall (Cisek *et al*., 2017) [14]. In temperate phages, the other type, have a different life cycle and infect their respective bacteria by initiating a lysogenic cycle, where the phage particle remains as dormant (prophage) state, replicates along with its host but occasionally excise from host chromosome and bursts into a virulent cycle under a specific trigger (e.g., ultraviolet, high temperature, ionizing radiation, antibiotics, or heavy metals) (Kutter and Sulakvelidze, 2004) [39].Lysogeny and dormant phage can be beneficial to host as they can encode genes for antimicrobial resistance or other factors for virulence (Lin *et al*., 2017) [40]. For that cause, only virulent phages should be used for the purpose of phage therapy (Kim and Bae, 2018; Wang *et al*., 2018) [37, 61].

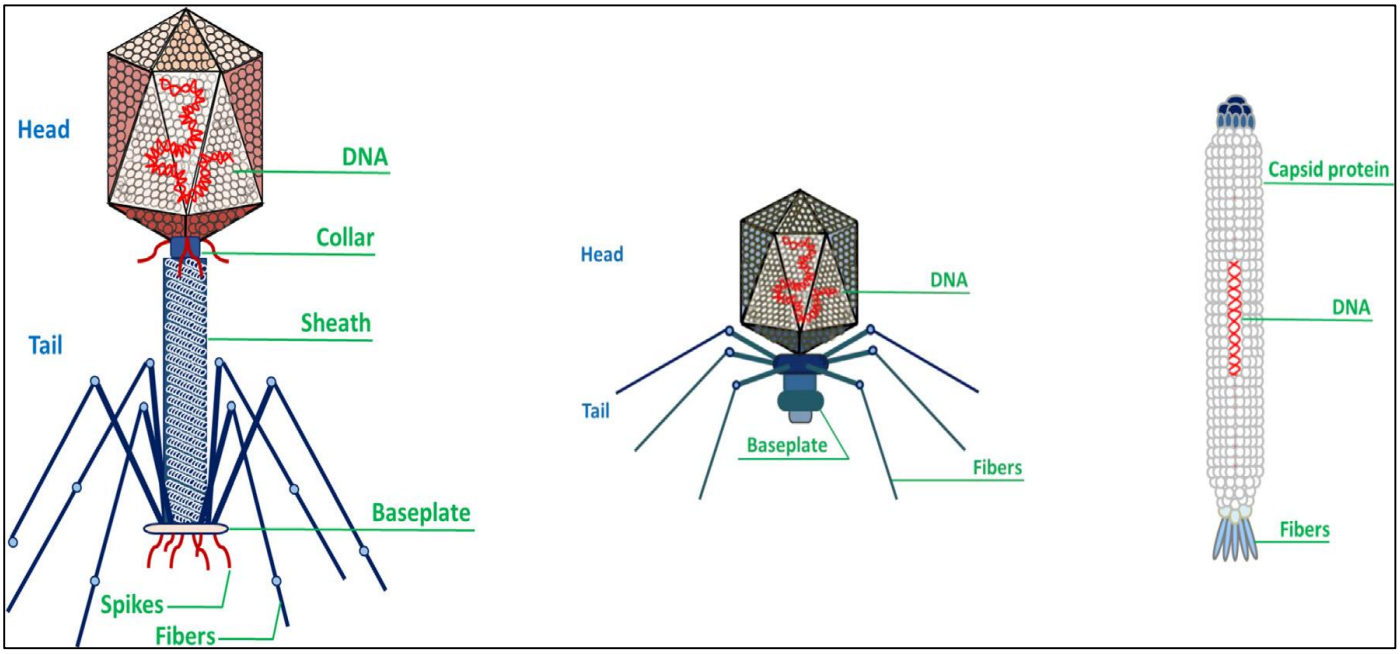

Fig 1: Structural representation of a typical phage (Ye *et al*., 2019) [67]

History

In the year 1896, Ernest Hanbury Hankin reported one factor within the waters of the Ganges and Yamuna rivers in India which could pass via a very fine ceramic filter. He prompts that this was accountable for decreasing the spread of cholera epidemics of India. Hankin published his observations in the annals of the Institute Pasteur in 1896 – which was the first primary proof of the presence of bacteriophages like virus in water and having antibacterial activities. In 1915, Frederick W. Twort, a UK physician, was the forerunner to explain the potential presence of an "ultra-microscopic virion" that would lysis bacteria. He came to this statement after meeting

difficulties with his vaccine preparations with contaminating bacteria. Twort observed "glassy and transparent" spots within the colonies of the contaminating microbes, which appeared to be zones of dead bacteria. He gave some attainable clarifications for this phenomenon, together with the chance of the presence of a microscopic virus, but he was unable to confirm his hypothesis (Twort, 1915) [59]. In 1915, The Lancet published an article written by Frederick Twort about ''the transmissible bacterial lyses''. It was the first publication on bacteriophages. (The Lancet, Jan.10th, 1914, pp.101). After 2 year in 1917, Felix d'Herelle, a French-Canadian scientist, observed a same phenomenon in faecal cultures of patients improving from bacillary dysentery. Unlike Twort, d'Herelle was convinced that the cause of bacterial death was the existence of an "invisible microbe", an ultra-microscopic virus which he called bacteriophage (d'Hérelle, 2007) [19]. After the discovery of bacteriophages, the idea of phage therapy was taken with enthusiasm and was implemented in many cases, but its application subsequently subsided after the discovery of antibiotics and their use in the second World War, flourishing only behind the "curtain" (Abedon *et al*., 2011) [1].

Phage therapy

Phage therapy is that the therapeutic use of whole bacteriophages or their product so as to treat numerous pathogenic bacterial infections in human beings or animals (Pires *et al*., 2017; Sun *et al*., 2019) [52, 57]. Phage therapy has a protracted history, yet there are a lot of problems associated with its clinical application. At the early-stage phage therapy was applied to cure human bacterial infections like pneumonia, urinary tract, septicaemia during 1940s; it was then overlooked by the people following discovery of antibiotics (Kot, 2018) [38]. However, the phage therapy came back again to researchers' visualizations due to counter-increasing antibiotic resistance within the last decade, that not solely energized the implementation of phage therapy for the clinical use, but additionally simplified its application to control pathogenic bacterial infection in natural environments (Dou *et al*., 2018) [21].

Advantage

Hypothetically, there aren't any bacterium that can't be killed by at least one bacteriophage (Kakasis and Panitsa, 2019) [34]. As bacteriophage has a very fine range of effect, that overcome the most important disadvantage associated with antibiotic treatment, i.e., the impact on whole microbiome with elimination of beneficial microbes (Domingo-Calap *et al*., 2016) [20]. Moreover, after inactivation of the host pathogens, once the host microbes reduce, the bacteriophage count conjointly declines, that equilibrates the microbial stability and diversity (Paez-Espino *et al*., 2016; Watts, 2017) [49, 63].

In divergence to conventional antibiotics, phages are supposed to have some other benefits. It is assumed that phages are pointedly safer and better tolerated, as they multiply only in the target host but can't infect human or animal cells. Furthermore, administration is much easy, as bacteriophages don't require frequent administrations just after one another over several days (Bogovazova *et al*., 1991) [7]. On the other hand, very limited doses are needed because of the upsurge in phages concentration in the infection site after the first administration.

Animal model study

Numerous animal studies have given promising outcomes of phage therapy in the clinical trials of infections with these pathogens.

- Watanabe *et al*. (2007) [62] demonstrated that phage therapy induced significant protection against *Pseudomonas aeruginosa* of gut-derived sepsis in a mouse model.
- Fukuda *et al*. (2012) [25] revealed the high efficacy in *Pseudomonas aeruginosa* elimination after phage eye-drop administration in a murine model of Pseudomonas keratitis.
- Hua *et al*. (2018) [29] demonstrated that intranasal administration of bacteriophages protected neutropenic mice from lethal carbapenem-resistant *Acinetobacter baumannii* lung infection.
- Hung *et al*. (2011) [30] reported that bacteriophages, orally or intraperitoneally administered, protect mice from death and reduce liver damage in an intragastric model of *Klebsiella pneumoniae* infection, meanwhile Wang *et al*. (2006) [60] figure out that intranasal phage treatment of *Klebsiella pneumoniae* lung infection in a murine model increased survival in a dose-dependent manner and reduced the bacterial burden in the infected mouse lungs.
- A fresh study of *Escherichia coli* pneumonia in a mouse model, conducted by Dufour *et al*. (2015) [22] revealed 100% survival rate of phage treated mice and pointed out a phage effectiveness which was similar to the antibiotic ceftriaxone with regard to the survival rate and bacterial load of the lungs.
- Bacteriophage therapy has confirmed satisfactory efficiency in murine bacteraemia models of vancomycin-resistant *Enterococcus faecalis* (Cheng *et al*., 2017) [13] and vancomycin-resistant *Enterococcus faecium* (Biswas *et al*., 2002) [6].

Studies in human model

There are some reports on successful bacteriophage therapy in humans. Advantageous effects of phage therapy of localized infections in burns, wounds and trophic ulcers were stated during and after the Second World War in various part of Europe (Morozova *et al*., 2018) [46].

- Indicatively, two recent case reports, one concerning a patient with *Pseudomonas aeruginosa* aortic graft infection and the other concerning a patient with *Pseudomonas aeruginosa* septicaemia, demonstrated a favourable outcome after bacteriophage therapy. (Chan *et al*., 2018) [10]
- Positive results were shown in several studies that used bacteriophages in patients with infected ulcers. patients with diabetic foot ulcers infected by *S. aureus* strains benefited from the administration of the Staphylococcus phage Sb-1 (Jault *et al*., 2019) [31], even when bacteriophage administration therapy was the only antimicrobial treatment (Rhoads *et al*., 2009; Fish *et al*., 2018) [54, 24].
- The potential effectiveness of bacteriophage therapy could be of great use especially in patients with chronic multi-drug resistant infections. Certainly, the most highly anticipated results are those of the Phagoburn project, which aims to evaluate the efficacy of phage therapy preparations against Escherichia coli and Pseudomonas aeruginosa in burn victims (phagoburn project).

Phage derived proteins and engineered phages

Apart from their utilization as whole phage therapy, the potential of phage applications in the fight against bacterial host can be expanded in many directions. As stated above, phages use their proteins called lysins or endolysins to hydrolyse and damage the bacterial cell wall components. These endolysins can be utilized as antibacterial, particularly against gram-positive bacteria, because the gram-negative bacteria having an outer membrane which protects the endolysin-susceptible peptidoglycan layer (Nelson *et al*., 2012) [47]. In order to improve the efficacy of endolysins against gram-negative bacteria, several studies have suggested explanations that selection of endolysins that have a natural tendency to destroy gram-negative bacteria by disordering the outer membrane, the combination of lysins with other agents that can destabilize the outer membrane or the genetic engineering of the endolysins (Oliveira *et al*., 2018; Pires *et al*., 2016) [48, 51].

Problems and Limitations of phage therapy

In the last century, since the encounter of bacteriophages, phage therapy has shown cheerful success levels, while some phage therapy studies had been proven unsuccessful. The key factors for this disappointment mainly consist in the unsuitable phage selection, preparation and storage.

- Identification of a therapeutic phages is very complex. However, before a phage is considered as a potential antibacterial agent, it has to be established that it is specific for a given bacterial spp. (Mattila *et al*., 2015).
- For clinical implementation of a phages, stabilization strategies should be optimized for each phage separately (Merabishvili *et al*., 2013).
- Emergence of bacterial resistance against phages is potentially possible, as bacteria have or can develop some mechanisms to prevent viral infections e.g., change or loss of receptor, hiding, secretion of substances etc. (Seed, 2015).
- Lysogenic phages incorporate their genome into the bacterial chromosome. Subsequently, they might be vehicles for horizontal exchange of genetic material and play a role in the diffusion of antibiotic resistance genes (Brabban *et al*., 2005; Maiques *et al*., 2007) [43].
- Bacteriophages and their products are non-self-antigens, and they can be recognized by the host immune system and induce responses that can diminish the benefit of phage therapy. (Kazmierczak *et al*., 2014).

Conclusion

Multidrug-resistant bacteria have opened a second window for phage therapy. Use of bacteriophages to overcome the risk of increasing antimicrobial is attractive, and some research publications shown that it might be a rational measure. But present knowledge is insufficient to continue the use of phages for therapeutic purpose. Till date, properly premeditated clinical trials to evaluate bacteriophage efficacy are very less. Moreover, the problem of how to prepare the designs for standardized and clinical use in bacterial control, how to avoid or limit the risk of emergence of bacterial resistance and the transmission of genetic material are yet to be solved. Still a hope that, with the help of new generation technologies combined with careful scientific applications bacteriophages can be one of the most powerful options for treatment of bacterial infections in human and animals as well as a potent weapon to combat antimicrobial resistance.

References

1. Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. Phage treatment of human infections. Bacteriophage 2011;1(2):66-85.
2. Adriaenssens E, Brister JR. How to name and classify your phage: an informal guide. Viruses 2017;9(4):70.
3. Aiewsakun P, Adriaenssens EM, Lavigne R, Kropinski AM, Simmonds P. Evaluation of the genomic diversity of viruses infecting bacteria, archaea and eukaryotes using a common bioinformatic platform: steps towards a unified taxonomy. The Journal of general virology, 2018;99(9):1331.
4. Antibiotics / antimicrobial Resistance | CDC [Internet]. 2018 [cited 2018 Apr 15]. Available from: https://www.cdc.gov/drugresistance/index.html
5. Bao HD, Olaniran A, Zhang XH, Zhang H, Zhou Y, Sun LC *et al*. Alterations in the diversity and composition of mice gut microbiota by lytic or temperate gut phage treatment. Applied microbiology and biotechnology 2018;102(23);10219-10230.
6. Biswas B, Adhya S, Washart P, Paul B, Trostel AN, Powell B *et al*. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant Enterococcus faecium. Infection and immunity 2002;70(1):204-210.
7. Bogovazova GG, Voroshilova NN, Bondarenko VM. The efficacy of Klebsiella pneumoniae bacteriophage in the therapy of experimental Klebsiella infection. *Zhurnal mikrobiologii, epidemiologii i immunobiologii*, 1991;(4):5-8.
8. Brabban AD, Hite E, Callaway TR. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodbourne Pathogens & Disease 2005;2(4):287-303.
9. Burch TR, Sadowsky MJ, LaPara TM. Effect of different treatment technologies on the fate of antibiotic resistance genes and class 1 integrons when residual municipal wastewater solids are applied to soil. Environmental science & technology 2017;51(24):14225-14232.
10. Chan BK, Turner PE, Kim S, Mojibian HR, Elefteriades JA, Narayan D. Phage treatment of an aortic graft infected with Pseudomonas aeruginosa. Evolution, medicine, and public health 2018(1):60-66.
11. Chen C, Guron GK, Pruden A, Ponder M, Du P, Xia K. Antibiotics and antibiotic resistance genes in bulk and rhizosphere soils subject to manure amendment and vegetable cultivation. Journal of environmental quality, 2018;47(6):1318-1326.
12. Chen Q, An X, Li H, Su J, Ma Y, Zhu YG. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environment international 2016;92:1-10.
13. Cheng M, Liang J, Zhang Y, Hu L, Gong P, Cai R *et al*. The bacteriophage EF-P29 efficiently protects against lethal vancomycin-resistant Enterococcus faecalis and alleviates gut microbiota imbalance in a murine bacteremia model. Frontiers in microbiology 2017;8:837.
14. Cisek AA, Dąbrowska I, Gregorczyk KP, Wyżewski Z. Phage therapy in bacterial infections treatment: one hundred years after the discovery of bacteriophages. Current microbiology 2017;74(2):277-283.
15. Cohen ML. Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science* 1992;*257*(5073):1050-1055.

16. Couch M, Agga GE, Kasumba J, Parekh RR, Loughrin JH, Conte ED. Abundances of tetracycline resistance genes and tetracycline antibiotics during anaerobic digestion of swine waste. Journal of environmental quality 2019;48(1):171-178.
17. Czaplewski L, Bax R, Clokie M, Dawson M, Fairhead H, Fischetti VA *et al*. Alternatives to antibiotics–a pipeline portfolio 2016.
18. Dewangan G, Kashyap DK, Giri DK. Bacteriophage and their applications: a review. Journal of Cell & Tissue Research 2017;17(2).
19. d'Hérelle F. On an invisible microbe antagonistic toward dysenteric bacilli: brief note by Mr. F. D'Herelle, presented by Mr. Roux. 1917. Research in microbiology 2007;158(7):553-554.
20. Domingo- Calap P, Georgel P, Bahram S. Back to the future: bacteriophages as promising therapeutic tools. Hla 2016;87(3):133-140.
21. Dou C, Xiong J, Gu Y, Yin K, Wang J, Hu Y, *et al* Structural and functional insights into the regulation of the lysis–lysogeny decision in viral communities. Nature microbiology 2018;3(11):1285-1294.
22. Dufour N, Debarbieux L, Fromentin M, Ricard JD. Treatment of highly virulent extraintestinal pathogenic Escherichia coli pneumonia with bacteriophages. Critical care medicine 2015;43(6):e190-e198.
23. Fang H, Han L, Zhang H, Long Z, Cai L, Yu Y. Dissemination of antibiotic resistance genes and human pathogenic bacteria from a pig feedlot to the surrounding stream and agricultural soils. Journal of hazardous materials 2018;357:53-62.
24. Fish R, Kutter E, Wheat G, Blasdel B, Kutateladze M, Kuhl S. Compassionate use of bacteriophage therapy for foot ulcer treatment as an effective step for moving toward clinical trials. In Bacteriophage Therapy Humana Press, New York, NY. 2018, 159-170
25. Fukuda K, Ishida W, Uchiyama J, Rashel M, Kato SI, Morita T *et al*. Pseudomonas aeruginosa keratitis in mice: effects of topical bacteriophage KPP12 administration. PloS one, 2012;*7*(10):e47742.
26. Garner E, Inyang M, Garvey E, Parks J, Glover C, Grimaldi A *et al*. Impact of blending for direct potable reuse on premise plumbing microbial ecology and regrowth of opportunistic pathogens and antibiotic resistant bacteria. Water research 2019;151:75-86.
27. Gill JJ, Hyman P. Phage choice, isolation, and preparation for phage therapy. Current pharmaceutical biotechnology 2010;11(1):2-14.
28. Hobbs Z, Abedon ST. Diversity of phage infection types and associated terminology: the problem with 'Lytic or lysogenic'. FEMS microbiology letters 2016;363(7).
29. Hua Y, Luo T, Yang Y, Dong D, Wang R, Wang Y *et al*. Phage therapy as a promising new treatment for lung infection caused by carbapenem-resistant Acinetobacter baumannii in mice. Frontiers in microbiology 2018;8:2659.
30. Hung CH, Kuo CF, Wang CH, Wu CM, Tsao N. Experimental phage therapy in treating Klebsiella pneumoniae-mediated liver abscesses and bacteremia in mice. Antimicrobial agents and chemotherapy 2011;55(4):1358-1365.
31. Jault P, Leclerc T, Jennes S, Pirnay JP, Que YA, Resch G *et al*. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. The Lancet Infectious Diseases 2019;19(1):35-45.
32. Jernigan JA, Hatfield KM, Wolford H, Nelson RE, Olubajo B, Reddy SC *et al*. Multidrug-resistant bacterial infections in US hospitalized patients, 2012-2017. *New* England Journal of Medicine 2020;382(14):1309-1319.
33. Jiao W, Du R, Ye M, Sun M, Feng Y, Wan J, Zhao Y *et al*. 'Agricultural Waste to Treasure'–Biochar and eggshell to impede soil antibiotics/antibiotic resistant bacteria (genes) from accumulating in Solanum tuberosum L. Environmental Pollution 2018;242:2088-2095.
34. Kakasis A, Panitsa G. Bacteriophage therapy as an alternative treatment for human infections. A comprehensive review. International journal of antimicrobial agents 2019;53(1):16-21.
35. Kaźmierczak Z, Piotrowicz A, Owczarek B, Hodyra K, Miernikiewicz P, Lecion D *et al*. Molecular imaging of T4 phage in mammalian tissues and cells. Bacteriophage 2014;4(2):e28364.
36. Keen EC. A century of phage research: bacteriophages and the shaping of modern biology. Bioessays 2015;*37*(1):6-9.
37. Kim MS, Bae JW. Lysogeny is prevalent and widely distributed in the murine gut microbiota. The ISME Journal 2018;12(4):1127-1141.
38. Kot W. Genome sequencing of dsDNA-containing bacteriophages directly from a single plaque. In *Bacteriophages* Humana Press, New York, NY. 2018, 179-184.
39. Kutter E, Sulakvelidze A. eds., Bacteriophages: biology and applications. Crc press 2004.
40. Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World journal of gastrointestinal pharmacology and therapeutics 2017;*8*(3):162.
41. Liu K, Sun M, Ye M, Chao H, Zhao Y, Xia B *et al*. Coexistence and association between heavy metals, tetracycline and corresponding resistance genes in vermicomposts originating from different substrates. Environmental Pollution 2019;*244:*28-37.
42. Lyon J. Phage therapy's role in combating antibiotic-resistant pathogens. Jama 2017;318(18):1746-1748.
43. Maiques E, Úbeda C, Tormo MÁ, Ferrer MD, Lasa Í, Novick RP *et al*. Role of staphylococcal phage and SaPI integrase in intra-and interspecies SaPI transfer. Journal of bacteriology 2007;189(15):5608-5616.
44. Mattila S, Ruotsalainen P, Jalasvuori M. On-demand isolation of bacteriophages against drug-resistant bacteria for personalized phage therapy. Frontiers in microbiology 2015;6:1271.
45. Merabishvili M, Vervaet C, Pirnay JP, De Vos D, Verbeken G, Mast J, Chanishvili N *et al*. Stability of Staphylococcus aureus phage ISP after freeze-drying (lyophilization). *PloS one*, 2013;*8*(7):e68797.
46. Morozova VV, Kozlova YN, Ganichev DA, Tikunova NV. Bacteriophage treatment of infected diabetic foot ulcers. In Bacteriophage Therapy Humana Press, New York, NY. 2018, 151-158.
47. Nelson DC, Schmelcher M, Rodriguez-Rubio L, Klumpp J, Pritchard DG, Dong S *et al*. Endolysins as antimicrobials. Advances in virus research 2012;83:299-365.

48. Oliveira H, São-José C, Azeredo J. Phage-derived peptidoglycan degrading enzymes: challenges and future prospects for in vivo therapy. *Viruses* 2018;*10*(6):292.
49. Paez-Espino D, Eloe-Fadrosh EA, Pavlopoulos GA, Thomas AD, Huntemann M, Mikhailova N *et al*. Uncovering Earth's virome. Nature 2016;536(7617):425-430.
50. Phagoburn: Evaluation of phage therapy for the treatment of burn wound infections [Internet]. [cited 2018 Apr 22]. Available from: http://www.phagoburn.eu/
51. Pires DP, Cleto S, Sillankorva S, Azeredo J, Lu TK. Genetically engineered phages: a review of advances over the last decade. Microbiology and Molecular Biology Reviews 2016;80(3):523-543.
52. Pires DP, Melo LD, Boas DV, Sillankorva S, Azeredo J. Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. Current opinion in microbiology 2017;39:48-56.
53. Projan SJ. Why is big Pharma getting out of antibacterial drug discovery? Current opinion in microbiology 2003;6(5):427-430.
54. Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. Journal of wound care 2009;18(6):237-243.
55. Seed KD. Battling phages: how bacteria defend against viral attack. PLoS Pathog 2015;11(6):e1004847.
56. Simmonds P, Aiewsakun P. Virus classification–where do you draw the line? Archives of virology, 2018;163(8):2037-2046.
57. Sun M, Ye M, Zhang Z, Zhang S, Zhao Y, Deng S *et al*. Biochar combined with polyvalent phage therapy to mitigate antibiotic resistance pathogenic bacteria vertical transfer risk in an undisturbed soil column system. Journal of hazardous materials 2019;365:1-8.
58. Tolstoy I, Kropinski AM, Brister JR. Bacteriophage taxonomy: an evolving discipline. In *Bacteriophage Therapy* Humana Press, New York, NY. 2018, 57-71.
59. Twort FW. An investigation on the nature of ultra-microscopic viruses. The Lancet 1915;186(4814):1241-1243.
60. Wang J, Hu B, Xu M, Yan Q, Liu S, Zhu X *et al*. Therapeutic effectiveness of bacteriophages in the rescue of mice with extended spectrum β-lactamase-producing Escherichia coli bacteremia. International journal of molecular medicine, 2006;17(2):347-355.
61. Wang Q, Guan Z, Pei K, Wang J, Liu Z, Yin P, Peng D. *et al*. Structural basis of the arbitrium peptide–AimR communication system in the phage lysis–lysogeny decision. Nature microbiology 2018;*3*(11):1266-1273.
62. Watanabe R, Matsumoto T, Sano G, Ishii Y, Tateda K, Sumiyama Y *et al*. Efficacy of bacteriophage therapy against gut-derived sepsis caused by Pseudomonas aeruginosa in mice. Antimicrobial agents and chemotherapy 2007;51(2):446-452.
63. Watts G. Phage therapy: revival of the bygone antimicrobial. Lancet (London, England) 2017;*390*(10112):2539-2540.
64. Weber-Dąbrowska B, Jończyk-Matysiak E, Żaczek M, Łobocka M, Łusiak-Szelachowska M, Górski A. Bacteriophage procurement for therapeutic purposes. Frontiers in microbiology 2016;7:1177.
65. WHO | WHO's first global report on antibiotic resistance reveals serious, worldwide threat to public health [Internet]. WHO. [cited 2018 Apr 15]. Available from: http://www.who.int/mediacentre/news/releases/2014/amr-report/en/
66. Williamson KE, Fuhrmann JJ, Wommack KE, Radosevich M. Viruses in soil ecosystems: an unknown quantity within an unexplored territory. Annual Review of Virology 2017;4:201-219.
67. Ye M, Sun M, Huang D, Zhang Z, Zhang H, Zhang S, Hu F, *et al*. A review of bacteriophage therapy for pathogenic bacteria inactivation in the soil environment. Environment international 2019;129:488-496.
68. Young R. Phage lysis: do we have the whole story yet? Current opinion in microbiology 2013;16(6):790-797.
69. Yu P, Mathieu J, Li M, Dai Z, Alvarez PJ. Isolation of polyvalent bacteriophages by sequential multiple-host approaches. Applied and environmental microbiology 2016;82(3):808-815.