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Using Phage as A Highly Specific Antibiotic Alternative Against *Methicillin Resistance Staphylococcus aureus* (*MRSA*)

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Misuse of antibiotics in humans and animals often leads to the development ofmultidrug resistance (MDR)bacteria. Resistance can occur within a few years of novel antibiotics being introduced. Lytic bacteriophage (phage) are a kind of virus that undergo a cyclical lifestyle wherein they infect and replicate through the use of a bacterial host cell and cause cell lysis. Phage recognizes specific receptors on their host cell to attach to,insert their DNA and take over their host's molecular machinery. These receptors only exist on the surface of specific bacterial host cells and are often not present on other non-specific bacteria and not especially on the surfaces of eukaryotic cells. The mechanisms by which phage can destroy bacteria are different from antibiotics; phage can lyse MDR resistant bacteria without being affected by hydrolytic enzymes or ribosomal variations that's mean unlike drug resistance mechanism which bacteria can destroy the drug before can inter the bacteria cell, the phage not effected with such enzyme.

Key words: Antibiotics, MRSA, Human and Animals.

Bacteriophages (phages) are viruses that insert their genomes into bacterial cells, rapidly taking control of the host's molecular machinery and produce progeny that are released by lysis of the bacterium. Phage therapy involves using phages as a treatment or prophylaxis application against infectious diseases caused by bacteria. The worldwide prevalence, persistence and incurabilityof pathogenic multidrug resistant bacteria is a serious public health concern that will bring about the post-antibiotic era. This result is primarily from the extensive use of antibiotics that puts selective pressure on bacteria to develop mechanisms of resistance[1]. Phage were first discovered separately by Fredrick Twort and Félix d'Herelle in the early 20th century and later used heavily among Eastern Europe countries and the form Soviet Unionfor treating and preventing bacterial infectious diseases[2-4].In more recent years an interest in phage therapy has been resurrected as a potential option to deal with MDR bacteria infections.

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MRSA

Staphylococci are gram-positive bacteria that cause various infectious diseasesin humans and animals.*Staphylococci* are classified using different methods, one of the main categories on the basis of coagulase.Coagulase is a major factor that causes blood clotting in the proximity of the bacteria and bacteria lacking in these factors often make poor pathogens.Accordingly, the only specie of Coagulase-positive *Staphylococci* in this category is *S. aureus*.*S. aureus* initially was susceptible to *Penicillin*, but with time and indiscriminate use of antibiotics, resistant strains

emerged along with ones that became resistant to more recent generations of β-lactam antibiotics. *Methicillin* was among the later generation drugs and became commonly used after the emergence of*Penicillin*-resistant *S. aureus*[5,6].Two subsets of *S.aureus* strains are further distinguished based on resistance and susceptibility to *Methicillin;* MRSA (*Methicillin* Resistance *Staphylococcus aureus*), and MSSA (*Methicillin* Sensitive *Staphylococcus aureus*) (Fig. 1).

Diseases caused by *S. aureus* include endocarditis, wound infection, carbuncle, toxic shock syndrome, osteomyelitis, etc.These bacteria produce a toxin that damage cells as well as being able to directly attack the cell to cause apoptosis using a *Fibronectin Binding Protein*[7].Drug resistance in MRSA isthe result of a *mecA* gene that produces *Penicillin-Binding Protein* (PBP) witha lower affinity[8]. The PBP is a peptidogly cansynthesis protein and involved in the synthesis of peptidoglycan, which is the major component of bacterial cell walls.

Phage

Nearly 10^8 phage particles per ml are present in the oceans and an estimated 10^{30} phage particles exist in the world.Phages can be divided based on features such as host range, absence or presence of an lipid envelope, physical characteristics (size and morphology of capsid and tails, if present), resistance to organic solvents, genome composition (single or double-stranded DNA or RNA) and if they have antigenic properties[9,10]. Based on classification by the International Committee of Taxonomy of Viruses (ICTV) a vast majority of phages are in a one category called *Caudovirales*[9], have one of three tail morphologies and ads DNA genome packaged a head-full into their capsid[11].Phages are further divided into three types based on their life cycle: lysogenic, lytic and chronic[12].The genome of a temperate phage, once inserted into the bacterial cytoplasm, integratesinto the host chromosome and can undergo a dormant state, even be replicated along with the host chromosome during binary fission and at a later time excise from the chromosome to form phage particles and utilize the lytic cycle[13]. Obligatorily lytic phages however inject their genome into the cytoplasm of the bacteria, hijack the cell and immediately convert it into producing progeny and shorty (or eventually a few hours later which is the case with T4-like phages due to lysis inhibition) releases them by destroying the bacteria through lysis. Chronic phage can replicate and release their progeny from the host without compromising the viability of their host.Phages can survive outside the host cell but utilization of the host is requiredfor replication[14]. Phage specificity can be used to further characterize bacterial strains through a method called phage typing[15, 16].

There are several problems to identifying phagesone of which is that phages cannot be cultured outside of ahost[17].Phage particleswill be comprised of a tail, a filamentous tube or polymorphous structure[11].The composition of a *Caudioviridae* includes acapsid, disk plates that make a tail sheath, a base plate and aspike for *Siphoviridae* or multiple tail fibers for *Podo*- and *Myoviridae*. In addition to phage therapy, phage can be used for industrial purposes, including phage display, detection of pathogenic bacteria via luminescence[18], provide bactericidal endolysin proteins that clear if not mitigate the prevalence of pathogens from food products[20], enhanced food safety applications in agricultural and aqua culture settings, as a prophylactic and in hospitals to control biofilm-forming pathogens and common bacterial infections associated with hospital visits[19].

Advantages of phage therapy

(A) Effective against MDR bacteria because they use significantly different mechanisms in comparison to antibiotics; (B) Phages are capable of evolving/mutating therefore, they can respond to phage-resistant bacteria; (C) Shifting of target organisms does not occur because it has high specificity and secondary infections are avoided[21]; (D) Phages ortheir protein products, such holin and lysozyme,do not negatively affect eukaryotic cells[22, 23];(E) Phages can replicated at the site of a bacterial infection; (F)Phage-resistant bacteria are likely susceptible to other phages[24].

Classification of phage and infection mechanism:

Phages are classified into 13 families according to nucleic acid type, morphology and the presence or absence of an envelope or lipid layer[22]. Most phages have a tail and are further classified into three families according to the morphological features of a tail: *Myoviridae* (a contractile tail most commonly found in T-even phages), *Siphoviridae* (long, flexible, non-contractile tail seen in T5) and

Podoviridae (extremely short tail; e.g., T7)[25,26]. Phage infections function much like other viruses wherein the ûrst step is to make contact with a receptor, usually a protein or sugar component on the bacterial surface. Phages are able to absorb to speciûc bacterial species and even to specific strains within a species; or genera within the same gram type which are called polyvalent phages[27]. The second step (for lytic phages specifically) is the injection of DNA into the bacterial cytoplasm, use the host RNA polymerase to express early mode genes from the phage genome, take over of the host by either degrading the host genome or ejecting it from the cell,replicate the phage genome, and synthesized multiple middle and late genes that encode proteins such as the capsid and tail, followed by the genomic DNA being packaged into the capsid, resulting in completed phage. In the last step, phages produce the endolysin that destroy the peptidoglycan of the cell wall allowing phage progeny to be released[28].

Table 1. Phage therapy: Pros and Cons

Researcher(s)	Title	Year	Result
Bruynoghe R. and Maisin J.	Essai de thérapeutique aumoyen du bacteriophage	1921	Phage treated *Staphylococcal* skin disease by injecting the phage near the site of the infection.
Smith, H. W., and Huggins M. B.	Successful treatment of experimental *Escherichia coli* infections in mice using phages: its general superiorityover antibiotics	1982	Successful treatment of *E. col i* infections in mice without collateral damage.
Smith, H. W., and M.B. Huggins.	Effectiveness of phages in treating experimental *E. coli* diarrhea in calves, piglets and lambs	1983	Used single doses of specific phage to treat diarrheal*E.coli* strains, reduced and/or stopped the diarrhea.
S ´Lopek S. et al	Results of bacteriophage treatment of suppurative bacterial infections in the years	1981–1986	Phage were used to treat 518 patients with antibiotic resistant bacterial infections; the success rate of curing patientswas between 75–100%.
Bogovazova, G. G., et al.	Immunobiological properties and therapeutic effectiveness of preparations from *Klebsiella b*acteriophages	1992	Demonstrated their phagesto besafe and effective against treating *invivo Klebsiella* infections.
Soothill, J. S. et al.	Bacteriophage prevents destruction of skin grafts by *Pseudomona s aeruginosa*	1994	Phage used as a bio-control against *P.aeruginosa* infections.
Thiel K.	Old dogma, new tricks 21st Century phage therapy	2004	Demonstrated how therapeutic phages can be produced inexpensively
Gill J. J. et al.	Efficacy and Pharmacokinetics of Bacteriophage Therapy in Treatment of Subclinical *Staphylococcus aureus* Mastitis in Lactating Dairy Cattle	2006	This study showedthe ability of adaily dose of lytic staph phage K to eliminate bovine *S aureus*intra- mammary infections during lactation in24 cows.
Trigo G. et al.	Phage Therapy is Effective against Infection by *Mycobacterium ulcerans* in a Murine Footpad Model	2013	*Mycobacteriophage* D29was evaluated for therapeutic efficacy in footpad mice

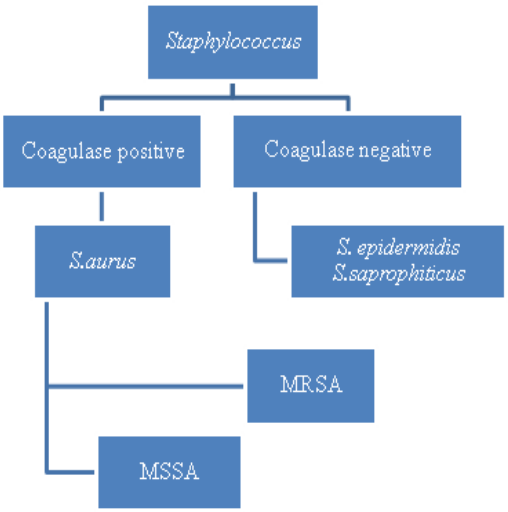

Fig. 1. Differentiation of *S. aureus* based on Coagulase

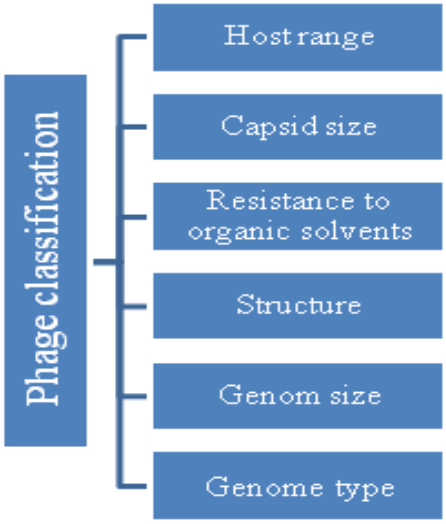

Fig. 2.

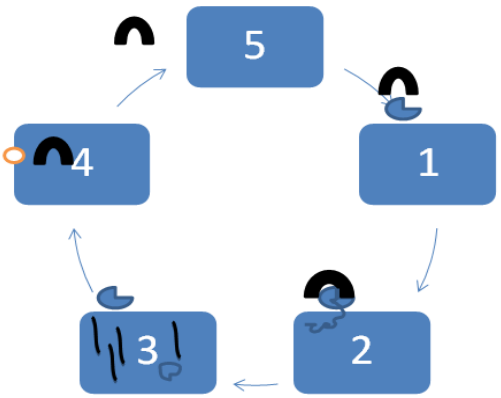

Fig. 3. Mechanism of phage=induced lysis of the bacteria: 1) Phage detects specific receptors and adsorbs to them. 2) In the second step, the adsorbed phage injects its genome into the bacterial cytoplasm. 3) Host molecular machinery recognizes promoters on phage genome causing transcription and translation of protein products such as capsid and enzymes meanwhile the phage genome is replicated. 4) Assembly of completed phage particles, lysis of bacteria with holin and exit out of the cell by diffusion. 5) Attach to new host and repeat cycle

Phage and genetic engineering

Most gram-negative bacteria have extracellular lipopolysaccharide components that are often shared amongst different strains. Through the use of genetic engineering, we could modify the properties of a phage's tail fibers that could allow it to bind to one or more ofthe shared components found in most lipopolysaccharide of gram-negative bacteria of interest resulting in a wider host range. In a study conducted by Lorena Rodríguez-Rubio three different proteins uses between lysostaphin and HydH5 resulted in increased performances of the 2 proteins compared to the wild type and caused lysis of the bacteria in plate and reduce the opaqueness of bacteria in liquid media[29].

Phage therapy vs. Antibiotic therapy against MRSA

The use of phage as an antimicrobial agent with similar efficacy to an antibiotic is one of the most exciting aspects in treating infections. A number of studies have shown the use of phage has no direct, negative effects on eukaryotic cells and the efficiency of phage can in some cases be greater than antibiotics. In addition, the problematic, disease-causing bacteria can be destroyed with no or minimal effect on neighboring and essential microbiotia. Smith et al. showed that a single intramuscular dose of one anti-K1 coliphage was more effective for treating mice challenged with *E. coli* intramuscularly than multiple intramuscular doses of *Tetracycline*, *Chloramphenicol* or *Trimethoprim* plus *Sulfafurazole*[30]. Chibani-Chennouû et al. showed that all of the administered phage passed through the gastro in testinal tract in adult mice and destroyed a diarrheal *E.coli* strain[31].Matsuzak et al. used four types of phage to treat 72 strainsof *MRSA* (*Methicillin Resistance*

Staphylococcus aureus) and showed that injections of $8*10^8$ bacteria intra-peritoneal,caused bacteremia and eventual death in mice but when administration simultaneously with puriûed phage ØMR11 (MOI ≥ 0.1) they suppressed *S. aureus*-induced lethality. High-doses of phage ØMR11 used on uninfected mice showed no adverse effects[32]. Miêdzybrodzki *et al* did a comparison between the cost of a phage therapy and antibiotic therapy and showed that the use of phage therapy in MRSA infections was less expensive to use than antibiotics such as *Vancomycin,Linezolide, Teicoplanin* and *Chinupristin + Dalfopristin*[33]. Clem attempted to use Staphphages to eliminate numerous MRSA strains and showed that many of their phages could not infect some of the strains and suggested a plausible phenomenon relating to the surface antigen properties such as clumping factor A, clumping factor B, *Fibronectin Binding Protein A, Fibronectin Binding Protein B, collagen adhesion*, *SdrC, SdrD, SdrE*, Protein A and *Methicillin* resistance surface proteins[34].Gu *et al* used the phage endolysine(LysGH15) as a prophylactic to protect mice against the MRSA infections. Their results demonstrated that 50 µg of LysGH15 was sufficient to protect mice against injections at double the minimum lethal dose of MRSA when administered 1 hour prior to the bacterial challenge[35].S. O'Flaherty et al discovered and tested two novellytic phages (DW2 and CS1) against their entire personal collection of *Staphylococci* species that cause mastitis-associated infections and demonstrated their phages capabilities of being used as a prophylactic[36]. According to the above studies we concluded that the use the phage is often cheaper and promising for thetreatment of MRSA infections.Data shown in Table-1.

Problems with Phage therapy and possible solutions

A) Most phage have limit detection of the host, so that a narrow range of hosts ability to identify with phage.A possible solution could be to use polyvalent phages that can identify a wide range of bacteria[37].

B) The presence of cellular debris and other by products from the host bacteria in phage preparations can cause negative immune responses. Using modern technology such as density gradient centrifugation can eliminate or greatly mitigate a patient's exposure to these elements[38].

C) Phage clear from the body rather fast if their host is not available,for more persistence can be used strains that delay detected and cleared by the immune system[39].

CONCLUSION

Numerous studies have used phage as an antimicrobial agent and have shown them to be effective at destroying target bacteria[33, 40-43]. Phage are highly specific and are only capable of lysing bacteria and have not been demonstrated to cause lysis or have direct negative immunological effects to eukaryotic cells[44].Phages specificity is due to their tail fibers' ability to bind to specific antigens on their host that aren't present on other non-target bacteria. In other words the use of phage to attack problematic bacteria is beneficial and not likely to have any negative effects on normal microbiota; an effect often not seen in conventional broad-spectrum antibiotics. Another advantage of phage is that they can replicate at the site of infection and easily overcome and out number the bacteria after a number of infection cycles. The likelihood of a naturally occurring phage being able to infect a eukaryotic cell is highly unlikely and probably even impossible without the extensive use of genetic engineering in a lab setting. The receptors on the outer membrane of eukaryotes are significantly foreign in comparison to those of bacteria, the phage wouldn't be able to penetrate the significantly thicker cell membrane, the inserted genome likely wouldn't survive within the cytoplasm as well take over the molecular machinery of the host within the nuclei[45].

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REFERENCES

1. Pfeltz R.F., W.B.J., The escalating challenge of vancomycin resistance in *Staphylococcus aureus. Curr. Drug. Targets Infect. Disord,* 2004; **4**: 273-294.
2. Alisky J, I.K., Rapoport A, Troitsky N. , Bacteriophages show promise as antimicrobial agents. *J Infect,* 1998; **36**: p. 5-15.
3. Weber-Dabrowska B, M.M., Górski A. , Bacteriophage therapy of bacterial infections: an update of our institute's experience. *Arch Immunol Ther Exp,* 2000; **48**: 547-51.
4. Chanishvili N, T.M., Chanishvili T. , Phages and experience for their application in the former Soviet Union. . IUMS Congress (Paris), 2002.
5. Hiramatsu, K., et al., The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol, 2001; **9**: p. 486-93.
6. Mark C. Enright, D.A.R., Gaynor Randle, Edward J. Feil, Hajo Grundmann, Brian G. Spratt,. , The evolutionary history of methicillin-resistant *Staphylococcus aureus (MRSA)*. P.N.A.S, 2002; **99**: 7687-7692.
7. Krut, O., Utermo hlen, O., Schlossherr, X., & Kro¨ and M. nke, , Strain Specific Association of Cytotoxic Activity and Virulence of Clinical *Staphylococcus aureus Isolates. Infection and Immunity,* 2003; **71**: p. 2716-2723.
8. Chambers, H.F., The changing epidemiology of *Staphylococcus aureus*. *Emerging Infectious Diseases*, 2001; **7**: 178-182.
9. Rohwer, F.E., R. ,, The phage proteomic tree: Genome-based taxonomy for phage. *J Bacterio*, 2002; **184**: 4529-4535.
1. Guttman, B., Raya, R., Kutter, K., *Bacteriophages: Biology and Applications.* 2004; **3**: 30-69
10. H.-W., A., 5500 phages examined in the electron microscope. *Arch Virol*, 2007; **152**: 227-243.
11. Deurenberg, R.H., Vink, C., Kalenic, S., Friedrich, A.W.,, C.A. Bruggeman, Stobberingh, E.E., , The molecular evolution of methicillin-resistant *Staphylococcus aureus. Clin. Microbiol. Infect*, 2007; **13**: 222-235.
12. Little, J.W., Lysogeny, prophage induction, and lysogenic conversion. 2005.
13. Jensen, E.C., Schrader, H. S., Rieland, B., Thompson, T. L., Lee, K. W., Nickerson, K. W. & Kokjohn, T. A. ,, Prevalence of broad-host range lytic bacteriophages of Sphaerotilus natans, *Escherichia coli* and *Pseudomonas aeruginosa*. *Appl Environ Microbiol,* 1998; **64**: p. 575-580.
14. Kakoma, K., Isolation and Characterisation of Bacteriophages and Their Potential Use for the Control of Bacterial Infections in Poultry. 2009.
15. Welkos, S., Schreiber, M. & Baer, H., Identification of Salmonella with the 0-1 bacteriophage. *Appl Microbiol*, 1974; **28**: p. 618-622.
16. Edwards, R.A. and F. Rohwer, Viral metagenomics. *Nat Rev Microbiol*, 2005; **3**(6): p. 504-10.
17. Schmelcer, M., Loessner, M., Application of bacteriophages for detection of foodborne pathogens. Bacteriophage, 2014.[https://www.landesbioscience.com/journals/bacteriophage/2014 bacteriophage0007R.pdf]
18. Hagens, S. and M.J. Loessner, Application of bacteriophages for detection and control of foodborne pathogens. *Appl Microbiol Biotechnol,* 2007; **76**(3): p. 513-9.
19. Carson, L., et al., The use of lytic bacteriophages in the prevention and eradication of biofilms of Proteusmirabilis and *Escherichia coli. FEMS Immunol Med. Microbiol,* 2010. 59: p. 447-455.
20. Azizian R., Azizi J. F., Askari H., Naser A., Karimi S., Sadeghifard N. et al, Bacteriophage as a Novel Approach to Inhibite and Remove Biofilms, *Scientific Journal of Ilam University of Medical Sciences*, 2013; **4**(4): 104-109.
21. Chernomordik, A.B., Bacteriophages and their therapeutic-prophylactic use. *Med.Sestra*, 1989; 6: p. 44-47.
22. Rashel, S.M.M., Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. J Infect Chemother, 2005. 11: p. 211-219.
23. Merril, C.R., Interaction of bacteriophages with animals. In Bacteriophage ecology, 2008: p. 332-352.
24. Salyers, A.A., and C. F. Amabile-Cuevas., Why are antibiotic resistance genes so resistant to elimination? Antimicrob Agents Chemothe, 1997. 41: p. 2321-2325.
25. Matsuzaki S, K.M., Kimura S, Tanaka S. , Vibriophage KVP40 and coliphage T4 genomes share a homologous 7-kbp region immediately upstream of the gene encoding the major capsid protein. *Arch Virol*, 1999; **144**: p. 2007-12.
26. Matsuzaki S, K.M., Kimura S, Tanaka S. , Major capsid proteins of certain Vibrio and Aeromonas phages are homologous to the equivalent protein, gp23, of coliphage T4. *Arch Virol,* 1999; **144**: 1647-51.
27. Ackermann HW., Tailed bacteriophages: the order Caudovirales. *Adv Virus Res*, 1998; **51**: p. 135-201.
28. Wang IN, S.D., Young R., Holins: the protein clocks of bacteriophage infections. *Annu Rev Microbiol,* 2000; **54**: p. 799-825.
29. Lorena Rodríguez-Rubio, e.a., Enhanced Staphylolytic Activity of the Staphylococcus

aureus Bacteriophage vB_SauS-phiIPLA88 HydH5 Virion-Associated Peptidoglycan Hydrolase: Fusions, Deletions, and Synergy with LysH5. *Appl Environ Microbiol,* 2012; 2241-2248.

30. Smith HW, H.M., Shaw KM. . J, The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *Gen Microbiol,* 1987; **133**: 1111-26.
31. Sandra Chibani-Chennouû, J.S., Anne Bruttin,Elizabeth Kutter, Shafiq Sarker,and Harald Bru ¨ssow, In Vitro and In Vivo Bacteriolytic Activities of *Escherichia coli* Phages: *Implications for Phage Therapy. Antimicrobial Agents and Chemotherapy,* 2004. 48: p. 2558–2569.
32. Shigenobu Matsuzaki, M.Y., Experimental Protection of Mice against Lethal *Staphylococcus aureus Infection by Novel Bacteriophage MR11. jid,* 2003; **187**: p. 613-624.
33. Miêdzybrodzki, R., Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment. *Postepy Hig Med Dosw*, 2007; **61**: 461-465.
34. Clem, A., Bacteriophage for the elimination of methicillin resistant *Staphylococcus aureus* (MRSA) colonization and infection. http://scholarcommons.usf.edu/etd/2485, 2006.
35. Jingmin Gu, W.X., LysGH15, a Novel Bacteriophage Lysin, Protects a Murine Bacteremia Model Efûciently against Lethal Methicillin-Resistant *Staphylococcus aureus* Infection. *Journal ofClinical Microbiology,* 2011; **49**: 111-117.
36. S. O'Flaherty, R.P.R., Isolation and characterization of two anti-staphylococcal bacteriophages specific for pathogenic *Staphylococcus aureus* associated with bovine infections. *Letters in Applied Microbiology,* 2005. **41**: p. 482-486.
37. S. O'Flaherty, Potential of the Polyvalent Anti-*Staphylococcus* Bacteriophage K for Control of Antibiotic-Resistant *Staphylococci* from Hospitals. *Appl. Environ. Microbiol*, 2005; **71**.
38. Carlton, R.M., Phage therapy: past history and future prospects. *Arch Immunol Ther Exp* (Warsz), 1999; **47**(5): p. 267-74.
39. al., M.C.e., Long-circulating bacteriophage as antibacterial agents. *Proc. Natl. Acad. Sci*, 1996; **93**: 3188-2192.
40. Gabriela Trigo, T.G.M., Phage Therapy Is Effective against Infection by Mycobacterium ulcerans in a Murine Footpad Model. *Neglected Tropical Diseases*, 2013; 7.
41. Chan, B.K., Phage cocktails and the future of phage therapy. *Future Microbiol,* 2013; **8**: 769-783.
42. Henriques, A., Reducing *Salmonella* Horizontal Transmission During Egg Incubation by Phage Therapy. *Foodborne Pathogens and Disease,* 2012.
43. McCallin S, Sarker S. A., Barretto C., Sultana S., Berger B., Huq S., Krause L. er al., Safety analysis of a Russian phage cocktail: From MetaGenomic analysis to oral application in healthy human subjects. virology, 2013.
44. Miernikiewicz P, D¹browska K, Piotrowicz A, Owczarek B, Wojas-Turek J, et al. (2013) T4 Phage and Its Head Surface Proteins Do Not Stimulate Inflammatory Mediator Production. PLoS ONE 8(8): e71036. doi:10.1371/journal.pone.0071036
45. Azizian R., Mousavinasab S. D., Ahmadi N. A., Bacteriophage as a Novel Antibacterial Agent in Industry and Medicine, *Journal of Paramedical Sciences* (JPS) 2013; **4**(4): 92-100.