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Bacteriophage Therapy against Antimicrobial Resistant Crisis

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Abstract

The most common virus on the earth is bacteriophage (or phages) that are present in all organisms. Their classification is currently being evaluated based on the phage's unique and antibacterial properties. The phage replicates within the host through a lytic or lysogenic process following infection and use of a bacterial cell machine. Phage has become an effective therapeutic drug against pathogens after twort and Filex d'Herelle discovery of bacteriophage in the 1900s, and subsequent research has been conducted. Nevertheless, bacteriophage therapy has become an unavoidable option for research due to the recent occurrence of bacterial antibiotics resistance. Around fifty years after antibiotic were found, antibiotics resistance is key risk for health care. Antimicrobial resistance is a rising big issue in global healthcare. The WHO, 1st report on antimicrobial resistances globally, has emphasized the threat of a forthcoming post antibiotics age, where little infection could be not treatable and once again will be fatal. Considering the present condition, producing therapeutic agent that are complementary to antibiotics play great role to fight against antibiotic resistance. The crisis requires development and implementation of new therapeutic agents against infections and phage therapy is suitable to control infectious diseases because safety of phage therapy. There is a perception with regards to phage therapy that phages are usually safe, on the bases of fact that they are ubiquitous in nature and our continued contact to phages in the environment and furthermore that they are widely used without adverse effects in many of the world, with this positive interpretation, the application of phage therapy must be verified by current research studies. Bacteriophage preparations contain detrimental substances, such as toxins of gram-negative bacteria, during the formulation process of bacteriophage and that can be remove by different purification methods. Phages effects normal flora GIT negligibly due to specificity in nature and they infect only a small number of bacterial species.

Keywords: Bacteriophage Therapy, Antibiotic Resistance, Phage Replication Mechanisms, Antimicrobial Resistance Crisis, Phage Therapy Safety and Specificity

1.0 Introduction

The therapeutics uses of bacteriophages make bacteriophages viruses not to ignored anymore. In recent era the emerging of antibiotics resistance bacteria, phages are proposed as a new class of antibacterial, like antibiotics.(Altamirano & Barr, 2019; Payne & Jansen, 2000). Bacteriophages are those viruses that attack bacterial cells and replicate within them. Phages are ubiquitous on earth,(Millard, Clokie, Letarov, & Heaphy, 2011).The literal meaning of bacteriophage is "bacterium eater," that's why they are used to treat bacterial diseases in human. Bacteriophages replicate by two primary cycles, lytic and lysogenic cycle. Lytic phages infect and their rapid replication kill bacteria cell quickly, whereas lysogenic phages are slowly integrating into their host bacterial genome. Phage therapy is describe as the therapeutic use of phages to treat bacterial infection(Viertel, Ritter, & Horz, 2014). About half century after the discovery of antibiotics, antibiotics resistance is major risk for health care.

2.0 Literature Review

Around fifty years after antibiotic were found, antibiotics resistance is key risk for health care. Antimicrobial resistance is a rising big issue in global healthcare. The WHO, 1st report on antimicrobial resistances globally, has emphasized the threat of a forthcoming post antibiotics age, where little infection could be not treatable and once again will be fatal(Kakasis & Panitsa, 2019). The CDC (Center for Disease Control) reports that 24,000 death each year in America results from infection with antimicrobial tolerant bacterial strains (CDC internet 2018)(Kakasis & Panitsa, 2019). The level of antimicrobials resistance in the united kingdom are mostly recorded in nations with elevated levels of MDR (multidrug resistance).(Prevention & Control, 2016). Pharmaceutical companies, on the other hand, have little enthusiasm in discovering and creating novel antimicrobials, because the antimicrobials market is much less profit-making than in other sectors. (Projan, 2003). Considering the present condition, producing therapeutic agent that are complementary to antibiotics play great role to fight against antibiotic resistance. (Czaplewski et al.). The crisis requires development and implementation of new therapeutic agents against infections and phage therapy is suitable to control infectious diseases(Duckworth, 1976).

In this review we discuss the therapeutic use of bacteriophage that attack bacteria and can kill or lyse bacteria. The phage therapy'' is an old concept that is recently regaining acceptance. (Kortright, Chan, Koff, & Turner, 2019).

3.0 Antimicrobial resistance crisis and its consequences.

The microbial world is made up of certain microbes that, are very to see with the human naked eye, and numerous and existing everywhere. only bacteria and virus are not microorganisms, there are massive groups of various kinds of microorganism. (Green et al., 2004; Whitman, Coleman, & Wiebe, 1998). the microbial community is also the base of the global ecosystem, and these microbes live in all environmental places on this globe, including each surface, space, as well as in human body. Most of these microbes are beneficial to their human hosts. However, some pathogenic, causing infections, and even fatal consequences. To prevent the disease of pathogenic microbes, an agent known as antibacterial

agents has been developed that are efficient in avoiding or eradicating the growth of pathogenic microbes. Many of these chemical agents derived from naturally present product where were really utilized by organism to prevent pathogenic microbes(D'Costa & King, 2011; Moellering Jr, 2011). many of these products have been modified by human to enhance its antimicrobial activity(D'Costa & King, 2011). These modified antimicrobial agents are effective against specific type of pathogenic microbes while some attack on broad range of microorganisms. These antimicrobial substances include antibiotics, antifungals, antihelminthic and antiseptic. The administration of antimicrobials to treat and prevent infectious diseases has activate pathogenic microbes to produce resistance against antimicrobial agents(Sykes, 2010). An example of this resistance is the prevalent development of antibiotics resistance against antibiotics since from use of first penicillin in WW2 (1939-1945). Firstly it was efficient towards a broad range of infections, today, Only about Eighty years after antibiotics discovery, there are a vast number of bacteria resistant to penicillin, its derivatives as well as many other antibiotics. (Mazel, 2004). Like bacterial pathogens many others microbes such as Plasmodium species, which cause malaria are now also producing resistance to all antimalarial agents(Dondorp et al., 2011). The increasing antimicrobial resistance is producing due to the extensive usage of antibiotics. This increasing resistance towards antimicrobials will lead to lethal epidemics which will be fatal and epidemics may be globally in extent and will be continue for years because there will be not treatment for resistant microbial epidemic.so this requires an urgent response to overcome this antimicrobial resistance in microbial world. Antibiotics resistance nowadays is a global problem. This problem will be overcome by the production of alternative agents to antibiotics. Phage therapy is best option to overcome this problem.(Michael, Dominey-Howes, & Labbate, 2014).

4.0 Bacteriophage

Bacteriophage can be characterized as obligate bacterial intracellular parasite lacking an independent metabolic mechanism. Bacteriophages are everywhere where bacteria present and it play a vital role in different biotic processes, that's why phages are most abundant because there bacterial host are present everywhere, we can say that bacteriophages are ubiquitous (Keen, 2015). Mostly phages (particularly 96% of the recently discovered) are categorized in different orders such as Caudovirale, the virus which have tail ,and have dsDNA and further they are categorized in classes of Podoviridae, Myoviridae, and Siphoviridae (Sharp, 2001). Phages are specific for specific strain or specie of bacteria. they infect or attack on specific type of bacteria. Bacteriophages are divided on the base of life cycle. Bacteriophages are divided into two groups virulent bacteriophages and temperate bacteriophages. The virulent phage have in which phages attaches to host and enter its genome and reproduce by using host cell machinery and finally release from host cell as progeny virion. (Kutter & Sulakvelidze, 2004). There are two types of proteins in lytic phages for lysis of host cell, (holins , lysins). The holin protein are used to rupture the bacteria cell membrane and work in synergy with endolysin, which are used for the degradation of cell wall(Cisek, Dąbrowska, Gregorczyk, & Wyżewski, 2017). The temperate phages have a different way to attack on bacteria and enter its genome into host and start lysogeny, and they integrated its genome into host chromosome. where the bacteriophage genome remains latent, where they replicate with host cell replication and also

passes to its daughter cells and accidently transfer into a lytic cycle under a particle circumstance. temperate phages can be useful to bacteria as they add some extra gene into bacteria and when this extra expressed it increases its virulence. Due to this drawback temperate phage is not suitable for therapeutic uses.(Clark, 2015)

4.1 Life Cycle of bacteriophages

The existence cycle of bacteriophages has been a terrific model for understanding how viruses affect the cells they infect, on the grounds that comparable strategies were discovered for eukaryotic viruses, that may cause immediate demise of the cell or set up a latent or chronic infection. Virulent phages usually lead to the death of cell via cell lysis. Temperate phages, however, remain part of a host's genome and are multiply with the host cell genome till that time as they are started to make progeny virus.

4.2 Lytic phages life cycle.

During the life cycle of lytic bacteriophage. the phage taken over the bacterial cell and produces new bacteriophages and inside the number of new phages increases and cell lysis occur. There are 5 different steps in the bacteriophage lytic cycle. Attachment is the 1st stage in cycle by which bacteriophage attaches to the receptors on bacterial cell. Mostly bacteriophages have limited host range which may attacks only one species of bacteria or one strain. This distinctive characteristic of phages is used for medication of specific bacterial diseases. The 2nd stage lytic cycle is penetration. The penetration of phages occurs through tightening of sheath of tail, which acts as needle of injection of viral genome into cell. Head and remaining components of phages remains outside of the cell. The 3rd stage is **synthesis** of new viral component. After entrance into host cell, the virus starts synthesizing of endonucleases to destroy the chromosome host bacteria and hijack the host cell to produce viral required component e.g. capsomere, tail sheath, tail fibers, base plates, and viral enzymes for the production of new viruses and in **maturation** stage, progeny virions are produced. To release these new phages, the phage protein (holins) disrupts the bacterial cell wall and final stage of the cycle is release. Matured virus release from the cell by a process called cell lysis and new virus freed into the environment to attacks new cell. (Fig.1)

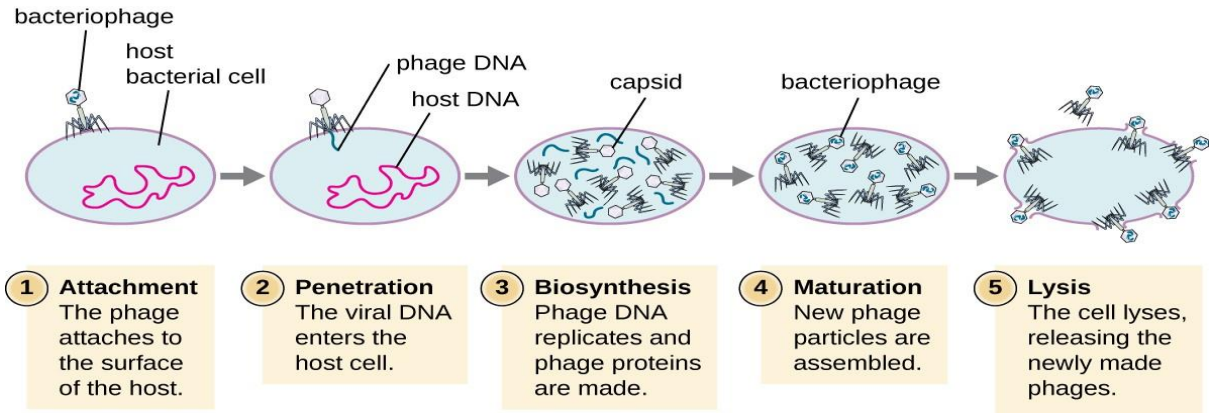

Fig.1. lytic phages life cycle

4.3 Temperate phage cycle.

Temperate phages are replicated by lysogenic cycle in lysogenic **cycle**, in this life cycle the bacteriophage DNA also enters to cell via (attachment and penetration). During the lysogenic cycle bacteriophage cannot kill host and the phage integrated its DNA into the chromosome of host and become component of host cell. The combined genome of bacteriophage called **prophages** and prophage with bacteria is called **lysogens**. **The process by which temperate phage infect bacteria is called lysogeny**. the phage remains latent inside the cell when the cell starts replication of own chromosome, it also replicates DNA of the bacteriophage and pass it to new descendant cell during procreation. The existence of the phage might change the phenotypes of bacteria, may it be capable to add additional gene (the genes which increase virulence). This changes in the host phenotypes is known as phage conversions or lysogenic conversions. Certain bacteria Vibrio cholerae is less virulent without prophage and phages infecting this bacterium adds gene in the genome and after gene expression it can improve the virulence of vibrio cholerae. In the case Vibrio cholera causes severe diarrhea. (Fig.2)

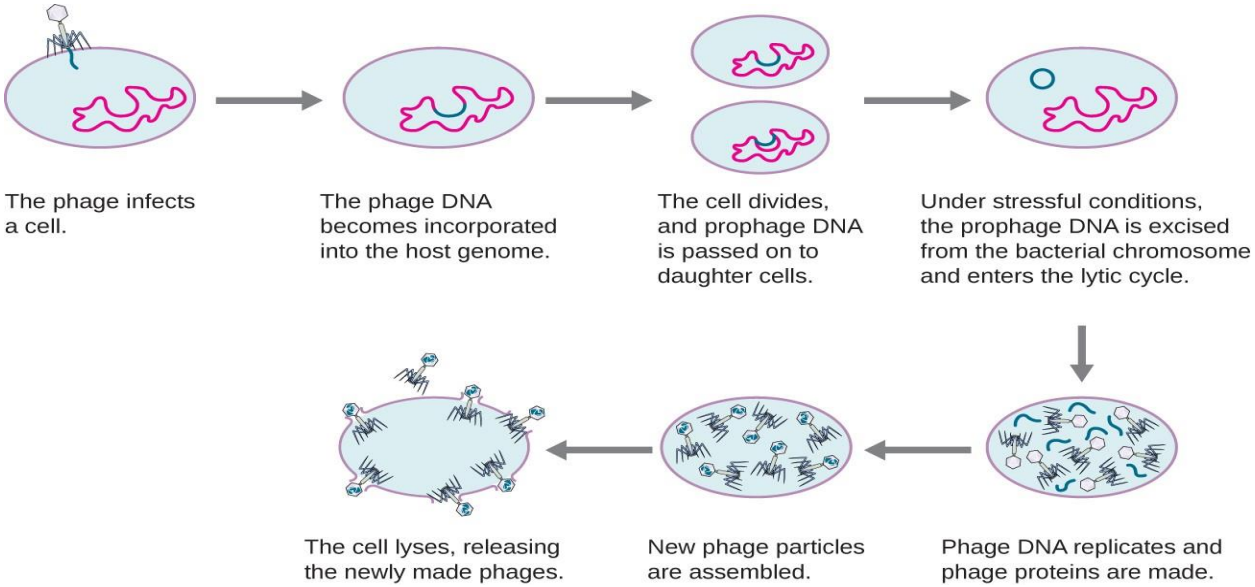

Fig.2. temperate phage has both lysogenic and lytic cycles.

5.0 Bacteriophage therapy discovery and history.

Bacteriophages are those viruses which attacks on bacteria. The history & discovery of bacteriophage is a lengthy discussion, including a disagreement over significance of bacteriophages. in 1896 a British microbiologist presented the phage antibacterial activity against v. cholerae which he observe in Jumna and Ganga river water in Hindustan, and he suggested that there are an unknown substance which passes over porcelain filter was responsible for this occurrence and for preventing of cholera spread in population. (Alexander Sulakvelidze, Alavidze, & Morris, 2001). After 2 years, a microbiologist Gamaleya detected the same phenomenon while working with B.subtilis. (Samsygina & Boni, 1984), and other microbiologists are also believed this phenomenon(Van Helvoort, 1992). However, none of the microbiologist do not discover further result until F.Twort, after 20 years of Hankins

observation a microbiologist from UK, reintroduce the subject again by reporting a same phenomenon and advances the assumption that it may have due to some agents like viruses. But, due to financial and some other reasons F.Twort, did not chase this findings(Lehmann, 1999) and after two years a French microbiologist Felix d'Herelle, discover bacteriophage "officially" at the institute of Pasteur in capital of France peris.

The rediscovery of phages by Felix d'Herelle, is associated with an epidemic of severe dysentery among French army reside at Maison's- Laffitte (on the border of French capital) in Aug-sep 1915, even though d'Herelle first observed the phenomenon of bacteriophage in 1910 while working on controlling measure of an outbreak of an animal's disease in Mexico city. Many troopers were hospitalized due the epidemic, and he was assigned to examine the epidemic. During investigation he made filtrates of the patients' fecal sample free from bacteria, mixed and incubated the filtrate with Shigella spp separated from patient. A small portion of the mixture was inoculated into lab animals, and a small portion was spreaded on the agar medium to study bacterial growth. He found the small, translucent spots on this agar medium, which he initially called taches, then taches the edges and it became a plaque (Summers, 1999). The finding of D'Herelle's were presented on Sep 1917 in the meeting of the School of Sciences and he presented the phenomenon that it was caused by a bacterium invading virus Felix d'Herelle and his wife Marie both suggested the name bacteriophage on 18 Oct 1916. (Felix d'Herelle isolate 1st bacteriophages in June 1916, about one year after the Maison's epidemic). The name bacteriophage was coming from bacterio means bacteria and phagein which means to eat. Bacteriophages are bacteria eater that is why the virus named bacteriophage. D'Herelle, thought of himself as the discoverer of bacteriophages, When he was aware of Twort 's prior finding, but believed that the discovery of Twort was distinct from his work, (Twort, 1920). Felix d'Herelle continues to research on phages and endorses the theory that phages are live virus rather than an enzyme. Many researchers believe that it was an enzyme, but it reveals to be a virus. Eventually the target dispute ended, and the discovery of phages was acknowledged by researchers and referred to as the (Herelle-twort phenomenon) and afterward as the phage phenomena.

5.1 Early studies of phage therapy.

Felix d'Herelle used bacteriophages for the treatment of dysentery after his discovery. it was the first attempt of phages to use as a therapeutic agent. The research was completed in 1919 at the Hospital in Paris under Dr. Victor-hutinal 's supervision.(Summers, 1999). The bacteriophage formulations and preparations was done by both d'Herelle and Dr.Hutinel, and hospital employees to confirm the phage safety before administering to a 11-years old boy with dysentery. after administration of single dose of Felix d'Herelle's antidysentery phage the patient subsides and after few days the boy fully recovered. The antidysentery phages efficacy was verified when 3 other patients were treated with a single dose of phage preparations, and then after twenty hours of administration they began recovering. the results of the study was not published immediately and after years the 1st report on phages preparation uses to treat human diseases came in 1921 from R.bruynoghe and J. Maisin. (Bruynoghe & Maisin, 1921), they were use bacteriophages for treatment of skin diseases. The phages were spread on surgically opened lesion and they reported reversion of the infection within 24 hours. other

scientists are encouraged and follow these results for further studies. F.d'Herelle and other microbiologists continued study on uses of bacteriophages as therapeutic uses e.g. F.d'Herelle used several phage preparation for the treatment of cholera and bubonic plague in Bharat(Monsur et al., 1970; Summers, 1999). After successful detection of bacteriophage the concept of bacteriophage therapy was taken vigorously and introduced in numerous situations, but its implementations diminished after the development and usage of antibiotic in the WW2. (Abedon, Kuhl, Blasdel, & Kutter, 2011). Usually, two centers are concerned with the historical research regarding bacteriophages therapy. The Polish Herzfeld Institute, and the Georgian Eliava Institute. Pyophage and Intestiphage are examples of modified cocktails that are still used in Georgia and Russia for bacterial infections. The Eliava Institute works on the preparations and medicinal usage of phage cocktails (e.g. pyophage and intestiphage). The Hirszfeld Institute promotes the development of more advanced approaches to clinical planning and formulation of bacteriophages. In addition, many companies started commercial production of bacteriophages against various bacterial infections (Alexander Sulakvelidze et al., 2001).

5.2 Preparation of phage for therapeutic uses.

In previous century, after discovery and isolation of phages, uses of bacteriophage therapy have shown positive success results. The main reasons for the failure of bacteriophage therapy occur due to an unsuitable bacteriophage selections, preparations and storing(Gill & Hyman, 2010). Bacteriophages are isolated from any environmental source for therapeutic uses. the major source of phage isolation is hospital settings, sewage and other wastes because bacteria is present in numerous number in these. (Latz et al., 2016). we can effortlessly isolate bacteriophage against infectious pathogens because bacteriophages are dependent on bacteria(Mattila, Ruotsalainen, & Jalasvuori, 2015).

We can isolate bacteriophage from any sample. easy way of isolation is to first sterilize the sample to remove undesired microbes and then pour it to bacterial culture to observe the pattern of plaque. (Gill & Hyman, 2010). detail information's of phage isolation can be found in a newly publish book by Sillankorva & Azeredo(Azeredo & Sillankorva, 2018). Later phage isolations it should be characterize and genetically sequenced for proper therapeutic uses. (Gill & Hyman, 2010). Preparation and selection of phage is critical for therapeutic uses. The important standards for the selection of appropriate bacteriophage is its specificity, effectiveness, and the evasion of negative effect, that's the selected bacteriophage must properly absorb and lytic for the bacteria. (Weber-Dąbrowska et al., 2016). The temperate or lysogenic phages is not suitable for therapeutic bacteriophage preparation and formulation. Temperate phage may increase the virulency of bacteria or provoke resistance against phage. Temperate phages have ability of transduction, It is the capability of transfer genetic material from one bacteria to another, hence the bacteria can obtain undesired gene. (Gill & Hyman, 2010).The one of the main issues for phage therapy is phage resistance inducement. Modification of the host of a phage have a major effects on success of bacteriophage therapy. (Goodridge, 2010).The formulations which contain two or more phages called cocktail which have broad host range. (Chan, Abedon, & Loc-Carrillo, 2013). The persistence of bacteriophages within the cell after administration is also critical for the efficacy of

bacteriophage therapy. (Goodridge, 2010). Any phage therapy products must be prepared according to Good Manufacturing Practices (GMP). GMP provide very stringent rule for isolations, sterilization and purification. Separate the phages from other components by filtration and centrifugation. Gram-negative bacteria lysate required more purification because it has endotoxins. (Pirnay, Merabishvili, Van Raemdonck, De Vos, & Verbeken, 2018).phage preparation also require proper storage. The phage should be properly stored after preparations. Phages are susceptible to various factors such as Temp, PH, organic materials, mechanicals stress because phages are consisting of protein. various methods, including encapsulation improve the phage preparations storage. (Malik et al., 2017).

In bacteriophage therapy the bacteriophage should be administered to desired site of infection and keep medicinal level for adequate period for effective bacteriophage therapy. Topical, enteral, inhaled, and injected phage administration route could be necessary. Enteral route is much more common in bacteriophage therapy. (Abedon & Thomas-Abedon, 2010). As an approximation about ten or more phages are required to diminish bacterial cells. (Abedon, 2018). The current therapeutic parameter trails are inconvenient for bacteriophage therapy and apparently, an designed background is required for the practical of bacteriophage therapy. (Pirnay et al., 2015)

5.3 Bacteriophage therapy safety

There is a perception with regards to phage therapy that phages are usually safe, on the bases of fact that they are ubiquitous in nature and our continued contact to phages in the environment and furthermore that they are widely used without adverse effects in many of the world. with this positive interpretation, the phage therapy must be confirmed by current scientific researches. (Henein, 2013). The safety of bacteriophage therapy can be potted in the potential impact of bacteriophage on body tissues and normal flora, their capability to change their targeted bacteria by the virulence gene expression or via transduction b/w bacteria, and in their ability to initiate immune reaction. (S. Abedon, 2017; Abedon et al., 2011). Additionally, bacteriophage preparation contain detrimental substances, such as toxins of gram negative bacteria, during the formulation process of bacteriophage and that can be remove by different purification methods (Gill & Hyman, 2010; Pirnay et al., 2018). Phages effects normal flora GIT negligibly due to specificity in nature and they infect only a small number of bacteria species(Loc-Carrillo & Abedon, 2011). The capability of bacteriophages to translate virulence genes or add undesired genes it can be prevented by avoid the usage of lysogenic bacteriophages. Moreover, recent bacteriophage genome sequencing tactics give us the chance to appropriately address this matter. (S. Abedon, 2017; Kakasis & Panitsa, 2019). A little number of phage clinical experiment have been supervised till to date. No harmful reaction have found in any of these trials. (Vandenheuvel, Lavigne, & Brüssow, 2015). Remarkably, the intravenous route of systemic administration appears to be safe, which is defined in historical review by two microbiologists. (Speck & Smithyman, 2016). the intravenous use of bacteriophages have many objection on the base on the quick elimination phages from blood by the reticuloendothelial system (Merril et al., 1996; Alexander Sulakvelidze et al., 2001) and the massive release of toxins by the speedy bursting of bacterial host and the likelihood of immune. reaction which induce the production of immunoglobulins against bacteriophages,

that decrease the effectiveness of bacteriophage therapy and other severe immune reactions such as hypersensitivity reactions. (Campbell, 2003). But In history of phage therapy anaphylaxis has never been reported (Speck & Smithyman, 2016).

5.4 Phage therapy for human use

Europe and former Soviet Union uses bacteriophage as therapeutic agent in their health care after discovery of bacteriophages. Bacteriophage therapy is currently examined according to scrupulous systematic criteria(E Kutter et al., 2010; Villarroel, Larsen, Kilstrup, & Nielsen, 2017). A scientist has reported lists of essential standards that is followed meticulously and presented in bacteriophage therapy researches (S. T. Abedon, 2017). Information important to the successful medical experiments are the proper characteristics & choice of bacteriophages and humans and targeted bacterial cell. Formulation, preparation, and effectiveness are also required for clinical trials, without the base of characterization and well-prepared target they have no value. Comprehensive reportage would enhance the quality of upcoming study and expansion of prior study. Additional concern is the choice of appropriate infection target for bacteriophage therapeutic uses(Harper, 2018). For single pathogen infections highly specific characters of bacteriophages is highly essential and specificity of phages is a main drawback in treatment of mix pathogen infections, for this type of infections the phage is delivered combined with a specific antibiotic. Such contemplations are important in clinical trial for patient safety, as elimination of one microbial pathogen and momentous growth of 2nd can cause incurable complications(Harper, 2018). It is currently believed that wide range bacteriophage are common in bacteriophage isolations approaches and this disagreement require more and more further researches(de Jonge, Nobrega, Brouns, & Dutilh, 2019).

5.5 Clinical Trials of bacteriophage therapy.

The lack of approved & sufficiently precise clinical trials is one of the recent challenges of advancing bacteriophage treatment into the hospital. Even more care needs to be taken with in planning and development of these trials, a development of research studies for phage therapy can discuss several other resemblances to standard clinical studies with pharmaceutical drugs so there are multiple factors remarkable with phages as phage dose. (Payne & Jansen, 2003). These would have self-replicating ability; its dosage may rise massively whenever bacteria of relevance have reached them. This leads to another application thought: bacteriophages need close interaction with bacteria also, and they will be less successful if distributed too widely. Commonly, however, systemic treatments were used to tackle this, as other approaches have been used successfully described. Phage cocktails display wide range action as well as reduce the chance of forming resistant, but this should be remembered which combined treatment massively enhances a task of evaluating therapeutic effect, a possibility for (gene transference) and the production of bacteriophage resistance. (Parracho, Burrowes, Enright, McConville, & Harper, 2012).

Several have claimed that exposures to phages occur every day in individuals and it is proof of their protection, but there are some issues that really should be discussed in clinical trials. First of these concerns the sterility and purification of the preparations for the phage. To

ensure compliance with good quality manufacture practices or equal quality reassurance principles, The removal of toxins and bacterial compounds from the products is important. From phage recognition to manufacturing methods Parracho et al. presented the suggested executive management team for bacteriophage products. (Parracho et al., 2012)Secondly, questions about the risk for toxic shock due to the bactericidal impact of the phages must be examined. Though not a concern was listed. (Speck & Smithyman, 2016). And antibiotic follow this process of destroying bacteria. (Dufour, Delattre, Ricard, & Debarbieux, 2017), which is a protection technique until human trials are needed.

Kutter et al. dealt in detail with earlier clinical trials including phage therapy that included those performed in Georgia that Poland. (E Kutter et al., 2010). Here are two genes editing clinicals trial that are used as guide in the research to focus on the defense of bacteriophage for the avoidance of leg ulcer. (Rhoads et al., 2009) and their safety and efficacy in serious sinus inflammation. (Wright, Hawkins, Änggård, & Harper, 2009). Rhoads and his coworkers tested safety of therapy in patient with leg ulcer in a particular phase I analysis and found zero adverse effect during bacteriophage application(Rhoads et al., 2009). The effectiveness and defense of phages against recurrent late-stage otitis triggered by MDR-P regulated pseudomonas aeruginosa. (Wright et al), explained P. aeruginosa These 2 are the first monitored clinical trial undertaken on human infections in the civilized world. Most recent, various clinical trial have been published. Three patients with serious bacterial prostatitis E. faecium administered phage due to a lack of targeted antibiotic treatment were also treated for Enterococcus faecium infection. Enterococcal lytic phages were designed by isolation from 134 cultures of wastewater and potable water. Before applying phage to patient, bacteriophage activity was examined by checking ready phage on isolated bacterial species from every patient. Rectally delivered the bacteriophages, and E. faecium has been removed from all patients following phage therapy and no progression of the disease has been shown. (Letkiewicz, Międzybrodzki, Fortuna, Weber-Dąbrowska, & Górski, 2009).

Staphylococcus aureus, the series of bacteriophage therapy were provided for nine patients who have diabetic foot ulcer and the patients' bone and soft tissue were infected by S. aureus. (Fish et al., 2016). All the antibiotic treatments were responded poorly or failed, and the only other acceptable treatment option was surgical removal of toe. Thus, commercially available staphylococcal bacteriophage Sb-1 was administered through topical route to the ulcerations once in week. All patients treated successfully by phage therapy in few weeks. This application of bacteriophage therapy was efficient and successful without no adverse events. (Fish et al., 2016).

Another 65-year-old patient with nosocomial eye corneal abscess and keratitis infected by S. aureus received phage therapy for treatment at Phage Therapy Center in Georgia. The commercially available phages SATA- 8505 was administered intravenously and topically for some weeks. The treatment was successful, and the elimination of S. aureus was confirmed with examination of signs and laboratory cultures after treatment. (Fadlallah, Chelala, & Legeais, 2015).

Another clinical trials of bacteriophage therapy were reported in Russia against K. pneumoniae. The outbreak of infections of pneumonia caused by K. pneumoniae in the neonatal and resulted in newborns infections and antibiotics were not successful to control the infections. The commercially available bacteriophage targeting K. pneumoniae was

administered orally for 4-5 days. The phage therapy successfully eliminated K. pneumoniae in all patients. (Aslanov, Lubimova, Dolgiy, & Pshenichnaya, 2018).

Another trial was done on a patient who had a tibial infection caused by K. pneumoniae and A. baumannii and the phages were administered intravenously, and it showed a speedy clinical improvement. The phage therapy show effectiveness and the surgical removal of the patient's leg was no longer needed (Nir-Paz et al., 2019).

6.1 Advantages of bacteriophage Therapeutic uses.

The following are the advantages associated with the use of phage therapy with features that improve protection, economy, and accessibility. Such fundamental benefits of phages are as follows.

Single dose usage.

Applying only one dose of bacteriophage can replicates & in this manner achieve successful therapy, i.e., substantial development of bacteriophage by auto-dose result in the killing of large numbers of bacteria. A single dose of bacteriophage is enough to kill targeted bacterial cell. (Abedon & Thomas-Abedon, 2010)

Transfer of bacteriophages among subjects.

it is the cross transfer of bacteriophages from cured subject to uncured subject. It may be beneficial in agriculture side. (Alisky, Iczkowski, Rapoport, & Troitsky, 1998; Barrow & Soothill, 1997)

Single phage hit kinetics.

Only single dose of phage is required to lyse single bacteria unlike other antimicrobial agents.5 Only a small number of bacteriophages are required for treatment but a large quantity of bacteriophage adsorption to bacterium are still important to significantly ease the target bacteria populations. (Abedon & Thomas-Abedon, 2010).

low influence on environment.

phages are mainly comprised of nucleic acid, protein and have relatively limited host range,(Ding & He, 2010) they have small impact on environmental bacteria. Bacteriophages are susceptible to some environmental factor, e.g., temperature, dehydration, or sunlight and quickly inactivated.

Bacteriophages are not like an antibiotic.

There, are several unnecessary uses of antibiotics that provide way to bacterial resistance and phages are not like antibiotics bacteria cannot produce resistance against phages. so Bacteriophages do not lead to antimicrobial resistance and the use of bacteriophages as an alternative to antibiotics may help with typical antibiotic medicinal applications (Rashmi, Chaman, & Bhuvneshwar, 2005).

low cost.

Bacteriophage production requires a mixture of the hosts growth and purifications.

And the price of hosts growth depend on the strain of bacteria, and as technology progresses, the cost of phage purification continues to decrease.(Gill & Hyman, 2010; Kramberger, Honour, Herman, Smrekar, & Peterka, 2010). the cost of bacteriophage productions and costs of discovery and characterization are not out of line. Bacteriophages are preparing for

therapeutic uses in very low cost unlike other antimicrobials drugs (Skurnik, Pajunen, & Kiljunen, 2007).

6.2 Disadvantages of phage therapy.

Disadvantages of phage therapy are categorized into four classifications:

- Bacteriophages selection,
- unfamiliarity with bacteriophages
- bacteriophage host-range limits,
- the "individuality" of bacteriophages as pharmaceutical,

All bacteriophages do not make good therapeutics agent.

Good therapeutics bacteriophages would have a high capability to kill bacteria in combinations with a low ability they can change the environment. phages are obligatory lytic, stable within normal storage condition and temperature, subject to adequate protection and effectiveness, and the sequence for the absence of unwanted gene such as toxins genes must be checked. (Krylov, 2001) Note that a bacteriophage that is lytic is described as attacking bacteria & injecting its DNA and releasing it via cells lysis from infected cell and is unable to show lysogeny. The usage of lysogenic phages as therapeutic is problematical due to slowly integrating into their host bacterial and converts bacteria into phage resistance bacteria and they encode bacterial virulence genes, which produce resistance against phage. (Krylov, 2001; Merabishvili et al., 2009). In addition to sidestepping toxin-carrying bacteriophages or temperate bacteriophages, the goal of phage characterization is to eliminate bacteriophage with low killing potential against targeting bacteria. These poor killing bacteriophages are due to the poor adsorption capacity, poor ability to stop bacterial defenses or poor replication characteristics. (Abedon & Thomas-Abedon, 2010) For therapeutic purposes, certain phages that exhibit poor pharmacokinetics are also less desirable, i.e. poor absorption, dissemination, and viability within the host cell. (Alisky et al., 1998) Bacteriophages which do not fulfil these conditions sufficiently cannot be used as therapeutic agents.

The problem of narrow host range.

Host range of bacteriophage is narrow it targets only some strain or much fewer, only some bacteria species. Treatment of bacteriophage started before the identification of the bacteria sensitivity to antimicrobial like specific bacteriophages and bacteriophages are often employ in combination with another antimicrobial agents, with other bacteriophages which called cocktails, the spectrum of cocktails bacteriophages products are much broader than the individual phage spectrum. (Goodridge, 2010; Kutateladze & Adamia, 2010). cocktail bacteriophages which have broad spectrum are more selective than typical phages Like as antibiotics they have spectrum some phages show broad spectrum, and some have narrow range. The spectrum of phages depends on host range some have large number of host and some have small host range.

Bacteriophages are not distinctive pharmaceutical.

Bacteriophages are living protein-based agents which can easily interact with body's immune system and multiply aggressively, and alter during development or uses, but bacteriophages are far away limited in these matters. E.g several protein-based therapeutics

may trigger an immune response, and antibiotics that destroy bacterial cells releases toxin in situ, readily multiply and modify live attenuated vaccines, even in the sense of affecting the body's own tissues. Other Protein-based agent, antibiotics, and whole vaccine are granted for uses with these properties. therefore, due to these reasons' bacteriophages should not be disqualified for having this similar characteristic. The Western authorities of health unfamiliar of bacteriophages, that it is an antimicrobials and phage therapy's is a biggest challenge. It is noted that various bacteriophage as drugs are not at least unique to them. Only a small number of bacteriophage products have passed regulatory criteria and have been categorized by the FDA as Generally Regarded as Safe (GRAS) and registered by EPA and approved for used by the USDA, (Elizabeth Kutter et al., 2010; A Sulakvelidze & Barrow, 2005) phages are viruses that's why misunderstood by the public as equivalent to viral pathogen which causes diseases. Thus, public oppositions have not emerged, and its maybe privileged that bacterial viruses are called bacteriophages.

7.0 Future of phages therapy

Infectious diseases specialists have warned that there is now a credible necessity to create completely new types of antimicrobial products, those which cannot produce resistant against them by the same method through which bacteria produce resistant against antibiotics. Phage therapy is that new class of antimicrobials which will effective against antibiotic resistance pathogens. There are three further aspects of bacteriophages that must be remarked.

Specificity toward host.

The specificity of phage is to some extent a disadvantage it require interaction of bacteriophage to target bacteria or the production of phage which attacks a broad range of bacteria and it is also is the advantage of phage that will not lyse the normal flora of body. So, phage therapy has no adverse effects or lyse normal flora of the guts and lung or genital areas. The further study is required for making the bacteriophages which have broad range of host.so with the improvement of phages. Phage therapy will be used for more diseases.

Genetic engineering.

By genetic engineering, the production of new phage is possible which have grater activity then normal phage. by doing this, scientists will have to answer the valid concern of supervisory agencies concerning genetically modified organisms. The regulatory obstacles may be well cost, given the powerful engineering instruments that are presently available. (Dalmasso, Hill, & Ross, 2014). bacteriophages exhibit an immense genetics, morphology and structural variety compare with others organism. Today, new high-output sequencing technology employed to metagenomics analysis methods allow several previously undiscovered bacteriophages to be discovered and identified. The very huge number of sequences obtained with these techniques reveals previously unseen bacteriophages genes that have little similarity to those formerly stored in database. This growing genetic variation is of particular importance and may open new opportunity to utilize as therapeutic.

Co-therapy of bacteriophages with antibiotic.

If a bacteria acquire resistance against a bacteriophage e.g receptor site mutation and in the endonuclease's enzyme, The mutation is unable to "teach" the bacteria to resist antibiotic that do not attack such structures. Likewise, whether the bacteria induce resistant an

antibiotic e. g. through ribosomal subunits mutations, such mutation is not likely to teach the bacteria to avoid the bacteriophage, which does not attack those structure. So, if the bacteria are expose to both agent, there are chances of expression of any resistance gene, and they will able it to stay alive. There is certain report which show that bacteria have a tendency of mutation against antibiotic once in each 106 divisions, whereas they have tendency of mutation against bacteriophages once in each 107 divisions. Hence the chances of bacteria mutating against an antibiotics and bacteriophage at same time will be the product of 107×106 and it will take 1013 bacterial divisions for occurrence of double mutations. Because of this small possibility, the co-therapy of bacteriophages and antibiotic may help avoid the antibiotic resistance development in bacteria, by this means significantly extending their medical value. Just as several types of anti-HIV drugs are provided to patients with AIDS to avoid the production of resistant strains of this virus, co-therapy of bacteriophages and antibiotic can be considerable therapeutic benefits. (Dalmasso et al., 2014).

it seems that bacteriophages have distinctive ability as therapeutics tool, in future phages will be use for the preventions (phages vaccine), diagnosis and treatment of specific diseases. these therapeutic applications are increasing in pharmaceuticals and clinical field. The conception of acceptable regulatory, supervision and protection protocol directing their future applications within the framework of prudently conducted clinical trial will helps in this aim.

Conclusion

It has a long history in bacteriophage therapy. This includes early excitement, dismissal due to large use of antibiotic and renewed interest due to the increased resistance to antibiotic. Despite promising record of bacteriophage therapy in some region of the globe, more innovative randomized dual-blind monitored trials are required to confirm the bacteriophage therapy safety & effectiveness. Problems such as the collection, isolations, preparations, purifications, storage, & pharmacology of bacteriophages must be specifically discussed and carefully investigated in depth. In addition, bacteriophage production regulations are another challenge to bacteriophage therapy adoption, as the new regulatory guidelines for therapeutic drugs look to be daunting for bacteriophage therapy product. (Fauconnier, 2018) Even With these barriers, bacteriophages have characteristics that make them alternative options to bacterial infections therapy in an era of growing antimicrobial resistance crises.(Czaplewski et al.; Loc-Carrillo & Abedon, 2011) . Besides naturals phages, endolysin derived from phages and engineered bacteriophage can act as powerful antimicrobials. (Nelson et al., 2012; Pires, Cleto, Sillankorva, Azeredo, & Lu, 2016). Like antibiotic, bacteriophage therapy is not really a mystic bullet, and neither is antibiotic therapy a alternative. Rather, it should be seen as a possible matching treatment that could not be used only against antibiotic-resistant bacteria, but when antibiotic are not sufficient, e.g. it is preferable when to eliminate a particular pathogen while keeping the microbiota intact, or in non-critical circumstances when antibiotics uses would be discouraged to minimize antibiotics resistance spread.

Declaration of conflicting interest

The authors declare that there is no conflict of interest in this work.

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