

Lytic bacteriophages and phage cocktails seems to be a future alternatives against multi-drug resistant bacterial infections

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Abstract:

Lytic bacteriophages have the efficacy to act and eradicate pathogenic bacteria as the attractive tool in near future. Bacteriophages specifically kill multidrug resistant bacteria even which have the capacity to form biofilms. The present review mainly focused on the efficacy of bacteriophages and cocktails as therapeutic agents against predominate MDR-bacteria and their biofilms which are isolated from septic wound infections. The body of evidence includes data from studies investigating bacteriophages from sewage samples as novel antibacterial and antibiofilm agents against pathogenic bacteria. The goal of this review is to present an overview on predominant bacteria from septic wound infection, biofilm forming capacity of bacteria, lytic effect of bacteriophages and phage cocktails with emphasis on the application of bacteriophages against septic wound causing bacteria.

Key words: Bacteriophages, Septic wound infection, MDR, Biofilms, Phage Cocktails

1. Septic wound infections

Infection is an important cause of mortality in general during bacterial infections, septic wound infections in particular. The rapidly emerging pathogens and the development of multidrug resistance required and review of isolation of pathogens and antibiogram as well. Exposed subcutaneous tissue provides a favorable substratum for wide variety of microorganisms to contaminate or colonize and if the effected tissue would be devitalized (ischemic, hypoxic or necrotic) and the host immune response is compromised where the conditions likely to be optimal for microbial growth (Howell-Jones et al. 2005; H et al. 2018; Shridhar and Dhanashree, 2019).

For instance, it is reasonable to predict that wounds with sufficient hypoxic and reduce environment are more successful to colonize variety of microorganisms in general bacteria in particular. However, the current wide spread opinion among wound care practitioners is that aerobic or facultative pathogens such as *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *Escherichia coli*, *Streptococcal Sps.* are the primary causes of delayed healing in either acute or chronic wounds. Acute wounds are caused by external damage to intact skin include surgical wounds, bites, burns, minor cuts, aberrations, more severe traumatic wounds such as lacerations and those caused by crush or gut sat injury. Irrespective of nature of injury, the acute wounds an expected to heal with in predictable time. Although treatment required for healing will vary according to the type, site and depth of the wounds (Bowler, Duerden, and Armstrong 2001; Bessa et al. 2015). In contrast, chronic wounds are more frequently caused by endogenous mechanisms associated with predisposing conditions such as integrity of dermal and epidermal tissue. However, pathophysiological abnormalities may predispose to form chronic wounds such as leg ulcers, foot ulcers, pressure sores including tissue perfusion or venous drainage and metabolic diseases such as diabetes. Both acute and chronic wounds are susceptible to contamination and colonization by number of aerobic and facultative anaerobes (Church et al. 2006; Karumidze et al. 2013; Sikka et al. 2014).

A wound infection usually occurs when virulence factors expressed by one or more microorganisms at the site, outcompete the host natural immune system, invasion and diminishments of microorganisms that enhance the local and systemic host response. In general characteristic local responses are purulent discharge (pus) or painful spreading of erythema indicative of cellulitis around the wound (Golkar 2013; Mave et al. 2017). The progression of a wound with an infected state likely to involve magnitude of microbial and host factors (Mengesha et al. 2014; Charan Kaur and Wankhede 2014; Jault *et al.*, 2018).

The detection methodology of wound infections little complex and is based on the detection of 1. Assumed causative agents, 2. Initial clinical findings 3. Level and type of tissue involved 4. The rate of progression 5. The type of therapy required. However, Janda, Abbott, and Brenden 1997; Founou, Founou, and Essack 2017 et al argued that the classification of such infections serves little clinical evidence, because the prognosis and treatment are consequently differentiated based on the microbial loads. The clinical significance of microbial load in delaying

wound healing (disease progression) was discovered by Bowler, Duerden, and Armstrong 2001. They reported that healing of ulcers and their progression as well as in decubitus ulcers progressed only when the bacterial load was very less than 10^6 CFU/mL.

Specific nutrients produced by one bacterium may encourage the growth of fastidious and potentially pathogenic microorganisms. Increased prevalence of multi-drug resistance in bacteria is one of the major problems for the health care of modern world. Nowadays due to improper and use of antibiotics which lead to the emergence of untreatable bacterial diseases globally. According to the current statistics, the number of death incidence were attributed to multidrug-resistant bacteria in India approximately 70 000, globally it was up to 300 million. If there is no action would be taken against drug resistance, then the estimated number of death rate up to 10 million in India as well as 100 trillion global death rates each year by 2050 (Godebo, Kibru, and Tassew 2013; Yakha et al. 2014; Mave et al. 2017).

Wound is referred to as the results from mechanical interruption of the skin surface either deliberately induced or hospital-acquired. Which leads to breaches provides the opening the door for the pathogens usually penetrated into the internal organs of the body and colonized by various bacterial species in particular leads to infection (15). These aberrations are exclusively associated with pathogens including *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *Proteus species*, *Coagulase negative staphylococcus aureus*, *Acinetobacter*, *Streptococcus species*, *Enterobacter species*, and *Enterococcus species*, *Candida* and *Acinetobacter* along with other microorganisms viz., fungi, protozoans, and viruses (Lessa et al., 2003; Naqvi et al., 2005; Yakha et al. 2014; Patil, Paramne, and Harsh 2016). The frequency of wound infections in India is in between 10-30 %. Various types of wound infections played a critical role in human health care burdens as well as mortality rates. In the present study, we focused mainly on three different wounds for the bacterial isolation and application of bacteriophages against the predominant MDR-bacteria which forms a biofilm (7,20).

Burn wound infections

Now a days ICU has become a major reservoirs for pathogenic microorganisms, responsible for septic wounds and also accountable for the pneumonia, and bacteremia there in the admitted wards. Burn wounds are very common incidences in India, majority of the microorganisms cause the

invasive infection in burn cases and globally its range cost greater than 50%. Burn patients are more susceptible to bacterial infections, because of the physical damage of the skin surface and reduction of the innate immunity (Lessa et al., 2003). Hence, the bacteria can colonize and initiates the infections on the internal structures of the skin barrier. Due to burns, the wound infections and other complications are enhanced including high loads of microorganisms therefore the mortality rate was reached up to 75 % (5,18). Multi-drug resistant *S. aureus* is a primary culprit of burn wound infections and is a major cause of hospital acquired infections (Bayram et al. 2012; Samutela et al., 2015). Moreover, is a predominant and have an increased mortality rate of the burn wounds. Particularly in India the pediatric mortality rate is reported about 7–12 %.

Post-operative wound infections

The post-operative wounds or surgical site infections are one of the burning problems in the field of surgery. These wounds defined as “infection in the tissues of the incision site and operative area that can commonly occur in between the 5 to 30th days after surgery (Daniela Guta, 2014; Mengesha et al. 2014). Post-operative wounds are majorly spread in the hospitals and are associated with many factors such as microbial contamination, virulence capacity of the microbes, the susceptibility of host immune system, and drug resistance of pathogens, site of wounds, microbial colonization and attachment at the infectious site. Either chronic or acute infections are accompanied by poly-microbial communities such as bacteria, viruses, fungi, and protozoa at the sidelines or drainages of the wounds (24). Even though post-operative infections are preventable but remain high risk because of poor operational settings in India due to lake of attention and lack of scientific knowledge with standardized criteria for the diagnosis, epidemiology and also emergence of antibiotic-resistance in bacteria. Ranging between 4 to 30 %, whereas in the case of the USA or European countries the incidence rate was 2.8 % and 2.5 % respectively. The intensity of the infection varied from patients type, hospital to location, clinicians and procedure applied (Sikka et al. 2012; Shaaban, Ghozlan, and El Maghraby 2012; Jerry et al. 2018).

Diabetic wound infections

Diabetes is a metabolic disorder that exhibits high glucose levels in the blood leads to the establishment of severe complications such as kidney damage, nerve impairment, heart strokes, Xerophthalmia. World health organization has revealed that diabetes is one of the 7th leading

contributor to death by 2030 (Seth et al. 2012; García-Quintanilla et al. 2013; Vijayaraghavan Shamsundar 2015; Bessa et al. 2015) and also the range of diabetics increased from 171 million to 550 million from 2000-2030. Diabetics are nearly 5% more susceptible to fungal and bacterial infections. Also elevated sugar levels that encourages the microbial growth, hinders the blood flow, lowered the healing rate of wounds, and aberrations.

Bowler, Duerden, and Armstrong 2001; García-Quintanilla et al. 2013; Sanchez et al. 2013; Castillo, Nanda, and Keri 2019 according to these reports from American Diabetic Association (ADA), approximately 25 % diabetics will suffer from wound infections during their lifetime. Foot and leg ulcers are the main cause of hospitalization and mortality of diabetic patients across the world. In India, 20 % of diabetic patients were hospitalized because of foot wounds raised because of microbial infections either aerobic or facultative anaerobes. However, the misuse or unnecessary uses of antibiotics lead to elevated levels of drug resistance in microorganisms of wound patients were noticed. *S. aureus* is one of the most common bacteria isolated from wounds of diabetic wound patients (Patil, Bandekar, and Patil,2012; Mathangi and Prabhakaran 2013; Shivshetty et al. 2014; Mahgoub, Elfatih, and Omer 2015).

Wounds provide the suitable environment for colonization, proliferation, and growth of microorganisms. The pathogenic microorganisms escaped from the first line of the defense system of the human body i.e. skin. From the breached portions of skin, microbes can infect the integral parts of the body. The breaches on the skin surface by any mechanical, surgical, accidental, burn to allow the microorganisms into the internal structures of the skin, leads to the development of infections. The skin is the largest sense organ which produces an innate immunity and protecting underlying tissues of the human body (Kotz et al. 2009; Newton-Esebelahie, and Omoregie R 2013; Pondei, Fente, and Oladapo 2013; Bessa et al. 2015; Esebelahie, Keswani et al. 2018). The main and important function of the skin is to provide protection against pathogenic microbes, which invades on the skin and controlling the microbial/bacterial colonization (Ndip et al. 2007; Dai et al. 2010; Charan Kaur and Wankhede 2014). By losing the skin integrity by any mechanical injuries, exposes the subcutaneous tissues to the environment leads to microbial colonization and proliferation. Mechanical disruption of the skin results in the wound and the major cause for the establishment of infections by microorganisms ranging from bacteria to fungi, parasites and a virus. The septic infections caused mostly by bacteria and break the protection barrier may

establish deep-seated infection (J. Michael Janda and Abbott 2002; Goswami et al. 2011; Singh et al. 2015; Mahgoub, Elfatih, and Omer 2015).

Generally wound infections suppress the immune system and rendering the patients highly susceptible to colonize by opportunistic organisms of exogenous and endogenous origins. Microbial infections are one of the most important factors for the upturn of morbidity and mortality of septic wound patients (Goswami et al., 2011; Pondei, Fente, and Oladapo 2013; Pallavali et al., 2017). Septic wound infection can have an antagonistic impact on the human body, quality of life as well as on the healing rate of the wounds. The wound infections are reported to be one-third of the hospital-acquired infections among surgical patients and accounts for 70-80 % of mortality rate and particularly in developing countries, irrespective to the nature of wound and site of infection (Mehta, Dutta, and Gupta 2007; Goswami et al. 2011; Guan et al. 2014).

Wounds may not subside easily and spread because of human habits and aggravate in patients having disorders such as diabetes, obesity and cardiovascular diseases. Infectious wounds are critical, painful, odorous, hypersensitive and lead to discomfort and inconvenience for the patient. Bacterial colonization in wounds is common and difficult to control especially hospital environment. Septic wounds which harbor multiple pathogenic bacteria are common and leading to sepsis (J. Michael Janda and Abbott 2002; Singh et al. 2015; Pallavali et al. 2017; Sheridan et al. 2018; Kumari, Rani, and Lakshmi 2018). Diagnosis of wound infections are of serious task, taking long time usually requiring sophisticated diagnostic equipment or qualified professionals (53). 10-33 % of septic wound infections were seen in India. Multidrug-resistant bacterial infections are increased day by day and these organisms showed resistance to most of the available antibiotics. Drug resistance is a common and natural mechanism in microorganisms because of inexorable spread of resistance genes. According to WHO reports all the regions of the world documented that among the resistance viz., *E. coli* and *K. pneumonia*, *S. aureus* and *P. aeruginosa* genera provided 50 % of the species labelled as resistant to potential antibiotic namely Cephalosporin, is one of the third generation drug and its mortality rate was very high. In Europeans the mortality rate of drug resistance showed 25000/ year. Similarly in the United States 23,000 deaths per year (Negi et al. 2015; El-Shibiny and El-Sahhar 2017). WHO advisory committee searched for new methods instead of usage of antibiotics. World health experts and scientist look forward to get new approaches against to MDR-bacteria. The advent and cumulative frequency of multi-drug resistance proportionate with few novel antibiotic drugs on the possibility

of untreatable infections. Public health agencies recently reported on the dramatic increase in drug-resistant pathogens, which compelled the World Health Organization to declare a new “pre-antibiotic era” in its 2014 surveillance report. There is an emergence to identify efficient non-antimicrobial therapies to combat the inexorable increase in MDR pathogens (Goswami et al. 2011; Godebo, Kibru, and Tassew 2013; Piracha et al. 2014; Mama, Abdissa, and Sewunet 2014; Vijayaraghavan Shamsundar 2015).

In recent times, bacterial infectious agents are attaining to get multidrug resistance nature (58). In this scenario; increased attention has been given to test the efficacy of bacteriophages as an alternative therapeutic agent. In this regard, phage therapy is recommended against infections caused by multidrug-resistant bacteria from septic wounds, predominant bacterial isolates which are responsible for septic wound infections are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. Bacteriophages are likely to be a lytic in nature against bacteria, it can be employed indirectly to detect pathogens or directly used as the biocontrol agents. Number of studies has proved that the phages suitable biocontrol agents employed against plant pathogens, animal pathogens, fungus, and bacteria. In comparison to the antibiotics, the bacteriophages, phage cocktails and their enzymes (lysins) can suitable therapeutic agents to control bacterial infections (Smith and Huggins 1982; Zhvania et al. 2017; Kvachadze et al. 2011; Pires et al. 2011; Azizian et al. 2013; Cohen et al. 2013; Mishra et al. 2014; Bessa et al. 2015; Yuan et al. 2019; Pallavali et al. 2019).

Microbiology of septic wounds

As per the Center for Disease Control and Prevention (CDCP) guidelines to prevent surgical site infection has recognized that *S. aureus*, *Coagulase-negative staphylococci*, *Enterococcus* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *P. aeruginosa*, *Proteus* spp. and *Enterobacter* spp. are the prevalent pathogens. In recent past number of studies were conducted and revealed that the most common isolates are from wound sepsis are: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella species* and *Acinetobacter species* (38,44,67). The control of wound infections has become major challenge due to development of resistance against to antibiotics and a greater incidence of infections caused by poly-microbial flora (Duerden, and Armstrong 2001; Ear 2002; Esebelahie, Newton-Esebelahie, and Omoregie R 2013; Bowler,; Bessa et al. 2015).

The successful management of bacteria in a wound is of great important. However, it is still a complex issue. Therefore our study evaluates the current situation in a particular geographical area, which is mostly helpful to the clinician and microbiologist involved, because it can make them aware of the real circumstance that they are dealing with presently massive progress of resistance to antibiotics, the improper use of antibacterial agents in an array of systemic antibacterial agents there is a requirement for perfect and predictive susceptibility pattern of bacterial isolates to help the clinician to indicate the choice of antibacterial management (Varaiya et al., 2008.; El-Shibiny and El-Sahhar 2017). Since, from 50 years, it has been an increase of bacterial infections directly proportion to resistant and with available antibiotics because of the evolution of antibiotic-resistance. The most concern of our study was bacterial pathogen viz *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *Escherichia coli* have become resistant to the majority of the available antibiotics, giving rise to multidrug-resistance (MDR), except remaining two or more accessible antibiotics (Vieira et al. 2012; Khairnar et al. 2013; Mandal et al. 2014; In et al. 2018; Jerry et al. 2018).

2. Multidrug resistant bacteria

In 1928, the penicillin antibiotic was discovered during IInd world war, followed by the discovery and commercial production of many other antibiotics. It is granted that any infectious disease is curable by antibiotic therapy. So, as the most of antibiotics has effective function. It has been estimated scale of one lakh tons/year worldwide and their use had a profound impact on microorganisms in general bacteria in particular on the earth. Drug resistance nature of bacterial pathogens to available antibiotics has become a great burden to the human health care (Chaudhry et al. 2013; Godebo, Kibru, and Tassew 2013; Bessa et al. 2015). Drug resistance is a common and natural mechanism in microorganisms. Since 1930's we are using antibiotics for treatment of various diseases has led to the emergence of multidrug resistant bacterial strains. WHO reported that, 60% of pathogens are attaining resistant to major available antibiotics, in coming years of time, all pathogens will acquire 100 % drug resistance. One of the reasons for better adaptation for microorganisms to antibiotics may be self-medication along with continual usages (Le et al. 2013; Allen et al. 2014; Baranovskiy and Boichenko 2014; Nakonieczna, Cooper, and Gryko 2015; Shahi and Kumar 2016; El-Shibiny and El-Sahhar 2017; Sváb et al. 2018; Cha et al. 2018; Fernandes and São-José 2018; Maciejewska, Olszak, and Drulis-Kawa 2018).

Multidrug resistance in bacteria is a severe health problem in these days because of improper use of antibiotics, increased the incidence of antibiotic-resistant pathogens is particularly in the hospital areas, resulting in a significant upturn of morbidity and mortality. Rigorous research activities are ongoing to develop alternate methods of treatment of infections caused by these microorganisms. Phage therapy seems to be a good option for this problem. The Bacteriophage Institute in Tbilisi (now the George Eliava Institute of Bacteriophage, Microbiology and Virology) is still researching phage therapy applications and supplies phage for the treatment of various bacterial infections (Pirnay et al. 2011; Cieplak et al. 2018). While several research groups continue to develop whole phage as alternative treatments, the isolation and optimization of purified phage components as antibacterial opens up new opportunities in the fight against harmful infections. That's why the phages have the therapeutic values since 1919s onwards. Phage therapy is one of the oldest bactericidal methods used in the Eastern Europe (Sangster, Hegarty, and Stewart 2014; Nakonieczna, Cooper, and Gryko 2015; Bowater 2016).

Moreover large numbers of pathogens have come with antibiotic resistance, some bacteria showed resistant to many antibiotics and chemotherapeutic agents. This phenomenon termed as "multidrug resistance". In principle multidrug resistance in bacteria may be generated by different mechanisms. However, bacteria can still resistance through mutations that make a target protein less susceptible to the agent. Nevertheless, the mutant will become more prevalent by clonal selection in the selective process. one of the most important task for selection is drug resistant. First we must identify the mutant of the respective enzyme, for this case high level prediction of drug resistant target enzymes from the plasmids that make drug of the bacteria to be resistant, and further these genes have to be spread widely on plasmids (81). Emerging multidrug resistant organisms not only by accumulation and also by absorbing the antibiotics from outside the environment. But, crucially the response of antibiotic attack to the bacteria by adaptation and pinnacle of evolution, "survival of the fittest" is the broad consequence of immense genetic plasticity of pathogens, which triggers the responses resulted by adaptive mutations. Acquisition of genetic elements or altering the gene expression that alternatively produces the resistance to virtually all the antibiotics currently available (Shahi and Kumar 2016; Jerry et al. 2018; Sváb et al. 2018; Cha et al. 2018). Therefore understanding the biochemical and genetic evidence of resistance is of paramount importance to design mechanism to perlitte the emergence or spread of resistance by therapeutic approaches against multidrug resistant organism (81,83).

In general the drug resistance is mediated by mobile genetic elements which provide the important and rapid mechanism of dispersion. However, the most commonly studied mobile genes are plasmids, transposons, transposable elements i.e. more recently recognized in bacteriophages (Agudelo Suárez et al. 2002; Patel, Shrivastava, and Kumar 2009). Interestingly these bacteria may accumulate multiple genes, each gene encodes for resistance to a single drug within the cell. Hence forth, the accumulation occurs typically on resistance plasmids. Another important mechanism proved that multidrug resistance may occur by the increased expression of genes which encode multidrug efflux pumps extruding multiple ranges of drugs (73,86).

3. Biofilms

Biofilms are densely packed communities of microorganisms growing on biotic and abiotic surfaces or surrounded by themselves by secreting extracellular polymers (Sutherland et al. 2004; Cassat, Smeltzer, and Lee 2014; Milho et al. 2019). Within a biofilm, the bacteria communicate with each other by producing chemotactic factors or pheromones. This phenomenon is called quorum sensing (90). If the availability of major nutrients, chemo taxis towards the motility of bacteria, surface adhesions and presence of surfactants are responsible to form biofilms, is one of the important survival strategies of pathogens (Zhu et al. 2002; Sharma et al. 2016). The formation of biofilms thought to begin where the bacteria sense environmental conditions suitable, that triggers the transition to leave on those surfaces. The structural and physiological complexity of biofilms has led to an idea enforced on coordinated and cooperated groups which are analogues to multicellular organisms (93). In humans, the biofilms are responsible for development of many disease, most of them are associated by use of medical devices (94). Major problems of biofilms are their inherent tolerance on defense mechanisms and antibiotic therapy. Therefore, there is an urgent need to manifest the alternative ways to prevent and control of biofilms associated infections (Sillankorva, Neubauer, and Azeredo 2008; Santos et al. 2009; Pirnay et al. 2011; Pires et al. 2011; Steier and Oliveira, 2019).

The microorganisms growing in a biofilms are intrinsically more resistant to antimicrobial agents than planktonic cells. The high dose of antimicrobial agents required to inactivate the biofilm growth. According to national institute of health (NIH) report more than 80 % of infections associated with biofilms such as dental plaques, urogenital tract infections, peritonitis, urogenital infections etc (98,99). Both gram positive and gram negative are capable to form biofilms which include *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*, *Klebsiella*

pneumoniae, *proteus species* (Mahgoub, Elfatih, and Omer 2015; Elkadi 2014). Pathogenic bacterial biofilms have been associated as a source of infection and often it's very difficult to treat with available antibiotics because they develop resistance to present antimicrobial treatment mechanisms and often acts as a source of a high number of bacterial communities. The bacteria which is encased in the biofilm showed the elevated drug resistance nature and even difficult to host immune system to clear (66,102,103).

Presently the numbers of strategies are used to eradicate the biofilms formation and minimizing the microbial load on the infectious sites. One of the widely employed methods for the treatment of biofilms is the bacteriophage therapy. The bacteriophages have been used for the treatment of bacterial diseases in plants, animals, and in humans. Phages are used to remove bacterial food contaminants in the stored food items proved by FDA (Food and Drug Administration, USA) was used to eradicate *Listeria monocytogenes* (104,105). The use of bacteriophages to control biofilms is one the best method because of its merits. Phages can replicate at the site of an infection, thereby increasing the number of progeny, where the bacterial load is predominant and where the biofilm films are formed. Moreover, single virion will produce the hundreds of progeny phages and some bacteriophages will produce degrading enzymes for the extracellular polysaccharide of the bacteria (71).

Bacterial communities in the extracellular matrix showed special features which are deviated from the planktonic bacterial cells such as, a) Intercellular signals between the community (Quorum sensing) usually which regulates the maturation and detachment of the biofilms to objects. b) Activation of secondary messengers, which plays a role in the formation of biofilms, in flagellar movements and production of extracellular polysaccharides. c) bap protein 12 plays a role in the formation of the matrix with the help of matrix scaffold proteins and create the suitable environment for the bacteria to live in the biofilm (Sanchez et al. 2013; Yazdi, Bouzari, and Ghaemi 2018; Yuan et al. 2019). Formation of biofilms depends on the many internal and external factors such as moist surfaces, energy sources on the site of the wound, type of bacterial association, availability of receptors for the bacterial attachment, temperature, and pH.

Biofilm formation stages:

Biofilm usually forms by the following four steps. Those are

- 1) Bacterial attachment to the suitable objects,
- 2) Micro colony formation by the association of different bacterial cells in the vicinity of the site of infection,
- 3) Maturation of the bacterial biofilms

by using available nutrients at the site of infection, 4) Bacterial dispersion or detachment of the biofilm is mostly by external enzymes such as proteases and nucleases, which degrades the external matrix of the biofilm (S. Kumari, Harjai, and Chhibber 2010; Kwiatek et al., 2015; Zhvania et al., 2017).

There are various methods available to detect biofilms: these include tissue culture plate, Tube method (110), Congo red agar method (111), bioluminescent assay (Liasi et al., 2009; Silva et al., 2014), Piezo electric sensors and fluorescent microscopic methods (114–116).

4. Bacteriophage therapy and its application

Now, over 60 years later, interest again is turning to phages, as adjunct therapies in the control of bacterial pathogens which emerged as resistant to antibiotics because of abundance increase of antibiotic resistance now again researcher's showing interest on used phages as therapeutic agents in medical, veterinary, poultry, agriculture and aquaculture applications (Wommack, and Radosevich 2003; Viertel, Ritter, and Horz 2014; Karthik 2014; Williamson, Zaczek, Weber-Dabrowska, and Górski 2015).

Antibiotic resistance is now observed in the following organisms throughout the world viz *Staphylococcus aureus*, *Salmonella*, *Mycobacterium tuberculosis*, *Acinetobacter*, *Escherichia coli*, *Streptococcus pneumonia*, *Campylobacter jejuni*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Clostridium difficile* (Ndip et al., 2007; Trigo et al., 2013; Sanchez et al., 2013; Sekulovic et al., 2014; Mendes et al., 2014; Mattila, Ruotsalainen, and Jalasvuori 2015; Leung et al., 2016; Chang, and Payne 2018; Sváb et al., 2018). This is evidenced that the phage particles do not disturb the normal intestinal flora with evidenced no side effects reported. It is also suggested that they can be use prophylactically and also in established infection because of their cell perpetuating nature, in the presence of susceptible bacterial infection, multiple administrations were not required (123,124).

Inspite of unlimited advantages, some of the inconveniences may also been observed (Pirnay et al., 2011a; Hall et al., 2012). They are, thus bacteriophages were not recommended for the patient condition is too critical. The phage preparations are not recommended to give Intraperitoneal and intravenous injections that contracted by illness and death. Usually phage preparations contain live bacteria. Rapid clearance of phages by spleen, liver and other filtering organs. Development of Lysogeny state would be a big problem. Antiphage antibodies is an another

problem because they won't be able to interfere with acute treatment lasting a week also. Because of these reasons the reoccurrence same bacterial infection progressed (12,67,70,126,127).

Bacteriophages when injected in diseased patients suffering from cutaneous boils caused by staphylococcus, results improvement within 48 hrs by reducing pain and fever. (Azeredo and Sutherland 2008; Crothers-Stomps et al. 2010; S. Sillankorva et al. 2010; Bowater 2016). Mershivalli and coligues 2009 proved that the phage cocktail consists of all the lytic phages exclusively used for burn wound patients infected by *Staphylococcus aureus* and *P. aeruginosa*. There is remarkable control of otitismedea caused by drug resistant *Pseudomonas aeruginosa* for which a clinical trial of the therapeutic phage preparations has been employed. So far phages have been reported effectively in treating bacterial diseases such as cerebrospinal meningitis, skin infections by *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *E. coli* etc. also cystic fibrosis, eye infections, neonatal sepsis, urinary track diseases and also cancers (Garbe et al., 2010; Wright Kvachadze et al., 2011; Tóthová et al., 2011; Schembri et al., 2015; Sharma et al., 2016; Pallavali et al., 2017; Waters et al., 2017; Fong et al., 2017; Brown-Jaque et al., 2018).

Mattila, Ruotsalainen, and Jalasvuori 2015 There is an important prospective reported by markoishivi et al, the phages impregnated polymers used to treat infections venousstatis skin ulcers. Similarly using in the same principle the combinational therapy has been implemented by using biodegradable polymers embedded with ciproflaxin and bacteriophages. The phages can also applied to treat various infections like urogenital system by direct infection or topical applications. As per as the phage therapy is concern MRSA is simply another stain treated by phages can be accomplished by local application. There is a need to provide the phage preparation for the systemic application in order to treat the patients suffering from immunocompromised state. With the grate increasing worldwide prevalence of antibiotic resistance, the bacteriophage endolycins represents a very promising novel alternative therapeutic against infections. Phage endolycins or lysins are enzymes that damage the cellular integrity by hydrolyzing peptidoglycan components. It is also evidenced that recently the phage lysins employed for the reduction of nasopharynx carriage of *K. pneumoniae* (Hall et al. 2012; Kęsik-Szeloch et al. 2013; Baranovskiy and Boichenko 2014; Dalmaso et al. 2016; Dalmaso et al. 2016; Maciejewska, Olszak, and Drulis-Kawa 2018)

Phage therapy has been tested successfully for protecting fish from experimentally induced bacterial infections in aquaculture and has been approved by the U.S.A. While several research groups continue to develop whole phage as alternative treatments, the isolation and optimization of purified phage components as antibacterial opens up new opportunities in the fight against vital infections. According to the scientific classification made by combination of three bacteriophage cocktails were more effective against the *Enterobacter cloaca* than the single bacteriophage. (140) the combined treatment of bacteriophage cocktail and polysaccharide depolymerase were best tools for the control of bacteriophages. Findings suggest that the combinational treatment of bacteriophages and chlorine is a promising method to control and remove bacterial biofilms from various surfaces (Fan et al.2012; Li and Zhang 2014; Zhang et al. 2018; Chen et al. 2018).

In contrast bacteriophages seems to have an abundant ability to target the bacteria growth, but high number of bacteria present with in biofilms which actually facilitates the action of bacteriophages by developing an efficient and rapid infection of the host and consequent amplification of bacteriophages. In general these phages have the intrinsic property to make the biofilm susceptible to their action i.e. they are known to induce enzymes which degrade the extracellular matrix. Some cultured biofilms are the better examples to support the replication of bacteriophages than other planktonic systems. It is perhaps and surprising that bacteriophages as the natural predators of the bacteria and are able to target the common form of bacterial life (Cornelissen et al. 2011; Azizian et al. 2013; Yan, Mao, and Xie 2014; Deshpande Kaistha and Kaistha 2017; D. P. Pires et al. 2017).

Biofilms are thought to underlie much of the resistance reported to antibiotics. As an outline of life cycles of bacterial biofilms it is exemplified that the *P. aeruginosa* is a motile bacterium has the capacity to produce vast amount of biofilms than the non-motile except *S. aureus* which forms extensive biofilms. The lytic bacteriophages replicates inside the host and release progeny particles are able to infect more number of bacteria. It has also been assumed that biofilms confer resistant to bacteriophages due to the impermeability of biofilm matrix. However, although they are utmost larger than antibiotics, bacteriophages are still very small then their bacterial host. Since, many of the bacteriophages they can infect the bacteria with in biofilm selectively, indeed one can make an argument that the phages have convoluted with biofilms and those infection of

biofilms would be expected. There are four mechanisms are under views to destruct the biofilms by phages.

1. Integrity of biofilms disrupted by destruction of cells producing the biofilm matrix.
2. Progeny bacteriophages diffuse through biofilms.
3. Biofilm matrix attacked by enzymes induced by bacteriophages from their host.
4. Persistent cells infected by bacteriophages which remain dormant until their reactive and lyse (Zhu et al. 2002; Ryan et al. 2011; Dalmasso et al. 2016; Zameer et al. 2016).

It is very important to note that different bacterial species produce different exopoly saccharides, thus depolymerase active against polysaccharides by one species may not digest that produced by others. Since the complexity (viability) in the exopolysaccharides is very lower than that of host where the bacteriophages have the broad activity to target the exopolysaccharides (141) which is evidenced from the young biofilms. However, block the activity of antibiotics, where amikacin showed 100 X the MIC activities enhancing the bacterial growth. Even though it has been shown to be sensitive at the MIC by disc susceptibility while growing planktonically.

Sharma et al. 2016 reported the onset of antibiotic resistance developed in the early stage of biofilms, thus the bacteriophages can kill bacteria in situation, where conventional antibiotic cannot do so (Santos et al. 2009; Oliveira et al. 2017). As a result, massive and often readily available and diversified bacteriophages isolated from natural environment, are almost target the bacteria including biofilms. It is further possible to optimize the bacteriophages for their intended application either initial selection or serial passage or other standard techniques. Hence, Bacteriophage possesses unique properties and show considerable promise in the control of biofilms. However, such applications are still evolving large scale production are still under development. Thus the identification is the most effective approach or to be required and speculative nature to reach the best practices for appropriate use.

The emergence of multidrug resistance in pathogenic bacteria has become a sweltering problem of modern medicine in worldwide, because increasing of immunocompromised patients due to transplantation and autoimmunity etc. Antibiotic discovery is a substantial success in the history of medicine. In general antibiotic resistance is a natural phenomenon of microorganisms particularly in bacteria. Tremendous and excessive use of antibiotics leads to the develop the acceleration mode of multi-drug resistance in bacterial

populations. However, treatment options for common infections were completely neglected and ran out.

In the present scenario, new classes of antimicrobial agents are needed to treat the infections but drug development pipeline is dry. As a result, now a day's, clinicians and microbiologist focused interest on the alternative therapeutics. To overcome this problem, very few therapeutic alternatives remain and pathogen resistant strains becoming more prevalent. Therefore, it is the high time to use non-antibiotics. In the early of 20th century Twort and d Herelle discovered the bacterial eaters independently and also Ernst Hankins (1896) reported the antibacterial activity in the Ganga and Jumna river water against *Vibrio cholera*.

Usually, these bacterial eaters were applied for inactivates the bacteria, many years ago, the innovation and extensive implementation of antibiotics as antimicrobial agents. Long term use of antibiotics extensively causes the severe problem to the global health care burdens and drug resistance of bacteria contributing to enhancing morbidity and mortality rates, where the patients are more susceptible to the bacterial and fungal infections (150–152).

Most of the septic wound infections caused by bacteria, but antibiotic-resistant bacteria are very common in hospital-acquired infections account for more than 80 % of the mortality. The most common bacterial species responsible for the septic wound infections viz. *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E.coli*, *CoNS*, *Enterobacter sps.*, *Enterococcus sps.* *Proteus sps.* *Acinetobacter sps.* To treat various bacterial infections traditional or a conventional option ahead, that is use of bacteriophages to treat the bacterial pathogens as an alternative, the replacement of drug resistance, use of bacteriophages become a choice of interest to public health. In this regard, recent studies have focused on the used bacteriophages indirectly to detect pathogenic bacteria or directly act as biocontrol agents. Most common nosocomial infections are spreading through burn wounds, diabetic surgical wounds or post-operative wounds. The mortality rate depends on the patient's age, immunological status of the subjects, and nature of the injury as well as the lodes of bio burden (J. M. Janda, Abbott, and Brenden 1997; Bowler, Duerden, and Armstrong 2001; Seth et al. 2012; Bayram et al. 2012; Baranovskiy and Boichenko 2014; Mama, Abdissa, and Sewunet 2014; Jafari and Karbasizade 2014).

Mandal et al. 2014; Founou, Founou, and Essack 2017 conducted a survey on the total antibiotic sales in selected countries from 2000 to 2010 discloses that India one of the economically growing country with the highest consumption of antibiotics approximate of 13 billion standard

units, followed by China (10 billion standard units) and the U.S. (more than 6 billion standard units), augmented intake of antibiotics were also reported from Australia and New Zealand (Carvalho et al. 2010; Mandal et al. 2014; Amatya, Rijal, and Baidya 2015; Mave et al. 2017).

World Health Organization conferring that 60 % of pathogens empowered resistance against the major antibiotics very often used against bacterial infections since from 10-20 years, because of their genetic constraint till to date, bacterial infections promoting their signals occupy the first place in the world in increasing deaths rate for about 18 million per year, for instance, death number recorded as 22 million individuals per year was of chronic diseases eg: Septic wounds. Antibiotics are capable for treatment of bacterial diseases albeit, due to many bacteria are developing and even developed resistance to antibiotics (García-Quintanilla et al. 2013; Shahi and Kumar 2016; Castillo, Nanda, and Keri 2019).

Allen et al. 2014; D. J.; Camelo-Ordaz, García-Cruz, and Sousa-Ginel 2015; Malik et al. 2017 were stated that the use of antibiotics leads to obfuscates treatment and creates important challenges in clinical prescription. That is why there is an actual and crucial medical need to develop substitute antimicrobial approaches that will kill specific problem causing bacteria without disturbing a normal, beneficial or gut micro biota (Tetz and Tetz 2018; Vila 2018; Cieplak et al. 2018). Therefore, they also suggested that one such potential alternative approach is required to use of lytic bacteriophages as an alternative medicine for treating bacterial infections caused by multidrug-resistant pathogens.

According to Kęsik-Szeloch et al. 2013; Baranovskiy and Boichenko 2014 self-medication and excessive administration of the antibiotics make less effective and provide side effects including the destruction of normal flora. Henceforth this is one of the main drawback for the development of new drugs viz. antibiotic but takes a lot of time for registration and as well as clinical trials owing high cost i.e. hundreds millions of dollars price. In spite of this, due to the fidelity of genetic changes and a wide range of mutations among pathogenic bacteria, rapidly of acquire resistance tack even to the synthetic drugs. The antibiotics action would not as specific as bacteriophages (“Mantle’s Transplant Spurs Interest in Organ Donation” 1995; Pirnay et al. 2011). Extensive use of antibiotics or chemical drugs showed the side effects like allergic reactions, kidney and hearing dysfunction, usually, the tetracycline can accumulate in the bones, disrupt the teeth growth and decaying of enamel and excessive antibiotic ingestion leads to anemia (20,161,162).

Recently Godebo, Kibru, and Tassew 2013; Mama, Abdissa, and Sewunet 2014; Mohammed et al. 2017; Nureye, Mohammed, and Assefa 2018 revealed that the most predominant bacterial isolates from burn wound patients were *S. aureus* (43.9%), *K. pneumoniae* (15.2%) and *E. coli* (13.4%). *S. aureus*, therefore, marked as more prevalent pathogen from their study, it was concluded that transmission might be due to it is nosocomial transmission or perhaps regarding as resident flora.

Mahgoub, Elfatih, and Omer 2015 worked on diabetic subjects revealed that the aerobic bacterial species isolated from wound infections were *S. aureus* (46%), *P. aeruginosa* (13.4%), *E. coli* (4.8%), *K. pneumoniae* (3.7%), *Enterococcus sps.* (8%), *Proteus sps.* (9.1%), *CoNS* (5.9%). As it was proved (Soares De Macedo and Santos 2005; Citron et al. 2007; Goswami et al. 2011; Godebo, Kibru, and Tassew 2013; Aisha Mohammed, Adeshina, and Ibrahim 2013; Pallavali et al. 2017) that the gram-positive isolate is *S. aureus* was predominant than *P. aeruginosa*. Like this way single species, microbial infections (77.3%) are frequent than the mixed infections (168).

Mengesha et al. 2014 reported that post-operative wounds were shown max percentage of *S. aureus* loads than (34.2%) followed by *K. pneumoniae* (24.8%), *CoNS* (15.4%), *Proteus spp* (12.8%), *P. aeruginosa* (9.4%), *E. coli* (5.1%) and *Citrobacter sps.* (3.4%) and about 102/123 (82.9%) and all the bacterial isolates noticed with multidrug resistance. Similarly, we also focused our research on the same lines. There is an emergency and need in search of new methods to contradict the MDR-bacteria.

Recently Bessa et al. 2015 described that the prevalent bacteria of chronic wound infections were *P. aeruginosa*, located on the deeper tissue regions of the wounds, whereas another predominant one was *S. aureus* is covered on the skin surfaces, (Di Giulio et al. 2018) due to multidrug resistance of bacteria, they employed graphene oxide as an alternative therapeutic agent to chronic bacterial infections. Also (170) proposed the novel therapeutic agent like i.e. bacteriophages used against the skin infections caused by *Acinetobacter baumannii* also resembles the multidrug resistance nature (171).

Similarly, one of the reports (Olira 2007; Goswami et al. 2011; Bandaru et al. 2012; Negi et al. 2015) described that the 20 % of post-operative wound infections acquired through nosocomial, encountered within 5 to 30 days of surgery. Due to poor surgical interventives in the

developing countries, post-operative wounds are infections were very common to exist. The total of 80 post-operative septic subjects had been considered for the study.

Garba Damen 2015; Amatya, Rijal, and Baidya 2015; Rao and Chakravarthy 2016; Almuhanha and Alnadwi 2018 describes the frequency of site-specific post-operative wounds are reported as 13 % and the frequencies monitored by bacterial isolates from post-operative wounds regulated by *S. aureus*, *Streptococci*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli* and all these isolates were showed resistance to penicillin co-trimoxazole. Perhaps they also noticed that diabetes a state of immuno compromising one of the predisposing factors to enhance and persistence of infections.

Bayram et al. 2012; Gupta et al. 2013; Dhopte, Bamal, and Tiwari 2017; Oliveira et al. 2018 studied and extensively described the factors responsible for the mortality rate of the burn wounds. The increasing of mortality rate with these infections among the 475 is 149 (31.1%). The significant factors for this mortality of burn patients were due to inhalation injury, positive cultures, and deep-seated wounds infections. They are also noticed that susceptible the female genders are more than male to induce wound infections (180). At the same time, The mortality rate is very high in wound infections created by mixed species *Acinetobacter* + *K.pneumoniae* (58.3%), and *Pseudomonas* + *Klebsiella* accounts for 31.5%, but *Acinetobacter* and *Pseudomonas* alone accounts for 31.5 %, 26.3 % respectively.

K. Gupta et al. 2013 worked on the bacteriological profile of post-operative wound infection of trauma patients, recognized from the US center for diseases control (CDC) of post-operative wounds showed as surgical site infections. There is an emergence of multidrug resistance at hospital environments leads to a challenge for the provision of high quality of health care. In this regard isolation of causative agents and wound infections are utmost important to treat with suitable drugs. Samples were collected by commercially available sterile swabs and after transportation in the laboratory, one set of swabs were immediately inoculated on the culture media (blood agar, MacConkey agar, EMB agar, Cetrinide agar, Mannitol salt agar, Nutrient agar, and Luria agar) and after incubation at 37 °C aerobically for 24 to 48 hrs, then gram stained and bacterial isolates were subjected to biochemical tests for identification and classification (Ndip et al. 2007; Khadri and Alzohairy 2010; Dessalegn et al. 2014; Akbar et al. 2014; Sawdekar, Sawdekar, and Wasnik 2015; Kishor et al. 2016; Aynalem Mohammed et al. 2017).

Some of the authors (Bowler, Duerden, and Armstrong 2001; Sikka et al. 2014; Dufour et al. 2016; Waters et al. 2017; Chhibber, Kaur, and Kaur 2018) detected that the biopsies from the wound tissue can be used to detect the causative agent of septic wounds instead the liquid pus or exudates. However, it is found to be difficult sample processing, need the local anesthesia and clinical experts for collection and analysis of the samples. Wound biopsies collected for the Quantitative analysis of bio burden of the bacteria which is not recommended for routine diagnosis (189,191,192).

Mave et al. 2017 described the elevated drug resistance of microbes responsible for the high mortality of 1.2 billion, but India is among resident Indians are more prone to extreme global bacteria diseases. The utility of antibiotic agents is radically increased from the last 20 years, as the progress drug resistance in bacteria; it is very difficult to formulate the concentration of antibiotics against the MDRs. (193) proved that inexorable spread of antibiotic resistance genes among pathogens.

Recent studies Pirnay et al. 2011b; Ghannad and Mohammadi 2012; Dufour et al. 2016; Castillo, Nanda, and Keri 2019; Milho et al. 2019 described the lytic activity now threaten the long term viability of antimicrobial therapy. Antibiotic resistance became a crisis situation. Besides this problem, non-antibiotic therapy walked out for serious consideration, among the preferable options specific phage therapy targeted bacterial pathogens it has essentially rediscovered by modern medicine which is cost effective. Bacteriophage LM33-P1 on the O25b *E.coli* which was highly resistant to the β - lactams, and Fluoroquinolones. They also recorded that phage LM33-P1 is efficient in invitro conditions and showed its burst size 320 plaque forming units. This intervention recorded similar invivo using different animal models, septicaemia, and UTI (188).

U. Patil, Bandekar, and Patil 2011; Hobizal and Wukich 2012; Saseedharan et al. 2018; Morozova, Vlassov, and Tikunova 2018 explained the facts from at ternary care hospital and described the multidrug-resistant bacteria from the diabetic foot infection. They found that the 289 bacterial species isolated from 178 tissue sample of 261 patients suffering from diabetic foot infections. Amongst them, 44.3% predisposed from single species and remaining were 55.7% were a mixed bacterial infection. This is one of the remarkable studies where mixed bacterial infections.

Osariemen Isibor et al. 2008; Godebo, Kibru, and Tassew 2013; Mengesha et al. 2014; Omole and Stephen 2014; Mahgoub, Elfatih, and Omer 2015 documented the similar reports on isolation of bacterial species from post-operative wounds and diabetic septic wound infections

revealed the prevalent bacteria were *S. aureus*, *P. aeruginosa*, *E. coli*, *K. aerogenes*, and also demonstrated that monomicrobial infections were prevalent (77%) in septic wound infections of diabetics and also noticed that both gram-positive and gram-negative bacterial isolates showed sensitivity towards both Gentamycin and Vancomycin antibiotics. All most all bacterial isolates showed the multi-drug resistance (82.9%), but with ciprofloxacin, gentamicin, streptomycin, augmentin, and ofloxacin are still effective against bacterial infections. They also noticed that males are much more prone to the wound infections than the females (Liasi et al. 2009; Akbar et al. 2014; Yakha et al. 2014; Charan Kaur and Wankhede 2014).

Chaudhary et al. 2015 demonstrated their results multidrug-resistant bacteria. Except for gentamycin which is one of the broad spectrum of antibiotic used for curing of superficial bacterial infections. In order to overcome the drug-resistant of bacterial pathogens, clinicians are gained and renewed interests on the old practices i.e. use of bacteriophages or phage cocktails were applied as biocontrol agents to target the bacterial pathogens (Fu et al. 2010; Pirnay et al. 2011b; Ghannad and Mohammadi 2012; Hall et al. 2012; Karumidze et al. 2013), though bacteriophage action was quite limited due to species-specific host range it does not affect the host natural flora. Clinical studies also have proven that phage therapy is one of the best ways to treat antibiotic-resistant bacterial infections and moreover, no side effects were reported.

Kutter et al. 2010; Abedon et al. 2011a; Nina Chanishvili 2012; Bolocan et al. 2016; (Dalmasso et al. 2016; El-Shibiny and El-Sahhar 2017 denoted that Georgia, Russia employed and suggested bacteriophages as targeting agents against the bacterial infections instead of antibiotics from the discovery of bacteriophages still described similarly the therapeutic application of bacteriophages against the *Escherichia coli* which causes a wide range of infections such as diarrhea, urinary tract infections, meningitis, pneumonie, and septicemia. With this limited information, they also described the latest research and potential role of bacteriophages as an ideal target against the *E. coli* related diseases.

Azizian et al. 2013 suggested that “the bacteriophages hold vast opportunities as a novel missile for fighting infectious bacterial infections”. Similarly, (Olira 2007; Pondei, Fente, and Oladapo 2013; Nilsson 2014; Jafari and Karbasizade 2014; Vijayaraghavan Shamsundar 2015; D. J. Malik et al. 2017; Speck and Wormald 2018) reported that “The extensive consumption of antibiotics both proper and improper way provided a selective impact on bacteria to evolve drug

resistance. The therapeutic use of bacteriophages also noted that ‘phages’ – viruses that adsorb, infect and kill bacteria are good old knowledge beginning new attentiveness, which may represent a worthwhile alternative to antibiotics in clinical settings”. Now a day’s most of bacterial diseases such as pyogenic infections, neonatal sepsis, dysentery, typhoid, salmonellosis post-operative wounds, skin infections, burn infections, diabetic foot ulcers, urogenital infections could be treated by using host-specific bacteriophages (Yadav et al. 2015; Deshpande Kaistha and Kaistha 2017 Cheng et al. 2018; Morozova, Vlassov, and Tikunova 2018; Castillo, Nanda, and Keri 2019).

Bedi, Verma, and Chhibber 2009 also disclosed the combinatorial therapy (amoxicillin and bacteriophages) which was extensively eradication of biofilms of *K. pneumoniae* B5055. Similarly (64) expressed their contribution on bacteriophages (phiIBB-PAA2 and phiIBB-PAP21 phages belonging to the family of *Podoviridae*) isolated against *Pseudomonas aeruginosa* PAO1 and ATCC 10145 controls the both planktonic as well as biofilms of the *P. aeruginosa* by using phage cocktail lytic action. (201,207) and his colleagues were proved that bacteriophages were isolated from the wastewater treatment plant against *Pseudomonas* infection. These results suggested that the potentiality of phages, especially phage cocktails, applied on to the surfaces of indwelling medical devices for mitigating biofilm formation by clinically significant bacteria (Donlan 2001; Azeredo and Sutherland 2008; Fu et al. 2010; Ryan et al. 2011; Nouraldin et al. 2016; Webber and Hughes 2017).

The emergence of antibiotic-resistant and lack of production of novel antibiotics by the pharmaceutical industries lead to a crucial and an essential to improve novel methodologies to compete MDRs, exclusively *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Earlier the Bacteriophage therapy had been applied for decades as a means of treating bacterial infections in some countries across the world and numerous encouraging results have been documented (Periasamy and Sundaram 2013; Bolger-Munro et al. 2013; Vandersteegen et al. 2013; Golkar and Jamil, 2013; Frampton et al. 2014; Cao et al. 2015; Kwiatek et al. 2015; Sváb et al. 2018).

Vinodkumar, Kalsurmath, and Neelagund, 2008; Golkar 2013 showed that bacteriophages are the suitable agents for the lysis of multidrug resistant *Pseudomonas aeruginosa*, isolated from the neonates over five year’s period Carvalho et al. 2010; Abedon et al. 2011b; Gutiérrez et al. 2011; Hall et al. 2012; Kęsik-Szeloch et al. 2013; Kęsik-Szeloch et al. 2013.

Skurnik and Strauch 2006; Hall et al. 2012; Keşik-Szeloch et al. 2013; Keşik-Szeloch et al. 2013; Coulter et al. 2014; Kumaran et al. 2018 showed their results on combinational therapy of bacteriophage and tobramycin against the biofilms of multidrug-resistant *P. aeruginosa* and *E. coli*. Usually, they applied the eradication of *E. coli* biofilms by T4 phage and for the control of *P. aeruginosa* by phage PB-1 and showed 99% of reduction of biofilms of both pathogens. Interestingly, they noticed the treatment with antibiotics alone showed 39 % on the *E. coli* and 60 % on the *P. aeruginosa* biofilms. This is one of the prominent studies which supported the use of bacteriophages in combination with tobramycin and is good enough to eliminate the biofilms of MDR-bacterial isolates.

Pallavali et al. 2017; Fong et al. 2017; Chhibber, Kaur, and Kaur 2018; Cieplak et al. 2018; Cha et al. 2018 proved that unlike antibiotics; bacteriophages are the suitable candidates for the destruction of pathogens in the intestine but does not cause any harm to the normal flora and demonstrated the broad spectrum antibiotic namely ciprofloxacin killed the all beneficial flora present in the intestine whereas bacteriophage cocktail of three different specific and selective phages (ECML-363, ECML-122, and ECML-359) targets and kills only pathogenic *E. coli* only.

Recent by Sanjay Chhibber et al 2018 suggested and proved the treatment facts of diabetic wounds (*S. aureus*) by liposome entrapped phage cocktail of MR-5 and MR-10 an efficient than the free phages used to eradicate the bio burden of diabetic wound infections. These results were recommended that the liposome-entrapped phage cocktail treatment is much more efficient than the free phages, employed on diabetic induced mice. Similarly, recent reports of Chen et al., 2018 explained the information on the phage cocktail was very efficient than the single phage action against the multidrug-resistant bacteria which causes diseases in pieces by *Aeromonas salmonicida*.

The respiratory infections of *Pseudomonas* were treated with bacteriophage PEV2 by formulating the aerosol formation by Spray drying method was reported by (223). This study successfully documented and proved that phage PEV2 is therapeutic agent against the respiratory tract *Pseudomonas* infection. After several inventions, recently one of the US-based company OmniLytics has developed the Agriphage stocks which consist of phages against *Xanthomonas campestris* and *Pseudomonas* for the eradication of bacterial diseases in crops of tomato and

pepper plants. Company OmniLytics states that the Agriphage is safe for the plants as well as for humans (120,138).

Yazdi, Bouzari, and Ghaemi 2018 declared that bacteriophage vB PmiS-TH has the highest lytic activity on both the planktonic and biofilms of the *Proteus mirabilis* which was isolated from urinary tract infections. Further (206,224) they suggested that the combinational therapy with antibiotics was also a good practice which preferred for the removal of older biofilms.

Crothers-Stomps et al. 2010; Khairnar et al. 2013; Madsen et al. 2013; D. Castillo et al. 2014; Stalin and Srinivasan 2016 and their coworkers were demonstrated that antibacterial efficacy of the bacteriophages against the *Vibrio spp.* in the aquaculture systems. The therapeutic potential of bacteriophages namely VhCCS-01, VhCCS-02, VhCCS-04, VhCCS-06, VhCCS-17 VhCCS-19, VhCCS-20 VhCCS-21 showed the perfect lysis on the *Vibrio* species.

Agsar et al. 2013 described the therapeutic potentials of the phage Φ DMPA-1, was isolated from the sewage showed maximum lytic action against the MDR-bacteria DMPA-1 isolated from the pyogenic skin infections. Similarly (229) demonstrated invitro and invivo bacteriophage therapy against the MDR-*E. coli*, was isolated from bed sore and foot ulcers of diabetic patients from Iran. Phage TPR 7 prepared from environmental water samples and showed the broad host range and bacteriolytic activity against the multi-drug resistant *E. coli* (46,179,190,197).

Phages are pervasive in environment and play a fundamental role in evolution and emergence of new pathogens. So, there is a need of development of alternative drugs, but it must be natural, non-toxic for controlling the bacterial pathogens and their biofilms. Bacteriophages are gladly accessible from the environmental samples. Contemporary attention in phages has been enthused. Bacteriophage or phage cocktail treatment against either single or multiple species biofilm, due to lytic natures of phages, there is a reduced number of adherent cells within the biofilms (31,98,140,230).

González et al. 2017 proved that two lytic phages, B_SauM_phiIPLA-RODI (phiIPLA-RODI) and vB_SepM_phiIPLA-C1C (phiIPLA-C1C), belonging to the *Myoviridae* family and exhibiting wide host ranges and reduced the cell number of *S. aureus* and *S. epidermidis*. Invitro lytic activity of phages against planktonic cells showed the 5 log units eradication of cells where

in case of single and dual species biofilms, adhered cells eradicates only 2 log units (Sillankorva, Neubauer, and Azeredo 2010; González et al. 2017; Fang, Jin, and Hong 2018).

The biofilm of *S. aureus* is treated with combinational therapy with both antibiotics and bacteriophages is suitable agents for the eradication of biofilms and also suggested that treatment with phage followed by antibiotics is preferable for the removal of biofilms suggested by (222). This bacteriophage, target the host cell enzyme also removes the exopolysaccharides and provides the space for the entry of antibiotic to kill the bacteria in which it is encased in the biofilms (Sutherland et al. 2004; Williams 2013; Yuan et al. 2019).

Kęsik-Szeloch et al. 2013; Mattila, Ruotsalainen, and Jalasvuori 2015; Sváb et al. 2018 noticed the 32 isolated bacteriophages has the lytic activity against the extended-spectrum beta-lactamases (ESBL) Enterobacteriaceae strains including *Klebsiella*. They were characterized by biophysical and molecular characterization and proven the bacteriolytic activity of these phages against the ESBL- *Klebsiella species*. (106) also demonstrated that the combinational therapy is one of the critical practice for the treatment of urinary tract infection caused by *Proteus mirabilis* bacteriophage vB_PmiS-TH belonging to *Siphoviridae* family has the burst size of 260 pfu and ampicillin would be selective on both planktonic and biofilms of the *P. mirabilis*.

Battaglioli et al. 2011; Delfan et al. 2012; Li and Zhang 2014; Kwiatek et al. 2015; Yazdi, Bouzari, and Ghaemi 2018 suggested that bacteriophages can be used for the wastewater treatment for which they isolated two bacteriophages from wastewater belonging to the families of *Myoviridae* and *Podoviridae*.

Future studies are still needed to focus to use bacteriophages as therapeutic agents in *in vivo* conditions. Bacteria have the ability to change with physical factors. Accordingly, some of the challenges presented on the available data are still compelling for future applications of bacteriophages as biocontrol agents (Nakonieczna, Cooper, and Gryko 2015; Deshpande Kaistha and Kaistha 2017; Zhou, Feng, and Zong 2018; Gutiérrez et al. 2019). Phage therapy is successful in *in vitro* and pre-clinical administration by *in vivo*. However, there is enormous data from more clinical trials to fully assist the development of bacteriophages used against various pathogenic infections as alternative therapeutics (Hospital et al. 2008; Kęsik-Szeloch et al. 2013; Abbasifar et al. 2014; Gu et al. 2018).

Le et al. 2014; Xia and Wolz 2014 demonstrated that phage-resistant bacterial evolution was due to their chromosomal aberrations. Since then they conferred that one of the main drawbacks of phage therapy is the evolution of phage resistant bacteria against the specific bacteriophages is due to the idles of DNA (234,239). It was proved by conducting an experiment on the *Pseudomonas aeruginosa* PA1 with phage PaP1 by using gene manipulation studies, transposon mutagenesis in murine models.

Xia and Wolz 2014 documented that twenty-two phages were isolated against the *Pseudomonas aeruginosa* from an African city and showed the large variety of phage types against the Pathogens, which suggests evolutionary relations with their specific bacteria. Zhu et al. 2002; Liu et al. 2014 predictable that expression of early and late- early proteins of Gp13 and Gp 21 proteins of phage LUZ19 leads to complete eradication of *Pseudomonas aeruginosa* PAO1.

Similarly (241) has also demonstrated the bacteriophage (M^{Sa}) recused the 97 % of mice of infection caused by methicillin-resistant *Staphylococcus aureus* A170. Both invitro and invivo phage therapy against the MDR-*S. aureus* stated that significantly reduced the abscess formation and bacterial loads. It has been revealed that the potential utility of bacteriophages against to treat both local and systemic *S. aureus* infections in humans.

Isolated and characterization of phage SPW against the bovine mastitis-causing *S. aureus* done by (144) and this study proved that the bacteriophage is the suitable candidates for the lysis of *S. aureus* in particular because of its wide host range and stability at various pH ranges, chloroform, and isopropanol and environment. Phage SPW belonging to the family *Myoviridae*. Brown, Brown, and Burlingham 1972; Vandersteegen et al. 2013; Gutiérrez et al. 2015; Kishor et al. 2016 revealed that uses of phages as a potential tool for the treatment or prophylaxis of *Staphylococcus aureus* associated diseases. Also (244) demonstrated that Phage ϕ MSP belonging to the *Siphoviridae* family. Albeit this bacteriophage was isolated and characterized from the veterinary sewage applied against the *S. aureus* and showed the perfect lysis. In spite of the above remarks, this study also showed that phage-infected bacteria produce some overexpressed proteins and some are under expressed proteins used as markers for selection.

Trung et al. 2005; Sridhar, Umavanitha, and Umamaheswari 2013; Bourdin et al. 2014; Sváb et al. 2018 emphasized that bacteriophages were isolated from the sewage samples against

the bacterial pathogens such as *E. coli*, *S. typhi*, *Klebsiella sps.*, *Shigella sps.*, and *Pseudomonas aeruginosa*. Similar to this study we also proved that bacteriophages against human pathogens are abundantly present in the sewage such and these phages may hold a lot of promise as the first choice of prophylaxis against the nosocomial multi drug-resistant bacterial infections with burst size, stability at various pH and temperature Sasikala and Srinivasan 2016; Yazdi, Bouzari, and Ghaemi 2018; Cieplak et al. 2018.

The invivo application of T4 Phages such as JS4, JSD.1, JSL.6, and JS94 was isolated from the stool specimens against diarrhea associated *Escherichia coli*, belonging to the family of *Myoviridae* showed by Sandra Chibani-Chennoufi et al 2004. They verified the lytic activity against the clinical isolate of *E. coli* by administrated by various routes. Murine intestinal *E. coli* isolates were lysed by the invitro application of phage cocktails (Chibani-Chennoufi et al. 2004; Seema Kumari, Harjai, and Chhibber 2009; Olszak et al. 2015; Sasikala and Srinivasan 2016).

Le et al. 2014; Hua et al. 2018 enlightened that Intraperitoneal route of injection of imipenem resistant *P. aeruginosa* were noticed the 100 % mortality within 24 hrs in the mice model. But the phage ØA392 application after bacterial challenge (60 min) was sufficient to rescue the 100 % of the murine model. This study also revealed that alone high dose of bacteriophages not causing any side effects or harm to animals.

According to the statements given by (Wagenaar et al. 2005; Capparelli et al. 2007; Barbosa et al. 2013; Sridhar, Umavanitha, and Umamaheswari 2013; Mattila, Ruotsalainen, and Jalasvuori 2015) now a day's phage therapy offering a substitute approach in contradiction of the developing multidrug-resistant bacterial infections. In order to prove that the isolation and identification of phages can be demonstrated by employing spot assay or by using plaque assay methods. One of the problems encountered during isolation of bacteriophage is the formation of tiny or pinpoint plaques on the double layer agar plates. To resolve this problem some of the remedies can be applied on with small plaques, the enhancement can be overcome by using sub lethal concentrations of antibiotics.

Silvio B Santos et al 2009 described the concepts for the use of the antibiotics (Ampicillin, Cefotaxime, Tetracycline) along with glycerol (5 to 20 %) to improve the plaque size on the double layer agar media (95,226). Luo et al. 2012; Higuera et al. 2013; Kęsik-Szeloch et al. 2013; De

Sordi, Lourenço, and Debarbieux 2018; Yazdi, Bouzari, and Ghaemi 2018 also evaluated the plaque size on the double layer agar plate by phages phi PVP-SE1, phi PVP-SE2 (*Salmonella enterica Enteritidis*), phi IBB-PF7A (*P. fluorescens*), and phi IBB-SL58B (*S. lentus*). The antibiotics and glycerol combinations increase the plaque size on the double layer plates. Sandeep 2006; Chhibber, Kaur, and Kaur 2018 similarly focused on the work done by bacteriophages i.e. enhanced the plaque size by three times with phage MR-5 against the *Staphylococcus aureus* ATCC 43300 using antibiotics i.e. highlighted the modification of traditional double layer agar method for the enhancement of plaque size for better observation of phages (6,255,257,259,260).

Klieve 2005; Sandeep 2006; Skurnik and Strauch 2006; Keşik-Szeloch et al. 2013; Bourdin et al. 2014; Leung et al. 2016 had been evaluated the different types of parameters for the amplification and purification of T4 like *Escherichia coli* bacteriophage for the recommendation as therapeutic agents against various bacterial infections. Fan et al. 2012; Husev 2013; Fernández et al. 2017a also demonstrated, isolated the 18 bacteriophages specific for *E.coli* from the hospital sewage canals. Total 18 phages, 10 phages have the broad host range (Abbasifar et al. 2014; Dalmaso et al. 2016).

Bacteriophages are not only used for the therapeutic agents against clinical bacterial pathogens and also used for the biocontrol agents (McVay, Velásquez, and Fralick 2007; Carvalho et al. 2010; Schmerer, Molineux, and Bull 2014; Hoyles et al. 2015). It was elucidated during sewage wastewater treatment and a novel approach for the pathogen reduction in the wastewater. This method of treatment does not cause any harm to the environment. Interestingly biological pollution of water can be measured by observing the multidrug-resistant bacteria in water such as *E. coli*, *Bacillus* spp., *Pseudomonas* spp., *Streptococcus* spp. etc. was supported by reports of Periasamy and Sundaram 2013. They showed the 100 % biocontrol of *E. coli* by using its specific phage within a 14 hrs of incubation, since in same lines of our research; they highlighted the potential role of bacteriophages in treatment for normal control of bacterial populations in the hospital wastewater treatment. At the same time another study from Lithuania by Klausa et al., 2003 and reported that T4 type phages are abundantly present in the municipal sewage. Therefore these studies highlight the generalized application of bacteriophages as biocontrol agents during wastewater treatments (267,269).

Multidrug resistance of bacterial species is not only the fixed problem for human beings, and birds but also the problem of poultries, veterinaries, etc. pathogenic *Escherichia coli* where severe effects on poultry industries across the globe. Reported by Zhang et al. 2018, he suggested that bacteriophage therapy for MDR-bacterial infections in the poultry industry is a paradigm of the medical era and also one of the best aspects for the diversified field in such a way they used T4 like phage Bp7 (*Myoviridae*) to counter the MDR-*Escherichia coli*.

C. Carvalho et al 2010 enlightened on the invivo efficacy of bacteriophage cocktail to reduce the bacterial lode on the poultry meat using phage phiCcolBB12, phage phiCcolBB35, and phage phiCcolBB37 as phage cocktails were employed to reduce the *Campylobacter coli* and *Campylobacter jejuni*. Carvalho et al. 2010 noticed that bacteriophages are used for the treatment of poultry meat infection caused by *Campylobacter coli*. Bacteriophages could be a potential antimicrobial agent against the MDR-*E. coli* instead of antibiotic therapy, exclusively the antibiotic therapy has been they facing an enhanced threat with antibiotic resistance Hobizal and Wukich 2012; Bolocan et al. 2016.

Interestingly Battaglioli et al. 2011; Piracha et al. 2014 is another research by reported that isolation of bacteriophages ϕ EB49 (*Myoviridae*), ϕ EB5, ϕ EB32 and ϕ EB47 (*Siphoviridae*) has the ability to generalize, transducing nature on the uropathogenic *E. coli* for providing a significant development of genetic tools for MDR therapy (Higuera et al. 2013; Amsa et al. 2014; Sasikala and Srinivasan 2016).

The human application of T4 bacteriophage to control diarrhea caused by *Escherichia coli* a novel approach conducted by Chibani-Chennoufi et al. 2004, from his studies, the application of T4 phage against the diarrheal by *E. coli* and human volunteers received a high dose of phages through the drinking water, no fecal phages was observed and also there are no T4 specific antibodies. This is one of the remarkable studies which supports the first human application of bacteriophages clinically (Frampton, Pitman, and Fineran 2012; Golkar 2013; Azizian et al. 2013; Viertel, Ritter, and Horz 2014; Elbreki et al. 2014; Broxmeyer, n.d.).

Vandersteegen et al. 2013 performed the biofilm degradation studies by using phage Romulus and Remus; belonging to *Myoviridae* family. Bacteriophages Romulus and Remus are specific phages of *S. aureus* and showed broad host range, and rapid initial adsorption of biofilm

degrading capacity (Kakoma 2009; Alkhulaifi 2017; Fong et al. 2017; D. J. Malik et al. 2017; Duplessis et al. 2018; Kumaran et al. 2018).

Hall et al. 2012 documented that four bacteriophages were isolated from the sewage samples against the *Pseudomonas aeruginosa*, namely bacteriophages PT7, 14/1, ϕ KZ belonging to the family of *Myoviridae* and phage PNM belonging to the *Podoviridae* family. They also performed both invitro and invivo studies in the wax moth larvae using the single and multiple combinations of bacteriophages against the *P. aeruginosa*. It was heightened that, their observations noted that phage therapy increased the life span of wax moth larvae infected with *P. aeruginosa*, and a phage cocktail was the most effective for the treatment.

Khalifa et al. 2018 suggested that the bacteriophage EFLK1; belonging to *Myoviridae* is isolated successfully against the phage-resistant strain of Vancomycin-resistant *Enterococcus faecalis* V583. This is the first study was proved even phage resistant bacteria can be killed by other phages from the same host because of genetic dominance.

Similarly (250) phage cocktails of two phages PA5 and KT28 showed the lytic activity against the *P. aeruginosa*, isolated from cystic fibrosis patients and executed in both invitro and invivo studies in *Galleria mellonella* larvae model. Cao et al. 2015 isolated bacteriophage vB_PaeP_PPA-ABTNL (PPA-ABTNL) (belonging to the *Podoviridae* family) from the hospital sewage against *P. aeruginosa*; the therapeutic potential of bacteriophages was proved by both invitro and invivo studies.

Hung et al. 2011 reported bacteriophage ϕ NK5 was isolated from a sewage sample against the bacterial abscesses caused by *Klebsiella pneumoniae* in the liver of murine sample. These results suggested that a low dose of ϕ NK5 is a potential therapeutic agent for *K. pneumoniae*-induced liver infection. Correspondingly, Waters et al. 2017 noticed the evolution of the multi-drug resistant bacteria and their increased rate of infection leads to phages as alternative agents against bacterial infections, i.e. old therapeutics gets renewed interests as biocontrol agents. To prove this, perspective murine study model with lung infections by *P. aeruginosa* LESB65 and NP22_2 was treated with Phage PELP20 and showed the total escape from the lung infection. This is one of the confirmatory works done and the study showed that phage therapy is an effective treatment against chronic (*P. aeruginosa*) lung infections, and also showed the efficacy against (*P.*

aeruginosa) biofilm-associated cystic fibrosis lung-like environment. These in vitro studies were revealed the prospective application of bacteriophages for the chronic lung infections (McNerney 1999; V. Verma, Harjai, and Chhibber 2010; Garbe et al. 2010; Ryan et al. 2011; Tóthová et al. 2011; Shen et al. 2012; Leung et al. 2016; Hua et al. 2018).

Kakoma 2009; Abedon 2015; Fong et al. 2017; D. J. Malik et al. 2017; Alkhulaifi 2017; Taha et al. 2018; Duplessis et al. 2018 reported that phage ZCKP1 was isolated from the freshwater against the *K. pneumoniae* isolated from the diabetic foot infection patients; phage ZCKP1 showed its lytic action on MDR- *K. pneumoniae*, which forms both planktonic and biofilm, has been proved the invitro action against the MDR-bacteria and invivo studies are still pending.

Eriksson et al. 2015 noticed that phages vB_KpnP_SU503 vB_KpnP_SU552; belonging to the Podoviridae family, Autographivirinae subfamily and genus Kp34 like virus. These phages vB_KpnP_SU503 (SU503), vB_KpnP_SU552A (SU552A) were isolated from the sewage samples worked about against the antibiotic-resistant *Klebsiella pneumoniae* Leung et al. 2016; Zhou, Feng, and Zong 2018.

Seema Kumari, Harjai, and Chhibber 2010 studies on Pa29, Pa30, Pa31, Pa33 and Pa34 bacteriophages isolated from sewage samples showed a lytic action on *Pseudomonas aeruginosa* PAO, but invivo conditions these phages do not show any lytic action on *P. aeruginosa* observed in murine burn model. From this study, they suggested that some mutations may occur in the bacterial receptors and phages may not recognize and responds to those particular receptors. For instance, an invivo application of bacteriophages need to study and the host-phage interaction probably and pharmacological interventions etc. (McNerney 1999; Burrowes et al. 2011; Shen et al. 2012; Henriksen et al., 2013; Bourdin et al. 2014; Reindel and Fiore 2017).

Seema Kumari, Harjai, and Chhibber 2011a from her other reports saying that bacteriophage action is higher than the silver nanoparticles and antibiotic gentamycin. These results strongly suggested that phage Kpn5 has therapeutic value in the treatment of burn wound infection as a single topical application of this phage was able to rescue mice from infection caused by *K. pneumoniae* B5055 in comparison to multiple applications of silver nitrate and gentamycin.

Cohen et al. 2013 documented that bacteriophage and bacteria were isolated from seawater. Therefore their results demonstrated that bacteriophage YC has the potential to treat

coral disease outbreaks triggered by the bacterial pathogen *V. coralliilyticus*, made it a suitable candidate for phage therapy management of the coral disease. These bacteriophages have a potential lytic role of aquaculture related bacterial pathogens (70,128).

Dias et al. 2013 reported ten bacteriophages (Ufv-aur2 to Ufv-aur11) were isolated from the sewage sample against to *Staphylococcus aureus* of mastitis. These phages showed dominant lytic action on the antibiotic-resistant *S. aureus*. These integral results suggested the bacteriophages used as therapeutic agents against the MDR-bacteria of veterinary bacterial diseases Verma et al. 2013; Sanchez et al. 2013; Karthik 2014; Mendes et al. 2014; Cassat, Smeltzer, and Lee 2014; Gutiérrez et al. 2015; Fernández et al. 2017).

Ryan et al. 2011 suggested that bacteriophage T4 and antibiotic cefotaxime combined therapy can eradicate the biofilms of *E. coli* and even alone phage therapy can clear the *E. coli* biofilm. (257,287) reported that phage CHOED isolated from the Chilean mussels against the *Vibrio anguillarum*, a fish species related vibriosis is one of the fatal hemorrhagic septicemia. Interestingly 100% of salmon fishes are survived by application of phage CHOED, which indicates that phage therapy is an appreciable and remarkable tool in aquaculture.

Mathur, Vidhani, and Mehndiratta 2003; Qiao et al. 2010; Periasamy and Sundaram 2013; Cao et al. 2015 reported that phage OMKO1 was isolated from the pond water against the *Pseudomonas aeruginosa*. They showed the phage treatment re-establishes the antibiotic sensitivity towards Erythromycin, Ceftazidime, Tetracycline, and Ciprofloxacin of MDR-*P. aeruginosa*. Bacteriophages therapy, on the other hand, restores the antibiotic sensitivity in the multidrug-resistant bacterial strains.

Ritchie and Klos 1977; Santos et al. 2009; Synnott et al. 2009; Kaur, Harjai, and Chhibber 2012; Rahmani et al. 2015; Zhang et al. 2018 demonstrated that the comparison of spot and plating assay methods, from their results it was clarified that the problem associated with the biology of phages and interactions between the phages and their bacterial hosts. In order to maintain the phage libraries, phages were selected by using efficiency of plating (EOP) method than the spot test, by virtue of comparison, employed *Escherichia coli* as reference (ECOR) host.

Chhibber, Kaur, and Kumari 2008; Bedi, Verma, and Chhibber 2009; Karumidze et al. 2013; Hoyle et al. 2015; Eriksson et al. 2015; Zhvania et al. 2017; Pallavali et al. 2017 showed

bacteriophage KLPN1 was isolated from the against the *K. pneumoniae* from the human sample. Their results were noticed that phages were suitable candidates for the application and treating a range of *K. pneumoniae* diseases. Further categorization of their gene products is henceforth required to conclude their practicality.

Zhou, Feng, and Zong 2018 similarly bacteriophages were isolated from the sewage showed the highest burst size and latent period. Semler, Lynch, and Dennis 2012; Lynch et al. 2013; Shan et al. 2014; Sharahi and Tehrani 2019 reported that, the phage JG068 was isolated against *Burkholderia cenocepacia* K56-2 showed the wide range towards *Burkholderia multivorans*, *B. cenocepacia* stabilize, and *Burkholderia dolosa*. The selective and suitability of these phages were target and biocontrol agents or as future therapeutics.

5. Conclusion:

Bacteriophages and phage cocktails surely provide the countless benefits to society mainly in science, agriculture, Aquaculture, veterinary science, also offered an excellent solution to multi-drug resistant bacterial infections. Phages and cocktails eradicate the biofilms of MDR-bacteria and provide protection from numerous bacterial infections. Along with phages, cocktails and also phage proteins have capacity to abolish the bacterial infections.

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