Reseach Progress and Future of Phage Therapy in Klebsiella Pneumoniae Infection

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Abstract. Bacteriophages are widely found in nature and can specifically lyse bacterial viruses. Because of the extensive use and abuse of antibiotics, bacterial resistance has become a global problem. Klebsiella pneumoniae is one of the most common clinical drug-resistant bacteria, and the incidence is increasing year by year. Because many strains of Klebsiella pneumoniae are multidrug-resistant, treatment has been difficult. And this resistance has been increasing in recent years, adding to this many new difficulties due to the misuse of antibiotics. The phage can be a new way treat Klebsiella pneumoniae by lysis, which is non-toxic, non-secondary drug resistance, highly specific and penetrating, however, it has not been widely used in clinical practice and only a few cases have been reported. Through the in-depth study of phages, can play an important role in drug-resistant Klebsiella pneumoniae. The paper will introduce the characteristic Klebsiella pneumoniae and phage therapy, and also summarize development of Current animal models and clinical trials of phage therapy in Klebsiella pneumoniae.

Keywords: Bacteriophage, phage therapy, Klebsiella pneumoniae, antibiotic resistance.

1. Introduction

Klebsiella pneumoniae is a very common opportunistic bacteria in hospitals and is one of the common bacteria in hospital infections, with high infection rates and mortality rates. With the increasing use of antibiotics, drug resistant Klebsiella pneumoniae has become more common. Antibiotic use is ineffective in clinical practice. The toxicity of Klebsiella pneumoniae is related to its surface capsule serotyping, and antibiotic resistance is related to the genome of Klebsiella pneumoniae. To treat Klebsiella pneumoniae, a old therapy called phage therapy was proposed again. Phage therapy is depended by characteristic that phage have the ability to kill the bacteria. This treatment is getting renewed attention, in the increasingly resistant.

Phages are specific adsorbents of receptors on the surface of bacteria that inject DNA into the host cytoplasm. Through the process of DNA replication and gene expression, they assemble offspring viral particles, and finally release offspring viruses through lysis of the host. Phage therapy has the following advantages:(1) Phages are non-toxic,e ability to attack very limited bacteria. Targeting a bacterium with almost specificity. So, it won't harm other bacteria; (3) Phages have good penetrability and are easy to reach many places that other drugs cannot reach. So, it will have better treatment outcomes. Klebsiella pneumoniae infection. Animal experiments have shown that bacteriophage therapy is effective, and it has been found that drug treatment time is more important than drug dosage. The earlier the use of bacteriophages, the better the treatment effect. However, formal clinical trials of phage therapy for Klebsiella pneumoniae have not yet they only attack bacteria, not human cells; (2) Phages do not develop secondary resistance similar to antibiotics, resulting in a short development cycle; (3) Phages have high specificity. Specificity is begun. At present, there are only a few reported cases where bacteriophage therapy is effective.

2. Klebsiella pneumoniae

Klebsiella pneumoniae is a kind of Gram-negative bacteria that can be commensal in many different kinds of environments. Many places such as soil, water, and animals are the colonization by this kind of bacteria [1]. It was widely distributed in many parts of the body of humans and animals,

such as the mouth, skin, urogenital tract and so on. Meanwhile, it is considered a kind of food-borne pathogen which was hidden in many foods [2]. Though it is not dangerous for the food safety, a host of Klebsiella pneumoniae for separation and identification of bacteriophage used in the experiment was isolated from the food many times. Also, Klebsiella pneumoniae is a very common kind of hospital opportunistic bacteria that spreads through people-people content. Newborns, elder people, and people who have immunity deficiency are the main group of the klebsiella pneumoniae inflection. It caused a very high death rate that is up to more than 50% [3]. Klebsiella pneumoniae infection accounts for about 3% to 7% of all bacterial infections in the hospital. It has become one of the important eight pathogens in the hospital [1]. The high death rate and the rate of infection of Klebsiella pneumoniae have become a little popular and attracted the wide attention of many researchers in the public. The high death rate is only one reason, the other reason for attraction of Klebsiella pneumonia is its antibiotic resistance. Klebsiella pneumonia appear to a strong antibiotic resistance in many medicines therapy, especially the aminoglycoside resistant Klebsiella strains. With the increasing use of antibiotics, antibiotic resistant Klebsiella pneumoniae has become more prevalent, presenting additional difficulties and obstacles to clinical treatment.

2.1. HvKp

Now in life, the most common kind of klebsiella pnumoniae is called Classic klebsiella pneumoniae (cKp). It caused nosocomial infections, leading to increased morbidity mortality [1]. It has relatively high antibiotic resistance to some extent. But in these years, a kind of super-bacteria belongs to klebsiella pnumoniae family that is called Hypervirulent klebsiella pneumoniae (hvKp). Hypervirulent Klebsiella pneumoniae can cause many diseases, including pneumonia, liver abscess, meningitis, osteomyelitis and sepsis, and all of them are associated with high morbidity and mortality. Clinical antibiotic therapy is not effective to treat this disease. And hvKp has the ability to infect many kinds of groups, including young strong people etc. Compared to cKp, the ability to infection of hvKp is stronger and the antibiotic resistance is greater. HvKp first appeared in the clinical report in Taiwan in the 1980s. From then on, cases of hvKp occurs in many countries in Asia, Europe, and America. Among them, Asia is an important location for hvKp transmission. In recent years, the incidence of HVKP-related diseases continues to increase globally. For example, In Taiwan, the annual incidence of hvKP-induced liver abscesses increased by nearly 60% between 1996 and 2004. In Korea in the mid-2000s, the incidence of liver abscess caused by Klebsiella pneumoniae increased from 3.3% to 78.2% [4]. When hvKp appears, the characteristics of this special bacteria attract a lot of attention from researchers and making it a medical problem of great concern. Capsular serotypes such as K1 and K2 serotypes, sequence types such as ST23 and CC23 sequence types, a virulence plasmid, and a pathogenicity island are all very important virulence factors of hvKp stains.

2.2. Antibiotic resistance of Klebsiella pneumoniae

According to the diversity of virulence factors, the mechanism of antibiotic resistance and clinical manifestation, Klebsiella pneumoniae strains also have highly diverse genomes, which can produce considerable phenotypic variation. Actually, K. The diversity of four different system groups is generally recognized as KPI, KpII-A, KpII-B and KPIII, they are thought to have differentiated into three distinct species: Klebsiella pneumoniae (KpI), quasi-klebsiella pneumoniae (KPII) and Klebsiella mutans (KPIII). More genomic analysis explains that Klebsiella pneumoniae represents a complex of several species and subspecies: Klebsiella pneumoniae, a subgenus of quasi-klebsiella pneumoniae. Quasi-pneumonia, quasi-klebsiella pneumoniae subgenus. Similar to pneumonia, variant Klebsiella subgenus. Smallpox, Klebsiella subgenus. The tropical, quasi-variant, and hyperviscous strains of Klebsiella pneumoniae Genes with toxic manifestations, such as Yersinia, RMPA, first reported in Southeast Asia, and community-acquired pyogenic liver abscesses are rarely shown in these high strains to exhibit antibiotic resistance gene profiles; Until now, opportunistic hospital-acquired infections have often been treated with antibiotics [5]. However, highly virulent and antibiotic-resistant strains of Klebsiella pneumoniae are emerging. Due to the highly diverse

genome of Klebsiella pneumoniae, the increasing use and abuse of antibiotics, drug-resistant klebsiella pneumoniae may be incurable.

3. Phage therapy

Phage is a kind of virus that is widespread in nature and capable of infecting microorganisms such as bacteria. It is a special group of various viruses that is a kind of virus that depends on the bacteria. Its infection with bacteria can lead to the death of bacterial lysis. Phages are the natural enemies of bacteria [6]. This method of using phages to treat bacterial infections is called 'phage therapy'. Now, due to a large number of inappropriate use of antibiotics, the problem of bacterial resistance is becoming increasingly serious. The phage therapy is a new way to solve this problem. It is a completely different treatment from antibiotic therapy. The resistance of the antibiotic has no effects on the phage therapy. Phage therapy has great promise to study, but it has great limitations.

3.1. Types of phage to use therapy

Phage has two common characteristics: lytic and temperate. Lytic phages follow only one dissolution pathway, which begins with specific adsorption to a receptor on the bacterial surface. This receptor can be a carbohydrate, protein, lipid, or other external characteristic. After receptor adsorption, DNA is injected into the host cytoplasm, which then proceeds through DNA replication and gene expression processes, assembly of progeny virions, and finally release of progeny viruses by lysing the host. Temperate phages infect in the same way as the infection process of lytic phages. But it has another way when it infects, viral gene expression is blocked by phage-encoded inhibitors, and the dormant prophage integrates into the host chromosome or forms a linear or circular self-replicating plasmid in this progress [7]. Though using medicine to transition the phages from a temperate state to a lytic state, the temperate phage is not stable. It may form a linear or circular self-replicating plasmid that can't 'kill the bacteria soon. The results are variable. So lytic phages are more suitable. And people always choose lytic phages to make treatment experiences.

3.2. Advantages and disadvantages of phage therapy

Phage therapy is an alternative treatment for multi-drug-resistant bacterial infections. Compared to other treatments, there are some unique advantages of therapy:(1) Phages are not toxic and they only attack bacteria and not human cells. They will not have negative effects. For some other medicines, it will cause some symptoms, such as dizziness, nausea, etc. Although phages are also thought to cause allergic reactions, there are no known cases of allergic reactions in clinical trials. Patients either get better in therapy or they don't. For now, phage therapy is very safe for people. (2) Phages do not develop secondary resistance similar to that of antibiotics. Developing new kinds of phages is much easier than developing new antibiotics. Obtaining a new phage takes only a few weeks, whereas obtaining a new antibiotic takes many years. (3) Phages are highly specific. Specificity is to attack only a very limited number of bacteria and to target one type of bacteria almost specifically. So, it will not damage other bacteria. The antibiotic will also accidentally injure bacteria that are good for our body's health, such as Escherichia coli, etc[6]. (4) Phages are highly penetrable and can pass through the endothelial barrier, epithelial barrier, intestinal mucosal barrier and blood-brain barrier. It is easier for phages to reach many places that other medicines can't get. So, it will have better treatment outcomes [7]. But there are also disadvantages of phage therapy: (1) The highly specific nature of phages also has the disadvantage that a phage will only kill bacteria if it matches a specific strain. As a result, his efficiency is not high, and once the bacteria mutate, they have to develop new species, and the economic cost is high. There are too many kinds of viruses to have specific phage drugs for each of them, so they cannot be brought to market. (2) The efficiency of phages is not stable. Phages have different reproductive effects on different types of bacteria, and some phages are even unable to infect certain bacteria. Too slow in efficiency has no effect on treatment. (3) Phage therapy causes an allergic response from the immune system. (4) Phages may be wiped out by the immune

system before they enter the infected body part. Because phages must be commensal with bacteria. They can't depend on themselves to survive. They enter a body with bacteria. The body may make reaction to wipe out the bacteria by immune system. Without bacteria as a carrier, phages cannot survive too.

4. Clinical trial of pneumoniae infection with phages

4.1. Phase I clinical trial of pneumoniae infection with phages

Through the current hypothesis and theory, phages have made progress in clinical trials for the treatment of pneumonia Kleber. Based on studies of bacteriophage properties, bacteriophages can be used to prevent and treat infections caused by Klebsiella pneumoniae, some of which have achieved remarkable therapeutic efficacy [8]. In 2008, the results of a bacteriophage treatment of a group of mice infected with Klebsiella pneumoniae B5055 were lobar pneumonia. Experiments showed that when bacteriophages grow exponentially in the presence of host bacteria, the bacteriophage particles eventually control the number of bacteria by limiting their further growth.

Now the and A study in 2021, evaluated the microbiology, histopathology, and survival results of ST258 infection, whether treatment alone or in combination with other drugs, can save mice with Klebsiella pneumoniae ST258, and found that drug time is more important than drug dose, 1 hour after the infection, after 8 hours after the treatment, 24 hours after the worst [9]. All of the concurrent saline-treated control mice died quickly.

4.2. Phase II clinical trial of pneumoniae infection with phages

Formal clinical trials of phage therapy for Klebber pneumonia have not yet begun. Only a few cases have been reported. In 2021, phage nebulized inhalation for pan drug-resistant Klebsiella pneumoniae lung infection was used to treat a patient with an extremely severe head injury and pandrug-resistant Kp lung infection [10]. The patient's symptoms of cough and sputum improved after phage treatment. Before treatment, the patient had bilateral pleural effusion accompanied by two lower lungs partial ataxia and reactive inflammation. After treatment, the bilateral pleural effusion was significantly reduced, and the inflammatory response was alleviated [7].

5. Prospects and limitations of phage therapy for Klebsiella pneumoniae infection

5.1. Prospects of phage therapy for Klebsiella pneumoniae infection

The prospects of phages for the treatment of Klebsiella pneumoniae infection has a lot. After the interaction between phage and bacteria, the bacteria can quickly develop phage tolerance. The production of phage-resistant bacteria is an important factor restricting the clinical treatment effect. Experiments have shown that there are still bacteria cultured after 1 day of treatment, and the cleavage spectrum of bacteria is different, indicating that the bacteria have changed, and the evolved bacteria cannot be killed by phage, which is also one of the main reasons for the failure of phage treatment to completely eliminate pathogens [11].

5.2. limitations of phage therapy for Klebsiella pneumoniae infection

The limitations of bacteriophages for the treatment of Klebsiella pneumoniae infection are as following: (1) Compared with Staphylococcus aureus and pseudomonas aeruginosa, there are few studies on Klebsiella pneumoniae phage at home and abroad. There have been multiple clinical reports of Staphylococcus aureus and Pseudomonas aeruginosa, while there have been no clinical reports of Klebsiella pneumoniae. A lot of unknowns will make the next experiment more difficult [12]. (2) Whether Klebsiella pneumoniae phage is safe. Whether the body's immune system will have a strong allergic reaction needs to be found. (3) There are few reports on administration methods,

dosage and efficacy evaluation. When people begin the next experiment about it, they should test this datas by themselves.

6. Conclusion

Although many research reports have shown significant therapeutic effects of bacteriophage therapy in various diseases, there is no data support from a double blind randomized controlled clinical trial. In clinical practice, replacing antibiotics with bacteriophage therapy requires consideration of the following issues. Firstly, how to expand the antibacterial spectrum of bacteriophages and apply them to more bacterial infections; secondly, how to prevent bacteria from resisting bacteriophages; thirdly, what medication method and dosage of bacteriophages are used for treatment; fourthly, how to avoid the cytotoxicity of bacteriophages. Although there are currently some reports on these issues, there is still a lack of systematic and comprehensive analysis to find accurate solutions. Phages have specific recognition of host bacteria, so their antibacterial spectrum is narrow. Sometimes, due to the large number of bacterial species that infect diseases, if the main pathogenic bacteria cannot be clearly identified and specific phages are used, the therapeutic effect may be significantly reduced. In addition, the safety of bacteriophage therapy also needs to be considered. When dealing with the problem of narrow bacteriophage antibacterial spectrum, using bacteriophage cocktails or combining bacteriophages with antibiotics to expand the bacteriophage antibacterial spectrum is a good choice.As an emerging antibacterial therapy, bacteriophage has been limited in clinical application, but with the development of bacteriophage research, the maturity of molecular biology technology and the accumulation of clinical experience, phage therapy will make greater progress, showing a broad space for development.

References

[1] Podschun, R.. (1998) Klebsiella spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors, Clinical Microbiology Reviews.

[2] Sofy, A.R.. (2021a) Characterization and full genome sequence of novel KPP-5 lytic phage against Klebsiella pneumoniae responsible for recalcitrant infection, MDPI.

[3] Chhibber, S., Kaur, S. and Kumari, S. (2008) Therapeutic potential of bacteriophage in treating Klebsiella pneumoniae b5055-mediated lobar pneumonia in mice, microbiologyresearch.org.

[4] Lee, C.-R. et al. (2017) Antimicrobial resistance of hypervirulent Klebsiella pneumoniae: Epidemiology, hypervirulence-associated determinants, and resistance mechanisms, Frontiers.

[5] Moradigaravand D, Martin V, Peacock SJ,et al. Population structure of multidrug-resistant Klebsiella oxytoca within hospitals across the United Kingdom and ireland identifies sharing of virulence and resistance genes with K. pneumoniae. Genome Biol Evol 2017;9:574–584

[6] Reardon, S. Phage therapy gets revitalized. Nature 2014, 510, 15–16. [CrossRef] [PubMed]

[7] Chhibber, S.; Kaur, S.; Kumari, S. Therapeutic potential of bacteriophage in treating Klebsiella pneumoniae B5055-mediated lobar pneumonia in mice. J. Med. Microbiol. 2008, 57, 1508–1513

[8] Dedrick RM, Guerrero-Bustamante CA, Garlena RA, et al. 2019. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat Med 25:730–733.

[9] Chhibber, S.; Kaur, S.; Kumari, S. Therapeutic potential of bacteriophage in treating Klebsiella pneumoniae B5055-mediated lobar pneumonia in mice. J. Med. Microbiol. 2008, 57, 1508–1513.

[10] Li lisha, Li Jianhui, He bin, et al. Bacteriophage in the treatment of drug-resistant Klebsiella pneumoniae pulmonary infection: clinical application and efficacy, Shanghai Jiao Tong University [J]2021,41(9)1272-1276.

[11] Kumari S, Harjai K, Chhibber S. 2010. Evidence to support the therapeutic potential of bacteriophage Kpn5 in burn wound infection caused by Klebsiella pneumoniae in BALB/c mice. J Microbiol Biotechnol 20:935–941

[12] Gill JJ, Hyman P. 2010. Phage choice, isolation, and preparation for phage therapy. Curr Pharm Biotechnol 11:2–14.