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Phage Therapy: An Emerging Strategy against Drug-Resistant Bacteria and Future Perspectives

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Abstract. The widespread use of antimicrobial agents has led to the increasingly severe global problem of drug resistance. Multidrug-resistant bacteria and even "superbugs" threaten human health, making the development of new antibacterial approaches extremely urgent. Against this backdrop, phage therapy has attracted much attention as an emerging strategy. From both historical and empirical standpoints, this paper examines the devastation wrought by unchecked Yersinia pestis during the Black Death. It proceeds to dissect the mechanisms of drug resistance in Gram-negative bacteria, elucidate the bactericidal action of phages, and introduce therapeutic strategies employing single phages, phage cocktails, and genetically modified phages. It lists successful cases to demonstrate the application effects of phage therapy. The analysis highlights the advantages of phage therapy in precise bactericidal action, low immunogenicity, etc.. It points out its challenges in large-scale production, regulation, bacterial drug resistance, and clinical cognition. Phage therapy can potentially combat drug-resistant bacteria, but it needs to overcome existing challenges. In the future, in-depth research should be carried out from optimizing production and improving regulation to promote its development.

Keywords: Phage therapy, Drug-resistant bacteria, Black Death, Antimicrobial agents

1. Introduction

The escalating global crisis of antimicrobial resistance is largely attributable to the widespread application of antimicrobial agents. The advent of multidrug-resistant bacteria, including so-called "superbugs," presents a formidable challenge to human health [1-3]. Against this backdrop, developing new antimicrobial drugs or exploring alternative therapies has become extremely urgent.

As viruses specifically attacking bacteria, bacteriophages have attracted widespread attention in the "post-antimicrobial era" due to their remarkable advantages, such as high host specificity and non-disruption of normal flora [4]. The World Health Organization (WHO) has identified phage therapy as a pivotal approach in its global action plan to combat antimicrobial resistance [3]. This article reviews phage therapy's principles, applications, advantages, challenges, and prospects, providing a theoretical basis for the clinical application of phage-based anti-infective treatment.

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2. The black death and the rise of drug-resistant bacteria

2.1. A historical review of the black death

In 1346, diseases broke out in the Mongol camp during the Mongol Empire's siege of Kaffa (a city near the northern shore of the Black Sea). The Mongols catapulted infected corpses into the town using trebuchets. They then sailed to Europe. At that time, Europeans disliked bathing, and the squalid environment facilitated rat survival. When Kaffa refugees reached Constantinople, rats carrying bacteria on their ships rapidly multiplied, turning Constantinople into a plague-stricken area. Unaware that the bacteria originated from rats, people continued sailing, introducing the plague to ports across the Mediterranean. The Black Death propagated rapidly, encompassing almost the entirety of the European landmass. The sheer volume of fatalities overwhelmed burial grounds, leading to the disposal of cadavers in fluvial systems, thereby exacerbating the transmission dynamics of the disease. In just five years, the plague spread like wildfire, claiming 25 million lives and reducing Europe's population by one-third. Patients developed swollen lymph nodes ("black tumors"), gangrenous skin, and died within 48 hours. Contemporary medicine was helpless—bloodletting, incense-burning, and even flagellation for "atonement" proved ineffective. The Black Death was a medical catastrophe caused by Yersinia pestis, a bacterium belonging to the genus Yersinia and the vector for bubonic, pneumonic, and septicemic plague. After invading the skin, Y. pestis multiplies locally, rapidly spreading via lymphatic vessels to regional lymph nodes, causing primary lymphadenitis. Bacteria and toxins proliferating in lymph nodes enter the bloodstream, leading to systemic infection, septicemia, and severe toxic symptoms.

2.2. Mechanisms of drug-resistant bacteria formation

The multifaceted mechanisms by which Gram-negative bacteria manifest drug resistance predominantly encompass the subsequent aspects [5]. First, the double-membrane structure serves as an essential resistance barrier for Gram-negative bacteria, which can prevent many antimicrobial agents from entering the bacterial cells and reduce the efficacy of drugs. Gram-negative bacteria have an outer membrane outside the cell wall, on which there are some special protein channels called porins. These porins allow some small-molecular substances to pass through, but their pore sizes are too small for some larger antibiotic molecules to pass through. In addition, the outer membrane of Gram-negative bacteria also contains some special lipids and proteins, which can bind to antibiotics, thereby preventing antibiotics from entering the cell interior. Second, efflux pumps actively expel antibiotics. Some special proteins on the cell membrane of bacteria are called efflux pumps. These efflux pumps utilize energy, like ATP, to actively transport antibiotics from the intracellular to the extracellular space. The presence of these pumps allows Gram-negative bacteria to expel any antibiotics that permeate the cell, preventing cellular damage, maintaining a low intracellular antibiotic concentration, and consequently, fostering drug resistance. Third, resistance genes carried by plasmids spread through horizontal transfer. The plasmid of Gram-negative bacteria is a small circular DNA molecule which can have some resistance genes. These resistance genes can encode some proteins that bind to antibiotics, preventing antibiotics from damaging the cells. In addition, the plasmids of Gram-negative bacteria can also transfer resistance genes to other bacteria using horizontal transfer, so that other bacteria also acquire drug resistance, leading to the spread of drug resistance.

Cefiderocol, a prime example, showcases a novel cephalosporin's stability against prevalent clinical carbapenemases and its siderophore-mimicking ability to penetrate the bacterial periplasmic

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space. However, this unique mechanism of action has also spurred bacteria to develop diverse resistance mechanisms [6]. For instance, in Enterobacterales, alterations in the structure of siderophore receptors affect the rate of cefiderocol entry into bacteria. At the same time, the production of carbapenemases remains the primary mechanism of bacterial resistance [6]. In Escherichia coli, NDM (New Delhi metallo-β-lactamase) is common, and an increase in its copy number is associated with the MIC value of cefiderocol. Meanwhile, defects in the siderophore receptors fiuA and cirA also reduce bacterial sensitivity to cefiderocol. Additionally, gene clusters regulating the iron transport system may influence the sensitivity to cefiderocol [6]. In Enterobacter cloacae, the body's anti-infective capacity affects drug resistance. At the same time, NDM and mutated cirA are essential for cefiderocol resistance, and AmpC enzymes exhibit unique hydrolytic activity against cefiderocol [6]. In Klebsiella pneumoniae, in addition to NDM and cirA mutations, some ESBLs with high hydrolytic activity against cefiderocol, even without siderophore receptor mutations, can achieve resistance in combination with the loss of porin OmpK35 [7].

3. Principles and mechanisms of phage therapy

3.1. Bacteriophage bactericidal mechanisms

Bacteriophages are a group of viruses specialized in infecting bacteria, and their bactericidal mechanisms mainly include the following steps [8]. Adsorption: Phages adhere to bacterial surface receptors via specific adsorption proteins. For instance, phage T4 employs its tail fiber proteins for specific adsorption to lipopolysaccharide molecules on the Escherichia coli cell wall. Injection: Following adsorption to the bacterial surface, phages inject their genetic material into the bacterial cell.Taking phage T4 as an example, its tail structure undergoes a conformational change, just like a syringe, to inject phage DNA into the bacterial cell. In contrast, the protein capsid of the phage remains outside the bacterial cell. Some phages even carry cell wall-degrading enzymes to dissolve the cell wall for penetrative injection. Proliferation: after the genetic material of the phage enters the bacterium, it uses the bacterium for replication and transcription. Also, it uses the bacterium to synthesize various proteins that the phage requires. Meanwhile, the phage DNA will continuously replicate to produce multiple genomic copies of progeny phages. Assembly: The newly synthesised phage proteins and DNA are assembled into complete progeny phages within the bacterial cell. First, components such as the head and tail of the phage are assembled, then the phage DNA is packaged into the head structure, and finally, the head and tail are assembled to form mature progeny phages. Egress: Upon reaching a critical intracellular concentration of progeny phages, phages typically synthesize lytic proteins. These proteins compromise the structural integrity of the bacterial cell wall and membrane, culminating in cell lysis and the subsequent liberation of progeny phages into the surrounding milieu.

3.2. Phage therapy strategies

Single-phage therapy can be used to treat bacterial infections. Phages typically act on only one type of bacterium, exhibiting high specificity and targeting. Single-phage therapy utilises this feature for treatment, enabling a precise attack on specific pathogens with strong penetrability. However, using a single phage makes its host range minimal. In treating infections caused by multiple bacteria, single-phage therapy may fail to control the disease entirely. Moreover, bacterial mutations can reduce the therapeutic efficacy of single phages.

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Phage cocktail therapy involves the combined use of multiple phages, which can expand the host range, enhance infection control, and reduce the risk of bacteria developing resistance to a single phage [1-3]. Phage cocktails present notable benefits in the therapeutic intervention of multidrug-resistant bacterial infections, owing to their capacity to induce lysis across diverse bacterial strains and consequently augment the efficacy of treatment regimens [1-3]. Engineered phages, especially those modified using the CRISPR-Cas system, can enhance the therapeutic effect of phage therapy. However, this also imposes specific technical requirements.

4. Successful cases of phage therapy

A 12-year-old boy with cystic fibrosis developed persistent pulmonary infection caused by pan-drug-resistant Achromobacter xylosoxidans after bilateral lung transplantation. Traditional antibiotic therapies (including tigecycline and imipenem) showed poor efficacy, and the patient required long-term oxygen dependency. Facing treatment bottlenecks, clinicians attempted personalized phage therapy. The initial therapeutic intervention consisted of nebulized administration of a three-phage cocktail, which unfortunately did not elicit a noteworthy clinical response. Subsequently, a modified approach was implemented, incorporating a fourth phage delivered via direct bronchoscopic instillation into the lungs, complemented by a fortnight of nebulization therapy. Despite the persistence of the pathogen in early diagnostic assays, the patient exhibited a progressive amelioration of respiratory function, culminating in the cessation of oxygen supplementation post-treatment. Follow-up data showed that A. xylosoxidans was no longer detected in bronchoalveolar lavage fluid one year after treatment, with significant improvement in lung function and no recurrence by 2020. This case demonstrates the potential of phage therapy in controlling refractory drug-resistant infections and suggests that phages may indirectly promote recovery by selecting less virulent drug-resistant strains [6].

5. Advantages and challenges of phage therapy

5.1. Advantages

Bacteriophages exhibit high specificity toward target bacteria, infecting only specific host bacteria. During treatment, they can precisely kill pathogenic bacteria without interfering with the host's normal flora. This contrasts with the broad-spectrum antibacterial activity of conventional antibiotics, substantially mitigating the potential for harm to the microbial ecosystem and the emergence of antimicrobial resistance [1-3].

Compared with traditional antibiotics, bacteriophages have lower immunogenicity and induce relatively weak immune responses in the body [1,4,8]. This makes bacteriophages more easily accepted by the body during treatment, reduces the risk of adverse reactions caused by immune responses, and improves treatment safety. Bacteriophages exhibit remarkable specificity in targeting and infecting bacteria, enabling precise lysis of drug-resistant strains and efficient eradication of bacteria within infected foci, thus underscoring their considerable potential and efficacy in combating drug-resistant bacterial infections [1-3].

5.2. Challenges

Large-scale production of bacteriophages faces numerous difficulties, such as complex manufacturing processes and high costs [1,2]. Meanwhile, bacteriophages have strict requirements for storage conditions—factors like temperature and pH value can affect their activity and stability,

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leading to significant storage challenges. These limitations hinder the widespread application of phage therapy [1,2].

There is a lack of regulatory frameworks for phage therapy, with no unified standardized production specifications or quality control standards [1,2]. This results in inconsistent quality of phage products, making it challenging to ensure their safety and efficacy, and posing risks for clinical application.

In their protracted co-evolutionary arms race, bacteria will progressively develop resistance mechanisms against bacteriophages, including impeding phage adsorption via alteration of surface receptor architecture and deploying dedicated anti-phage defense systems [1-3]. This may lead to the reduction of the effect of bacteriophage therapy or even treatment failure, which requires continuous development of new bacteriophages or optimization of treatment plans to deal with it.

As a new treatment method, bacteriophage therapy has relatively few clinical application data, and doctors and patients have little understanding and acceptance of it. At the same time, bacteriophage therapy has obvious personalized characteristics. Administering personalized therapy necessitates bespoke approaches tailored to individual patient physiologies, thereby placing considerable demands on both healthcare infrastructure and specialized expertise, which consequently impedes its widespread adoption in clinical settings.

6. Conclusion

This article reviews phage therapy, first pointing out that the abuse of antimicrobials has led to the continuous evolution of drug-resistant bacteria, which has become a significant challenge in global public health. Based on a review of the historical background of the plague, the article analyzes the complex resistance mechanisms of Gram-negative bacteria. It emphasizes the practical dilemma of declining efficacy of traditional antibiotics. As particular viruses capable of targeting and lysing bacteria, bacteriophages have bactericidal mechanisms covering adsorption, injection, replication, assembly, and release processes. This study elucidates diverse phage therapy modalities, encompassing monophage, phage cocktail, and genetically modified phage strategies. By presenting a representative case of a pediatric lung transplant recipient successfully treated for pan-drug-resistant Achromobacter xylosoxidans infection, it showcases the potential applications and efficacy of phage therapy in real-world clinical scenarios. Phage therapy has significant advantages in precise bactericidal action and low immunogenicity. Still, it also faces practical challenges such as harrowing production, lack of regulatory mechanisms, and the potential for bacteria to develop resistance to phages. In the future, efforts should be made to strengthen the phage resource reserve and standardised research to promote its transition from the laboratory to the clinic. With the continuous accumulation of technology and clinical experience, phage therapy is expected to become a necessary alternative approach to solve the problem of antibiotic resistance and provide a new direction for treating infectious diseases.

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