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# Why Phage Can't be the End of Superbug on its Own?

**Yaoyang Zhong ***

Shenzhen College of International Education, Futian District, Shenzhen, Guangdong, China
*** Corresponding author Email:** zhongyaoyang2007@hotmail.com

**Abstract:** The emergence of ultra-resistant superbug due to the widespread use and even abuse of antibiotics has caused people to start dying from common wound infections. It has become a global consensus to crack the superbug problem as soon as possible. In this battle, a virus called "phage" discovered a century ago has become a research hotspot again because of its characteristic of engulfing bacteria. Especially, the emergence of "phage display" technology has made the targeted evolution of viruses possible, and phage has become a potential weapon against superbug. By reviewing the discovery history of phage, dissecting the relationship between phage and bacteria as well as the molecular mechanism of interaction, this paper found that phage and bacteria are not in a simple parasitic relationship with each other, and that bacteria will evolve various active and passive defense mechanisms against phage infection, while phage will further evolve their responses against the various defense mechanisms of bacteria. In this invasion-defense-counter-defense arms race, phage and bacteria interact with each other and coexist in co-evolution. Phage not only indirectly affects human health by influencing the pathogenicity of bacteria, but also directly affects human health by interacting with human immune cells. The specificity and replication variability of phage, the large number of phage-bacteria, the complexity of phage-bacteria interactions and their unknown evolutionary mechanisms, and the insecurity of phage entry into the human body also contribute to the fact that the efficacy of phagotherapy, which has a century-long history, has always been uncertain and not reliable for large-scale application. Therefore, this study concludes that phage cannot defeat superbug alone, providing a new way of thinking about how humans can finally overcome the superbug problem.

**Keywords:** Phage; Bacteria; Phage-bacteria Interaction.

## 1. Introduction

If there are any two types of microorganisms that have the greatest impact on humans, it would have to be bacteria and viruses. Some of them coexist, harmoniously with us, helping us in digestion, and breakdown of nutrients. But there are also microorganisms destroy human health, making humans sick or even die [1]. Globally, there are probably millions of deaths per year due to bacterial infections and tens of millions of deaths per year due to viruses. (WHO, Global Antimicrobial Resistance and Use Surveillance (GLASS) Report (2022)). There are no specific drugs for viruses, while for bacteria we have many kinds of antibiotics.

However, the misuse and overuse of antibiotics has led to the emergence of drug-resistant bacteria and has caused more serious harm to people, costing more human and financial resources. In the face of drug-resistant bacteria, one antibiotic, alone, is not effective, and two, three or even more antibiotics have to be used simultaneously. The increase in dosage increases toxicity and raises the probability of adverse reactions. Drug-resistant bacteria that are difficult to remove by antibiotics may also invade other parts of the body, causing concurrent infections in other tissues such as septicemia, intracranial infections complicated by other infections, etc., which can be extremely hazardous to life.

The emergence of superbugs, which are resistant to a wide range of antibiotics, exposes humans to the triple threat of high virulence, high drug resistance and high infectivity, with no cure once infected. Global deaths from superbug have reached 700,000 per year (WHO, Global Antimicrobial Resistance and Use Surveillance (GLASS) Report (2022)). In 2017, WHO announced that 12 of the most urgently needed classes of new antimicrobial drugs to be developed were anti-superbug. In 2019, WHO announced the top 10 global health threats, and antibiotic resistance is once again on the list.

At the moment of crisis, phage, which has been neglected for more than a hundred years, has returned to human vision, and is even regarded by some scientists as a superhero to put an end to superbug. Compared with antibiotics, phages have obvious advantages in solving the problem of bacterial drug resistance. Phage can specifically target one or several kinds of bacteria and cause the bacteria to lyse and die, and it is only effective for the corresponding pathogenic bacteria, will not destroy the normal flora, and has fewer side effects. Therefore, phagotherapy is used as an emergency treatment for superbug infections in many countries. The United States and the United Kingdom have also taken the lead in restarting phage I/II clinical trials.

This paper summarizes the history of phage discovery, various theories of phage-bacteria relationship, phage infection mechanism and molecular mechanism of phage-bacteria interaction, and explores the limitations of existing research on phage, the impact of phage on human body as well as phage on bacterial pathogenicity. It is thus found that phage and bacteria are not simply in a parasitic relationship, but co-evolve in interaction. Some studies have demonstrated that the role of phage is one of the key factors in the emergence of superbug. Therefore, this paper proposes that phage itself cannot be the terminator of superbug.

## 2. Literature Review

Phages, having coexisted extensively with bacteria in nature for billions of years, belong to a class of viruses that parasitize various, microorganisms including bacteria, fungi, and actinomycetes. They are widely distributed in most prokaryotic organisms, and consist primarily of proteins and nucleic acids [2].

## 2.1. Discovery of Phage

It has been academically recognized that phage was discovered in 1915 and 1917 by Frederick Twort, a British bacteriologist, and Félix D'Hérelle, a dual French-Canadian microbiologist [3].

However, tracing the literature, there had been studies highly suggestive of phage before them [4]. In 1896, Ernest H. Hankin, a British bacteriologist who studied the cholera epidemic in the Ganges River Basin, found there was a huge difference in the number of Vibrio cholerae between the entrance and the exit of the Ganges River in the city of Agra. The number of Vibrio cholerae in the upstream water was 100,000/mL, while in the downstream it was only 90/mL, from which he hypothesized there might be substances killed Vibrio cholerae. In 1898, Russian bacteriologist Niko-lay Gamaleya observed a similar phenomenon in his study of Bacillus subtilis. In 1901, Emmerich and Low reported that autolysis of bacterial cultures occurs during the treatment of infections as well as during storage after prophylactic inoculation was provided, and that it was possible to cure the same species of Vibrio cholerae with such an autolytic substance, and autolyzed material could cure experimentally infected animals caused by the same bacteria.

In 1915, Frederick Twort published the discovery of phage in the prestigious international journal Lancet. This was the official discovery of phage. In an accidental experiment, he found that coccobacillus colonies grew on solid medium without living cells, and some of the moss areas underwent glassy transformation. This transparent transformation could be repeated stably: when a small spot of this transparent colony was inoculated onto other new coccobacillus colonies, the colonies also turned into transparent areas. Frederick Twort hypothesized that there was probably an ultramicroscopic microvirus smaller than bacteria or amoebas that could grow in the bacterial cytoplasm to form a no definite individual or enzyme with a "growth force". A factor turned the colony into a hyaline zone that could cause acute infections with cocci. The transparent areas that Twort saw were what are now called "phage spots," and he pointed out that the factor that made the colonies transparent had the power to grow, which is now called a virus.

In 1917, Félix D'Hérelle published a paper officially reporting the discovery of an invisible virus that could alter the pathogenicity of bacteria in dysentery bacilli found in the stools of dysentery patients. He found that the invisible virus could cause melting of liquid cultures of the bacteria, producing a clear area on solid agar plates. The virus could also multiply, and the lysis of bacterial cells was the result of its multiplication. D'Hérelle called them ultravirus, which can invade bacteria, multiply inside them and consume them. D'Hérelle named these ultramicroviruses phages, i.e., bacteria-feeders. He also called the transparent areas on solid agar plates that caused bacterial death phage plaque, and proposed phage plaque counting as a way to quantify invisible phages. D'Hérelle also found that the titer of phage plaque increased during the recovery period of dysentery patients, and hypothesized that phages were a natural factor in the fight against infectious diseases, calling them exogenous agent of immunity. Later, D'Hérelle clearly stated that phages are infectious agents, intracellular parasites, and that the antigenicity and host spectrum specificity of phages are characterized by a race.

Phage has been closely linked to the fight against bacterial infections from the very beginning of their discovery. Many theories and techniques of molecular biology are closely related to phage research. For example, the "mutation and selection theory", the confirmation that DNA is the genetic material, the revelation of gene expression regulatory pathways, and the establishment and development of genome sequencing technology.

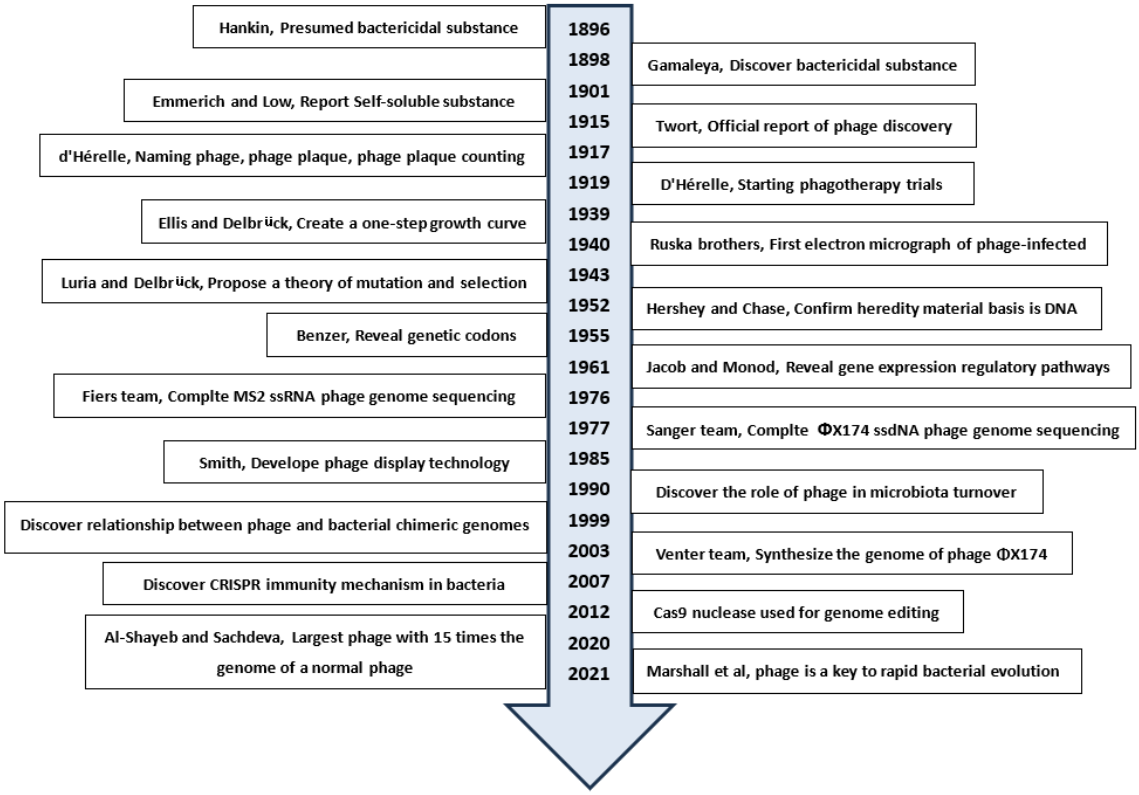


**Figure 1.** History of phage research
(Note: Self-organized drawing)

## 2.2. Characterization of Phage

Phages are characterized by two main features: structural simplicity and diversity.

Like other viruses, infectious phages consist of a protein coat and a core, with some phages having a nucleic acid-only core and others consisting of other components together with nucleic acid. Phages must rely on bacteria in order to reproduce, and it is hypothesized that every second, about $10^{24}$ bacteria are infected worldwide. In the process of repeated infections and struggles, phages continue to evolve [4].

Phages have a wide range of diversity. It is estimated that the number of phage particles in the world is estimated to be 10 times the number of all bacteria, exceeding the total number of all other organisms, including bacteria. From rivers, lakes, jungles and grasslands to human skin, mouth and intestines, phages are ubiquitous and closely related to the natural environment and human health. Currently, nearly 2900 species of phages have been observed by electron microscopy [5]. The more common ones are T-series phage (a general term for seven types of E. coli phages T1-T7), λ phage, P1 phage, Mu phage, φX174 phage, φ29 phage, PRD1 phage, Qβ phage, P2 phage, Sf6 phage, and so on. Each phage has a different way of infecting bacteria and its effect on bacteria.

**Table 1.** Phage classification

| Classification basis | Category | Peculiarity | |
|---|---|---|---|
| **Phage-host relationship** | **Mild phages (lysogenic phages)** | A class of phages that, after binding to the host bacteria, integrates their own genes into the chromosomes of the host bacteria, becomes part of the host bacteria's genes, and multiplies with the reproduction of the host bacteria. | |
| | **Potent phages** | A class of phages that, after binding to sensitive host bacteria, rapidly proliferate in the host bacteria and eventually lyse them. | |
| **Genetic** | **DNA phages** | **Double-stranded DNA phages** | A class of phages that can infect bacteria and replicate their own DNA virus within bacteria. Most phages are DNA phages. |
| | | **Single-stranded DNA phages** | |
| | **RNA phages** | **Double-stranded RNA phages** | A class of phages that can infect bacteria and replicate their own RNA virus within bacteria. |
| | | **Single-stranded RNA phages** | |
| **Morphological structural characteristics** | **Tailed phages** | 1.A class of phages whose shell consists of a head and a tail, which are connected by the neck. The head is usually icosahedral symmetrical and whose tail is spiral symmetrical.<br>2.Except for Rφ6 double-stranded DNA, which is circular and super spiral, most of phages with tails are double-stranded linear DNA.<br>3.A class of phages with the largest number and the largest change in shell structure and the most common of which include T4, T7, P2, P22, λ, and φ29. | |
| | **Tailless phages (Spherical phages)** | A class of phages with simple shells but quite different in nucleic acid groups. Some are double-stranded DNA molecules, such as PM2 phages. Some are positive polarity single-stranded circular DNA molecules, such as φX174 phages. Some are positive polar single-stranded RNA molecules, such as MS2. Others are multi-segment double-stranded RNA molecules, such as φ6. | |
| | **Filiform phages (Rod phages)** | A class of phages whose core is composed of single-stranded circular DNA. Both M13 and FD belong to filamentous phages. | |
| **Note:** Self-organized tabulation | | | |

Phages are mainly categorized by morphology and genomics. According to the morphological and structural characteristics, phages can be divided into three major groups: tailed phages, tailless phages (also known as spherical phage) and filamentous phages (also known as rod phage). According to the relationship between phage and host, they can be divided into two major categories: mild phages and virulent phages. According to the genetic material, phages are divided into double-stranded DNA phages, single-stranded DNA phages, single-stranded RNA phages and double-stranded RNA phages. The vast majority of phages are double-stranded DNA phages.

## 2.3. Mechanisms of Phage Infection

Phages are virus that lack an independent infection system and need to utilize the cellular machinery of the host bacterium for their own reproduction to complete the replication and life cycle of their progeny. Phages can specifically infect host bacteria. The host bacteria of phages are usually some bacterial strains or subgroups within a certain species, and some phages can infect some bacteria within several related species.

Phages infect host bacteria in two ways. Mild phages infect phages via the lysogenic pathway, and virulent phages infect phages via the lytic pathway. The whole process of phage infection of bacteria is precisely regulated, and the specific mechanism of the related molecular regulation is unclear. A common strategy used during phage infection is to produce proteins that regulate or target specific host proteins immediately after genome entry into the bacterial cell to evade various bacterial defense mechanisms or alter the host payload mechanism.

After infecting a bacterium, a mild phage integrates its own nucleic acids onto the chromosome of the infected bacterium, does not reproduce itself, but coexists with the bacterium, replicates with the bacterial chromosome, and is assigned to the chromosome of the zygote bacterium as the bacterium divides, thus establishing a lysogenicity cycle. Phage genes that are integrated into the bacterial chromosome are called prophages. Bacteria with prophages on their chromosomes

are called lysogenic bacteria. Passing on phage genes as lysogenic bacteria divide is called the lysogenic state. Therefore, mild phages are also known as lysogenic phages.

Potent phages directly destroy the bacteria, which multiply rapidly after infecting bacteria and can be divided into five processes, which are adsorption, invasion and decapsulation, biosynthesis, assembly, and release.

Adsorption is the contact of the phage's adsorptive organs (tail filaments, substrates, and spines) with specialized sites in sensitive host cells. Phage is very specific to his hosts, and sensitive cells are not adsorbed everywhere in the cell, but at specific sites, and the degree of adsorption varies. For example, the receptor site of E. coli also varies according to the phage. After adsorption, the tail filament shrinks and the infection process begins; the phage not only inserts its tail pith into the cell wall, but also injects DNA into the cell as if it were an injection. The time from adsorption to invasion is very short, e.g., T4 takes only 15s. As soon as the phage invades the bacteria, proliferation begins, i.e., replication of nucleic acids and protein synthesis. First, the host's RNA polymerase is utilized for transcription, and the phage mRNA is translated by the host's protein synthesis system to form a series of new early proteins. Then phage nucleic acid and late protein replication begins. When all phage structural components have been synthesized, the maturation process (i.e., "assembly") begins. The process begins with the coalescence of the DNA, with the subunits of the head being assembled into the head, and the tail and the tail filament being formed separately. The head subunits are assembled into the head, and the tail and tail filaments are formed separately. The phage then assembles itself from head to tail, and finally the tail filaments are mounted to form a complete phage. The final stage of phage multiplication is lysis to release daughter phages. Each phage invades sensitive cells, the number of phages that can be finally assembled and released from each cell is called the lysis volume, which is relatively fixed. For example, T4 is 100, ΦX174 is 1,000, f2 is 10,000, and T2 is 200; the phage of glutamate-producing bacteria is 50 to 150.

**Table 2.** The process by which potent phages infection in bacteria

| Stage of infection | Stage name | Infection process |
|---|---|---|
| **Stage1** | **Adsorption** | The cervifolial protein of the phage binds to surface-specific receptors of the host cell, as indicated by attachment to the host cell. Different phages have different specific receptors on the surface of host cells. For example, the adsorption-specific receptors of T2 and T5 phages are lipoproteins, and the adsorption-specific receptors of T3, T4 and T7 are lipopolysaccharides. |
| **Stage2** | **Invasion and shelling** | Phage tail filament contraction scientific research allows the tail tube to touch the cell wall of the bacteria, and the lysozyme carried by the tail tube can cleave the peptidoglycan on the cell wall of the host bacteria, thereby injecting nucleic acids into the cell, and the protein shell remains outside the bacterial body. |
| **Stage3** | **Biosynthesis** | DNA replication, transcription, and protein synthesis with host cells. |
| **Stage4** | **Assembly** | Within the host cell, the parts of the phage bind in a certain way to assemble mature virions. |
| **Stage5** | **Release** | Upon 0ffspring phage phages maturation, most phages can synthesize an enzyme that inhibits cell wall synthesis within the host, resulting in a gradual thinning of the host cell wall and eventual lysis. |
| **Note:** Self-organized tabulation | | |

## 2.4. Mechanisms of Phage-Bacteria Co-evolution

**The "Red Queen Hypothesis" theory.** "The Red Queen Hypothesis is a classic theory in co-evolutionary studies. The theory was proposed by Van Valen in 1973, based on the story of Alice in Wonderland. The Red Queen in the story says, "The proposed must keep running to keep you in place." In the process of co-evolution, both parties evolve, and if one party evolves at a slower rate, it is eliminated. The process of co-evolution is like "rowing a boat against the current. If you don't advance, you will retreat." Both phage and bacteria must evolve to adapt to each other's evolution during the co-evolutionary process.

Phage and bacteria have been struggling with each other for a long time in nature, and bacteria has evolved a variety of mechanisms to resist phage infection, and phages has also acquired a large number of mechanisms to resist bacterial resistance, which are constantly changing with the long-term phage-bacteria co-evolution. The advantages of phage such as short reproduction period, small genome, easy cultivation, and controllable conditions make phage an excellent target for experimental evolutionary studies. However, although the evolutionary process of phage-bacteria in nature can be inferred from genome sequences, human knowledge of phage-bacteria evolutionary process and evolutionary mechanisms is still very limited [6].

It was found that the phage-bacteria co-evolutionary process can be divided into two phases in the medium and long-term co-evolutionary studies of phage-bacteria in vitro experiments.

In the first 200-250 generations, phage-bacteria co-evolution is characterized by an **"arms race" mode.** Co-evolution greatly accelerates the rate of phage-bacteria molecular evolution, resulting in population diversity, i.e., the ability of bacteria to tolerate phages gradually increases, and at the same time, phages are able to infect a greater number of bacterial species [7].

After more than 250 generations of co-evolution, phage-bacteria co-evolution has become a **"fluctuating selection mode"**. Although the early "arms race" mode will lead to the emergence of generalism phage and pan-tolerant bacteria, both of them will have fitness cost, and phage-bacteria with different genotypes will appear alternately, and the host spectrum of phage and the resistance of bacteria will not increase all the time. resistance will not always increase [8].

Since phage depend on host bacteria for their reproduction, phage can benefit from the promotion of host bacteria survival and reproduction even if bacteria-phage struggle during co-evolution, maintaining a mutually beneficial bacteria-phage symbiosis. Bacteria-phage exhibit several symbiotic and mutually beneficial phenomena [9].

The lysogenic phenomenon has a unique role in the bacteria-phage arms race. After entering the lysogenic state, lysogenic phage is able to maintain a relatively long-term symbiosis with the host bacteria and realize mutual benefit through the integration and excision of phage and bacterial genomes. Lysogenic transitions have no more particular value to the phage itself other than preventing other phage from infecting the host bacterium and allowing it better space for replication, but some phages encode functional molecules that can enhance the adaptability and survivability of their host bacteria.

An unusual and even more intriguing type of long-term bacterial-phage coexistence is known as active lysogeny, in which the integrated prophage is able to regulate the expression of host bacterial genes, leading to the prophage being referred to as a "phage-regulated switch" During active lysis, the prophage integrates into the coding region or a neighboring regulatory region of a bacterial gene with a core function and inactivates the bacterial gene. When needed, the prophage that blocks the expression of the target gene can be precisely cut away again to restore the expression of the target gene without triggering lysis of the host bacteria. This active regulation by prophages is categorized into two types: reversible and irreversible. The strategy of reversible active lysogen regulation of bacterial genes is similar to a switching mechanism, in which prophage cleavage and complete reintegration are completely reversible, and the cleaved prophage exists in free form in the cytoplasm and can be reintegrated into the target gene under conditions that allow the bacterial gene to be inactivated again. This approach is not only capable of escaping phagocytosis by host cells by regulating the expression of bacterial receptor-related genes, but also of repairing genes and altering bacterial mutation rates by regulating bacterial mismatches [10]. Another type of active lysogen regulation is irreversible excision, where the prophage acts as a controllable switch that, once excised, will no longer be integrated and will eventually be lost or degraded. While lysogenic switching improves host fitness mainly through horizontal transfer of phage genes, the active lysogenic principle is a more responsible phenomenon that alternately drives the process of bacterial-phage co-evolution through effective phage-bacterial lysis and cut-off. In this process, phage integration into bacterial core genes may lead to reduced bacterial fitness, which is then gradually restored through mutual adaptation between phage and bacteria.

## 2.5. Trends in Phage Research

George Smith, one of the winners of the 2018 Nobel Prize, invented the phage display technology, which makes it possible to induce targeted evolution of virus infecting bacteria into new proteins. On the basis of further analyzing the phage-bacteria interaction mechanism, engineering phage and recombinant phage through synthetic biology to improve phagotherapy effect and safety are gradually becoming a research hotspot to solve the problem of phage application disadvantage. The receptor binding protein (RBP) of phage is the primary determinant of host specificity [11]. Cutting-edge research is utilizing the property of RBP to assist phage in host recognition and artificially narrowing the phage host spectrum. The development of high-throughput sequencing technology promotes the integration of multi-omics technologies for research to comprehensively and systematically understand the mechanisms of phage-host interactions, e.g., to study the regulation of phage gene expression and how phages overcome bacterial immune defense mechanisms. Existing studies have shown that phages usually hijack the transcriptional machinery of host bacteria to regulate their own and host bacterial gene expression, but the structural basis of phage protein-mediated transcriptional regulation, especially transcriptional repression, is not yet clear.

Researchers at the University of California, Berkeley, have searched a massive DNA database and found 351 giant phages. The genomes of these phages are more than four times larger than the average genome of viruses that feed on single-celled bacteria, with the largest measuring 735,000 base pairs, almost 15 times as long as the average phage, and carrying many of the genes found in ordinary bacteria for use against bacterial hosts. Giant phages fill the gap between non-living phages and bacteria and archaea [12]." We are exploring Earth's microbial communities and sometimes unexpected things happen. These viral bacteria are part of biology and can replicate entities, but we know very little about that." Gil Banfield, professor of earth and planetary sciences and environmental sciences, policy and management at the University of California, Berkeley, who is a senior researcher and one of the researchers, said it appears that there must be some successful survival strategy that has prompted the gradual fusion of the genomic dividing line between bacteria and viruses into a genetic continuum that raises intriguing questions about the nature of the evolution of life on Earth.

# 3. Discussion

## 3.1. Molecular Mechanisms of Bacterial Defense Against Phage Infection

The main defense strategy mechanism of host bacteria against phage infection involves the whole process of phage infection of bacteria in both passive and active defense.

**(1)Passive defense**

**Bacterial biofilm physical barrier.** Bacterial biofilms are clustered biocellular structures secreted by bacteria that surround the outer surface of the bacteria, and the main components include extracellular polysaccharide matrix, lipoproteins, and fibronectin. The dense structure of bacterial biofilm can conceal some phage receptors and resist phage infection. Extracellular polysaccharides not only enhance bacterial resistance to antibiotics, but also prevent phage from entering the cell. In addition, the biofilm barrier usually contains protein hydrolases and cellulose endonucleases, which can directly inactivate phages.

**(2)Active defense**

**Blocking phage adsorption.** Some bacteria prevent phage adsorption by mutating, masking, or degrading the receptor molecules. Others alter the bacterial surface receptor molecules through reversible genetic modification, temporarily silencing the phage receptor gene and thus altering the bacterial surface receptor to resist phage infection. Some bacteria competitively prevent phage from binding to their receptors through the synthesis or utilization of receptor inhibitors. Some bacteria, such as Escherichia coli, mediate

their resistance to phage infection with a quorumsensing (QS) system, which represses the expression of genes encoding genes involved in flagellum formation and reduces the adsorption sites for the Escherichia coli phage.

**Blocking the invasion of phage DNA**

**Sie system.** Sie, Super infection exclusion, is the primary mechanism that prevents phage DNA from entering the host cell and was originally discovered in mild phages. When a host cell is infected by a phage and the same type of phage re-infects the host cell, this mostly membrane-anchored protein or proteins associated with membrane components come into play, thus preventing re-infection by the same type of phage.

**Cutting phage DNA**

**RM system.** RM, Restriction Modification, is a bacterial immune system that is widely directed against targeted DNA. Over 90% of sequenced bacterial and archaeal genomes contain the RM system, which consists of two enzymes: restriction endonucleases and methyltransferases. Bacteria cleave exogenous DNA by endonucleases, and bacteria protect their own DNA from cleavage by methyltransferases, which modify the methylation of adenine and cytosine in the endonuclease recognition sequences in their own DNA.

**CRISPR-Cas system.** CRISPR, clustered regularly interspaced short palindromic repeats, is present in nearly half of all bacteria and all archaea. CRISPR sequences are also currently used as engineering tools for genetic modification and gene silencing. When a phage infects a bacterium, some bacteria insert an interphase sequence near the leading region of the CRISPR sequence that is identical to the phage sequence. When the same phage is infected again, the CRISPR-Cas-related gene product of the bacterium uses the interphase sequence to mediate the production of a CRISPRRNA precursor transcript, which is processed into small CRISPRRNA molecules. PRRNA molecules that utilize an RNA interference-like mechanism to initiate bacterial disintegration of invading phage nucleic acids. The CRISPR-Cas system improves bacterial fitness while increasing the overall level of phage resistance in the population.

**BREXT System.** BREXT, bacteriophage exclusion, is a recently discovered phage resistance system in Bacillus cereus. It is present in about 10% of the bacterial population genes. The system does not affect phage adsorption but prevents phage DNA replication. The system recognizes its own sequences or foreign sequences by methylation of non-transcribed TAGGAG motifs in bacterial genes, which makes the BREX system phage-infectious. Unlike restriction modifications, phage DNA is not cleaved or degraded.

Blocking the release of phage particles

**Abi System.** Abi, Abortive Infection, is a system that prevents other bacteria from being infected by a phage when the phage is successfully injected with DNA and its propagation is blocked, resulting in a failure of propagation and release of the progeny phage. In contrast to other resistance mechanisms, this system destroys the phage while ultimately leading to the death of the host bacteria, but by this sacrifice of individuals the bacterial population can be protected.

**TA System.** TA, toxin-antitoxin, which is prevalent in prokaryotes, also belongs to the Abi system. Take Mycobacterium tuberculosis as an example. When infected by phage, the expression of the toxin mazF in the toxin-antitoxin system on the chromosome of Mycobacterium tuberculosis is strengthened, while the expression of the antitoxin mazE is suppressed, and the bacterium will die under the toxicity of the mazF protein, resulting in abortive infection.

## 3.2. Molecular Mechanisms of Strain in Phage Bacterial Defense Mechanisms

In the face of bacterial defensive resistance, phages have also evolved a series of corresponding changes.

**(1) Adaptation to new receptors**

Bacteria can randomly express receptors at different times and under different conditions. Accordingly, phage can modify the tail receptor binding protein (RBP) to bind to the receptors expressed at different times and under different conditions to increase the infection rate. When the receptor on the bacterial cell membrane changes, the phage tail RBP also changes accordingly, recognizes and binds to the new receptor, and adsorbs again; when the bacterial cell membrane forms an envelope outside the cell membrane and masks the receptor, the phage generates hydrolytic enzymes to degrade the envelope, exposing the receptor and thus adsorbing again.

**(2) Responding to Abi and TA systems**

Phages bypass the Abi and TA systems through genetic mutations. For example, mutations in genes involved in nucleotide metabolism in some phages of the genus Lactococcus inactivate phosphorylated cytokines that act on metabolic processes, thereby bypassing the Abi system; phages can encode molecules that functionally substitute for bacterial antitoxins, counteracting the toxin activity.

**(3) Responding to CRISPR-Cas and RM Systems**

Phages achieve escape from bacterial degradation by reducing restriction sites and by inhibiting modifying enzymes. For example, it protects itself from killing by inhibiting CRISPR-Cas effector complex formation, or by methylating genes by the host or itself to protect newly synthesized DNA.

**(4) Maintenance of the solvated state**

Phage infection of the host releases arbitrium peptide, and the concentration of extracellular arbitrium peptide rises continuously, leading to phage lysogenic state. After phage integration, it can hydrolyze and label isolated phage repressor genes, which can prevent the stable expression of proteins and maintain the lysogenic state.

## 3.3. Effect of Phage on Bacterial Pathogenicity

Differences in the potential pathogenicity of bacteria depend largely on the presence of virulence-related genes and whether these genes are expressed, and many of the genes encoding bacterial virulence are located in mobile genetic elements, such as plasmids, phages, transposons, virulence islands, and integrons. The role of phage as vectors for virulence gene transmission has mutated the evolution of pathogenic bacteria and phages themselves. On the one hand, virulence-related genes of phage can be transferred to host bacteria to enhance their virulence or adaptability [13]. or transform them from non-pathogenic strains to pathogenic strains, which plays an important role in bacterial evolution, the generation of pathogenic strains, and bacterial pathogenesis. On the other hand, the survival of phage depends on the survival of lysogenic bacteria. In their interaction with the host bacteria, lysogenic bacteria contribute to the preservation of the phage genome, further dissemination of relevant genes, and promote the evolutionary advantage of the phage, thus potentially reducing bacterial pathogenicity.

In a new study, researchers from the University of

Pittsburgh School of Medicine have found, for the first time ever, that phages are key to initiating the rapid evolution of bacteria, leading to the emergence of drug-resistant 'superbugs'. The researchers also found that, contrary to the prevailing theory in the field of evolutionary microbiology, the process of adaptation and diversification of bacterial colonies does not begin with a homogeneous group of clones, the cause of most early adaptations was not random point mutations. Instead, phage was responsible for giving winning strains an evolutionary advantage early on [14]. Bacteria grow so quickly; it only takes a few days for bacterial strains to acquire new traits or become resistant to antimicrobial drugs.

Dr. Vaughn Cooper, a professor of microbiology and molecular genetics at the University of Pittsburgh, one of the researchers, said, "Essentially, parasitism becomes a weapon. Phage provides the means for winning strains to win. Phage kills more sensitive bacterial strains, giving their bacterial strains a survival advantage."

### 3.4. Interaction of Phage and Human Body

Viriota, the viral component of the human microbiota, are very large in number, and phage is the most abundant one, with about 1015 phages in the human gut alone. The effect of phage on human body has two aspects. On the one hand, phage affects the structure and function of bacteria through its infectious effect on host bacteria, which in turn affects the human body. On the other hand, phages not only infect bacteria, but also directly interact with human immune cells. It has been demonstrated that phages can pass from the intestinal lumen through the epithelium into the intestinal basal lamina, a site where immune cells are enriched, and come into direct contact with immune cells. In addition to the phage itself, the DNA and RNA components of the phage are able to bind to the receptors of immune cells and influence the immune response. As mobile elements in the microbiome, prophages have the potential threat of transferring drug-resistant genes from non-curing bacteria to the genomes of pathogenic bacteria. In addition, prophages are capable of transferring harmful genetic material such as genes encoding virulence factors [15]. The mechanisms of how phages maintain the human microecological structure and influence human health are unknown [16].

### 3.5. Limitations of Existing Studies

There is no direct evidence in either academia or clinical medicine that phage can end superbug.

In academia, although phage display technology makes phage directed evolution into superbug terminators possible, the technology itself is flawed. The phage display process must go through bacterial transformation, phage packaging, and in some cases, such as the realization of the function of some proteins requires folding, translocation, membrane insertion and complexation, the display system must also go through the process of transmembrane secretion, which greatly restricts the capacity of the constructed libraries and the diversity of molecules. Some molecules that are toxic to cells, such as biotoxin molecules, are difficult to be effectively expressed and displayed in phage display technology. As a result, the existing research on phage is faced with many uncontrollable factors, such as sampling, sequencing, and analyzing errors, and the understanding of phage-bacteria co-evolutionary mechanism and characteristics is still extremely limited.

In clinical medicine, the efficacy and reliability of phagotherapy for superbug infections is still debatable [17], and there are no comparative studies using phage alone versus antibiotic treatment [18]. Phagotherapy is still dominated by safety assessments and case-by-case treatment, and is only an adjunct to antibiotic therapy [19]. The main reason is the lack of large-sample randomized controlled clinical trials of phage therapies [20], the lack of data on treatment personalization, the variable therapeutic efficacy of existing phage-fixed combinations, and the lack of susceptibility testing of pathogenic bacteria to therapeutic phage preparations. This is also the main reason why phagotherapy has not been practiced on a large scale after a century, despite the fact that Félix D'Hérelle started applying phages against bacteria as early as 1917.

## 4. Conclusion

Phage cannot be the terminator of superbug alone.

Firstly, phage feeds on bacteria and depend on them for survival, co-evolving and co-surviving with them. The long-term antagonistic evolution of phage with bacteria determines the characteristic of phage not completely hunting the host.

Second, due to bacterial tolerance, when bacteria sense the presence of phage, they will activate their defense mechanism to limit the further spread of phage. It is difficult for phage to remove pathogenic bacteria completely, and it is possible that some phage-tolerant strains may be activated again after phage kill most of the strains, resulting in incomplete effect, which requires joint immune-boosting, combining antibiotics, or other bactericidal methods.

Third, phage is host-specific. Not only is there specificity between different bacteria, but also between different strains of the same bacteria. As a result, it is difficult to cover the complex and diverse clinical superbug by a few phages, and a phage can only terminate a few strains of a bacterium, and the uncovered strains will replace the original strains to become the new dominant strains, leading to relapse.

Fourth, phage has the potential to cause allergic reactions, and when phage is injected into the human body, the immune system may have an allergic reaction to the phage, leading to adverse reactions. There is insufficient evidence of the effectiveness of phage in terminating superbug.

Therefore, as of now, phagotherapy has not been widely and scientifically conducted and evaluated. Randomized controlled clinical trials have shown poor phage efficacy, and those with good phage efficacy are often comparisons between patients before and after treatment, or combined treatments in which phage is used as an adjunct, making it difficult to accurately assess the independent effects of phage in the fight against superbug.

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