

An overview: *Staphylococcus aureus* phage therapy

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Abstract

Staphylococcus aureus (*S. aureus*) is a common and a frequent virulent pathogen in humans. *S. aureus*, a ubiquitous bacterium among humans, can cause severe infections such as cellulitis, sepsis and osteomyelitis. The emergence of antibiotics in the 1940s led to believe that bacterial infections would cease to exist. In 1959, however, bacteria started to gain resistance to antibiotics, including *S. aureus*. Despite the many attempts of developing new antibiotics, bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRSA) were unaffected. This has led to a renewed interest in phage therapy, a non-antibiotic approach to treat bacterial infections. Here, I will give an overview of the current state of *S. aureus* phage therapy.

Introduction

At the beginning of the 20th century, phage therapy was being developed as a treatment against human and animal bacterial infections. Bacteriophages, also known as phages, are viruses which specifically kill bacteria without interfering with cell lines from other organisms. This is because phages have a high specificity against certain cellular target hosts.

This form of treatment, however, was overlooked in the Western World after the emergence of antibiotics in the 1940s (Lin D.M. et al., 2017; Chanishvili N., 2012; Wittebole X. et al., 2014). At that time, antibiotics were seen as the 'wonder drugs' in the mid-20th century. Prior to antibiotics, infectious diseases accounted for high mortality and morbidity rates worldwide. Infectious diseases such as tuberculosis, syphilis, smallpox, cholera, pneumonia etc. are nowhere near as high as seen today than they were before the use of antibiotics. And thus with the emergence of antibiotics, it was believed that these diseases would cease to exist, and thereby improve the quality of life (Zaman S.B. et al., 2017; Adedeji W.A. et al., 2016).

Yet in 1959 methicillin was introduced to treat infections caused by penicillin-resistant *S. aureus*. After two years there were reports from the United Kingdom of *S. aureus* isolates that had required resistance against methicillin. Soon other methicillin-resistant *S. aureus* isolates from other European countries, and

later from the United States were discovered. Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital-acquired infections that are becoming more difficult to treat because of the rising resistance to all antibiotics, and will be an even bigger cause of public health concern (Enright M.C. et al., 2002).

This has led to a renewed interest in phage therapy, a non-antibiotic approach to treat bacterial infections. Phages are the most abundant microorganism on earth and are the natural regulators of bacterial populations. They are able to induce bacterial lysis in Gram-negative as well as in Gram-positive bacteria, including MRSA (Sulakvelidze A., 2011; Wittebole X. et al., 2014).

In order to implement phage therapy against *S. aureus*, *S. aureus* specific phages are needed. Fortunately, Staphylococcal phages are plentiful, of which most are present as prophages. Prophages are viral forms integrated in bacterial genomes where they reside in their host until conditions favor their reactivation, after which they activate the lytic cycle (**Fig. 4**). Prophages contribute to the virulence and pathogenesis of *S. aureus* by encoding virulence factors. In order to implement phage therapy, phages which are lytic (**Fig. 4**), specific to *S. aureus* and are lacking pathogenic factors are suitable for *S. aureus* phage therapy (Azam A.H. et al., 2019; Fortier L.C. et al., 2013).

In this review, I will discuss the development and possible application of phage therapy against *S. aureus*. How come phage therapy may be a novel alternative to antibiotics, as well as some of its downsides such as phage resistance. And in order to evaluate resistance of *S. aureus* to phages, it is of importance to get to know the mechanisms behind the attachment of phages to *S. aureus* and elimination of *S. aureus*.

Classification and morphology of *S. aureus* phages

In early studies by Rippon (1956), *S. aureus* phages have been classified on the basis of their lytic spectrum, serological group and host range. This resulted in Eleven serological groups of phages indicated by the letters A – L (Rees P.J. et al., 1980). All *S. aureus* infecting phages belong to the Caudovirales. Caudovirales is an order of viruses also known as tailed bacteriophages. The tail is a special organelle used for host recognition, cell wall penetration and injection of the genome into the host. Caudovirals are divided into three families: *Myoviridae*, *siphoviriae* and *podoviridae*, which is based on the tail morphology. Serological group D belongs to the family *Myoviridae*, characterized by having an icosahedral capsid and the longest straight contractile tail out of the three families. Serological groups A, B and F are phages that belong to *Podoviridae*, which possess a short, blunt non-contractile tail and a small icosahedral capsid. Serological group G belongs to the *Siphoviridae* family and is characterized by having a small icosahedral capsid long flexible non-contractile tail (**Fig. 1**) (Fokine A. et al., 2014; Azam A. H. et al., 2019).

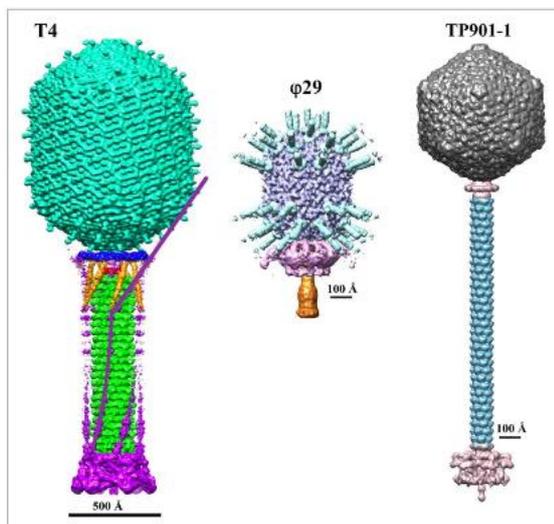


Figure 1. Structure of *Myoviridae* phage (T4), *Podoviridae* phage (ϕ 29) and *Siphoviridae* phage (TP901-1) (Fokine A. et al., 2014).

As said earlier, all three of the Caudovirals families contain icosahedral capsids, which in turn contain a tightly packed linear double-stranded DNA. The DNA, or rather the genome, of the phages extends its size from 20 kb up to 125 kb. The three classes of Caudovirals all have a different sized genome, *Myoviridae* containing the largest one (>125 kb), *podoviridae* harboring the smallest genome (<20 kb) and *Siphoviridae* showing an in-between genome size (40 kb).

Genomes of *Siphoviridae* show five functional modules which are ordered as follows: lysogenic cycle, DNA metabolism, DNA packaging and capsid formation, tail formation and lastly, host cell lysis. If virulence factors are present, they generally are located downstream of the lysis module. Although *Podoviridae* genomes show similar functional modules as *Siphoviridae*, they do differ in the fact that some modules are either not well defined or are overlapping. Genome of *Myoviridae* are also organized into modules of conserved genes such as replication and structural elements, but these modules are more likely to be interrupted by regions encoding genes of unknown function

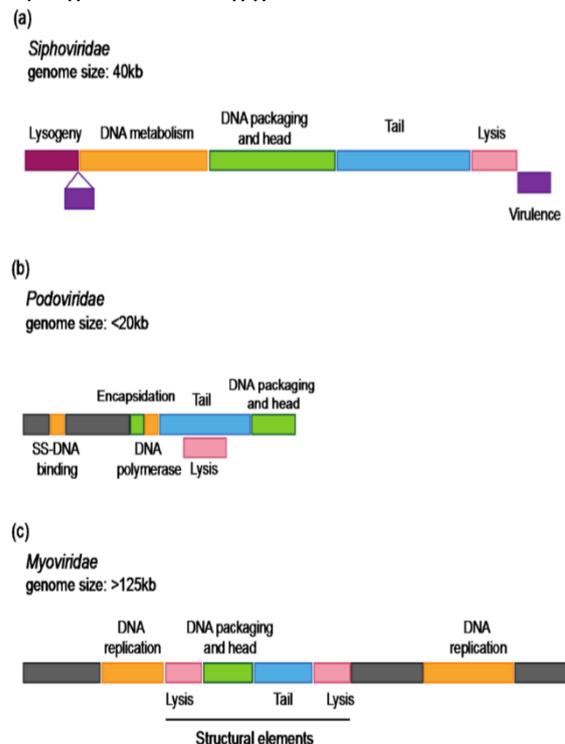


Figure 2. organization of the functional modules of *staphylococci* phages genomes indicated by five colored boxes. Red: lysogeny, orange: DNA metabolism, green: DNA packaging and capsid morphogenesis, blue: tail morphogenesis, pink: cell host lysis, purple: virulence genes, grey: region encodes genes of unknown function. Note that only *Siphoviridae* possess virulence genes. (Deghorian M. et al., 2012).

The tail of the phages is connected to the phage capsids via a dodecameric portal, also known as a connector. This connector allows the genome packaging to enter the prohead, or rather the capsid, during the virion assembly and to exit during the infection process.

proteins seal the connector, preventing the genome from exiting the capsid. The head completion proteins also provide a binding site for the tail to bind to (Fokine A. et al., 2014). Since it may not have been clear what a phage life cycle looks like, an illustration (Fig. 4) has

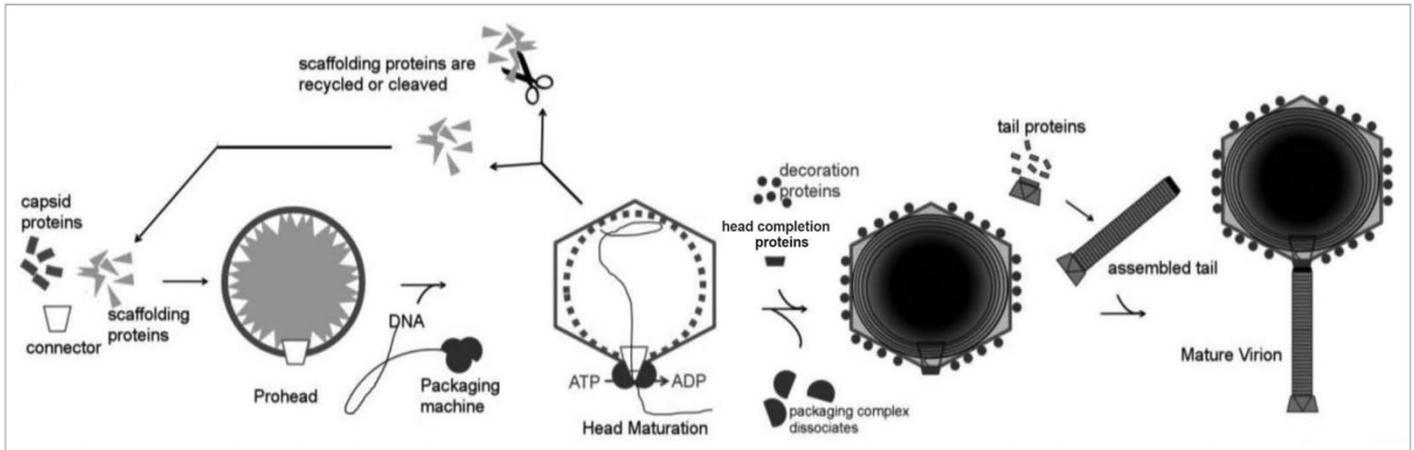


Figure 3. Internal scaffolding proteins help with the assembly of the phage capsid. This leads to the formation of the capsid precursor called prohead. The DNA packaging machine ensures that phage DNA enters the prohead. While the phage DNA is being packaged, the prohead undergoes structural rearrangement (head maturation), such as attachment of decoration proteins to their outer surface, to become the mature capsid. The head completion proteins assemble into a ring below the portal (connector), which allows the tail to connect to the connector. Now a mature virion (phage) has formed (Fokine A. et al., 2014).

As illustrated in Fig. 3, scaffolding proteins help with the assembly of the phage capsids and ensure the correct geometry of the capsid. During the completion of the capsid, DNA translocation motor package the phage genome into the capsid. The DNA translocation motor is driven by ATP hydrolysis. So as the DNA is being packaged, the capsid undergoes structural rearrangements and thus becomes a mature capsid. After the genome has been fully packed, the DNA translocation motor is removed from the capsid and head completion

been added to this paragraph. The *Podoviridae* and *Myoviridae* undergo a different life cycle compared to *Siphoviridae*. *Podoviridae* and *Myoviridae* both undergo a lytic cycle, while *Siphoviridae* undergo a lysogenic cycle. All three Caudovirales, however, attach to the bacterial host via a specific receptor on the surface of the bacterium, after which they inject their genetic material into the cell. Lysogenic phages insert their genome into the host chromosome, this prophage DNA gets replicated along with the host chromosome. Only after certain signals,

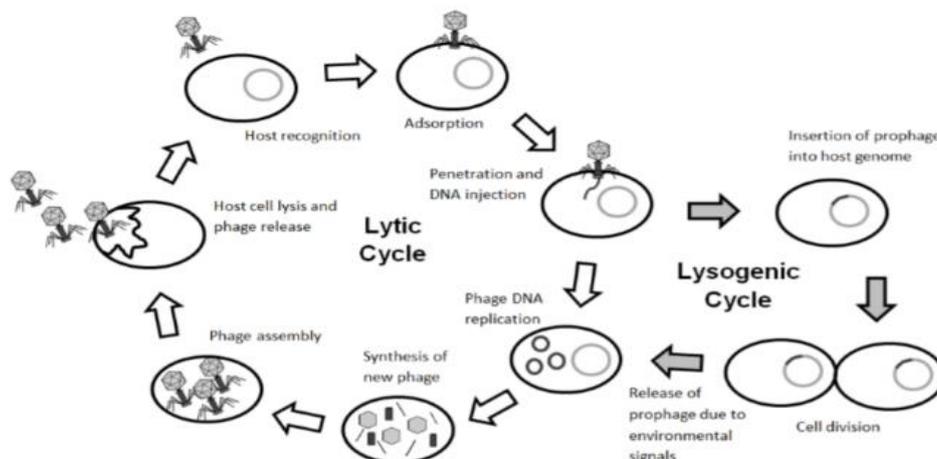


Figure 4. Lytic and Lysogenic phage life cycle. Lytic phages undergo the lytic cycle; after host recognition and adsorption, lytic phages inject their DNA into the host. After which the phage DNA is replicated, undergoes transcription and translation, which leads to synthesis of phage proteins and phage assembly. The last step of the lytic cycle is lysis of the host cell leading to the release of the phages. Lysogenic phages undergo the lysogenic cycle; after host recognition and adsorption, lysogenic phages inject their DNA into the host and incorporate their DNA into the host genome. This phage DNA is now called a prophage. Release of prophage can be induced by environmental signals, leading to phage DNA replication, synthesis of phage proteins, phage assembly and eventually lysis of the host cell and phage release (Doss J. et al., 2017).

such as oxidative stress or DNA damage, favor expression of phage DNA, this in turn will activate the lytic cycle and thus the newly assembled phages will be released into the environment. Lytic phages, on the other hand, do not integrate their genome into the host genome. They use the host cell to provide molecular building blocks and enzymes required to replicate the phage genetic material and to assemble new phages. Proteins such as endolysin and holin lyse the cell from the inside out, allowing the newly assembled phages to escape into the environment (Doss J. et al., 2017).

For the purpose of a phage therapy, direct elimination of the bacterial infections is needed, and thus lytic phages are desired. This leaves two families of *S. aureus* phages, *Myoviridae* and *Podoviridae* respectively. Of the two families, *Myoviridae* have been shown to have a broad host range, including other staphylococcal species. On the other hand, *Podoviridae* are unsuccessful to infect certain strains susceptible to *Myoviridae*, including some *S. aureus* strains. Therefore *Myoviridae* may be the best candidates for phage therapy because of their broad host range (Azam A.H. et al., 2019).

Binding of phages to *S. aureus*

Host recognition, or rather binding of phages to molecules on the cell wall of the host, is mediated by phage receptor-binding proteins (RBPs), which are located at the distal end of the phage tail (Xia G et al., 2011). Binding of RBPs to the host, overall, is extremely specific, which essentially means that RBPs determine which bacteria to target and to infect. These proteins vary greatly in complexity and composition between phages. Therefore, it is of

importance to understand the interaction between RBPs and the molecules on the bacterial surface, known as phage receptors (Takeuchi I. et al., 2016). **Fig. 5** illustrates these phage receptors on the bacterial surface, to which different types of phages bind.

Many Gram-positive bacteria, including *S. aureus*, carry unique anionic glycopolymers, which consist of carbohydrates, also known as peptidoglycan-anchored wall teichoic acid (WTA). WTAs are one of the most abundant molecules on the surface of the bacteria and play a crucial role in cell shape, regulation of cell division and adsorption of phages to the cell wall. Additionally, WTAs play an important role in pathogenesis and antibiotic resistance (Xia G et al., 2011; Brown S. et al., 2014).

Since binding of phages to its host is crucial for any successful phage infections, some of the adsorption steps will be described.

The first stage of phage infection is adsorption to a host cell by RBPs. This usually involves a reversible interaction with the host cell receptors, such as WTAs. After this an irreversible binding will take place through

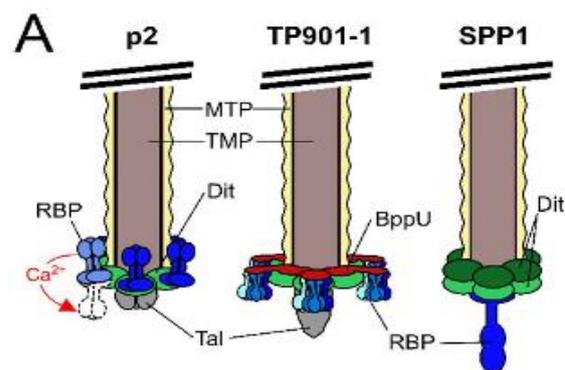


Figure 6. A simplified model of the baseplate at the distal end of the phage tail. (Dunne M. et al., 2018).

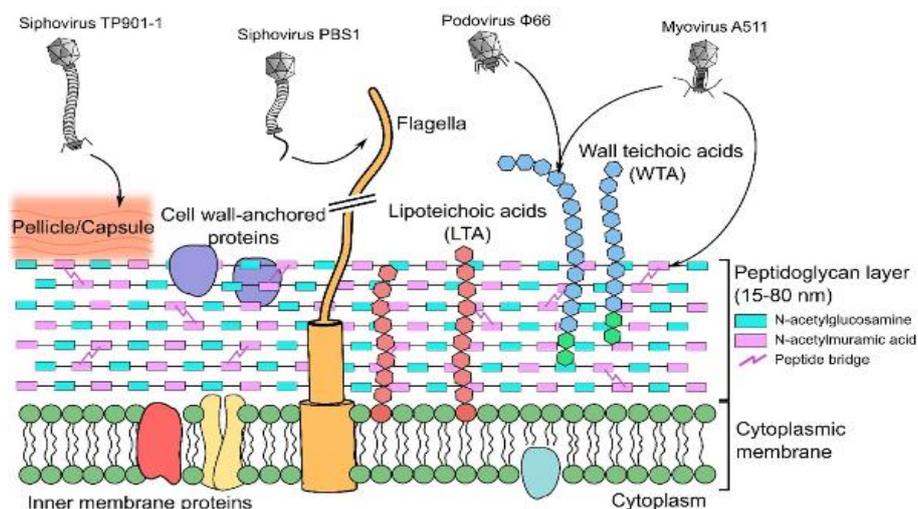


Figure 5. Gram-positive phage receptors. A layer of peptidoglycan, containing different types of phage receptors, is surrounding the bacterial cytoplasmic membrane. Phage receptors are: Wall teichoic acids, peptidoglycan, flagella, lipoteichoic acids and a pellicle layer. (Dunne M. et al., 2018).

either tighter binding to the initial receptors, or through binding to secondary receptors. Subsequently of phage binding to the host, host cell wall degradation, penetration and DNA transfer into the host will take place.

Some essential proteins for the binding of the phage to its host has been illustrated in **Fig. 6**. The distal Tail Protein (Dit) is protein which forms a hexameric hub around the distal end of the phage tail onto which baseplate components, such as RBPs, are attached to. It is to be noted that the Dit protein is specific to the *Siphoviridae*, though the *Myoviridae* and *Podoviridae* possess a similar structure, the structure is more complex consisting of more proteins (Vegge C.S. et al., 2005). Tail-Associated Lysin (Tal) is connected to the Dit. Many Tal proteins feature peptidoglycan hydrolase activity, which ensures for the degradation of thick peptidoglycan layers of the Gram-positive bacteria and thus establish for an efficient cell wall penetration and phage infection. As previously indicated, RBPs differ greatly in their morphology depending on the infection process and type of host receptor recognized. Not only the morphology of the RBPs differ between phages, also the total amount of RBPs which the phage carries differ. Phages using protein-based receptors usually feature a single, high affinity and specific RBP. These protein-based receptors usually bind to surface proteins such as WTAs. Phages binding to saccharide receptors, on the other hand, feature a higher RBPs count to compensate for the lower affinity these RBPs have. Not only do RBPs bind to external receptors, such as WTAs, they also bind to the peptidoglycan layer (PG). The PG of Gram-positive bacteria is a polymer composed of N-acetylglucosamine and N-acetylmuramic acid, to which some phages can bind. Many phages do not just bind to the PG, instead they bind to PG alongside with other cell wall receptors (Dunne M. et al., 2018).

Elimination of *S. aureus*

Elimination of *S. aureus*, or rather lysis of *S. aureus*, can be proceeded via two mechanisms. Phages which harbor single-stranded DNA encode for a lysis effector, this lysis effector in turn inhibits the biosynthesis of bacterial peptidoglycan, resulting in an indirect degradation of the bacterial cell wall. Caudovirales, however, possess a double-stranded genome, thus no further elaboration of the initial mechanism will be done. On the other hand, phages possessing double-stranded

DNA encode for proteins, holins and endolysin respectively. Holins are small membrane proteins that accumulate in the host membrane until the holins form a pore and the membrane becomes permeable. The formation of pores, by holins, occurs at a specific time which is programmed into the holine gene. Directly after the pore-forming, endolysins will be able to pass through the bacterial membrane and access the cell wall, resulting in lysis of the bacterial cell wall. Consequently, lysis of the bacterial cell wall results in osmotic lysis of the bacteria, accelerating the elimination process. This process of cell lysis takes place as soon as the phages have been fully assembled and matured, meaning the lytic cycle has been completed (Gutiérrez D. et al., 2018; Wang I.N. et al., 2000). **Fig. 6** visualizes the operation of holins and endolysins.

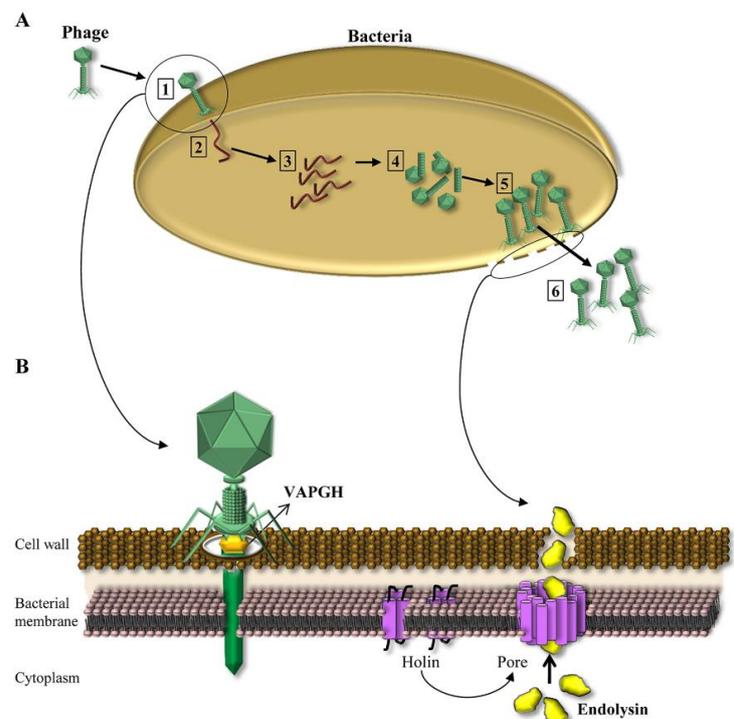


Figure 6. A) lytic cycle of phages. Virion-associated peptidoglycan hydrolases are components of the phage which are involved in the initial steps of infection. They ensure degradation of peptidoglycan, allowing the genome of the phage to enter the bacterial cell. B) Holins and endolysin are produced at the end of the lytic cycle. Holins form pores in the bacterial membrane after which endolysins are able to reach the bacterial cell wall and degrade the peptidoglycan (Gutiérrez D. et al., 2018).

Application of phages therapy

In Eastern Europe and the Soviet Union phage therapy in humans is not uncommon. Phage therapy is usually performed after bacterial

infections is unresponsive to antibiotics. As Weber-Dabrowska et al. showed, an overall success rate of 85% has been achieved with phage therapy against sepsis caused by either a single pathogen (*S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*) or by mixed bacterial flora (involving the pathogens listed above as well as *Proteus mirabilis*, *Morganella morganii* and *Enterobacter*). The phages directed to a specific pathogen were given orally three times a day at a dose of 10 mL (Weber-Dabrowska B. et al., 2003; Azam A.H. et al., 2019). Despite a success rate of 85%, phage therapy has just recently received attention in the Western World. This may be due to the fact that literature on phage therapy safety has gained attention by western medicine only recently, thus most of the information on phage therapy safety is relatively new, in contrast to the widespread literature on antibiotics (Lin D.M. et al., 2017).

In order to make a suitable phage therapy, suitable phages, which are able to bind to and eliminate the bacterial infection, must be selected. As of today, phage therapy relies on two different approaches. The first approach makes use of multiple phages which feature a broad antibacterial spectrum, resulting in a broad range of elimination of bacteria. This approach is suitable for infections such as infected burn wounds, often infected with more than one bacterial strain type (Lin D.M. et al., 2017; Abedon S.T. et al., 2011). The second approach, in contrast, isolates pathogenic bacteria from the infection. This makes it possible to test the pathogenic bacteria against several well-known phages, this ensures that only pathogenic bacteria will be affected. This second approach will be the most appropriate for phage therapy against *S. aureus*, since a limited host range is desired, so other non-pathogenic bacteria will not be affected (Abedon S.T. et al., 2011). Although phage therapy may seem very potent as of today compared to antibiotics, it has its downfalls too. Both the ups and downs will be highlighted below.

As stated earlier, side effects of antibiotics are well documented, side effects of phage therapy not so much. Though some studies have shown that phages entering the blood circulation led to activation of the innate immune system, which in turn attempts to remove the administered phages. Other studies have shown phages to be well tolerated by the host and even reduce inflammation (Capparelli R. et al., 2007). This,

again, indicates that future investigations in specific phage strains and different types of patients are needed.

One of the strong point of phage therapy is its specificity towards both bacterial species and strain compared to antibiotics. It is well known that broad-spectrum antibiotics frequently cause diarrhea and *Clostridium difficile* overgrowth and colonization of a bowel (Wawruch M. et al., 2002). Phage therapy, though literature of side effects of phage therapy is limited, has been reported to show less perturbation in the gut microbiome compared to antibiotics, while still being effective against pathogens in the gut. This specificity of the phages however, comes with the downside that it may be less effective against infections that are colonized with more than one type of strain. A solution to this would be to generate a phage cocktail that is effective against a range of known pathogens. This, however, is dependent on the knowledge of which pathogens are present. Thus in this case antibiotics may be a more viable option. Another advantage of a possible phage therapy would be the penetration and elimination of Biofilm (Lin D.M. et al., 2017). A biofilm is a microbial community in which cells stick to each other and a surface. This microbial community is embedded in a matrix of extracellular polymeric substance, which makes it hard for antibiotics to penetrate it (Archer N.K. et al., 2011). Phages, on the other hand, carry enzymes such as polysaccharide depolymerase (Fig. 7) allowing the phages to penetrate the extracellular polymeric substance

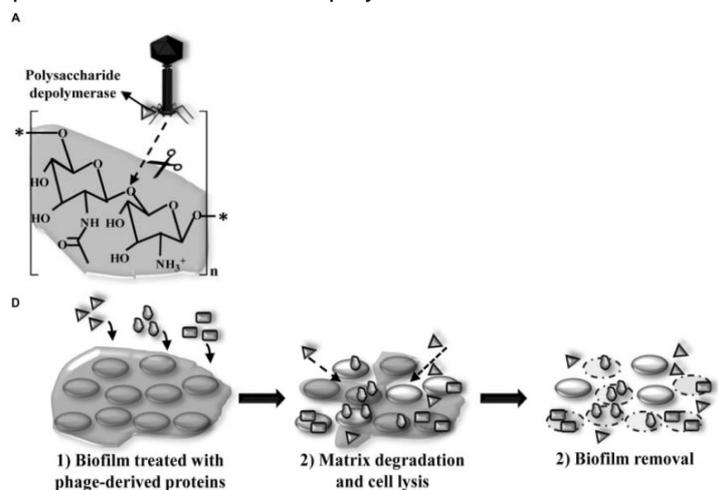


Figure 7. A) Polysaccharide depolymerase breaking down β -bonds of the biofilm extracellular matrix of staphylococcal species. D) Biofilm treated with exogenous phage proteins, such as polysaccharide depolymerase and endolysins (Gutiérrez D. et al., 2016).

and access the bacteria which reside within the biofilm (Lin D.M. et al., 2017; Olsen N.M.C. et al., 2018).

This leads us to the most potent cause of increasing interest in phage therapy; antibiotic resistance in bacteria. As early as in the 1960's, *S. aureus* had been reported to be already resistant to penicillin and methicillin (Enright M.C. et al., 2002). In a study conducted in mice by Matsuzaki S. et al. has shown that *S. aureus* phages (ϕ MR11) are able to multiply, and eradicate a lethal dose of *S. aureus*, including MRSA (Matsuzaki S. et al., 2003; Kazmierczak Z. et al., 2014). This leads us, unfortunately, to an upcoming threatening remark; bacteriophage resistance.

Phage resistance of *S. aureus*

As early as 1947 it had been shown that coagulase-positive strains of staphylococci could inactivate different types of phages, whether the phages were lytic or not (Chatterjee A.N., 1969).

In order to be resistant to phages, bacteria have developed several mechanisms to either block pathways and thus preventing phages to enter the bacterium, or block phage DNA injection into the host genome.

To prevent the infection process, the resistant bacteria inhibit the adsorption of phages to cell surface by either changing their phage receptors or by blocking the phage receptor. In order to block, or mask a phage receptor (e.g. WTA receptor), *S. aureus* has been shown to overproduce surface protein A, and thus limiting phage adsorption to the cell surface. Protein A binds covalently to the peptidoglycan subunit of the cell wall, which for some phages is needed for adsorption to the cell wall (**Fig. 5**) (Nordström K. et al., 1974). A similar study, performed by Wilkinson B.J. et al. showed that encapsulated *S. aureus* strains displayed a both sparser reversible and irreversible phage adsorption to *S. aureus* (Wilkinson B.J. et al., 1979).

Though not all encapsulated Staphylococci strains show inhibition of phage adsorption (Moller A.G. et al., 2019).

Another way for bacteria to prevent phage infection is by degrading the injected phage DNA before it has the chance to be integrated into the bacterial genome and eventually become translated.

Restriction-modification systems (R-M systems) operate in such a way (**Fig 8**). R-M systems consist of several intracellular

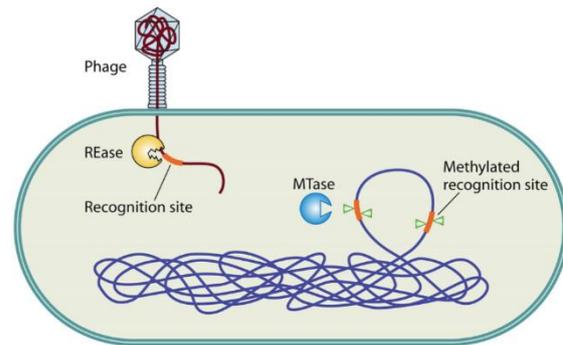


Figure 8. The R-M system recognizes the infecting phage DNA with the help of REase (restriction endonuclease). MTase methylates host DNA and thus the host DNA will not be cleaved by the restriction endonuclease (Vasu K. et al., 2013).

enzyme activities; restriction endonucleases, modification methyltransferases and DNA-binding proteins. Restriction endonuclease cleaves unmodified (phage) DNA, it recognizes the methylation status of the infection DNA. Modification methyltransferases modify host DNA and thus protecting its own DNA from cleavage. Lastly, specific DNA-binding proteins recognize short recurring DNA patterns (sequence motifs) that are targeted for cleavage or modification (Wilson GG et al., 1991; Moller A.G. et al., 2019). Four types of R-M systems are known of which all the four have been found in staphylococci. The four types of R-M systems differ in their cleavage site and methylation target site, with the exception of type IV R-M system since it does not have a modification methyltransferase. All of the four R-M systems have been found in *S. aureus*, with type 1 being the most abundant and type III the least abundant in *S. aureus* (Moller A.G. et al., 2019). A third way bacteria are able to gain immunity to phage infection is by a system called CRISPR. Just like R-M systems, CRISPR cleaves foreign DNA. But unlike R-M systems, which target specific DNA patterns, CRISPRs acquire 26 – 72 base pair sequences of DNA from either infecting phages or plasmids (small circular DNA strand in the cytoplasm of a bacterium). Thereafter, small RNAs are coded by these acquired base pair sequences to prevent subsequent infections by phages and plasmids with a similar DNA sequence (Levin B.R. et al., 2013; Moller A.G. et al., 2019). This may imply that, eventually, phages are not able to eliminate bacteria anymore. But some phages, however, have acquired adaptations for these restriction invasions. Antirestriction

mechanisms counter the R-M systems by altering and blocking the restriction site or by inhibiting the R-M systems activity. R-M systems inhibition occurs through the binding of specific antirestriction proteins (Moller A.G. et al., 2019). Anti-CRISPR mechanisms prevent CRISPR from binding DNA target sites or prevent cleavage of foreign DNA. Anti-CRISPR mechanisms either interact with CRISPR protein subunits and using non-steric or steric modes of inhibition. Or by binding to Cas3, preventing cleavage of foreign, phage DNA (Bondy-Denomy J. et al., 2015).

Discussion

Treatment of staphylococcal infections, including *S. aureus*, with antibiotics is becoming more and more difficult with the rise of multidrug-resistant bacteria, such as MRSA and Vancomycin-resistant Enterococci, also known as VRE (van Duin D. et al., 2017). With antibiotics no longer being a viable option as a treatment for some bacterial infections, interest in phage therapy has risen again. Previous literature, as early as 1919, has already shown that phage therapy is a viable option as a treatment for pathogens such as *Shigella dysenteriae* (Lin D.M. et al., 2017). However, the need for a better understanding of the interactions between different strains of phages, humans and the bacteria is needed. This since there are inconsistencies between studies regarding the host range, interactions with the immune system and potential horizontal gene transfer. Even phages of the same strain show some differences in their affinity to specific hosts, this may be due to the fact that phages may have co-evolved within their specific geographic region (Lin D.M. et al., 2017). This regional specificity could be helpful

in finding phages which show great affinity to pathogens within a specific region, however, future studies will be needed to specify these specific region.

Another point of concern is phage resistance of bacteria. As described earlier, some bacteria are able to inhibit adsorption of phages to their cell wall by either changing the phage receptor or by blocking the phage receptor (Moller A.G. et al., 2019; Nordström K. et al., 1974). A solution to this would be the use of different phages by trial and error to discover phages that are able to attach to those bacteria. This is, however, a similar procedure to what has been done with antibiotics, which has led to multidrug resistant bacteria. Other mechanisms of which bacteria may gain resistance to phages have been found too. Mechanisms such as R-M systems and CRISPR operate on the same principle; both splice foreign, or rather phage DNA and thus inhibit the DNA to inject itself into the host genome. Fortunately, some phages have been found to possess antirestriction and anti-CRISPR mechanisms, by either altering their phage DNA and preventing R-M systems from binding to DNA target sites, or by production of proteins which prevent CRISPR systems from binding to DNA target sites. mechanisms like anti-CRISPR, however, have yet to be found in *S. aureus*. (Moller A.G. et al., 2019).

Although research has to be done on phage therapy safety in humans and phage resistance, phage therapy looks like a promising alternative to antibiotics. Especially since not only do bacteria evolve to gain resistance to invading mechanisms such as antibiotics and phages, phages also co-evolve in order to keep themselves alive, thus making it a promising alternative to fight multi-drug resistant bacteria.

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