



Phage Therapy: A Potential Novel Therapeutic Treatment of MRSA

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Abstract

The emergence of multi-drug resistant bacterial strains, especially in the clinical setting has renewed interest in alternative therapeutic treatment methods. The utilization of prokaryotic viruses in phage therapy has demonstrated potential as a novel treatment method against multidrug resistant bacterial infections. As the post-antibiotic era quickly approaches the development and standardization of phage therapy is critically relevant to public health. This review serves to highlight the development of phage therapy against methicillin resistant *Staphylococcus aureus* (MRSA), an antibiotic resistant bacterial strain responsible for severe clinical infections.

Keywords: Bacteriophage therapy; MRSA; Antibiotic resistance; Virulence factors

Phage Therapy: History and Current Relevance

Phages were discovered independently in 1915 by the British microbiologist Felix Twort and in 1917 by French-Canadian microbiologist Felix d'Herelle. Responsible for the systematic investigation of the nature of bacteriophages, d'Herelle in 1921 first utilized phages for the treatment of dysentery in Paris, France [1]. This treatment resulted in the rapid recovery of patients and brought relevance to phage therapy as a clinical treatment method. Continued study and clinical use led to d'Herelle becoming the leading expert on phage therapy in this period. Throughout the early 20th century d'Herelle and other microbiologist isolated phages for the treatment of pathogenic bacteria such as *Shigella dysenteriae*, *Salmonella typhi*, *Escherichia coli*, *Pasteurella multocida*, *Vibrio cholerae*, *Yersinia pestis*, *Pseudomonas aeruginosa*, *Neisseria meningitis* and various strains of *Streptococcus* [2]. By 1931 d'Herelle had established phage therapy centers across the world, in the United States, France, and

Soviet Georgia. While phage therapy showed promise, it was weighed down by a few major problems. These problems included host range, genetic variation, and the inability to consolidate the value of phage in prevention of infectious disease. These problems eventually led to the fall of phage therapy. In this time period, phage therapy was poorly incorporated into the field of medicine, lacking theories that could be integrated with other notions of conventional medicine [3]. The discovery of antibiotics as an efficacious treatment method against bacterial infections led to a decreased interest in the development of phage therapy. While phage therapy maintained some traction in the Eastern world, it was all but forgotten in the Western world. At the turn of the 21st century the field of medicine faced a new challenge. The overuse and eventual abuse of antibiotics over the span of the 20th century led to the prevalence of antibiotic-resistant bacterial strains [4-6]. The inability to treat these bacterial infections with standard antibiotics makes them a significant threat to public health. The

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continued prevalence of antibiotic-resistant bacterium has led to the need for new novel antimicrobial agents, renewing interest in phage therapy as a potential novel therapeutic treatment. When considering phage therapy in this modern era there are three major characteristics of phages that lead to their consideration as a potential treatment method: 1) Host specificity: Phage targets bacteria with high specificity. This characteristic ensures that phage treatment would only infect the target bacteria while natural micro biota is unaffected. 2) Genetic engineering: In the early stages of phage therapy genetic engineering was not an available option. With current advances in science, we are now able to engineer phages to express traits of potential value. 3) Phages are ideal candidates for co-therapy with antibiotics: Co-therapy involves the use of both antibiotics and phage therapy for the treatment of multidrug resistant bacteria. The advancement of science since the discovery of phages in the early 20th century has led to a greater understanding of phages and an increased ability to utilize them for the benefit of public health [7-11]. As we now enter the 21st century, the prevalence of antimicrobial resistance (AMR) in bacteria has increased on account of the massive and sometimes inappropriate use of antibiotics. Antibiotic-resistant bacterial infections account for over 2.8 million infections and 35,000 deaths annually in the United States alone [5]. The continued occurrence and prevalence of antibiotic-resistant bacterial strains is considered a serious threat to global health and the economy [12-18]. The Institutes of Medicine estimates that the annual cost of antibiotic-resistant bacterial infections in the United States is approximately

4 to 5 billion USD [19,20]. Increased prevalence of antibiotic-resistant bacterial strains as well as a decrease in antibiotic development is a critical issue in the field of medicine [21,22]. Estimates from the United Kingdom project that antibiotic-resistant bacteria could result in losses to approximately 100 trillion USD worldwide by 2050, with a potential death toll up to 10 million per year [19]. Considering this, development of novel treatment methods for antibiotic-resistant bacterial infections is crucial for the preservation of international health and economy. In recent research into antibiotic alternatives, bacteriophages and their components have gained relevance as potential novel therapeutic treatment methods [7-11]. Phage therapy utilizes phage particles that specifically infect and lyse bacterial cells. A major benefit of phage therapy is host specificity; phages only infect prokaryotic cells and cannot infect eukaryotic cells. The development of new alternative treatment methods for bacterial infections are subject to technical and regulatory challenges. Challenges of alternative treatment methods such as phage therapy include activity spectrum, pharmacokinetics, immune response, manufacturing logistics, regulation, quality control, and market acceptance [23]. While these alternative treatments may not be able to replace antibiotics completely, it has been suggested that use in unison with antibiotics could be a potentially viable method for treatment of multidrug resistant bacterial strains [24-26]. This review will focus on the development of phage therapy specifically against methicillin-resistant *Staphylococcus aureus* (MRSA), a serious threat to public health (Table 1).

Table 1: Distinguishing features between CA-MRSA and HA-MRSA.

Feature	CA-MRSA	HA-MRSA
At-Risk Population	Young, healthy individuals with no exposure to healthcare facilities	Individuals with previous contact to healthcare facilities. (e.g., Hospitals, Nursing Homes)
Risk Factors	Frequent skin-to-skin contact, use of intravenous drugs, HIV, crowded or unsanitary living conditions	Long hospital stays, frequent antibiotic usage, intravenous tubing, compromised immune system, invasive procedures, devices, and surgery
Infection Type	Mild to moderate skin and soft tissue infections	Severe, invasive disease in patients or individuals in frequent contact with healthcare facilities
Infection Locations	Skin and soft tissues, lung	Bloodstream, lung, surgical site, prosthetic implant
Antibiotic Resistance Pattern	Susceptible to many antibiotics except Beta-lactams	Multi-resistant to several antibiotics

Methicillin-resistant *S. aureus* (MRSA) is one of the most common and clinically relevant examples of an antibiotic resistant bacteria [5,27]. MRSA is the result of a *S. aureus* infection that has developed resistance to antibiotics commonly used for the treatment of these infections. MRSA is the result of misappropriate use of antibiotics over the span of the 20th century. MRSA is categorized into two general types, healthcare associated MRSA

(HA-MRSA) and community associated MRSA (CA-MRSA). Most MRSA cases fall under HA-MRSA infections associated with invasive procedures or devices such as surgeries, intravenous tubing, or artificial joints. Contamination of these devices can lead to deadly MRSA infections and outbreaks in healthcare facilities. In general, those exposed to MRSA in the healthcare setting are typically more susceptible to adverse outcomes as a result of

acquiring this infection due to the compromised state of their health [5,27]. CA-MRSA is not as a common and occurs in healthy communities. CA-MRSA infections are commonly associated with skin-to-skin contact among individuals. Conditions that can place individuals at risk for acquiring these infections are group sports

that induce skin-to-skin contact, working in child-care, intravenous drug use, or crowded living conditions. The ability of *S. aureus* to develop resistance paired with the inappropriate use of antibiotics of has created a potentially deadly pathogen (Figure 1).

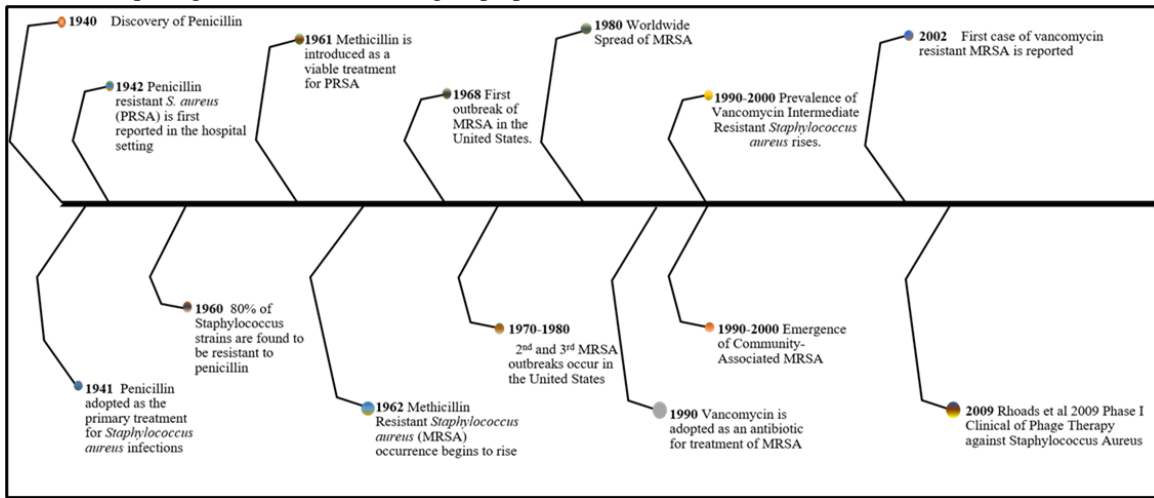


Figure 1: A chronological map outlining several important aspects of *S. aureus* treatment, evolution, and impact.

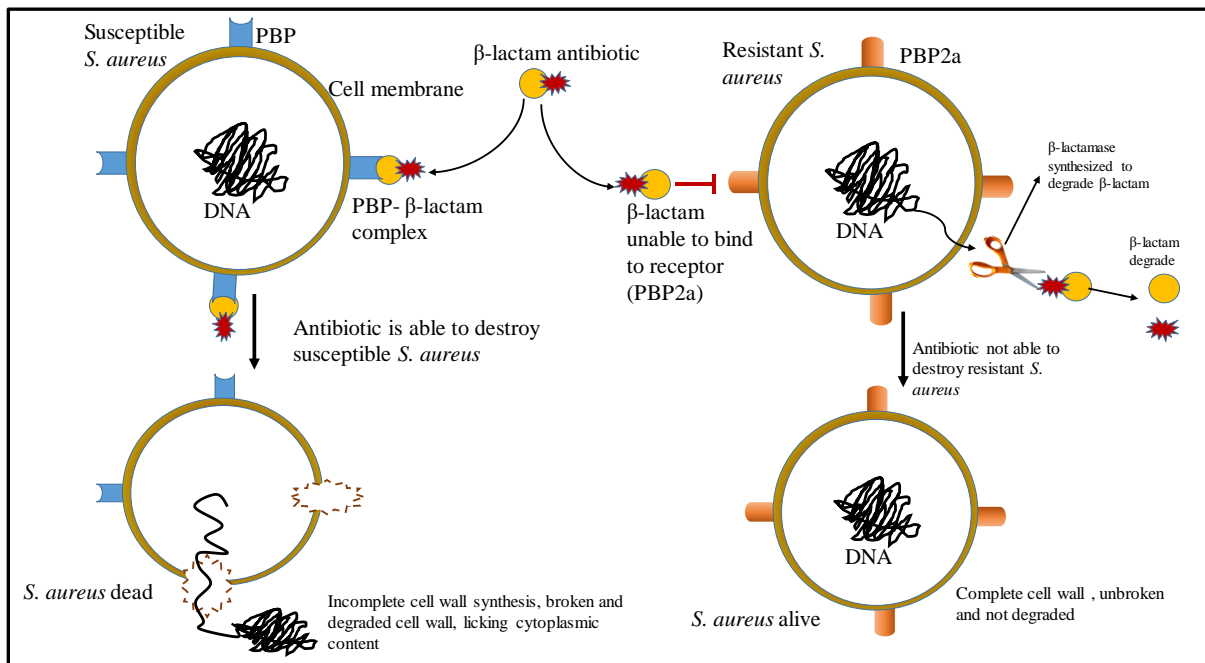


Figure 2: Diagrammatic illustration of the mechanism of inhibition of antibiotic resistance of MRSA.

Figure 1 outlines several notable timepoints in the history of *S. aureus* of the mid-20th to early 21st centuries. In 1940, the discovery of penicillin as a miracle drug offered an unlimited hope to bacterial control, however, within the space of two years, *S. aureus* developed resistance to penicillin [7,28-30]. By 1960 over 80% of *S. aureus* strains had developed resistance to penicillin [28,30]. Methicillin was introduced in 1961 as an alternative

treatment of *S. aureus*. Only a year later, *S. aureus* developed resistance to this antibiotic as well [29]. The first outbreak of MRSA was recorded in 1968, this was followed with the second and third outbreaks between 1970 and 1980 [29]. By 1980 MRSA had spread worldwide. In 1990, vancomycin became the drug of choice against MRSA, however there was an observed rise in intermediate vancomycin resistance, leading to the occurrence of

complete vancomycin resistance in 2002 [29,31]. Since 2002, MRSA prevalence coupled with a decrease in antibiotic development created a serious risk for public health. Several researchers have delved into antibiotics against MRSA; however, none have reached clinical applicability [32,33]. In 2009, a group of researchers set out to examine the safety of bacteriophage-based formulations for the treatment of wounds caused by *S. aureus* [34]. In a phase I clinical trial they reported that there were no safety concerns with the use of bacteriophage treatment, nonetheless, they encouraged a vigorous test for efficacy of the phage preparations in a phase II trial [34]. As we continue to discuss MRSA and the significant hazard it possesses to public health, it is essential that we discuss and explain what makes this pathogenic bacterium so difficult to treat on a molecular level.

MRSA and Antibiotic Resistance

As previously mentioned, resistance to standard antibiotic treatment is a major obstacle in the treatment of MRSA infections, but it is important to understand the genetic factors that facilitate this resistance. MRSA infections are resistant to beta-lactam antibiotics such as penicillin and semi-synthetic antibiotics such as methicillin that were the standard treatment of *S. aureus* prior to prevalence of MRSA [30]. To understand the virulence factors that allow for MRSA's antibiotic resistance it is important to understand the evolution of *S. aureus* infections. As figure 1 outlines, *S. aureus* has gradually developed resistance to antibiotics starting with penicillin in the form of penicillin resistant *S. aureus* (PRSA) which was first reported in 1942 [7,29]. The virulence factor present in PRSA was determined to be the gene blaZ [7,35]. This gene inhibits the binding of penicillin binding proteins (PBPs) that function to disrupt peptidoglycan cross linking during cell wall synthesis [28] (Figure 2).

As shown in Figure 2, inhibition is achieved through the production of beta-lactamase enzymes and structural alteration of the PBP receptor [28]. The development of resistance resulted in methicillin, a semisynthetic derivative of penicillin, becoming the new standard antibiotic treatment. *S. aureus* and PRSA eventually developed new resistance mechanisms against methicillin resulting in MRSA [7,29]. Methicillin resistance is the result of the development of a mobile genetic element called the staphylococcal cassette chromosome mec (SCCmec) [29]. The SCCmec genetic element contains the gene mecA along with several other functional genetic elements. The gene mecA is fundamentally responsible for inhibition of methicillin binding to the PBP 2a receptor resulting in resistance [29,36]. Methicillin utilizes the PBP 2a receptor for the disruption of peptidoglycan cross linking during cell wall synthesis. This structural change influenced by mecA results in methicillin resistance. There exists an allosteric control of *S. aureus* penicillin binding protein 2a that allows for methicillin resistance. In β -lactam susceptible *S. aureus*, the trans peptidase

activity of their PBPs is absent. Consequently β -lactam permanently acrylates the active site serine [37]. However, MRSA PBP 2a is impervious to β -lactam acylation, hence the dd-transpeptidation reaction is carried out, thus producing the cell wall of the bacteria. As shown in Figure 3, the PBP 2a enzyme, a dimeric molecule is shown bound to a peptidoglycan moiety during the bacterial cell wall synthesis process (Figure 3).

The PBP 2a structure (3ZG5) was extracted from the RCSB website (<https://www.rcsb.org/structure/3ZG5>), with the PDB ID, 3ZG535. Molecule - ligand interactions were analyzed using Biovia Discovery Studio 2021 Client (BIOVIA Discovery Studio Visualizer- <https://discover.3ds.com/discovery-studio-visualizer>). As shown in figure 2A, the dimeric molecule binds to peptidoglycan via a minor cleave found in both monomers (chain A, and chain B). PBP 2a establishes hydrogen bonds with peptidoglycan moiety at the following amino acids in the binding site; ARG151 (bond distance, 2.33Å), THR165 (bond distance, 3.36Å), THR216 (bond distance, 2.75Å), SER240 (bond distance, 2.76Å), ARG241 (bond distance, 2.85Å), TYR373 (bond distance, 2.52Å), GLY166 (bond distance, 3.73Å), HIS293 (bond distance, 3.84Å). It also interacts with the peptidoglycan molecule via alkyl hydrophobic interactions in PRO258 (bond distance, 5.31Å) and MET372 (bond distance, 4.00Å). The ability of MRSA to acquire mobile genetic elements carrying a variety of virulence factors has led to significant variation amongst MRSA strains [29]. Virulence factors that have been highlighted in literature include Pantone-Valentine leucocidin (PVL), PSM cytolysins, and toxic shock syndrome toxin-1 [29,39]. These exotoxins are responsible for MRSA's increased virulence and exceptional ability to evade the immune system [29]. The rapid development of resistance as well as the multifaceted nature of this resistance poses a significant challenge in the development of novel antibacterial agents. While these genetic components serve to develop resistance against several antibiotics, some genetic factors induce physical states that alter the effectiveness of antibiotics such as biofilm development [40]. The formation of biofilms by *Staphylococcus* spp. is a crucial adaptation for bacterial survival, thus protecting it from harsh environmental factors, antibiotics and even the bacterial host's immunity [40]. In *Staphylococcus epidermidis*, discovery of poly-N-acetylglucosamine (PNAG) and polysaccharide intercellular adhesin (PIA) was the first factor shown to mediate biofilm formation [41,42]. The discovery of multiple biofilm formation factors in *S. aureus* such as the LPXTG-cell wall-anchored biofilm-associated protein (BAP), fibronectin binding protein (FnBP), cell wall anchored clumping factor A (ClfA), cell wall-anchored clumping factor B (ClfB), *S. aureus* surface protein G (SasG), *S. aureus* surface protein C (SasC), *S. aureus* protein A (Spa) as well as other genes such as ebpS, fib, and icaA elucidated the mechanisms of action involved in antibiotic resistance employed

by *S. aureus* via its biofilm formation [42]. Some cytoplasmic proteins have also been implicated in biofilm phenotypes [43-45].

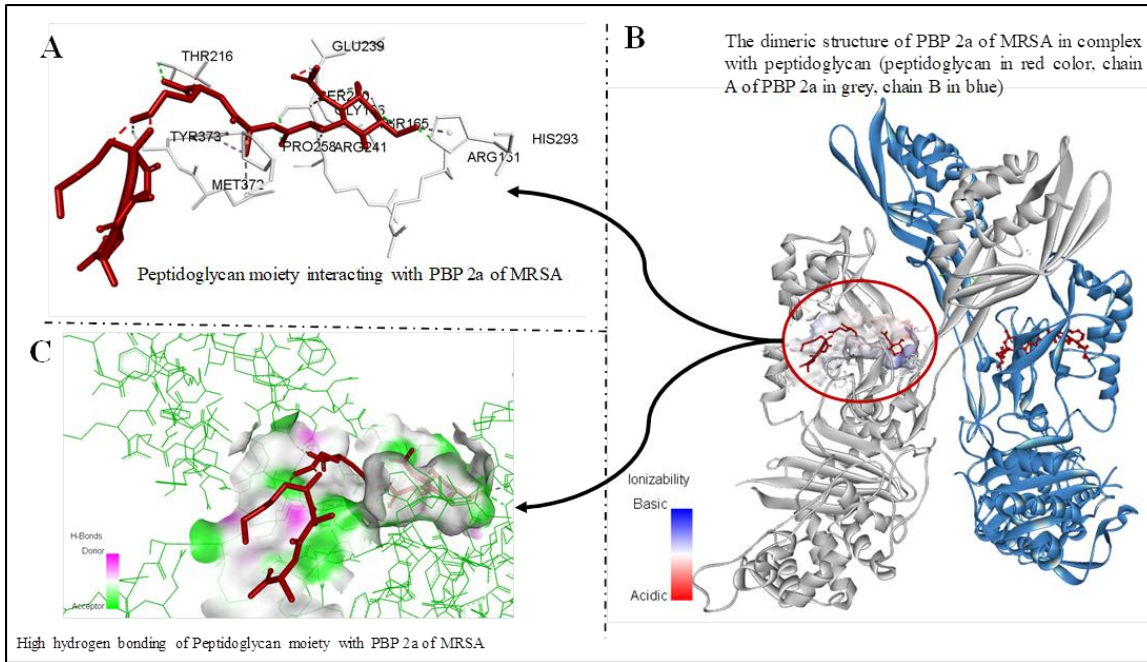


Figure 3: The dimeric PBP 2a binds to peptidoglycan moiety from MRSA.

Table 2: A brief comparative description of antibiotic and potential phage treatments against *S. aureus*. Chart outlines antibacterial mechanisms as well as bacterial resistance mechanisms.

Antibiotic	Protein	Antimicrobial Mechanism	Bacterial Mechanism	Resistance	Resistance Gene
Penicillin	Beta-lactamase	Binding to penicillin binding proteins (PBPs) disrupts peptidoglycan cross linking during cell wall synthesis resulting in lysis.	Production of Beta-lactamase enzymes and alteration of PBP [28]		blaZ [28,35]
Methicillin	Beta-lactamase 2a	Binding to PBP 2a disrupts peptidoglycan cross linking during cell wall synthesis resulting in lysis.	Structural change of PBP 2a [36]		mecA [28]
Vancomycin (glycopeptides)	D-ala: D-lac ligase D-ala: D-ser ligase	Interaction with uncross-linked peptidoglycan pentapeptides results in inhibition of the extracellular steps of peptidoglycan synthesis.	Alteration of structure of cell wall peptidoglycan precursors [31]		vanA vanB [28,31]
Phage	Protein	Antimicrobial Mechanism	Bacterial Resistance		
<i>S. aureus</i> Phages Trsa205 [70] Trsa207 [70] Trsa220 [70] Trsa222 [70] SA003 [71] MR003 [71] LS2a [106] Regional Phages [88,103]	Tailspike Proteins	Phage tailspike proteins specifically target receptors on the membrane to initiate penetration, replication, synthesis, assembly, and release, resulting in lysis of the bacterial host [47-49].	The evolutionary rate of bacteria to develop resistance to phage treatment is significantly slower than bacterial development of antibiotic resistance [7].		

Identification of Phages against MRSA

Phages are characterized by a narrow host range and may infect only one species or strain of bacteria within a species. The development of phage therapy for specific bacterial strains requires the identification, isolation, and characterization of phages that exhibit lytic lifestyles in the desired bacterial target. Lytic phages offer the greatest therapeutic potential due to their consistent lethal effects on their host [46]. The lytic lifestyle is comprised of five stages: attachment, penetration, biosynthesis, maturation, and lysis. In the attachment stage, phages utilize their tailspike proteins to interact with specific bacterial surface receptors of the membrane. This interaction has been observed at the molecular level in a variety of phage families [47-49]. As previously mentioned, phages are characterized by a narrow host range and may infect only one species or strain of bacteria within a species [50]. This specificity is unique and can be exploited for targeted treatment of bacterial infections in phage therapy and identification of bacterial pathogens in phage typing [51,52]. Following attachment to the host cell membrane, the phage utilizes its tail machinery to penetrate the cell membrane and inject its viral genome [53,54]. The biosynthesis step of this mechanism is carried out through synthesis of virus encoded endonucleases to degrade the bacterial chromosome. The virus then utilizes the functions of the host cell to replicate, transcribe, and translate viral components for the assembly of a progeny [55]. Assembly of the newly synthesized virions, termed maturation, is followed by the disruption of the host cell membrane by phage proteins holin or lysozyme [56]. This disruption leads to the lysis of the host cell and the release of the progeny to infect other bacterial cells. According to literature all known phages associated with the Staphylococci family of bacteria belong to the order of Caudovirales and are primarily members of the families Siphoviridae and Myoviridae [57-60]. The use of phage typing for the identification of *S. aureus* infections in the clinical setting served to develop a library of phages specific to this bacterial genus. This library is integral in the screening of phages for lytic activity against MRSA. As the prevalence of MRSA increases, the ability to identify *S. aureus* phages that carry out lytic lifestyles in MRSA is a vital step in the development of viable therapeutic treatments. Isolation of phages from the order Caudovirales followed by characterization and in vitro testing is a viable method for identification of *S. aureus* phages with lytic lifestyles within MRSA [61-66]. Phages are known to be abundant in any ecosystem in which their bacterial host is present [64]. Literature has been able to utilize samples, primarily from healthcare facility sewage, for the isolation of *S. aureus* phages [61-65]. Characterizations of these isolates through double-layer plaque assay (DLA) and electron-microscopy has resulted in the identification of *S. aureus* phages belonging to the Siphoviridae and Myoviridae families [61-64]. Phages of the order Caudovirales

are classified structurally into three families of tailed bacterial viruses: Myoviridae (long contractile tails), Siphoviridae (long non-contractile tails), and Podoviridae (short non-contractile tails) [63]. All three families of Caudovirales feature non-enveloped protein shell heads containing a single linear dsDNA molecule. The dsDNA genomes of these phages encode from 27-600 genes clustered according to function arranged in large operons. Caudovirales are found in over 140 prokaryotic genera representing most branches of the bacterial phylogenetic tree. With a wide variety of host ranges some members of this order can infect members of multiple genera of bacteria while others show high specificity [63]. A major obstacle in the development of phage therapy is the host range of phages. Infection specificity of phages can often lead to difficulties in the development of efficacious phage therapy methods. The host range of *S. aureus* phages against clinically isolated MRSA strains can be determined through in vitro assays. Against isolates of clinical and community related MRSA infections, phage host ranges have shown wide variation as naturally expected. We contribute this wide variation to the high specificity between phages and their target host. Literature has been able to identify a variety of phages with host ranges suitable for phage therapy against MRSA [67-72]. Phages that are selected for treatment of MRSA infections should exhibit a broad host range against clinically relevant strains. Literature has outlined several polyvalent phages that could be utilized for phage therapy [46]. A phage that has exhibited a broad host range against MRSA is the phage MR003 [71]. This phage, a member of Caudovirales family, has been observed to infect 97% of clinical and community MRSA strains [71]. This host range is significantly higher than other *S. aureus* phages that typically infect anywhere from 20% to 73% of MRSA strains [70-72]. The host specificity of phage MR003 is hypothesized to be a result of the genomic structure of the tailspike and baseplate structures of the virus. Comparative genomic studies of MR003 to common *S. aureus* phage SA012 revealed that these two phages share homology in ORF117 and ORF119, responsible for receptor binding to host cells. It was determined that differences in the tailspike and baseplate structures seem to be the key contributing factor to the broad host specificity in MR003 [73]. Another relevant phage is phage 812. In vitro studies have shown this phage's ability to kill 95% of 782 clinical *S. aureus* isolates [74]. Phage 812 is closely related to phage K that is known to demonstrate a large host range against MRSA. Phage K has also been shown to be effective against MRSA strains that are vancomycin resistant and teicoplanin resistant [46]. In vitro study demonstrated that 39 out of 53 clinically isolated strains were sensitive to phage K and that insensitive strains could be treated with variants of phage K [75]. Genomic studies of phages that are potential candidates for phage therapy against MRSA infections could be useful in identifying factors that influence host range [71-73]. A method utilized to increase the host range of phage

treatments against MRSA is phage cocktails. Phage cocktails address the challenge of limited host ranges through the incorporation of multiple phages with varying host ranges in solution. This method has been shown to increase the infectivity of phages against MRSA [70]. An experimental phage cocktail of four *S. aureus* phages that infected 37.5%, 26.7%, 21.4%, and 19.6% of clinical MRSA isolates respectively resulted in a cocktail that had the ability to infect 66% of clinical MRSA isolates [70]. Phage cocktails allow for the lysing of MRSA bacterial strains without the host range limitations associated with individual phage treatments. While phage cocktails provide a greater ease of use a potential downfall of this method is the greater complexity in manufacturing and possible clinical outcomes [76]. While individual phage therapy only requires the isolation of one specific phage, phage cocktails require the isolation and purification of multiple phages which in turn increases the complexity of manufacturing.

Development of MRSA Phages as Therapeutic Agents

Biological considerations

Phage therapy, first used almost a century ago, is driven by the continued occurrence and prevalence of antibiotic resistant bacterial strains. While the discovery of antibiotics negated the need for new antimicrobial agents in the 20th century, antibiotic resistance in the 21st century has renewed the need for new antimicrobial agents. The rise of phage therapy as a potential novel therapeutic method is facilitated by our improved understanding of phage biology, genetics, immunology, and pharmacology. Aspects of phage therapy that once hindered its efficacy have now been standardized to improve treatment success. Regulatory requirements of phage therapy call for strictly lytic phages, confirmed antimicrobial activity against the target pathogen, and the removal of contaminating bacterial debris and endotoxins [77]. Identification of the bacterial host cell receptor for any therapeutic phage is also key in the long-term success of phage therapy. Identification of these receptors can provide insight into phage resistance, evolutionary trade-offs, and use of co-therapies that are less likely to generate phage resistant hosts. Phages that feature lytic lifestyles are ideal for the success of phage therapy. The use of temperate or lysogenic phages is highly inadvisable in phage therapy as their ability to lysogenize cells is hindered by the rise of homoimmunity in a bacterial population and the possibility of lysogenic conversion [78,79]. Lysogenic conversion can lead to bacterial populations gaining new, often pathogenic genetic traits, such as phage-encoded toxins or antimicrobial resistant determinants [79]. Despite these drawbacks and potential hazards, temperate or lysogenic phages have shown potential to be utilized through genetic manipulation of their life cycle [46]. Research has

demonstrated that two distinct mutations, *vir* and *clear plaque*, can essentially change temperate phages into obligately lytic phages [46]. Both mutations effect the repressor protein of the phage, inhibiting its ability to become a prophage or carry out lysogenic conversion. A *vir* mutant has already been successfully utilized in an animal study, showing promise for this method [46].

While lytic phages are considered the standard for phage therapy, there are still some concerns about their abilities. Scientific understanding of phage has been greatly advanced since their discovery a century ago. However, our knowledge of phages is still limited. The genomes of lytic phages can contain greater than 50% hypothetical genes with no known function as well as encode auxiliary proteins that alter bacterial physiology in ways that are not fully known [80]. The number of genes and auxiliary proteins that we are currently unaware of makes abortive infections a major concern. Abortive infection is a method of bacterial defense in which the bacterial cell upon infection kills itself to ensure the replication of a phage is stopped. This mechanism could possibly lead to the bacterial host acting as a reservoir inside the human body for phage DNA with unknown function. This concern is also shared with mutant phages such as *vir* and *clear plaque*, especially considering that temperate phages typically carry a wide range of virulence factors [46]. Continued research of phage genetics is key in ensuring the safety of phage therapy.

Comparison to Antibiotics

Phages and antibiotics both serve as antibacterial agents functioning to lyse or inhibit the persistence of bacterial infections. While both agents have similar function, they feature several key differences that determine their appropriateness for situational usage. The use of antibiotics has been observed to have adverse health effects in some situations [81]. Adverse health effect of antibiotics includes instances of anaphylaxis, nephrotoxicity, cardiotoxicity, hepatotoxicity, neurotoxicity, and several gastrointestinal and hematological complications [81]. The most common adverse effect of antibiotic treatment is allergic reaction, which is prominent in children. These allergic reactions are most commonly the product of high tissue concentrations [82-84]. The safety of phage therapy has not been as extensively studied, especially in western medicine. However, new studies have deemed phage therapy practices such as oral administration as safe [82-88]. In terms of oral administration, the translocation of phage across the intestinal epithelium into the blood has been suggested as beneficial to the host [89]. The benefit of this translocation is the downregulation of immune response to indigenous gut microbiota antigens through the inhibition of interleukin-2, tumor necrosis factor, and interferon gamma production [89]. This down regulation in addition to phage host specificity protects the natural gut microbiota. The protection of natural gut microbes is a typical criticism of antibiotics. The immunological response to phage

therapy may be beneficial in healthy patients however literature disputes the safety of treatment in patients with compromised immune systems [90-92]. Immunological response is especially significant in the context of MRSA infections that are prominent in patients who are immunocompromised. Patient to patient variation in the study of phage therapy has been an area of concern. While transduction may be beneficial to natural gut microbes, there is concern that this characteristic could also be related to disruption of normal intestinal barrier function. This disruption could potentially lead to disorders such as Crohn's disease, inflammatory bowel disease (IBS), and type 1 diabetes [93]. Literature has determined that there is variation in inflammatory response to phage therapy based on the site of infection [94]. The study of phage therapy is relatively new and there are many characteristics such as immunological response and physiological response that require further study to comprehensively assess the safety of phage therapy. Host specificity is a defining characteristic of phage therapy. The broad use of antibiotics has been documented for its adverse effects on the human gut microbiome that sometimes lead to diarrhea and *C. difficile* infection [95]. Other potential outcomes of antibiotic perturbations in the gut microbiome include asthma, obesity, and diabetes [96-101]. Phage therapy is highly specific to bacterial species and strain, resulting in less irritation of the natural gut microbes while still effectively reducing presence of pathogens [99,100]. As discussed in the host range section of this review, the specificity of phages can sometimes lead to inability to treat an infection colonized by multiple bacterial species. A common clinical example of this scenario is burn victims who typically suffer infections colonized by more than one singular bacterial strain [101]. The development of phage cocktails that are effective against a range of bacteria present in an infection can increase the host range of treatment, which in turn results in a more effective treatment of the infection. It is important note that the success of phage cocktail treatment is dependent on the ability to identify the pathogens present. While phage cocktails address complex infections and the limitations of host specificity, they result in major logistical challenges [76]. Phage cocktails present limitations in development, large-scale production, and distribution; a distinct advantage of broad-spectrum antibiotics. Another specific advantage of phage therapy in comparison to antibiotics is the rate of resistance development by target species. Phages, as naturally occurring organisms, actively adapt to ensure persistence and survival. However, even with these adaptations, bacteria can often still develop resistance against phages. For this reason, specifically, phage cocktail therapies have been of elevated interest as they make the emergence of a resistant bacterial cell substantially less likely [102]. In addition to the use of cocktail therapies, it is also important to understand that phages, unlike antibiotics, require little to no development and are readily available from the environment. Considering this even in cases of

bacterial resistance, there are a multitude of phage species that our target pathogen is likely not resistant to. An interesting characteristic of phage therapy is the relationship between geographic location and phages used for treatment. Studies have shown that phages show high specificity to bacterial targets from their indigenous region [88,103]. These studies utilized Russian *E. coli* phage cocktails for the treatment of microbiologically determined *E. coli* diarrhea in Bangladesh [88]. The treatment resulted in no improvement of clinical outcome. Results suggested that phage cocktails are better adapted to local bacteria populations, and that bacterial host range can be restricted both spatially and temporally [104]. A suggested solution to this challenge is the development of phage cocktails with regional specificity for the clinical setting [105]. In the context of MRSA infections, as well as other antibiotic resistant bacterial strains, this means that the phages that can be used to target these bacteria are likely found in the same environment [106]. While this high specificity provides challenges in production that are not common with broad-spectrum antibiotics it does have some benefits. Regions that have limited access to antibiotics would greatly benefit from the ability to isolate phages that could be utilized for specific phage therapy of regionally prevalent pathogens. Utilization of phage therapy in these regions would also positively impact the economic burden that the cost of antibiotic treatment entails. Antibiotics have been a cornerstone of clinical treatment for over a century, but the increased prevalence of antibiotic resistant bacterial strains has required the development of new novel therapeutic treatments. The limited adverse effect, target specificity, and abundance of phages in the natural world make phage therapy a potentially viable therapeutic treatment (Table 2).

Clinical Challenges of Phage Therapy against MRSA

The lack of validated and adequately controlled clinical trials is a current challenge of progressing phage therapy into standard clinical practice [108]. The pharmacological characteristics of phages hinder their standardization in clinical trials. A major pharmacological concern is the self-replicating nature of phages, unlike conventional drug treatments, phage therapy requires awareness of various novel kinetic phenomena [109]. Determining dosage is particularly challenging since phages have the potential to exponentially increase upon infection of the target bacteria. Experimental design of clinical trials utilizing phages requires standardization and guidance using tailored pharmacokinetic models for specific systems. The establishment of these models as standard practice would greatly advance the use of phage therapy in clinical trials [109]. Another challenge in the clinical use of phages for the treatment of bacterial infections is the delivery of phage virions to the location of the infection. Phages require direct



contact with the target bacteria to carry out infection and lysis. Broad distribution of phage in the body cannot effectively treat the target infection. Literature has exhaustively examined methods of delivery in animal models, revealing that administration of phages into the intramuscular, subcutaneous, or intraperitoneal have shown significant influence on the success of phage therapy [107,110,111]. Intraperitoneal injection of phage MR11, an *S. aureus* phage, demonstrated the ability to eradicate MRSA infections in mice models [111]. Animal trials have demonstrated the abilities of phage therapy as a novel therapeutic against MRSA as well as worked towards standardization of dosages for adequate treatment. Dose-response studies in white rabbits have demonstrated the effectiveness of phage therapy against *S. aureus* via subcutaneous injection. This study concluded that high concentrations of the phage L2Sa, a *S. aureus* phage, was shown to prevent abscesses caused by infection [107]. While phage immunotherapy has shown promise, combination therapy or phage cocktails also offer a broad range of activity against bacterial infections. Phage cocktails as previously described in this review consist of a combination of several phages with various host ranges. This combination addresses the limitations of monotherapies host range and reduces the potential development of phage resistance in bacteria. While phage cocktails feature a broader host range, it has been shown that they greatly increase the challenge of assessing inflammatory response, potential gene transfer, and development of multi-phage resistance [112]. Further study and standardization of phage cocktail therapy is required to fully determine their effectiveness as well as efficacy. While there are still many questions regarding the effectiveness of phage therapy treatments, groups such as the Center for Innovative Phage Applications and Therapeutics (IPATH) have taken major steps forward in furthering the development of phage-based therapeutics. IPATH utilizes phage therapy to treat patients with life-threatening multi-drug resistant infections through the Food and Drug Administrations (FDA) compassionate use program. Additionally, IPATH serves to rigorously evaluate phage therapy in clinical trials with the eventual goal of combating the global antimicrobial resistance crisis. Hopefully continued study of phages and their clinical efficacy can continue to provide meaningful insight into the progress of phage-based therapy. Utilizing clinical studies from groups such as IPATH will be crucial in the further development of clinical trials for phage therapeutics.

Human Clinical Trials

Human clinical trials for phage therapy against MRSA are limited due to the challenges previously mentioned. Standardization of clinical trials requires preliminary studies to determine adequate dosage, delivery, and host response. The use of animal models has been largely beneficial to the progression of standardized phage

therapy methods [107,111,112]. As previously mentioned and important to note, while the western worlds use of phages as therapeutics is limited, the eastern world has considered phage therapy a viable option [113]. Not specific to MRSA, phage treatments against drug resistant bacterial species have shown effectiveness with a reported ~85% success rate [114]. The select phage therapy clinical trials that have been conducted show promise for the use of phages against MRSA infections and will be briefly discussed throughout this section. Referenced throughout literature as one of the pioneering clinical trials against MRSA, Rhoads et al in 2009 focused on the treatment of venous leg ulcers in humans [34]. This trial treated ulcers with bacteriophages targeted against *Pseudomonas aeruginosa*, *S. aureus*, and *Escherichia coli*. Results of this phase I trial concluded that there were no adverse events attributed to the phage therapy and that between test and control groups there was no significant difference ($p>0.05$) in the frequency of adverse events, rate of healing, or frequency of healing [34]. Phase I was successful in demonstrating the safety of phage therapy in humans [34]. While Rhoads et al showed promise, phase II trials of phage therapy must be carried out to determine efficacy. While several clinical trials focused on phage therapy are underway, the results and conclusions of these trials have not been released yet to the public. However, groups such as IPATH, have been utilizing phages for compassionate treatments that have yielded positive outcomes [115-118]. Compassionate treatments, unlike clinical trials, are not designed to provide results to evaluate the efficacy of a treatment but instead to benefit patients who have exhausted all standard medical options. Compassionate treatment cases have provided insight into the potential effectiveness of phage therapy in humans. While compassionate treatment covers a wide variety of diseases, the most frequently reported bacterial infections targeted are resistant *S. aureus* strains [119]. In 2006, compassionate phage therapy was effective in the treatment of two individuals with radiation burns infected with resistant *S. aureus* [120]. Following antibiotic treatment, infections continued to persist leading the individuals to seek compassionate alternative treatment. Patients were treated with a phage cocktail preparation called Phage Bio Derm. This treatment utilizes a lytic phage cocktail with various host specificity suspended in a biodegradable polymer mixture. This treatment results in *S. aureus* inhibition and eradication of the infection. This treatment protocol highlights the non-invasive manner with which phages can be utilized for treatment of persistent infections especially in the case of skin infections. Where antibiotics and other treatment methods rely on sometimes invasive measures, this phage cocktail can simply be applied as a film to the skin where the infected ulcer is present. There are several other notable examples of compassionate phage treatments showing success. In 2016 compassionate phage treatment was carried out on six individuals with perfuse toe ulcers infected with MRSA. These

infections that were non-responsive to all antibiotic treatment responded to treatment with staphylococcal phage Sb-1. In all six patients, phage treatment effectively eradicated all MRSA cells [121]. Recent interest in phage therapy has resulted in the increased involvement of pharmaceutical companies in phage research and clinical trials. Novolytics (UK) has recently announced that phage cocktail gels that target MRSA are in the developmental stage [122]. This phage cocktail would serve to treat nasal carriage of MRSA as well as skin infections and indwelling medical devices [122]. In addition to Novolytics several other companies including Armata Pharmaceuticals Inc., Intralytix Inc., Adaptive Phage Therapeutics Inc., Pherecydes Pharma, and Locus Biosciences Inc. have also announced continued development of phage-based therapeutics. While phase II and phase III trials have not been announced for phage therapy treatment of MRSA infections, it can only be assumed that they are on the horizon. Continued research into phage pharmacokinetics, stability, delivery, partnered with the development of novel formulations and exhaustive clinical trials will eventually allow phage therapy to reach widespread clinical application.

Conclusion

The increased prevalence and occurrence of antibiotic-resistant bacteria is a major threat to public health especially the notorious antibiotic resistant *S. aureus*. While antibiotic dose-response has been standardized, consideration of MRSA phages varied replication factors is crucial for the determination of standard relative dosage for ‘killing’ titers. Additionally, MRSA phages multiplication is incumbent on host availability, for this reason, an initial “killing titer” might tremendously increase after phage administration through the phage’s replicative process. An added dimension in the phage biology is its ability to co-evolve with its host, this added advantage over antibiotics enhances the need to study MRSA phages as therapeutic tools against the bacteria. Hence, a clearer insight of MRSA phage biology, pharmacokinetics and pharmacodynamics will provide the requisite avenue for broad application of phage therapy. It is undoubtable that an alternative treatment method for these antibiotic-resistant bacteria such as MRSA is essential to counteract human infections as well the economic burden they present. MRSA being one of the most prevalent antibiotic resistant bacterial strains is an immediate and serious threat to public health. The utilization of lytic *S. aureus* phages for the treatment of MRSA shows potential as a therapeutic treatment method. Literature has outlined the potential benefits of phage therapy against MRSA due to their host specificity, wide diversity, and success in animal and limited clinical trials. While phage therapy against MRSA requires further study, literature to this date suggests that phage therapy shows favorable potential as a novel therapeutic treatment.

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