Chapter

Bacteriophages as Anti-Methicillin Resistant *Staphylococcus aureus* Agents

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Abstract

Staphylococcus aureus is a colonizing microorganism of the nasal region of both humans and animals and represents an important opportunistic pathogen. The acquisition of the mecA and mecC genes by *S. aureus* led to the emergence of methicillin resistance (MRSA), becoming a public health problem in both human and animal areas. In addition to resistance to β-lactam antibiotics, MRSA strains have multidrug resistance to antimicrobials, significantly limiting therapeutic options, making it crucial to have effective alternatives for treating staphylococcal infections. In this context, the use of lytic bacteriophages, which are viruses that infect and lyse bacteria, as well as the use of their by-products, such as endolysins, has shown potential in the control of *S. aureus*, including MRSA. Due to the specificity of bacteriophages to infect particular prokaryotic hosts, these viruses represent an antibacterial resource for the control of public health relevant microorganisms, especially antibiotic-resistant bacteria.

Keywords: Methicillin resistant *Staphylococcus aureus*, MRSA, phage, phage therapy, phage by-products

1. Introduction

1.1 The role of *S. aureus* in human and animals

Among the different relevant bacterial genus in Veterinary and Human Medicine, *Staphylococcus* is one of the most frequent opportunist pathogens. The species belonging to this genus present themselves as Gram positive cocci and are related to different communitarian and nosocomial infections, in both humans and animals. The members of *Staphylococcus* spp., especially *Staphylococcus aureus*, are constituents of the normal microbiota of the skin, mucous membranes, and upper respiratory tract of humans [1]. Although *S. aureus* is not considered part of the microbiota of dogs, indexes of 5% [2], 10% [3, 4], and even 20% [5] of nasal colonization by the bacterium were described in canines. Similarly, the cats also are included among the pet target-species potentially colonized by *S. aureus* due to their close proximity to humans, as pets [6]. In the context of proximity, the coexistence between man and dogs is still closer in order of canine aptitudes additional to the condition of the pet, as guide dogs, hunting dogs, guard dogs, among others. Thus,

the pets share daily routines with their owners, establishing affective bonds that emphasizes the importance of the control of transmissible diseases inter-species.

Historically, the first publications related to the human carriage of *S. aureus*, emerged in mid-1940s [7] and showed the relevance of the bacteria in the human infections. On the other side, the approach to this theme in the vet sphere was only evidenced from the year 2000. Regardless, *S. aureus* has zoonotic potential [8], being even more relevant when the bacteria is methicillin-resistant (Methicillin Resistant *S. aureus* or MRSA). The transmission of this emerging zoonotic pathogen among pets and humans [9], including veterinary staff, has been demonstrated [10, 11], implying problems in the public health sphere [12]. In addition, the risk of zoonotic transmission of *S. aureus* may impact directly in the relation between humans and animals, harming the strength of the affective bond. Additionally, the expressive occupational health risk to veterinary professionals must also be considered [13].

1.2 Infections related to *S. aureus* and Methicilli Resistant *S. aureus* (MRSA)

S. aureus is one of the most structured species in order of the high frequency as etiological agent of infections, as well as the growing prevalence of its resistance to antimicrobials [14]. The health complications arising of the infection by *S. aureus* in humans and animals are diversified and depend on intrinsic factors to the bacteria (virulence factors as extracellular enzymes, capsular polysaccharides, surface-associated proteins), as well as the conditions inherent to the host. Clinically, they can limit themselves to localized skin infections, but can cause severe illnesses as septicemia, respiratory tract infections, osteomyelitis, endocarditis, besides food poisoning [9]. Along with the severity of the bacterial infections, the other factor that compromises the recovery of the infected individuals is the bacteria antimicrobial resistance profile. The higher the degree of resistance, the higher will be the restriction to therapeutic alternatives to the treatment of the infection, there may not even be an effective drug. In this regard, the World Health Organization (WHO) suggested in 2017, a list of resistant bacteria considered more relevant in order of antibiotics shortage to treat the diseases. The specialists grouped the pathogens accordingly with the bacterial species and the resistance type shown, resulting in three priority tiers: critical, high, and medium, being Methicillin-Resistant *S. aureus* considered high priority [15].

1.3 Perspectives to MRSA infections treatment

Alternatively, with the development of the new antibiotics to supplant the resistance, there is the possibility of using viral agents to control unwanted bacteria. Viruses termed “bacteriophages” or “phages” are the most abundant agents in the environment and are host-specific, i.e., they infect only prokaryotes that have their own specific receptors for their adsorption. The absence of such receptors makes phage binding to the target cell as well as subsequent infection impossible, characterizing the specificity of these viruses [16, 17]. Phages are easily recovered from soil, sewage, and feces and their numbers are about 3 to 10 times higher than bacterial counts even though variations exist between ecosystems [18, 19]. Like other viruses, bacteriophages are obligate intracellular, and are characterized according to the replication cycle exhibited after infection of the bacterial host. The cycle can be lytic or lysogenic, but only phages that exclusively perform the lytic cycle are of interest for use as therapeutic agents, since they will promote cell lysis at the end of the cycle [18].

2. Methicillin resistant *Staphylococcus aureus* (MRSA)

2.1 What is MRSA?

The *Staphylococcus* genus consists of a variety of opportunistic pathogens of variable relevance in veterinary medicine, being the coagulase-positive *S. aureus* and members of the group *Staphylococcus intermedius*, particularly *Staphylococcus pseudointermedius,* the most important clinically [13]. In human medicine, *S. aureus* can cause clinical manifestations ranging from mild skin and soft tissue infections to severe bloodstream infections. A remarkable skill of this genus is its capacity to acquire antibiotic resistance [20], mainly from the irrational increase in the intensity of its use [21]. Methicillin resistant *S. aureus* (MRSA) are resistant to an important range of antibiotics [20]. The resistance to methicillin, conferred by the presence of the *mec*A or *mec*C gene, is of particular relevance. These genes, located in Staphylococcal Chromosomal Cassette (*SCCmec*) confer the methicillin resistance [22] and codify the production of a penicillin-binding protein (PBP) with low affinity to beta-lactams antibiotics, such penicillin, cephalosporins, and carbapenems [20, 23].

2.2 Laboratory detection of MRSA

Phenotypic tests for laboratory identification of *Staphylococcus* species are relatively simple, with the employment of the catalase and coagulase tests, both positive. However, definitive confirmation requires the employment of additional tests or the Matrix Assisted Laser Desorption Ionization - Time Of Flight Mass Spectrometry (MALDI-TOF) [24], since both *S. aureus* and *S. pseudointermedius* (in addition to other species of staphylococci, such as *S. lugdunensis*) are coagulase positive. The detection of *mec*A and *mec*C genes by polymerase chain reaction (PCR) is also a complementary alternative for the correct identification of methicillin resistant species [25]. Alternatively, phenotypic tests to confirm methicillin resistance are often performed because they have low cost and reliable results. In this context, the behavior of the bacteria is evaluated by disk-diffusion on Mueller Hinton agar with 30 μg Cefoxitin disk for *S. aureus* (MRSA) [24]. The test consists of preparing a bacterial suspension in sterile 0.9% NaCl with density equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL). Next, a cotton swab is soaked in the freshly prepared solution and is seeded on the Mueller Hinton agar surface. After application of the antimicrobial disks and appropriate incubation (35°C/24 hours), the behavior against the antibiotics is verified according to the measurements of the inhibition halos formed around the tested disks [21], and it is interpreted according to the current reference guidelines used in each health service.

2.3 MRSA colonization and MRSA infection

Historically MRSA was described in humans in 1961 [26], while MRSA colonization and infection in animals was first reported in 1972 in asymptomatic dogs in Nigeria and a case of bovine mastitis in Belgium [23]. Around 25–30% of the human population is asymptomatically colonized by *S. aureus* in their nostrils [22, 27]. Humans and animals with nasal colonization by *S. aureus* and MRSA are considered to be at higher risk for developing infections and transmission of bacteria and, since colonization usually precedes infection [26]. In this sense, there is a great public health concern because domestic animals are potential reservoirs of these pathogens, with subsequent transmission to humans. The

colonization of people in contact with colonized animals has been described. In addition, it has been shown that transmission can occur from animal to human as well as from human to animal [20]. The epidemiological success of *S. aureus*-related pathogens depends not only on its ability to produce virulence factors but also on its *fitness*, that is, its ability to grow and persist in its hosts, promoting colonization [28].

It is now well established that MRSA isolates are often non-susceptible to different classes of antibiotics and are considered multidrug-resistant (MDR) when resistance is observed for at least three different classes of antimicrobials [25]. The great adaptability of this pathogen is due to its expressive genetic plasticity, in which approximately 25% of the *S. aureus* chromosome consists of mobile genetic elements, such as chromosomal cassettes, transposons, plasmids, and bacteriophages, which can be acquired through horizontal transfer [29].

When human MRSA infections persist, worsen, or recur despite surgical treatment, additional use of systemic antibiotic therapy is required [27]. Different clinical treatment options are available to combat MRSA infections, including vancomycin. Although this drug is the main therapeutic option, there are several limitations in its use, such as the achievement of optimal serum concentration, long-term treatment, renal toxicity, and restricted route of administration (intravenous) [30]. In the veterinary field, there is no effective therapy to treat MRSA infections, so prevention and control measures are critical to contain the further spread of MRSA [21]. While this challenge remains unresolved, successful treatment of infections may require the development of new antibiotics and the use of bacteriophages and phage-derived lytic proteins [29] as alternative therapeutic resources.

2.4 Bacteriophages as anti-MRSA agents

With the emergence of MRSA, staphylococcal infections have become difficult to control. MRSA is typically resistant to beta-lactams and can even present resistance to other antimicrobials [20], thus requiring new therapeutic alternatives. In this sense, phage therapy resurfaces as a promising tool for the control of unwanted bacteria, since it consists of the use of viruses, called bacteriophages, capable of infecting and killing prokaryotes without harming human or animal cells.

2.4.1 What are bacteriophages?

Bacteriophages, also known as phages, are viruses that infect and lyse prokaryotes. They are considered the most numerous infectious entities on the planet, being found in different environmental matrices, such as sewage, water, soil, among others [31]. Phages have been proposed as an alternative resource to the problem of resistant bacteria since they infect bacterial cells and, at the end of their reproduction cycle, promote the lysis of the host bacterium [18, 32]. After their discovery in 1917, phages were successfully used for the treatment of several bacterial infections [31]. However, the advent of antibiotics and their industrial-scale production, coupled with the lack of adequate studies and the poor understanding of phage biology at the time, resulted in the abandonment of studies related to these viruses as therapeutic agents in most institutions. A few places followed up on these studies, such as Eastern Europe, mainly Russia, Georgia, and Poland. Truly, the production and use of phages for prophylaxis and therapy never stopped in the last two countries mentioned [33]. From these countries emerged the main research in the phage therapy field.

Subsequently, the indiscriminate use of antibiotics enabled progressive bacterial resistance, leading to the resumption of studies with phages. Thus, bacteriophages and their products, such as enzymes released at the end of their replication cycle, were once again considered as therapeutic agents [32]. Phage therapy is the use of bacteriophages to eliminate bacterial pathogens, and fortunately, innovative research techniques have made several advances in the field possible. One of the most important discoveries has been the distinction between the replication cycles carried out by phages. The replication of these viruses occurs mainly through two cycles: the lysogenic and the lytic.

2.4.2 Phage replication: Lytic and lysogenic cycles

Frequently, *S. aureus* displays prophages inserted into its DNA and this viral genetic material contributes to bacterial adaptability once it encodes virulence and fitness factors [34]. Although most phages that infect *S. aureus* are temperate, i.e. lysogenic, some of them are strictly lytic and present potential for use as anti-staphylococcal agents. According to the International Committee on Taxonomy of Viruses (ICTV), phages with DNA genetic material belong to the order *Caudovirales* which comprises nine different families: *Siphoviridae, Myoviridae, Podoviridae, Herelleviridae, Drexlerviridae, Demerecviridae, Chaseviridae, Autographiviridae* and *Ackermannviridae* [35]. The phages described so far capable of infecting *S. aureus* belong to the first three families, of which *Myoviridae* and *Podoviridae* involve *S. aureus* phages whose cycle is exclusively lytic [36]. Phages from these families are characterized by having an icosahedral capsid, where the genetic material is located, and are differentiated by the type of tail they have, which can be long and flexible (*Siphoviridae*), long and retractable (*Myoviridae*) or short (*Podoviridae*) [18].

Regardless of the type of cycle (lytic or lysogenic) performed by the bacteriophage, the replication process will begin by the adsorption of the virus to receptors on the surface of the host cell wall. During the infection of Gram-positive bacteria, as is the case of *Staphylococcus* spp., proteins present in the fibers of the viral tail interact with the teichoic acids of the cell wall, and the teichoic acids found in *S. aureus* are distinct from those observed in other *Staphylococcus,* thus allowing the specific binding of the phage [37]. The absence of this receptor in the bacteria renders the phage unable to bind and start its replication cycle, giving the virus the characteristic of being host specific. After the irreversible binding of the phage to the bacterial proteins, the bacterial cell wall undergoes the action of enzymes associated with the phage tail tip complex, forming a pore in the bacterial wall through which the genetic material of the virus is ejected into the cell. In *Staphylococcus* phages of the *Myoviridae* family the ejection of the viral DNA is facilitated by the contraction of the tail sheath [38]; in *S. aureus Siphoviridae* phages occurs the action of enzymes associated with the phage tail tip complex [39] and in *S. aureus Podoviridae* phages are the putative cell wall-degrading enzymes located in the tail spike [40]. Once the viral DNA is inside the host, either the lytic or the lysogenic cycle will be performed according to the characteristics of the phage.

The lysogenic cycle is characterized by phages that are able to infect and integrate their genetic material into the DNA of the bacteria, thus forming a prophage. The ability to integrate its genetic material with the bacteria is due to the presence of genes that encode the integrase protein, an enzyme that mediates the recombination between the phage's DNA and that of the host [41]. Subsequently, proteins are produced that induce viral latency, implying a pause in the transcription of gene products, allowing the prophage to exist with the bacteria for several bacterial

generations without major consequences. Furthermore, the prophage induces immunity in the bacteria against infection via new phages. Bacteriophages that exhibit this type of replication cycle are not suitable in the context of phage therapy, since at the end of the viral cycle the death of the bacteria will not necessarily occur. In addition, bacteriophages that perform the lysogenic cycle may be responsible for producing toxic substances and carrying resistance genes [32], implying benefits for the bacteria.

On the other hand, in the lytic cycle there is no integration of the phage genetic material to the prokaryote DNA. At the end of this viral replication cycle, when the new virions are already formed and ready to be released, there is the production of enzymes capable of lysing the bacteria cell wall, inducing bacterial rupture and death for the release of new virions. Therefore, phages whose replication cycle is lytic are the most suitable for use in phage therapy, precisely because they cause bacterial lysis [18]. The schematic representation of the lytic and lysogenic cycles in *S. aureus* is shown below (e.g., **Figure 1**).

2.4.3 History of phage therapy in S. aureus infections

The attempt to use phages for the treatment of infections caused by *S. aureus* began soon after the discovery of phage therapy, and it is likely that the first use was in six patients with skin diseases in 1921. After the discovery of antibiotics, the studies related to phage therapy were abandoned and the few that continued, conducted in Georgia, Russia, and Poland, included efforts to treat staphylococcal infections [31]. Although the main studies target the use of phage therapy in humans, phages have also been proposed for use in veterinary medicine. The first case of application of this therapy in animals was associated with d'Herelle, one of those responsible for the discovery of phages. In 1919, he used the viruses to contain an outbreak of lethal typhoid fever in chickens. After analyzing several dead animals, d'Herelle was able to identify *Salmonella* Gallinarum and after isolated a lytic bacteriophage for the bacterium in question [42]. In another study, *S. aureus* phages were tested in mice, but the results were unsatisfactory because the virus used was not able to protect against a lethal dose of the bacteria [42].

Studies with phages for the control of staphylococcal infections were continued in some regions of the world. In the United States (1952), a laboratory (Delmont Laboratories) licensed, for human use, a bacterial lysate produced from the

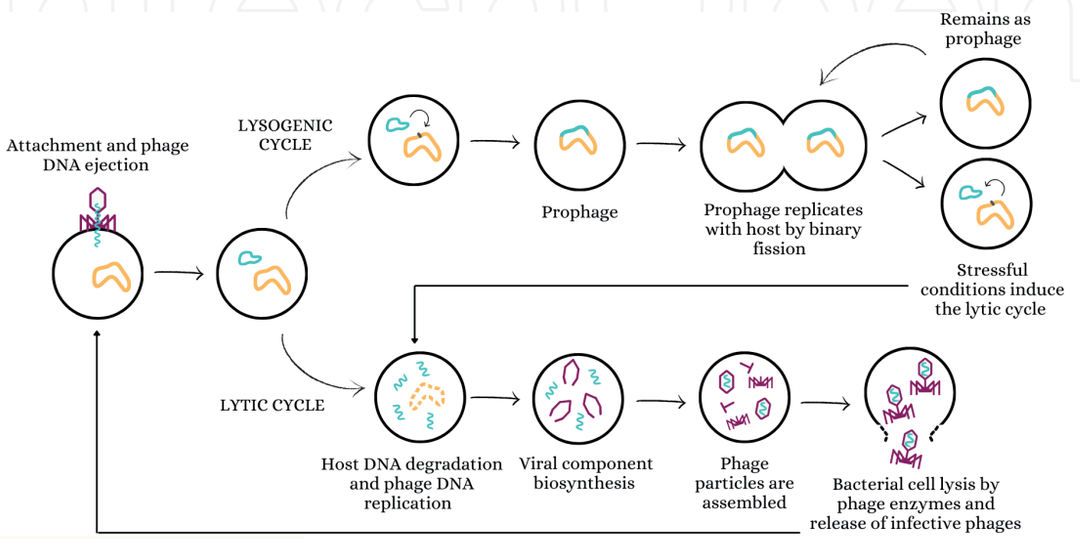

Figure 1.
Lytic and lysogenic cycles.

infection of bacteriophages in two virulent strains of *S. aureus.* Several years later, in 1986, the same product was licensed for veterinary use for the treatment of recurrent canine pyoderma but is no longer marketed for human use. This lysate, whose commercial name is "Staphage Lysate SPL", consists of bacterial cell wall fragments, intracellular components released during bacterial lysis, culture media ingredients, and viable bacteriophages. In 1981, it was demonstrated to be able to protect 80–100% of infected mice compared to the group not treated with SPL [43]. In dogs, SPL has been used effectively to treat chronic staphylococcal blepharitis as well, where weekly injections were administered to control the disease without adverse effects on the animals [44].

Because of the resistance of *S. aureus* to antimicrobials, some studies have sought to evaluate the activity of phages and their products against MRSA isolates. In 2008, one study evaluated the potential use of phages to eliminate or reduce nasal colonization by *S. aureus*, concluding that decolonization may be beneficial for certain patient groups, and phages were able to effectively combat induced infections in animal experiments [45]. A recent review concluded that phages are effective as topical antimicrobials against *S. aureus*, being able to combat MRSA in skin infections regardless of whether they are used with or without combination to topical antibiotics [46]. In addition to the phage itself being used as an antimicrobial agent, its products, such as lytic enzymes (endolysins), are also the subject of investigation. Phages and their products can be administered orally, inhaled, intravenously, subcutaneously, and topically, as suspensions for ocular use or application to bacteria-infected burns. The use of bacteriophages in therapeutics has advantages, mainly the high viral specificity that allows them to bind only to bacterial cells with the specific receptors, not affecting human or animal cells, thus avoiding significant side effects. Furthermore, phages can be used in the control of bacteria that show resistance to antibiotics [32]. Additionally, these viruses can adapt to the resistance mechanisms developed by bacteria, evolving in parallel to their host.

2.4.4 Commercial phage products anti-staphylococcal

Commercial products containing phages or enzymes produced by them are manufactured and available in some countries, mainly in Russia and Georgia, but also in Canada, the Netherlands and the Czech Republic. The following table (e.g., **Table 1**) gathers different commercial phage products, the target bacteria of each product, their main uses and the manufacturer [47–50].

In recent years, different studies involving commercial phage products with anti-staphylococcal activity have been undertaken. Most of them were related to *S. aureus Myoviridae* phages and demonstrated very promising results. Among them, it was shown that 100% (10/10) of multidrug resistant *S. aureus* isolates were lysed by Fersisi phage cocktail; 90% (9/10) were lysed by Instesti bacteriophage and 80% (8/10) by Pyo phage cocktail, showcasing the high lytic activity of commercial phage cocktails of Eliava BioPreparations, Georgia [51]. Similarly, 95% of clinical isolates of staphylococci, including 3 MRSA and 17 Methicillin susceptible *S. aureus* (MSSA) were sensitive to the action of Pyofag® polyvalent bacteriophage (Pharmex Group LLC, Ukraine for NeoProbioCare Inc.) Moreover, the same commercial phage cocktail was able to control furuncles in a patient with skin lesions by topical application of Pyofag®, as well as orally and nasally, for 14 days [52].

Some commercial products with the same name, but produced by different manufacturers, are proposed for the control of *S. aureus* in skin and wound infections, including Pyophage (polyvalent purified) cocktails from Microgen (Russia) and Pyophage from Eliava BioPreparations (Georgia). One study evaluated the performance of both cocktails against 20 MSSA and 31 MRSA clinical isolates

Product name	Active against	Informations/use	Manufacturer
Complex Pyo bacteriophage	*Staphylococcus, Streptococcus, Enterococcus, P. mirabilis, P. vulgaris, K. pneumoniae, K. oxytoca, P. aeruginosa,* enteropathogenic *E. coli*	Mix of sterile lysate phages. Used for the treatment of diseases of the eyes/ear/ nose, throat, infections of respiratory tract, lungs, surgical sites, urogenital, enteric, septic diseases. operational and newly infected wounds, for the prevention of hospital-acquired infections.	Microgen (Russia)
Fersisi bacteriophage	*S. aureus, S. epidermidis, S. pyogenes, S. sanguis, S. salivarius, S. agalactiae*	Sterile filtrate of phage lysates. Used for the treatment of otolaryngological diseases; infections of skin, urogenital, gynecologic, enteric, pyo-inflammatory disease in children (including newborns).	Eliava BioPreparations (Georgia)
Gladskin Acne, Gladskin Eczema, Gladskin Rosacea, Gladskin Shaving Irritation	*S. aureus,* Metichillin Resistant *S. aureus* (MRSA)	Endolysin XZ.700. Used for the treatment of skin disorders (acne, eczema, rosacea, psoriasis).	Micreos (Netherlands)
Intesti bacteriophage	*S. flexneri* serotypes 1,2,3,4, *S.* Paratyphi A and B, *E. coli*, *S.* Typhimurium, *S. enteritidis, P. vulgaris, S.* Cholerasuis, *S. sonnei, S.* Oranienburg, *P. mirabilis*	Mix of sterile filtrates of phage lysates. Used for the treatment of enteric infections.	Eliava BioPreparations (Georgia)
Intesti-bacteriophage	*S. flexneri* serotypes 1,2,3,4,6, *S. sonnei*, *S.* Paratyphi A and B, *S.* Typhymurium, *S.* Cholerasuis, *E. coli*, *S.* Oranienburg, *S. enteritidis, P. vulgaris, P. mirabilis, Enterococcus, Staphylococcus, P. aeruginosa*	Mixture of sterile filtrates of phage lysates. Used for the treatment of bacterial dysentery, dyspepsia, disbacteriosis, enterocolitis, colitis, salmonellosis.	Microgen (Russia)
Pyophage	*S. aureus, S. pyogenes, S. sanguis, S. salivarius, S. agalactiae, E. coli, P. aeruginosa, P. mirabilis, P. vulgaris*	Mix of sterile lysate phages. Used for the treatment of infections of upper respiratory tract, dermatological, surgical site, ocular urogenital, gastrointestinal, purulent septic infections in children, for prevention of post- operational complications and hospital infections.	Eliava BioPreparations (Georgia)

Product name	Active against	Informations/use	Manufacturer
Pyofag® polyvalent bacteriophage	*S. pyogenes, S. aureus, E. coli, P. aeruginosa, P. vulgaris, P. mirabilis*	Solution in vial with bacteriophages. Used for the treatment of pyoinflammatory diseases of ears, throat, nose, oral cavity, eyes, surgical infections, burn wounds; urogenital, gynecologic, and enteric infections.	Pharmex Group LLC (Ukraine) for NeoProbioCare Inc. (Canada)
Stafal ®	*S. aureus,* MRSA, including biofilms	Polyvalent bacteriophages of the family *Myoviridae* and genus *Kayvirus.*	Bohemia Pharmaceuticals (Czech Republic)
Staphefekt ™	*S. aureus* and MRSA	Endolysin XZ.700. Used for treatment of inflammatory skin conditions such as eczema, acne, rosacea, psoriasis.	Micreos (Netherlands)

Table 1.
Commercially available anti-S. aureus phage products.

and concluded that both products had greater than 75% coverage, but Microgen's Pyophage was extremely effective against MRSA, killing 97% of the bacterial isolates. Genomic analyses of the *S. aureus* phages contained in these commercial products revealed great similarities (*Myoviridae, Kyavirus* genus), however Microgen's cocktail additionally featured a *S. aureus Podoviridae* component that possibly contributed to the higher coverage observed against MRSA [53].

In a recent study, the action of Stafal® (a preparation with polyvalent bacteriophages active *on S. aureus)* on planktonic cells as well as on biofilms produced by MSSA and MRSA was demonstrated. Bacterial cells immersed in the biofilm required high phage concentrations and longer exposure time to be destroyed compared to planktonic forms [54]. It is likely that this occurred because of the difficulty of the phage to access the host cell surface within the biofilm matrix. Still, the phages were active on the biofilms, whereas antimicrobials are known to be ineffective due to the limitation of their diffusion through the extracellular polymeric substances matrix. Similarly, enzymes encoded by bacteriophages called endolysins have shown promising advances against bacterial biofilm formation. Such enzymes are responsible for lysis of the host bacterial cell wall promoting the release of viral progeny at the end of the replication cycle of lytic phages [55]. Experimental assays showed that the phage-derived lysine named "LysH5" was able to remove *S. aureus* biofilm, even eliminating persistent cells (subpopulation of cells that showed high resistance to antibiotics). During treatment of staphylococcal biofilm with LysH5 (0.15 μM), complete inhibition in biofilm formation was also seen in certain *S. aureus* isolates [56].

Commercially, the recombinant endolysins Staphefekt SA.100 and Staphefekt XDR.300 (Micreos Human Health BV, Netherlands) which act on *S. aureus* (including MRSA) are available for use. A few clinical studies have been conducted with Staphefekt SA.100 and all have demonstrated remission and/or improvement of chronic *S. aureus* skin infections (folliculitis, rosacea, and eczema) [57, 58], reinforcing the utility of this therapeutic resource. Moreover, it is believed that endolysins may be better therapeutic alternatives than bacteriophages themselves since bacteria have the possibility to develop resistance to the phage. On the other

hand, it is necessary to consider that endolysins present limitations, such as: i) induction of inflammatory response of cytokines and neutralizing antibodies that imply the reduction of the half-life time (*in vivo*); ii) their systematic use *in vivo* will provoke an immune response that will promote the loss of the lytic activity of the enzyme [59]; iii) lower activity on Gram-negative bacteria due to the presence of the external membrane in the cell wall [60].

Other commercially available products are: Bronchophage, Otophage, Phagodent, Phagoderm, Phagogyn, Phagovet, Vetagyn (Micromir, Russia); ENKO bacteriophage, SES Bacteriophage, Staphylococcal bacteriophage (Eliava BioPreparations, Georgia); Dysentery bacteriophage, *E. coli* bacteriophage, *E. coli-Proteus* bacteriophage, *Klebsiella* purified polyvalent bacteriophage, Sextaphag® polyvalent pyo bacteriophage, *Streptococcus* bacteriophage (Microgen, Russia); Phagestaph, Phagyo, Septaphage (Biochimpharm, Georgia); and Intestifag® polyvalent bacteriophage (Pharmex Group LLC, Ukraine for NeoProbioCare Inc., Canada). Detailed information can be found in related sources [47–50].

2.4.5 Non-commercial anti-S. aureus bacteriophages

Fortunately, since the year 2000, different studies have contributed to a better understanding of phages as anti-*S. aureus* therapeutic agents. For example, the efficacy of the bacteriophage named ØMR11 against a lethal infection caused by *S. aureus* in mice was evaluated. Initially, the phage was isolated, had its bacteriolytic activity determined, and finally, *in vivo* infection experiments were performed by introducing *S. aureus* intraperitoneally, including MRSA strains, causing bacteremia and eventual death of the mice. After peritoneal administration of the isolated phage in infected animals, suppression of *S. aureus*-induced lethality occurred [61]. Similarly, the use of cloned lysins encoded by the phage ØMR11 was efficient in cell lysis, including MRSA. These lysins are enzymes produced at the end of the replication cycle of bacteriophages and are responsible for degrading the bacterial wall and releasing virions. After sequencing the phage ØMR11 the possible genes related to lysins were identified, these were cloned, and their protein products were purified on a large scale. The results showed high activity of lysins against MRSA isolates both in mice contaminated intranasally and subsequently treated with the intranasal lysins, as well in animals infected intraperitoneally, showing that the enzyme can be used for the control of *S. aureus* in humans and domestic animals [62].

A cocktail containing two bacteriophages, designated K and 44AHJD, was tested against clinical isolates of *S. aureus*, showing 85% of lytic action on the bacteria. The *in vivo* efficacy of the cocktail was evaluated through the murine nasal colonization model. Efficient decolonization was verified after eight days of intranasal administration in animals treated with the phage cocktail, while the control group (received only the bacteria) and the group treated with placebo remained colonized [63]. Although different studies have already demonstrated the efficiency of phages on *S. aureus*, few clinical trials have been conducted to validate their efficacy and safety. According to the records of clinical trials involving *S. aureus* and bacteriophages, in progress or already concluded [64] it appears that they are scarce and that few countries, mainly the U.S., have invested in clinical trials that corroborate the use of phages in clinical practice (e.g., **Figure 2**). The lack of large clinical studies that can effectively consolidate the use of phages *in vivo* is an obstacle to be overcome.

The Clinical Trials platform, a database of clinical studies conducted worldwide, reports the existence of eight studies related to the use of bacteriophages against *S. aureus* [64]. These are intended for the use of viruses for the treatment of ulcers infected by *S. aureus* in diabetic patients, prevention, and treatment of infection by *S. aureus* and other bacteria in burn patients, use for patients with covid-19

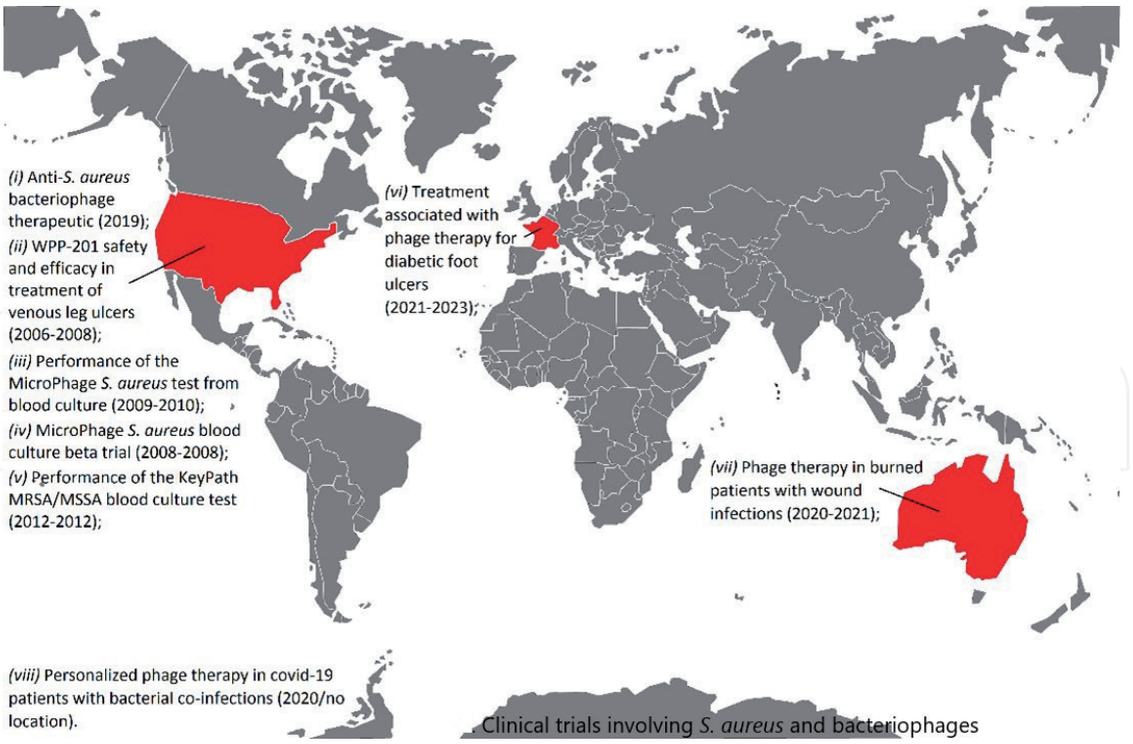

Figure 2.
Clinical trials involving S. aureus *and bacteriophages. Available at: www.pngwing.com*

affected with pneumonia or bacteremia/septicemia due *S. aureus* infection, use in patients with serious or immediate risk of life, and patients with venous leg ulcers. In addition, three studies that use phages as a diagnostic method. When considering regulatory measures for the application of phages as therapeutic agents, it is likely that, initially, such viruses are more easily used prophylactically in order to reduce the frequency of infections. In contrast, phage therapy aimed to eradicate systemic bacterial infections will inevitably be more complex [65].

2.4.6 Advantages and challenges of phage therapy

Among the principal attractive aspects of phage therapy, the main ones are: i) high specificity of the virus for the bacteria providing freedom from side effects on cells that are not targeted by the therapy; ii) activity against different bacteria, including multidrug resistant bacteria; iii) reduced treatment costs compared to antibiotic therapy; iv) prevention to the growth of secondary pathogens; v) ability to degrade bacterial biofilm by lysing bacteria; vi) high body distribution and vii) high efficacy compared to antimicrobials [32]. On the other hand, there are some limitations to the use of phages in therapy, among them: i) the possibility of antibody production by the immune system; ii) the difficulty of measuring the application dose; iii) the possibility of gene transfer among pathogens through phages, which may be responsible for passing pathogenic determinants and virulence factors, resulting in a possible resistance of bacteria; iv) the ability of bacteria to develop resistance against bacteriophages; v) elucidation of the correct route of administration and treatment time and vi) accurate and rapid diagnosis of the microorganism that is provoking the illness [32].

Fortunately, for all the limitations previously indicated, there are already studies that aim to circumvent these problems. For example, viral genome sequencing avoids the use of phages that are lysogenic or contain toxic and resistant genes. Along with this is the progressive search for new phages to be used if antibodies are produced by the immune system, or to replace phages for which the bacteria have become resistant. In addition, it is already known that viruses can mutate and adapt to resistance

mechanisms created by bacteria. In other words, after the creation of barriers that make it impossible for the phage to replicate in the bacteria, changes occur in the viruses that allow their replication cycle to continue, even with the presence of the bacterial adaptations [32]. Further in this context, the use of new diagnostic resources allows the rapid differentiation of the disease-causing bacteria, in addition to the use of cocktails with different phages for the same bacterium, enhancing even more the specificity and avoiding the manifestation of resistance [32, 66].

3. Conclusions

MRSA represents a global threat due to its progressive resistance to antimicrobials, as well as the future prospect of no effective antibiotics. The use of lytic bacteriophages and their by-products are promising alternatives for bacterial control, since they infect and lyse the pathogen without the inconvenience of side effects, as well as contributing to lower consumption of antimicrobials, reflecting in the reduction of antibiotic resistance rates. The study of phages has always occurred in countries such as Georgia and Russia, where phage-based commercial products are relevant antibacterial alternatives. Although different *in vivo* studies have already evidenced the efficacy of phage therapy in prophylaxis and treatment of staphylococcal infections, including those caused by MRSA, some aspects should be considered before its clinical use. Among them, the restriction and scarcity of clinical trials along with the lack of robust randomized clinical trials evaluating the safety and efficacy of phage therapy are important limitations for the therapeutic use of these viruses. We highlight the need to foster studies in the area of phage therapy, especially given the scenario of increasing multi-resistant bacteria worldwide and the scarcity of new antimicrobial drugs.

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