Trends in Microbiology

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Review

The phages of staphylococci: critical catalysts in health and disease

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The phages that infect *Staphylococcus* species are dominant residents of the skin microbiome that play critical roles in health and disease. While temperate phages, which can integrate into the host genome, have the potential to promote staphylococcal pathogenesis, the strictly lytic variety are powerful antimicrobials that are being exploited for therapeutic applications. This article reviews recent insights into the diversity of staphylococcal phages and newly described mechanisms by which they influence host pathogenicity. The latest efforts to harness these viruses to eradicate staphylococcal infections are also highlighted. Decades of research has focused on the temperate phages of *Staphylococcus aureus* as model systems, thus underscoring the need to broaden basic research efforts to include diverse phages that infect other clinically relevant *Staphylococcus* species.

Highlights

The phages of staphylococci have recently been identified as major constituents of the skin microbiome.

Detailed genomics analyses have shed light on the remarkable proteomic diversity of staphylococcal phages.

Recent studies reveal new mechanisms by which temperate *Staphylococcus aureus* phages cooperate with the host to enhance fitness/virulence and promote their mutual survival in a changing environment.

Emerging case studies and clinical trials demonstrate the utility of lytic staphylococcal phages in eradicating *S. aureus* infections.

Cutting-edge technologies and alternative approaches are being used to overcome some of the challenges associated with the routine application of therapeutic phages as antistaphylococcal agents.

Phages impact multiple domains of life

The viruses of prokaryotes, collectively known as phages, have profound impacts on the ecosystems within, on, and around us. As the most abundant entities on the planet [1], they reside in diverse environments alongside their prokaryotic hosts and can outnumber them by an order of magnitude or more [2]. Phages are major causes of microbial mortality, key conduits of gene exchange, and critical catalysts of prokaryotic evolution (Box 1 and Figure 1). Thus, their influence prevails upon life on Earth at multiple scales, driving a range of environmental and biological processes, such as global biogeochemical cycles [3] and climate change [4], as well as human health and disease [5]. Beyond these impacts in natural systems, phage research has historically provided a fertile ground for discovery and innovation. Indeed, a handful of phages that infect *Escherichia coli* were used as early model systems to lay the foundations of modern molecular biology [6–8]. Moreover, early insights into phage–host dynamics have enabled the development of transformative technologies, such as restriction-enzyme-facilitated assembly of recombinant DNA [9–11] and the directed evolution of peptides and antibodies using phage display [12]. More recently, the discovery of adaptive antiphage immunity, mediated by clusters of regularly-interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (*cas*) genes [13,14], formed the basis of a revolutionary genome-editing technology which has accelerated the pace of genetics research and opened new frontiers in agriculture and medicine [15–17].

Presently, phages continue to inspire broad interest, particularly those that infect bacterial pathogens, as, in addition to controlling pathogen survival and evolution in their natural environments, such phages provide powerful weapons for the treatment of antibiotic-resistant infections. This article focuses on a representative group – the phages of staphylococci. *Staphylococcus* species are ubiquitous residents of human skin that play critical roles in health and disease (Box 2), and recent research has revealed that staphylococcal phages are also dominant constituents of the skin microbiome [18]. The sections that follow review the latest insights into staphylococcal phage diversity and newly discovered mechanisms by which they regulate fitness and virulence of the host. Recent efforts to harness a subset of these viruses for therapeutic applications are

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Box 1. Impacts of phages on microbial mortality and evolution

Phages are parasites that rely upon prokaryotic hosts to facilitate their own reproduction – they attach to a specific host, inject their genetic material, and exploit the host's enzymes and energy stores to replicate in a process that typically leads to cell lysis and death. The majority of known phages follow two life cycle pathways – lytic and lysogenic (see Figure 1A in main text). While strictly lytic phages replicate immediately upon infection, the lysogenic (i.e., temperate) variety may choose to integrate into the host's chromosome and persist as a prophage indefinitely, until a stressor or other environmental cue triggers its excision and replication. With an estimated 10^{31} phage particles on the planet [1], phages abound in terrestrial and marine environments, and have even been found in the atmosphere, riding upon dust particles and debris [81]. It is estimated that in the oceans alone, 10^{23} phage infections occur every second, which collectively cause the removal and turnover of 20–40% of all marine prokaryotes each day [3]. Thus, phage predation imposes a tremendous selective pressure upon their hosts, and in response, prokaryotes have evolved a variety of mechanisms to survive this perpetual threat. These include passive mechanisms that involve the acquisition of random mutations that lead to phage resistance, and active mechanisms provided by innate and adaptive immune systems, such as restriction–modification and CRISPR-Cas, respectively. Only recently has research begun to shed light on the remarkable abundance and diversity of such prokaryotic immune systems [80].

In addition to propagating their own genetic material, phages have the potential to package and spread nonphage DNA via various mechanisms of transduction (see Figure 1B in main text). The best characterized of these are generalized and specialized transduction. During generalized transduction, phages package DNA from plasmids and/or the bacterial genome bearing sequences homologous to phage packaging sites and deliver these to the next host. During specialized transduction, inaccurate excision of the prophage genome leads to packaging and delivery of chromosomal genes contiguous with phage DNA. Notably, new mechanisms of transduction have recently come to light using *S. aureus* and its phages as a model host–virus system (refer to main text for more details).

also highlighted. The goal of this review is to offer a holistic perspective of the complex relationships between staphylococcal phages and their hosts and bring to light key areas that require further investigation.

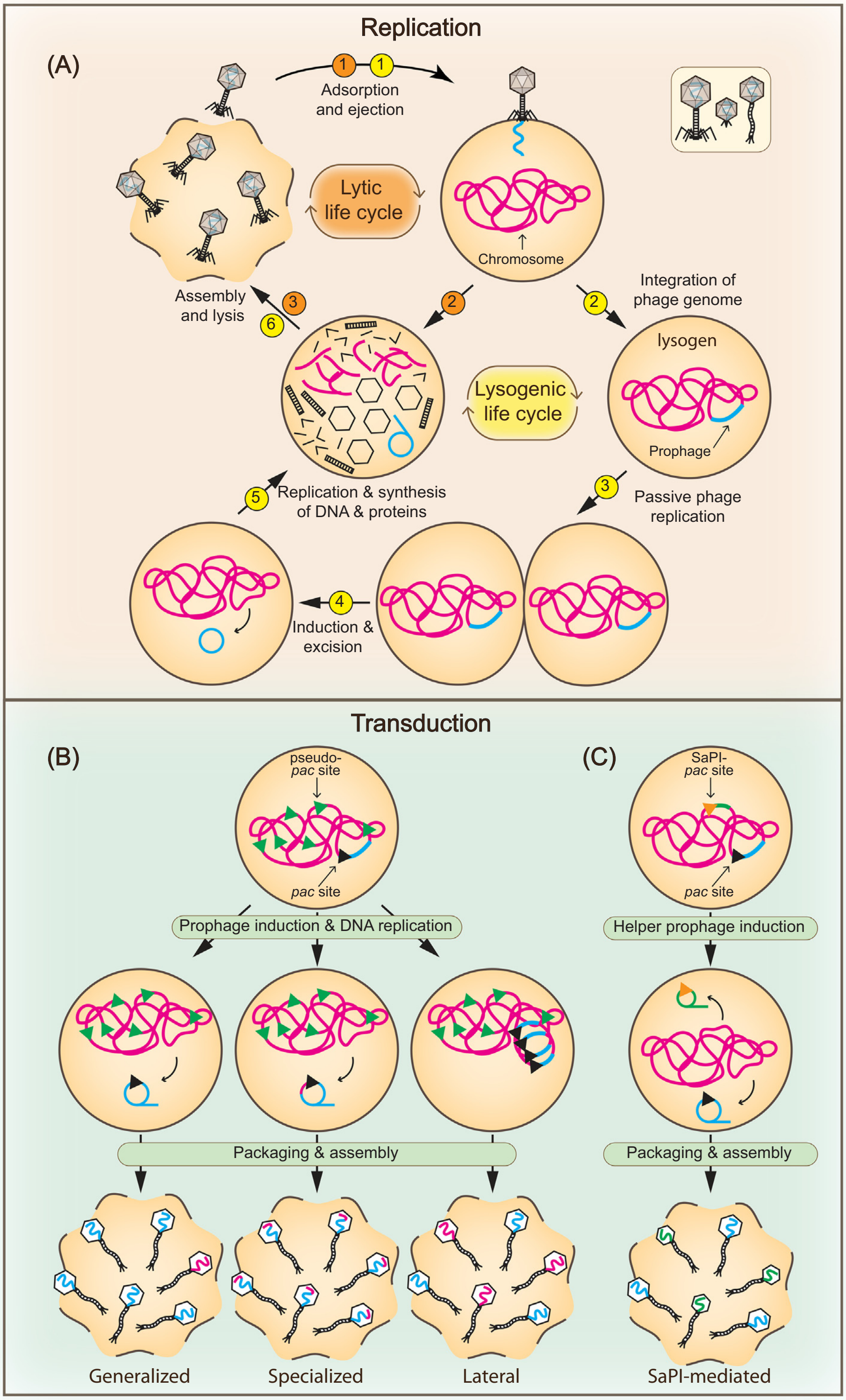
Staphylococcal phage diversity

Since the discovery of phages over a century ago [19,20], the staphylococcal phage collection has steadily grown to over 200 sequenced isolates and countless examples integrated within sequenced staphylococcal genomes. All known staphylococcal phages have icosahedral capsids (i.e., heads), tails of varying proportions, and genomes of double-stranded DNA. Thus, they are restricted to the Caudovirales order of tailed phages and have historically been placed within three families: Myoviridae, Siphoviridae, and Podoviridae [21]. As a recent update, the International Committee on Taxonomy of Viruses (ICTV, https://talk.ictvonline.org/taxonomy) has shifted Myoviridae members to a newly-defined family, Herelleviridae [22]. Members of these families exhibit myovirus, siphovirus, and podovirus morphologies (Figure 2). This relatively narrow phenotypic distribution is not surprising given that approximately 96% of the thousands of phages that have been imaged to date belong to these three categories [23]. In recent years, a mounting interest in resolving their structures at higher resolutions has led to remarkable functional insights that would otherwise be difficult to obtain using traditional genetic and biochemical analyses. For example, recent cryo-electron microscopy analyses of representatives from all three morphological classes have shed light on the complex choreography of conformational changes required to complete DNA ejection [24–26].

While early attempts to further classify staphylococcal phages have taken into account limited functional and genomic aspects [27], the recent advent of next-generation sequencing technologies has enabled more comprehensive classification based on total shared gene and protein content. Such analyses have revealed that members of each morphological group encapsulate genomes of discrete lengths, and phages within the same size class are more related to each other than with those in other size classes [28–30]. In addition, their genomes exhibit a modular organization in which genes that perform similar functions (e.g., DNA replication, integration, packaging, head, tail, and lysis) reside proximal to one another. However, within this organizational context, they

Glossary

Infective lytic particle: a phage particle packaged with a phage genome that is capable of lytic replication.
Lysogen: a prokaryotic cell harboring a prophage.
Lysogenic conversion: a change in the properties of a prokaryotic host caused by the integration of a lysogenic phage.
Lysogenic/temperate phage: a phage that has the capacity to choose between the lytic or lysogenic life cycle pathways.
Obligately lytic/virulent phage: a phage that reproduces strictly through the lytic life cycle pathway.
Prophage: the genome of a lysogenic phage that has been incorporated into the host chromosome or remains dormant as an episomal phage and has the ability to become an **infective lytic particle** if specifically activated (induced).
Prophage induction: the process by which a prophage is triggered to enter the lytic cycle pathway.
Transducing particle: a phage-like particle packaged with nonphage DNA.
Transduction: the horizontal transfer of genetic information mediated by a phage.



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Box 2. Prevalence and pathogenic potential of staphylococci

About 40 different *Staphylococcus* species have been identified [82], and of these, *S. aureus* and *S. epidermidis* have the highest pathogenic potential in humans. In clinical settings, staphylococci are distinguished by their ability to produce the enzyme coagulase and have historically been divided into two broad categories – coagulase-positive and coagulase-negative species (CoPS and CoNS, respectively). While *S. aureus* is a CoPS that colonizes approximately 30% of the human population and is restricted to the nasal cavity [83], *S. epidermidis* is a CoNS that colonizes 100% of humans and resides on all skin surfaces [18]. However, despite its relatively narrower distribution, *S. aureus* is a more aggressive pathogen and remains a leading cause of a variety of diseases, from mild skin and soft-tissue infections to severe, life-threatening infective endocarditis, bacteremia, and musculoskeletal infections [84]. Further, while nasal colonization is typically asymptomatic, it constitutes a major risk factor for recurring or more serious *S. aureus* infections. In contrast, *S. epidermidis* is largely considered a skin commensal that provides a variety of benefits, such as preventing colonization by *S. aureus* [85], producing antimicrobial peptides that target skin pathogens [86], and stimulating the human immune system to facilitate pathogen defense [87,88]. However, *S. epidermidis* is also an important opportunistic pathogen as it is a leading cause of sepsis in preterm infants [89] and remains the most common cause of infections associated with indwelling medical devices [90]. Importantly, pathogenic staphylococci that have evolved resistance to multiple antibiotics pose a major threat to global public health [91].

Here, it is worthwhile mentioning a critical distinction regarding the basis for their pathogenicity – while *S. epidermidis* pathogenesis relies upon factors that have evolved to promote survival within the skin environment, such as genes that facilitate adhesion and biofilm formation [90], *S. aureus* uses an arsenal of toxins and other virulence factors to promote its colonization, dissemination, persistence, and immune evasion [92]. Thus, while *S. epidermidis* pathogenicity has been aptly described as 'accidental' [90], *S. aureus* is considered a true pathogen. Nonetheless, *S. epidermidis* and its more benign CoNS cousins are not entirely without fault as they harbor a reservoir of antibiotic-resistance genes which can be transferred to *S. aureus* and other pathogenic species [93]. Importantly, these virulence and fitness factors are disseminated through a variety of mechanisms, and chief among these is via phage infection (refer to main text for more details).

also possess extensive mosaicism, wherein regions of high homology in related members are interrupted by small deletions or insertions containing one or a few unique open reading frames that are dedicated to similar tasks. This mosaic genetic architecture, observed as a static snapshot of select representatives, reveals the dynamic nature of the phage population in which related members are likely engaged in continual genetic exchange through various modes of recombination. The most comprehensive genomic analysis performed to date compared the sequences of 205 phages

Figure 1. Primary pathways of phage replication (top) and transduction (bottom). (A) The lytic and lysogenic pathways of phage replication are shown. The first step in both pathways involves the attachment of phage receptor-binding proteins to specific receptor(s) on the host cell surface and subsequent ejection of phage DNA (cyan). **Obligately lytic/virulent phages** are obliged to follow the lytic life cycle, in which phage DNA and proteins are replicated and synthesized, respectively (rolling-circle DNA replication is shown as an example). The DNA is packaged, phage particles are assembled, and the host cell is finally lysed to allow for phage escape. **Lysogenic/temperate phages** have the capacity to choose whether to enter into the lytic cycle or lysogenic cycle following DNA ejection. During the lysogenic cycle, the phage genome is integrated into the host chromosome (magenta), at which point the phage becomes a prophage, and the cell becomes a lysogen. The prophage genome is then replicated passively along with the host genome, until a stressor or other environmental signal triggers phage entry into the lytic cycle, a process known as induction. Inset shows the morphologies of the most commonly-encountered phages – myovirus (left), podovirus (center), and siphovirus (right). (B) The three major pathways of transduction are shown using a lysogenic staphylococcal phage as an example. Following **prophage induction**, the phage packaging (*pac*) site (black triangle) serves as a primary recognition sequence for the phage packaging machinery and enables the high-frequency production of infective lytic particles containing phage genomes (cyan). During generalized transduction (left), the occasional packaging of host-derived DNA bearing *pac* site homologs (pseudo-*pac* sites, green triangles) results in the infrequent production of transducing particles containing host-derived DNA (magenta). During specialized transduction (middle pathway), imprecise excision of the prophage genome leads to packaging of a DNA hybrid containing the phage genome along with host genes adjacent to the phage integration site. Lateral transduction (right) occurs in specific staphylococcal prophages in which DNA replication begins prior to excision – this process results in the high-frequency packaging of both phage DNA and host DNA encoded hundreds of kilobases from the prophage integration site. (C) The pathway for SaPI (*Staphylococcus aureus* pathogenicity island) transduction is shown. Upon induction of the helper prophage (cyan), both prophage and SaPI (green) excise and replicate. A SaPI-specific packaging site (orange triangle) directs the high-frequency packaging of SaPI DNA into transducing particles composed of phage structural proteins. Some SaPIs encode proteins that alter the capsid architecture, resulting in the production of transducing particles with unusually small capsids.

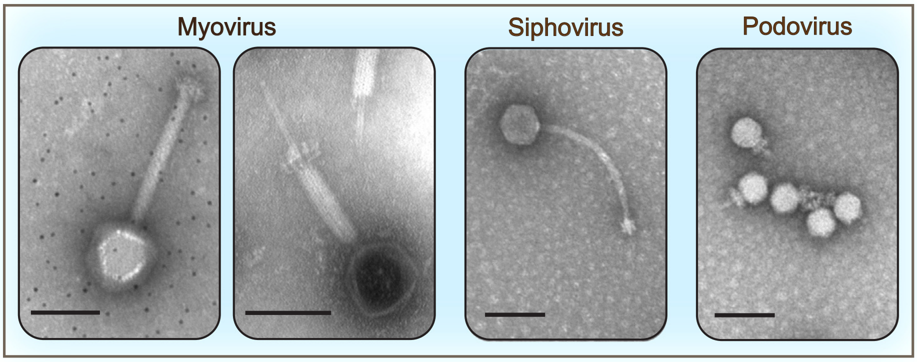

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Figure 2. Transmission electron microscope images of *Staphylococcus epidermidis* phages representing the morphological diversity of all known staphylococcal phages. Herelleviridae (formerly Myoviridae) members have myovirus morphology, with rigid, retractable tails with inner and outer sheathes. Siphoviridae phages have siphovirus morphology with flexible, noncontractile tails. Podoviridae members have podovirus morphology, with short, noncontractile tails. Phages were isolated from municipal wastewater in Tuscaloosa, AL, USA. Scale bar = 100 nm.

that infect 11 different *Staphylococcus* species, including nine non-*aureus* staphylococci [30]. Based on a shared gene content cutoff of 35%, these phages were grouped into four distinct clusters (A–D) plus an unrelated singleton (Table 1). This collection encodes 20 579 predicted proteins which can be divided into 2139 unique phage protein families, thus illustrating their extensive proteomic diversity [30]. However, the true diversity of the staphylococcal phage contingent has yet to be fully appreciated as the majority of isolates (~66%) have siphovirus morphology, while the podoviruses remain in the minority (~8%). Also, across all families, well over half of the phages were isolated using *S. aureus* as host, while phages specific to *S. epidermidis* and other non-*aureus* species remain severely under-represented.

Siphoviruses play pivotal roles in staphylococcal pathogenesis

Staphylococcal phages of the family Siphoviridae, particularly those within cluster B, have evolved a variety of mechanisms to conspire with the host to promote its fitness and pathogenicity. The majority of these phages encode integrase and repressor genes and are thus predicted, or experimentally demonstrated, to be lysogenic [30]. Further, their genomes harbor fitness/virulence factors which, when integrated, benefit the host. Staphylococcal **prophages** (see Glossary) can also cause host genome rearrangements that enhance pathogenicity [31], and following their entry into the lytic cycle, prophages have the potential to package and disseminate virulence/fitness factors encoded on nonphage DNA. Notably, as many as four

Table 1. Staphylococcal phage diversity and genomic classification[a]

Cluster (number of representatives)	Subclusters	Morphology	Genome length (kilobases)	Number of predicted genes	Genes with unknown functions (%)
A (16)	A1-A2	Podovirus	16–18	20–22	40
B (132)	B1-B17	Siphovirus	39.6–47.8	42–79	50
C (53)	C1-C6	Myovirus	127.2–151.6	164–249	60
D (3)	D1-D2	Siphovirus	89–93	129–142	65
SPβ-like (1)	N/A	Siphovirus	127.7	177	70

[a]Data are summarized from [30].

prophages can reside in a single staphylococcal strain [32], thus compounding these effects. Insights into these mechanisms have been gleaned through decades of research using *S. aureus* and its temperate phages as model organisms, and the most recent revelations are reviewed in the sections that follow.

Lysogenic conversion

Temperate phages insert into the host genome using phage-encoded integrases which recognize specific attachment sequences within the phage and bacterial genomes (*attP* and *attB*, respectively) and catalyze recombination between them. *attB* sites in staphylococci have been found in intergenic regions, within noncoding RNAs, and overlapping several protein-coding genes, including *geh*, *hlb*, and *comG* [30,33]. Although integration at these sites can cause a loss-of-function mutation through negative **lysogenic conversion**, the simultaneous addition of genes encoded within the prophage via positive lysogenic conversion often compensates for any potential loss in fitness or virulence of the host. As a classic example, *hlb* codes for β-hemolysin, a secreted membrane-damaging toxin that lyses red blood cells and promotes *S. aureus* pathogenesis [34], and although **lysogens** harboring a prophage insertion in *hlb* can no longer produce the enzyme, the introduction of other toxins encoded in the prophage (such as staphylokinase and enterotoxin A) results in a net increase in virulence [35].

Some staphylococcal prophages have evolved various mechanisms to coexist with the host without impinging on its fitness via negative lysogenic conversion. In addition to sporadically inserting into alternative integration sites [36], many staphylococcal prophages have recently been found to persist extrachromosomally, either stably as plasmidial phages, or transiently as episomal phages [37,38]. The reversible excision and reintegration of episomal prophages in the absence of lytic replication is termed ‘active lysogeny’, and has been proposed as a mechanism by which temperate phages can act as regulatory switches to control the expression of bacterial genes [39]. Indeed, a recent report showed that a member of the widely distributed family of *hlb*-converting phages can excise transiently to allow for β-hemolysin production when lysogens are grown under conditions that are relevant to *S. aureus* pathogenesis [40].

As many as 14 distinct protein families with virulence-associated motifs have been identified across cluster B members, and a single staphylococcal prophage can bestow up to five such virulence factors to the host through positive lysogenic conversion [30]. The most common and best-characterized of the prophage-encoded virulence factors include tissue-destroying toxins such as Panton–Valentine leucocidin and exfoliative toxin A, as well as members of the 'immune evasion complex', staphylococcal kinase, enterotoxin A, chemotaxis inhibitory protein (CHIP), and staphylococcal complement inhibitor (SCIN) [27]. These factors are typically encoded in a discrete module between the genes that facilitate lysis and integration, and accordingly, their expression appears to be linked with the lysis–lysogeny decision. Highlighting the collaborative nature of the relationship between these prophages and their hosts, phage-encoded toxin expression is also subject to control by host-encoded regulatory factors [27]. Although only ~55% of cluster B members encode identifiable virulence factors, about half of their collective genes have unknown functions, thus leaving room for the possibility that more may yet be discovered [30].

It is important to note that virulence determinants are not restricted to the Siphoviridae of cluster B. For example, a recent report identified a major virulence factor in prophage SPβ-like, a singleton with a ~127 kb genome [30,41]. The virulence gene, called *sasX*, encodes a surface-anchored protein that promotes bacterial aggregation and immune evasion, and its spread has been associated with a recent Asian MRSA (methicillin-resistant *S. aureus*) epidemic. In addition to residing within hospital-acquired MRSA strains, SPβ-like is also found in *S. epidermidis* RP62a and other coagulase-negative species (CoNS) [42], and thus plays a key role in moving SasX across the species barrier.

Transduction

Phage-mediated horizontal gene transfer, known as **transduction**, occurs through various mechanisms that rely upon the machinery which phages use to package their own DNA. Although, in theory, both lytic and temperate phages of staphylococci have the potential to facilitate transduction, the process has been demonstrated and studied in depth in only a handful of cluster B members. Staphylococcal prophages enter into the lytic cycle spontaneously at low frequencies and at higher rates in response to exogenously applied stressors, such as DNA-damaging agents or subinhibitory concentrations of antibiotics [27]. Following induction, the prophage genome is typically excised, circularized, and then replicated in a process which produces several contiguous copies of the phage genome (i.e., concatamers) linked in a head-to-tail orientation [43]. Genomes are then processed and packaged by terminases, motor proteins composed of small (TerS) and large (TerL) subunits which bind phage-specific packaging sites (*pac* or *cos*) and cleave concatemeric copies of the phage genome, respectively, while translocating genome monomers into preassembled capsids (termed procapsids) [44]. *pac*-type phages use a headful mechanism of packaging during which a single *pac* site initiates genome cleavage and packaging, and the process is terminated with a second cleavage event after the capsid is filled to capacity (slightly more than 100% of the genome). In contrast, *cos*-type phages require two *cos* sites, spaced one genome length apart, to initiate and terminate packaging, respectively. While phage-derived DNA is the primary target for packaging, nonphage DNA can also become packaged, resulting in the production of **transducing particles** alongside **infective lytic particles**.

For decades, all transduction was thought to occur via two well-described mechanisms – generalized and specialized transduction (Box 1); however, recent research has revealed a third mechanism known as lateral transduction which, thus far, has been demonstrated only in staphylococci (Figure 1B) [45]. Lateral transduction relies upon the unique temporal program of events that occur after induction in a subset of *pac*-type *S. aureus* prophages: rather than excising immediately after induction, these prophages exhibit a delayed excision which allows for some replication and packaging to take place while the phage remains integrated in the bacterial chromosome [45]. This results in the packaging of several headfuls of bacterial DNA spanning several hundred kilobases flanking the prophage integration site. While packaging is initiated from the phage-encoded *pac* site, subsequent headfuls are processed from the adjoining bacterial genome. The mechanism is coordinated such that it allows for the simultaneous production of both transducing particles and infective lytic particles at high titers. Thus, lateral transduction is a high-frequency event, occurring at least 1000 times more frequently than generalized transduction.

Although a relatively rarer event, generalized transduction has long been recognized as a major route through which pathogenic staphylococci acquire resistance to multiple antibiotics [46]. In addition to disseminating antibiotic-resistance genes through plasmid transfer, generalized transduction has more recently been implicated in the spread of some staphylococcal cassette chromosome *mec* (SCC*mec*) elements [47–50], genomic islands of 20–60 kilobases that harbor a variety of virulence/fitness factors, including the *mecA* or *mecC* genes which confer resistance to methicillin and other β-lactam antibiotics [51]. The traditional view is that generalized transduction arises as a consequence of errors during the phage packaging process; however, there is mounting evidence to support the new notion that transduction is a trait that has evolved in some temperate phages as a way to maximize the mutual survival of both phage and host populations. For example, a recent study showed that different antibiotics may influence the source of DNA that becomes packaged in the phage capsid [52]. In addition, other studies showed that, in mixed *S. aureus* populations in which only a fraction of cells carry a transducing phage, the lysogens can scavenge DNA from nonlysogen neighbors by maintaining low levels of phage induction [53,54]. This process, termed 'autotransduction', relies upon the assumption that lysogens carrying the same (or related) prophage(s) remain immune to reinfection by lytic particles and can thus benefit from the continuous supply of stolen DNA delivered by transducing particles.

Staphylococcal phages within cluster B also facilitate the spread of *S. aureus* pathogenicity islands (SaPIs), mobile genetic elements 15–18 kilobases in length that harbor a variety of virulence factors, such as TSST-1, the toxin responsible for toxic shock syndrome, and enterotoxin B, responsible for food poisoning [55]. SaPIs are the founding members of a larger class of phage-inducible chromosomal islands (PICIs), molecular parasites that have evolved a variety of mechanisms to hijack the components of specific 'helper' phages to facilitate their own reproduction and spread. Similarly to prophages, SaPIs reside within specific attachment sites in the bacterial chromosome and remain under the control of their own repressor proteins. Following infection by the helper phage, or induction of an endogenous helper prophage, SaPIs excise, circularize, and replicate (Figure 1C). Most SaPIs encode a TerS homolog, which recognizes the SaPI-specific *pac*-site and works together with the phage-encoded TerL to direct packaging of SaPI DNA into phage-like particles built from phage virion proteins. This process not only interferes with phage reproduction but also results in the high-frequency packaging of SaPIs and their subsequent transfer to diverse hosts, including non-*aureus* staphylococci [56], and remarkably, *Listeria monocytogenes* [57]. In addition to spreading their own genes, SaPIs can direct the transfer of distally encoded virulence factors proximal to sequences homologous to the SaPI *pac*-site, a process termed 'island-mediated generalized transduction' [58]. While traditionally thought to be facilitated exclusively by *pac*-type phages, intra- and inter-*genus* SaPI transfer has recently been shown to occur via *cos*-type phages as well [57,59]. Although these mobile elements are widely distributed in staphylococci, little is known about their function and impacts in non-*aureus* species.

Myoviruses and podoviruses constitute powerful antistaphylococcal agents

In contrast to staphylococcal siphoviruses, which have a high potential to promote pathogenesis, the podovirus and myovirus varieties (members of clusters A and C, respectively) are devoid of recognizable integrases and virulence factors [30] and are thus deemed suitable for use as antimicrobial agents. Indeed, soon after their discovery, phages were exploited as therapeutics to treat a variety of infections [60]. Although this practice was largely abandoned following the advent of conventional antibiotics, phage-based therapies are regaining global interest in the midst of the burgeoning antibiotic-resistance crisis. Building on decades of research in animal models which demonstrate the safety and effectiveness of whole-phage antimicrobials (for examples, see [60]), recent years have witnessed increasing case reports and clinical trials in which phages are used in patients suffering from *S. aureus* infections (Table 2). In these studies, wild-type phages are administered alone or in cocktails, and in most cases, alongside antibiotics. Remarkably, the majority of cases report complete eradication of *S. aureus* and/or overall patient improvement. Myoviruses are more commonly used in therapeutic applications as they typically exhibit broader host ranges [61]; however, podoviruses are increasingly being recognized for their unique benefits. For example, their compact genomes leave little room for virulence-associated genes [62], and their protein-priming mechanism of DNA replication [63] likely precludes the accidental packaging

Table 2. Application of staphylococcal phages to treat *S. aureus* infections in humans (2016–2020)

Treatment	Phage morphology[b]	Type of study	Infection type/# patients	Major findings	Refs
Phage Sb-1	Myo	Case report	Diabetic toe ulcers/6	Ulcers in all patients resolved over an average of 7 weeks	[94]
AB-SA01[a] in combination with antibiotics	Myo	Case report	Bacteremia/1	Within days of treatment, blood cultures became sterile and tested negative for staphylococcal DNA Leukocyte counts returned to normal	[95]
AB-SA01 in combination with antibiotics	Myo	Case report	Severe staphylococcal sepsis with prosthetic valve endocarditis/1	Blood cultures were negative at onset of therapy, and the C-reactive protein, temperature, and white cell count results showed downward trends within 24 h	[96]
Cocktail of three phages	NS	Case report	Chronic nonhealing wound/5	All wounds became sterile by day 13 of treatment Complete healing or significant decrease in wound depth observed in all patients	[97]
AB-SA01 in combination with antibiotics	Myo	Case report	Left ventricular assist device/1	Sternal cultures became negative for *S. aureus* at weeks 1, 2, and 4 (end of therapy) Wound appearance improved with reduced purulence and healthy granulation tissue	[98]
AB-SA01	Myo	Phase I clinical trial	Recalcitrant chronic rhinosinusitis/9	Intranasal irrigation with cocktail with doses up to 3×10^9 PFU[b] for 14 days was safe and well tolerated, with promising preliminary efficacy observations	[99]
Phages Sa30, CH1, SCH1, and/or SCH111 in combination with antibiotics	Myo and Podo	Case report	Critical infections related to cardiothoracic surgery/5	Eradication of *S. aureus* in four of five patients	[100]
Phage SaGR51F1 in combination with antibiotics	NS	Case report	Chronic prosthetic joint infection/1	Infection was eradicated following treatment	[101]
Phages PP1493 and PP1815 in combination with antibiotics	NS	Case report	Prosthetic knee infection/1	*S. aureus* was eradicated following phage treatment; however, the patient developed a superinfection with other bacterial pathogens, which led to amputation	[102]
Phages PP1493 and PP1815 and PP1957 in combination with antibiotics	Myo and Podo	Case report	Relapsing prosthetic knee infection/3	All patients showed clinical improvement	[103]
AB-SA01 and/or phage SaGR51ø1 in combination with antibiotics	Myo	Case report	Ventricular assist device infection/1 and prosthetic joint infection/1	Infections resolved with phage therapy	[104]
AB-SA01 in combination with antibiotics	Myo	Clinical trial	Bacteremia/13	AB-SA01 (at 1×10^9 PFU/ml) was administered intravenously, twice daily for 14 days. No adverse reactions to treatment were observed. 62% of patients showed clinical improvement by day 14, while 38% died within the first 28 days	[105]

[a]Cocktail of three phages.
[b]Abbreviations: Myo, myovirus; NS, not specified; PFU, plaque-forming unit; Podo, podovirus.

and spread of nonphage DNA. Notably, recent studies have shown that Podoviridae phages enhance the overall effectiveness of antistaphylococcal therapeutic cocktails [64,65].

Despite these successes, many challenges impede the routine use of whole-phage therapeutics. For instance, phages typically exhibit narrow host ranges, and bacteria readily evolve phage resistance, thus necessitating the use of complex cocktails consisting of phages with multiple mechanisms of action. This practice leads to safety concerns, as the preponderance of uncharacterized genes in phage genomes have the potential to promote bacterial virulence or cause other undesirable side-effects. Further, high doses of phages may elicit an unwanted immune response [66], and the pharmacodynamics and pharmacokinetics of phages in the human body are poorly understood. Beyond the biology, there are also practical considerations. For example, therapeutic phages must be purified according to current good manufacturing practices, which may impinge on the economic feasibility of large-scale phage production [67]. Finally, there are regulatory hurdles to surmount, as the approval pathway for conventional antimicrobials is unsuitable for whole-phage therapeutics. Undoubtedly, the routine implementation of phage therapy will require interdisciplinary collaborations across multiple sectors, including academia, industry, and government entities.

Fortunately, many of these shortcomings are currently being addressed using cutting-edge tools and alternative approaches. Indeed, recent advances in genetics and synthetic biology have enabled the engineering of lytic phages, which are largely refractory to classical genetic techniques. For example, studies have shown that CRISPR-Cas systems can be used as counter-selection tools to introduce mutations in lytic phages that infect diverse organisms, including staphylococci [68]. In addition, synthetically constructed phage genomes harboring desired mutation(s) can be introduced directly into the host and subsequently packaged into infective lytic particles in a process known as ‘rebooting’ [69]. While the latter strategy is ideal for Gram-negative bacteria, which are more receptive to transformation, a recent study showed that cell-wall-deficient *L. monocytogenes* can be used to reboot diverse phages of Gram-positive bacteria, including staphylococci [70]. These powerful tools not only enable the basic characterization of phage genes with unknown functions, but also allow for the creation of ‘designer phages’ that are optimized for therapeutic applications [71]. Optimizations include the broadening/tuning of host range and the introduction of genetic payloads that enhance phage antibacterial activity. Host range is dictated not only by the availability of the cell surface receptor required for phage attachment, but also by the absence of internal barriers (such as restriction–modification or CRISPR-Cas systems) which block various steps of phage replication [61]. Therefore, broadening the host range of staphylococcal phages may entail the introduction of additional receptor-binding proteins, and/or proteins that counter antiphage defenses. In order to enhance the antibacterial activity of therapeutic phages, a recent approach that has garnered significant attention involves the introduction of CRISPR-Cas systems programmed to target and degrade specific DNA sequences within the pathogen of interest [72,73]. As a proof-of concept, such phages were shown to selectively eliminate *S. aureus* strains harboring targeted virulence factors and/or plasmids encoding antibiotic-resistance genes [72]. As a clever twist to this approach, a recent study showed that SaPIs stripped of toxin genes and outfitted with a CRISPR-Cas system can block or cure *S. aureus* infections in mice [74]. Finally, as an alternative approach to using whole phages, phage-derived lysins (cell wall hydrolases) have shown significant promise as antistaphylococcal agents in animals and humans [75–77]. The latter has the potential to overcome both safety concerns and regulatory hurdles associated with the use of whole-phage therapeutics.

Concluding remarks and future perspectives

Through their shaping and molding of our resident *Staphylococcus* communities, the phages of staphylococci undoubtedly have profound impacts on human health and disease. Although staphylococcal phages have been studied for decades, and many mechanistic insights have come to light, research has historically focused on the temperate phages of *S. aureus* as model organisms, thus our knowledge is far from complete (see Outstanding questions). There is a critical need to explore the diversity and impacts of lytic staphylococcal phages and all varieties that infect non-*aureus* species. Indeed, the identification of a Siphoviridae singleton in the collection [30], the recent revelation that lytic staphylococcal phages may promote biofilm formation [78], and the recent discovery of a giant staphylococcal myovirus capable of intraspecies generalized transduction [79], provide evidence that the true diversity of staphylococcal

Outstanding questions

What is the extent of genomic diversity in lytic staphylococcal phages, and all varieties that infect non-*aureus* species?

What are the roles of the ~50% of genes in staphylococcal phage genomes that encode hypothetical proteins?

How do prophages impact fitness and pathogenic potential of non-*aureus* staphylococci, including the largely beneficial species?

To what extent do lytic staphylococcal phages facilitate inter-*species* and inter-*genus* transduction?

To what extent are the different forms of transduction subject to regulation by the phage or host?

What are the functions and impacts of the phage-inducible chromosomal islands in non-*aureus* staphylococci?

What are the molecular and genetic determinants that dictate the host range of staphylococcal phages, on the sides of both the phage and the host?

To what extent do lytic/therapeutic phages enhance virulence and pathogenicity of the host?

How do phage–host dynamics play out in the context of the microbiome and naturally impact our resident *Staphylococcus* communities?

What are the pharmacokinetics and pharmacodynamics of therapeutic staphylococcal phages when deployed in high doses through various routes of entry?

phages and the scope of their impacts remain poorly understood. Further, as a complement to focusing on the phages themselves, it is necessary to consider the immune systems targeted against them, as the latter constitute formidable forces that propel phage evolution and also threaten to undermine the effectiveness of phage-based antimicrobials. Indeed, recent years have witnessed a surge of interest in discovering and characterizing new antiphage immune systems in diverse organisms [80]. It is anticipated that the methodical examination of phage–host interactions with increasing focus on nonmodel organisms will not only accelerate the pace of generating new insights but also fuel the development of future technologies.

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Declaration of interests

There are no interests to declare.

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