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Review

Advances in the field of phage-based therapy with special emphasis on computational resources

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Abstract

In the current era, one of the major challenges is to manage the treatment of drug/antibiotic-resistant strains of bacteria. Phage therapy, a century-old technique, may serve as an alternative to antibiotics in treating bacterial infections caused by drug-resistant strains of bacteria. In this review, a systematic attempt has been made to summarize phage-based therapy in depth. This review has been divided into the following two sections: general information and computer-aided phage therapy (CAPT). In the case of general information, we cover the history of phage therapy, the mechanism of action, the status of phage-based products (approved and clinical trials) and the challenges. This review emphasizes CAPT, where we have covered primary phage-associated resources, phage prediction methods and pipelines. This review covers a wide range of databases and resources, including viral genomes and proteins, phage receptors, host genomes of phages, phage–host interactions and lytic proteins. In the post-genomic era, identifying the most suitable phage for lysing a drug-resistant strain of bacterium is crucial for developing alternate treatments for drug-resistant bacteria and this remains a challenging problem. Thus, we compile all phage-associated prediction methods that include the prediction of phages for a bacterial strain, the host for a phage and the identification of interacting phage–host pairs. Most of these methods have been developed using machine learning and deep learning techniques. This review also discussed recent advances in the field of CAPT, where we briefly describe computational tools available for predicting phage virions, the life cycle of phages and prophage identification. Finally, we describe phage-based therapy's advantages, challenges and opportunities.

Keywords: phage therapy, host–phage interaction, antibiotic resistance, lytic proteins, bacterial infection, CAPT

Introduction

With the discovery of penicillin in 1928 by Alexander Fleming, the era of modern antibiotics started, which led to significant achievements in controlling infections [1]. Several drug-resistant bacterial strains have emerged in the last few decades, which poses a global health and economic burden [2]. Due to the emergence of multidrug-resistant bacteria, antibiotics' effectiveness has gradually decreased. In 2017, the WHO published a list of pathogens on a global priority basis that includes 12 species of bacteria based on their level of resistance and available therapies [3]. Due to antimicrobial resistance, an estimated 10 million people could die each year by 2050 [4]. The antibiotic discovery process has slowed as the rate of antibiotic resistance rapidly increases, indicating that the golden period of antibiotics may have ended [5, 6]. Therefore, alternative treatment regimens are needed, including a reappraisal of bacteriophage therapy [2]. Bacteriophages (phages) are viral predators that specifically target bacteria and the most prevalent organisms in our environment, and they can be crucial for preserving the microbial population [7, 8]. In the current era of antibiotic resistance, phage therapy is a rapidly growing effective treatment against emerging drug-resistant bacterial strains [9, 10]. Currently, phage therapy is a therapeutic alternative in treating various bacterial infections in humans, animals and plants. Several clinical trials have been conducted over the last few years to design phage-based therapy or bacteriophage cocktails which could be used as a better treatment against several diseases such as skin infections, gastrointestinal disorders, cystic fibrosis, urinary tract infections (UTIs), bone and joint infections [4, 11–13]. In addition, several phage products are approved for targeting plant pathogens and food safety, such as Agriphage and Biolyse_BP [14].

Moreover, due to current advancements in high-throughput sequencing technologies, a massive amount of genomic and metagenomic data is available for discovering novel therapeutic phages. Identifying host-specific phages with a traditional experimental approach is very tedious and time-consuming. A plethora of computational methods and repositories have been developed to identify putative host ranges and understand phage–host interactions since predicting specific therapeutic phages is crucial in the success or failure of phage therapy [15]. In this review, we have compiled computational repositories and tools for predicting phage–host sequences and studying their interactions. In addition, we have incorporated various tools for phage-virion protein identification, phage's lifecycle and prophage prediction.

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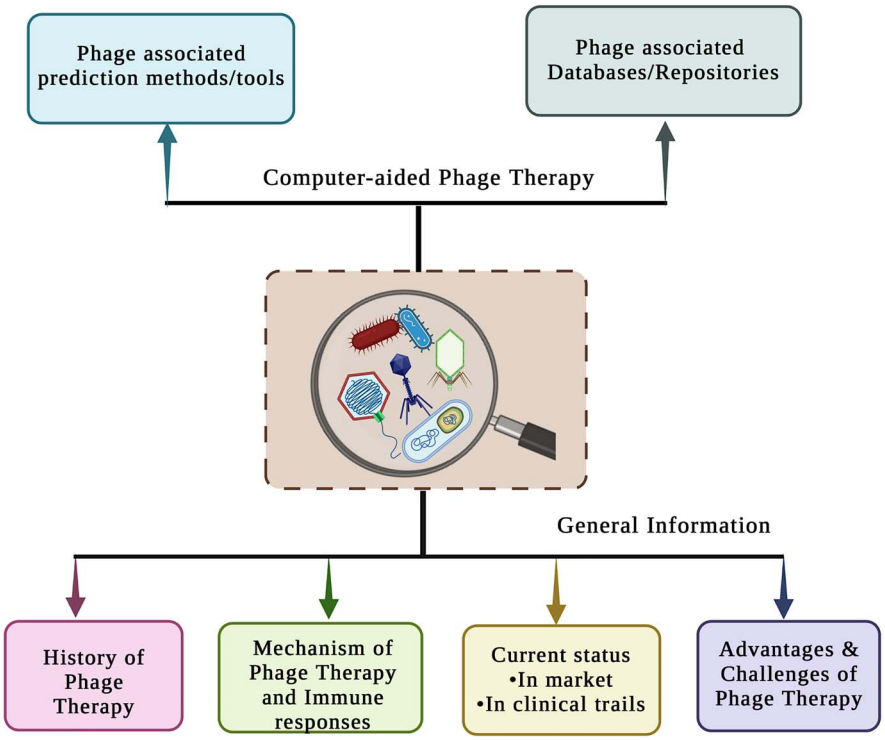

Figure 1. Overall structure of review.

Notably, these computational pathways are beneficial in creating innovative candidate therapy-ready phages. Moreover, to gain the future implication of phage therapy as an alternative treatment, we have summarized approved commercialized phage products and successful experimental clinical trials conducted to design new phage therapies for treating drug-resistant infections. The structure of our review is depicted in Figure 1.

History of phage therapy

Phage therapy is not new; it has a history older than antibiotics and is divided into four periods, i.e. early passion, critical rationalism, phase of decline and withdrawal, and recent interest [16], as depicted in Figure 2. The first foundation step in the history of phage therapy was taken in 1896 by a British bacteriologist, Ernest Hanbury Hankin. He demonstrated that the cultures of cholera-inducing bacteria were obtained from Indian rivers (Ganga and Yamuna) being destroyed by a biological entity [17]. In 1917, Felix d'He'relle coined the term 'bacteriophage' from the Greek word phage, meaning to devour, meaning 'bacterium eater' [10] and made the first attempt to treat chickens infected with *Salmonella gallinarum* using phages. After the discovery of bacteriophages, they were successfully used to treat other bacterial infections; during 1921–1930, bacteriophages were used to control cholera and plague epidemics in India, Africa, China and Vietnam [18]. After that, the first commercial anti-cholera phage-based drug was introduced in 1968. In addition, Bruynoghe and Maisin show the clinical use of phage to treat wound infections such as cutaneous furuncles caused by *Staphylococcus* species [18]. Despite phage therapy's successful and positive results, the nature of phages always remains controversial [16, 19]. With the discovery and widespread success of antibiotics, phage products were withdrawn from the market [20, 21].

However, this success of antibiotics has not continued for a very long time due to the development of resistance which causes significant clinical problems [22, 23]. In the 2000s, human trials of phages started [24], and the phage therapy gained global recognition in 2016 after successfully treating a person suffering from a resistant strain of *Acinetobacter baumannii* with intravenous phage cocktails, which fully recovered from the coma. With this, several clinical trials on phage therapy have been started and used to treat infections in animals and plants. Various companies commercialized phage and phage-based products in the market [4, 10, 18, 25, 26].

Mechanism of phage infection

Bacteriophages are diverse entities that infect bacteria with more than 10^{31} phages that were found in all-natural environments [27]. Phages have a small morphological structure that consists of a head, neck and tail [28]. They have double or single-stranded DNA/RNA genetic material that carries essential genes required for their replication inside the host, while the function of 80% of known genes is undefined [22, 29]. Phages have a narrow host range and lack complete replisome; therefore, they rely entirely on the host's machinery to synthesize phage particles for their propagation and survival [30, 31]. The phage infection process in bacteria involves a multi-step pathway following the two most found life cycles, i.e. lytic and lysogenic, as depicted in Figure 3. First, phage adsorbs on a pore on the host cell surface with the attachment to specific receptors via tail fibers; after that, they inject their genetic material into the cell via translocation by creating a pore on the host cell membrane [22]. Finally, replication occurs in the lytic cycle after the injection of genetic material, and the phage progeny is released by the rapid lysis of the infected bacterial cell.

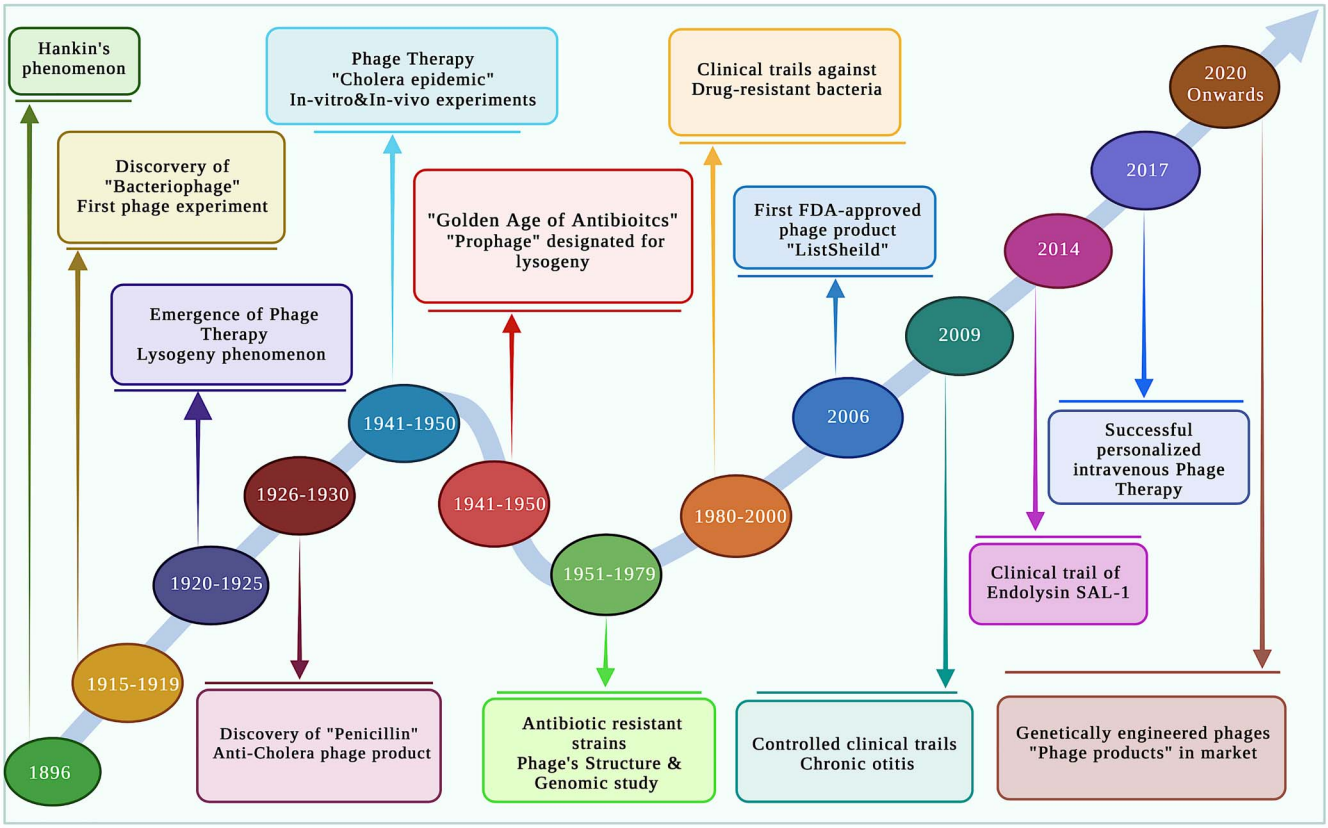

Figure 2. History and trends of phage therapy.

In the lysogenic cycle, phages remain in a state of lysogeny where they integrate their genetic material into the host genome as a prophage or exist as plasmids in the host cell without killing the host [2]. The genes necessary for lytic growth are switched off. When environmental conditions persist, or in the case of stress (such as nutritional stress and DNA damage), the prophage is excised from the host genome and starts to follow a lytic cycle [10]. Both types of phages in the environment are prominent; however, temperate phages are inadvisable for therapeutic purposes because of the lysogenic conversion through which bacteria acquire new genetic traits, such as phage-encoding toxins that can enhance their virulence [32]. The lysogeny genes are removed with the advancement of genetic engineering from the temperate phages. They can be used for therapy against bacterial infections [33] caused by *Clostridium diffificile* [34] and *Mycobacterium abscessus*, for which no lytic phages have been discovered [35].

Computer-aided phage therapy

In silico frameworks for designing and developing phage-based therapy for treating drug-resistant bacterial infections follow a multi-step process that leverages multiple tools designed for different tasks. The primary requirement for designing such a framework is the identification of candidate lytic phages and the target bacterial strains for which an alternate treatment is required. The choice of the phage is crucial for developing an effective treatment. Additionally, one must determine whether a single phage is intended to be employed or a cocktail of phages is to be used. The selected phages must be capable of disrupting the biofilm of the host cells and must undergo a lytic life cycle to lyse the host cell. Several databases/resources and computational frameworks have been developed for storing phage and host-associated datasets and predicting phage, host and their interactions. These bioinformatic tools facilitate the researchers to identify complete and partial phage and bacterial host genome sequences from metagenome samples obtained from advanced sequencing techniques. In addition, genome annotation tools are used for the functional annotation of phage isolates [36–40]. After the selection of candidate phages and bacteria, their interactions are to be predicted to determine the host ranges for the candidate phages, identifying the bacterial strains that can be infected by the chosen phages via penetration of the genome in the host cell via different mechanisms. Experimental verification of such interactions is very accurate, but such techniques are not scalable as each interaction requires specific conditions for the maintenance and storage of phages and bacteria. *In silico* tools help bridge the gap between performance and throughput and facilitate the researchers to determine host ranges, attachment mechanisms and receptor specificity. A wide range of computational tools have been developed which employ sequence similarities, machine-learning/deep-learning models or both to predict phage-host interactions leveraging features like genome sequences and protein sequences [41–45]. Additionally, phage virion proteins (PVPs) and phage life cycles play a crucial role in influencing the phage–host infection process. Thus, the prediction and study of such methods are necessary to develop a deeper understanding of the interactions between phages and their hosts.

Databases and repositories

In the past, due to the development of next-generation sequencing technologies, there was a huge increment in genomic data. Over the last few years, several repositories have been developed

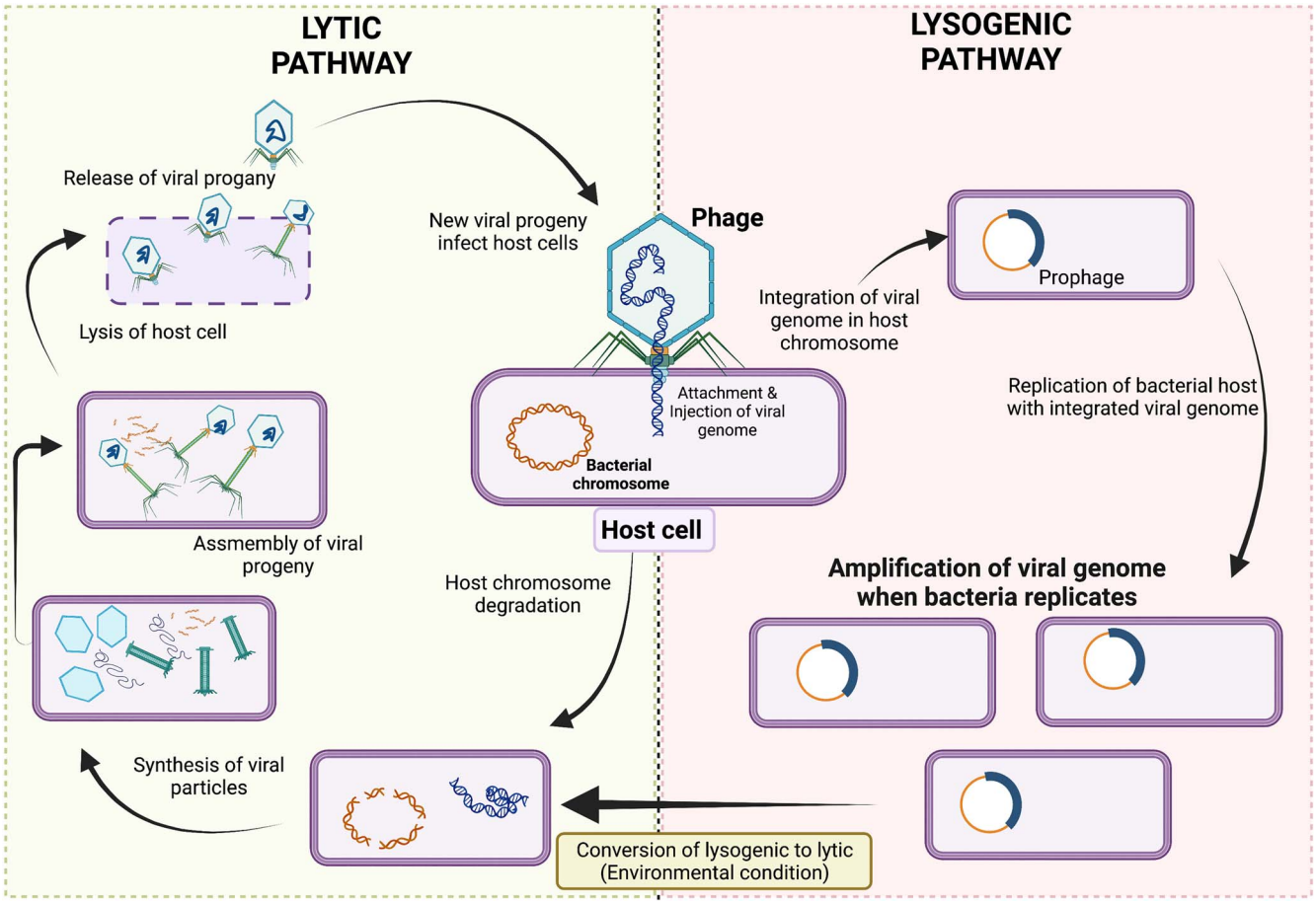

Figure 3. Overall mechanism of phage infection in host cell.

for studying phages based on their structural and functional properties, and their relationships and interactions have been created. Genomic sequences are the fundamental feature used in designing tools for phage-based therapy. Platforms like National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) and European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena/browser/home) maintain databases containing information about genome sequences and taxonomies. KEGG virus (https://www.genome.jp/kegg/genome/virus.html) is a resource repository that includes information on viruses and cellular organisms from an evolutionary perspective and has a collection of databases dealing with genomes, biological pathways, diseases, drugs and chemical substances. Information about gene regulation in phages is also interesting for multiple reasons. It helps in novel downstream tasks such as designing better phages for biotechnological purposes [46, 47] and bionanotechnology applications [48, 49].

phiSITE [50] is a database of gene regulation in bacteriophages that contains about 700 experimentally confirmed or predicted regulatory elements like promoters, operators, terminators and attachment sites from 32 bacteriophages. The data have been collected manually from multiple sources like EMBL, UniProt, NCBI taxonomy database, NCBI genome, ICTVdb and Pubmed Central. It provides full search and graph visualization of phage genomes. Phage lytic proteins serve as a novel alternative class of enzyme-based antibiotics called enzybiotics. PhReD [51] and PhaLP [52] are open-access databases that serve as repositories for phage receptors and lytic proteins, respectively. In addition, prophages have been widely used to study the interactions between phages and bacterial hosts. PhageWeb [53] is a computational tool for identifying prophages and consists of a collection of prophage sequences. Another important target for the observation and study of phages is the human intestines. The human intestines are the most diverse microhabitats comprising an eclectic mixture of organisms. The Gut Phage Database (GPD) [54] addresses these shortcomings. GPD is a database of 142 809 gut phage genomes that characterize phage–host relationships that are experimentally validated is pivotal in devising effective phage-based medication. Several platforms and databases contain information regarding such interactions as Microbe Versus Phage [55], Virus-Host DB [56], PhagesDB [57], VHRdb [58], etc. These databases primarily contain pairs of phage and microbes that denote an interaction. In Table 1, we collected databases/resources that can be utilized to develop novel methods for predicting viral host ranges and classifying phage–host interactions and serve as the backbone for devising phage-based therapies.

In silico tools for predicting phage candidates

Bacteriophages constitute a significant portion of living organisms. Therefore, studying their interactions and influence on other organisms is vital in developing a deeper understanding of biological phenomena. In the past, several computational tools were created to assay phages' multiple properties and relationships. These can be categorized into the following five categories: (i) phage identification tools, (ii) bacteriophage host prediction, (iii) phage-virion-protein identification, (iv) phage life cycle prediction and (v) prophage identification tools.

Table 1. List of phage-related repositories and resources in public domain

Name (reference)	Year	Description (link)
ENA [59]	2010	An archive of genomic and nucleotide sequencing information https://www.ebi.ac.uk/ena/browser/home
phiSITE [50]	2010	Database of gene regulation in bacteriophages http://www.phisite.org/main/
NCBI Virus [60]	2014	Contain genome sequence of bacteriophages https://www.ncbi.nlm.nih.gov/labs/virus/vssi/
VirusMentha [61]	2014	A resource that includes tools to analyze selected viral proteins https://virusmentha.uniroma2.it/about.php
PhReD* [51]	2016	Repository phage receptors http://www.ualberta.ca/phred
Virus-Host DB [56]	2016	Contains genome of hosts and phage and their interaction https://www.genome.jp/virushostdb/
Actinobacteriophage Database (PhagesDB) [57]	2017	Genome of phages that infect bacterial hosts (Actinobacteria) https://phagesdb.org/
PhageWeb database* [53]	2018	Compilation of sequences of prophages http://computationalbiology.ufpa.br/phageweb/database.php
Microbe Versus Phage (MVP) [55]	2018	A phage-microbe interaction database http://mvp.medgenius.info/
Viruses.STRING [62]	2018	Protein–protein interaction for virus-virus and virus-host. https://apps.cytoscape.org/apps/stringapp
PhaLP [52]	2021	A database of phage lytic proteins https://www.phalp.org/
Gut Phage Database (GPD) [54]	2021	A database of Gut phage genomes obtained from metagenome https://www.sanger.ac.uk/data/gut-phage-database/
Viral Host Range database (VHRdb) [58]	2021	Viruses infecting archaea, bacteria, and eukaryotes https://viralhostrangedb.pasteur.cloud/about/
mMGE [63]	2021	A database for human metagenomics, plasmids and phages https://mgedb.comp-sysbio.org
KEGG Virus [64]	2021	It contains information about viruses and cellular organisms https://www.genome.jp/kegg/genome/virus.html

Phage identification tools

Metagenomic sequencing has enabled fast and efficient sequencing of prokaryotic cells and viruses from metagenomic bins. However, studying the properties of phages in isolation remains challenging due to the inability of proper isolation, preservation and purification techniques for phages. Identifying phage genomes from these mixed sequences is crucial for developing most downstream phage analyses. In the last two decades, several computational tools have been developed for predicting phages. As listed in Table 2, DeepVirFinder [65] uses deep CNNs models to identify genomic motifs from viral sequences for making predictions to distinguish phage and prokaryotic genomes. MetaPhinder [40] predicts genomic fragments of phages using whole genome sequences and is capable of distinguishing bacteria and prophages. They demonstrate that their approach outperforms BLAST and other methods based on comparing k-mers. Metaviral SPAdes [66] utilize the variations in coverage depth between viruses and bacteria chromosomes for identifying viral genomes in metagenomic assembles. Phage_finder [39] and PhaMers [67] help identify phage samples in metagenomic samples. Seeker [68] uses deep learning models for the alignment-free identification of phage sequences. VIBRANT [69] uses a hybrid model comprising machine learning and protein similarity. DeePhage distinguishes between virulent and temperate phage-derived lines in metavirome data with a deep learning approach. VirMiner [36], virMine [70] and VirFinder [38] are tools that are used for phage contig prediction in metagenomic samples. VirSorter [37] and VirSorter2 [71] are tools used to detect viral signals (DNA/RNA) in microbial sequence data. VirSorter uses probabilistic models in both reference-dependent and independent manner to maximize novel virus detection, whereas VirSorter2 uses genome-informed database advances with multiple automatic classifiers for detecting the range of viruses.

Bacteriophage host prediction

Increased bacterial resistance to antibiotics is a global healthcare challenge that urges alternative treatment classes. Determining the bacterial host against bacteriophage is a quintessential step to leveraging the bactericidal effects of phages and developing phage-based medications. The lack of annotated hosts for phages sequenced from metagenomic bins poses the problem of identifying putative bacterial hosts for the bacteriophages. Therefore, developing computational methods for phage–host interaction prediction has been an important topic of interest among researchers. The interaction of a phage with its putative host is highly intricate. It is governed by ecological co-evolutionary processes wherein both organisms compete in a constant arms race for survival. The host evolves and adapts continuously to prevent viral infection and the phage in response, evolving to sustain its growth. Numerous studies developed methods for predicting phage–host interactions by using these features. Broadly these methods can be categorized into three categories, i.e. alignment based on sequence similarity [41,91,96], alignment-free methods [42–44,97], and integrated approaches and others [45,86,93,98,99] as represented in Table 2.

Phage-virion-protein identification

Phage virions are composed of proteins that contain genetic material (DNA or RNA), which are responsible for binding to the surface of the bacterial host to enable the phage to insert its genome into the cell [100]. Specific virion proteins are responsible for successful infection, and subsequent cell lyses. Therefore, identifying PVPs is pivotal in understanding the interactions between phages and host bacteria for developing phage-based antibacterial medications. Several computational tools have been developed to aid the process of identification and classification of virion proteins, like PVP-SVM [101], PVPred-SCM [102], PhageWeb [103] PhaNNs [104] (Table 3). Most of these tools employ machine learning and

Table 2. List of computational tools for identification of phage, bacterial hosts and interacting phage–host pairs

Name (reference)	Year	Description (link)
Phage identification tools		
Virsorter[a] [37]	2015	Prediction of viral signals using microbial data https://de.iplantcollaborative.org/de/
PhaMers [67]	2017	Screening of novel bacteriophage from hot springs https://github.com/jondeaton/PhaMers
VirFinder [38]	2017	Virtual screening of viral sequences from metagenomic data https://github.com/jessieren/VirFinder
VirusSeeker [72]	2017	Pipeline for virus identification http://pathology.wustl.edu/virusseeker/index.htm
VirNet [73]	2018	Identification of viral reads in NGS data of metagenomes https://github.com/alyosama/virnet
MARVEL [74]	2018	Prediction of bacteriophage sequences in metagenomic bins https://github.com/LaboratorioBioinformatica/MARVEL
ViraMiner [75]	2019	Prediction of viral genome using human samples https://github.com/NeuroCSUT/ViraMiner
VirMine [70]	2019	Detection of viral sequences using metagenomic samples https://github.com/putonti/virMine
Seeker [68]	2020	Deep-learning approach for identification of bacteriophage https://seeker.pythonanywhere.com/predict/
DeepVirFinder [65]	2020	Identification of viruses using metagenomic data https://github.com/jessieren/DeepVirFinder
Virsorter2 [71]	2021	Detect viral signals in various DNA/RNA viruses https://bitbucket.org/MAVERICLab/virsorter2/src/master/
Prediction of phage-specific hosts		
HostPhinder[a] [44]	2016	Prediction of bacterial hosts of phages https://cge.cbs.dtu.dk/services/HostPhinder/
WIsH [43]	2017	Identification of prokaryotic hosts using phage contigs https://github.com/soedinglab/wish
Viral Host Predictor [76]	2018	Predicting reservoir hosts using evolutionary information https://bioinformatics.cvr.ac.uk/software/viral-host-predictor/
Host Taxon Predictor (HTP) [77]	2018	Prediction of taxon of host of newly discovered phages https://github.com/wojciech-galan/viruses_classifier
PHERI [78]	2020	Pipeline for phage host exploration https://github.com/andynet/pheri
Virus Host Predict [79]	2020	Prediction of host taxonomic information using viral genome https://github.com/youngfran/virus_host_predict
vHulk [80]	2020	Deep learning method for bacteriophage host prediction https://github.com/LaboratorioBioinformatica/vHULK
HoPhage[a] [81]	2021	A tool for the identification of host from phage fragments data http://cqb.pku.edu.cn/ZhuLab/HoPhage/data/
HostG [82]	2021	A tool predict hosts of prokaryotic viruses https://github.com/KennthShang/HostG
CrisprOpenDB [83]	2021	Bacterial host predictions using CRISPR spacer streamlining https://github.com/edzuf/CrisprOpenDB
VPF-Class [84]	2021	Prediction of taxonomic class and host of phages https://github.com/biocom-uib/vpf-tools
PHIST [85]	2021	Prokaryotic host prediction using metagenomic viral sequences https://github.com/refresh-bio/phist
Bacteriophage-Host Prediction [86]	2021	Bacteriophage–host prediction from receptor-binding proteins https://github.com/dimiboeckaerts/BacteriophageHostPrediction
PHP [87]	2021	Prokaryotic virus host prediction tool https://github.com/congyulu-bioinfo/PHP
RaFAH [41]	2021	Viruses of bacteria and archaea prediction method https://sourceforge.net/projects/rafah/
Prediction of phage–host interaction pairs		
ILMF-VH [88]	2019	Predicting virus–host association https://github.com/liudan111/ILMF-VH
PredPHI [89]	2020	Identification of bacteriophage–host interaction https://github.com/xialab-ahu/PredPHI
VirHostMatcher-Net [45]	2020	Prediction of virus–prokaryote interactions https://github.com/WeiliWw/VirHostMatcher-Net
DeepVHPPI [90]	2021	Prediction of virus–host interactions for novel viral sequences https://github.com/QData/DeepVHPPI
SpacePHARER [91]	2021	Phage-host interactions via prediction of phage genomes https://github.com/soedinglab/spacepharer
PHIAF [92]	2022	Prediction of phage–host interactions with GAN-based data https://github.com/BioMedicalBigDataMiningLab/PHIAF
PHISDetector [93]	2022	*In silico* detection of phage–host interaction signals http://www.microbiome-bigdata.com/PHISDetector/
CHERRY [94]	2022	Virus–prokaryotic host interactions prediction tool https://github.com/KennthShang/CHERRY
PhageTB [95]	2022	Ensemble approach for predicting phage–host interactions https://webs.iiitd.edu.in/raghava/phagetb/

[a]Currently not working.

deep learning models for identifying virion proteins, and some tools use a combination of base models to develop ensemble learning models.

Phage life cycle prediction

Bacteriophages need to infect host bacterial cells to reproduce and carry out their life functions. The infection and reproduction cycles of phages are denoted as the life cycle of phage. Phages primarily exhibit two life cycles: lytic (or the virulent cycle), wherein they burst and kill their host cell, and lysogenic (or the temperate cycle), where they take over the host cell without killing it. Identifying the life cycle type exhibited by a phage is a necessary step when choosing phages for therapeutic use. Phages undergoing a lytic cycle can destroy the bacterial cells upon infection, while the ones that experience a lysogenic cycle remain dormant. These lysogenic (or temperate) phages are also responsible for horizontal gene transfer [114] and can transfer undesirable features like antibiotic resistance into the population. A few computational tools have been developed for the prediction of the life cycles of phages. For instance, PhageAI [115] is a tool containing information about more than 10 000 phages and their life cycles. It builds on a linear SVM classifier using nucleotide sequence embeddings based on the Word2vec skip-gram model for predicting the phage life cycle (https://phage.ai/). Phage Classification Tool Set (PHACTS [116]) is another tool developed for predicting phage life cycles. PHACTS uses a similarity search algorithm to construct a training set of phages described by their proteomes with annotated life cycles and then trains a machine learning classifier for making predictions (http://www.phantome.org/PHACTS/). BACterioPHage LIfestyle Predictor (BACPHLIP [117]) uses conserved protein domains within a phage genome for the prediction of the life cycle of phages (https://github.com/adamhockenberry/bacphlip).

Table 3. List of computational tools developed for predicting phage virion proteins

Name (reference)	Year	Description (link)
iVIREONS[a] [105]	2012	Deep learning-based models to classify viral structural proteins http://vdm.sdsu.edu/ivireons
PBVP[a] [106]	2015	An ensemble tool that uses hybrid feature for prediction of phage virions http://pbvp.weka.cc/
PHPred2.0 [107]	2018	Prediction of phage proteins and their subcellular localizations http://lin-group.cn/server/PHPred2.0/
PVP-SVM [101]	2018	Support vector machine-based PVP predictor http://www.thegleelab.org/PVP-SVM/PVP-SVM.html
PVPred [108]	2018	Identification of bacteriophage virion proteins http://lin-group.cn/server/PVPred
PhagePred[a] [103]	2018	A tool for predicting phage virion proteins using Naïve Bayes. http://bigroup.uestc.edu.cn/bacteriophage
Pred-BVP-Unb [109]	2020	Identification of phage virion proteins within a vast volume of proteins. https://github.com/Muhammad-Arif-NUST/BVP_Pred_Unb
PVPred-SCM [102]	2020	A scoring card method to identify phage virion proteins http://camt.pythonanywhere.com/PVPred-SCM
Meta-iPVP [110]	2020	Sequence-based meta-predictor to identify phage virion proteins http://camt.pythonanywhere.com/Meta-iPVP
PhANNs [104]	2020	Classification of phage structural proteins http://edwards.sdsu.edu/phanns
iPVP-MCV [111]	2021	Identification of phage virion proteins using an ensemble model https://github.com/taigangliu/iPVP-MCV
VirionFinder [112]	2021	Prediction of complete and partial prokaryote virus virion proteins. https://github.com/zhenchengfang/VirionFinder
SCORPION [113]	2022	Machine learning-based approach for predicting phage virion proteins https://github.com/saeed344/SCORPION

[a]Currently not working.

Table 4. Computational resources developed for identifying prophage or phage sequences in bacterial genomes

Name (reference)	Year	Description (link)
Phage_finder [39]	2006	Identification and classification of prophage http://phage-finder.sourceforge.net/
Prophinder[a] [119]	2008	Prediction of prophage in prokaryotic genomes http://aclame.ulb.ac.be/prophinder
PHAST [120]	2011	Annotation and graphically mapping of prophage http://phast.wishartlab.com/
PhiSpy [121]	2012	Prediction of prophages by ranking genomic regions https://sourceforge.net/projects/phispy/
PHASTER [122]	2016	An updated version of PHAST https://phaster.ca/
PhageWeb [53]	2018	Homology based identification of phage regions http://computationalbiology.ufpa.br/phageweb/
Prophage Hunter [123]	2019	Virtual screening of prophages in bacterial genomes https://pro-hunter.bgi.com/
ProphET [124]	2019	Prophage sequence prediction tool https://github.com/jaumlrc/ProphET
Phigaro [125]	2020	Predict and annotate prophage https://github.com/bobeobibo/phigaro
Prophage Tracer [126]	2021	Precisely tracing prophages in prokaryotic genomes https://github.com/WangLab-SCSIO/Prophage_Tracer
PhageBoost [127]	2021	Machine learning based data mining for predicting prophage regions http://www.phageboost.dk
DEPhT [128]	2022	Prophage discovery tool https://pypi.org/project/depht/
DBSCAN-SWA [129]	2022	An integrated tool for rapid prophage detection https://github.com/HIT-ImmunologyLab/DBSCAN-SWA/

[a]Currently not working.

Prophage identification tools

Prophages are integrated phage forms in host bacterial genomes that contribute to interstrain genetic variability. The presence of genes associated with viruses is reported to be prophage encoded as the integration of phages in the bacterial genomes leads to the transfer of genes and their spread in the bacterial population [118]. The identification of prophages enables the study of the interaction between phages and their hosts effectively and helps characterize hosts of related phages. Thus, they can be used in developing new phage-based therapies. Numerous tools have been developed that utilize alignment-based methods, homology searches, machine learning and deep learning models to identify prophages in bacterial genomes (Table 4). The complete overview of the bioinformatics pipeline for designing phage therapy is shown in Figure 4.

Clinical usage of phage-based therapy

Phage therapy for systematic use in clinical medicine from individual case experiences requires rigorous clinical trials. These trials need extensive knowledge of translational endpoints to help better define the critical aspects of phage therapy, including the route of administration, dosage, pharmacokinetics, pharmacodynamics, clinical conditions that benefit from phage therapy and optimal use in combination with antibiotics [30]. Phages have been used to treat clinical conditions, including UTIs, skin and surgical wound infections, dysentery, external otitis, septicemia and typhoid fever [18]. Over the last few years, several clinical trials have been carried out, but only a few are currently completed [4, 130, 131] (Table 5). The first phase I safety clinical trial, regarding the oral administration of *Escherichia coli* phage T4 in drinking water, was carried out in 2005, which showed neither release of antibodies nor phages in patient serum, suggesting that oral phage administration is safe [132]. In 2006, a case study in the UK was reported where phage suspension (approximately 103 PFU of phage in 0.2 ml of saline) was used to cure *Pseudomonas aeroginosa* infection of the transplanted skin after the failure of antibiotics [16781080]. In the USA, the first FDA-approved phase I clinical trial was conducted in 2009 to evaluate the safety of the phage cocktail ('WPP-201') developed by Intraltix Inc. for the treatment of venous leg ulcers [24]. In 2013, the largest randomized controlled phase I/II clinical trial, 'PhagoBurn', with good manufacturing practices and good clinical practices, was conducted in Europe to treat patients with burn wound infections using a cocktail of 12 lytic phages [133, 134]. From 2009 to 2011, a randomized, double-blind, placebo-controlled study was conducted in collaboration with Nestle (Switzerland) and Dhaka Hospital, Bangladesh, to test the safety and efficacy of oral administration of T4-like phage cocktail in children with acute bacterial diarrhea [135].

Moreover, in 2016, a study showed the use of *Staphylococcal* phage Sb-1 to treat diabetes and staph-infected toe wounds in nine patients, and the wounds were healed by the middle of

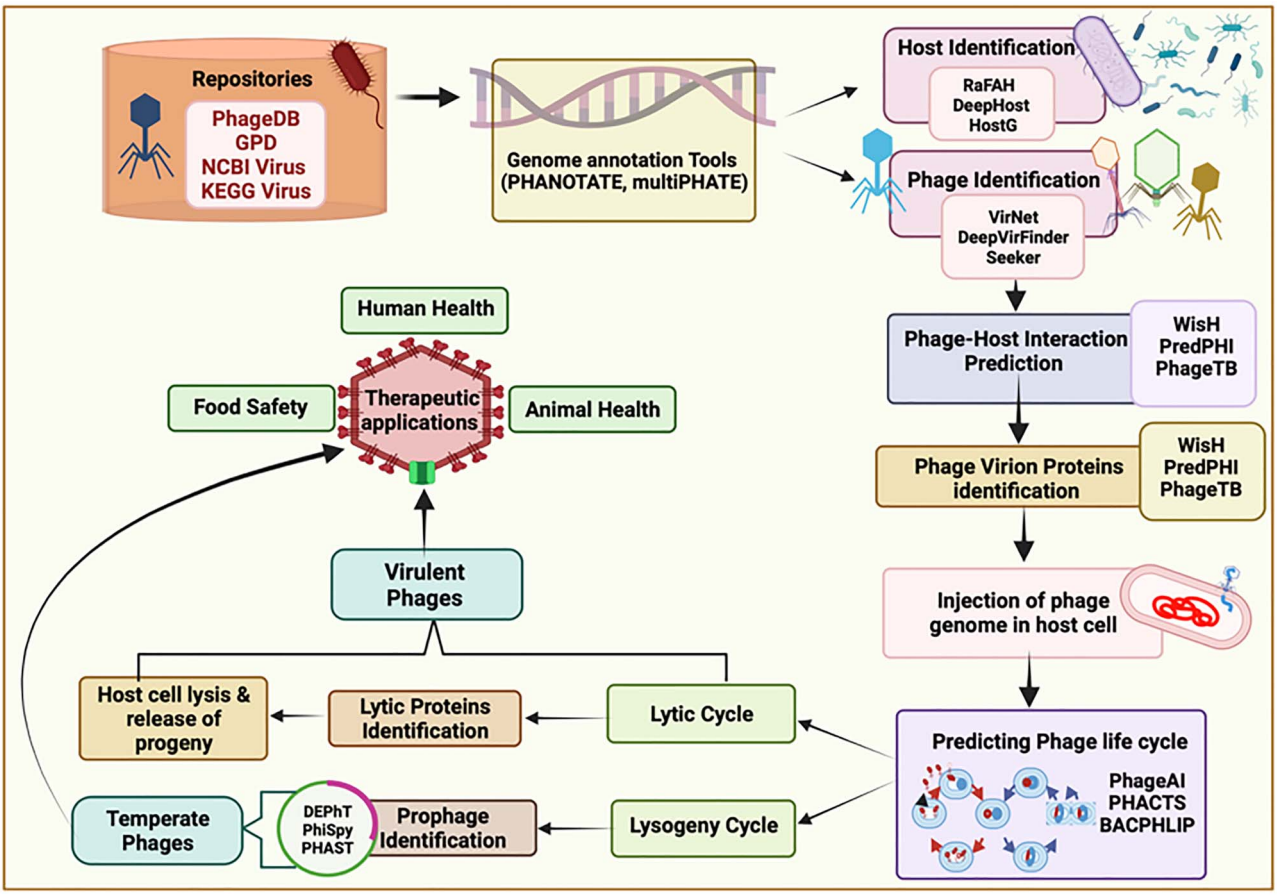

Figure 4. Schematic representation of bioinformatics tools for designing phage therapy.

Table 5. A list of clinical trials of phage therapy with brief description

National clinical trial number	Year	Disease or condition	Phage used for treatment	Age group
NCT00001540	1996	HIV infections	Bcateriophage phi X174	Child, adult, older adult
NCT00089180	2004	Skin carcinoma	Liposomal T4N5 Lotion	19 years and older
NCT00663091	2006	Venous leg ulcers	WPP-201 bacteriophage mixture	16 years and older
NCT00814151	2008	Bacteremia and staphylococcal infections	MicroPhage *S. aureus*/MSSA/MRSA Blood Culture test (Protoype)	18 years and older
NCT01184339	2009	Bacteremia	MicroPhage *S. aureus*/MSSA/MRSA Blood Culture test	18 years and older
NCT01818206	2012	Cystic fibrosis	Cocktail of 10 bacteriophages	6 years and older
NCT03269617	2016	Gastrointestinal disorders	Bacteriophage mixture	18 years to 65 years
NCT02757755	2016	Healthy adults	AB-SA01 (bacteriophage cocktail targeting *Staphylococcus*)	18 years to 60 years
NCT03140085	2017	Urinary tract infections	PYO phage and antibiotics	18 years and older
NCT0451221	2018	Mild gastrointestinal symptoms	Bifidobacterium probiotic and PreforPro	18 years to 65 years
NCT04191148	2019	Urinary tract infections	LBP-EC01	18 years and older
NCT04737876	2020	Healthy adults	BX002-A (cocktail against *Klebsiella pneumonia*)	18 years to 65 years

the seventh week of the phage application [136]. Not only single phages but also phage cocktails have been used to treat bacterial infections. A case report on a 61-year-old man with acute kidney injury and elevated serum creatinine levels was treated with a purified phage cocktail (BFC1), where his symptoms disappeared and his kidney function recovered. Blood culture showed no further bacterial growth [137]. Overall outcomes from these clinical trials are that there are no or fewer side effects of phage therapy, as concluded from animal pre-clinical studies, which require high phage titers, and their efficacy depends on the type of infection. More recently, several clinical trials have been registered on the web server (https://clinicaltrials.gov/) and summarized in Table 5.

Pros and cons of phage-based therapy

Phage therapy has advantages over conventional antibiotics and has been reviewed extensively by Loc-Carrillo and Abedon [138]. Several past studies reported several advantages and

Table 6. Possible pros and cons of phage-based therapy

Pros	Cons
'Specificity' binds to particular receptors of host cell.	'Narrow host range' not useful in case of early infection.
'Bactericidal effect' lyse the host cell after infection.	'Immunogenicity' elicits unintended immune response.
'Minimum side effects' no risk to normal microbiome.	'Release of endotoxins' lead to inflammatory responses.
'Auto-dosage' replication capability at the site of infection.	'Lack of phage pharmacokinetic data' administration route and dosage form remain unclear.
'Narrow potential to induce resistance' co-evolution of phages and bacteria.	'Lack of clinical trials' to evaluate the safety and efficacy.
'Cost-effective treatment' production and isolation of phages is of low cost.	'Knowledge barrier' lack of comprehensive familiarity with phages in treating diseases.
'Biofilm clearance' phages can penetrate and lyse biofilms.	'Limit to intracellular pathogens' invader pathogens are inaccessible by phages.
'Rapid discovery' phages are abundant in the natural environment.	'Neutralization' inactivated by neutralized antibodies and removed by mononuclear phagocyte system.
'Ecologically friendly drugs' phages possess no or low intrinsic toxicity.	'Ethics and regulation of phage therapy' no official safety guidelines/protocols and regulatory framework.

disadvantages related to phage therapy. Phages show a narrow antibacterial spectrum and bactericidal effects, are easy to discover, are effective against resistant strains of pathogens, pose no risk to the typical microbiome in the body, are effective against biofilms and have a low level of toxicity. Cost-effective are some of the pros of phage-based therapy [7, 9, 18, 23, 138–145]. Even though phage therapy has recognizable advantages over conventional antibiotic treatment, it still poses some drawbacks that need to be addressed for its acceptance in modern clinical practice. For example, phages are limited to intracellular pathogens and can evoke immunogenicity due to the release of endotoxins, narrow host range, lack of phage pharmacokinetic data and less public awareness. Moreover, there are no well-established approval pathways and regulatory agencies compared to antibiotics. In order to establish phage-based therapies, separate legal authorities must be required to document rules and regulations to formulate phage-based products. In addition, educational campaigns should be conducted to create awareness among people of the usefulness and acceptability of phages and phage-based products [4, 18, 23, 131, 138, 146–156]. In this section, we have reported several pros and cons of phage therapy, summarized in Table 6.

Advantages of computer-aided methods in phage therapy

Viruses are abundant and diverse. Due to advancements in high-throughput sequencing technologies, an enormous amount of viral genome sequencing data has been generated. The current experimental methods for understanding phage biology during host infection require a lot of time and resources. Computational tools are necessary to extract meaningful information from phage genomic data efficiently. The significant advantages of computer-based methods over traditional approaches in phage therapy are described as follows:

- The interaction of phages with the host is an essential step in the development of the phage infection process, i.e. the acquaintance of the host for the novel prokaryotic viruses is essential to understand the dynamic relationship between microbes. Experimental approaches, such as single-cell tagging, have been used to characterize the host specificity of phages [157]. However, these experimental methods are expensive, tedious and time-consuming. Also, these methods cannot keep pace with the exponential growth of sequenced phages. Therefore, in the past, a number of computational tools have been developed for predicting the phage–host interactions for newly discovered phages [41,94].
- Lytic phages are generally preferred over temperate phages while designing phage therapy. Therefore, identifying the lifecycle of phages is essential for understanding their role in the ecosystem and phage therapy. Conventional laboratory techniques based on plaque clearance or turbidity were used to identify the lifecycle of phages [158] though these methods are impractical for the newly discovered phage genomes [159]. In the past, researchers tried to develop artificial intelligence-based methods (such as PHACTS [116], BACPHILP [117], and PhageAI [115]) have been developed for the prediction phage life cycle. However, there is a need to develop more highly accurate bioinformatics methods to identify the phage life cycle accurately.
- PVPs are essential in recognizing and binding to the host cell's receptors, leading to lyses after infection [100]. Identifying and understanding the mechanism of PVPs are essential in developing phage-based therapy. While traditional lab methods such as mass spectrophotometry and protein arrays are well known for detecting and characterizing PVPs [160], these methods are costly and require rigorous labor work. Consequently, there is a need for computational tools to identify PVPs correctly and to understand the mechanism of their action.
- In phage therapy, it is necessary to know the host range for our choice of therapeutic phages in order to enhance their bactericidal effects. Laboratory experiments are the gold standard for identifying host ranges but are limited to a small number of virus and bacterial hosts that can be cultured. At the same time, it becomes difficult for those challenging to cultivate in lab conditions such as growth media and temperature. These methods can also be scientifically challenging due to the absence of infection signs or inconclusive [161]. Alternatively, the *in silico* methods require less time and resources to identify the putative host range for the phages [162].
- The computational pipeline will help the phage biologist to explore the virosphere for a comprehensive analysis of natural viral diversity, their interactions, lifestyle, and the evolutionary arms race between phages and their hosts. In addition, these methods will aid in prioritizing the candidate

phages for experimental design and testing. Thus, directly increasing the experimental efforts by proposing a hypothesis that can be tested.

Discussion and conclusion

Phage therapy has been used to treat various human infections, such as skin infections like wounds and burns, sepsis, eye, ear or dental infections, gastrointestinal tract infections, UTIs and respiratory diseases [163, 164]. Still, there are several knowledge gaps in selecting phages for therapy, frequency and route of administration, phage resistance, stability and storage of phage products, dosage, and pharmacokinetic and pharmacodynamic properties [165, 166]. A thorough investigation of immunological responses in phage therapy is also required. However, recent advancements in formulations, purification and phage genomics contributed to the efficacy of phage therapy required to develop phages or their products for treating infections [167]. Recently, to control antimicrobial resistance, WHO has launched the 'One Health' approach, an interdisciplinary idea that aims to improve lives by integrating areas in human health, environmental health, animal health and biodiversity [168] (https://onehealthinitiative.com/). Phages, from their discovery, have been globally used to treat diverse bacterial infections with great success; therefore, they are considered a new alternative therapy under one health approach to controlling pathogens in food, humans, plants and animals to prevent the spread of antibiotic resistance in humans and their overuse [169].

Phage cocktails instead of monophages have emerged as a new approach to treat infections caused by multiple strains of bacteria or whose causative agents have not been identified, as they contain various phages of different specificity, thus increasing the efficacy of phage therapy [170]. Not only the whole phage has been used in therapy, but phages-derived proteins and enzymes are also used, as the whole intact phage can be immunogenic and elicit unwanted immune responses. However, the isolation of these proteins is a challenge for developing therapy and is under experimental trials to replace the intact phages. With the help of genetic engineering and recombinant DNA technologies, the therapeutic potential of phages could be increased dramatically by expanding the host range of phages, preventing the transfer of virulence genes, removing lysogens from temperate phages to make them lytic and using in-therapy [10, 169]. Phage display is primarily used in designing phage-based vaccines and antigen expression to provoke an immune response and create immunological memory [171]. Phage adjuvants are a less explored area, which enhances the phage activity or inhibits the development of phage resistance with the use of adjuvants like Dnase, which degrade extracellular DNA, preventing bacterial aggregation, sugar alcohols such as sorbitol xylitol that inhibit bacterial growth by accumulating into biofilms as toxic [172] and with a synergistic antimicrobial that inhibits cell division or growth of bacteria and high production of phage particles [173]. These combination therapies need more *in vitro* assessments and experimental animal studies to understand the potential mechanism of interaction of phages with adjuvant and establish co-dosing regimens [22]. With the advancement in sequencing technologies, several computational tools have been developed, with their own advantages and disadvantages. Some of the machine-learning-based tools are highly reliable and accurate, for example, 'PhageTB', 'BACPHLIP' and 'DBSCAN-SWA' for the prediction of phage–host interaction, lifecycle and prophage identification, respectively.

Future prospects

The antibiotic-resistant era calls for an alternative approach where phage therapy has been slowly taking place in novel treatment methods for multidrug-resistant infections. The comprehensive study of phage biology highlights its potential application in various aspects of humankind. However, phages have broad applications in therapeutics; still, several gaps need to be resolved before phages can bloom in clinical practices, including the screening of phages, effective dosage form, stable phage products, the kinetics of phage action, etc. [31]. The lack of well-controlled clinical trials, which is the most common and necessary step, is a significant obstacle to the success of phage therapy [174]. Most clinical trials are done with antibiotics, making it challenging to identify phage therapy's effectiveness and safety evaluation alone [22]. Therefore, in the future, more robust clinical trials with phages or phage products are required for the emergence of phage therapy. Additionally, we need to select the best phage candidates for the therapy, phage enzymes or proteins and phage host ranges for an effective outcome. With the increase of the genomic and bioinformatics era, many computational approaches have been developed to select suitable phages accurately and efficiently against pathogens that are difficult to cultivate in lab conditions. Consequently, computational methods provide perfect ways to inspect viral diversity, phage–host interactions and lifecycle. However, these methods have several computational challenges that need to be overcome. The foremost issue that needs to be addressed is the reference databases since the computational tools and methods depend on the databases to obtain the datasets which somehow biased toward viruses and their hosts that are culturable and well studied [175].

Key Points

- The emergence of drug-resistant and extreme drug-resistant strains of bacteria.
- Infections due to drug-resistant bacteria can be treated using phage-based therapy.
- Databases contain sequences of phages and bacteria that are growing at an exponential rate.
- Computer-aided phage therapy is essential to discover novel and efficient phages.
- Compilation of phage-associated computational, genomics and proteomics resources.

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Authors' contributions

N.B., S.A., A.D. and G.P.S.R. performed the conception and design of the study. N.B., A.D., S.A. and G.P.S.R. performed the writing,

reviewing and draft preparation of the manuscript. All authors have read and approved the manuscript.

References

1. Malik DJ, Sokolov IJ, Vinner GK, *et al.* Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. *Adv Colloid Interface Sci* 2017;**249**:100–33.
2. Venturini C, Petrovic Fabijan A, Fajardo Lubian A, *et al.* Biological foundations of successful bacteriophage therapy. *EMBO Mol Med* 2022;**14**:e12435.
3. Tacconelli E, Carrara E, Savoldi A, *et al.* Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;**18**:318–27.
4. Furfaro LL, Payne MS, Chang BJ. Bacteriophage therapy: clinical trials and regulatory hurdles. *Front Cell Infect Microbiol* 2018;**8**:376.
5. Hover BM, Kim SH, Katz M, *et al.* Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant gram-positive pathogens. *Nat Microbiol* 2018;**3**:415–22.
6. Ling LL, Schneider T, Peoples AJ, *et al.* A new antibiotic kills pathogens without detectable resistance. *Nature* 2015;**517**:455–9.
7. Clokie MR, Millard AD, Letarov AV, *et al.* Phages in nature. *Bacteriophage* 2011;**1**:31–45.
8. Naureen Z, Dautaj A, Anpilogov K, *et al.* Bacteriophages presence in nature and their role in the natural selection of bacterial populations. *Acta Biomed* 2020;**91**:e2020024.
9. Lin DM, Koskella B, Lin HC. Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. *World J Gastrointest Pharmacol Ther* 2017;**8**:162–73.
10. Gordillo Altamirano FL, Barr JJ. Phage therapy in the postantibiotic era. *Clin Microbiol Rev* 2019;**32**(2):e00066–18.
11. Leitner L, Sybesma W, Chanishvili N, *et al.* Bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomized, placebo-controlled, double-blind clinical trial. *BMC Urol* 2017;**17**:90.
12. Wright A, Hawkins CH, Anggard EE, *et al.* A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. *Clin Otolaryngol* 2009;**34**:349–57.
13. Febvre HP, Rao S, Gindin M, *et al.* PHAGE study: effects of supplemental bacteriophage intake on inflammation and gut microbiota in healthy adults. *Nutrients* 2019;**11**(3):666.
14. Sillankorva SM, Oliveira H, Azeredo J. Bacteriophages and their role in food safety. *Int J Microbiol* 2012;**2012**:863945.
15. Gill JJ, Hyman P. Phage choice, isolation, and preparation for phage therapy. *Curr Pharm Biotechnol* 2010;**11**:2–14.
16. Summers WC. Bacteriophage therapy. *Annu Rev Microbiol* 2001;**55**:437–51.
17. Kochhar R. The virus in the rivers: histories and antibiotic afterlives of the bacteriophage at the sangam in Allahabad. *Notes Rec R Soc Lond* 2020;**74**:625–51.
18. Wittebole X, De Roock S, Opal SM. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence* 2014;**5**:226–35.
19. Northrop JH. Concentration and purification of bacteriophage. *J Gen Physiol* 1938;**21**:335–66.
20. Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. *Front Microbiol* 2010;**1**:134.
21. Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and future. *Curr Opin Microbiol* 2019;**51**:72–80.
22. Luong T, Salabarria AC, Roach DR. Phage therapy in the resistance era: where do we stand and where are we going? *Clin Ther* 2020;**42**:1659–80.
23. Kortright KE, Chan BK, Koff JL, *et al.* Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. *Cell Host Microbe* 2019;**25**:219–32.
24. Rhoads DD, Wolcott RD, Kuskowski MA, *et al.* Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. *J Wound Care* 2009;**18**:237–43 240-233.
25. Nale JY, Clokie MR. Pre-clinical data and safety assessment of phage therapy in humans. *Curr Opin Biotechnol* 2021;**68**:310–7.
26. Pirnay JP, Kutter E. Bacteriophages: it's a medicine, Jim, but not as we know it. *Lancet Infect Dis* 2021;**21**:309–11.
27. Mushegian AR. Are there 10(31) virus particles on earth, or more, or fewer? *J Bacteriol* 2020;**202**(9):e00052–20.
28. Ackermann HW, Prangishvili D. Prokaryote viruses studied by electron microscopy. *Arch Virol* 2012;**157**:1843–9.
29. Hatfull GF, Hendrix RW. Bacteriophages and their genomes. *Curr Opin Virol* 2011;**1**:298–303.
30. Hatfull GF, Dedrick RM, Schooley RT. Phage therapy for antibiotic-resistant bacterial infections. *Annu Rev Med* 2022;**73**:197–211.
31. Caflisch KM, Suh GA, Patel R. Biological challenges of phage therapy and proposed solutions: a literature review. *Expert Rev Anti Infect Ther* 2019;**17**:1011–41.
32. Fortier LC, Sekulovic O. Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence* 2013;**4**:354–65.
33. Monteiro R, Pires DP, Costa AR, *et al.* Phage therapy: going temperate? *Trends Microbiol* 2019;**27**:368–78.
34. Hargreaves KR, Clokie MR. Clostridium difficile phages: still difficult? *Front Microbiol* 2014;**5**:184.
35. Dedrick RM, Guerrero-Bustamante CA, Garlena RA, *et al.* Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. *Nat Med* 2019;**25**:730–3.
36. Zheng T, Li J, Ni Y, *et al.* Mining, analyzing, and integrating viral signals from metagenomic data. *Microbiome* 2019;**7**:42.
37. Roux S, Enault F, Hurwitz BL, *et al.* VirSorter: mining viral signal from microbial genomic data. *PeerJ* 2015;**3**:e985.
38. Ren J, Ahlgren NA, Lu YY, *et al.* VirFinder: a novel k-mer based tool for identifying viral sequences from assembled metagenomic data. *Microbiome* 2017;**5**:69.
39. Fouts DE. Phage_Finder: automated identification and classification of prophage regions in complete bacterial genome sequences. *Nucleic Acids Res* 2006;**34**:5839–51.
40. Jurtz VI, Villarroel J, Lund O, *et al.* MetaPhinder-identifying bacteriophage sequences in metagenomic data sets. *PLoS One* 2016;**11**:e0163111.
41. Coutinho FH, Zaragoza-Solas A, Lopez-Perez M, *et al.* RaFAH: host prediction for viruses of bacteria and archaea based on protein content. *Patterns (N Y)* 2021;**2**:100274.
42. Ahlgren NA, Ren J, Lu YY, *et al.* Alignment-free d_2^* oligonucleotide frequency dissimilarity measure improves prediction of hosts from metagenomically-derived viral sequences. *Nucleic Acids Res* 2017;**45**:39–53.
43. Galiez C, Siebert M, Enault F, *et al.* WIsH: who is the host? Predicting prokaryotic hosts from metagenomic phage contigs. *Bioinformatics* 2017;**33**:3113–4.
44. Villarroel J, Kleinheinz KA, Jurtz VI, *et al.* HostPhinder: a phage host prediction tool. *Viruses* 2016;**8**(5):116.

45. Wang W, Ren J, Tang K, *et al.* A network-based integrated framework for predicting virus-prokaryote interactions. *NAR Genom Bioinform* 2020;**2**:lqaa044.
46. Hasty J, McMillen D, Isaacs F, *et al.* Computational studies of gene regulatory networks: in numero molecular biology. *Nat Rev Genet* 2001;**2**:268–79.
47. Skiena SS. Designing better phages. *Bioinformatics* 2001; **17**(Suppl 1):S253–61.
48. Shu D, Huang LP, Hoeprich S, *et al.* Construction of phi29 DNA-packaging RNA monomers, dimers, and trimers with variable sizes and shapes as potential parts for nanodevices. *J Nanosci Nanotechnol* 2003;**3**:295–302.
49. Taton TA. Bio-nanotechnology: two-way traffic. *Nat Mater* 2003; **2**:73–4.
50. Klucar L, Stano M, Hajduk M. phiSITE: database of gene regulation in bacteriophages. *Nucleic Acids Res* 2010;**38**:D366–70.
51. Bertozzi Silva J, Storms Z, Sauvageau D. Host receptors for bacteriophage adsorption. *FEMS Microbiol Lett* 2016;**363**(4):fnw002.
52. Criel B, Taelman S, Van Criekinge W, *et al.* PhaLP: a database for the study of phage lytic proteins and their evolution. *Viruses* 2021;**13**(7):1240.
53. de Sousa AL, Maues D, Lobato A, *et al.* PhageWeb - web Interface for rapid identification and characterization of prophages in bacterial genomes. *Front Genet* 2018;**9**:644.
54. Unterer M, Khan Mirzaei M, Deng L. Gut phage database: phage mining in the cave of wonders. *Signal Transduct Target Ther* 2021;**6**:193.
55. Gao NL, Zhang C, Zhang Z, *et al.* MVP: a microbe-phage interaction database. *Nucleic Acids Res* 2018;**46**:D700–7.
56. Mihara T, Nishimura Y, Shimizu Y, *et al.* Linking virus genomes with host taxonomy. *Viruses* 2016;**8**:66.
57. Russell DA, Hatfull GF. PhagesDB: the actinobacteriophage database. *Bioinformatics* 2017;**33**:784–6.
58. Lamy-Besnier Q, Brancotte B, Menager H, *et al.* Viral host range database, an online tool for recording, analyzing and disseminating virus-host interactions. *Bioinformatics* 2021;**37**:2798–801.
59. Leinonen R, Akhtar R, Birney E, *et al.* The European Nucleotide Archive. *Nucleic Acids Res* 2011;**39**:D28–31.
60. Brister JR, Ako-Adjei D, Bao Y, *et al.* NCBI viral genomes resource. *Nucleic Acids Res* 2015;**43**:D571–7.
61. Calderone A, Licata L, Cesareni G. VirusMentha: a new resource for virus-host protein interactions. *Nucleic Acids Res* 2015;**43**:D588–92.
62. Cook HV, Doncheva NT, Szklarczyk D, *et al.* Viruses.STRING: a virus-host protein-protein interaction database. *Viruses* 2018; **10**(10):519.
63. Lai S, Jia L, Subramanian B, *et al.* mMGE: a database for human metagenomic extrachromosomal mobile genetic elements. *Nucleic Acids Res* 2021;**49**:D783–91.
64. Kanehisa M, Furumichi M, Sato Y, *et al.* KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res* 2021;**49**: D545–51.
65. Ren J, Song K, Deng C, *et al.* Identifying viruses from metagenomic data using deep learning. *Quant Biol* 2020;**8**:64–77.
66. Antipov D, Raiko M, Lapidus A, *et al.* Metaviral SPAdes: assembly of viruses from metagenomic data. *Bioinformatics* 2020;**36**: 4126–9.
67. Deaton J, Yu FB, Quake SR. Mini-metagenomics and nucleotide composition aid the identification and host Association of novel bacteriophage sequences. *Adv Biosyst* 2019;**3**:e1900108.
68. Auslander N, Gussow AB, Benler S, *et al.* Seeker: alignment-free identification of bacteriophage genomes by deep learning. *Nucleic Acids Res* 2020;**48**:e121.
69. Kieft K, Zhou Z, Anantharaman K. VIBRANT: automated recovery, annotation and curation of microbial viruses, and evaluation of viral community function from genomic sequences. *Microbiome* 2020;**8**:90.
70. Garretto A, Hatzopoulos T, Putonti C. virMine: automated detection of viral sequences from complex metagenomic samples. *PeerJ* 2019;**7**:e6695.
71. Guo J, Bolduc B, Zayed AA, *et al.* VirSorter2: a multi-classifier, expert-guided approach to detect diverse DNA and RNA viruses. *Microbiome* 2021;**9**:37.
72. Zhao G, Wu G, Lim ES, *et al.* VirusSeeker, a computational pipeline for virus discovery and virome composition analysis. *Virology* 2017;**503**:21–30.
73. Abdelkareem AO, Khalil MI, Elaraby M, *et al.* VirNet: Deep Attention Model for Viral Reads Identification.In: *2018 13th International Conference on Computer Engineering and Systems (ICCES)*. 2018;**623**:626.
74. Amgarten D, Braga LPP, da Silva AM, *et al.* MARVEL, a tool for prediction of bacteriophage sequences in metagenomic bins. *Front Genet* 2018;**9**:304.
75. Tampuu A, Bzhalava Z, Dillner J, *et al.* ViraMiner: deep learning on raw DNA sequences for identifying viral genomes in human samples. *PLoS One* 2019;**14**:e0222271.
76. Babayan SA, Orton RJ, Streicker DG. Predicting reservoir hosts and arthropod vectors from evolutionary signatures in RNA virus genomes. *Science* 2018;**362**:577–80.
77. Galan W, Bak M, Jakubowska M. Host taxon predictor - a tool for predicting taxon of the host of a newly discovered virus. *Sci Rep* 2019;**9**:3436.
78. Andrej Baláž MK, Budiš J, Szemeš T, *et al.* PHERI - Phage Host Exploration pipeline. *bioRxiv* 2020.
79. Young F, Rogers S, Robertson DL. Predicting host taxonomic information from viral genomes: a comparison of feature representations. *PLoS Comput Biol* 2020;**16**:e1007894.
80. Amgarten D, Iha BKV, Piroupo CM, *et al.* vHULK, A New Tool for Bacteriophage Host Prediction Based on Annotated Genomic Features and Deep Neural Networks. *bioRxiv* 2020.
81. Tan J, Fang Z, Wu S, *et al.* HoPhage: an ab initio tool for identifying hosts of phage fragments from metaviromes. *Bioinformatics* 2021;**38**:543–5.
82. Shang J, Sun Y. Predicting the hosts of prokaryotic viruses using GCN-based semi-supervised learning. *BMC Biol* 2021;**19**:250.
83. Dion MB, Plante PL, Zufferey E, *et al.* Streamlining CRISPR spacer-based bacterial host predictions to decipher the viral dark matter. *Nucleic Acids Res* 2021;**49**:3127–38.
84. Pons JC, Paez-Espino D, Riera G, *et al.* VPF-class: taxonomic assignment and host prediction of uncultivated viruses based on viral protein families. *Bioinformatics* 2021;**37**:1805–13.
85. Zielezinski A, Deorowicz S, Gudys A. PHIST: fast and accurate prediction of prokaryotic hosts from metagenomic viral sequences. *Bioinformatics* 2021;**38**(5):1447–9.
86. Boeckaerts D, Stock M, Criel B, *et al.* Predicting bacteriophage hosts based on sequences of annotated receptor-binding proteins. *Sci Rep* 2021;**11**:1467.
87. Lu C, Zhang Z, Cai Z, *et al.* Prokaryotic virus host predictor: a Gaussian model for host prediction of prokaryotic viruses in metagenomics. *BMC Biol* 2021;**19**:5.
88. Liu D, Ma Y, Jiang X, *et al.* Predicting virus-host association by Kernelized logistic matrix factorization and similarity network fusion. *BMC Bioinformatics* 2019;**20**:594.
89. Li M, Wang Y, Li F, *et al.* A deep learning-based method for identification of bacteriophage-host interaction. *IEEE/ACM Trans Comput Biol Bioinform* 2021;**18**:1801–10.

90. Zheng N, Wang K, Zhan W, *et al.* Targeting virus-host protein interactions: feature extraction and machine learning approaches. *Curr Drug Metab* 2019;**20**:177–84.
91. Zhang R, Mirdita M, Levy Karin E, *et al.* SpacePHARER: sensitive identification of phages from CRISPR spacers in prokaryotic hosts. *Bioinformatics* 2021;**37**:3364–6.
92. Li M, Zhang W. PHIAF: prediction of phage-host interactions with GAN-based data augmentation and sequence-based feature fusion. *Brief Bioinform* 2022;**23**(1):bbab348.
93. Zhou F, Gan R, Zhang F, *et al.* PHISDetector: a tool to detect diverse in silico phage-host interaction signals for virome studies. *Genomics Proteomics Bioinformatics* 2022;**20**(3):508–23.
94. Shang J, Sun Y. CHERRY: a computational metHod for accuratE pRediction of virus-pRokarYotic interactions using a graph encoder-decoder model. *Brief Bioinform* 2022;**23**:bbac182.
95. Suchet Aggarwal AD, Patiyal S, Choudhury S, *et al.* An Ensemble Method for Designing Phage-Based Therapy Against Bacterial Infections. *bioRxiv* 2022.
96. Edwards RA, McNair K, Faust K, *et al.* Computational approaches to predict bacteriophage-host relationships. *FEMS Microbiol Rev* 2016;**40**:258–72.
97. Mock F, Viehweger A, Barth E, *et al.* VIDHOP, viral host prediction with deep learning. *Bioinformatics* 2021;**37**:318–25.
98. Alguwaizani S, Park B, Zhou X, *et al.* Predicting interactions between virus and host proteins using repeat patterns and composition of amino acids. *J Healthc Eng* 2018;**2018**:1391265.
99. Hauser R, Blasche S, Dokland T, *et al.* Bacteriophage protein-protein interactions. *Adv Virus Res* 2012;**83**:219–98.
100. Kabir M, Nantasenamat C, Kanthawong S, *et al.* Large-scale comparative review and assessment of computational methods for phage virion proteins identification. *EXCLI J* 2022;**21**:11–29.
101. Manavalan B, Shin TH, Lee G. PVP-SVM: sequence-based prediction of phage virion proteins using a support vector machine. *Front Microbiol* 2018;**9**:476.
102. Charoenkwan P, Kanthawong S, Schaduangrat N, *et al.* PVPred-SCM: improved prediction and analysis of phage virion proteins using a scoring card method. *Cell* 2020;**9**(2):353.
103. Pan Y, Gao H, Lin H, *et al.* Identification of bacteriophage virion proteins using multinomial Naïve Bayes with g-gap feature tree. *Int J Mol Sci* 2018;**19**(6):1779.
104. Cantu VA, Salamon P, Seguritan V, *et al.* PhANNs, a fast and accurate tool and web server to classify phage structural proteins. *PLoS Comput Biol* 2020;**16**:e1007845.
105. Seguritan V, Alves N, Jr, Arnoult M, *et al.* Artificial neural networks trained to detect viral and phage structural proteins. *PLoS Comput Biol* 2012;**8**:e1002657.
106. Zhang L, Zhang C, Gao R, *et al.* An ensemble method to distinguish bacteriophage Virion from non-virion proteins based on protein sequence characteristics. *Int J Mol Sci* 2015;**16**:21734–58.
107. Cheng, *et al.* Prediction of bacteriophage proteins located in the host cell using hybrid features. *Chemom Intel Lab Syst* 2018;**180**:64–9.
108. Tan JX, Dao FY, Lv H, *et al.* Identifying phage virion proteins by using two-step feature selection methods. *Molecules* 2018;**23**(8):2000.
109. Arif M, Ali F, Ahmad S, *et al.* Pred-BVP-Unb: fast prediction of bacteriophage virion proteins using un-biased multi-perspective properties with recursive feature elimination. *Genomics* 2020;**112**:1565–74.
110. Charoenkwan P, Nantasenamat C, Hasan MM, *et al.* Meta-iPVP: a sequence-based meta-predictor for improving the prediction of phage virion proteins using effective feature representation. *J Comput Aided Mol Des* 2020;**34**:1105–16.
111. Haitao Han WZ, Ding C, Liu T. *iPVP-MCV: A Multi-Classifier Voting Model for the Accurate Identification of Phage Virion Proteins*. Symmetry 2021;**13**(8):1506.
112. Fang Z, Zhou H. VirionFinder: identification of complete and partial prokaryote virus Virion protein from virome data using the sequence and biochemical properties of amino acids. *Front Microbiol* 2021;**12**:615711.
113. Ahmad S, Charoenkwan P, Quinn JMW, *et al.* SCORPION is a stacking-based ensemble learning framework for accurate prediction of phage virion proteins. *Sci Rep* 2022;**12**:4106.
114. Harrison E, Brockhurst MA. Ecological and evolutionary benefits of temperate phage: what does or doesn't kill you makes you stronger. *Bioessays* 2017;**39**(12):1700112.
115. Piotr Tynecki AG, Kazimierczak J, Jadczuk M, *et al.* PhageAI - Bacteriophage Life Cycle Recognition with Machine Learning and Natural Language Processing. *bioRxiv* 2020.
116. McNair K, Bailey BA, Edwards RA. PHACTS, a computational approach to classifying the lifestyle of phages. *Bioinformatics* 2012;**28**:614–8.
117. Hockenberry AJ, Wilke CO. BACPHLIP: predicting bacteriophage lifestyle from conserved protein domains. *PeerJ* 2021;**9**:e11396.
118. Canchaya C, Fournous G, Brussow H. The impact of prophages on bacterial chromosomes. *Mol Microbiol* 2004;**53**:9–18.
119. Lima-Mendez G, Van Helden J, Toussaint A, *et al.* Prophinder: a computational tool for prophage prediction in prokaryotic genomes. *Bioinformatics* 2008;**24**:863–5.
120. Zhou Y, Liang Y, Lynch KH, *et al.* PHAST: a fast phage search tool. *Nucleic Acids Res* 2011;**39**:W347–52.
121. Akhter S, Aziz RK, Edwards RA. PhiSpy: a novel algorithm for finding prophages in bacterial genomes that combines similarity- and composition-based strategies. *Nucleic Acids Res* 2012;**40**:e126.
122. Arndt D, Grant JR, Marcu A, *et al.* PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 2016;**44**:W16–21.
123. Song W, Sun HX, Zhang C, *et al.* Prophage Hunter: an integrative hunting tool for active prophages. *Nucleic Acids Res* 2019;**47**:W74–80.
124. Reis-Cunha JL, Bartholomeu DC, Manson AL, *et al.* ProphET, prophage estimation tool: a stand-alone prophage sequence prediction tool with self-updating reference database. *PLoS One* 2019;**14**:e0223364.
125. Starikova EV, Tikhonova PO, Prianichnikov NA, *et al.* Phigaro: high-throughput prophage sequence annotation. *Bioinformatics* 2020;**36**:3882–4.
126. Tang K, Wang W, Sun Y, *et al.* Prophage Tracer: precisely tracing prophages in prokaryotic genomes using overlapping split-read alignment. *Nucleic Acids Res* 2021;**49**:e128.
127. Siren K, Millard A, Petersen B, *et al.* Rapid discovery of novel prophages using biological feature engineering and machine learning. *NAR Genom Bioinform* 2021;**3**:lqaa109.
128. Gauthier CH, Abad L, Venbakkam AK, *et al.* DEPhT: a novel approach for efficient prophage discovery and precise extraction. *Nucleic Acids Res* 2022;**50**:e75.
129. Gan R, Zhou F, Si Y, *et al.* DBSCAN-SWA: an integrated tool for rapid prophage detection and annotation. *Front Genet* 2022;**13**:885048.
130. Rohde C, Wittmann J, Kutter E. Bacteriophages: a therapy concept against multi-drug-resistant bacteria. *Surg Infect (Larchmt)* 2018;**19**:737–44.

131. Expert round table on acceptance and re-implementation of bacteriophage therapy, Sybesma W, Rohde C, *et al.* Silk route to the acceptance and re-implementation of bacteriophage therapy-part II. *Antibiotics (Basel)* 2018;**7**:35.
132. Bruttin A, Brussow H. Human volunteers receiving Escherichia coli phage T4 orally: a safety test of phage therapy. *Antimicrob Agents Chemother* 2005;**49**:2874–8.
133. Jault P, Leclerc T, Jennes S, *et al.* Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by Pseudomonas aeruginosa (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect Dis* 2019;**19**: 35–45.
134. Voelker R. FDA approves bacteriophage trial. *JAMA* 2019; **321**:638.
135. Sarker SA, Sultana S, Reuteler G, *et al.* Oral phage therapy of acute bacterial Diarrhea with two Coliphage preparations: a randomized trial in children from Bangladesh. *EBioMedicine* 2016;**4**:124–37.
136. Fish R, Kutter E, Wheat G, *et al.* Bacteriophage treatment of intransigent diabetic toe ulcers: a case series. *J Wound Care* 2016;**25**:S27–33.
137. Jennes S, Merabishvili M, Soentjens P, *et al.* Use of bacteriophages in the treatment of colistin-only-sensitive Pseudomonas aeruginosa septicaemia in a patient with acute kidney injury-a case report. *Crit Care* 2017;**21**:129.
138. Loc-Carrillo C, Abedon ST. Pros and cons of phage therapy. *Bacteriophage* 2011;**1**:111–4.
139. Kudrin P, Varik V, Oliveira SR, *et al.* Subinhibitory concentrations of bacteriostatic antibiotics induce relA-dependent and relA-independent tolerance to beta-lactams. *Antimicrob Agents Chemother* 2017;**61**(4):e02173–16.
140. Hampton HG, Watson BNJ, Fineran PC. The arms race between bacteria and their phage foes. *Nature* 2020;**577**: 327–36.
141. Golkar Z, Bagasra O, Pace DG. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *J Infect Dev Ctries* 2014;**8**:129–36.
142. Carlton RM. Phage therapy: past history and future prospects. *Arch Immunol Ther Exp (Warsz)* 1999;**47**:267–74.
143. Weber-Dabrowska B, Mulczyk M, Gorski A. Bacteriophage therapy of bacterial infections: an update of our institute's experience. *Arch Immunol Ther Exp (Warsz)* 2000;**48**: 547–51.
144. Abedon ST. Kinetics of phage-mediated biocontrol of bacteria. *Foodborne Pathog Dis* 2009;**6**:807–15.
145. Sulakvelidze A, Alavidze Z, Morris JG, Jr. Bacteriophage therapy. *Antimicrob Agents Chemother* 2001;**45**:649–59.
146. Dy RL, Richter C, Salmond GP, *et al.* Remarkable mechanisms in microbes to resist phage infections. *Annu Rev Virol* 2014;**1**: 307–31.
147. Roach DR, Leung CY, Henry M, *et al.* Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host Microbe* 2017;**22**:38–47.e4.
148. Davis BM, Waldor MK. Filamentous phages linked to virulence of Vibrio cholerae. *Curr Opin Microbiol* 2003;**6**:35–42.
149. Nau R, Eiffert H. Modulation of release of proinflammatory bacterial compounds by antibacterials: potential impact on course of inflammation and outcome in sepsis and meningitis. *Clin Microbiol Rev* 2002;**15**:95–110.
150. Tetz GV, Ruggles KV, Zhou H, *et al.* Bacteriophages as potential new mammalian pathogens. *Sci Rep* 2017;**7**:7043.
151. Domingo-Calap P, Delgado-Martinez J. Bacteriophages: protagonists of a post-antibiotic era. *Antibiotics (Basel)* 2018;**7**(3):66.
152. Pirnay JP, De Vos D, Verbeken G, *et al.* The phage therapy paradigm: pret-a-porter or Sur-mesure? *Pharm Res* 2011;**28**: 934–7.
153. Gill JJ, Pacan JC, Carson ME, *et al.* Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical Staphylococcus aureus mastitis in lactating dairy cattle. *Antimicrob Agents Chemother* 2006;**50**:2912–8.
154. Goodridge LD. Designing phage therapeutics. *Curr Pharm Biotechnol* 2010;**11**:15–27.
155. Lin J, Du F, Long M, *et al.* Limitations of phage therapy and corresponding optimization strategies: a review. *Molecules* 2022;**27**(6):1857.
156. Gorski A, Miedzybrodzki R, Wegrzyn G, *et al.* Phage therapy: current status and perspectives. *Med Res Rev* 2020;**40**: 459–63.
157. Dzunkova M, Low SJ, Daly JN, *et al.* Defining the human gut host-phage network through single-cell viral tagging. *Nat Microbiol* 2019;**4**:2192–203.
158. Hyman P. Phages for phage therapy: isolation, characterization, and host range breadth. *Pharmaceuticals (Basel)* 2019;**12**(1):35.
159. Camarillo-Guerrero LF, Almeida A, Rangel-Pineros G, *et al.* Massive expansion of human gut bacteriophage diversity. *Cell* 2021;**184**:1098–1109.e9.
160. Lavigne R, Ceyssens PJ, Robben J. Phage proteomics: applications of mass spectrometry. *Methods Mol Biol* 2009;**502**:239–51.
161. Hanna LF, Matthews TD, Dinsdale EA, *et al.* Characterization of the ELPhiS prophage from Salmonella enterica serovar Enteritidis strain LK5. *Appl Environ Microbiol* 2012;**78**:1785–93.
162. Versoza CJ, Pfeifer SP. Computational prediction of bacteriophage host ranges. *Microorganisms* 2022;**10**(1):149.
163. Morozova VV, Vlassov VV, Tikunova NV. Applications of bacteriophages in the treatment of localized infections in humans. *Front Microbiol* 2018;**9**:1696.
164. Abedon ST, Kuhl SJ, Blasdel BG, *et al.* Phage treatment of human infections. *Bacteriophage* 2011;**1**:66–85.
165. Suh GA, Lodise TP, Tamma PD, *et al.* Considerations for the use of phage therapy in clinical practice. *Antimicrob Agents Chemother* 2022;**66**:e0207121.
166. Al-Ishaq RK, Skariah S, Busselberg D. Bacteriophage treatment: critical evaluation of its application on World Health Organization priority pathogens. *Viruses* 2020;**13**(1):51.
167. Hermoso JA, Garcia JL, Garcia P. Taking aim on bacterial pathogens: from phage therapy to enzybiotics. *Curr Opin Microbiol* 2007;**10**:461–72.
168. Aslam B, Khurshid M, Arshad MI, *et al.* Antibiotic resistance: one health one world outlook. *Front Cell Infect Microbiol* 2021;**11**:771510.
169. Pires DP, Costa AR, Pinto G, *et al.* Current challenges and future opportunities of phage therapy. *FEMS Microbiol Rev* 2020;**44**: 684–700.
170. McCallin S, Sarker SA, Sultana S, *et al.* Metagenome analysis of Russian and Georgian Pyophage cocktails and a placebo-controlled safety trial of single phage versus phage cocktail in healthy Staphylococcus aureus carriers. *Environ Microbiol* 2018;**20**:3278–93.
171. Aghebati-Maleki L, Bakhshinejad B, Baradaran B, *et al.* Phage display as a promising approach for vaccine development. *J Biomed Sci* 2016;**23**:66.
172. Chhibber S, Bansal S, Kaur S. Disrupting the mixed-species biofilm of Klebsiella pneumoniae B5055 and

Pseudomonas aeruginosa PAO using bacteriophages alone or in combination with xylitol. *Microbiology (Reading)* 2015;**161**: 1369–77.

173. Kamal F, Dennis JJ. Burkholderia cepacia complex phage-antibiotic synergy (PAS): antibiotics stimulate lytic phage activity. *Appl Environ Microbiol* 2015;**81**:1132–8.

174. Tkhilaishvili T, Winkler T, Muller M, *et al.* Bacteriophages as adjuvant to antibiotics for the treatment of periprosthetic joint infection caused by multidrug-resistant Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2019;**64**(1):e00924–19.

175. Khot V, Strous M, Hawley AK. Computational approaches in viral ecology. *Comput Struct Biotechnol J* 2020;**18**:1605–12.