Chapter

Role of Phage Therapy in COVID-19 Infection: Future Prospects

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Abstract

The pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan City, China, in 2019. After that, the outbreak has grown into a global pandemic and definite treatment for the disease, termed coronavirus disease 2019 (COVID-19), is currently unavailable. The slow translational progress in the field of research suggests that a large number of studies are urgently required for targeted therapy. In this context, this hypothesis explores the role of bacteriophages on SARS-CoV-2, especially concerning phage therapy (PT). Several studies have confirmed that in addition to their antibacterial abilities, phages also show antiviral properties. It has also been shown that PT is effective for building immunity against viral pathogens by reducing the activation of NF kappa B; additionally, phages produce the antiviral protein phagicin. Phages can also induce antiviral immunity by upregulating expression of defensin 2. Phages may protect eukaryotic cells by competing with viral adsorption and viral penetration of cells, virus mediated cell apoptosis as well as replication. Moreover, by inhibiting activation of NF-κB and ROS production, phages can down regulate excessive inflammatory reactions relevant in clinical course of COVID-19. In this chapter, we hypothesize that the PT may play a therapeutic role in the treatment of COVID-19.

Keywords: phage therapy, phage display, NF-κB, bacteriophage, SARS-CoV-2, COVID-19

1. Introduction

Phages are infections caused by bacterial viruses and they are the most bountiful elements on the Earth [1], due to the introduction of antibiotics their application in clinical practice was immediately overcome in Western countries [2]. Patients with antibiotic-resistant infections are traveling from different spots to Georgia and Poland for phage treatments [3]. Despite all the success cases of patients, phage therapy is still faces significant obstacles, particularly administrative issues. In European countries and United States several on-going efforts are being led for the acceptance of phage therapy [4]. In this chapter, we will first discuss the early and current state of phage therapy, address the major challenges faced by phage therapy treatment in Covid-19 infection and the future prospects in this field [5].

1.1 Early studies of phage therapy (PT)

The efficacy of the phage treatment was confirmed when three patients having the same infection dysentery treated with one dose of the anti dysentery phages and recovered within 24 hours of treatment but his study was not published [1]. However, treatment of infectious diseases of humans reported in 1921 by Richard Bruynoghe and Joseph Maisin, who used bacteriophages to treat staphylococcal skin disease [6]. In addition, d'Herelle used various phage preparations in India to treat thousands of peoples suffering with cholera and bubonic plague [7].

Phages mediate immune regulatory and immunotherapeutic trials that are significant in balancing the immunological homeostasis in human [8]. It was suggested that the viability of PT in autoimmune diseases and it can also be used to for the treatment of infection caused by SARS-CoV-2virus [9]. To determine the infection in mass population, a single sewage test is enough to examine the whole population has been infected or not because RNA of SARS-CoV-2 remains stable with the capsid [10]. However, it has been found from accessible information that although the concentration of the virus in sewage water is high but the transmission risk via this route is very low. This information can play a major role in managing COVID-19 [11].

Phage display technique of producing antibodies was developed for MERS-CoV and effectively applied in light of the fact that bacteriophages have the potential to produce recombinant antibodies (Ab) rapidly [12]. Another Yin-Yang biopanning technique features the chance of utilizing crude antigens for the isolation of monoclonal Ab by phage display method [13]. Before using these expensive techniques, production of artificial Ab was primarily done by using animals but it is a slow process and less cost effective than using bacteriophage display techniques [14]. Bacteriophage could be used to decrease the mortality rate due to Covid-19 pandemic, and for the production of artificial Ab against SARS-CoV-2 in the early stages of infection [15, 16].

2. Interactions between phages and the immune system

It is well known that the immune system plays an important role in phage clearance from animal and human bodies [17]. Components of the mono nuclear phagocyte system (MPS) in the spleen and liver are major sites of phage accumulation. The MPS has been credited for the quick expulsion of administered wild-type phage λ from the human circulatory system [18]. In addition, these phages can directly interact with immune cells by either interacting with cell surface molecules or receptors or through phage transcytosis [19]. Besides the take-up of phages, by Ag presenting cells (APC; e.g., dendritic cells) prompts the activation of B-cells and the exhibition of specific Ab against the phage as shown in **Figure 1** [20].

3. Mode of action

In spite of the huge number of publications on phage therapy, there are only few reports in which the pharmacokinetics of therapeutic phage preparations is depicted [21]. Phages get into the circulatory system of experimental animals (after giving a single dose orally) within 2 to 4 h and they reached into the internal organs within 10 hours and can remains in the human body up to several days [22]. In any case extra exploration is required in order to obtain rigorous

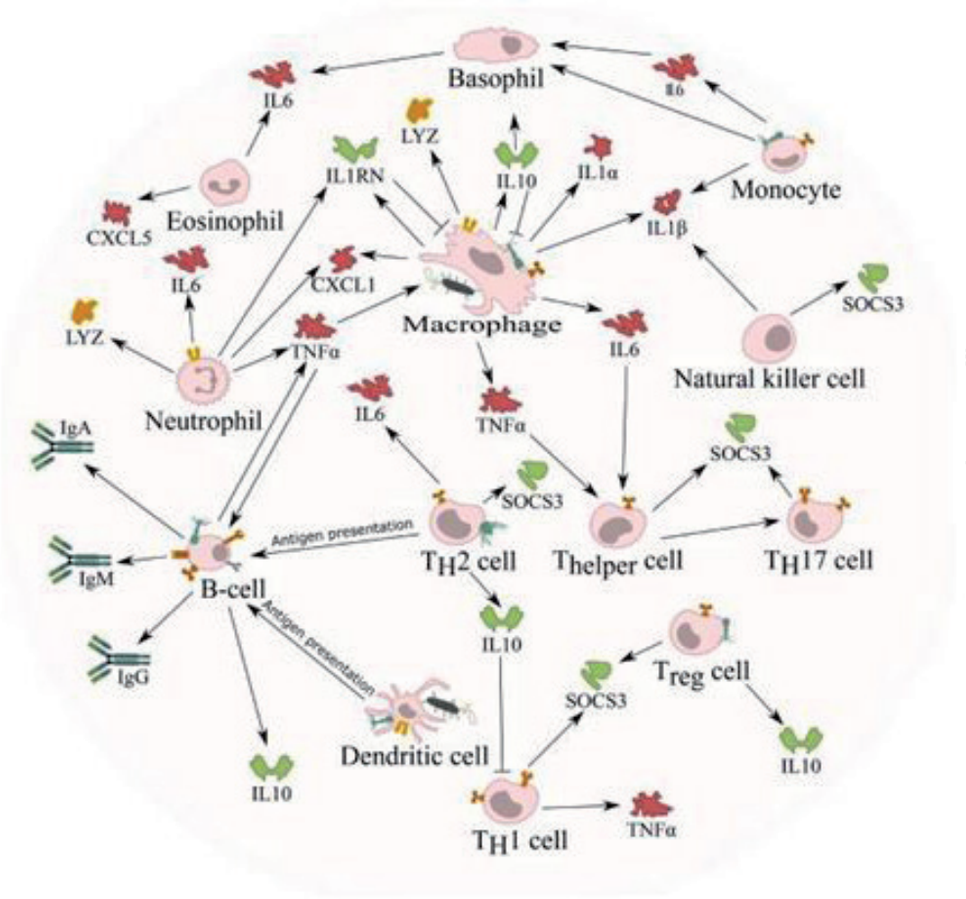

Figure 1.
Interaction of bacteriophage with mammalian immune cells (Belleghem et al. [20]).

pharmacological information concerning lytic phages, including full-scale toxicological research [23]. However, after few years studies reveal that not all phages replicate correspondingly and that there are significant differences in the replication cycles of lytic and lysogenic phages as shown in **Figure 2** [11]. Moreover, it is possible that numerous therapeutic phages act through a common path; however, it may also possible that some therapeutic phages have some distinctive unidentified genes or some unknown mechanisms responsible for lysis of their target bacteria [24]. In a study conducted by *Sulakvelidze et al*. More interpretation of these and common mechanisms is likely to produce

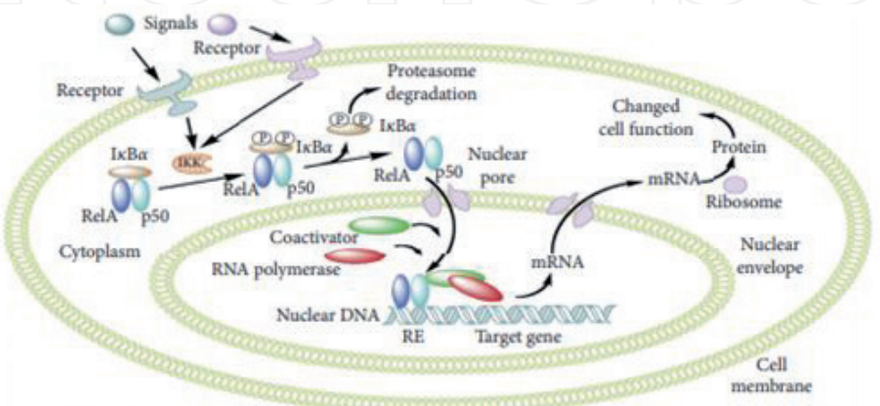

Figure 2.
Mechanism of phage action in bacterial cell (Mishra et al. [11]).

information useful for genetically engineering which was helpful in effective therapeutic phage preparations for the treatment of Coronavirus [7].

3.1 How can ms2 bacteriophage help to fight against coronavirus?

MS2 Bacteriophage is contemplating as a control to study molecular biology processes. It includes viral RNA replication, translation method, and physiology of infected cells. MS2 RNA coding for viral polypeptides includes protein A, coat protein, and RNA replicase complex. The structure of the MS2 virus comprises of Protein A and coat protein makeup.MS2 Bacteriophage can be used as an internal control in RT-PCR testing for COVID-19 to prevent false negative results and to verify the efficacy of the sample preparation and absence of inhibitors in the PCR reaction [25].

4. Phage therapy in humans

Human phage therapy has been practiced in France since 1919, d'Hérelle carried out very extensive studies especially in fowl typhoid and in cholera. In 1921 Bruynoghe and Maisin, Belgium reported that injecting phages targeting *Staphylococcus* near the base of cutaneous boils (furuncles and carbuncles), prompted improvement within 48 hours includes reduction in irritability [26–28].

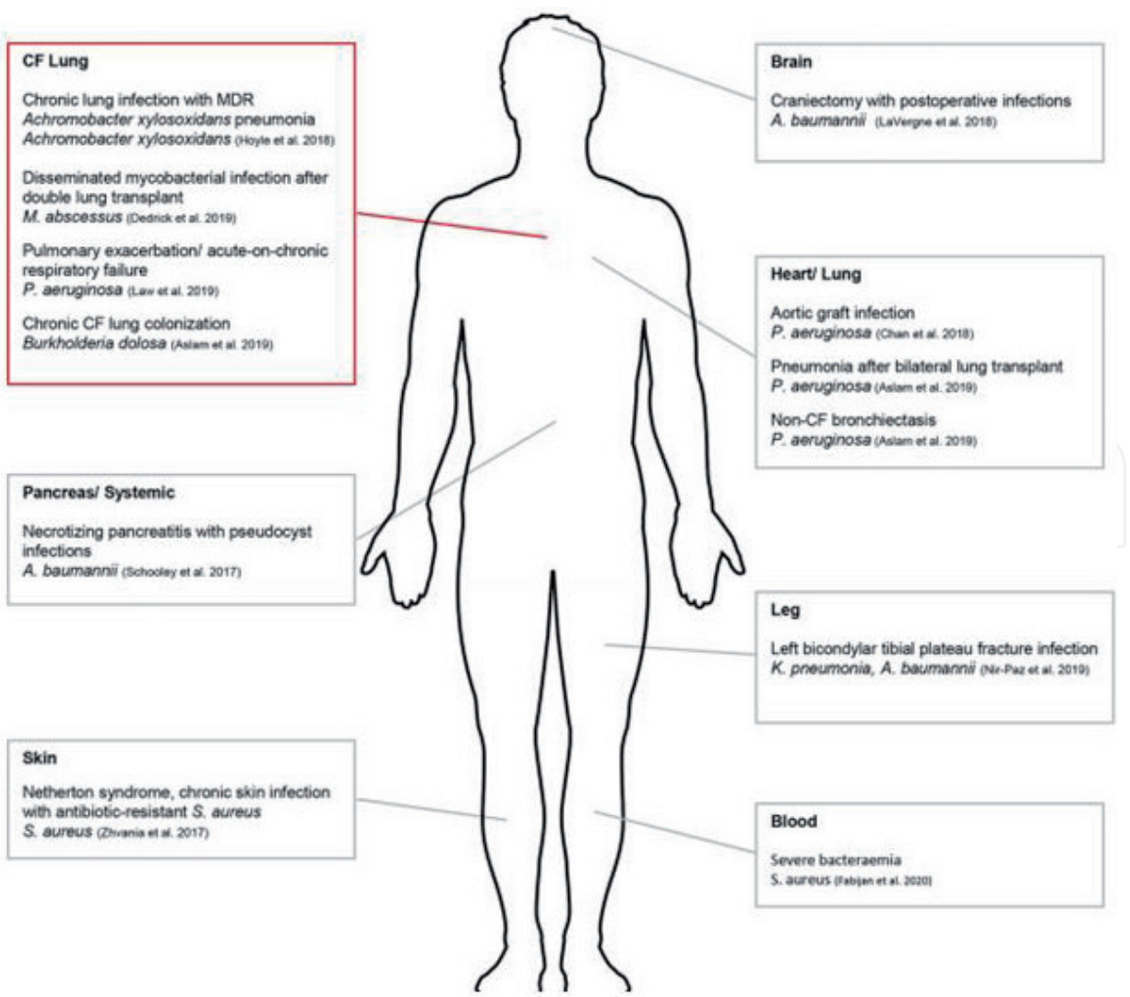

Figure 3.
Schematic diagram indicating areas where phage therapy had been applied clinically (Ng et al. [33]).

A study conducted in by *G. Lang* revealed the utilization of bacteriophage in seven patients with chronic orthopedic infections with antibiotic resistant organisms. He was able to fix two out of seven cases of hip prostheses (after removal of the prostheses) infected by Gram-negative bacteria, one case of tibial osteomyelitis because of the infection caused by*Proteus spp.*, *Staphylococcus aureus* and *Klebsiella spp*.; one instance of septic arthritis of the knee caused due to *Enterobacter* spp. and *Staphylococcus aureus,* one case of septic non-union of the femur due to pan-drug resistant (PDR) *Providencia* [29, 30]. *Henri de Montclos* expressed that phage appear to be safe for human cells though potentially there could be problems associated with their modes of preparation. He also stated about propagation on media produced from animal tissues [31].

The Pasteur Institute stopped making therapeutic cocktails of phages but few French physicians have continued to use phages therapeutically and obtaining their phages from Russia or Georgia. Infections through *Staphylococcus* appear to be the most common target which was treated by phages. In 2011 *Abedon et al*. reported successful phage therapy in two patients from France and Australia who had strong history of antibiotics treatment and other therapies [32]. There are many body places, where phage therapy have been applied and investigated as shown in **Figure 3** [33].

5. A future for phages

The research on phages and their possible antiviral properties are fundamental and should be approved by meticulous *in-vitro* and *in-vivo* studies. If lab research shows some promising results, then it could be possible to have clinical research and randomized stage from one to three human trials to prove their therapeutic utility. Phage therapy may likewise hold promise as a treatment for SARS-CoV-2 [11].

The bacterial growth rate might potentially be diminished by the aerosol use of bacteriophages that prey on the original species of bacteria responsible to cause respiratory failures [34]. This can occur in a self-administrative manner, similar to prey–predator regulation in ecosystem. The remarkable development of the bacteriophage population should allow for a fast clearance, particularly in situations where the bacterial population has already grown significantly [35].

In a study conducted by *Prazak et al.* in 2020, they found the evidences that pneumonia can be treated by nebulized bacteriophages. Target bacteria that commonly cause respiratory problems and selection of bacteriophages can be quickly identified through screening method and by group of experts. Prophylactically administered bacteriophages decreased lung bacterial burdens and improved endurance of antibiotic resistant *S. aureus* infected animals with regards to ventilator-associated pneumonia [36]. It should be ensured to have the right selection of bacteriophages that target both the optimal bacteria and should be most effective against bacterial population growth. The bacteriophages should not interfere with the patient's innate or adaptive immunity. It is also very necessary to rule out that patient does not have antibodies toward bacteriophages used, nor develops any antibodies toward bacteriophages to clear off the bacteriophage earlier than to SARS-CoV-2. If required, quantitative microbiome sequencing can be used potentially in phage therapy [16].

Another obstruction could be a risk of particular species of micro-organism which may develop resistance to the bacteriophage [37]. However, this would be significantly less serious than the drug resistance problem as it would just reduce the efficacy of that one bacteriophage and there is the chance of the bacteriophage also adapting to overcome any resistance to it. They are much specific to one species of bacteria and there is very minor possibility of the bacteriophage damaging any

beneficial bacteria but still these things need to be verified through clinical trials. It has to be noted that decrease bacterial growth in critical time of illness allows the patient more time to recover from the SAR-CoV-2 infection [16].

6. Development of a phage display panning strategy

The phage display technology is based on the integration of a gene encoding a peptide or a protein fused with the phage coat proteins was first described by George Smith in 1985 [38]. The most broadly used coat proteins for display are the PVIII and PIII proteins; however, other coat proteins likewise been utilized for display. As a result of its high copy number (~2700 copies), the PVIII protein has been just utilized for the display of small peptides due to conformational issues hampering capsid formation. The PIII system, on the other hand, with its low copy number (5 copies), allows the display of larger molecules such as recombinant antibodies [12]. The first phage display system as shown in **Figure 4** displaying antibodies was explained by *Mc Cafferty et al*. in 1990. They effectively showed variable regions of antibody on phages by using immunoglobulin variable genes of hybridomas and B cells [39]. After its innovation, phage display technology has been extensively used for the research and discovery of antibodies or peptides against a large variety of antigens in many fields of application such as toxicology, drug discovery, immunization, epitope mapping and virus or toxin neutralization by using phage peptide and antibody libraries. Phage display technology has been intensively used for the production of neutralizing antibodies as shown in **Table 1** [40].

Various antibody libraries of different methodologies and strategies have been screened against SARS-CoV-2 spike protein and its receptor binding domain (RBD). Few studies have focused on screening previously developed libraries against SARS-CoV and MERS-CoV and finding cross-reactive antibodies. Others have performed screenings against semisynthetic or synthetic antibody libraries [41]. Phage displayed single-domain antibody was previously developed from llama, which simultaneously neutralizes the S antigen of SARS-CoV and also help in the neutralization of S antigen of the pseudotyped virus SARS-CoV-2 as a bivalent human IgG Fc-fusion protein. A selected antibody has high affinity to RBD, for this a library

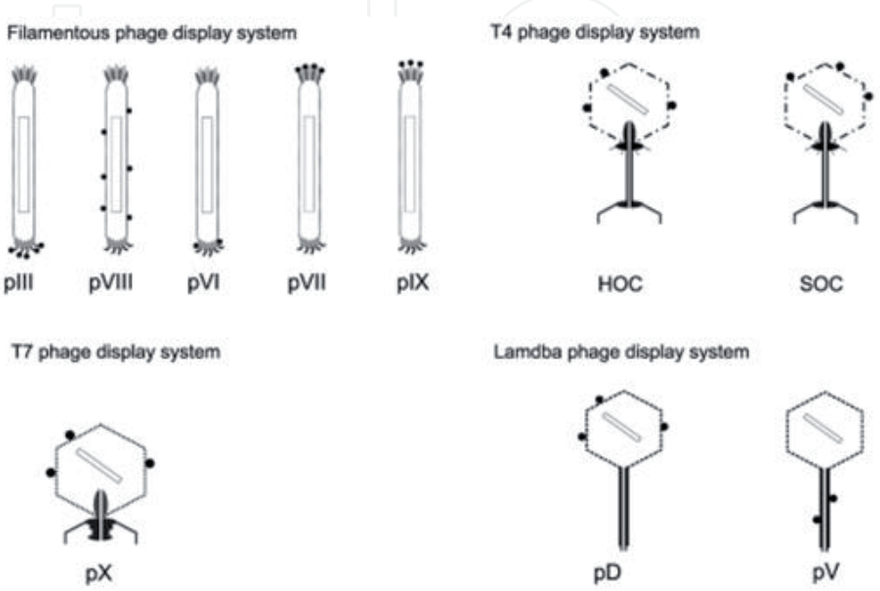

Figure 4.
Schematic presentation of phage display systems (Bazan et al. [12]).

Methods	Original antibody	Reformated antibody	Target region	In vitro/in vivo mode
Phage display	Single-domain antibody from llama	Bivalent human IgG Fc fusion protein	SARS-CoV-2 Spike antigen	Pseudotyped virus and SARS-CoV-2 virus neutralization assay
	Synthetic human Fab library	CDR3 Diversification by mutations	SARS-CoV-2 Spike antigen RBD	Pseudotyped virus neutralization assay
	Single-domain antibody	Grafting naive CDR regions into the framework region of an allele in human antibody heavy chain variable region	RBD domain and the S1 subunit of SARS-CoV-2	Pseudotyped virus neutralization assay
	Naive human scFv antibody	Human IgG1 antibody (4A3)	SARS-CoV-2 RBD	Pseudotyped virus neutralization assay
	Domain library	Fused with human Fc	SARS-CoV-2-RBD	Pseudotyped virus and SARS-CoV-2 virus neutralization assay

Table 1.
*Phage display strategies for neutralizing antibody development (*BalcioĞlu et al*. [15]).*

constructed and screened against the RBD domain of the SARS-CoV-2 spike antigen known as phage displayed synthetic human Fab library [42].

ELISA and pseudo typed virus neutralization assay. A phage-displayed single-domain antibody library has been developed by grafting naive CDR regions into the framework region of an allele in the human antibody heavy chain variable region. They made affinity selection against the RBD domain and the S1 subunit of SARS-CoV-2 and chose several neutralizing antibodies, including a "cryptic" epitope located in the spike's trimeric interface.A site directed screening was performed in a naive human scFv antibody library and domain antibody library by phage display against SARS-CoV-2 RBD. After several rounds of screening, they obtained 9 enriched clones from the domain antibody library and a single clone from the scFv antibody library. The scFv clone was reformatted into a human IgG1 antibody, while the domain antibody clones were fused with human Fc tag. A potential neutralizing effect of these recombinant antibody structures revealed with pseudotyped virus neutralization assay [15].

The future of phage therapy is not necessarily to replace current therapies, rather there is potential for clinical applications to enhance and provide another treatment for infections. Research in this area is likely to grow at an exponential rate. However, the full potential of phage therapy can only be accomplished when there is transparency and an eagerness to share knowledge as well as resources. Preferably, phage libraries should be freely accessible through a network of collaboration, information on preparation and delivery methods for phages implied for clinical usage should be well documented. Phage articulation and delivery are also critical considerations in order to direct activity to targeted areas and maximize efficacy. In fact, use of phage therapy already appears to be as of now gives off an impression of being composed in different nations, and by major public health institutes such as Therapeutic Goods Administration (TGA) (Australia), Food and Drug Authority (FDA) (United States of America) and the European Medicines Agency (EMA) (Europe). Importantly, a universal code of ethics should

be established and regulatory bodies reach a consensus on the exchange of information, usage of phages as treatment and reporting of treatment outcomes. Due to the critical nature of the rise of multiple drug resistance (MDR), expanding the urgency for phage therapy to be implemented as standard consideration, alternative therapies to be translated into clinical applications need to be expedited. A concerted effort with both national and international partners could see phage therapy being translated into standard care in the next 5 years [33].

7. Phages as potential inducers of antiviral immunity

There are also data suggesting that phages may drive antiviral immunity by inducing antiviral cytokines, for example, IFN-α and IL-12. An experimental study that phage RNA may induce IFN-α in human granulocytes [43]. Recently, *Sweere et al.* demonstrated that Pf phages (and phage RNA) endocytosed by leukocytes trigger TLR3-dependent pattern recognition receptors and inhibit TNF-driving type I IFN production [44]. The phage-dependent virucidal sign in the lungs could be happen in the phage has capable enough to penetrate the body organ through various routes; therefore phage therapy has been applied successfully in respiratory tract. Intriguingly a fine respiratory microbiome including bacteriophages, during the event of viral pathogens even such as Corona virus is also related with quite low percentage of phages. Recent data indicate that Lactobacillus, *E. coli* and *Bacteroides* phages and phage DNA may stimulate IFN-γ production via TLR9 activation. IFN-γ is another potent antiviral cytokine. Although, the increase TNF level might cause significant risk of virus replication. Hence, a therapeutic agent could regulate TNF production to keep the values at normal level for patient could be appreciated. Pre-clinical studies suggest that viral pneumonia may be cured by anti-TNF therapy. As increased levels of TNF are in blood samples and tissue from patients with COVID-19 may be inhibit TNF production through phage, which is confirmed by other author's previous reports that showed phage may down regulate TNF-α level in serum and lungs of mice with experimental acute pneumonia. Interestingly, clinical phage therapy may reduce TNF production when its pre-treatment level is high and increase it in low responders [45, 46].

These informations might be considered as a relevant argument for phages as a potential agent that could help to decrease TNF levels, allowing for appropriate antiviral immune responses in COVID-19 while reducing the risks of excessive immunosuppression. Different Phages may also interact with TLR [47]. TLR2 is involved in antiviral responses as a result of recognition of the repeating protein subunit patterns common to many viral capsids. [*Induction of Antiviral Immune Response through Recognition of the Repeating Subunit Pattern of Viral Capsid Is Toll-Like Receptor 2 Dependent*]. Other antiviral effects could be mediated by the A5/80 Staphylococcal phage through its ability to increase the expression of the IL-2 gene. IL-2 drives NK cell activity, which is important in defines against viral infections [47]. Phage can also induce antiviral immunity by up regulating expression of defense in IL-2, and recently shown that the T4 phage may induce a marked up regulation of gene coding for hBD2, a multifunctional peptide expressed mainly in epithelial cells with antiviral activity. Virus replication disrupt by the peptide through the binding of the virus by hBD2, decrease viral replication and modulation of signaling pathway essential for virucidal effects, even do the recruitment of immune cells contributing to antiviral activity leading to down regulation of cytopathic effects in human alveolar and laryngeal epithelial cells [48]. In some experiment studies in mice have revealed a co-relation between beta-defensin expression and pulmonary immunity. Moreover, participation of hBD2 in antiviral defenses in the respiratory tract has been confirmed in human disorders [49].

It was advised that phages could be reintroduced for the treatment of not only bacterial, but also other infections such as viral and fungal infections (Adv, *Epstein–Barr virus, Aspergillus fumigatus, Candida albicans*). It showed that there is evidence that proof phage could be comprised in current treatment being studied for re-purposing in the therapeutic treatment of COVID-19. According to *Gorskiet et al.* phage in COVID-19 could be in an adjunct antiviral therapy, which is quite similar to the current trend ofcombined phages with antibacterial treatment in bacterial infections. In other way, a standard phage therapy could be considered for the treatment of bacterial complications of COVID-19, which occur in >40% of patients [45, 50]. Phages may act as shield for eukaryotic cells by competing with surface assimilation and viral penetration of cells; virus mediated, programmed cell death as well as viral replica. Phages may also arouse antiviral immunity during contributing to a equal immune response. Moreover, by inhibiting activation of NF-κB and ROS production, phages can down regulate extreme inflammatory reactions relevant in pathology and clinical course of COVID-19. The data presented in this which was judged are often preliminary but suggest that further studies centered on the potential of phage therapy as at least an adjunct treatment of COVID-19 are warranted. Both general and remote safety of phage therapy was corroborating in human viral diseases. Therefore, extensive studies comprising relevant clinical trials are needed to prioritize applicability of phage to help fighting against COVID-19 pandemic [45].

8. Production of industrial phage propagation strains

The development of new page-based resources using traditional methods can be an on-going issue that may require hundreds of species to be treated with plasmids, active prophages, perhaps other mobile genomic elements. However, given the recent breakthroughs in synthetic biology and advances in re-integration with genetic engineering methods, this need not be the case. Even a given phage infects a particular type of bacterium strain from the affected species depending on the bacterial characteristics and the phage [45].

Metabolic compatibility of a bacterium with a phage to support the propagation of the phage in an already existing infection appears to be specific to certain species, but is sometimes extended to more than one species of bacteria of the same or different genera. Definitions of phage acquisition differentiation encoded by a variety of similar species include genes that include phage receptors or their means of integration and restriction-modification systems associated with the phage. In addition, bacteria, encrypt phage defense process but these mechanisms fortify the bacterium itself from infection through certain pathogens or through the propagation of phage, or induce apoptosis to protect people from the spread of the disease [45].

The distinct indicators of phage determinants are reversible between the strains of given species. Bacteria can gain or lose sensitivity to an appropriate phage or the ability to support the phage development by mutation-recombination-, or horizontal genetically modified changes in their phage orientation or phage defense determinants [51]. There are so many genes which are analogous with phage resistance or susceptibility exposure is carried by mobile genetic elements. Key features of Phage that are important of a metabolically-compatible host include the interaction of phage receptor-binding proteins and receptors on the surface of the bacterial cell, alignment of the phage genome with the bacterial restriction-modification mutation system, or the ability to prevent bacterial action by bacterial restriction-modification systems or by encoding efficient anti-restriction mechanisms. In addition, to infect bacteria, phage reproduce effectively, protein-induced phage allows them

to overcome bacterial phage-resistant strains, such as anti-CRISPR proteins and proteins that inhibit the action of Abi systems or toxin-antitoxin (TA) [51, 52].

The structure of each phage and its infectivity for a specific host are determined by the genetic makeup of the phage. The only factors determined by phage handling are considered to be some epigenetic alterations, which are patterns related to host DNA methylation [52]. They have a significant impact on the functioning of new host infections by a phage; play a very important role in horizontal gene transfer through bacteriophages. Therefore, in addition to the species-specific metabolic pathways specific to supporting the efficient propagation of a given phage and which should be equipped with surface receptors for this phage attachment, envelope structures of cell susceptible to the action of the phage lytic protein, and a block-conversion modification system that will allow in this case to infect the desired set of phage in clinics [45, 53].

Removal of such strains of genetic determinants of other phage defense mechanisms (e.g., CRISPR/Cas, Abi, or TA loci), if there is a genetic mutation, can extend the number of phages it can propagate to its cells to phage infecting the strains of the same species and uses the same receptors, but is in capable to overcome the suitable defense. The discovery of sensitivity to several specific phages upon the abolishment of various bacterial phage defense systems has been demonstrated in a number of cases. A good future strategy for finding the therapeutic phage propagation strains of desired properties may be the construction of a bacterial chassis of selected clinically relevant pathogenic species. In synthetic biology, the chassis refers to the microorganism that serves as the basis for genetic engineering and to support them by providing resources for basic tasks, such as replication, transcription, and translation mechanisms [45, 53–55].

The common strains of bacterial chassis that will serve as the basic platforms for construction of industrial phage propagation should have genomes reduce their complexity and unnecessary genetic content by the depletion of most of the transposable element as well as virulence and phage resistance determinants method called as a top-down strategy of the genome reduction process [54]. In addition, they ought to be prepared for the introduction or exchange of genomic modules which enable these strains to function as microbial cells in the use of selected treatment phage. Methods to allow the elimination of mobile genetic elements and other genes are used for genetic reshuffling recombineering, oligo-mediated allelic replacement, or genome editing using CRISPR/Cas-assisted selection of clones for model bacteria, or even on a genomic-wide scale. A repertoire of engineering tools that enhances genomic deceptive ability in bacteria other than *E. coli* uses new and ever-evolving techniques, providing ways to classify genomes belong to particular genera represented by problematic bacterial pathogens, including potential phage propagation strain [45].

The results of studies on micro-organisms that were cured of some or most of the recombinogenic or mobile genetic elements (including prophages) indicate many more benefits. The strain, *Escherichia coli* K-12 with a genomic reduction by approximately 15% by the removal of mobile DNA and cryptic virulence genes. Due to these changes this strain preserved good growth profile and protein production as well as the accurate propagation of recombinant genes and plasmids that could not be stably propagated in other strains [56]. Apart from phage capacity in combating different bacterial infections, emerging evidence suggests role of phages in viral infections as well treatment. Many viral illnesses do not have specific treatment and same antiviral drugs have been used for different viral diseases [57]. Thus, in our opinion, the construction for the propagation of therapeutic phages, of chass is strains equipped with certain phage susceptibility determinants and depleted of phage resistance determinants as well as certain mobiles genetic elements or virulence determinants will not only ensure the safety of therapeutic phage preparations, but will also reduce the cost of phage production substantially [58].

This reduction will be a result of:

i. It helps in reducing the number of strains required for the production of different types of phages.

ii. No need of evaluating phage preparations for the composition (temperate phages and toxins) of undesired elements.

iii. It helps to increase the fitness and stability of these strains in the commercially production of therapeutic phages.

In addition, to single fundamental strain establish for a microbial species can serve as a platform for the enrichment of its genome with several gene cassettes required for the propagation of several phages. Further work to remove additional undesired genomic elements from the genomes of these strains is in progress [45, 59].

9. Phage-based vaccines

Phage-based vaccines offer significant potential advantages by building up a stage approach with the ability to quickly switch the vaccine in response to mutations in the Coronavirus. Also, vaccines based on phages are self-fulfillment, which means they automatically activate and boost immune response, with the ability to show multiple antigens. The therapeutic use of phage in humans is well understood and has a favorable safety measures. Recent studies recommended the immune response to SARS-CoV2 could be transient and need frequent booster vaccinations to manage defensive levels of immunity [60, 61].

The possible advantages of phage-based vaccines incorporate flexibility for route of administration (mucosal and intramuscular), including a potential oral drop, adaptability to virus mutations, quick progression and cost-effectiveness. These advantages, along with the known safety profile, offer hope as a potential tool in reducing this pandemic. Moreover, countries have the potential to increase productivity rapidly. Researchers are fully committed to combat the impact of this public health crisis [60].

9.1 Benefits of phage-based vaccine over other vaccine technologies

- It offers the phages with excellent safety measurements.
- Quickly adaptation of new vaccines to potential mutations in coronavirus.
- Lower cost of manufacturing in comparison with alternative vaccine approaches.
- Self-adjuvanting to provoke immune response

10. Challenges to build phage libraries

The main challenges in treating phage are:

1. Doctors need to know exactly what type of bacteria is causing the infection.

2. They must have various specific phages target that strain, easily available, in fact from a large phage library that can be tested to find the right phage a cocktail that matches the bacteria.

To address latter problem, many pharmaceutical companies are reluctant to committed resources to improve treatment and therapy. That is because phage treatment is almost 100 years old, making it difficult to patent and raise income to allow for the initial cost of development. Lack of regulatory permission to manage the treatment of the page is problematic. Phage cocktails need to be customized for each patient's infection and regularly organized as the bacteria mutate and improve resistance as shown in **Figure 5**. Regulatory agencies such as the US Food and Drug Administration (FDA) currently do not have the necessary review and approval mechanisms to be able to accept your identity and adapt to a greater degree. The experimental design that benefits from genomic sequence and mass spectrometry will soon meet the need for rapid and accurate microbial identification. A second barrier to phage treatment, the need for easily accessible therapeutic phage, could ultimately be met to some extent by the U.S Medical Research Centre and different groups around the world are presently building phage libraries as shown in **Table 2** [63].

Looking forward, other technological innovations could help make the phage treatment more specific and help with patent issues. For an example, phages can at last be developed using CRISPR/Cas9 genetic engineering strategies to kill only

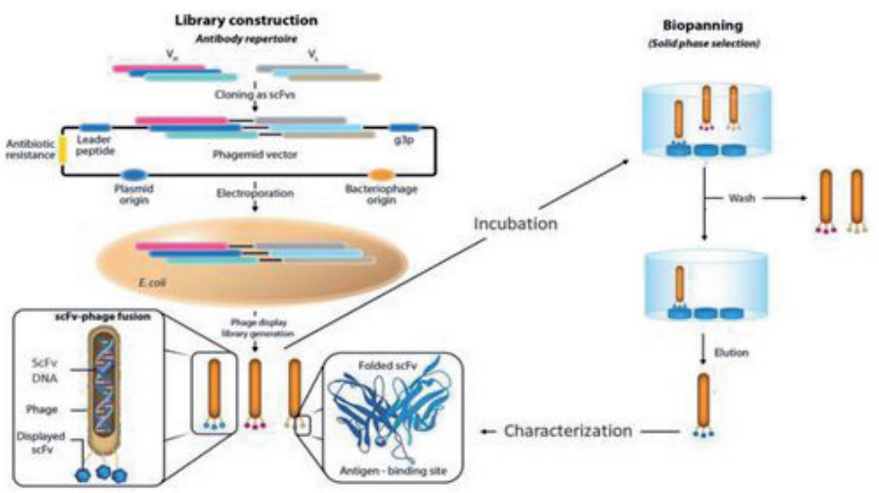

Figure 5.
Phage display method to build library of peptides and proteins variants (source: Almagro et al. [62]).

Library Name	Company/ Laboratory	Repertoire	Display Format	Size
XFab1	Xoma	Naive	Fab	3.1×10^{11}
XscFv2	Xoma	Naive	scFv	3.6×10^{11}
HAL9/10	TU-IB (b)	Naive	scFv	1.5×10^{10}
KNU-Fab	KNU (c)	Naive	Fab	3.0×10^{10}
pIX V3.0	Janssen Bio	Synthetic	Fab	3.0×10^{10}
HuCAL PLATINUM	MorphoSys	Synthetic	Fab	4.5×10^{10}
Ylanthia	MorphoSys	Synthetic	Fab	1.3×10^{11}
PHILO Diamond	ETH Zurich	Synthetic	scFv	4.1×10^{10}
ALTHEA Gold Libraries	GlobalBio/ADL	Semisynthetic	scFv	2.1×10^{10}

Source: Almagro et al. *[62].*

Table 2.
Example of Phage display antibody libraries.

resistant micro-organism. Some agencies there may also be eligible for patents on separate phage or phage cocktails, making them a viable commercial investment [63].

No matter what the future holds for the treatment of the phages, most experts agree that the phage treatment will never completely replace antibiotics. Instead, this method can be used in combination with antibiotics, or as a last resort to protect patients with diseases that have not responded to other treatments. Given the alarming increase in the number of life-threatening multidrug- resistant diseases in recent years, the need to investigate the potential role of phage and other alternative to antibiotic treatments is urgently required [64, 65].

11. Conclusion

The progressing SARS-CoV-2 related COVID-19 pandemic is persistently emerging worldwide and signifying the greatest spotlight on public health, education, travels, and monetary conditions in the current world. As irresistible situations have no borders because there is no single specific therapy that may give effective responses toward COVID-19. Thus, a worldwide activity intends to make phage therapy worldwide overall accessible is required. This obviously requires an active joint effort between countries for overcoming logistic and administrative challenges and among clinicians and researchers for filling current knowledge gap and encouraging advances in the field.

How would it be advisable for us to deal with the current infection prevention and control a strategy which also works after the epidemic? How could we react to similar contagious diseases in the future? These are open questions which require further discussion and research. While phages may have the potential to play a role in the current pandemic, it is also very important to understand that there is no magic stick for this pandemic. The current situation highlights the urgency for adhering to clinical pharmacology, therapeutic, preventative and diagnostics interventions to optimize COVID-19 therapies. The instant and cell free production of synthetic phages, whether designed or not? This had considerable advantages over classically produced natural phages. Implementation of the right patient, right drug, right dosage, and right timing approach helps to reduce the rate of infection. Finally, adaptive designs for COVID-19 will lead to the development of more vigorous infectious disease research infrastructure and funding to help mitigate future pandemics.

Acknowledgements

We would like to thanks Microbiologists and Junior Residents from the Department of Microbiology, Baba Raghav Das Medical College Gorakhpur, UP for providing support in the preparation of this article. We would like to thanks to our laboratory technicians (Mr. Umesh Chaudhary, Mr. Akhilanand Rai, Mr. Jagmohan Prasad) for their support.

Authors' contributions

This work was carried out in collaboration among all authors. Scientific data collection was performed by authors AKS, VG, and AK. The first draft of the manuscript was written by author VG; data was provided and corrected by authors AKS, and AK. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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