Potential of Bacteriophage Therapy: A Double Edge Sword to Combat COVID-19 and Associated Pulmonary Bacterial Infections

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Bhargava et al.: Bacteriophage Therapy in COVID-19 Pandemic

The Coronavirus Disease 2019 pandemic has wreaked havoc on global health infrastructure and personnel, resulting in enormous misery, deaths and economic stagnation. Severe Acute Respiratory Syndrome-Coronavirus-2 respiratory infections are frequently worsened by secondary bacterial infections and co-infections due to prolonged hospitalizations; resulting in irreversible lung damage, respiratory failure, cardiac arrest and death. The high mortality rate of Coronavirus Disease 2019 patients is primarily due to multi drug resistant microbial (viral/bacterial) infections, unrestrained inflammatory response and delayed antibody production. The superfluous use of broad spectrum antimicrobial drugs as the last resort has further aggravated the Coronavirus Disease 2019 crisis by contributing to the global antimicrobial resistance. To overcome these hurdles for effective treatment of Coronavirus Disease 2019 and associated bacterial infections, phage therapy seems to be promising due to a lack of effective antiviral drugs and antimicrobial-resistant superadded bacterial infections. Prior studies suggest that when phages, their cocktails and endolysins are administered alone or in synergism with antibiotics through nebulization or through intravenous and intraperitoneal injections have exhibited greater antibacterial potential to combat even Multidrug-Resistant pulmonary bacterial infections. Bacteriophages and phagicin have also shown potent antiviral activity by triggering the production of antiviral cytokines. Many studies have also indicated phage mediated antiviral immunity by lowering Nuclear Factor Kappa B activation and reactive oxygen species production. Phage display technique can serve as a promising approach for Coronavirus Disease 2019 vaccine development through production of Severe Acute Respiratory Syndrome-Coronavirus-2 specific antibodies. This review illustrates the potential of phage therapy as a double edged sword to combat both Coronavirus Disease 2019 as well as associated bacterial infections.

Key words: Bacteriophages, viral infections, Coronavirus Disease 2019 , pulmonary bacteria, secondary infection, co-infection

In the last two decades, there have been six significant viral outbreaks which include Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV) (2002), Hemagglutinin Type 1 and Neuraminidase Type 1 (H1N1) Influenza (2009), Middle East respiratory syndrome coronavirus (MERS-CoV) (2012), Ebola virus (2013), Zika virus (2015) and SARS-CoV-2 also known as Coronavirus Disease 2019 (COVID-19) that have contributed to the worldwide turmoil. Out of these, four of them (SARS-CoV, H1N1 Influenza, MERS-CoV and SARS-CoV-2) resulted in severe viral respiratory disease[1]. Clinical presentation for COVID-19 infection ranges from asymptomatic to severe viral pneumonia with respiratory failure, often resulting in death[2]. The COVID-19 virus is estimated to have caused approximately 196 million infections and 4.2 million deaths and the number of infections and death toll is still mounting[3]. The emphasis during viral pandemic is primarily on viral infection treatment; however, associated bacterial infections that develop in patients after or during the primary infection quite often turns unnoticed. So far, multiple studies have examined

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the epidemiological and clinical features of COVID-19, but data regarding associated bacterial infections is still quite limited[4].

Respiratory infections due to viruses predispose patients to bacterial infections. The 1918 Spanish flu outbreak, the 2003 SARS-CoV epidemic and the 2009 H1N1 Influenza pandemic were also associated with bacterial infections, which have resulted in increased morbidity and mortality[5,6].

According to a recent study, 7 % of COVID-19 positive cases developed an extremely heterogeneous bacterial infection, with a higher prevalence in intensive care settings due to nosocomial infections and invasive ventilation[7]. Fu et al. reported secondary bacterial infection in 13.9 % of Intensive Care Unit (ICU) patients[4]. A retrospective analysis conducted in Wuhan, China, stated that the proportion of secondary bacterial infection in lungs was 86.3 %, bloodstream 34.3 % and urinary tract 7.8 % respectively, in COVID-19 patients[2]. In addition, another retrospective analysis revealed that 15 % of hospitalized COVID-19 patients developed secondary infections, which contributed to 50 % of the death toll[8].

Diagnosis of superadded bacterial infections in this pandemic is highly complicated. Hence, broad-spectrum antibiotics are used prophylactically to reduce their risk, ultimately contributing to the prevalence of Anti-Microbial Resistance (AMR) globally[7]. However, in this challenging time and with the advent of Multi-Drug Resistant (MDR) bacterial infections, bacteriophages can be introduced as they are highly specific, self-limiting, self-replicating, naturally abundant, display low toxicity, evolve naturally and thus ultimately undergo degradation within patient's body[9,10].

PULMONARY BACTERIAL INFECTIONS DURING COVID-19

Secondary bacterial infections occur in addition to the primary infection, where bacterial pneumonia follows acute viral influenza[11]. Co-infections occur simultaneously due to multiple pathogens and are the most prevalent complications during a pulmonary viral pandemic, resulting in mixed viral and bacterial pneumonia features[12]. COVID-19 patients with comorbidities and extended hospitalization in ICU are susceptible to nosocomial infections including Ventilator-Associated Pneumonia (VAP), followed by bacteremia with sepsis and also SARS-CoV-2-associated immune dysfunction. This association between COVID-19 and superinfection can be

probably due to major lung impairment triggered by viral reproduction resulting in cytokine storm and inflammatory reactions. The impaired immune response caused by primary virus-related disease promotes secondary bacterial infections and co-infections, leading to a high rate of mortality and morbidity. It is projected that 1 in 7 COVID-19 patients admitted in hospitals are predisposed to secondary infections[13]. The antibiotics used in high doses as a last resort for such bacterial illnesses can be counter-productive due to several side effects and emerging drug resistance. Secondary bacterial infections in COVID-19 are predominantly caused by *Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Acinetobacter baumannii*[14,15]. Several theories have been elucidated for the development of concomitant bacterial infection in patients with a primary pulmonary viral infection, including immunological host modifications, structural disruption promoting easy dissemination and impaired clearance of mucus within the lungs[1]. Secondary bacterial infections are facilitated by the impaired mucociliary function of the upper respiratory tract caused by primary pulmonary viral insult[16]. Bacterial spread is facilitated by thickened mucus, which prevents immune cell penetration, as well as immunosuppression of the host's immune system as a result of primary viral infection[17]. Secondary bacterial infection further damages the epithelial cell layer, inhibiting its repair and regeneration, thus fostering disease severity, morbidity and mortality[18]. In addition, surfactant disruption and respiratory tract cell sloughing can provide access and essential nutrients enabling bacteria to proliferate rapidly[19]. The addition of bacterial infection augments airway inflammation and alveolar consolidation, increasing the severity of the disease[20].

AMR BACTERIAL PREDISPOSITION TO PRIMARY VIRAL INFECTION

AMR is a global problem, with 2.8 million people infected and 35 000 people dying each year in the United States[21] and the indiscriminate use of antibiotics during COVID-19 will exacerbate the matter. MDR bacteria are becoming more common and our ability to eliminate them is dwindling, thereby increasing our susceptibility to bacterial infections, especially during pandemics. Antiviral and anti-inflammatory properties of azithromycin and doxycycline have been empirically used to treat COVID-19 infections; besides, they may also counter co-infection, but their injudicious use may further lead to the emergence of MDR strains. Secondary

infections and co-infections have irreversible effects during viral pandemics, especially in high-risk groups, including immunodeficient or immunosuppressed groups[22]. Patients vulnerable to pulmonary viral infections (Influenza, SARS and COVID-19) face the greatest risk of being infected with superbugs[11,23,24]. One of the classical reports of bacterial infection arising concurrently or immediately following comes from the 1918 influenza pandemic, in which bacterial co-infection was responsible for the majority of fatalities[5]. Approximately 3 00 000 people died worldwide due to the 2009 H1N1 pandemic, with bacterial pneumonia accounting for 30-55 % of cases[25]. Respiratory viruses associated with bacterial co-infection reported are influenza, Human Parainfluenza Virus (HPIV), Human Metapneumovirus (HMPV), rhinovirus, adenovirus and Syncytial Respiratory Virus (SRV) [26,27]. Human coronavirus NL63, Human Bocavirus (HBoV), H1N1 and H5N1 influenza viruses, SARS, coronavirus associated with MERS and COVID-19 are examples of emerging pulmonary viruses where bacterial infections result in complications[26]. Research conducted in Wuhan, China, in hospitalized patients with COVID-19 reported that MDR *Acinetobacter baumannii* and *Klebsiella pneumoniae* induced secondary infection[2], which resulted in further complications. A weakened immune system is a significant risk factor for MDR bacterial infections in patients with severe COVID-19[28]. Another study reported that 33 % of COVID-19 patients acquired MDR Enterobacteriaceae, Vancomycin Resistant *Enterococci* (VRE), *Enterococcus faecium* and MDR *Pseudomonas aeruginosa*[29]. Patel *et al*. also reported the dissemination of MDR gram-negative bacteria among COVID-19 patients in Maryland, USA[30]. A list of bacterial infections with the virus during COVID-19 and other previous viral pandemics is shown in Table 1[31-59]. As broad-spectrum antibiotic prophylaxis does not support severe secondary and co-infections in COVID-19 patients, alternative antibacterial therapies such as bacteriophages can be used to avoid further complications and counter global AMR.

BACTERIOPHAGES: A RESURGENT ARSENAL

Bacteriophages, also known as phages, are ubiquitous viruses that selectively infect, replicate inside the bacterial cell and kill it without affecting any host eukaryotic cells[60]. The administration and exploitation of phages for treating pathogenic bacteria dates back to a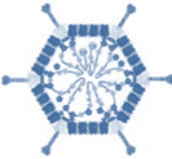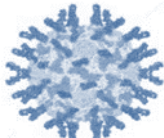 century. The Sacred River Ganges of India is one of the major repositories of bacteriophages, particularly at Gomukh[61]. In addition, they have been found in rivers, sewage, wastewater and hospitals worldwide, as well as human and animal gastrointestinal tract and wherever their host survives[62]. The resurgence of bacteriophage therapy as a potent weapon to combat AMR during pandemic times appears to be a rational measure in light of the rapid rise in MDR pathogens globally, as well as a decrease in the discovery of new antibacterial compounds. Hence, they can be utilized either alone or in combination with antibiotics to treat resistant bacteria[63].

Bacteriophage therapy utilizes phages that specifically docks on host-pathogen for its replication, resulting in the release of phage progeny via lysis of its host[64]. Bacteriophages replicate via two types of cycles, i.e. lytic and lysogenic cycles. Bacteriophages attach and invade susceptible specific bacteria in these two groups via specific bacterial receptors (fig. 1A)[65]. Specific phages infect and take over the replication process of their exclusive host cells (bacteria) during the lytic cycle, producing viral genomes and proteins (fig. 1E). Following that, phage assembly and packaging culminate in the release of new progeny via cell lysis, which would further colonize other bacterial hosts (fig. 1F)[66]. In the lysogenic cycle, bacterial biochemical machinery is infiltrated, where viral genetic material is incorporated (fig. 1B) into the host genome and the virus chromosome is conveyed to daughter cells via cell division (fig. 1C)[60]. The incorporated viral DNA (prophage) remains inactive but is replicated with each cell division of its host[67]. The prophage becomes activated under the correct environment, initiating the lytic cycle and releasing new progeny[68].

BACTERIOPHAGE THERAPY: A DOUBLE EDGE SWORD AGAINST COVID-19 PANDEMIC

Phage therapy was originally designed to kill bacteria. Previously, little was known about the biology of phages and their interaction with bacteria[64]. With the advancement of biomedical technology new details about bacterial and viral biology (fig. 2) has been revealed and a strong resurgence of phage therapy has been observed due to the emergence of AMR during pandemics on a global platform[69]. Bacteriophages have great potential to tackle bacterial infections either alone or in combination with antibiotics[61] and through its lytic enzymes as well as can be used against viruses via phage display technique, thus leading to a prospective roadmap to tackle COVID-19 and associated bacterial

TABLE 1: PULMONARY VIRAL AND ASSOCIATED BACTERIAL INFECTIONS

Virus	Associated Bacteria	Infection type	References
Influenza	*Staphylococcus aureus*, MRSA	Co-infections	[31-34]
	Streptococcus pneumoniae	Co-infections	[35]
	Streptococcus pyogenes	Secondary infections	[36]
	Haemophilus influenzae	Co-infections	[37]
	Chlamydophila pneumoniae	Co-infections	[38]
	Mycoplasma pneumoniae	Co-infections	[38]
	Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Burkholderia cepacia, Enterobacter aerogenes, Legionella pneumophila	Secondary infections	[39]
Metapneumovirus	*Streptococcus pneumonia, Haemophilus influenzae, Enterococcus spp., Brucella spp., Streptococcus pyogenes*	Secondary infections	[40]
Respiratory syncytial virus	*Pseudomonas aeruginosa*	Secondary infections	[41]
Adenovirus	*Haemophilus influenzae, Chlamydia trachomatis*	Co-infections	[42]
Parainfluenza	*Streptococcus pneumoniae, Haemophilus influenzae*	Secondary infections	[43]
	Streptococcus agalactiae	Co-infections	[44]
Rhinovirus	*Streptococcus pneumoniae, Mycoplasma pneumoniae*	Co-infections	[45]
	Staphylococcus aureus	Co-infections	[46]
SARS	*Chlamydophila pneumoniae*	Co-infections	[6]
	Mycoplasma pneumoniae	Co-infections	[6]
	MRSA	Secondary infections	[47,48]
MERS	*Mycobacterium tuberculosis*	Co-infections	[49]
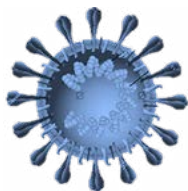
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Acinetobacter baumannii, Klebsiella pneumoniae	Co-infections	[50]
Mycoplasma pneumoniae, Legionella pneumophila, Streptococcus pneumoniae	Secondary infections	[51]
Staphylococcus aureus	Co-infections	[52]
Haemophilus influenza, Staphylococcus aureus	Co-infections	[53]
Enterobacter cloaca, Acinetobacter baumannii	Secondary infections	[54]
Pseudomonas aeruginosa, Staphylococcus aureus	Secondary infections	[55]
Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli	Secondary infections	[56]
Klebsiella pneumoniae, Klebsiella oxytoca, Staphylococcus aureus, Enterobacter cloacae, Enterobacter aerogenes, Pseudomonas aeruginosa	Secondary infections	[57]
Klebsiella pneumoniae, Acinetobacter baumannii	Secondary infections	[58]
Staphylococcus aureus, Klebsiella oxytoca, Stenotrophomonas maltophilia, Haemophilus influenza & Haemophilus parainfluenzae	Co-infections	[59]
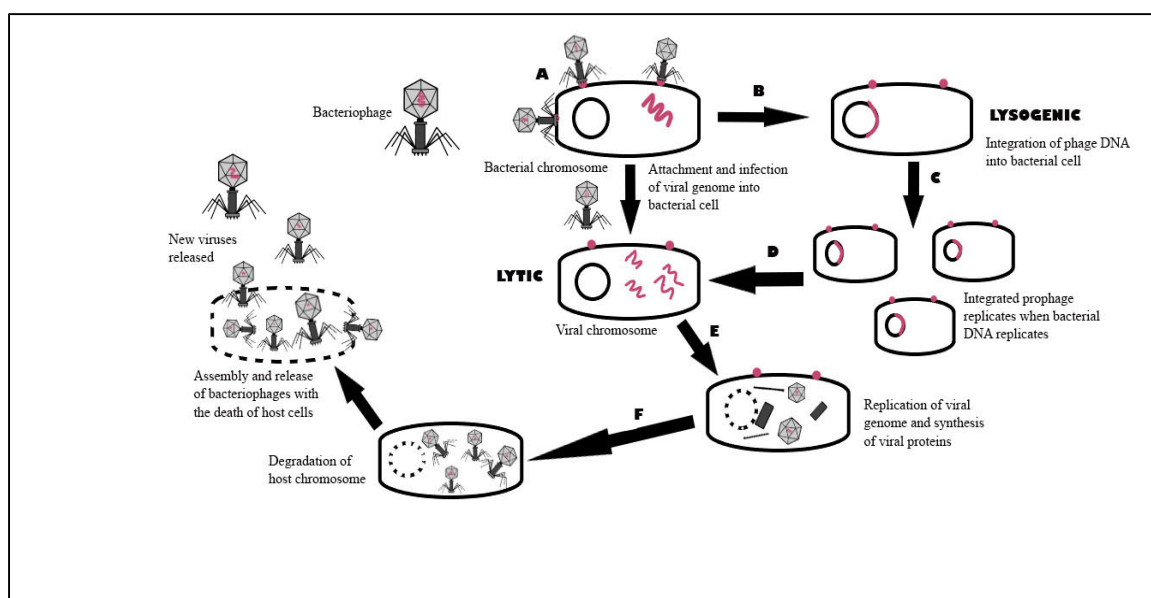
COVID-19

Fig. 1: Mechanism of action (Lytic and lysogenic cycle) of bacteriophages

infections during COVID-19 pandemic.

Phage therapy against secondary pulmonary bacterial infections:

As AMR infections continuously rise, adjunct therapeutic options are required, inspiring renewed interest in bacteriophage therapy. Waters *et al.* reported high effectivity of phage therapy against recalcitrant chronic lung infection caused by *Pseudomonas aeruginosa*[70]. A recent study investigated the use of pre optimized phages as an alternative therapy for lung infections caused by carbapenem-resistant *Acinetobacter baumannii* strains in COVID-19 patients and showed improvement in infection by reduction of bacterial load[71]. A case report describes a favorable phage therapeutic response in a cystic fibrosis patient, confirming its efficacy against *Staphylococcus*

aureus[72]. The phage therapeutics studies including bacteriophages and lysins for most frequent pulmonary bacteria causing secondary infections are listed in Table 2. Recently, the U.S. Food and Drug Administration (FDA) have approved clinical trials for personalized intravenous phage therapy intended for COVID-19 patients[73] suffering with bacteremia, pneumonia or septicemia due to MDR bacterial co-infections by *Pseudomonas aeruginosa*, *Acinetobacter baumannii* or *Staphylococcus aureus*[74-100].

Phage synergism with antibiotics:

Bacteriophages have been used synergistically with antibiotics to treat AMR infections. The use of phages in synergism with antibiotics has proven to be extremely efficient in the treatment of antibiotic-resistant opportunistic bacteria that cause polymicrobial
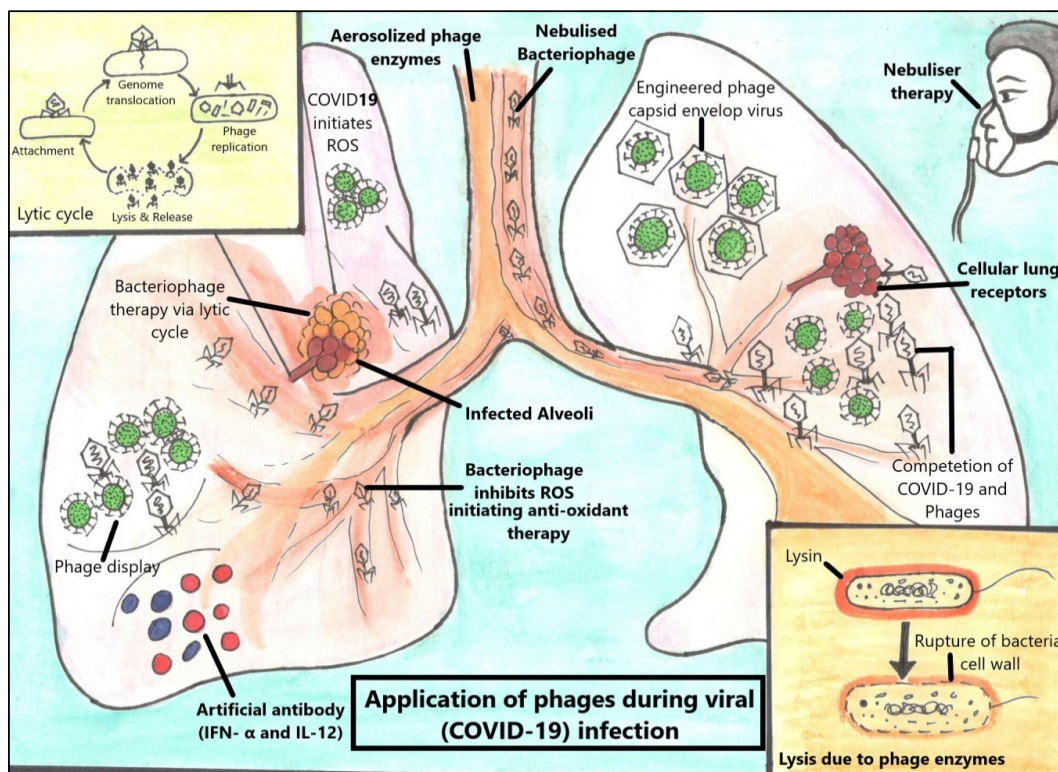
Fig. 2: Future perspectives of bacteriophage therapy during COVID-19 and associated pulmonary bacterial infections

TABLE 2: PHAGE THERAPY AGAINST SECONDARY PULMONARY BACTERIAL INFECTIONS

Secondary Bacterial Infection	Subject	Phages/Lysin +/- Antibiotic	Phage Family	Administration	Reference
Multi-Drug Resistant *Pseudomonas aeruginosa* Pneumonia	Murine	Phage cocktail PaAH2ФP (103), PsBAP5Ф2 (130) and PAФ134+Meropenem	Myoviridae	Intratracheal/Intraperitoneal injection	[74]
Pseudomonas aeruginosa Ventilator-associated Pneumonia	Human	AB-PA01 (Cocktail of four lytic phages)+antibiotics	Myoviridae and Podoviridae	Intravenous and nebulization	[75]
Imipenem resistant *Pseudomonas aeruginosa* bacteremia	Mice	Phage ØA392 and phage Ø1093cocktail	Myoviridae	Intraperitoneal injection	[76]
Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Proteus and *Escherichia coli*	Human	Pyophage (Cocktail of phages)	*Podoviridae & Myoviridae*	Nebulization & sanitizing of nose and throat	[77]
Burkholderia cepacia chronic lung infections	*Galleria mellonella* larvae	ФKS12, ФKS14+Meropenem, Ciprofloxacin, and Tetracycline	Myoviridae	Hamilton syringe injection	[78]
Burkholderia cepacia complex respiratory infections	Mice	ФKS4-M, ФKS14, Ф KS12	Myoviridae	Intraperitoneal & Aerosolized	[79]
Burkholderia cepacia and *P. aeruginosa* Pneumonia	*In vitro*	ФKS4- M, ФKS14, and cocktails of ФKZ/D3 and ФKZ/D3/KS4-M	Myoviridae	Spray-dried respirable powders delivered from an Aerolizer® dry powder inhaler (DPI)	[80]
K. pneumoniae B5055-mediated lobar pneumonia	Mice	ФSS	Podoviridae	Intraperitoneal injection	[81]

Multidrug-Resistant *Klebsiella pneumoniae* ST258 *bacterimia*	Mice	Pharr ΦKpNIH-2	Podoviridae Siphoviridae	Intraperitoneal injection	[82]
Carbapenem-resistant *Acinetobacter baumannii* (CRAB)	Human	Phage cocktail (ΦAb124+ΦAb121)	Podoviridae & Myoviridae	Nebulization	[83]
Multidrug resistant *A. baumannii* (MDRAB)	Mice	ΦPD-6A3/Endolysin Ply6A3	Podoviridae	Intraperitoneal injection	[84]
Carbapenem-resistant *Acinetobacter baumannii* (CRAB) associated pneumonia	Mice	ΦSH-Ab 15519	Podoviridae	Intranasal	[71]
Extensively drug-resistant *Acinetobacter baumannii* (XDRAB) bacteraemia	Mouse	ΦvB_AbaM_3054 ΦvB_AbaM_3090	Myoviridae	Intraperitoneal injection	[85]
Acinetobacter baumannii Bacteraemia	Mouse	Endolysin PlyF307	Myoviridae	Intraperitoneal injection	[86]
Acinetobacter baumannii and *Pseudomonas aeruginosa*	*Caenorhabditis elegans*	Engineered Endolysins (Artilysins) OBPgp279 and PVP-SE1gp146	Myoviridae	Bacteriophage suspension	[87]
Extraintestinal *Escherichia coli* Ventilator-associated Pneumonia	Mice	Φ536_P1 and Φ536_P7	Myoviridae	Intranasal Instillation	[88]
Streptococcus pneumoniae Community- Acquired Pneumonia	*In vitro*	ΦSPSL1	Siphoviridae	Bacteriophage suspension	[89]
Streptococcus pneumoniae	*In vitro*	ΦCp-1	Podoviridae	Bacteriophage suspension	[90]
Penicillin-resistant *Streptococcus pneumoniae*	*In vitro*	Endolysins Pal and Cpl-1	Podoviridae	Bacteriophage suspension	[91]
Streptococcus pneumoniae bacteremia	Mice	Chimeric phage lysin Cpl-711 (Cpl-1 and Cpl-7S)	Podoviridae	Intraperitoneal injection	[92]
Staphylococcus aureus	Human	ΦAB-SA01	Myoviridae	Intravenous	[93]
Methicillin-resistant *Staphylococcus aureus* induced ventilator-associated pneumonia	Rat	Cocktail (ΦK, Φ3A, 2002 and 2003)	Myoviridae	Nebulized bacteriophages ("aerophages")	[94]
Methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus* causing acute Pneumonia	Mouse	AB-SA01 Component Phages	Myoviridae	Intraperitoneal (IP) injection	[95]
Staphylococcus aureus	*In vitro*	ΦSA5+Gentamicin	Myoviridae	Bacteriophage suspension	[96]
Methicillin-resistant *Staphylococcus aureus* bacteremia	Mouse	LysGH15	Myoviridae	Intraperitoneal injection	[97]
MRSA *Staphylococcus aureus* septicemia	Mouse	Chimeric lysin (ClyS)+Oxacillin	Myoviridae	Intraperitoneal injection	[98]
Staphylococcus aureus	Mouse	Recombinant phage endolysin, SAL-1	Myoviridae	Intravenous injection	[99]
Mixed infections by MRSA, Vancomycin-Intermediate *S. aureus* (VISA), *Streptococcus pyogenes* and *S. pneumoniae*	Mice	Endolysin PlySs2+Mupirocin	Siphoviridae	Intraperitoneal injection	[100]

biofilm-associated diseases[101]. Aslam *et al.* reported clinical experience with phage therapy combined with antibiotics in three patients with life-threatening MDR infections caused by *Pseudomonas aeruginosa* and *Burkholderia dolosa*, two of whom improved[102]. According to a researcher, treatment of phage combined with ceftazidime appears to eradicate aortic graft infection caused by *Pseudomonas aeruginosa* with no evidence of recurrence[103]. An *ex vivo* study was performed on a human airway epithelial cell line model to test the efficacy of combined bacteriophage and ciprofloxacin treatment in preventing *Pseudomonas aeruginosa* infection, where co-administration efficiently prevented bacterial regrowth and maintained epithelial cell integrity[104].

Role of bacteriophage lytic enzyme:

Over time, many phage-derived hydrolytic enzymes, such as endolysins and ectolysin, have been discovered that disrupt the bacterial peptidoglycan cell wall, eventually killing the bacteria[105]. The bacterial cell wall comprises peptidoglycan polymer chains comprised of a disaccharide repeat of glycan strands (N-acetyl glucosamine and N-acetylmuramic acid, linked by β $(1\rightarrow4)$ glycosidic bonds). Lysins are glycosidases and when employed exogenously in pure forms, they cause immediate osmotic lysis and bacterial death. They are also referred to as enzybiotics due to their antibacterial activity. Lysins kill bacteria rapidly upon contact and they are specific to the target pathogen[106]. Furthermore, lysin does not disrupt the natural microbiome. Thus, resistance development is highly improbable and it can be used alone or synergistically with antibiotics[107]. Larpin *et al.* demonstrated *in vitro* optimal bactericidal activity of phage lysin PlyE146 against *Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains, making it a promising therapeutic agent against infections caused by these bacteria[108]. Lysins can be an effective therapeutic tool against bacterial infections after standardizing the dose and regimen.

Potential role of phage therapy against SARS-CoV-2:

Phagicin is synthesized during viral (bacteriophage) replication and can also be extracted by disrupting the phage particles[63]. Phagicin has been shown to exhibit antiviral properties (interfering with viral DNA intracellular replication) against the Herpes Simplex Virus (HSV) and Vaccinia viruses[109]. As a result, its antiviral properties make it suitable for use against other pathogenic viruses, including SARS-CoV-2. According to a researcher, a genetically engineered bacteriophage capsid envelops the influenza virus, inhibiting it from adhering to lung tissues and thus preventing infection[110]. Because the influenza virus shares few genomic similarities to that of COVID-19; hence, the same could be applied to it. Antiviral drugs for COVID-19 (favipiravir and remdesivir) are not particularly efficient and their mode of action is by inhibiting Ribonucleic Acid (RNA)-dependent RNA polymerase[111]. Therefore, they do not halt virus attachment to the host cell and do not prevent the initial stage of infection (entry of the virus into the host cell), causing disruption of the alveolar epithelium (Pneumocyte type II)[112]. Also, when phages are introduced after a primary viral infection, they often compete with the pathogenic virus for the cellular receptors and restrict their infectivity[113]. Nuclear Factor Kappa B (NF-κB) is a widely expressed transcription factor induced by SARS-CoV-2 and is involved in inflammatory and immunological responses[114]. However, bacteriophages significantly reduce or abolish the triggering of NF-κB activation[115]. A respiratory pathogen (bacteria and/or viruses) infecting the lung is often associated with inflammation and cell death caused by excessive production of Reactive Oxygen Species (ROS)[116]. However, phage and phage proteins inhibit ROS production and exhibit antimicrobial activities via anti-oxidant therapy[117]. Another data suggests the exploitation of phages where they drive antiviral activity by promoting the production of antiviral cytokines like Interferon Alpha (IFN-α) and Interleukin-12 (IL-12)[115].

Phage display: a technique to counter bacterial and viral infection

COVID-19 pandemic has enforced us to explore alternative therapies to combat SARS-CoV-2 and prevent associated microbial infections. Phage display is an alternative to hybridoma technique for manufacturing therapeutic Monoclonal Antibodies (MABs) against a specific viral or bacterial antigen[118]. The phage display technique can be utilized in two ways: to decrease the pulmonary bacterial infection and/or to efficiently produce antibodies against pulmonary viral (COVID-19) infections[119]. It is a method of producing phage-displayed vaccines in which a protein gene of interest is encoded into the phage coat protein, leading the phage to exhibit the protein on the exterior while carrying it on the inside. Thus, it can provide SARS-CoV-2 positive patients more time to build their unique

immune response against COVID-19, allowing them to escape the damage caused by an overly sensitive immune system[65]. Phage display technique has been used to isolate specific mAbs against viruses such as influenza A[120] and human immunodeficiency virus (HIV)[121]. A latest report suggests CR3022 (a SARS-CoV-1/2 antibody) isolated from a phage display library exhibited potent SARS-CoV-2 neutralizing activity arising from destabilization of the spike trimer[122]. The use of biopanning technique has led to the recognition of human monoclonal antibodies (hmAbs) from eight large phage-displayed VH, scFv and Fab libraries including mAb, IgG1 ab1 targeted against the receptor binding domain (RBD) of the spike protein of SARS-CoV-2. The IgG1 ab1 effectively neutralized live SARS-CoV-2 in human angiotensin-converting enzyme 2 (ACE2) expressing transgenic mice model[123]. The studies thus emphasize the importance of phage display to tackle global health issues in the era of COVID-19 and future pandemics of constantly emerging and re-emerging microbes. It is rightly said that 'A diamond can cut a diamond', likewise in this prevailing pandemic of COVID-19, when even antiviral medicines are not very effective, bacteriophages can be a promising tool to combat SARS-CoV-2 infections.

CHALLENGES IN PHAGE THERAPY

Bacteriophages are used to treat bacterial infections as an alternative to antibiotics with an emerging crisis of AMR. Phage cocktails are therefore used to treat a broad range of secondary bacterial infections. But, the guidelines for the use of bacteriophages have yet to be properly formulated, as most of the countries have not yet approved invasive phage therapy in humans. Phage derived enzymes can also be used, but the dose of administration requires proper calibration. Moreover, rapid identification of susceptible phages is required for implementation of phage therapy, especially against secondary infections and AMR bacteria. Phage libraries, screening platforms and phage banks need to be established for effective phage therapies. There is also lack of awareness about bacteriophages therapy because of limited clinical trials demonstrating its effectiveness and therapeutic cGMP preparation (current Good Manufacturing Practice)[124]. Also, phage resistance has been observed during single phage application, but it can be surpassed when a cocktail of phages is used in a sequential approach of administration along with standardized formulation and dosage[60].

CONCLUSION

The COVID-19 pandemic is a striking health crisis throughout the globe threatening the survival of humanity. COVID-19 patients after prolonged hospitalizations in ICUs are more vulnerable to SARS-CoV-2 infection when paired with super added pulmonary infections like ventilator-associated pneumonia, bacteremia with sepsis caused by MDR nosocomial pathogens resulting in respiratory failure and death. Broad-spectrum antibiotics are generally recommended to keep these infections at bay especially in ICUs, which is further stimulating AMR. The irrational use, ill-effects of antibiotics and a lack of treatment options for MDR bacterial infections has led to resurgence of phage therapy alone or in synergism with antibiotics as one of the most promising option for treating bacterial infections in this era of AMR. Bacteriophages and their lytic enzymes have shown great potential in the treatment of bacterial infections and studies have also indicated phage mediated antiviral immunity by lowering NF-κB activation, ROS generation, along with the production of antibodies via phage display vaccines. Bacteriophage therapy can thus serve as a double edge sword to avert an emerging healthcare crunch from COVID-19 and multi drug resistant pulmonary bacterial infections. However, further exploration is needed to ensure safety protocols and efficacy in order to meet the challenges in phage therapy and develop appropriate regulations for its clinical use in prevention and management of current and future pandemics.

Conflict of interests:

The authors declared no conflicts of interest.

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