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MINI-REVIEW

# Phage therapeutics: from promises to practices and prospectives

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## Abstract

The rise in multi-drug resistant bacteria and the inability to develop novel antibacterial agents limits our arsenal against infectious diseases. Antibiotic resistance is a global issue requiring an immediate solution, including the development of new antibiotic molecules and other alternative modes of therapy. This article highlights the mechanism of bacteriophage treatment that makes it a real solution for multidrug-resistant infectious diseases. Several case reports identified phage therapy as a potential solution to the emerging challenge of multi-drug resistance. Bacteriophages, unlike antibiotics, have special features, such as host specificity and do not impact other commensals. A new outlook has also arisen with recent advancements in the understanding of phage immunobiology, where phages are repurposed against both bacterial and viral infections. Thus, the potential possibility of phages in COVID-19 patients with secondary bacterial infections has been briefly elucidated. However, significant obstacles that need to be addressed are to design better clinical studies that may contribute to the widespread use of bacteriophage therapy against multi-drug resistant pathogens. In conclusion, antibacterial agents can be used with bacteriophages, i.e. bacteriophage-antibiotic combination therapy, or they can be administered alone in cases when antibiotics are ineffective.

**Key points**

• *AMR, a consequence of antibiotic generated menace globally, has led to the resurgence of phage therapy as an effective and sustainable solution without any side effects and high specificity against refractory MDR bacterial infections.*

• *Bacteriophages have fewer adverse reactions and can thus be used as monotherapy as well as in conjunction with antibiotics.*

• *In the context of the COVID-19 pandemic, phage therapy may be a viable option.*

**Keywords** Bacteriophage therapy · Multi-drug resistance (MDR) · Phage cocktail · Extreme-drug resistance (XDR) · Combination therapy · Antimicrobial resistance (AMR)

## Introduction

"The role of the infinitely small in nature is infinitely great…". The prevailing global pandemic COVID-19 reminds us of this quote by the Father of Microbiology-Louis Pasteur. The current pandemic makes us realize and reimagine the immense power of the tiniest microorganisms. Phages are viruses that infect prokaryotic (bacteria) cells but have no effect on eukaryotic (human or animal) cells; hence, they can be employed to manage

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infections caused by bacteria (Domingo-Calap and Delgado-Martínez 2018). The administration of bacteriophages for treatment traces back almost a century, and the widespread availability of effective and safe antibacterial medications following WWII led to scepticism of bacteriophage use until the 1990s (Abedon et al. 2011).

Over the last three decades, the global spread of multidrug resistant (MDR), pan-drug resistant (PDR), and extreme-drug resistant (XDR) bacteria, as well as the decreased availability of new effective antibacterial agents, has rekindled the scientific community's interest in bacteriophage as an alternate antibacterial agent (Perros 2015; WHO 2018). Phage particles are natural and versatile. Furthermore, owing to their (phages) lack of affinity for eukaryotic cells, their modifications contribute to their prospective use in gene therapy and medicinal applications (Hashemi et al. 2010; Robertson et al. 2011; Yata et al. 2014; Bardy et al. 2016). According to Biset et al. (2020) and Folliero et al. (2020), the most commonly reported resistant bacteria were *Escherichia coli* (56.67%), *Klebsiella pneumoniae* (50%), *Enterobacter cloacae* (100%), *Staphylococcus aureus* (45.45%), Coagulase-negative *Staphylococcus aureus* (76.47%), *Enterococcus* spp. (97.50%), *Acinetobacter baumanni*i (100%), and *Pseudomonas aeruginosa* (96.80%). If corrective measures are not implemented, MDR infection mortality may exceed 10 million by 2050 (WHO 2020).

Antibiotic resistance (AR) has been continuously increasing and has primarily been addressed from the perspective of human misuse, whereas in veterinary and agricultural context, their overuse leading to MDR has not received the requisite attention. Antimicrobial resistance (AMR) poses a significant threat to the ecosystem, which must be acknowledged and addressed when designing effective AMR plans (O'Neill 2014). The global problem of MDR bacterial infections necessitates immediate actions, and one such infection control option could be bacteriophage therapy (Chanishvili and Aminov 2019). Besides, in addition to engineered phages, bacteriophage-produced lytic enzymes also exhibit properties that can be marketed in medical and industrial sectors.

With the current advent of AMR throughout the world and the dried pipelines of pharmaceutical industries, phage therapy could be the answer. In this review, we emphasize the aspects of phage therapy that make it a promising solution to MDR infectious diseases and also delineate some of the hurdles that must be addressed in the designing and implementation of clinical research to determine the effectiveness of phage therapy in humans against MDR infections and briefly discuss the prospects of phage therapy in COVID-19 pandemic. Most of the mini-reviews that have been published before discuss how phages were discovered? Where were they practiced before? Why they were abandoned and their advantages compared to antibiotics? To break the cycle of resistance and circumvent this silent global epidemic, the pharmacokinetics of phage therapy must be adopted which is being used in some instances that we have summarized in this manuscript based on different clinical infections. The clinical cases outlined in this manuscript focuses on major systemic diseases in which phage therapy experiments have contributed to the fight against resistant bacterial infections caused by MDR bacteria. This manuscript has not nullified antibiotics use but has stipulated an approach where bacteriophages can be used synergistically, as evident from some practical clinical case studies.

## Understanding the phenomenon of AMR to antibiotics

In a natural ecosystem, antibiotics play a critical part in the regulatory processes of the microbes (Aminov 2009), where they serve as signalling molecules in small quantities (Davies et al. 2006). However, when used in an appropriate concentration, antibiotics act as bacteriolytic and bacteriostatic agents in the management of bacterial infections (Leekha et al. 2011). Antibiotics are also commonly used at sub-therapeutic concentrations in the livestock feed for metaphylaxis (Manyi-Loh et al. 2018). Antibacterial agents, which are imprudently used in the medical, aquaculture, agricultural, and other industries, are hotspots for their continual entry into the ecosystem, leading to the selection and amplification of antibiotic-resistant genes (ARG) (Yadav and Kapley 2021). ARG is incorporated into the commensal flora at this stage, and the expense of "fitness" of an organism carrying ARG is reduced (Beceiro et al. 2013). As a result, even in the absence of antibacterial agent-induced selective pressure, AR bacterium persists (Andersson and Hughes 2011; Brown and Wright 2016). The pool of amplified ARG is subsequently discharged into other compartments of the ecosystem, along with the accompanying antibacterial medications, from whence it is further distributed to even more distant ecological compartments via horizontal gene transfer (HGT) processes [Fig. 1] (Aminov 2011; Duran et al. 2016; Brown et al. 2017).

## Mechanism of action of bacteriophage therapy

Bacteriophages bind to and adsorb on specific receptors on the surface of their hosts (bacteria) before introducing their genetic material to begin viral propagation; their relationship ranges from parasitic to mutualistic (Weinbauer 2004;

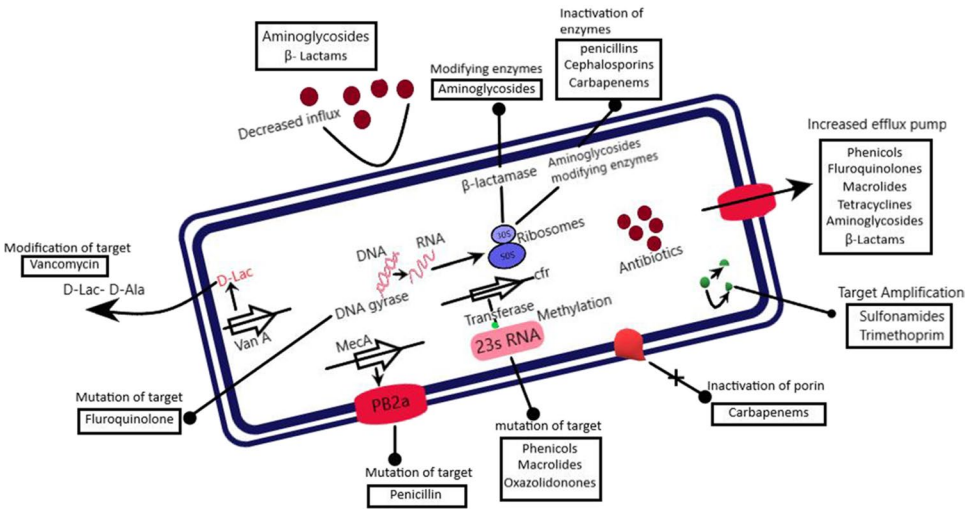


**Fig. 1** Illustration of the mechanism of antimicrobial resistance in a bacterial cell

Sime-Ngando 2014). In lytic infection, the phage adheres to the bacterial surface [Fig. 2A], injects its chromosomes into the bacterial cell, and reproduces naturally [Fig. 2E] with the release of new virions (virulent phage) [Fig. 2F]. During the lysogenic cycle, phage genetic material integrates into bacterial chromosomes [Fig. 2B], allowing bacteria to continue reproducing normally [Fig. 2C] along with phage genetic material (prophage) resulting in the release of temperate phages. Thus, virulent phages outperform temperate phages in terms of therapeutic potential.

Antibiotics gained popularity due to their broad-spectrum activity (Fair and tor 2014), however, with the increase in the understanding of the human microbiome, this broad-spectrum killing potential is rapidly revealing itself as a significant disadvantage (Cho and Blaser 2012). Being natural bacteria predators, bacteriophages have an advantage over antibiotics as they are specific, targeting only their host bacteria, implying a gentler approach to local microflora (Divya Ganeshan and Hosseinidoust 2019). However, another factor to consider is the interaction of the polyvalent lytic phages with the commensal flora (Ly-Chatain 2014). This is especially true in the case of gut flora if the phage is administered orally. Therefore, the impact of bacteriophages on any microbiome,

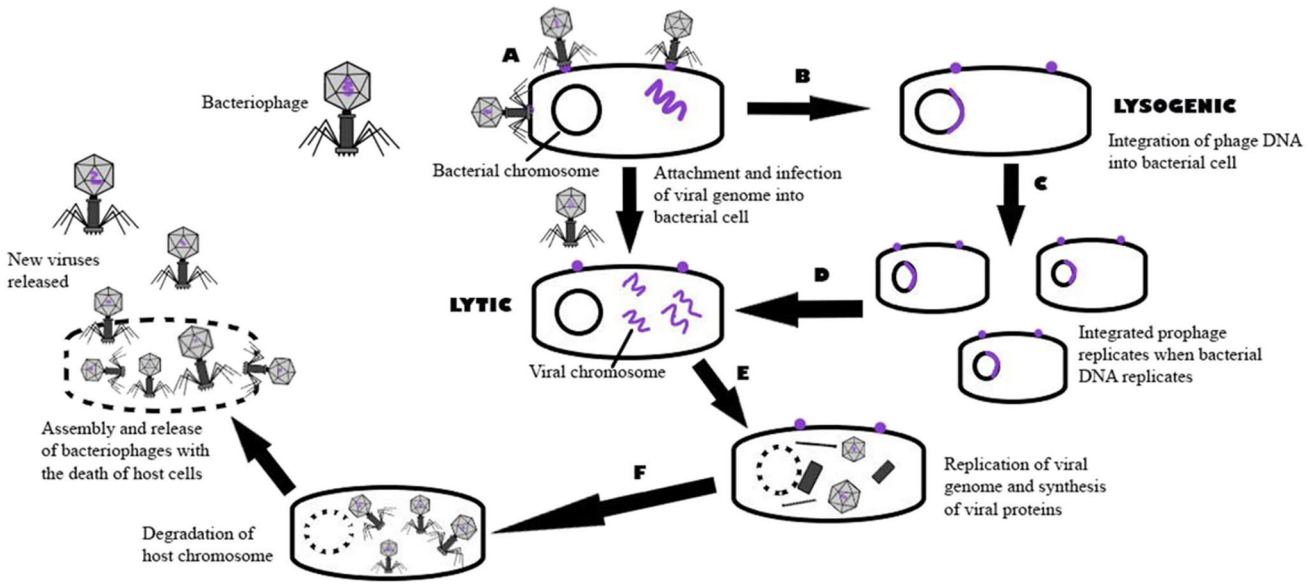


**Fig. 2** Mechanism of action of bacteriophage therapy: After bacteriophage infection (A), phage DNA (purple) is either conventionally replicated and processed as new virions at the cost of the host cell (virulent phage in the lytic cycle; left, A, E and F) or reproduces with host DNA (temperate phage in the lysogenic cycle; right, B, C and D) (Fabijan et al. 2020)

particularly gut microbiota, must be regarded as an essential component that governs the microbiome and should be further researched (Sutton and Hill 2019).

Drug resistance is a worrisome and widespread problem worldwide, with the emergence of MDR, PDR and XDR strain containing novel ARGs and the paucity of new medicines for treating bacterial diseases (Fair and Tor 2014). Bacterial defence mechanisms evolved as a result of the fight for survival between bacteria and the phages that infect them; yet, phage counteracting mechanisms are little understood and unexplored (Rostøl and Marraffini 2019). Phages can evolve in tandem with their hosts, increasing their chances of infecting their host. Lysogenic phages/temperate phages insert their DNA into the bacterial genome and may act as vehicles for HGT and ARG dissemination, making them ineffective in acute infections (Principi et al. 2019; Lin et al. 2017). Hence for bacteriophage therapy, lytic phages should be employed. ARG can be carried by phages and spread to other bacterial pathogens; however, utilizing DNA sequencing methods assures that therapeutic phage is clear of any virulent genes (Taati Moghadam et al. 2020).

## Bacteriophage therapy—successful case studies

Many experimental data have demonstrated the potential role of phage-antibiotic synergistic effects and efficacy of bacteriophage alone on different models for different indications. The concept of bacteriophage therapy, which was used to treat bacterial diseases at the turn of the twentieth century, has garnered attention in the current period of rising AMR (Schooley et al. 2017; Furfaro et al. 2018; Aslam et al. 2019). However, due to the unavailability of validated and adequately controlled clinical trials, phage therapy faces many challenges in its progression in the present scenario. Additional care needs to be taken in the organising and designing such trials where therapeutic variables such as the dose (Payne and Jansen 2003) and concoction of phage cocktails are required (Aslam et al. 2019). Some of those systemic studies of antimicrobial agents and chemotherapy, of diseases caused by MDR organisms, successful treatments with adjunctive bacteriophage therapy are described below (Fig. 3).

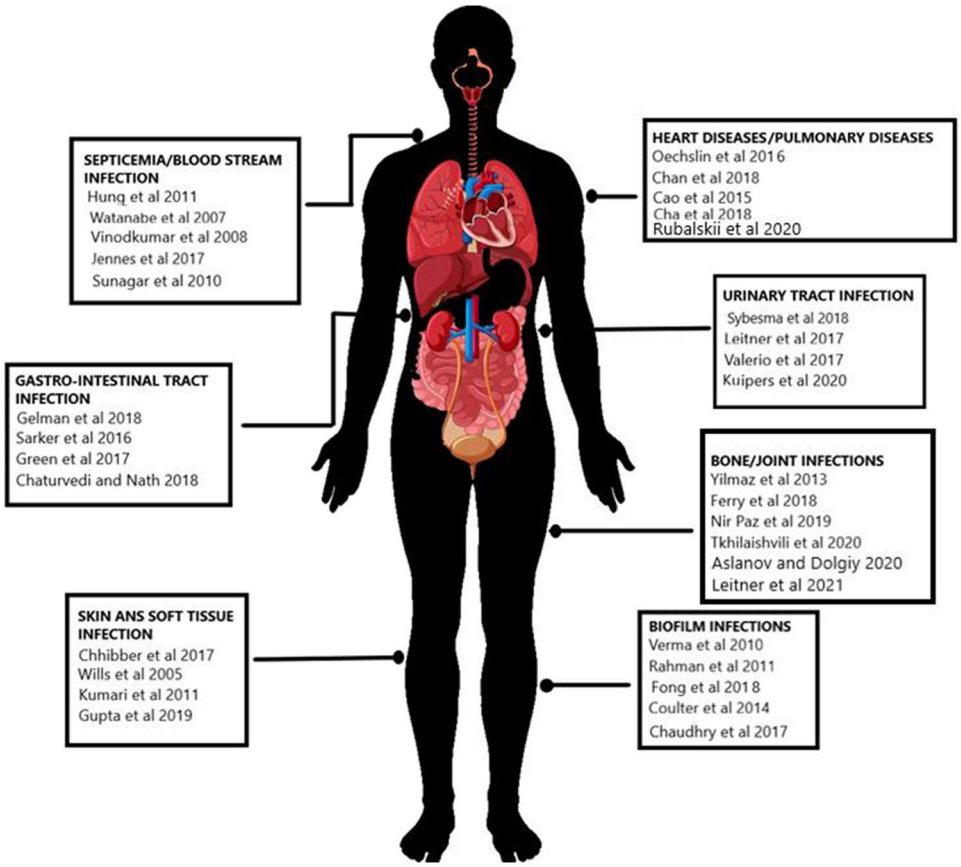


**Fig. 3** Current summary of phage therapy clinical trials targeting some major diseases

## Bone and joint infections

Bone and joint infection (BJI) pose significant management challenges and cause severe morbidity [Table 1]. If ignored, BJIs induce acute sepsis with bone and joint deterioration, severe pain, sinuses, and permanent impairment, necessitating exceptional microbiological examination to allow for targeted antibiotic therapy (Colston and Atkins, 2018). A recent study suggested that local phage administration into the joints, with hardware removal, systemic antibiotics, and antibiotic spacers, successfully managed MDR *P. aeruginosa* infection of prosthetic joint (PJ) and osteomyelitis (Tkhilaishvili et al. 2020). In vitro, the authors discovered phage-antibiotic synergistic effect against *P. aeruginosa* biofilm. Several other studies in the publication endorse the use of supplementary bacteriophage therapy in the management of resistant BJIs. Ferry et al. (2018) reported a recent case study of recurring *S. aureus* PJ infection treated with a phage cocktail and antibiotic combination instilled locally. Another case report documented treatment via bacteriophage and antibiotics of left tibial infection caused due to XDR *A. baumannii* and MDR *K. pneumoniae*, thereby resulting in tissue healing and limb preservation (Nir-Paz et al. 2019). Yilmaz et al. (2013) investigated the therapeutic efficacy of MRSA (methicillin-resistant *Staphylococcus aureus*) and *P. aeruginosa* induced rat tibiae infection utilizing a phage cocktail and an antibiotic in combination and/or alone, resulting in the reduction of bacterial colony-forming units. Another animal model study for the management of *S. aureus* osteomyelitis was undertaken, where the potential of bacteriophage therapy alone in the treatment of chronic infections caused by MDR bacteria was demonstrated by Kishor et al. (2016). As a result, phage application in orthopaedic surgery as an adjuvant to antibiotic therapy or alone against drug-resistant bacteria holds a lot of promise. BJIs have a significant impact on healthcare resources due to growing urbanization and an ageing population, necessitating well organized, novel multidisciplinary collaboration for successful therapy.

## Urinary tract infections

The prevalence of urinary tract infection (UTI) is high, resulting in significant loss of man hours, financial burden on society, and a strain on health-care infrastructure. UTI complications frequently result in sepsis and can be fatal if caused by AMR pathogens, and various studies demonstrate the effectiveness of bacteriophages in the management of it [Table 2] (Bhargava et al. 2021). Leitner et al. (2017) reported lytic activity, as well as the resilience of phages to resistance, can be a useful option for the treatment of the world's ever-increasing AR. To treat recurrent UTI caused by ESBL (extended spectrum beta-lactamase)-positive *K. pneumoniae* in a subject with an indwelling urostomy and uretral stent, Kuipers et al. (2020) employed a personalized phage-antibiotic combination. Valerio et al. (2017) investigated the efficacy of bacteriophages and antibiotics alone or in combination in managing *E. coli*-induced UTI and also the susceptibility of the bacteria to the screened antibacterial agents in the presence and absence of bacteriophages. The effectiveness of combination therapy is determined by the AR, the bacteria in question, and the type of antibiotic employed (bactericidal or bacteriostatic). In another case study, bacteriophage therapy against *P. aeruginosa* UTI in humans was documented where combination therapy of phage and antibiotic was well tolerated resulting in treatment of the patient (Khawaldeh et al. 2011). The high bacterial inactivation efficiency of phage alone or in conjunction with antibiotics, as well as their self-limitation property, lay the groundwork for future comprehensive studies into UTI and AMR management and control.

## Biofilm infections

Biofilm formation by multiple microorganisms causes persistent tissue and foreign body infections which become highly resistant to the antibiotics [Table 3]. Bacteria in biofilm flourishes in slime-encased aggregations that affect millions of individuals, and it is sometimes difficult to treat, resulting in a chronic infection that medical practitioners struggle to control (Harper et al. 2014). As biofilms are difficult to treat in general, a novel technique is required; currently, antibiotics and basic disinfectants have shown a limited ability to successfully remove biofilms. Fong et al. (2019) conducted an experimental investigation on an animal model in which *P. aeruginosa* biofilm-induced frontal sinusitis was reduced by phage cocktail and concluded that it was effective and safe. Bacteriophages reproduce within the host bacterium and synthesize enzymes that disintegrate the bacterial biofilm extracellular polymeric substance, resulting in pathogen eradication. In their research, Coulter et al. (2014) documented that a combination of bacteriophage and antibiotics resulted in the complete eradication of biofilm and the reduction of bacteria resistant to both phages and antibiotics. Similar studies conducted using antibiotics combined with phage have resulted in the eradication of *Klebsiella pneumoniae* and *Staphylococcus aureus* biofilms (Verma et al. 2010; Rahman et al. 2011). Chaudhry et al. (2017) conducted an in vitro experiment in which *P.*

**Table 1** An overview of bacteriophage clinical studies for the treatment of bone and joint infections

| Case study | Description | Administration | Result | References |
|---|---|---|---|---|
| Bacteriophage therapy for chronic PJI infection of the knee and chronic femoral osteomyelitis | After a gunshot injury, an 80-year-old patient with T2DM2 and CKD was diagnosed with PJI and chronic Osteomyelitis due to MDR *Pseudomonas aeruginosa* infection | Phage[#]- Local instillation Antibiotics (colistin, meropenem and ceftazidime) —Intravenous | Bacteria eradicated successfully following combination therapy | Tkhilaishvili et al. 2020 |
| Bacteriophage therapy for PJI during DAIR | An 80-year-old obese woman with T2DM2 and mild chronic kidney injury with a history of relapsing PJI of right hip resulted in a postoperative purulent discharge with MSSA and MDR *Pseudomonas aeruginosa* | Phage (1493 & 1815)-Direct injection into joints Antibiotics (daptomycin, clindamycin and amoxicillin)—Oral | A successful clinical outcome resulted in complete eradication of infection | Ferry et al. 2018 |
| Synergistic effect of bacteriophages and antibiotics on patient with trauma-related left tibial Infection | A 42-year-old patient with bacterial osteomyelitis infected with XDR *Acinetobacter baumanni* and MDR *Klebsiella pneumonia* | Phage (AbKT21phi3; MK278859 and KpKT21phi1; MK278861) and antibiotics (meropenem and colistin)—Intravenous | No positive culture was found after 8 months of combined therapy | Nir-Paz et al. 2019 |
| Bacteriophage therapy for the treatment of implant-related infections (orthopaedic surgery) due antibiotic resistant bacteria | Implant related osteomyelitis was identified in a rat model due to MRSA and *Pseudomonas aeruginosa* | Phage (Sb-1 and vB_PsaP PAT14)-direct injection into the medullary canal Antibiotics (teicoplanin, imipenem, cilastatin and amikacin)-Intraperitoneal | Biofilm dissolved for both types of bacteria | Yilmaz et al. 2013 |
| Phage therapy for chronic osteomyelitis in the experimental rabbit model | Chronic osteomyelitis was established on distal end of femur in a rabbit model due to MRSA | Phage cocktail (SA-BHU1, SA-BHU2, SA-BHU8, SA-BHU15, SA-BHU21, SA-BHU37, SA-BHU47)—Intralesionally | A successful clinical outcome resulted in wound healing and site sterilization | Kishor et al. 2016 |
| Bacteriophage therapy for the treatment of PKI | A 79-year-old female with a resistant *Staphylococcus epidermidis* PKI was treated with phage therapy after debridement and implant retention surgery | Phage (PM448)-intraarticular instillation Antibiotic (daptomycin)-Intravenous | The patient recovered via intraarticular bacteriophage therapy with no adverse effect and evidence of clinical recurrence | Doub et al. 2021 |

*PJI* prosthetic joint infection, *PKI* prosthetic knee infection, *T2DM2* type 2 diabetes mellitus, *CKD* chronic kidney disease, *DAIR* debridement along with antibiotics and implant retention, *MDR* multi-drug resistant, *XDR* extreme drug resistance, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MSSA* methicillin-sensitive *Staphylococcus aureus*. Phage[#]: Collection (No identification given) received from George Eliava Institute of Bacteriophages, Microbiology and Virology (Tbilsi, Georgia)

**Table 2** An overview of bacteriophage clinical studies for the treatment of urinary tract infections

| Case study | Description | Administration | Result | References |
|---|---|---|---|---|
| Bacteriophage therapy for UTI: A randomised, placebo-controlled clinical trial | Patients with prostrate TUR were screened for UTI with recurrent bacteriuria (*Enterococcus spp., Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus spp. and Streptococcus spp.*) | Phage cocktail (Pyo-bacteriophage)-Intravesical suprapubic | 7 days of phage treatment resulted in sterile urine culture | Leitner et al. 2017 |
| Case study of effective treatment of chronic relapsing UTI via phage therapy | A 58-year-old renal transplant patient developed a post-transplant UTI due to ESBL *Klebsiella pneumoniae* (MDR) leading to epididymitis | Phage[#]-Oral, Intravesical and bladder irrigation Antibiotic (meropenem)-Intravenous | Sterile urine culture obtained, following combination therapy | Kuipers et al. 2020 |
| Bacteriophage and antibiotic efficacy in the inactivation of UTI-causing bacteria | In-vitro efficacy of combination therapy and alone was tested in the urine sample for the evaluation of *Escherichia coli* infections | Phage (ECA2) and antibiotics (ampicillin, piperacillin, kanamycin, tetracycline, chloramphenicol and ciprofloxacin)- Direct inoculation | Significant bacterium inactivation was obtained after 8 h of treatment | Valerio et al. 2017 |
| Treatment of refractory UTI by phage therapy | Intra-abdominal resection and pelvic irradiation for adenocarcinoma followed by bilateral ureteric stent placement resulted in *Pseudomonas aeruginosa* infection in a 67-year-old woman | Phage (Pyophage #051,007)-Intravesical Antibiotic (meropenem and colistin)-Intravenous | Combined therapy resulted in sterile urine culture | Khawaldeh et al. 2011 |
| Determination of intravesical phage therapy for the treatment of UTI | A placebo-controlled, double-blind clinical trial was conducted on men over the age of 18 who had acute UTI but no indications of systemic illness via uropathogens (*Enterococcus spp., Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus spp. and Streptococcus spp.*) | Phage (Pyophage cocktail R-022600)-intravesical instillation | Intravesical phage therapy was non-inferior to antibiotic treatment but not superior to bladder irrigation (placebo) in terms of efficacy and safety | Leitner et al. 2021 |

*UTI* urinary tract infection, *TUR* transurethral resection, *ESBL* extended-spectrum β-lactamase; Phage[#]: Solution (No identification given) received from Eliava Institute in Tbilisi, Georgia

**Table 3** Overview of bacteriophage clinical studies for the treatment of biofilm infections

| Case study | Description | Administration | Result | References |
|---|---|---|---|---|
| Bacteriophage therapy for the treatment of paranasal biofilm using an animal model | In sheep frontal sinuses, *Pseudomonas aeruginosa* infection was simulated for the development of biofilm and the establishment of infection | Phage cocktail (CT-PA containing Pa193, Pa204, Pa222 and Pa223)-Intranasal | Statistically significant reduction in the bacterial community of biofilm was obtained | Fong et al. 2019 |
| Effect of combination therapy on resistant bacteria forming a biofilm | Combination treatment against In-vitro biofilm cultures of *Escherichia coli* and *Pseudomonas aeruginosa* | Phage (T4-ATCC11303-B4 and PB-1-ATCC15692-B3) and antibiotic (tobramycin)—Direct inoculation | Significant biofilm reduction as 99% antibiotic-resistant cells of *Escherichia coli* and 60% antibiotic-resistant cells of *Pseudomonas aeruginosa* decreased | Coulter et al. 2014 |
| Bacteriophages induce structural changes in biofilms that eradicate them effectively | *Klebsiella pneumoniae* biofilm was developed In-vitro in wells of microtitre plates | Phage (KPO1K2 and NDP) and antibiotic (ciprofloxacin)—Direct inoculation | The highest reduction in bacterial load observed after 6 h of combined treatment | Verma et al. 2010 |
| An experimental study for an anti-biofilm activity via antibiotic and bacteriophage | In microtiter plates, *Staphylococcus aureus* was cultured in vitro, which resulted in biofilm formation | Phage (SAP-26) and antibiotic (azithromycin, vancomycin and rifampicin)—Direct inoculation | Reduction of 65% with rifampicin, 60% with azithromycin and 40% with vancomycin when exposed to combined therapy | Rahman et al. 2011 |
| Synergistic effect of phages and antibiotics for the destruction of biofilms | In microtiter plates, *Pseudomonas aeruginosa* biofilm has been grown in vitro to mimic the in-vivo environment | Phage (NP1 & NP3) and antibiotic (ceftazidime, ciprofloxacin, colistin, gentamycin & tobramycin)-Direct inoculation | As compared to other drugs, the synergistic effect of phages and drugs was highest with tobramycin | Chaudhry et al. 2017 |

*aeruginosa* biofilms were treated alone and in combination with bacteriophages and bacteriolytic antibiotics. The findings delineated that combination therapy of bacteriophage and antibiotics for the treatment of biofilm infections are more effective and a better therapeutic option. Furthermore, Rahman et al. (2011) demonstrated in their study that combining a bacteriophage and an antibiotic effectively combats *Staphylococcus aureus* biofilm. In another investigation paired lytic bacteriophage with an antibacterial agent, which resulted in increased biofilm eradication compared to either alone, reinforcing the concept that when combined, they are more efficient (Verma et al. 2010). Bacteriophages have tremendous potential for the management of biofilms; nevertheless, such applications are still in their early phases because the processes of killing by drugs and phages differ significantly, necessitating additional experimental investigation.

## Heart/pulmonary infections

The emergence of MDR bacteria has entailed the search for novel therapeutic designs or strategies [Table 4]. Heart and pulmonary infections are highly challenging as they often lead to septicemia. If left ignored, they might affect other organs as well. When pathogenic microorganisms evolve and gain resistance to potential treatments, novel therapeutic approaches would preferably target them, resulting in infection management. As an illustration of such a technique, Chan et al. (2018) isolated a lytic bacteriophage against MDR *P. aeruginosa* with multi-drug systemic porin M on its outer membrane, where phages bind. It culminated in the process of evolution in which the phage altered the bacteria's efflux pumping mechanism, making the pathogen susceptible to several classes of antibacterial drugs. Antibiotics alone are frequently ineffective in treating these resistant infections due to AR, poor biofilm permeability, and other factors; however, phages are host specific, killing the intended bacterium, and the development of phage resistance may lead to an increase in antibiotic sensitivity (Chan et al. 2018). In a patient with *Pseudomonas aeruginosa,* Oechslin et al. (2017) demonstrated that a single dose of bacteriophage had a high synergistic activity with an antibiotic and phage-resistant bacteria had decreased infectivity. Phage therapy, when used alone or in combination with antibiotics, warrants further clinical investigation to improve the efficacy of existing methodologies or develop innovative approaches. Cao et al. (2015) found that administering phage intranasally to mice with a *Klebsiella pneumoniae* pulmonary infection reduced pathogenicity and pro-inflammatory cytokine levels. An in vivo investigation was conducted in mice in which MDR *A. baumannii* lung infection was treated with a newly isolated phage cocktail, resulting in a higher rate of survival after infection as compared to the untreated group (Cha et al. 2018). As AR among bacteria is a problematic issue that necessitates the creation of next-generation treatment approaches in which phage can thrive and soon be at the forefront of clinical care in the management of heart and pulmonary infections.

## Gastrointestinal infections

The causes of gastrointestinal infection (GI) are many; however, bacterial causes are often responsible for severe cases of infectious diarrhoea than other infectious aetiologies [Table 5]. The gut microbiota offers various benefits to the healthy host; yet, perturbations in it may have a negative impact on an individual's health and impair protection against colonization (Casals-Pascual et al. 2018). Over the last decade, a variety of global resistance concerns in bacterial GIs have emerged, necessitating immediate attention and innovative therapeutic interventions. Sarker et al. (2016) from Bangladesh conducted a randomized trial in which they administered two coliphage formulations orally to children suffering from acute diarrhoea. The findings demonstrated that coliphage movement along the gut was uneventful but failed to amplify, concluding that a higher dose of phage is required. Another research study conducted by Gelman et al. (2018) employed a single dose of specific bacteriophage against Vancomycin-Resistant Enterococcus (VRE) which induced severe microbial peritonitis and reported a favourable response. They further stipulated that in the case of fulminant AR infections, antibiotics combined with phages will provide significant benefit in both the immediate and delayed outcomes, with a good survival rate. Chaturvedi and Nath (2018) reported that when *K. pneumoniae* (MDR)-specific phages were administered orally in the gut of albino mice, they eliminated MDR bacteria that had the potential to cause additional nosocomial infections upon translocation. Furthermore, Green et al. (2017) demonstrated that phages isolated from the environment could be effective in combating even the most serious of infections caused by *Escherichia coli* superbugs found in intestinal tracts of immunocompromised patients, which when translocate, pose a considerable threat. Despite the favourable findings of phage therapy, multiple studies have demonstrated that phage–host interactions are more complicated and that the majority of attention is focused on them and less on the phage–human interaction. As a result, more research is needed to make this medication broadly available for human usage.

**Table 4** Overview of bacteriophage clinical studies for the treatment of heart and pulmonary diseases

| Case study | Description | Administration | Result | References |
|---|---|---|---|---|
| Bacteriophage therapy for an aortic graft infection | A 76-year-old patient underwent surgery for an aortic aneurysm with Dacron graft, which resulted in MDR *Pseudomonas aeruginosa* infection | Phage (OMKO1) and antibiotics (ceftazidime)-direct injection into the site (mediastinal fistula) | Infection eradicated with no signs of recurrence in 18 months | Chan et al. 2018 |
| Synergistic effect of bacteriophages and antibiotics for endocarditis | Experimental aortic endocarditis caused by *Pseudomonas aeruginosa* was induced in a rat model | Phage cocktail (PP1131) and antibiotic (ciprofloxacin)-intravenous | Combination therapy resulted in killing bacterial vegetation within 6 h and treating 64% of rats | Oechslin et al. 2017 |
| Bacteriophage therapy for the treatment of pneumonia | Seven-week-old female mice were inoculated intra-nasally with MDR *Klebsiella pneumoniae* | Phage (1513)-intranasal | After therapy lung lesions improved and bacterial count decreased | Cao et al. 2015 |
| Evaluation of therapy and efficacy of bacteriophage cocktail in an in-vivo nasal and lung infections | In the six-week-old female mice, clinical MDR *Acinetobacter baumannii* was inoculated for the establishment of infection | Phage cocktail (PBAB08, PBAB25, PBAB68, PBAB80, PBAB93)-intranasal | Decreased bacterial concentration in lung resulted in a 60% survival rate of mice | Cha et al. 2018 |
| Phage therapy for critical Infections associated with Cardiothoracic surgery | Eight patients with immunosuppression after organ transplantation were infected via MDR *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* | Phage (CH1, Enf1, PA5, PA10, KPV811, KPV15, Sa30, SCH1, SCH111, ECD7, V18)-local, intraoperatively, inhalation and intranasal<br>Antibiotics (cefepime, daptomycin, linezolid, tobramycin, ceftazidime, colistin, meropenem, co-triamoxazole, rifampicin, flucloxacillin, sultamicillin and clindamycin)—oral and intravenous | No major adverse effects, seven out of eight people got their target bacteria eradicated | Rubalskii et al. 2020 |

MDR: Multi-drug resistant

**Table 5** Overview of bacteriophage clinical studies for the treatment of gastrointestinal infections

| Case study | Description | Administration | Result | References |
|---|---|---|---|---|
| Clinical coliphage analysis in Bangladesh to treat acute bacterial diarrhoea | In the hospital, 6–24-month-old children with acute watery diarrhoea due to the pathogenic *Escherichia coli* underwent phage therapy for 4 days | Phage cocktail (T4 containing AB2, 4, 6, 11, 46, 50, 55; JS34, 37, 98, D1.4 and Microgen Coli Proteus)- Oral | 93% of patients treated with coliphage recovered from diarrhoea within 6 days | Sarker et al. 2016 |
| Combined bacteriophage treatment for septic peritonitis | Female mice inoculated with a lethal dose of VRE disseminated intraperitoneally to intra- and extra-peritoneal organs | Phage (EFDGI and EFLK1) and antibiotics (ampicillin)-Intraperitoneal | 100% successful treatment via bacteriophage cocktail alone for critically ill mice and 60% the success rate for combination therapy | Gelman et al. 2018 |
| Bacteriophage therapy for intestinal MDR bacteria eradication | Due to increased antimicrobial resistance in gut bacteria, the effect of single-dose of bacteriophage on MDR *Klebsiella pneumoniae* isolated from albino mouse faeces | Phage[#]-Oral | The colony-forming unit of *Klebsiella pneumoniae* gradually decreased as the days progressed, leading to full eradication in 6 days | Chaturvedi and Nath 2018 |
| Bacteriophage therapy against MDR strain for the treatment of bacteremia | An immunocompromised mouse model was developed where colonised MDR *Escherichia coli* was translocated from the gastrointestinal tract to the bloodstream | Phage (HP3)-Intraperitoneal | Gastrointestinal bacteremia decreased dramatically after phage therapy relative to untreated mice | Green et al. 2017 |

*MDR* multi-drug resistant, *VRE* vancomycin-resistant *Enterococcus faecalis;* Phage[#]: Customized Phage cocktail (No identification given)

**Table 6** Overview of bacteriophage clinical studies for the treatment of septicemia/bloodstream infections

| Case study | Description | Administration | Result | References |
|---|---|---|---|---|
| A case report of bacteriophage therapy for the treatment of septicaemia in a patient | A 61-year-old man with peritonitis infection and other complications developed large necrotic pressure sores by MDR *Pseudomonas aeruginosa* leading to septicaemia | Phage (BFC1)-Intrevenous and Topical | Fever disappeared, CRP level dropped, and blood culture turned negative for *P. aeruginosa* | Jennes et al. 2017 |
| Experimental phage therapy for the treatment of liver abscesses and bacteremia | The mice intragastrical inoculation of *Klebsiella pneumoniae* led to the development of liver abscesses, necrosis of liver tissues and bacteremia | Phage (NK5)-Oral or Intraperitoneal | The bacterial count was eliminated virtually from both blood and liver tissues | Hung et al. 2011 |
| Bacteriophage therapy for the treatment of septicaemia | One-month-old BALB / c mice were intraperitoneally inoculated to mimic in-vivo neonatal septicaemia with clinical MDR *Pseudomonas aeruginosa* | Phage (CSV-31)-Intraperitoneal | 100% survival rate with minimal signs of illness after 24 h of treatment | Vinodkumar et al. 2008 |
| Treatment of bacteremia by phage therapy in an immunocompromised and stable host | A diabetic and non-diabetic six-week-old BALB/c mouse was induced with *Staphylococcus aureus* bacteremia | Phage (GRCS)-Intraperitoneal | 90% survival rate for diabetic and 100% survival rate for non-diabetic after treatment | Sunagar et al. 2010 |
| Efficacy of bacteriophage therapy for sepsis in mice model | Each mouse was inoculated orally and intraperitoneally with *Pseudomonas aeruginosa* to induce gut-derived sepsis | Phage (KPP10)-Oral, Intravenous and Intraperitoneal | 92.3% of phage treated mice survived as compared to 41.7% phage untreated mice | Watanabe et al. 2007 |

MDR: Multidrug-resistant; CRP: C-reactive protein

## Septicemia/bloodstream infections

Bloodstream infection (BSI) due to bacteria (bacteremia) is a severe multisystem disease that is strenuous to treat due to its high mortality rates and manifestation [Table 6]. Bacteremia can have several serious health repercussions, and the haematogenous spread of bacteria may result in diseases like endocarditis or osteomyelitis (Holland et al. 2016; Agarwal and Aggarwal 2016). Jennes et al. (2017) described the first contemporary report of bacteriophage monotherapy against MDR *P. aeruginosa* septicaemia in a patient, which resulted in the eradication of the pathogen. Hung et al. (2011) concluded in an experimental study of phage therapy that liver abscess and bacteremia in mice due to *K. pneumoniae* could be a prospective mode of therapeutic intervention. In a study conducted by Vinodkumar et al. (2008), a single dosage of lytic phage administered to mice suffering from MDR *Pseudomonas aeruginosa*-induced septicemia resulted in its redemption. The phage strain utilized in this study displayed broad-spectrum lytic activity against other isolated MDR strains of *Pseudomonas aeruginosa*, implying that phage therapy might be employed as a stand-alone treatment for AR infections. In an original research work, Sunagar et al. (2010) investigated the effect of bacteriophage therapy on fatal *S. aureus*-induced bacteremia in non-diabetic and streptozotocin-induced-diabetic mice. They concluded that bacteriophages could also be used to prevent *Staphylococcus aureus* infections in immunocompromised patients. Furthermore, Watanabe et al. (2007) ascertained the effectiveness of phage in an animal model of *P. aeruginosa*-induced sepsis. The results of this experiment showed that newly isolated lytic phage strain administration was extremely effective against sepsis caused by *P. aeruginosa.* Although bacteriophage therapy is thought to have significant potential in the treatment of a variety of topical and localized infections, this interest has not extended to the treatment of BSIs, which is surprising given that phages are likely to be safe and efficient when delivered in a controlled manner.

## Skin and soft tissue infections

Skin and soft tissue infections (SSTIs) are caused by microbial infiltration of the epidermis, dermis, subcutaneous tissue, superficial fascia, or muscles and can present with a wide range of symptoms, etiological agents, and severity (Ki and Rotstein 2008). The advent of AR bacteria has complicated the management of SSTIs, with MRSA, VRE, and ESBL positive isolates of *Escherichia coli* and *Klebsiella spp*. being the most common (Moet et al. 2007), necessitating a reconsideration of the use of phage for its treatment [Table 7]. Chhibber et al. (2017) investigated the efficiency

**Table 7** Overview of bacteriophage clinical studies for the treatment of skin and soft tissue infections

| Case study | Description | Administration | Result | References |
|---|---|---|---|---|
| Transfersomal phage cocktail treatment against SSTIs in a rat model | The posterior portion of both thighs of 4–6 week old female rats was intramuscularly injected with *Staphylococcus aureus* | Phage (MR-5 & MR-10)-Intramuscular | 100% survival rate was observed for both 30 min and 12 h post-infection | Chhibber et al. 2017 |
| Comparison of bacteriophage and antibiotics for the treatment of burn wound infection | A BALB/c mouse was infected with *Klebsiella pneumoniae*, which resulted in the establishment of the burn wound | Phage (Kpn5) or antibiotic (silver nitrate and gentamycin)-Topical | Survival rate (63.3%) via phage was higher than other two agents (56.66% & 53.33%) and untreated phage group | Kumari et al. 2011 |
| A clinical trial for the treatment of wounds via a bacteriophage cocktail | Patients aged between 12 to 60 years with chronic non-healing wounds caused by *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* | Phage[#]-Topical | Seven patient infections eradicated, while the remaining 13 wound sizes decreased significantly | Gupta et al. 2019 |
| Bacteriophage therapy against abscess induced in a rabbit model | *Staphylococcus aureus* was injected into the thigh area of adult New Zealand rabbits, progressing to wound infection | Phage (LS2a)-subcutaneous | Abscess in 90% of rabbits cured completely | Wills et al. 2005 |

SSTIs: Skin and soft tissue infections; Phage[#]: Cocktail of phages (No identification given)

of a transfersomal phage cocktail in the treatment of MRSA-induced SSTIs. They concluded that using transfersome as a delivery vehicle improves the stability and persistence of the enclosed bacteriophages in vivo. Similarly, when an animal model burn wound was infected with *Klebsiella pneumoniae*, bacteriophage was applied topically, which reduced mortality and resulted in an insignificant decrease in phage titre, indicating its stability (Kumari et al. 2011). An original clinical study reported by Gupta et al. (2019) stated that topical bacteriophage application for treatment of chronic non-healing wounds is highly effective. Chronic wounds are often recalcitrant to medication due to MDR pathogens and biofilm formation; however, phage cocktail was an excellent alternative to drugs (Jault et al. 2019). Wills et al. (2005) reported a wound infection caused by *S. aureus*, where staphylococcal phage managed to prevent abscess formation when injected simultaneously with the bacterium. Phage multiplied in the tissues. Therefore, the authors concluded that phages might also be valuable prophylaxis against staphylococcal infection (Wills et al. 2005). Superficial bacterial infections impose significant emotional and economic burdens on healthcare systems worldwide and are frequently worsened by AR, for which phage therapy is a viable potential antibacterial option (Abedon et al. 2011).

## Phage and antibiotic interaction

Antibiotic resistance is a cause of concern, and phage has been advocated as one feasible therapeutic alternative for therapy and antibiotic potency enhancement. Bacteriophage is presently the recommended therapeutic modality, either alone or combined with an antibacterial agent, with the latter is expected to boost efficiency (Gkartziou et al. 2021). The combination of phage with antibiotics could have a variety of outcomes, including additive, synergistic, ineffective, or antagonistic effects (Abedon 2019; Gu Liu et al. 2020). Principi et al. (2019) suggested that bacteriophages reduce the minimum inhibitory concentration of AR bacteria to the level of sensitive bacteria. Antibacterial agents are frequently chosen based on antibiotics susceptibility data and the patient's medical condition so that when phages are coupled, a "phage adjuvation" effect can be produced (Gu Liu et al. 2020). Antibiotic concentration must also be optimized since they increase the rate of bacterial cell mutation when used at sub-lethal concentrations, rendering coupled strain-specific phage useless (Saha and Mukherjee 2019). Several phage-antibiotic combinations have been assessed in vitro. However, due to inconsistent results with the combinatorial treatment, a customized in vitro assessment approach is required for optimal therapeutic effect in vivo against distinct bacterial species (Torres-Barceló et al., 2018). Currently, phage therapy does not replace antibiotics, but with the emergence of MDR, the concurrent use of personalized phage alone or in combination with antibiotics may be the way to the future. However, because some antibiotics can interfere with phage therapy by killing their host (bacterium) and blocking their reproduction, more rigorous sophisticated preclinical clarifications are essential prior to in vivo administration (Abedon 2019). There are significant gaps in how bacteriophages, bacteria, and antibiotics interact. Antibiotics can modify phage features such as growth rate, infectivity, and burst size, and their fate is mostly unknown and unexplored (Cairns et al. 2017). Torres-Barceló et al. (2018) findings imply that the effects of antibacterial medications on bacteriophages, and the combined effects of phages and antibiotics on bacteria, can alter substantially as interactions evolve. Although multiple studies have demonstrated a synergistic link between phages and antibiotics, the focus is often on bacteria, with little knowledge about the influence on phages. Therefore, further research is required to test it in its entirety.

## Engineered phages and its enzymes

In various medical and biotechnological areas, innovations in genetic engineering and molecular biology for the application of phages have been observed. One of the phage modifications, "modus operandi", is based on the integration of bacteriophage coat protein genes with foreign molecules (Bardy et al. 2016). It results in the creation of numerous bacteriophage variants. Currently, engineered bacteriophages have generated little proof of robust efficacy (Nair and Khairnar 2019). It has contributed to inconsistent outcomes in the treatment of diseases, as it has been less explored. Contrary to antibiotics, modified phages conflicted results are due to their unknown detailed molecular composition (Pizarro-Bauerle and Ando 2020). When bacteriophages are introduced into the food chain, they progress into the environment uncontrollably. When they infect bacteria, altering those would alter associated microbiota that may or may not be governable (Nair and Khairnar 2019). Engineered bacteriophages can pose an issue in terms of public acceptance due to a lack of knowledge and understanding (Sybesma et al. 2018). They are themselves living entities that are allowed to thrive on their host, which is thriving on another living organism. Hence, phage therapy via modified phages further derails its approval as a mainstream treatment option. For its concrete establishment in the world of medicine, there is a need for thorough research with extensive clinical trials (Pizarro-Bauerle and Ando 2020; Kutter et al. 2010; Miedzybrodzki et al. 2012; Carvalho et al. 2017). Unlike antibiotics, phages have genomes and replicate while parasitising on their host; engineering them may result in further complications and would lead to the addition of

clauses of legislation regarding the ethical concerns related to genetic modifications (Abdelkader et al. 2019).

Bacteriophage recombinant lytic proteins can be used as enzybiotics (Schuch et al. 2002). For gram-positive bacteria, external administration of endolysin results in cell disruption (Young 2013). Moreover, for gram-negative bacteria, lysins are unable to cross the bacterial outer cytoplasmic membrane (Fischetti 2018). Therefore, endolysin therapy is futile since it can also lead to the release of a large amount of endotoxin. Furthermore, it is a significant constituent of lipopolysaccharide, which, when released in the bloodstream of an infected host, would result in gram-negative septic shock causing hemodynamic and metabolic anomalies (Bardy et al. 2016; Prins et al. 1994). Also, when lysins are put up for therapeutic use, the production of neutralising antibodies is induced, which subsequently hinder their antibacterial activity on multiple administrations. Hence their use as an antimicrobial agent in human treatment raises concerns as they possess relatively short plasma life, immunogenicity and possible toxicity, proinflammatory response to bacterial debris and its inadequacy to lyse intracellular bacteria (Vazquez et al. 2018). Furthermore, the transfer of toxin-producing genes by genetically engineered phages puts them on the downside. However, new strategies are being implemented to safely use such phages after the risk of recombination and horizontal gene transfer among bacteriophages is thoroughly investigated. The successful launch of modified bacteriophages cannot be implemented by putting health, safety and environment at risk (Bardy et al. 2016).

## Challenges and future perspectives

Currently, no framework (Verbeken et al. 2014) exists that could define phage as a medicinal product for human use. However, institutes in Georgia, Poland, provide customized phage cocktails to chronically ill patients for whom all other options of authorized treatment get exhausted (Yilmaz et al. 2013). Although in other parts of the world, bacteriophage therapy is still controversial, hence a dedicated legal framework is essential for its smooth introduction into western medicine. Regulatory cells in western countries have been debating about norms and steps to pioneer guidelines for phage therapy (Vandenheuvel et al. 2015). For safety purposes, legislation nowadays is heavily controlled for the production and administration of drugs by having strict quality control procedures. For the safety of bacteriophage therapy, a monitoring system needs to be implemented for data collection and analysis to follow the development of bacterial resistance to phages along with the installation of dedicated public structures that could pioneer more clinical trials (Sybesma et al. 2018; Kortright et al. 2019; Pirnay et al. 2018; Svircev et al. 2018). For bacteriophage stability and effectiveness, good manufacturing practice level facilities are necessary for phage production and prospective research investigations (Brown et al. 2017; Kutter et al. 2010). This would allow for the storage of bacteriophage concoctions in retail pharmacies. Bacteriophage therapy is currently being developed in two directions. The first is the development of broad-host-range "off-the-shelf" solutions that may be utilised for infections caused by specific bacteria, and the second is a customized approach, such as the isolation and production of specific phage cocktails against a specific strain isolated from a patient. Bacteriophages isolated from the environment are the initial step toward bacteriophage therapeutics; many research investigations are currently underway to increase efficiency and optimise biofilm disruption, employing genetically altered phages (Bradley et al. 2016; Haellman and Fussenegger 2016; Wang et al. 2011) in situations when antibiotics become inadequate (Tagliaferri et al. 2019; Aslam and Schooley 2019). For further commercialization of phage and its products, there is a need for extensive research of bacteriophage metagenomics and metaproteomics.

Metagenomics is a concept that refers to the examination of genetic data from environmental samples in order to identify microbial communities (de Abreu et al. 2021). A high-throughput sequence (HTS) based functional metagenomics technique is useful for identifying and understanding resistance mechanisms detecting ARG and examining the internal dynamics (DNA or RNA level) of the cell (Churko et al. 2013; Sukhum et al. 2019). Because of the significant growth in data creation, new bioinformatics tools have been developed to deal with the massive volume of sequencing reads gathered during genome sequencing investigations (Pereira et al. 2020). There are two types of metagenomic analyses: sequence-based and functional-based (de Abreu et al. 2021). Numerous fragment sequences are generated and analysed using software, allowing for the development of structural and functional diversity in a microbiome by finding genes and metabolic pathways of bacterial genomes (Bharti and Grimm 2021). Genomic analysis also determines the relationship between the bacteriophage and its host, genomic content, and genetic linkage between the most sensitive and most resistant strains obtained to evaluate phage therapy viability by ensuring they did not encode for toxins and/or lysogenic characteristics (Haines et al. 2021). Furthermore, genetic analysis of isolates with polar opposite phage sensitivity could provide additional insight into resistance mechanisms, contributing to cocktail formulation design. Pirnay (2020) further discussed in "the future Earth 2035", in which he shared Dr John Iverian's experience and work on the technology Phage BEAM (Bedside Energized Anti-Microbial). First, a metagenome analysis of the entire sample was performed, and the results were stored

in the "Phage Xchange" server, where an intricate artificial intelligence-driven algorithm predicted the sequence of the phage that would most likely infect the infecting bacteria. Then the predicted phage genome data was transferred to the Phage-BEAM device, which manufactured the phage genome and, eventually, the phage itself for clients using patented technology (Pirnay 2020).

Antibiotic overuse and abuse in the medical, agriculture, and aquaculture sectors has aided the world into a silent pandemic of AMR. The most common cause of opportunistic infections worldwide is a group of ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*) organisms, the majority of which are MDR isolates (Mulani et al. 2019). The upsurge may be seen in the number of bacteriophage therapy publications (more than 600) covered by PubMed from 2016 to 2020, compared to around 285 articles published between 2010 and 2015 (Azam et al. 2021). It is worth noting that these data from the recent decade reflect a significant increase over those from two decades before when there were few papers published. The rise in the number of articles published in the last two decades indicates that the scientific industry's focus is returning to phage therapy; however, physicians are wary of phages because they are a living organism that is introduced into a sick patient, and their complete mechanism of action is still underexplored.

## Emerging prospect of phage therapy in COVID-19 patients

COVID-19 is one of the most prominent disaster-induced pandemics of our century (World Health Organization 2020). The prevalence, occurrence and manifestations of bacterial infections in patients already diagnosed with SARS-CoV-2 (COVID-19) are currently not well documented and pose many questions (Cox et al. 2020; Huttner et al. 2020; Langford et al. 2020). It has been reported that over 70% of patients were administered broad-spectrum fluoroquinolones and third-generation cephalosporins, despite a significant low rate of bacterial infections (Langford et al. 2020). While antibiotics are inadequate for the treatment of COVID-19, patients with suspected or confirmed secondary bacterial infections and/or co-infections are still administered antibiotics. This assumption, however, poses the question of antibiotic overuse and eventual global bacterial resistance. The increasing number and our decreasing ability to eradicate AMR bacteria not only makes us more prone to bacterial infections but also weakens us during viral pandemics (Vaillancourt and Jorth 2020). New antibiotics or alternative therapies for secondary bacterial infections are required for the recurrent waves of COVID-19 and the imminent future pandemic. Alternative therapies such as phage therapy can be explored as it shows promise. Although data for COVID-19 is still scarce, an integrative approach is proposed where bacterial infections, together with delayed production of antibodies results in a significant contributing factor to COVID-19 mortality rate. Therefore the implementation of phage therapy might lead to microbiota homeostasis accelerated development of therapeutic antibodies via "Phage display" (Blanco-Picazo et al. 2020) and also decrease the pathogenic bacterial load in the respiratory tract of the infected persons (Wojewodzic 2020). Also, phages tend to compete with virus for cellular receptors when introduced after a primary viral infection and thus reduce the harmful activities of it (Meek and Takahashi 1968). Inflammation and cell destruction resulting from excessive reactive oxygen species (ROS) are often associated with a respiratory virus infecting the lungs (Gorski et al. 2020). Phage and phage proteins, however, inhibit the development of ROS and thus demonstrate antiviral activities by anti-oxidant therapy (Miedzybrodzki et al. 2005; Centifanto 1968). The same types and dosage of drugs are used in both techniques, the prophylactic and therapeutic: but the prophylactic use of antibiotics is not approved by most health institutions and policy makers globally due to the reported rise in AMR rates, which correlate with the overuse and misuse of antibiotics (Manohar et al. 2020; Holshue et al., 2020; Wang et al. 2020). However, that is not the case with bacteriophages as they do not affect any eukaryotic cells and therefore can be used both prophylactically and therapeutically (Adhya et al. 2014; Lin et al. 2017).

## Conclusions

With the emerging AR bacterial infections, the activity of bacteriophages against resistant bacteria with no major serious side effects makes them a promising solution in recalcitrant bacterial infections since bacteriophages are specific for their host. As reflected from prior clinical case studies, instead of replacing antibiotics altogether, the combination of both, *i.e.* bacteriophage and antibiotic or phage alone, could result in potentially viable therapeutic options against bacterial pathogens. Enhanced bacterial clearance, more efficient adsorption into biofilms and a lower likelihood of the development of bacteriophage resistance are the potential advantages of bacteriophage therapy. Furthermore, they have pronounced prospective for treating secondary bacterial infections or co-infections during viral pandemics like the prevailing Covid-19 pandemic. We are in the midst of a significant shift in the medical industry, and we are failing to combat various diseases caused by AMR organisms. Looking at the current scenario, more research that sheds

light on the nature of host-phage interactions in the context of commensal flora is required that would aid in elucidating and accelerating the concept of bacteriophage therapy and advocating its regulatory approval in modern medicine.

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