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Review

# Wastewater as a fertility source for novel bacteriophages against multi-drug resistant bacteria

Najwa M. Alharbi [a,*], Mashayed M. Ziadi [b]

[a] College of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[b] King Abdulaziz University, Jeddah, Saudi Arabia

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ABSTRACT

Antibiotic resistance is a common and serious public health worldwide. As an alternative to antibiotics, bacteriophage (phage) therapy offers one of the best solutions to antibiotic resistance. Bacteriophages survive where their bacterial hosts are found; thus, they exist in almost all environments and their applications are quite varied in the medical, environmental, and industrial fields. Moreover, a single phage or a mixture of phages can be used in phage therapy; mixed phages tend to be more effective in reducing the number and/or activity of pathogenic bacteria than that of a single phage.
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* Corresponding author.
*E-mail addresses:* Nmaalharbi@kau.edu.sa (N.M. Alharbi), Mashayed.ziadi@gmail.com (M.M. Ziadi).
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## 1. Introduction

One of the major and most important discoveries of modern medicine is the use of antibiotics to treat infections (Pirmoradian and Hooshmand, 2019). The "golden time" of antibiotic discovery extended between the 1930s to the 1960s, resulting in a significant

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increase in different types of antibiotics (Nathan and Cars, 2014). Unfortunately, the rate of discovery and development of new antibiotics has not kept up with the demand to confront microbes with ever emerging antibiotic resistance. This failure in discovering and/or improving new antibiotics and their overuse are major factors contributing to the emergence of antibiotic/antimicrobial resistance (Nathan, 2004). Antimicrobial resistance applies to a broad range of microorganisms, including fungi, bacteria, parasites, and viruses, all of which can change or mutate over time leading to resistance. Through natural selection, these mutated and resistant microorganisms can develop into what are referred to as "superbugs." Resistant infections may spread to others and even cause death; therefore, antibiotic/antimicrobial/multidrug resistance is a major global public health concern and imposes high costs on individuals and society (https://www.who.int/features/qa/75/en/)

Antimicrobial resistance occurs naturally, but it can be accelerated by unsuitable and/or overuse of medications. For example, the use of antibiotics to treat viral infections such as a cold or influenza, the sharing of antibiotics, prescribing the wrong medicine, using low-quality medications, and poor infection prevention and control all support the development and spread of drug resistance. The lack of awareness on the part of various governments in tackling these issues, poor monitoring, and a reduced arsenal of tools to diagnose, treat, and prevent infections are all factors hindering the control of antimicrobial resistance (https://www.who.int/features/qa/75/en/).

Because of the expanding number of drug-resistant bacteria now commonly found in hospitals, the need to identify alternative therapies to antibiotics is essential (Finch et al., 2011; Sommer and Dantas, 2011). Recently, much attention has been paid to bacteriophage (phage) therapy, especially in treating bacterial diseases (Golkar et al., 2014). Bacteriophages are viruses that infect and ultimately destroy bacteria in a specific manner (Rahmani et al., 2015), including bacteria in humans without harming human cells (Dubey et al., 2016). D'Hérelle coined the term bacteriophage, which means "bacteria eater" and defined "the agent's bactericidal ability" (Lin et al., 2017). Bacteriophages are the most pervasive organisms on Earth, with a number of almost $10^{31}$, which is hypothesized to outnumber the stars in outer space (Weitz et al., 2013).

Phages act as natural antibacterial agents owing to their specificity and ability to control bacterial populations through the induction of bacterial lysis (Wittebole et al., 2014). They are active against gram-positive (Matsuzaki et al., 2003; Biswas et al., 2002) and gram-negative bacteria (Vinodkumar et al., 2005; Wang et al., 2006), including multidrug resistant (MDR) bacteria (Matsuzaki et al., 2003; Wang et al., 2006).

## 2. Importance of phages

Phage therapy has many advantages over traditional antibiotic treatment (Doss et al., 2017). First, the resistance developed by pathogenic bacteria against phages occurs at a much slower rate than resistance toward antibiotics (Oliveira et al., 2015). Second, the process of isolating phages is inexpensive, relatively simple (Parasion et al., 2014), and fast; the process can frequently be carried out within a few days or weeks (Sulakvelidze et al., 2001). On the other hand, improving an antibiotic (e.g., against antibiotic resistant bacteria) is a long process and can takes years (Silver and Bostian, 1993; Chopra et al., 1997). Third, bacteriophages can survive in very severe environments and do not lose their virulence until they have significantly reduced the mass of the host bacteria (Schmelcher and Loessner, 2014). Fourth, most bacteriophages have high specificity for their particular host and replicate at the infection site, minimizing any side-effects on the natural flora of the human body. This is not the case with antibiotics (Smith and Huggins, 1982; Veiga-Crespo et al., 2007). Fifth, in addition to being harmless to natural human microbiota, phages are also an appropriate treatment choice because they lack the ability to infect eukaryotes cells (Parasion et al., 2014). This safety feature has been tested and proven with only minor side effects reported (Haq, Chaudhry et al., 2012). Thus, phage therapy exhibits potentially countless benefits as well as applications in the fields of medicine and veterinary science (Doss et al., 2017). Even in agriculture, phage therapy could be used to decontaminate many types of ready-to-eat foods such as milk, vegetables, as well as meats product(Endersen et al., 2014).

Insufficient research has been conducted on phages to fully explore their capabilities and diversity in natural environments, especially considering that the number of phages worldwide is ten-fold greater than the number of bacteria cells (Weitz et al., 2013). Until 2016, only about 1910 phage genomes had been sequenced compared to 67,807 bacterial genomes reported in the NCBI database. Most of these phages have not been studied experimentally, leaving a fertile field for exploration (Jurczak-Kurek et al., 2016).

Phage therapy involves the use of intact bacteriophages to treat bacterial infections. The possibility of therapeutic use of bacteriophages has received renewed attention in recent years due to increased difficulties in the treatment of infections caused by bacterial strains resistant to antibiotics, and an increase in knowledge of phages (Nilsson, 2014). Research on phages dates back to Frederick Twort's discovery of them in 1915 (Twort, 1915), and continues today with the discovery and isolation of several phages around the world. Most of the results of this research carried out over several years were positive, including many successful *in vivo* and clinical treatments for bacterial species resistant to antibiotics.

## 3. Sources of bacteriophages

Phages can be found wherever the bacterial host exists. That might include salt and fresh water, soil, and the human body (Wang and Zhang, 2010; Lin et al., 2010; Zheng et al., 2013; Zhan et al., 2015; Brown et al., 2016). Sewage water is one of the most receptive media for phage growth because it has high amount of organic and non-organic material suitable for bacterial host proliferation, therapy, phage isolation. (Lobocka et al., 2014). Researchers attempting to exploit phage-based methods to clean wastewater, have devised treatments to use where effluent and sludge are either released into the environment or reused (Shende et al., 2017). These treatments are a viable way of dealing with the problems of environmental wastewater as they help minimize the number of pathogenic bacteria and foaming in activated sludge plants (Wittebole et al., 2014).

Several studies have reported on the isolation of phages from sewage since 1986. In this review, we have focused on phages isolated from sewage water as it is a rich source for phages and phage hosts. We observed that a single phage or a mixture of phages can be used in phage therapy. Recent studies have primarily used a mixture of phages, which exhibited more effective results than using a single phage (Jamal et al., 2017), as discussed in more detail below.

### 3.1. Cocktail phage therapy

A mixture of phages that cover the variety of clinically important strains are used in cocktail phage therapy (Pirnay et al., 2011; Merabishvili et al., 2018). The use of a diverse mix of phages allows for the potential treatment of a variety of pathogens, which

broadens the actions of phages and circumvents the development of phage-resistant mutants (Costa et al., 2019). For example, *Pseudomonas aeruginosa* (Friman et al., 2016), *Klebsiella pneumoniae* (Gu et al., 2012), and *Acinetobacter baumannii* (Regeimbal et al., 2016) often develop phage-resistant mutations. The use of a number of different phages in a single cocktail would help to overcome this problem.

The first application of cocktail phage therapy to treat a mycobacterium infection was reported for a 15-year-old patient with cystic fibrosis, who also had a common mycobacterial abscess infection. After lung transplantation, the patient was treated with a cocktail of three phages engineered with lytic phage derivatives. Over a period of 6 months, the infectious *Mycobacterium abscessus* strain was killed and clinical improvement was observed in the patient (Dedrick et al., 2019).

In another study, researchers experimented with using a mixture of bacteriophages, phSE-5 and ELY-1, isolated from a sewage water network in Aveiro, Portugal. The phage cocktail was used to treat a mix of pathogenic Enterobacteriaceae consisting of *Escherichia coli* and *Salmonella typhimurium.* The phage mix exhibited greater lytic activity against *S. typhimurium* than against *E. coli* (Costa et al., 2019). However, further experiments are needed to determine whether using a phage mixture is necessary, or if using a single phage would suffice.

In a study conducted by Manohar et al. (2019) three bacteriophages were obtained from wastewater in India, namely myPSH2311 infecting *E. coli*, myPSH1235 infecting *K. pneumoniae*, and myPSH1140 infecting four different *Enterobacter* species. Mixtures of these phages displayed widespread host activity *in vitro*; hence, multiple phage cocktails (EK, KL2, EL3, and EKL4) were used in this experiment. The EK1 cocktail, which included *Klebsiella* phage and *Escherichia* phage, reduced bacterial cell culture numbers of both *K. pneumoniae* and *E. coli*. The KL2 cocktail, which included *Enterobacter* phages and *Klebsiella* phage, minimized the bacterial cell counts of *E. cloacae* and *K. pneumoniae*. The third cocktail, EL3, which included *Enterobacter* and *Escherichia* phages, induced a double decline of *Enterobacter cloacae* and *Escherichia coli* cell numbers. Finally, the EKL4 phage cocktail, which comprised all of the previous phages, diminished the bacterial cell numbers of *Escherichia coli, K. pneumoniae*, and *Enterobacter cloacae*. These results demonstrated the efficiency of phage cocktail treatment compared to that of single phage treatment.

Geng et al. (2020) isolated two lytic phages, vbsm-A1 and vbsp-A2, from raw sewage from three cattle farms in Xinjiang, China. Phage vbsp-A2 induced the lysis of 16 *Staphylococcus aureus* strains, whereas vbsm-A1 induced the lysis of 23 *S. aureus* strains. The two phages could infect an abundant number of hosts, especially phage vbsm-A1. Interestingly, using a cocktail of vbsm-A1 and vbsp-A2 showed greater therapeutic efficacy for *S. aureus.*

Finally, the effect of a cocktail of lytic phages isolated from sewage was evaluated as a possible antimicrobial candidate against *K. pneumoniae,* which is resistant to the antibiotic gentamicin. The isolated phages, ϕkpav12, ϕkpav10, ϕkpav08, ϕkpav04, and ϕkpav03, displayed antimicrobial efficiency and inhibited *K. pneumonia* growth by 80% after incubation for 18 h. This study suggests the possibility of utilizing a phage cocktail against multidrug-resistant bacteria, for example, as an alternative to using gentamicin in the treatment of carbapenem-resistant infections caused by *K. pneumonia* (PARAN et al., 2020).

### 3.2. Single phage therapy

#### 3.2.1. Application to environmental issues

More phage therapy studies have been reported using a single phage isolated from wastewater than a cocktail of wastewater phages. In most studies, single phage efficiency was tested against a wide range of hosts. The use of single phages has been approved to help minimize environmental issues, such as biofilm formation and foaming at the top of sewage water tanks, which complicate the treatment process and threaten surrounding environments. The degradation of biofilms by phage lysis of the bacterial polysaccharide layer was demonstrated in a pioneering study using the lytic phage SF153b, isolated from sewage, against two strains of *Enterobacter agglomerans* (Hughes et al., 1998). These results demonstrated a valuable environmental application of phage enzymes.

In India, phage isolation from a wastewater plant treatment addressed the problem of foam formation (Khairnar et al., 2014), which is mainly caused by filamentous bacteria, such as *Nocardia* species. The isolated bacteriophages, NOC1, NOC2, and NOC3, halted the growth of a wide range of filamentous *Nocardia* species, especially NOC2 phages. This finding may inform future treatment approaches aimed at controlling foam formation in wastewater using a biological method rather than a physical or chemical method.

#### 3.2.2. Application to nosocomial infections

Nosocomial infection is another issue that threatens public health. Microbes involved in nosocomial infections, such as *S. aureus* and *Pseudomonas* spp., are rarely and poorly controlled by antibiotics. Research examining the potential of phage therapy in treating nosocomial infections has been carried out. For example, Synnott et al. (2009) isolated antibiotic-resistant *S. aureus* strains from cow milk and two staphylococcal phages, SA039 and SA012, from local sewage water. *In vitro* treatment with the SA039 phage produced clear plaques on 13 *S. aureus* isolates, whereas the SA012 phage produced clear plaques on 8 isolates, and was the only phage to produce a clear plaque on the non-virulent *S. aureus* strains (Synnott et al., 2009). Clearly, both phages displayed efficiency against a wide host range and may be used in phage therapy following further investigation. The threat of *S. aureus* to human health motivates further studies in the area of phage therapy. In another study, *S. aureus* was obtained from hospitalized patients and cultured with a sample of hospital sewage. One phage contained in the sewage showed lytic activity against *S. aureus* (Hallajzadeh et al., 2019). In a recent study on *S. aureus* phages, scientists in Egypt identified two lytic phages (vB_SauM_ME126 and vB_SauM_ME18), which were isolated from five different wastewater sources. These phages exhibited activity against multidrug-resistant (MDR) *S. aureus*, managing to destroy the bacteria at a relatively high rate (Gharieb et al., 2020).

*Acinetobacter baumannii* and *Klebsiella pneumoniae* also cause nosocomial infections, typically associated with respiratory problems and coupled with urinary and open wound infections. In 2010, a study at Panjab University, India, was conducted on five bacteriophages isolated from sewage samples. Each phage exhibited specificity against *K. pneumoniae* strain B5055, which is known to cause infection in burn wounds and is resistant to certain antibiotics. In the same year, the results of a subsequent *in vivo* study demonstrated the therapeutic action of the previously identified five phages in treating wound infections in mice (Kumari et al., 2010). In 2019, a study was conducted at Shahid Beheshti University, Tehran, Iran (Soleimani Sasani et al., 2019), in which samples were collected from a sewage water treatment plant that treated hospital wastewater. The authors reported the isolation of the lytic bacteriophage vb_kpns-Teh.1 and its specificity against *K. pneumoniae*, which accounts for approximately 8% of nosocomial infections. Yang et al. (2019) isolated an additional novel virulent bacteriophage, φAbp2, from wastewater in China and showed that it had strong activity against MDR *A. baumannii* strains. The φAbp2 phage also showed a relatively wide host range against local *A. baumannii* strains (Yang et al., 2019); however, further research

is required to examine the possibility of using these phages to treat infections *in vivo*.

In an additional study targeting drug-resistant bacteria causing hospital infections, Manohar et al. (2019) isolated three phages from wastewater. The phage *Escherichia* myPSH2311 displayed activity against six strains of *E. coli*, and morphological observations indicated that it belongs to the phiEco32 virus. The phage *Enterobacter* myPSH1140 displayed activity against four different phenotypes of *Enterobacter*. Finally, the phage *Klebsiella* myPSH1235 displayed activity against *K. pneumoniae*. In this study, each single phage could initiate a lytic cycle in its host; however, combined use in a cocktail may be more efficient.

Eleven new phages were isolated from the Main Sewage Treatment Plant (GOS) in Lodz, Poland. Seven of the eleven phages infected *Aeromona hydrophila*, which is commonly found in contaminated food and water, and the other four phages infected *Pseudomonas fluorescens*. Of the 11 recently isolated phages, only six (60AhydR15PP, 50AhydR13PP, 67PfluR64PP, 71PfluR64PP, 22PfluR64PP, and 25AhydR2PP) display phage therapy potential (Kazimierczak et al., 2019).

*Pseudomonas aeruginosa* is ubiquitous in the environment and is related to nosocomial infections, causing severe infections in plants, humans, and other animals. The high resistance of *P. aeruginosa* to antibiotics motivates scientists to find alternative treatments. In a major advance, lytic-bacteriophages were discovered in wastewater and their *in vitro* and *in vivo* activities were examined using MDR *P. aeruginosa* as a host. The bacteria were cultured from the wounds of burn patients in Pakistan. *In vivo* efficacy of the bacteriophage was specified using rabbit as an incision model. The results clearly support the possible use of this phage as a treatment option against MDR *P. aeruginosa* infections (Khalid et al., 2017). An *in vitro* study was conducted to examine the host range of a single *P. aeruginosa* phage, AZ1, obtained from sewage against 34 bacterial strains. Approximately six strains were sensitive: *P. aeruginosa-37*, *P. aeruginosa-2995*, *P. aeruginosa-2949*, *A. xylosoxidans*, *E. coli CR-061*, and *P. aeruginosa-2941*. However, a few bacterial strains were found to be resistant to phage AZ1, such as *P. aeruginosa*-2830, *S. aureus*-2895, *S. aureus*-2975, *E. coli*-3183, *E. coli*-3021, and *S. aureus*-2938, indicating that the phage-host range is very narrow (Jamal et al., 2017). Furthermore, Yuan et al. (2019) reported that bacteriophage vB_PaeM_LS1, isolated from local hospital sewage, displayed therapeutic activity against *P. aeruginosa*.

A review of the literature in 2020, found that the phage MA-1, isolated from wastewater (Adnan et al., 2020), exhibited activity against *P. aeruginosa*-2995, *P. aeruginosa*-2949, *P. aeruginosa*-3007, *P. aeruginosa*-3098, *P. aeruginosa*-3117, and *P. aeruginosa*-3088. These results indicate that phage MA-1 can eradicate *P. aeruginosa* cells and the formation of associated biofilms; however, the use of a cocktail of phages may be advised to avoid resistance. de Melo et al. (2019) targeted *P. aeruginosa* by isolating the bacterium from sick animals and using it as a host for phage isolation. Phage brsp1 was successfully isolated and shown to minimize the growth of two *P. aeruginosa* strains. Nevertheless, the bacterial count resumed thereafter, which might indicate the development of phage resistance, once again suggesting that a phage cocktail might be more effective.

Some nosocomial infectious microorganisms contribute to urinary tract infection, which is one of the most common medical conditions in hospitalized patients and the community. Most of these pathogenic microbes are highly resistant to several common antibiotics. Thus, using phages as therapeutic agents in these cases may be a suitable solution. Major bacterial species known to play a role in urinary tract infections, such as *E. coli*, *Citrobacter freundii*, and *Proteus* spp., can be used as core hosts to experimentally obtain specified phages from sewage samples. *C. freundii*, an Enterobacteriaceae family species, is one of the key contributing factors to nosocomial urinary tract infections in children, more so than in adults, and it is also known for its antibiotic resistance. The Lk1 phage, isolated from sewage water in Pakistan, showed lytic potential toward *C. freundii*; thus, this phage may be useful as a safe disinfectant agent to control dangerous bacteria in the natural environment as well as in health care settings (Chaudhry et al., 2014).

*Proteus mirabillis* is also a common cause of urinary tract infection and it is highly resistant to medications. Fifty strains of *P. mirabillis* were isolated from patients suffering from the use of catheters. Simultaneously, Maszewska et al. (2016) isolated bacteriophages from urban wastewater from the sewage water plant in Zgierz and Lodz, Poland. About 48 phages specific to *P. mirabilis* were obtained; however, their ability to lyse biofilm varied depending on the bacterial strain. Approximately 70% of the fifty strains were destroyed individually by ten phages. Additional *in vivo* studies are needed to support the suitability of these phages in phage therapy.

A team of researchers isolated the lytic bacteriophage φjs4 from sewage water and demonstrated its activity against *E. coli*. The virulent phage sjs4, which was effective against a previous strong *E. coli* biofilm strain, was isolated using strains of uropathogenic *E. coli* (UPEC). The application of phage φjs4 significantly reduced cell numbers within 3 h of application, and almost completely eliminated the cells within 36 h of incubation. These results support the efficacy of antibacterial phages from UPEC, and suggest that φjs4 may be a potential therapeutic alternative to antimicrobials on non-living and living organisms' surfaces (Gudina et al., 2018).

### 3.2.3. Application to the food industry

Alongside the medical field, the food industry is an attractive field for the application of phage therapy because there is a high demand for safe methods to decontaminate food. Several studies have successfully screened for phages that target the most common food-borne microorganisms, such as *E. coli*, *Bacillus* sp., *salmonella* sp., and *Yersinia* sp. Two phages against *E. coli* O157:H7, BECP2 and BECP6, were isolated from a sewage sample from Seongnam, Korea. In a biofilm environment, such as commercial milk, these phages can be used as effective agents for reducing the growth of *E. coli* O157:H7(Lee and Park, 2015).

The lytic activity of a phage isolated from multiple samples of Indian sewage water using *E. coli* MTCC as the host was reportedly narrow, only inhibiting the growth of *E. coli* MTCC (Singh et al., 2016). Testing the phage against additional bacterial species may broaden its application. Additional phages affecting *E. coli* were screened from Malayer dairy factory wastewater in Iran. Isolated phages exhibited lytic activity against six selected *E. coli* strains propagated in chicken meat (Faraji Kafshgari et al., 2019). Thus, these phages can potentially be used as a food preservative. Wastewater samples were collected from four different areas of Buffalo Farm in Hisar, India. The filtrate contained an LUVAS phage, which indicated a positive spot test against mastitis caused by *E. coli* (Swati et al., 2019).

*Salmonella* spp. are known to cause food-borne disease. Researchers collected samples from sewage in India and successfully isolated twelve phages against *Salmonella* bacteria, including three distinct phages: 41-India, 35-India, and 38-Indea (Karpe, Kanade et al., 2016). These phages may prove useful in developing methods to sanitize most food types that are susceptible to *Salmonella* growth, such as chicken, meat, and eggs. In 2017, a group of researchers in India cultured both food-borne bacteria and their phages from dairy farm sewage (Shende et al., 2017). The isolated bacteria were *B. subtilis* and *E. coli*, which were successfully used as primary hosts for phages EHR1, BsHR1, BsHR2, EHR2, EHR3, and BsHR3. The study found that the phages were more active against

**Table 1**
Phages isolated from sewage water, their bacterial host(s), and use in single or cocktail therapy.

| Phages | Year | Host | Single or cocktail therapy | Reference |
|---|---|---|---|---|
| vbsm-A1 vbsp-A2 | 2020 | *Staphylococcus aureus* | Cocktail | Geng et al. (2020) |
| ϕKPaV04, ϕKPaV08, ϕKPaV12) (ϕKPaV03, ϕKPaV10 | 2020 | *Klebsiella pneumonia* | Cocktail | Paran et al. (2020) |
| phage MA-1 | 2020 | *Pseudomonas aeruginosa* | Single | Adnan et al. (2020) |
| vB_SauM_ME18 and vB_SauM_ME126 | 2020 | *Staphylococcus aureus* | Single | Gharieb et al. (2020) |
| SSE1 | 2020 | *Shigella dysenteriae* | Single | Lu et al. (2020) |
| Pa1 and Pa2 | 2020 | *Pseudomonas aeruginosa* | Single | Marei (2020) |
| RSBMS-2 and RSBMT-1 | 2020 | *Bacillus cereus* | Single | Rovik and Pramono (2020) |
| *E. coli* phage | 2019 | *Escherichia coli* (PTCC: 1330) | Single | Faraji Kafshgari et al. (2019) |
| *phage Mycobacterium abscessu* | 2019 | *Mycobacterium abscessus* | Cocktail | Dedrick et al. (2019) |
| phage vB_KpnS-Teh.1 | 2019 | *Klebsiella pneumoniae* | Single | Soleimani Sasani et al. (2019) |
| phage vB_StuM_MH-1 | 2019 | *Staphylococcus aureus* (MRSA) | Single | Hallajzadeh et al. (2019) |
| phage BrSP1 | 2019 | *Pseudomonas aeruginosa* | Single | de Melo et al. (2019) |
| EK1, KL2, EL3 EKL4 | 2019 | *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae* | | Manohar et al. (2019) |
| 60AhydR15PP, 50AhydR13PP, 67PfluR64PP, 71PfluR64PP, 22PfluR64PP, 25AhydR2PP | | *Aeromonas Pseudomonas* | Single | Kazimierczak et al. (2019) |
| vB_PaeM_LS1 | 2019 | *Pseudomonas aeruginosa* | Single | Yuan et al. (2019) |
| *E. coli* phage | 2019 | *Escherichia coli* | Single | Swati et al. (2019) |
| Escherichia myPSH2311 Enterobacter myPSH1140 Klebsiella myPSH1235 | 2019 | *Escherichia coli Enterobacter* sp. *Klebsiella pneumoniae* | Single | Manohar et al. (2019) |
| Yersinia enterocolitica phage | 2018 | *Yersinia enterocolitica* | Single | Daneshgar et al. (2018) |
| Phage | 2018 | *Enterococcus faecalis, Escherichia coli* and *Salmonella typhi* | Single | Gautam et al. (2018) |
| phage (ΦJS4) | 2018 | *Escherichia coli* | Single | Gudina et al. (2018) |
| *Pseudomonas aeruginosa* phage | 2017 | *Pseudomonas aeruginosa* | Single | Khalid et al. (2017) |
| *E. coli* phage | 2017 | *Escherichia coli - E. coli* KP005067 | Single | Megha et al. (2017) |
| Phage AZ1 | 2017 | *Pseudomonas aeruginosa* | Single | Jamal et al. (2017) |
| phages EHR1, BsHR1, BsHR2, EHR2, EHR3, and BsHR3. | 2017 | *Bacillus subtilis Escherichia coli* | Single | Shende et al. (2017) |
| 48 phage isolates specific to 31*P. mirabili*s strains and 3 isolates for one *P. vulgaris* | 2016 | *Proteus mirabilis* and *Proteus vulgari* | Single | Maszewska et al. (2016) |
| *E. coli* phage | 2016 | *Escherichia coli* (MTCC-40,43 and 1585) | Single | Singh et al. (2016) |
| *E. coli* phage | 2016 | *Escherichia coli* | Single | Dallal et al. (2016) |
| phage_35-India, Salmonella phage_41-India Salmonella phage_38-India | 2016 | *Salmonella* bacteria | Single | Karpe et al. (2016) |
| BECP2 and BECP6 | 2015 | *Escherichia coli* O157:H7 | Single | Lee and Park (2015) |
| *E. coli* phage | 2015 | *E. coli* SBSWF27 *E. coli* PTCC1399 | Single | Maal et al. (2015) |
| NOC2 –NOC1 NOC3 | 2014 | *Nocardio* sp | Single | Khairnar et al. (2014) |
| LK1 | 2014 | *Citrobacter freundii* | Single | Chaudhry et al. (2014) |
| Kpn5, Kpn12, Kpn13, Kpn17 and Kpn22 | 2010 | *Klebsiella pneumoniae* strain B5055 | Single | Kumari et al. (2010) |
| SA039 and SA012 | 2009 | *Staphylococcus* | Single | Synnott et al. (2009) |
| SF153b | 1998 | *Enterobecter agglomerans* 53b and *Serratia marcescens* | Single | Hughes et al. (1998) |
| SF153b | 1998 | *Enterobacter agglomerans* 53b | Single | Hughes et al. (1998) |
| EK1, KL2, EL3 | 2019 | *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae* | Single | Manohar et al (2019) |
| EKL4 | 2019 | *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae* | Cocktail | Manohar et al (2019) |

*B. subtilis* than *E. coli*, and *Bacillus* phages could better accommodate variations in temperature and pH. These advantages might make the *Bacillus* phages more suitable for further use in the food industry and as medical therapy agents. At this time, however, there are no *in vivo* studies that support this hypothesis.

Researchers isolated bacteriophages from raw hospital wastewater using the food-borne bacteria *Yersinia enterocolitica* (Daneshgar et al., 2018). The phages were active at temperatures ranging between 4 and 70 °C; however, the phages became inactive at 80 °C. The phages were also tested against other bacterial strains and showed wide host ranges. Both *Biodiversitas* and *Bacillus cereus* are also considered food-borne disease agents that secrete toxins into food. A recent study screened phages against these two species and found two groups of phages with wide and narrow host ranges; the best results were obtained using RSBMS-2 and RSBMT-1 phages (Rovik and Pramono, 2020).

Morphological features of the SSE1 phage, isolated from a sewage water aeration tank, were associated with phages in Myoviridae family of bacteriophages (Lu et al., 2020). The SSE1 phage was highly specific against *Shigella dysenteriae*. Thus, in clinical applications, SSE1 may play a role in the potential treatment of dysentery *Shigella* infection that spreads via contaminated food and food handlers. Table 1 summarizes the most abundant phages isolated from sewage, the year discovered, their host(s), and whether they are used in single phage or cocktail therapy.

## 4. Concluding remarks

Phage therapy represents a remarkable era in modern science. Applications are varied and suitable to diverse disciplines, including environmental, agricultural, industrial, pharmaceutical, and

medical fields. Phages may someday replace, or at least fully supplement, the action of antibiotics and play a major role in preventing the development of superbugs. Phages have multiple distinguishing properties that make them attractive therapeutic agents, including their specificity for bacterial species while sparing eukaryotic cells. This helps ensure their safety for human use, which has also been confirmed by large-scale clinical trials. In addition to quickly adjusting to newly emerging bacterial threats, phages are available in all environments, their cost of isolation is low, and their incubation period is short.

We recommend that multidisciplinary groups work on further developing phage therapy treatments using rigorous *in vitro*, *in vivo*, and clinical trials as well as chemical tests to evaluate the toxicity rate and sensitivity for each newly isolated bacteriophage. We also recommend establishing a worldwide database for isolated phages and their bacterial hosts to support their applications in industrial, medical and environmental fields.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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