Bacteriophages of *Klebsiella* spp., their diversity and potential therapeutic uses

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Klebsiella spp. are commensals of the human microbiota, and a leading cause of opportunistic nosocomial infections. The incidence of multi-drug resistant (MDR) strains of *Klebsiella pneumoniae* causing serious infections is increasing, and *K. oxytoca* is an emerging pathogen. Alternative strategies to tackle infections caused by these bacteria are required as strains become resistant to last-resort antibiotics such as colistin. Bacteriophages (phages) are viruses that can infect and kill bacteria. They and their gene products are now being considered as alternatives or adjuncts to antimicrobial therapies. Several *in vitro* and *in vivo* studies have shown the potential for lytic phages to combat MDR *K. pneumoniae* infections. Ready access to cheap sequencing technologies has led to a large increase in the number of genomes available for *Klebsiella*-infecting phages, with these phages heterogeneous at the whole-genome level. This review summarises our current knowledge on phages of *Klebsiella* spp. and highlights technological and biological issues relevant to the development of phage-based therapies targeting these bacteria.
INTRODUCTION

*Klebsiella* spp. belong to the family *Enterobacteriaceae* and are non-motile, capsulate, Gram-negative bacilli. *Klebsiella pneumoniae* is a commensal bacterium found in the gastrointestinal and respiratory tracts, and on the skin of healthy individuals. It is also ubiquitous in the environment. It is an opportunistic pathogen capable of causing a wide range of community-acquired and nosocomial infections such as urinary tract infections (UTIs), respiratory tract infections, and infections of wounds and soft tissue (Podschun & Ullmann, 1998). It has, in recent years, become one of the world’s leading causes of nosocomial infections with an increasing mortality rate, particularly in immunocompromised individuals, neonates and the elderly. It is also increasingly implicated in severe community-acquired infections such as pneumonia and meningitis (Shon, Bajwa & Russo, 2013). Due to its widespread distribution and genetic make-up, *K. pneumoniae* has rapidly become a global threat to public health (World Health Organization, 2018). Similar to *K. pneumoniae*, *Klebsiella oxytoca* is an opportunistic pathogenic in humans, and is becoming increasingly associated with nosocomial infections, particularly in immunocompromised patients (Broberg, Palacios & Miller, 2014). It is also acquiring antimicrobial resistance genes and is detected throughout the UK (Eades et al., 2016; Moradigaravand et al., 2017b). Consequently, it is now considered the second most clinically important pathogen of the genus *Klebsiella* (Broberg, Palacios & Miller, 2014).

50 Given the reduction in the effectiveness of antimicrobial therapeutics to treat *Klebsiella*-associated infections, alternative strategies must be developed in response. This literature review will focus on bacteriophages (phages) of *Klebsiella* spp. and their potential for use as alternative antimicrobial agents.
Antibiotic resistance and *Klebsiella* spp.

Antibiotic resistance is defined as the ability of a bacterium, such as *K. pneumoniae*, to resist the effects of antimicrobial drugs that it was previously sensitive to. The development of antibiotic resistance is a result of the evolutionary process of natural selection, by which pathogenic bacteria are able to overcome the selection pressure applied to them during antimicrobial treatment, rendering the drug less effective (Huang et al., 2017). The first antibacterial agents were discovered between 1910 and 1935 (Ehrlich, 1910; Domagk, 1935; Fleming, 2001) with the most famous being the discovery of penicillin by Alexander Fleming in 1929. Hailed as ‘magic bullets’ in the fight against infection, these first antibacterial agents paved the way for the discovery of almost all classes of antibiotics in use today (Aminov, 2010).

Despite early evidence of the possibility for future antibiotic resistance (Abraham & Chain, 1988) and warnings that unrestricted use could reduce their effectiveness (Fleming, 1945), antimicrobials have been taken for granted. The continued overuse of antibiotics in both healthcare and agricultural settings over the course of the last century has contributed to the evolution and emergence of antibiotic-resistant strains of *Klebsiella* spp. and other Gram-negative bacteria.

*K. pneumoniae* strains are frequently resistant to extended-spectrum beta-lactams such as penicillins and cephalosporins. Extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* are able to target the beta-lactam ring structure within antibiotic compounds, neutralising their antimicrobial activity (Gürntke et al., 2014). Pathogenic ESBL-producing *K.*
*pneumoniae* derive their antibiotic resistance enzymes most commonly from acquired genes, such as SHV-5 and CTX-M-15 (Gürntke et al., 2014; Moradigaravand et al., 2017a). Ever since ESBL-producing *K. pneumoniae* were first described by Knothe et al. (1983) in Germany, *K. pneumoniae* carrying CTX-M-15 have spread throughout the world and are associated with a steadily increasing incidence of both nosocomial infections and, more recently, community-acquired infections with an increasing mortality rate (Khan et al., 2010; Chong et al., 2011; Mshana et al., 2013; Valenza et al., 2014).

ESBL-producing *K. pneumoniae* strains remain susceptible to the carbapenem class of antibiotics, which includes imipenem and meropenem. However, there is increasing incidence of *K. pneumoniae* infections caused by strains that have become resistant to even carbapenems. These multi-drug resistant (MDR) organisms are thought to have evolved in response to the increased use of carbapenems against ESBL-producing *K. pneumoniae*, with several independently evolved genetic elements conferring carbapenem resistance. *Klebsiella pneumoniae* carbapenemase (KPC) was first discovered in the United States (Yigit et al., 2001) and has since spread to all other parts of the world (Campos et al., 2016).

In Europe, KPC was found to be the most common carbapenemase resistance gene in *K. pneumoniae*-associated hospital-acquired infections (45%), followed by oxacillinase-48 (OXA-48-like) (37%), New Delhi metallo-beta-lactamase (NDM) (11%) and Verona integron-encoded metallography-beta-lactamase (VIM) (8%) (Grundmann et al., 2017). In the UK, confirmed cases of KPC, OXA-48-like, NDM, and VIM rose from 0 to 1 cases in 2007 to 661, 621, 439, and 86 cases, respectively, in 2015 (Public Health England, 2016). The spread of OXA-48-like
*K. pneumoniae* strains has occurred mostly in the Mediterranean and Northern Africa and is primarily spread via ST101 strains as a result of travel in the regions, whereas ST395 is associated with clonal outbreaks throughout Europe (Potron et al., 2013).

NDM carbapenemase-producers originated in India, primarily in strains of *Escherichia coli* and *K. pneumoniae*, and have spread throughout the world as a direct result of travel to and from the Indian subcontinent (Nordmann et al., 2011; Johnson & Woodford, 2013). Nordmann et al. (2011) showed that more than half of NDM isolates from the UK were from patients with a history of travel to India or Pakistan. The UK appears to have the highest concentration of NDM isolates in Europe currently (Glasner et al., 2013).

While MDR *K. pneumoniae* is itself a problem, its ubiquitous presence in both animal and human hosts, as well as in the environment, combined with its ability to acquire and maintain antimicrobial resistance plasmids and to pass those plasmids on to other Gram-negative bacteria, puts it into a relatively unique position to be able to amplify the spread of antimicrobial resistance genes throughout the world (Wyres & Holt, 2018). The contribution of *K. pneumoniae* to the antimicrobial resistance crisis is difficult to quantify. However, a recent population genomics study has shown that within- and between-hospital spread of carbapenem-resistant *K. pneumoniae* is the major driver of expansion of these bacteria within Europe, with carbapenemase-resistant isolates concentrated in clonal lineages ST11, ST15, ST11 and ST258/ST512 and their derivatives (David et al., 2019). *K. pneumoniae* (and likely *K. oxytoca*) will continue to play a key role in the development of antimicrobial resistance and is, therefore, a prime target for novel antibacterial therapeutics (Wyres & Holt, 2018).
**Risk factors for *Klebsiella* infections**

Primarily an opportunistic pathogen prevalent in the hospital setting, *K. pneumoniae* has become a common cause of hospital-acquired infections, such as UTIs and bloodstream infections (BSIs), in which antibiotic-resistant strains are becoming more difficult to treat and are associated with an increased mortality rate. Perhaps the most ubiquitous risk factors for all forms of hospital-acquired *K. pneumoniae* colonisation and infection are patient exposure to antibacterial agents and the length of hospital stay. Indeed, there consistently appears to be a positive correlation between the length of time a patient is required to stay in hospital, and the chance of acquiring a *K. pneumoniae* infection simply due to the increased exposure to healthcare-associated pathogens with time (Nouvenne et al., 2014; Shaikh et al., 2015; Liu et al., 2018). Moreover, a considerable number of studies aimed at identifying risk factors associated with such infections recognise previous antibiotic treatment as an important factor, particularly the widespread use of cephalosporins in the case of ESBL-producing *K. pneumoniae* infection (Tuon et al., 2011), and carbapenems, fluoroquinolones, glycopeptides and aminoglycosides for infections caused by carbapenemase-producing *K. pneumoniae* (Liu et al., 2018).

Not surprisingly, invasive procedures such as surgical intervention and catheterisation are also strongly associated with the acquisition of *K. pneumoniae* infection. Patients who are subject to invasive procedures, such as the installation of a central venous catheter, for example, are likely to be immunocompromised individuals who have been hospitalised for a severe underlying health condition. These patients are, therefore, particularly susceptible to opportunistic infections which could lead to a BSI, in the aforementioned example, soft tissue and wound infections in
patients subject to surgical procedures, or even severe cases of pneumonia or meningitis in neonates (Tumbarello et al., 2006; Yu et al., 2016).

Clinical features of disease may also be an important risk factor in the development of *K. pneumoniae* infection. Meatherall et al. (2009) identified chronic liver disease and cancer as being the most significant factors involved in the development of *K. pneumoniae* bacteraemia; several studies have evidenced a link between diabetes mellitus and invasive *K. pneumoniae* infection as a result of poor glycaemic control and subsequent bacterial resistance to phagocytosis (Silva et al., 2006; Tuon et al., 2011; Lee et al., 2016). Nouvenne et al. (2014) suggested an association between cardiovascular, respiratory, renal and neurological diseases, and colonisation and infection by carbapenem-resistant *K. pneumoniae*.

*K. oxytoca* is the causative agent of paediatric antibiotic-associated haemorrhagic colitis, caused by overgrowth of the bacterium with the release of cytotoxin when the intestinal microbiota is disturbed by antibiotic treatment (Zollner-Schwetz et al., 2008; Herzog et al., 2014). Likely due to a combination of improved detection methods (Eades et al., 2016), increased international travel (Moradigaravand et al., 2017b), contaminated hospital equipment (Moradigaravand et al., 2017b), increasing numbers of immunocompromised patients and more complex treatment regimens, *K. oxytoca* is being isolated more frequently from neonatal intensive care units than in the past, and is now also being found in a range of clinical samples from adult patients admitted to critical care centres. *K. oxytoca* is showing multidrug resistance and appears to have higher drug resistance compared with *K. pneumoniae*, though this requires further analyses (Singh, Cariappa & Kaur, 2016).
Virulence factors of *Klebsiella* spp.

*K. pneumoniae*, despite being considered an opportunistic pathogen, possesses an arsenal of virulence factors that enable the bacterium to both infect its host and resist the host immune response allowing it to cause severe disease. The most studied virulence factors associated with *K. pneumoniae* are the capsule, lipopolysaccharide (LPS), fimbriae and siderophores.

The capsule is an extracellular matrix made up of strain-specific polysaccharides that surrounds the bacterium forming a thick fibrous structure. The capsular polysaccharides produced by *K. pneumoniae* are called K antigens and, given that the polysaccharide produced depends on the strain of *K. pneumoniae*, they have been traditionally used to identify the strain using serological techniques (Wyres et al., 2016). The role of the capsule in human disease has been studied extensively and it is thought to have a defensive role by providing protection against phagocytic immune cells, blocking complement-mediated lysis and reducing levels of proinflammatory cytokines (Yoshida et al., 2000; Cortés et al., 2002). Indeed, the virulence of *K. pneumoniae* is greatly reduced in the absence of a capsule, as shown by infection of mice with acapsular mutants (Lawlor, Handley & Miller, 2006), and greatly increased in so-called hypervirulent strains which produce more capsular material resulting in a hypermucoviscous phenotype (Shon, Bajwa & Russo, 2013).

The LPS is composed of an O antigen, an oligosaccharide core and lipid A, and protrudes from the bacterial membrane (Follador et al., 2016). The primary role of LPS in *K. pneumoniae* infection is protection from the complement-mediated lysis of bacterial cells by binding of the
complement component C3b away from the bacterial membrane, preventing the formation of the membrane attack complex C5b-9. This is carried out by the O antigen of the LPS which, when absent, makes *K. pneumoniae* more susceptible to complement-mediated bacterial lysis (Merino et al., 1992).

*K. pneumoniae* expresses fimbriae which are membrane-adhesive protrusions involved in the adhesion of the bacterium to host surfaces facilitating its invasion. Two main types of fimbriae are exhibited by *K. pneumoniae*: type 1 fimbriae which are filamentous, and type 3 fimbriae which are helix-like in shape (Paczosa & Mecsas, 2016). Moreover, the expression level of each type varies depending on the surface to which the bacteria attach. Type 1 fimbriae are expressed in the urinary tract and the bladder, but not in the gastrointestinal tract or the lungs (Struve, Bojer & Krogfelt, 2008). Struve et al. (2008, 2009) speculate that the downregulation of type 1 fimbriae may be because it reduces the ability of *K. pneumoniae* to penetrate the intestinal mucus layer in the gastrointestinal tract, as is seen with *E. coli*, whereas in the lungs, selection against fimbriated cells occurs due to rapid elimination by phagocytes. Type 3 fimbriae bind to extracellular matrices and medical devices, and are important for the development of biofilms (Struve, Bojer & Krogfelt, 2009).

Finally, *K. pneumoniae* must acquire iron from the environment to grow and multiply. There is very little free iron to be found in mammalian hosts, so the bacterium must express siderophores. These are molecules that have a higher affinity for iron than mammalian iron transport molecules, such as transferrin, enabling the bacteria to obtain iron for rapid growth and subsequent invasion. The primary siderophore expressed by *K. pneumoniae* is enterobactin,
expressed in the majority of pathogenic strains; however, salmochelin, yersiniabactin, colibactin and aerobactin can also be expressed. Indeed, hypervirulent strains of *K. pneumoniae* are able to express multiple siderophores and are particularly associated with the expression of salmochelin, yersiniabactin, colibactin and/or aerobactin (Holt et al., 2015).

**Genetic diversity of clinically relevant *Klebsiella* spp.**

In keeping with the diversity of its virulence factors, antibiotic resistance mechanisms and clinical presentations, strains of *K. pneumoniae* also possess highly diverse and flexible genomes capable of producing considerable phenotypic variation. Indeed, the diversity of *K. pneumoniae* is such that the species is widely accepted to exist as four distinct phylogroups: KpI, KpII-A, KpII-B and KpIII, which have been shown to have diverged into three distinct species: *K. pneumoniae* (KpI), *K. quasipneumoniae* (KpII) and *K. variicola* (KpIII) (Holt et al., 2015).

In their whole-genome sequencing and pangenome-wide association study, Holt et al. (2015) found that severe community-acquired infections were more often caused by phylogroup KpI that expressed siderophores and ‘regulators of mucoid phenotype genes’ *rmpA* and *rmpA2*, which regulate capsule production. Moreover, their study also confirmed the presence of SHV, OKP, and LEN beta-lactamases as core chromosomal genes of all phylogroups, whereas acquired antibiotic-resistance genes were more commonly found in KpI and KpII commensal isolates compared to either hospital-acquired or community-acquired infection isolates, suggesting that antibiotic resistance plays more of a role in opportunistic hospital-acquired infections caused by commensal *K. pneumoniae*, whereas more severe community-acquired
infections are caused by strains enriched with virulence factors such as siderophores and increased capsular production.

Hypermucoviscous strains of *K. pneumoniae* – i.e. those that exhibit virulence genes such as yersiniabactin and *rmpA* – were first described in Southeast Asia and are commonly associated with community-acquired pyogenic liver abscess (Jun, 2018). These hypervirulent strains very rarely exhibit the antibiotic resistance gene profiles commonly associated with opportunistic hospital-acquired infections, and until recently have remained treatable with antibiotics (Lee et al., 2017). However, *K. pneumoniae* isolates with combined hypervirulence and antibiotic resistance are emerging. Given the highly diverse genome of the species and the increasing selective pressures being applied to them in the form of antibiotics, hypervirulent antibiotic-resistant *K. pneumoniae* is threatening to become untreatable (Holt et al., 2015; Lee et al., 2017).

A pangenome study of *K. oxytoca* strains isolated from bloodstream infections in the UK and Ireland showed that *K. oxytoca* has a highly diverse population, composed of several distinct phylogroups (KoI, KoII, KoV, KoVI) (Moradigaravand et al., 2017b). It shares numerous antimicrobial genes and mechanisms with *K. pneumoniae*. *K. oxytoca* has been far less studied than *K. pneumoniae*, and extensive studies of its global epidemiology are required (Moradigaravand et al., 2017b).

**PHAGES OF KLEBSIELLA SPP.**

A phage is a virus that infects bacteria and, as such, is found in all environments where bacteria would normally thrive. Viruses were initially suggested as a possible cause of clear zones on
bacterial culture plates by William Twort in 1915, and in 1917 Felix d'Herelle confirmed this
discovery, coining the term ‘bacteriophage’ (Twort, 1915; Anonymous, 2011). Prior to the
discovery of the first antimicrobial agents, phages were considered the cure for bacterial
infections and d’Herelle performed the first experimental phage therapy using an oral phage
solution to treat dysentery (d’Herelle, 1918). However, after the discovery of antimicrobial
compounds such as penicillin, the therapeutic uses of phages were largely left alone due to the
subsequent success of the antibiotic era. Phages remained useful, however, for scientific research
as tools to improve our understanding of molecular biology, horizontal gene transfer, and
bacterial evolution, and as diagnostic tools (Clokie et al., 2011). More recently though, given the
rise in the number of MDR infections caused by bacteria such as *K. pneumoniae*, the use of
phages has again come to the forefront as a potential alternative to current antimicrobial
chemotherapies.

**Life cycles**

Phages primarily have two distinct life cycles they are able to adopt in order to reproduce: the
lytic cycle and the lysogenic cycle. Both life cycles begin with the attachment of a phage to the
surface of the bacterial host, followed by the subsequent injection of the phage’s genetic material
into the cell. In the lytic life cycle, the viral genome produces proteins that initiate the
degradation of the bacterial genome, allowing the viral genetic material to take control of the
host cellular machinery for the sole purpose of replicating the viral genome, synthesising viral
proteins and assembling those proteins into viable phage particles that are released from the
bacterial cell in large numbers, destroying the host. The phages that are released are then able to
continue infecting bacteria nearby. In the lysogenic life cycle, the viral genetic material is
incorporated into the bacterial DNA, forming a prophage, and is replicated passively upon replication of the bacterial genome without destroying the host. Prophages in the lysogenic cycle are able to enter the lytic cycle under certain conditions (e.g. in the presence of environmental stressors), and begin actively replicating and producing viable phages at the expense of the host (Wittebole, De Roock & Opal, 2014).

Although the lytic/lysogenic phage life cycle is a well-established concept in phage biology, we now know there are multiple phage life cycles. Pseudolysogeny is the process by which the phage genome enters a bacterial host but neither stably establishes itself as a prophage nor initiates a destructive replicative response, remaining inactive and possibly awaiting more desirable environmental conditions for viral replication (Siringan et al., 2014). Chronic infection, resulting in the shedding of phage particles over long periods of time without destruction of the host cell, can occur with infection of filamentous phages in *Mycoplasma* (Lee et al., 2017). Finally, the carrier state life cycle occurs when a heterogeneous population of bacteria, containing individuals both sensitive and resistant to a given lytic phage, leads to the destruction of sensitive bacteria and the survival of resistant bacteria creating a stable equilibrium between viral and bacterial propagation (Siringan et al., 2014).

In the context of using phages as a therapeutic alternative to antimicrobial chemotherapy, those that reliably employ the lytic life cycle to reproduce are most suitable given that the end result is the destruction of bacterial host cells. Additionally, phages that are able to switch between multiple life cycles may not make reliable treatment options due to the possibility of dormancy and subsequent re-establishment of bacterial infection. This is just one aspect of comprehensive
phage characterisation that is an important consideration when choosing appropriate phage treatments.

Phage characterisation

Phages of *K. pneumoniae* have been isolated from a variety of sources worldwide including wastewater, sewage, seawater and human intestinal samples, and belong to one of three families of the order *Caudovirales* (Table 1). These families make up the entirety of the order and are described as non-enveloped, tailed phages, with icosahedral heads containing double-stranded DNA: *Myoviridae* are characterised by long, straight, contractile tails; *Siphoviridae* by long, flexible, non-contractile tails; and *Podoviridae* by short, non-contractile tails (Fokine & Rossmann, 2014).

Genomic comparisons of lytic *K. pneumoniae* phages of the order *Caudovirales* highlight a variety of useful similarities and differences. The expression of polysaccharide depolymerases, for example, has been observed in several recently discovered phages of *K. pneumoniae* (Kęsik-Szeloch et al., 2013; Chhibber, Nag & Bansal, 2013; Jamal et al., 2015) and these enzymes have a role in the degradation of the capsule surrounding the exterior of the bacterium. The breakdown of the capsule by phage depolymerases has been purported to combat *K. pneumoniae* biofilms (Taha et al., 2018) and increase the susceptibility of the bacterium to antibiotics, phage infection and the immune system (Kęsik-Szeloch et al., 2013). Additionally, phage depolymerase action can be observed in the laboratory with the production of ‘haloes’ around clear zones of lysis on bacterial culture plates after infection of *K. pneumoniae* with phage particles. This has become
the basis for important laboratory methods used in the characterisation of novel phages, revealing phage specificity and host range (Hughes et al., 1998).

Moreover, differences observed among Myoviridae, Podoviridae and Siphoviridae can be useful for preliminary identification. For example, sequence analysis reveals that Myoviridae tend to have a much larger genome size and a lower GC content compared to Podoviridae and Siphoviridae (Table 1). Restriction analysis, which uses bacterial restriction enzymes to digest phage DNA, can also help to estimate the size of the phage genome in addition to identifying those that are already known to science prior to extensive characterisation, and analysis by transmission electron microscope is able to reveal morphological characteristics such as phage tail structures (Kęsik-Szeloch et al., 2013). Phylogenetic analyses show several Klebsiella phages belong to accepted genera within the Siphoviridae, Podoviridae and Myoviridae, while others belong to new lineages with – as yet – no standing in viral taxonomy (Figure 1, Supplementary Material).

Specificity and host range

To infect its host, a lytic phage must first attach itself to a susceptible bacterial cell. It achieves this by recognising and binding a specific receptor on the surface of the host cell. This interaction between the phage tail structure and host receptor allows the phage to both identify susceptible bacteria, and position itself for injecting its genetic material into the cell. Adsorption to the host can occur via any external structure depending on the phage and host, but in Gram-negative bacteria, such as K. pneumoniae, these can include the capsule, pili, outer membrane
proteins, sugar moieties, or LPS (Bertozzi Silva, Storms & Sauvageau, 2016). This process, therefore, determines host range, i.e. the breadth of hosts that any given phage can infect.

D’Andrea et al. (2017) showed that their newly discovered lytic phage φBO1E was able to specifically target KPC-producing *K. pneumoniae* of the pandemic clonal group 258 (CG258) clade II lineage, but not those of the closely related clade I lineage, due to the recognition and targeting of specific capsular polysaccharides present on strains belonging to clade II. In contrast, Verma et al. (2009a) found that the lytic phage KPO1K2, specific for *K. pneumoniae* B5055, could infect multiple strains of *K. pneumoniae* as well as some strains of *E. coli* and, therefore, has a relatively broad host range compared to the clade-specific phage φBO1E.

It is generally considered, in the context of their therapeutic use, that lytic phages with a broad host range (e.g. at genus or species level) are more beneficial in combatting bacterial infection than those with a narrow host range (e.g. at strain level). Phages with a narrow host range are inappropriate for presumptive or prophylactic treatment, for example, and would rely on identification of an infective agent prior to treatment. Additionally, even phages considered to have a broad host range would generally have a narrower spectrum of activity compared to antibiotics (Loc-Carrillo & Abedon, 2011). Therefore, efforts to increase the spectrum of activity of phage treatment has led to the development of phage cocktails, to increase the host range by using multiple phages in a single treatment (Gu et al., 2012), and even the hybridisation of phage tail structures to increase the host range artificially (Yosef et al., 2017).

**THERAPEUTIC POTENTIAL OF KLEBSIELLA PNEUMONIAE PHAGES**
There are a number of considerations to be made when selecting phages suitable for use as therapeutic antimicrobial agents. Firstly, phages must be effective in killing *K. pneumoniae*. During phage characterisation, *in vitro* assessments of phage lysis and burst size are carried out on cultures of *K. pneumoniae*. Phages that produce rapid lysis of a bacterium and release large numbers of phage particles will produce large clear plaques. Moreover, phages with a broad host range are generally considered more useful than those with narrow host range so that multiple strains may be targeted at once (Harper, 2018). Secondly, lytic phages, due to the nature of their life cycle, clear bacteria quickly and efficiently compared to lysogenic phages, which integrate their genetic information into the host genome and remain dormant for an unspecified amount of time. In addition, lysogenic phages may transfer genes into the host that can confer toxin production and antibiotic resistance traits to the bacterium, thus making the infection more virulent and difficult to treat (Harper, 2018).

**In vivo experimentation**

Following *in vitro* investigations, the safety and effectiveness of any new therapeutic candidate must be measured in a suitable animal or insect model prior to human trials. In the case of *K. pneumoniae* phage research, mouse models have been used to investigate the effect of phage treatment against wound and soft tissue infections (Kumari, Harjai & Chhibber, 2010b), pneumonia (Chhibber, Kaur & Kumari, 2008), liver abscesses (Hung et al., 2011) and bacteraemia (Vinodkumar, Neelagund & Kalsurmath, 2005), closely mirroring the spectrum of disease caused by the bacterium in humans. More recently, *Galleria mellonella* larvae have been used to test the efficacy of lytic phages and phage-encoded products to clear *K. pneumoniae*
infections (Majkowska-Skrobek et al., 2016; Manohar, Nachimuthu & Lopes, 2018; Thiry et al., 2019).

Kumari and colleagues have carried out a series of murine-based experiments aimed at identifying the therapeutic potential of the *K. pneumoniae* phage Kpn5. Isolated as one of five phage candidates (Kpn5, Kpn12, Kpn13, Kpn17, and Kpn22) from samples of sewage (Kumari, Harjai & Chhibber, 2010a), Kpn5 was found to be the most effective, compared to the other four, when used to treat burn wound infections caused by *K. pneumoniae B5055* in BALB/c mouse models (Kumari, Harjai & Chhibber, 2009). When administered by intraperitoneal injection, Kpn5 produced an average 96.66% survival rate compared to the negative controls which had a survival rate of 0% (Chhibber, Kaur & Kumari, 2008). Additionally, when compared to topical treatments with both natural products (honey and aloe vera gel) (Kumari, Harjai & Chhibber, 2010c) and antimicrobial agents (silver nitrate and gentamicin) (Kumari, Harjai & Chhibber, 2011), Kpn5 was found to be superior in both cases, providing a higher level of protection and reduced mortality rates. However, despite the promising results that this research group has produced, the authors note the possibility of *K. pneumoniae* forming resistance to Kpn5, as highlighted in their *in vitro* experiments, and provide no data on phage host range, having used only a single strain of *K. pneumoniae* throughout their studies.

The delivery method of phage treatment is also an important consideration. For example, intraperitoneal injection is rarely used in human treatment given the relative ease of intravenous injection in most cases. In experiments carried out to treat murine lobar pneumonia, Cao et al. (2015) determined that intranasal delivery of phage 1513 was able to produce a survival rate of
80% in the Swiss-Webster mouse model, compared to 0% of negative controls, 2 h after nasal inoculation of MDR \textit{K. pneumoniae} 1513 as well as visibly reduced lung injury, in comparison to negative controls. Chhibber et al. (2008) demonstrated that intraperitoneal injection of phage SS administered immediately after intranasal inoculation of \textit{K. pneumoniae} B5055 into BALB/c mice resulted in complete clearance of bacteria in 5 days, compared to 10 days in untreated mice, although the authors state that even a short delay of 6 h post inoculation rendered treatment ineffective. Singla et al. (2015) found that phage KPO1K2, encased in a liposome, was effective in treating lobar pneumonia induced in BALB/c mice by intranasal inoculation of \textit{K. pneumoniae} B5055, even when phage treatment was delayed by up to 3 days.

Although there is a difference in the choice of phage in these published reports, and so studies cannot be compared directly, it does highlight the importance of investigating differing delivery methods of phage treatment, not only in a logistical sense, but also in elucidating the most efficient method of delivery according to the type of infection and the length of incubation prior to treatment. Moreover, these studies have each measured the \textit{in vivo} effect of phage treatment against only one strain of \textit{K. pneumoniae}, providing no information regarding phage host range. Further experiments should, therefore, seek to determine whether the host range of their respective phages is broad enough to be considered useful for therapeutic purposes.

While several studies have reported successful use of \textit{K. pneumoniae} phages to clear infections in murine and \textit{Galleria} models, the effects of phage infection on the microbiome (i.e. microbiota, metabolome) must now be considered when assessing phages (individually or as phage cocktails) as a viable treatment or patient decontamination measure. Hsu et al. (2019) showed that infection
with lytic phages caused an increase in phage resistance (28% to 68%) in a known bacterial population common to the human gut microbiota. Quantitative shifts in sensitive and non-sensitive strains were seen, highlighting the system-level effect of phage infection. Phage infection did not necessarily clear the target species but instead modulated the ecosystem towards a more stable gut environment. Phages inducing simultaneous knockdown of *Enterococcus faecalis* and *Bacteroides fragilis* populations had little effect on the microbiota compared with *Escherichia coli* and *Clostridium sporogenes* phages, which caused significant decreases (10⁶ per gram stool) in *Bacteroides vulgatus*, *Proteus mirabilis* and *Parabacteroides distasonis* populations, and 10⁸ per gram stool decreases in *Akkermansia muciniphila* and *Bacteroides fragilis* populations. Perturbation of the microbiota by phages also affected the metabolome. Abundance of 17% of examined compounds was altered significantly in the presence of phages. During initial phage infection, Hsu et al. observed a 10-, 17-, and 2-fold reduction in tryptamine, a microbiome-associated metabolite known to play a role in accelerating gastrointestinal transit in mice (Bhattarai et al., 2018). This led them to suggest phage infection could be used to modulate the microbiome in a targeted manner to influence systemic health.

**Combination therapy**

A number of *in vitro* experiments have identified the possibility of bacterial resistance arising as a result of phage therapy (Kumari, Harjai & Chhibber, 2010a; Gu et al., 2012; Cao et al., 2015; Chadha, Katare & Chhibber, 2016; Tabassum et al., 2018). To reduce the emergence of phage-resistant strains of *K. pneumoniae* during treatment, research has begun to explore combination therapy either using phage cocktails, or by combining phage treatment with antibacterial drugs.
Gu et al. (2012) generated a phage cocktail (i.e. a combination of phages that have different but overlapping host specificities) made up of three lytic phages (GH-K1, GH-K2 and GH-K3) specific to *K. pneumoniae* strain K7. The authors found that co-culture of K7 with the phage cocktail produced fewer phage-resistant variants of K7 and a more efficient reduction in bacterial load compared to cultures treated with a single phage. Moreover, when treating bacteraemic mice, produced by intraperitoneal injection of K7, the phage cocktail produced a significantly lower blood bacterial count and enhanced mouse survival rates compared to mice treated with individual phages. A similar phenomenon was seen by Chadha et al. (2016), who aimed to resolve *K. pneumoniae* B5055 burn wound infections in BALB/c mice and found that their phage cocktail (made up of Kpn1, Kpn2, Kpn3, Kpn4 and Kpn5) induced a greater decrease in bacterial load compared to treatment with individual phages and a complete bacterial clearance in a shorter time.

Finally, in combining a lytic phage with ciprofloxacin against *K. pneumoniae* biofilms, Verma et al. (2009b) demonstrated a reduction in the development of both phage-resistant and ciprofloxacin-resistant *K. pneumoniae* strains, as well as having an enhanced effect against bacterial biofilms compared to individual treatments.

**Human trials**

The progression of phage research from *in vivo* experimentation to clinical trials involving humans has generated some friction among regulatory bodies in Western countries. However, countries in Eastern Europe and the former Soviet Union have routinely used phages in their healthcare systems for many years (Kutter et al., 2010). For example, the Eliava Institute of
Bacteriophages, Microbiology and Virology in Georgia, and the Hirszfeld Institute of Immunology and Experimental Therapy in Poland both produce and supply phage therapeutic products specifically for routine human use (Furfaro, Payne & Chang, 2018).

In the West, regulatory issues surrounding the use of phages as therapeutic agents has hindered progress somewhat. It is not that there are specific regulations that prevent the use of phages in this way, but rather a lack of regulation that has placed limitations on progress. The unique nature of phages compared to traditional therapeutic agents, as evolving and self-replicating biological entities, requires them to have new rules and regulations regarding their safety, production and use. It is this lack of regulation in the EU and the UK, combined with a lack of interest from pharmaceutical companies, and the concept of personalised medicine often associated with phage therapeutics, which in itself is a new method of infection control, that makes approval for human trials a lengthy and difficult process (Debarbieux et al., 2016).

However, it should be noted that the Belgian government has introduced a pragmatic framework that facilitates tailored phage therapy (magistral phage regulatory framework) and allows non-authorized phage products to be prepared by a pharmacist, for a given patient in line with a prescription from a physician and complying with relevant standards (Pirnay et al., 2018).

Phages are very occasionally and only under exceptional circumstances used therapeutically in the wider EU under the umbrella of Article 37 (Unproven Interventions in Clinical Practice) of the Declaration of Helsinki (Pirnay et al., 2018).

Despite these regulatory hurdles, a limited number of human trials have been carried out in relation to phage therapy, although none have specifically targeted *K. pneumoniae*. Rhoads et al.
(2009), based in the USA, carried out a phase I clinical trial on 42 patients with chronic venous leg ulcers to investigate the safety of a phage preparation specific to *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*. The authors reported no adverse effects of phage treatment. In the same year, Wright et al. (2009), based in the UK, carried out a phase I/II clinical trial to determine the safety and efficacy of their phage product targeting *P. aeruginosa* in chronic otitis. Their study involved 24 patients with chronic otitis and showed a reduction in *P. aeruginosa* counts and, again, no adverse effects of phage treatment. Although consisting of a small sample size, the apparent success of these first human trials did little to prompt changes to the regulatory obstacles currently associated with phage therapy.

**Future directions**

Phage therapy shows promise as a potential response to the continued development and spread of MDR *K. pneumoniae*. *In vitro* and *in vivo* studies have confirmed the potential for phages to be used individually, as phage cocktails and in combination with current antimicrobial chemotherapeutic drugs. Moreover, the routine use of phage therapy in Eastern Europe, and the results from the small number of human trials that have been carried out in the West, suggest that phages are generally considered safe for use in humans. However, the lack of progress toward amending EU and UK regulations to account for phage therapy has hampered progress. The focus of future direction in the area of phage research must be to overcome this obstacle.

**USING PHAGE-DERIVED GENE PRODUCTS**

Another avenue of phage research aimed at finding therapeutic solutions to MDR *K. pneumoniae* is the potential to use specific phage gene products rather than phages themselves to combat
This kind of treatment could be advantageous in that it would be easier and quicker to gain clinical approval of a recombinant protein product over the direct use of phages. Indeed, phage-derived recombinant proteins may be used to directly combat infections caused by bacteria such as *K. pneumoniae*, or as part of a combinatory approach to complement or enhance current antimicrobial regimes.

### Phage proteins

In the lytic life cycle of an infecting phage particle, there are a number of proteins that the phage can use to ensure successful adsorption, infection, replication and release of progeny. In terms of potential antimicrobial agents against *K. pneumoniae*, there are a number of biologically interesting proteins to consider. Peptidoglycan hydrolases and polysaccharide depolymerases are normally present on the tail spikes of a phage particle and are involved in successfully infecting a bacterium after adsorption. Polysaccharide depolymerases degrade the macromolecular carbohydrates that make up the capsule surrounding the bacterial cell wall, whereas peptidoglycan hydrolases break down the peptidoglycan layer to penetrate the cell wall and access the cytoplasm to allow the phage to deposit its genetic material (Drulis-Kawa, Majkowska-Skrobek & Maciejewska, 2015).

Holins, endolysins and spanins are proteins that are produced after the infection of a bacterium, and are involved in the process of cell lysis whereby assembled phage particles ‘burst’ from the cell in order to spread and continue the infection cycle. Holins are hydrophobic transmembrane proteins that mediate the permeabilisation of the inner cell membrane. This cannot independently cause cell lysis; however, it allows endolysins and spanins to translocate from the cytoplasm,
where endolysins degrade the peptidoglycan layer in between the inner and outer cell membranes, and spanins disrupt the outer cell membrane present on Gram-negative bacteria. This is followed by bacterial cell lysis via osmolysis (Drulis-Kawa, Majkowska-Skrobek & Maciejewska, 2015).

Polysaccharide depolymerases

The capsule of *K. pneumoniae* is an important virulence factor and allows the bacterium to avoid phagocytosis and complement-mediated lysis. It is, therefore, a prime target for recombinant phage-derived proteins and has been studied extensively. For example, tail tubular protein A (TTPA), a structural tail protein of phage KP32, was shown to have additional polysaccharide depolymerase activity. Pyra et al. (2017) cloned and expressed TTPA in *E. coli* and determined its enzymatic activity by agar spot tests on lawns of *K. pneumoniae* PCM2713, which produced translucent zones of reduced growth. Subsequent microscopic analysis of treated and untreated *K. pneumoniae* revealed cells treated with TTPA were stripped of their capsules. In a similar process of cloning, expression and agar spot-testing, Pan et al. (2017) discovered nine polysaccharide depolymerases expressed by phage ΦK64-1, each of which demonstrated activity against a specific capsular type of *K. pneumoniae* which corresponded to the broad host range of the phage itself. This is interesting because not only does it confirm the role of enzymes such as polysaccharide depolymerases in the determination of phage-host specificity, but it also lends the idea of artificially generated cocktails of recombinant enzymes that can target a wide range of *K. pneumoniae* strains.
A number of *in vivo* experiments have also been carried out investigating the effect of polysaccharide depolymerases on *K. pneumoniae* infection. Majkowska-Skrobek et al. (2016) identified, cloned and expressed a KP36-derived capsule depolymerase, depoKP36, which produced haloes on lawns of *K. pneumoniae* in agar spot-tests. The authors tested the ability of depoKP36 to treat infection caused by *K. pneumoniae* in *Galleria mellonella* and found that 100% died without treatment, up to 40% survived when treated with depoKP36 post infection, and depoKP36 treatment of bacteria prior to infection resulted in only a 23% death rate. These results suggest that the decapsulating action of depoKP36 on *K. pneumoniae* produced a decreased ability of the bacterium to resist the host immune response. This was confirmed in subsequent research (Majkowska-Skrobek et al., 2018).

**Endolysins**

Endolysins have been studied extensively for use against Gram-positive bacteria, due to the absence of an outer cell membrane found in Gram-negative bacteria such as *K. pneumoniae* which would normally hinder the action of the enzyme in the absence of spanins. However, recent research has produced some promising results regarding the use of endolysins against Gram-negative bacteria also. Maciejewska et al. (2017) produced a recombinant endolysin from the *K. pneumoniae* phage KP27 and analysed its peptidoglycan-degrading activity on a range of Gram-negative bacteria, including strains of *K. pneumoniae, P. aeruginosa, Salmonella enterica* and *E. coli*, by co-incubation of bacteria and endolysin. The recombinant enzyme successfully lysed all strains of bacteria that were tested. However, the outer membrane of bacteria was permeabilised prior to endolysin treatment. This suggests that any potential endolysin-based
infection control agents require mixing with outer-membrane-permeabilising agents to be effective against *K. pneumoniae* (Maciejewska et al., 2017).

To overcome the need for additional outer-membrane-permeabilising agents during treatment of Gram-negative bacterial infections, artificial lysins (Artilysins) have been developed by the fusion of a phage endolysin with an outer-membrane-destabilising peptide (Briers et al., 2014b). Artilysins specific for *K. pneumoniae* have yet to be developed, but they have been successfully created for use against *P. aeruginosa* (Briers et al., 2014a) and *Acinetobacter baumannii* (Defraine et al., 2016). This technology opens up the possibility of developing artificial endolysins for use in human therapy against not only MDR *K. pneumoniae* but also MDR Gram-negative infections.

**Further research**

Recombinant polysaccharide depolymerases and artificial endolysins have the potential to be used as therapeutic agents in the fight against MDR *K. pneumoniae*. Polysaccharide depolymerases are able to degrade the capsule, an essential virulence factor of *K. pneumoniae*, which could find uses such as boosting the host immune response against the bacterium, and breaking down biofilms to allow current antibiotics to more easily access bacterial cells. Artificial endolysins have the potential to work against infection as an independent antimicrobial agent. Further research is required in this area to fully realise the potential of such phage-derived recombinant proteins, and in doing so the mechanisms by which they are able to inhibit bacterial growth and/or eliminate infection may lead to new breakthroughs. Importantly, an obvious advantage over phage therapy is that recombinant protein products for use in humans have well-
defined and established rules and regulations regarding their production, safety and use in the EU
and UK, whereas phage therapy does not.

CONCLUDING REMARKS

The increasing incidence of hospital-acquired and community-acquired infections caused by
MDR *K. pneumoniae* and hypervirulent *K. pneumoniae*, respectively, is rapidly becoming a
global threat to public health. The emergence of strains that are both MDR and hypervirulent is
even more of a concern. *K. pneumoniae* is becoming as much of a threat today as its non-
resistant counterparts were over a century ago prior to the discovery of antimicrobial compounds
such as penicillin. In response, research efforts have begun to look back in time at a once-
abandoned approach to bacterial infection, namely phage therapy. It is becoming increasingly
clear that there is potential for phages and their gene products to become novel sources of
antimicrobial strategies against MDR bacteria for which current treatment regimens are simply
becoming ineffective at countering. However, the field of phage therapy is still very much in its
infancy and is fraught with difficulties, both novel and familiar.

Safety

One of the major obstacles facing phage therapy are the novel safety implications regarding the
use of self-replicating biological entities in humans. For example, it is evident that phages are
capable of carrying antibiotic resistance (Colavecchio et al., 2017) and toxin-encoding (Strauch,
Lurz & Beutin, 2001) genes that could be transferred to the target bacterium via the process of
transduction. Proper characterisation is, therefore, important when considering phages for
The nature of a lytic phage is to increase its number at the expense of bacterial hosts. While this is the primary aim of phage therapy, little research has been conducted regarding the potential side-effects of this phenomenon. This is an important consideration because phages with a broad host range, or those within a phage cocktail, are often considered more appropriate for phage therapy. It is evident from the recent work of Hsu et al. (2019) that introduction of even a single phage into the mouse microbiota can have effects on the microbiome. What effect might therapeutic use of phages have on the normal microbiota of a human? Might it be safer to use individual phages, with a narrow host range, to minimise disruption of the commensal microbiota? If so, phage therapy will rely on very specific identification of infecting bacteria, and having the correct phage available for treatment. Or perhaps this particular side effect may be deemed acceptable, as is the case with current antibiotic regimens. Additionally, the number of clinical trials that have assessed the safety of phage therapy in humans is limited, and those that have occurred have consisted of small sample sizes and often rely on patient-generated data (Furfaro, Payne & Chang, 2018).

Practicality

The second barrier that must be overcome are the practical issues associated with phage therapy in the EU and UK. As discussed earlier, the regulations required to govern the safety, production and use of virus-based infection control mechanisms do not currently exist. The last attempt at tackling these regulatory hurdles came in the form of a phase II clinical trial funded by the
Launched in 2013 and achieved in 2017, PhagoBurn was the world first prospective multicentric, randomised, single blind and controlled clinical trial of phage therapy ever performed according to both Good Manufacturing (GMP) and Good Clinical Practices (GCP)” (European Commission, 2017). Although the project attempted to define appropriate practices for phage therapy during its assessment of efficacy and tolerability of phage-treated burn-wound infections (Jault et al., 2019), only temporary allowances were made. While recommendations for subsequent clinical trials were given, no further regulatory improvements have been attempted.

Moreover, if regulations are updated to account for phage therapy, where would producers of phage products stand in relation to intellectual property? Can naturally occurring biological entities be patented and sold, or would this be reserved for phage cocktails and phage–drug combinations that exhibit ‘unnatural’ antimicrobial properties? Indeed, in terms of personalised medicine, phage cocktails may require production within the healthcare setting to suit a specific patient’s needs. In this case, would the ingredients of a phage cocktail need to be individually patented and sold, or could cocktails be developed with the pliability for patient-specific modifications later? In the absence of profitable, patented technology, pharmaceutical companies may be reluctant to fund the research and development of such treatments.

Phage resistance

Finally, it could be argued that the issues surrounding phage therapy may be abrogated by using phage gene products instead. Being more akin to conventional antimicrobial therapeutics, they would be subjected to the well-established drug development processes and standards of
production and safety that are currently in place. However, the use of both phages and their gene
products against bacterial infection may still be subject to the age-old problem of bacterial
resistance. Indeed, some of the studies outlined in this literature review suggest, or provide
evidence of, the possibility of resistance against phage therapy, although this phenomenon has
yet to be observed *in vivo*.

The first warnings regarding the development of antibiotic resistance (Fleming, 1945; Abraham
& Chain, 1988) went unheeded, resulting in the spread of MDR bacteria such as *K. pneumoniae*,
and are the grounds upon which phage therapy has become a renewed topic of research. The
development of novel antimicrobial agents is, therefore, not enough to combat infection and
bacterial resistance in the long term. Strategies regarding the use of any novel antimicrobial
treatments must be developed to minimise the risk of the development of resistance. In terms of
phage therapy, such strategies might involve using combination treatments: for example, phage–
drug combinations or complex phage cocktails designed to minimise the selection pressures
applied against bacteria during treatment.

Prevention should be the primary focus of healthcare-associated infection control procedures.
The implementation or improvement of policies aimed at reducing the risk of patients developing
bacterial infections must be concurrent with the development of novel antibacterial therapeutics
to minimise the spread of resistance to treatment. Such procedures may include hand and
environmental decontamination, safe installation and maintenance of medical devices, prompt
removal of medical devices that are no longer needed, screening and decolonisation programmes,
and cautious use of antimicrobial agents.
Future research

The future of phage research is a promising one. Phages are perhaps the most numerous of all biological entities on the planet and as such could be the most valuable source of therapeutic solutions. As we further elucidate the interactions between phage and bacterium, as predator and prey, advances in our understanding of the molecular mechanisms defining such interactions may afford us new information and ideas that can be applied to infection control. Indeed, phage research has already led to the development of artificial phage-derived antibacterial proteins – Artilysin (Briers et al., 2014b) – and the artificial alteration of phage host range to infect a greater range of bacteria than is naturally possible is just beginning to come to fruition (Yosef et al., 2017).

Furthermore, recent technological advances have seen next-generation sequencing (NGS) become increasingly used in phage research, providing a more robust platform from which to launch detailed phage characterisation, screening of harmful genes and evaluating potentially useful gene products (Philipson et al., 2018). Further technological advancements and categorisation of information attained from methods such as NGS can only lead us onwards, providing new solutions to old problems.

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REFERENCES

Abraham EP, Chain E. 1988. An enzyme from bacteria able to destroy penicillin. 1940. Reviews of Infectious Diseases 10:677–678.

Aleshkin AV, Ershova ON, Volozhantsev NV, Svetoch EA, Popova AV, Rubalskii EO, Borzilov AI, Aleshkin VA, Afanas’ev SS, Karaulov AV, Galimzyanov KM, Rubalsky OV, Bochkareva SS. 2016. Phagebiotics in treatment and prophylaxis of healthcare-associated infections. Bacteriophage 6:e1251379. DOI: 10.1080/21597081.2016.1251379.

Aminov RI. 2010. A brief history of the antibiotic era: lessons learned and challenges for the future. Frontiers in Microbiology 1. DOI: 10.3389/fmicb.2010.00134.

Anonymous. 2011. On an invisible microbe antagonistic to dysentery bacilli. Note by M. F. d’Herelle, presented by M. Roux. Comptes Rendus Academie des Sciences 1917; 165:373–5. Bacteriophage 1:3–5. DOI: 10.4161/bact.1.1.14941.

Bertozzi Silva J, Storms Z, Sauvageau D. 2016. Host receptors for bacteriophage adsorption. FEMS Microbiology Letters 363. DOI: 10.1093/femsle/fnw002.

Bhattarai Y, Williams BB, Battaglioli EJ, Whitaker WR, Till L, Grover M, Linden DR, Akiba Y, Kandimalla KK, Zachos NC, Kaunitz JD, Sonnenburg JL, Fischbach MA, Farrugia G, Kashyap PC. 2018. Gut microbiota-produced tryptamine activates an epithelial G-protein-coupled receptor to increase colonic secretion. Cell Host & Microbe 23:775-785.e5. DOI: 10.1016/j.chom.2018.05.004.
Briers Y, Walmagh M, Grymonprez B, Biebl M, Pirnay J-P, Defraine V, Michiels J, Cenens W, Aertsen A, Miller S, Lavigne R. 2014a. Art-175 is a highly efficient antibacterial against multidrug-resistant strains and persisters of *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* 58:3774–3784. DOI: 10.1128/AAC.02668-14.

Briers Y, Walmagh M, Van Puyenbroeck V, Cornelissen A, Cenens W, Aertsen A, Oliveira H, Azeredo J, Verween G, Pirnay J-P, Miller S, Volckaert G, Lavigne R. 2014b. Engineered endolysin-based “Artilysins” to combat multidrug-resistant gram-negative pathogens. *mBio* 5:e01379-01314. DOI: 10.1128/mBio.01379-14.

Broberg CA, Palacios M, Miller VL. 2014. *Klebsiella*: a long way to go towards understanding this enigmatic jet-setter. *F1000prime Reports* 6:64. DOI: 10.12703/P6-64.

Brown TL, Petrovski S, Hoyle D, Chan HT, Lock P, Tucci J. 2017. Characterization and formulation into solid dosage forms of a novel bacteriophage lytic against *Klebsiella oxytoca*. *PloS One* 12:e0183510. DOI: 10.1371/journal.pone.0183510.

Cai R, Wang Z, Wang G, Zhang H, Cheng M, Guo Z, Ji Y, Xi H, Wang X, Xue Y, Ur Rahman S, Sun C, Feng X, Lei L, Tong Y, Han W, Gu J. 2019. Biological properties and genomics analysis of vB_KpnS_GH-K3, a *Klebsiella* phage with a putative depolymerase-like protein. *Virus Genes*. DOI: 10.1007/s11262-019-01681-z.

Campos AC, Albiero J, Ecker AB, Kuroda CM, Meirelles LEF, Polato A, Tognim MCB, Wingeter MA, Teixeira JJV. 2016. Outbreak of *Klebsiella pneumoniae* carbapenemase-producing *K pneumoniae*: A systematic review. *American Journal of Infection Control* 44:1374–1380. DOI: 10.1016/j.ajic.2016.03.022.

Cao F, Wang X, Wang L, Li Z, Che J, Wang L, Li X, Cao Z, Zhang J, Jin L, Xu Y. 2015. Evaluation of the efficacy of a bacteriophage in the treatment of pneumonia induced by
multidrug resistance *Klebsiella pneumoniae* in mice. *BioMed Research International* 2015:752930. DOI: 10.1155/2015/752930.

Carl G, Jäckel C, Grützke J, Hertwig S, Grobbel M, Malorny B, Rau J, Käsbohrer A, Hammerl JA. 2017. Complete genome sequence of the temperate *Klebsiella pneumoniae* phage KPP5665-2. *Genome Announcements* 5. DOI: 10.1128/genomeA.01118-17.

Casjens SR, Gilcrease EB, Huang WM, Bunny KL, Pedulla ML, Ford ME, Houtz JM, Hatfull GF, Hendrix RW. 2004. The pKO2 linear plasmid prophage of *Klebsiella oxytoca*. *Journal of Bacteriology* 186:1818–1832. DOI: 10.1128/jb.186.6.1818-1832.2004.

Chadha P, Katare OP, Chhibber S. 2016. In vivo efficacy of single phage versus phage cocktail in resolving burn wound infection in BALB/c mice. *Microbial Pathogenesis* 99:68–77. DOI: 10.1016/j.micpath.2016.08.001.

Chhibber S, Kaur S, Kumari S. 2008. Therapeutic potential of bacteriophage in treating *Klebsiella pneumoniae* B5055-mediated lobar pneumonia in mice. *Journal of Medical Microbiology* 57:1508–1513. DOI: 10.1099/jmm.0.2008/002873-0.

Chhibber S, Nag D, Bansal S. 2013. Inhibiting biofilm formation by *Klebsiella pneumoniae* B5055 using an iron antagonizing molecule and a bacteriophage. *BMC Microbiology* 13:174. DOI: 10.1186/1471-2180-13-174.

Chong Y, Yakushiji H, Ito Y, Kamimura T. 2011. Clinical and molecular epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a long-term study from Japan. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 30:83–87. DOI: 10.1007/s10096-010-1057-1.
Ciacci N, D’Andrea MM, Marmo P, Demattè E, Amisano F, Di Pilato V, Fraziano M, Lupetti P, Rossolini GM, Thaller MC. 2018. Characterization of vB_Kpn_F48, a newly discovered lytic bacteriophage for *Klebsiella pneumoniae* of sequence type 101. *Viruses* 10. DOI: 10.3390/v10090482.

Clokie MR, Millard AD, Letarov AV, Heaphy S. 2011. Phages in nature. *Bacteriophage* 1:31–45. DOI: 10.4161/bact.1.1.14942.

Colavecchio A, Cadieux B, Lo A, Goodridge LD. 2017. Bacteriophages contribute to the spread of antibiotic resistance genes among foodborne pathogens of the *Enterobacteriaceae* family - A review. *Frontiers in Microbiology* 8:1108. DOI: 10.3389/fmicb.2017.01108.

Connor TR, Loman NJ, Thompson S, Smith A, Southgate J, Poplawski R, Bull MJ, Richardson E, Ismail M, Thompson SE-, Kitchen C, Guest M, Bakke M, Sheppard SK, Pallen MJ. 2016. CLIMB (the Cloud Infrastructure for Microbial Bioinformatics): an online resource for the medical microbiology community. *Microbial Genomics* 2:e000086. DOI: 10.1099/mgen.0.000086.

Cortés G, Borrell N, de Astorza B, Gómez C, Sauleda J, Albertí S. 2002. Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of *Klebsiella pneumoniae* in a murine model of pneumonia. *Infection and Immunity* 70:2583–2590. DOI: 10.1128/iai.70.5.2583-2590.2002.

Cui Z, Shen W, Wang Z, Zhang H, Me R, Wang Y, Zeng L, Zhu Y, Qin J, He P, Guo X. 2012. Complete genome sequence of *Klebsiella pneumoniae* phage JD001. *Journal of Virology* 86:13843. DOI: 10.1128/JVI.02435-12.

D’Andrea MM, Marmo P, Henrici De Angelis L, Palmieri M, Ciacci N, Di Lallo G, Demattè E, Vannuccini E, Lupetti P, Rossolini GM, Thaller MC. 2017. φBO1E, a newly discovered lytic...
bacteriophage targeting carbapenemase-producing *Klebsiella pneumoniae* of the pandemic clonal group 258 clade II lineage. *Scientific Reports* 7:2614. DOI: 10.1038/s41598-017-02788-9.

David S, Reuter S, Harris SR, Glasner C, Feltwell T, Argimon S, Abudahab K, Goater R, Giani T, Errico G, Aspbury M, Sjunnebo S, Feil EJ, Rossolini GM, Aanensen DM, Grundmann H. 2019. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nature Microbiology*:1–11. DOI: 10.1038/s41564-019-0492-8.

Debarbieux L, Pirnay J-P, Verbeken G, De Vos D, Merabishvili M, Huys I, Patey O, Schoonjans D, Vaneechoutte M, Zizi M, Rohde C. 2016. A bacteriophage journey at the European Medicines Agency. *FEMS Microbiology Letters* 363:fnv225. DOI: 10.1093/femsle/fnv225.

Defraine V, Schuermans J, Grymonprez B, Govers SK, Aertsen A, Fauvart M, Michiels J, Lavigne R, Briers Y. 2016. Efficacy of Artilysin Art-175 against resistant and persistent *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy* 60:3480–3488. DOI: 10.1128/AAC.00285-16.

Domagk G. 1935. Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. *DMW - Deutsche Medizinische Wochenschrift* 61:250–253. DOI: 10.1055/s-0028-1129486.

Drulis-Kawa Z, Mackiewicz P, Kęsik-Szeloch A, Maciaszczyk-Dziubinska E, Weber-Dąbrowska B, Dorotkiewicz-Jach A, Augustyniak D, Majkowska-Skrobek G, Bocer T, Empel J, Kropinski AM. 2011. Isolation and characterisation of KP34--a novel φKMV-like bacteriophage for *Klebsiella pneumoniae*. *Applied Microbiology and Biotechnology* 90:1333–1345. DOI: 10.1007/s00253-011-3149-y.

Drulis-Kawa Z, Majkowska-Skrobek G, Maciejewska B. 2015. Bacteriophages and phage-derived proteins--application approaches. *Current Medicinal Chemistry* 22:1757–1773.
Eades C, Davies F, Donaldson H, Hopkins K, Hill R, Otter J, Khadani T, Pavlu J, Holmes A, Turton J, Woodford N. 2016. GES-5 carabapenemase-producing *Klebsiella oxytoca* causing clinical infection in a UK haematopoetic stem cell transplantation unit.

Ehrlich P. 1910. *Die experimentelle Chemotherapie der Spirillosen*: Springer.

Eriksson H, Maciejewska B, Latka A, Majkowska-Skrobek G, Hellstrand M, Melefors Ö, Wang J-T, Kropinski AM, Drlis-Kawa Z, Nilsson AS. 2015. A suggested new bacteriophage genus, “Kp34likevirus”, within the *Autographivirinae* subfamily of *Podoviridae*. *Viruses* 7:1804–1822. DOI: 10.3390/v7041804.

European Commission. 2017. Final Report Summary - PHAGOBURN (Evaluation of phage therapy for the treatment of *Escherichia coli* and *Pseudomonas aeruginosa* burn wound infections (Phase I-II clinical trial)).

Fleming A. 1945. Penicillin: nobel prize lecture.

Fleming A. 2001. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae*. 1929. *Bulletin of the World Health Organization* 79:780–790.

Fokine A, Rossmann MG. 2014. Molecular architecture of tailed double-stranded DNA phages. *Bacteriophage* 4:e28281. DOI: 10.4161/bact.28281.

Follador R, Heinz E, Wyres KL, Ellington MJ, Kowarik M, Holt KE, Thomson NR. 2016. The diversity of *Klebsiella pneumoniae* surface polysaccharides. *Microbial Genomics* 2:e000073. DOI: 10.1099/mgen.0.000073.

Furfaro LL, Payne MS, Chang BJ. 2018. Bacteriophage therapy: clinical trials and regulatory hurdles. *Frontiers in Cellular and Infection Microbiology* 8:376. DOI: 10.3389/fcimb.2018.00376.
Gao S, Linden SB, Nelson DC. 2017. Complete genome sequence of *Klebsiella pneumoniae* phages SopranoGao, MezzoGao, and AltoGao. *Genome Announcements* 5. DOI: 10.1128/genomeA.01009-17.

Glasner C, Albiger B, Buist G, Tambić Andrasević A, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H, European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. 2013. Carbapenemase-producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin* 18. DOI: 10.2807/1560-7917.es2013.18.28.20525.

Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, Cantón R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Monnet DL, European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. 2017. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE): a prospective, multinational study. *The Lancet. Infectious Diseases* 17:153–163. DOI: 10.1016/S1473-3099(16)30257-2.

Gu J, Liu X, Li Y, Han W, Lei L, Yang Y, Zhao H, Gao Y, Song J, Lu R, Sun C, Feng X. 2012. A method for generation phage cocktail with great therapeutic potential. *PloS One* 7:e31698. DOI: 10.1371/journal.pone.0031698.
Gürntke S, Kohler C, Steinmetz I, Pfeifer Y, Eller C, Gastmeier P, Schwab F, Leistner R. 2014. Molecular epidemiology of extended-spectrum beta-lactamase (ESBL)-positive *Klebsiella pneumoniae* from bloodstream infections and risk factors for mortality. *Journal of Infection and Chemotherapy: Official Journal of the Japan Society of Chemotherapy* 20:817–819. DOI: 10.1016/j.jiac.2014.08.012.

Harper DR. 2018. Criteria for selecting suitable infectious diseases for phage therapy. *Viruses* 10. DOI: 10.3390/v10040177.

d’Herelle F. 1918. Sur le rôle du microbe filtrant bactériophage dans la dysentérie bacillaire. *Comptes rendus de l’Académie des Sciences* 167:970–972.

Herzog KAT, Schneditz G, Leitner E, Feierl G, Hoffmann KM, Zollner-Schwetz I, Krause R, Gorkiewicz G, Zechner EL, Högenauer C. 2014. Genotypes of *Klebsiella oxytoca* isolates from patients with nosocomial pneumonia are distinct from those of isolates from patients with antibiotic-associated hemorrhagic colitis. *Journal of Clinical Microbiology* 52:1607–1616. DOI: 10.1128/JCM.03373-13.

Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Nhu NTK, Schultz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR. 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proceedings of the National Academy of Sciences of the United States of America* 112:E3574-3581. DOI: 10.1073/pnas.1501049112.

Hoyles L, Murphy J, Neve H, Heller KJ, Turton JF, Mahony J, Sanderson JD, Hudspith B, Gibson GR, McCartney AL, van Sinderen D. 2015. *Klebsiella pneumoniae* subsp. pneumoniae-
bacteriophage combination from the caecal effluent of a healthy woman. *PeerJ* 3:e1061. DOI: 10.7717/peerj.1061.

Hsieh P-F, Lin H-H, Lin T-L, Chen Y-Y, Wang J-T. 2017. Two T7-like bacteriophages, K5-2 and K5-4, each encodes two capsule depolymerases: isolation and functional characterization. *Scientific Reports* 7:4624. DOI: 10.1038/s41598-017-04644-2.

Hsu BB, Gibson TE, Yeliseyev V, Liu Q, Lyon L, Bry L, Silver PA, Gerber GK. 2019. Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. *Cell Host & Microbe* 25:803-814.e5. DOI: 10.1016/j.chom.2019.05.001.

Hsu C-R, Lin T-L, Pan Y-J, Hsieh P-F, Wang J-T. 2013. Isolation of a bacteriophage specific for a new capsular type of *Klebsiella pneumoniae* and characterization of its polysaccharide depolymerase. *PloS One* 8:e70092. DOI: 10.1371/journal.pone.0070092.

Huang W, Wang G, Sebra R, Zhuge J, Yin C, Aguero-Rosenfeld ME, Schuetz AN, Dimitrova N, Fallon JT. 2017. Emergence and evolution of multidrug-resistant *Klebsiella pneumoniae* with both *bla*KPC and *blaCTX-M* integrated in the chromosome. *Antimicrobial Agents and Chemotherapy* 61. DOI: 10.1128/AAC.00076-17.

Hughes KA, Sutherland IW, Clark J, Jones MV. 1998. Bacteriophage and associated polysaccharide depolymerases--novel tools for study of bacterial biofilms. *Journal of Applied Microbiology* 85:583–590.

Hung C-H, Kuo C-F, Wang C-H, Wu C-M, Tsao N. 2011. Experimental phage therapy in treating *Klebsiella pneumoniae*-mediated liver abscesses and bacteremia in mice. *Antimicrobial Agents and Chemotherapy* 55:1358–1365. DOI: 10.1128/AAC.01123-10.
Jamal M, Hussain T, Das CR, Andleeb S. 2015. Characterization of Siphoviridae phage Z and studying its efficacy against multidrug-resistant Klebsiella pneumoniae planktonic cells and biofilm. *Journal of Medical Microbiology* 64:454–462. DOI: 10.1099/jmm.0.000040.

Jault P, Leclerc T, Jennes S, Pirnay JP, Que Y-A, Resch G, Rousseau AF, Ravat F, Carsin H, Le Floch R, Schaal JV, Soler C, Fevre C, Arnaud I, Bretaudeau L, Gabard J. 2019. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *The Lancet. Infectious Diseases* 19:35–45. DOI: 10.1016/S1473-3099(18)30482-1.

Johnson AP, Woodford N. 2013. Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. *Journal of Medical Microbiology* 62:499–513. DOI: 10.1099/jmm.0.052555-0.

Jun JB. 2018. *Klebsiella pneumoniae* liver abscess. *Infection & Chemotherapy* 50:210–218. DOI: 10.3947/ic.2018.50.3.210.

Karumidze N, Kusradze I, Rigvava S, Goderdzishvili M, Rajakumar K, Alavidze Z. 2013. Isolation and characterisation of lytic bacteriophages of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Current Microbiology* 66:251–258. DOI: 10.1007/s00284-012-0264-7.

Kęsik-Szeloch A, Drulis-Kawa Z, Weber-Dąbrowska B, Kassner J, Majkowska-Skrobek G, Augustyniak D, Lusiak-Szelachowska M, Zaczek M, Górski A, Kropinski AM. 2013. Characterising the biology of novel lytic bacteriophages infecting multidrug resistant *Klebsiella pneumoniae*. *Virology Journal* 10:100. DOI: 10.1186/1743-422X-10-100.

Khan E, Schneiders T, Zafar A, Aziz E, Parekh A, Hasan R. 2010. Emergence of CTX-M Group 1-ESBL producing *Klebsiella pneumoniae* from a tertiary care centre in Karachi, Pakistan. *Journal of Infection in Developing Countries* 4:472–476.
Knothe H, Shah P, Krcmery V, Antal M, Mitsuhashi S. 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection 11:315–317.

Koberg S, Brinks E, Fiedler G, Hüsing C, Cho G-S, Hoeppner MP, Heller KJ, Neve H, Franz CMAP. 2017. Genome Sequence of Klebsiella pneumoniae bacteriophage PMBT1 isolated from raw sewage. Genome Announcements 5. DOI: 10.1128/genomeA.00914-16.

Komisarova EV, Kislichkina AA, Krasilnikova VM, Bogun AG, Fursova NK, Volozhantsev NV. 2017. Complete nucleotide sequence of Klebsiella pneumoniae bacteriophage vB_KpnM_KpV477. Genome Announcements 5. DOI: 10.1128/genomeA.00694-17.

Kumari S, Harjai K, Chhibber S. 2009. Efficacy of bacteriophage treatment in murine burn wound infection induced by klebsiella pneumoniae. Journal of Microbiology and Biotechnology 19:622–628.

Kumari S, Harjai K, Chhibber S. 2010a. Isolation and characterization of Klebsiella pneumoniae specific bacteriophages from sewage samples. Folia Microbiologica 55:221–227. DOI: 10.1007/s12223-010-0032-7.

Kumari S, Harjai K, Chhibber S. 2010b. Evidence to support the therapeutic potential of bacteriophage Kpn5 in burn wound infection caused by Klebsiella pneumoniae in BALB/c mice. Journal of Microbiology and Biotechnology 20:935–941.

Kumari S, Harjai K, Chhibber S. 2010c. Topical treatment of Klebsiella pneumoniae B5055 induced burn wound infection in mice using natural products. Journal of Infection in Developing Countries 4:367–377.
Kumari S, Harjai K, Chhibber S. 2011. Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by \textit{Klebsiella pneumoniae} B5055. \textit{Journal of Medical Microbiology} 60:205–210. DOI: 10.1099/jmm.0.018580-0.

Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon ST. 2010. Phage therapy in clinical practice: treatment of human infections. \textit{Current Pharmaceutical Biotechnology} 11:69–86.

Labudda Ł, Strapagiel D, Karczewska-Golec J, Golec P. 2017. Complete annotated genome sequences of four \textit{Klebsiella pneumoniae} phages isolated from sewage in Poland. \textit{Genome Announcements} 5. DOI: 10.1128/genomeA.00919-17.

Lawlor MS, Handley SA, Miller VL. 2006. Comparison of the host responses to wild-type and cpsB mutant \textit{Klebsiella pneumoniae} infections. \textit{Infection and Immunity} 74:5402–5407. DOI: 10.1128/IAI.00244-06.

Lee C-H, Chen I-L, Chuah S-K, Tai W-C, Chang C-C, Chen F-J, Chen J-F. 2016. Impact of glycemic control on capsular polysaccharide biosynthesis and opsonophagocytosis of \textit{Klebsiella pneumoniae}: Implications for invasive syndrome in patients with diabetes mellitus. \textit{Virulence} 7:770–778. DOI: 10.1080/21505594.2016.1186315.

Lee C-R, Lee JH, Park KS, Jeon JH, Kim YB, Cha C-J, Jeong BC, Lee SH. 2017. Antimicrobial resistance of hypervirulent \textit{Klebsiella pneumoniae}: epidemiology, hypervirulence-associated determinants, and resistance mechanisms. \textit{Frontiers in Cellular and Infection Microbiology} 7:483. DOI: 10.3389/fcimb.2017.00483.

Lin T-L, Hsieh P-F, Huang Y-T, Lee W-C, Tsai Y-T, Su P-A, Pan Y-J, Hsu C-R, Wu M-C, Wang J-T. 2014. Isolation of a bacteriophage and its depolymerase specific for K1 capsule of
Klebsiella pneumoniae: implication in typing and treatment. The Journal of Infectious Diseases 210:1734–1744. DOI: 10.1093/infdis/jiu332.

Liu P, Li X, Luo M, Xu X, Su K, Chen S, Qing Y, Li Y, Qiu J. 2018. Risk factors for carbapenem-resistant Klebsiella pneumoniae infection: A meta-analysis. Microbial Drug Resistance (Larchmont, N.Y.) 24:190–198. DOI: 10.1089/mdr.2017.0061.

Liu Y, Mi L, Mi Z, Huang Y, Li P, Zhang X, Tong Y, Bai C. 2016. Complete genome sequence of IME207, a novel bacteriophage which can lyse multidrug-resistant Klebsiella pneumoniae and Salmonella. Genome Announcements 4. DOI: 10.1128/genomeA.01015-16.

Loc-Carrillo C, Abedon ST. 2011. Pros and cons of phage therapy. Bacteriophage 1:111–114. DOI: 10.4161/bact.1.2.14590.

Lu Y, Shi H, Zhang Z, Han F, Li J, Sun Y. 2015. Isolation and characterization of a lytic bacteriophage φKp-lyy15 of Klebsiella pneumoniae. Virologica Sinica 30:66–68. DOI: 10.1007/s12250-014-3523-x.

Maciejewska B, Roszniowski B, Espaillat A, Kęsik-Szeloch A, Majkowska-Skrobek G, Kropinski AM, Briers Y, Cava F, Lavigne R, Drulis-Kawa Z. 2017. Klebsiella phages representing a novel clade of viruses with an unknown DNA modification and biotechnologically interesting enzymes. Applied Microbiology and Biotechnology 101:673–684. DOI: 10.1007/s00253-016-7928-3.

Majkowska-Skrobek G, Łątka A, Berisio R, Maciejewska B, Squeglia F, Romano M, Lavigne R, Struve C, Drulis-Kawa Z. 2016. Capsule-targeting depolymerase, derived from Klebsiella KP36 phage, as a tool for the development of anti-virulent strategy. Viruses 8. DOI: 10.3390/v8120324.
Majkowska-Skrobek G, Latka A, Berisio R, Squeglia F, Maciejewska B, Briers Y, Drulis-Kawa Z. 2018. Phage-borne depolymerases decrease Klebsiella pneumoniae resistance to innate defense mechanisms. Frontiers in Microbiology 9:2517. DOI: 10.3389/fmicb.2018.02517.

Manohar P, Nachimuthu R, Lopes BS. 2018. The therapeutic potential of bacteriophages targeting gram-negative bacteria using Galleria mellonella infection model. BMC microbiology 18:97. DOI: 10.1186/s12866-018-1234-4.

Meatherall BL, Gregson D, Ross T, Pitout JDD, Laupland KB. 2009. Incidence, risk factors, and outcomes of Klebsiella pneumoniae bacteremia. The American Journal of Medicine 122:866–873. DOI: 10.1016/j.amjmed.2009.03.034.

Merino S, Camprubí S, Albertí S, Benedí VJ, Tomás JM. 1992. Mechanisms of Klebsiella pneumoniae resistance to complement-mediated killing. Infection and Immunity 60:2529–2535.

Mijalis EM, Lessor LE, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae Myophage Miro. Genome Announcements 3. DOI: 10.1128/genomeA.01137-15.

Moradigaravand D, Martin V, Peacock SJ, Parkhill J. 2017a. Evolution and epidemiology of multidrug-resistant Klebsiella pneumoniae in the United Kingdom and Ireland. mBio 8. DOI: 10.1128/mBio.01976-16.

Moradigaravand D, Martin V, Peacock SJ, Parkhill J. 2017b. Population structure of multidrug-resistant Klebsiella oxytoca within hospitals across the United Kingdom and Ireland identifies sharing of virulence and resistance genes with K. pneumoniae. Genome Biology and Evolution 9:574–584. DOI: 10.1093/gbe/evx019.
Mshana SE, Hain T, Domann E, Lyamuya EF, Chakraborty T, Imirzalioglu C. 2013. Predominance of *Klebsiella pneumoniae* ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. *BMC infectious diseases* 13:466. DOI: 10.1186/1471-2334-13-466.

Nguyen DT, Lessor LE, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* Siphophage Sushi. *Genome Announcements* 3. DOI: 10.1128/genomeA.00994-15.

Nishimura Y, Yoshida T, Kuronishi M, Uehara H, Ogata H, Goto S. 2017. ViPTree: the viral proteomic tree server. *Bioinformatics (Oxford, England)* 33:2379–2380. DOI: 10.1093/bioinformatics/btx157.

Nordmann P, Poirel L, Walsh TR, Livermore DM. 2011. The emerging NDM carbapenemases. *Trends in Microbiology* 19:588–595. DOI: 10.1016/j.tim.2011.09.005.

Nouvenne A, Ticinesi A, Lauretani F, Maggio M, Lippi G, Guida L, Morelli I, Ridolo E, Borghi L, Meschi T. 2014. Comorbidities and disease severity as risk factors for carbapenem-resistant *Klebsiella pneumoniae* colonization: report of an experience in an internal medicine unit. *PloS One* 9:e110001. DOI: 10.1371/journal.pone.0110001.

Oh HK, Cha K, Hwang YJ, Cho J, Jo Y, Myung H. 2019. Complete genome sequence of a novel bacteriophage, PBKP05, infecting *Klebsiella pneumoniae*. *Archives of Virology* 164:885–888. DOI: 10.1007/s00705-018-04121-9.

Paczosa MK, Mecsas J. 2016. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiology and molecular biology reviews: MMBR* 80:629–661. DOI: 10.1128/MMBR.00078-15.

Pan Y-J, Lin T-L, Chen Y-Y, Lai P-H, Tsai Y-T, Hsu C-R, Hsieh P-F, Lin Y-T, Wang J-T. 2019. Identification of three podoviruses infecting *Klebsiella* encoding capsule depolymerases that
digest specific capsular types. *Microbial Biotechnology* 12:472–486. DOI: 10.1111/1751-7915.13370.

Pan Y-J, Lin T-L, Chen C-C, Tsai Y-T, Cheng Y-H, Chen Y-Y, Hsieh P-F, Lin Y-T, Wang J-T. 2017. *Klebsiella* phage ΦK64-1 encodes multiple depolymerases for multiple host capsular types. *Journal of Virology* 91. DOI: 10.1128/JVI.02457-16.

Pan Y-J, Lin T-L, Lin Y-T, Su P-A, Chen C-T, Hsieh P-F, Hsu C-R, Chen C-C, Hsieh Y-C, Wang J-T. 2015. Identification of capsular types in carbapenem-resistant *Klebsiella pneumoniae* strains by wzc sequencing and implications for capsule depolymerase treatment. *Antimicrobial Agents and Chemotherapy* 59:1038–1047. DOI: 10.1128/AAC.03560-14.

Park E-A, Kim Y-T, Cho J-H, Ryu S, Lee J-H. 2017. Characterization and genome analysis of novel bacteriophages infecting the opportunistic human pathogens *Klebsiella oxytoca* and *K. pneumoniae*. *Archives of Virology* 162:1129–1139. DOI: 10.1007/s00705-016-3202-3.

Philipson CW, Voegtly LJ, Lueder MR, Long KA, Rice GK, Frey KG, Biswas B, Cer RZ, Hamilton T, Bishop-Lilly KA. 2018. Characterizing phage genomes for therapeutic applications. *Viruses* 10. DOI: 10.3390/v10040188.

Pirnay J-P, Verbeken G, Ceyssens P-J, Huys I, De Vos D, Ameloot C, Fauconnier A. 2018. The magistral phage. *Viruses* 10. DOI: 10.3390/v10020064.

Podschun R, Ullmann U. 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews* 11:589–603.

Potron A, Poirel L, Rondinaud E, Nordmann P. 2013. Intercontinental spread of OXA-48 beta-lactamase-producing *Enterobacteriaceae* over a 11-year period, 2001 to 2011. *Euro Surveillance* 18:9–22.
Provasek VE, Lessor LE, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of carbapenemase-producing Klebsiella pneumoniae Myophage Matisse. Genome Announcements 3. DOI: 10.1128/genomeA.01136-15.

Public Health England. 2016. Carbapenemase-producing Enterobacteriaceae: laboratory confirmed cases, 2003 to 2015. Available at https://www.gov.uk/government/publications/carbapenemase-producing-enterobacteriaceae-laboratory-confirmed-cases-2003-to-2013 (accessed July 31, 2019).

Pyra A, Brzozowska E, Pawlik K, Gamian A, Dauter M, Dauter Z. 2017. Tail tubular protein A: a dual-function tail protein of Klebsiella pneumoniae bacteriophage KP32. Scientific Reports 7:2223. DOI: 10.1038/s41598-017-02451-3.

Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. 2009. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. Journal of Wound Care 18:237–238, 240–243. DOI: 10.12968/jowc.2009.18.6.42801.

Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. 2015. Risk factors for acquisition of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae in North-Indian hospitals. Saudi Journal of Biological Sciences 22:37–41. DOI: 10.1016/j.sjbs.2014.05.006.

Shang A, Liu Y, Wang J, Mo Z, Li G, Mou H. 2015. Complete nucleotide sequence of Klebsiella phage P13 and prediction of an EPS depolymerase gene. Virus Genes 50:118–128. DOI: 10.1007/s11262-014-1138-9.

Shen J, Zhou J, Chen G-Q, Xiu Z-L. 2018. Efficient genome engineering of a virulent Klebsiella bacteriophage using CRISPR-Cas9. Journal of Virology 92. DOI: 10.1128/JVI.00534-18.
Shon AS, Bajwa RPS, Russo TA. 2013. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. *Virulence* 4:107–118. DOI: 10.4161/viru.22718.

Silva N, Oliveira M, Bandeira AC, Brites C. 2006. Risk factors for infection by extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in a tertiary hospital in Salvador, Brazil. *The Brazilian Journal of Infectious Diseases: An Official Publication of the Brazilian Society of Infectious Diseases* 10:191–193.

Šimoliūnas E, Kaliniene L, Truncaite L, Klausa V, Zajančkauskaite A, Meškys R. 2012. Genome of *Klebsiella* sp.-infecting bacteriophage vB_KleM_RaK2. *Journal of Virology* 86:5406. DOI: 10.1128/JVI.00347-12.

Singh L, Cariappa MP, Kaur M. 2016. *Klebsiella oxytoca*: An emerging pathogen? *Medical Journal, Armed Forces India* 72:S59–S61. DOI: 10.1016/j.mjafi.2016.05.002.

Singla S, Harjai K, Katare OP, Chhibber S. 2015. Bacteriophage-loaded nanostructured lipid carrier: improved pharmacokinetics mediates effective resolution of *Klebsiella pneumoniae*-induced lobar pneumonia. *The Journal of Infectious Diseases* 212:325–334. DOI: 10.1093/infdis/jiv029.

Siringan P, Connerton PL, Cummings NJ, Connerton IF. 2014. Alternative bacteriophage life cycles: the carrier state of *Campylobacter jejuni*. *Open Biology* 4:130200. DOI: 10.1098/rsob.130200.

Solovieva EV, Myakinina VP, Kislichkina AA, Krasilnikova VM, Verevkin VV, Mochalov VV, Lev AI, Fursova NK, Volozhantsev NV. 2018. Comparative genome analysis of novel podoviruses lytic for hypermucoviscous *Klebsiella pneumoniae* of K1, K2, and K57 capsular types. *Virus Research* 243:10–18. DOI: 10.1016/j.virusres.2017.09.026.
Strach E, Lurz R, Beutin L. 2001. Characterization of a Shiga toxin-encoding temperate bacteriophage of *Shigella sonnei*. *Infection and Immunity* 69:7588–7595. DOI: 10.1128/IAI.69.12.7588-7595.2001.

Struve C, Bojer M, Krogfelt KA. 2008. Characterization of *Klebsiella pneumoniae* type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence. *Infection and Immunity* 76:4055–4065. DOI: 10.1128/IAI.00494-08.

Struve C, Bojer M, Krogfelt KA. 2009. Identification of a conserved chromosomal region encoding *Klebsiella pneumoniae* type 1 and type 3 fimbriae and assessment of the role of fimbriae in pathogenicity. *Infection and Immunity* 77:5016–5024. DOI: 10.1128/IAI.00585-09.

Tabassum R, Shafique M, Khawaja KA, Alvi IA, Rehman Y, Sheik CS, Abbas Z, Rehman SU. 2018. Complete genome analysis of a *Siphoviridae* phage TSK1 showing biofilm removal potential against *Klebsiella pneumoniae*. *Scientific Reports* 8:17904. DOI: 10.1038/s41598-018-36229-y.

Taha OA, Connerton PL, Connerton IF, El-Shibiny A. 2018. Bacteriophage ZCKP1: A potential treatment for *Klebsiella pneumoniae* isolated from diabetic foot patients. *Frontiers in Microbiology* 9:2127. DOI: 10.3389/fmicb.2018.02127.

Teng T, Li Q, Liu Z, Li X, Liu Z, Liu H, Liu F, Xie L, Wang H, Zhang L, Wu D, Chen M, Li Y, Ji A. 2019. Characterization and genome analysis of novel *Klebsiella* phage Henu1 with lytic activity against clinical strains of *Klebsiella pneumoniae*. *Archives of Virology*. DOI: 10.1007/s00705-019-04321-x.

Thiry D, Passet V, Danis-Wlodarczyk K, Lood C, Wagemans J, De Sordi L, van Noort V, Dufour N, Debarbieux L, Mainil JG, Brisse S, Lavigne R. 2019. New bacteriophages against
emerging lineages ST23 and ST258 of *Klebsiella pneumoniae* and efficacy assessment in *Galleria mellonella* larvae. *Viruses* 11. DOI: 10.3390/v11050411.

Tumbarello M, Spanu T, Sanguinetti M, Citton R, Montuori E, Leone F, Fadda G, Cauda R. 2006. Bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae*: risk factors, molecular epidemiology, and clinical outcome. *Antimicrobial Agents and Chemotherapy* 50:498–504. DOI: 10.1128/AAC.50.2.498-504.2006.

Tuon FF, Kruger M, Terreri M, Penteado-Filho SR, Gortz L. 2011. *Klebsiella* ESBL bacteremia-mortality and risk factors. *The Brazilian Journal of Infectious Diseases: An Official Publication of the Brazilian Society of Infectious Diseases* 15:594–598.

Twort FW. 1915. An investigation on the nature of ultra-microscopic viruses. *The Lancet* 186:1241–1243. DOI: 10.1016/S0140-6736(01)20383-3.

Valenza G, Nickel S, Pfeifer Y, Eller C, Krupa E, Lehner-Reindl V, Höller C. 2014. Extended-spectrum-β-lactamase-producing *Escherichia coli* as intestinal colonizers in the German community. *Antimicrobial Agents and Chemotherapy* 58:1228–1230. DOI: 10.1128/AAC.01993-13.

Verma V, Harjai K, Chhibber S. 2009a. Characterization of a T7-like lytic bacteriophage of *Klebsiella pneumoniae* B5055: a potential therapeutic agent. *Current Microbiology* 59:274–281. DOI: 10.1007/s00284-009-9430-y.

Verma V, Harjai K, Chhibber S. 2009b. Restricting ciprofloxacin-induced resistant variant formation in biofilm of *Klebsiella pneumoniae* B5055 by complementary bacteriophage treatment. *The Journal of Antimicrobial Chemotherapy* 64:1212–1218. DOI: 10.1093/jac/dkp360.
Villa L, Feudi C, Fortini D, Brisse S, Passet V, Bonura C, Endimiani A, Mammina C, Ocampo AM, Jimenez JN, Doumith M, Woodford N, Hopkins K, Carattoli A. 2017. Diversity, virulence, and antimicrobial resistance of the KPC-producing *Klebsiella pneumoniae* ST307 clone. *Microbial Genomics* 3:e000110. DOI: 10.1099/mgen.0.000110.

Vinodkumar CS, Neelagund YF, Kalsurmath S. 2005. Bacteriophage in the treatment of experimental septicemic mice from a clinical isolate of multidrug resistant *Klebsiella pneumoniae*. *The Journal of Communicable Diseases* 37:18–29.

Volozhantsev NV, Myakinina VP, Popova AV, Kislichkina AA, Komisarova EV, Knyazeva AI, Krasilnikova VM, Fursova NK, Svetoch EA. 2016. Complete genome sequence of novel T7-like virus vB_KpnP_KpV289 with lytic activity against *Klebsiella pneumoniae*. *Archives of Virology* 161:499–501. DOI: 10.1007/s00705-015-2680-z.

Wang C, Li P, Niu W, Yuan X, Liu H, Huang Y, An X, Fan H, Zhangxiang L, Mi L, Zheng J, Liu Y, Tong Y, Mi Z, Bai C. 2019. Protective and therapeutic application of the depolymerase derived from a novel KN1 genotype of *Klebsiella pneumoniae* bacteriophage in mice. *Research in Microbiology* 170:156–164. DOI: 10.1016/j.resmic.2019.01.003.

Wittebole X, De Roock S, Opal SM. 2014. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence* 5:226–235. DOI: 10.4161/viru.25991.

World Health Organization. 2018. Global antimicrobial resistance surveillance system (GLASS) report. Early implementation 2017-2018.

Wright A, Hawkins CH, Anggård EE, Harper DR. 2009. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clinical otolaryngology: official journal of ENT-
UK; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery 34:349–357. DOI: 10.1111/j.1749-4486.2009.01973.x.

Wu L-T, Chang S-Y, Yen M-R, Yang T-C, Tseng Y-H. 2007. Characterization of extended-host-range pseudo-T-even bacteriophage Kpp95 isolated on Klebsiella pneumoniae. Applied and Environmental Microbiology 73:2532–2540. DOI: 10.1128/AEM.02113-06.

Wyres KL, Holt KE. 2018. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Current Opinion in Microbiology 45:131–139. DOI: 10.1016/j.mib.2018.04.004.

Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R, Thomson NR, Holt KE. 2016. Identification of Klebsiella capsule synthesis loci from whole genome data. Microbial Genomics 2:e000102. DOI: 10.1099/mgen.0.000102.

Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC. 2001. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrobial Agents and Chemotherapy 45:1151–1161. DOI: 10.1128/AAC.45.4.1151-1161.2001.

Yosef I, Goren MG, Globus R, Molshanski-Mor S, Qimron U. 2017. Extending the host range of bacteriophage particles for DNA transduction. Molecular Cell 66:721-728.e3. DOI: 10.1016/j.molcel.2017.04.025.

Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Yamaguchi K. 2000. Role of bacterial capsule in local and systemic inflammatory responses of mice during pulmonary infection with Klebsiella pneumoniae. Journal of Medical Microbiology 49:1003–1010. DOI: 10.1099/0022-1317-49-11-1003.
Yu J, Tan K, Rong Z, Wang Y, Chen Z, Zhu X, Wu L, Tan L, Xiong W, Sun Z, Chen L. 2016. Nosocomial outbreak of KPC-2- and NDM-1-producing Klebsiella pneumoniae in a neonatal ward: a retrospective study. *BMC Infectious Diseases* 16:563. DOI: 10.1186/s12879-016-1870-y.

Zollner-Schwetz I, Högenauer C, Joainig M, Weberhofer P, Gorkiewicz G, Valentin T, Hinterleitner TA, Krause R. 2008. Role of Klebsiella oxytoca in antibiotic-associated diarrhea. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 47:e74-78. DOI: 10.1086/592074.
| Phage         | Family          | RefSeq/GenBank accession no. | Genome size (bp) | Source                | Reference                          |
|--------------|-----------------|------------------------------|------------------|-----------------------|------------------------------------|
| vB_KpnM_KpV477 | Myoviridae      | NC_031087                    | 168272           | Clinical sample       | Komisarova et al. (2017)           |
| ZCKP1        | Myoviridae      | MH525123                     | 159925           | Freshwater            | Taha et al. (2018)                 |
| KP15         | Myoviridae      | NC_014036                    | 174436 Irrigated fields | Kęsik-Szeloch et al. (2013) |
| JD001        | Myoviridae      | NC_020204                    | 48814            | Seawater              | Cui et al. (2012)                  |
| 0507-KN2-1   | Myoviridae      | NC_023343                    | 159991           | Sewage                | Hus et al. (2013)                  |
| Matisse      | Myoviridae      | NC_024750                    | 176081           | Sewage                | Provazek et al. (2015)             |
| Miros        | Myoviridae      | KE001919                     | 176055           | Sewage                | Miyakis et al. (2015)              |
| PKO111       | Myoviridae      | NC_031095                    | 168758           | Sewage                | Park et al. (2017)                 |
| PMBT1        | Myoviridae      | LT307058                     | 175206           | Sewage                | Koberg et al. (2017)               |
| vB_KpnM_KB57  | Myoviridae      | NC_028659                    | 142987           | Sewage                | Volodosiantsev et al. *            |
| vB_Kpn_F48   | Myoviridae      | MG746602                     | 170784           | Sewage                | Ciacci et al. (2018)               |
| GH_K2        | Myoviridae      | Not Available Unknown        | Sewage           | Ga et al. (2012)      |                                    |
| Kpn1         | Myoviridae      | Not Available Unknown        | Sewage           | Chakma, Katare & Chhibber (2016) |
| Kpn2         | Myoviridae      | Not Available Unknown        | Sewage           | Chakma, Katare & Chhibber (2016) |
| Kpn3         | Myoviridae      | Not Available Unknown        | Sewage           | Chakma, Katare & Chhibber (2016) |
| Kpn4         | Myoviridae      | Not Available Unknown        | Sewage           | Chakma, Katare & Chhibber (2016) |
| vB_KpnM_BIS47 | Myoviridae      | NC_026868                    | 166313           | Unknown Fan et al.*   |
| vB_KleM-RaK2 | Myoviridae      | NC_019526                    | 345809           | Unknown Simölinnäs et al. (2012) |
| Mineola      | Myoviridae      | MH333064                     | 166130           | Unknown Böeckman et al.* |
| May          | Myoviridae      | MG428891                     | 159631           | Unknown Nguyen et al.* |
| Menilow      | Myoviridae      | MG428900                     | 157281           | Unknown Newkirk et al.* |
| vB_KpnM_KpV79 | Myoviridae      | MF663761                     | 47760            | Unknown Komisarova et al.* |
| vB_KpnM_KpV52 | Myoviridae      | KX237516                     | 47405            | Unknown Komisarova et al.* |
| 1611E-K2-1   | Myoviridae      | MG197981                     | 47797            | Unknown Lin et al.*    |
| KP179        | Myoviridae      | MH729874                     | 162030           | Unknown Kozlova et al.* |
| KP1          | Myoviridae      | MG751100                     | 167989           | Unknown Kim*          |
| 3 LV-2017    | Myoviridae      | KY271397                     | 35100            | Unknown Villa et al. (2017) |
| 4 LV-2017    | Myoviridae      | KY271398                     | 33540            | Unknown Villa et al. (2017) |
| Kpn112       | Myoviridae      | KJ021043                     | 35560            | Unknown Chandekar et al.* |
| K64-1        | Myoviridae      | NC_027799                    | 346002           | Untreated water (Pan et al. (2015)) |
| KP15         | Myoviridae      | KY000080                     | 167034           | Wastewater Alshkin et al. (2016) |
| KP27         | Myoviridae      | NC_020080                    | 174413           | Wastewater plant Kęsik-Szeloch et al. (2013) |
| KP34         | Podoviridae     | NC_013649                    | 43809            | Roadside ditch Kęsik-Szeloch et al. (2013) |
| P13          | Podoviridae     | Not Available Unknown        | 45976            | Sewage Shang et al. (2015) |
| vB_KpnP_KpV475 | Podoviridae   | NC_031025                    | 42201            | Clinical sample Solovieva et al. (2018) |
| vB_KpnP_KpV74 | Podoviridae     | KY385423                     | 44094            | Clinical sample Solovieva et al. (2018) |
| vB_KpnP_KpV48 | Podoviridae     | KX237516                     | 44623            | Clinical sample Solovieva et al. (2018) |
| KP12         | Podoviridae     | NC_013647                    | 41119            | Roadside ditch Kęsik-Szeloch et al. (2013) |
| Kpn5         | Podoviridae     | Not Available Unknown        | 45976            | Sewage Shang et al. (2015) |
| Kpn12        | Podoviridae     | Not Available Unknown        | 24800            | Sewage Kumari, Harji & Chhibber (2010a) |
| Kpn13        | Podoviridae     | Not Available Unknown        | 24000            | Sewage Kumari, Harji & Chhibber (2010a) |
| Kpn17        | Podoviridae     | Not Available Unknown        | 24000            | Sewage Kumari, Harji & Chhibber (2010a) |
| Kpn32        | Podoviridae     | Not Available Unknown        | 24000            | Sewage Kumari, Harji & Chhibber (2010a) |
| phiNKS       | Podoviridae     | Not Available Unknown        | 29000            | Sewage Hung et al. (2011) |
| Phage SS     | Podoviridae     | Not Available Unknown        | Sewage Chhibber, Kaur & Kumari (2008) |
| Hemu1        | Podoviridae     | MK203841.1                   | 40352            | Sewage Teng et al. (2019) |
| vB_KpnP_BIS33 | Podoviridae     | KY652725                     | 41697            | Sewage plant Labudda et al. (2017) |
| vB_KpnP_BIS3  | Podoviridae     | KY652725                     | 41335            | Sewage plant Labudda et al. (2017) |
| vB_KpnP_PRA33 | Podoviridae     | KY652723                     | 40605            | Sewage plant Labudda et al. (2017) |
| F19          | Podoviridae     | NC_023567                    | 43766            | Unknown Chen et al.*  |
| K11          | Podoviridae     | NC_011043                    | 41181            | Unknown Savalia et al.* |
| Pylus        | Podoviridae     | MIB899585                    | 70408            | Unknown Powell et al.* |
| vB_KpnP_PME121 | Podoviridae    | MHS85638                     | 39906            | Unknown Wang et al. (2019) |
| SH-Kp 152234 | Podoviridae     | KY450753                     | 40578            | Unknown Zhi et al.*   |
| KP8          | Podoviridae     | MG922974                     | 73679            | Unknown Bokovaya et al.* |
| SH-Kp 152410 | Podoviridae     | MG035588                     | 40945            | Unknown Xu et al.*    |
| KP-Rus2015   | Podoviridae     | KX856662                     | 43557            | Unknown Mienia et al.* |
| KN4-1        | Podoviridae     | LC413194                     | 41219            | Unknown Pan et al. (2019) |
| KN1-3        | Podoviridae     | LC413194                     | 41059            | Unknown Pan et al. (2019) |
| KN1-1        | Podoviridae     | LC413193                     | 40236            | Unknown Pan et al. (2019) |
| lipskk3      | Podoviridae     | MK134560                     | 40539            | Unknown Shi et al.*   |
| Phage     | Family          | RefSeq/GenBank accession no. | Genome size (bp) | Source          | Reference          |
|-----------|-----------------|------------------------------|------------------|-----------------|--------------------|
| phiKpS2   | Podoviridae     | KX587949                     | 44024            | Unknown         | Shen et al. (2018) |
| myPSH1235 | Podoviridae     | MG972768                     | 45135            | Unknown         | Manohar, Nachimuthu & Lopes (2018) |
| 2044-307w | Podoviridae     | MF285615                     | 40048            | Unknown         | Zhao*              |
| 6 LV-2017 | Podoviridae     | KY271400                     | 19260            | Unknown         | Villa et al. (2017) |
| vB_KnP5IME205 | Podoviridae | KU183906                     | 41310            | Unknown         | Bai et al.*        |
| vB_Kp.5   | Podoviridae     | Not Available                | Unknown          | Unknown         | Karumidze et al. (2013) |
| vB_Kp.6   | Podoviridae     | Not Available                | Unknown          | Unknown         | Karumidze et al. (2013) |
| vB_KnP_KP289 | Podoviridae | NC_028977                    | 41054            | Untreated sewage| Volozhantsv et al. (2016) |
| NUH-K2044 | Podoviridae     | NC_025418                     | 43871            | Untreated water | Lin et al. (2014) |
| K5        | Podoviridae     | NC_028800                     | 41698            | Wastewater      | Schneider et al.*  |
| phiBO1E   | Podoviridae     | KM576124                     | 43865            | Wastewater      | D’Andrea et al. (2017) |
| KPV811    | Podoviridae     | KY000081                     | 42641            | Wastewater      | Aleshkin et al. (2016) |
| vB_KpI    | Podoviridae     | NC_026688                     | 40114            | Wastewater plant| Alvez et al.*      |
| vB_Kp2    | Podoviridae     | NC_026664                     | 43963            | Wastewater plant| Alvez et al.*      |
| vB_KnP5SU503 | Podoviridae | NC_026816                     | 43099            | Wastewater plant| Eriksson et al. (2015) |
| vB_KnP5SU552A | Podoviridae | NC_028870                     | 43595            | Wastewater plant| Eriksson et al. (2015) |
| AltoGao   | Podoviridae     | MF612071                     | 43012            | Wastewater plant| Gao, Linden & Nelson (2017) |
| SoproGao  | Podoviridae     | MF612073                     | 61644            | Wastewater plant| Gao, Linden & Nelson (2017) |
| KLPN1     | Siphoviridae    | NC_028760                     | 49057            | Human caecum    | Hoyles et al. (2015) |
| KPp5665-2 | Siphoviridae    | MF695815                     | 39241            | Mastitis milk   | Carl et al. (2017)  |
| 1513      | Siphoviridae    | NC_028786                     | 49462            | Sewage          | Cao et al. (2015)  |
| PKP126    | Siphoviridae    | NC_031053                     | 50934            | Sewage          | Park et al. (2017) |
| Sushi     | Siphoviridae    | NC_028774                     | 48754            | Sewage          | Nguyen et al. (2015) |
| vB_KnP5_KpV522 | Siphoviridae | KX237515                     | 51099            | Sewage          | Komisarova et al.* |
| TK1       | Siphoviridae    | MH888453                     | 49861            | Sewage          | Tabassum et al. (2018) |
| IME207    | Siphoviridae    | NC_031924                     | 47564            | Sewage          | Liu et al. (2016)  |
| vB_Knp5s_GH-K3 | Siphoviridae | MB645311.1                    | 49427            | Sewage          | Gu et al. (2012), Cai et al. (2019) |
| 48ST307   | Siphoviridae    | KY271402                     | 52338            | Unknown         | Villa et al. (2017) |
| KPN N95   | Siphoviridae    | MG835858                     | 59214            | Unknown         | Jeon et al.*        |
| YM1601-N133_KPN_BP | Siphoviridae | MF415413                     | 59100            | Unknown         | Jeon et al.*        |
| YM15101-N53_KPN_BP | Siphoviridae | MF415412                     | 49090            | Unknown         | Jeon et al.*        |
| KPN N441  | Siphoviridae    | MF415411                     | 59087            | Unknown         | Jeon et al.*        |
| KPN U2874 | Siphoviridae    | MF415410                     | 59100            | Unknown         | Jeon et al.*        |
| Seifer    | Siphoviridae    | MH817999                     | 58197            | Unknown         | Salazar et al.*    |
| SH-Kp 160016 | Siphoviridae | KY575286                     | 49710            | Unknown         | Zhi et al.*         |
| Sugarland | Siphoviridae    | MG459987                     | 111103           | Unknown         | Eriksson et al.*    |
| vB_Kp5-BME260 | Siphoviridae | KX485404                     | 123490           | Unknown         | Xing et al.*        |
| NJK15     | Siphoviridae    | MH633487                     | 40468            | Unknown         | Hao et al.*         |
| NJ53      | Siphoviridae    | MH633486                     | 49387            | Unknown         | Hao et al.*         |
| NJ52      | Siphoviridae    | MH633485                     | 50132            | Unknown         | Hao et al.*         |
| TAH8      | Siphoviridae    | MB633484                     | 49343            | Unknown         | Hao et al.*         |
| phiK02    | Siphoviridae    | NC_005857                     | 51601            | Unknown         | Casjens et al. (2004) |
| NJ51      | Siphoviridae    | MB445453                     | 49292            | Unknown         | Zhu et al.*         |
| JY917     | Siphoviridae    | MG894052                     | 37655            | Unknown         | Hao et al.*         |
| vB_Knp5s_BME279 | Siphoviridae | MF614100                     | 42518            | Unknown         | Zhao et al.*        |
| 1LV-2017  | Siphoviridae    | KY271401                     | 29880            | Unknown         | Villa et al. (2017) |
| 2LV-2017  | Siphoviridae    | KY271396                     | 44400            | Unknown         | Villa et al. (2017) |
| 2b LV-2017 | Siphoviridae    | KY271395                     | 44279            | Unknown         | Villa et al. (2017) |
| 5LV-2017  | Siphoviridae    | KY271399                     | 47014            | Unknown         | Villa et al. (2017) |
| vB_Kp3    | Siphoviridae    | KT367887                     | 48493            | Unknown         | Alvez et al.*        |
| phiKp5-lyy15 | Siphoviridae | Not Available                | Unknown          | Unknown         | Lu et al. (2015)    |
| vB_Kp.1   | Siphoviridae    | Not Available                | Unknown          | Unknown         | Karumidze et al. (2013) |
| vB_Kp.3   | Siphoviridae    | Not Available                | Unknown          | Unknown         | Karumidze et al. (2013) |
| vB_Kp.4   | Siphoviridae    | Not Available                | Unknown          | Unknown         | Karumidze et al. (2013) |
| KOX1      | Siphoviridae    | KY780482                     | 50526            | Wastewater      | Brown et al. (2017) |
| phase Z   | Siphoviridae    | Not Available                | Unknown          | Unknown         | Jamal et al. (2015) |
| KP56      | Siphoviridae    | NC_029099                     | 49818            | Wastewater plant| Kęsik-Szeloch et al. (2013) |
| MezoGao   | Siphoviridae    | MF612072                     | 49077            | Wastewater plant| Gao, Linden & Nelson (2017) |
| GH-K1     | Unknown         | Not Available                | Unknown          | Sewage          | Gu et al. (2012)    |
| PBK05     | Unknown         | Not Available                | 30240            | Unknown         | Oh et al. (2019)    |
| Kpp95     | Unknown         | Not Available                | ~175000          | Unknown         | Wu et al. (2007)    |

*No paper associated with the RefSeq/GenBank record(s).
Figure 1. Phylogenetic placement of dsDNA *Klebsiella* phages within the order *Caudovirales.* Placement of 109 genomes (Table 1) within ViPTree version 1.9 (Nishimura et al., 2017) was checked on 6 August 2019. Those sequences (n = 84) that clustered together in groups of three or more were analysed with their nearest phylogenetic relatives using ViPTreeGen v1.1.2 (--ncpus 8 --method ‘bioinj’) and a non-redundant set of genomes (fasta file of input sequences and newick-format file available in Supplementary Material) to generate the tree shown (annotated using https://itol.embl.de and Adobe Illustrator). Taxonomy of phages was checked via https://talk.ictvonline.org/taxonomy/ (release 2018b); accepted species names are written in italics. A phylogenetic tree showing the placement of the remaining 25 *Klebsiella* genomes within ViPTree version 1.9 is available in Supplementary Material.