Isolation and chacterisation of *Klebsiella* phages for phage therapy

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Abstract
Klebsiella is a clinically important pathogen causing a variety of antimicrobial resistant infections, in both community and nosocomial settings, particularly pneumonia, urinary tract infection and septicaemia. We report the successful isolation and characterisation of 30 diverse *Klebsiella*-infecting phages. The isolated phages are diverse, spanning six different phage families and nine genera. These phages comprise of both lysogenic and lytic lifestyles. Individual *Klebsiella* phage isolates infected 11 of 18 different *Klebsiella* capsule types across all six *Klebsiella* species tested. Our *Klebsiella*-infecting lytic phages are suitable for phage therapy, based on the criteria that they encode no known toxin or antimicrobial resistance genes. However, none of the characterised phages were able to suppress the growth of *Klebsiella* for more than 7 hours. This indicates that for successful phage therapy, a mixed cocktail of multiple phages is necessary to treat *Klebsiella* infections.

Background
Bacteria of the *Klebsiella* genus are able to cause a variety of infections in both community and nosocomial settings including pneumonia, urinary tract infection, septicaemia, wound infection, and infections in vulnerable populations including neonates and intensive care patients [1]. *Klebsiella* is one of the most numerous secondary infection agents in COVID-19 patients, particularly those who had undergone invasive ventilation [2-4]. Additionally, sub-clinical carriage of *Klebsiella* is linked to cardiovascular disease risk [5, 6]. *Klebsiella pneumoniae* is the most reported problematic pathogen from the genus, which has given rise to hypervirulent clones with extended virulence factors, making them more deadly. Other species of *Klebsiella*, such as *K. oxytoca* [7] and *K. variicola* [8], are emerging pathogens, causing infections in immunocompromised patients. Whilst the arsenal of virulence factors employed by *Klebsiella* make them efficient pathogens, it is the high prevalence of antimicrobial resistance mechanisms that complicates treatment and leads to high mortality [9].

Antibiotic or antimicrobial resistance represents a current threat to global health and security, fueled by our intensive use of antibiotics in both medicine and agriculture. Resistance prevalence in *Klebsiella* has increased exponentially to most available antimicrobial drugs, and there have been cases of pan-resistant *Klebsiella* described [10, 11]. This poses a difficulty in treating these infections with existing antibiotics that are available in clinical settings [12]. *Klebsiella* is termed an ESKAPE pathogen, on the World Health Organisation priority pathogens list [13]. The ability of the *Enterobacteriaceae* to gain and transfer an increasing number of antimicrobial-resistance genes, particularly in health-care settings, represents a critical threat to human health [14]. The economic cost of *Klebsiella* outbreaks in individual health-care settings are high; in 2015 a Dutch hospital estimated the cost of an outbreak of multidrug-resistant (MDR) *K. pneumoniae,* infecting 29 patients, was $804,263 [15]. Alongside the economic consequences of these MDR *Klebsiella* infections, there is increased risk of mortality [15, 16]. One potential alternative treatment to antibiotics are bacteriophages.

Bacteriophages are natural killers of bacteria and phage therapy is emerging as a potential weapon against MDR bacterial infections [17, 18]. The discovery of phage candidates for therapeutic use has been accelerated by modern sequencing technologies, facilitating viral metagenomics, in which numerous phage populations can be assembled from environmental metagenomes or predicted from bacterial genomes as prophages [19]. These *in-silico* approaches provide a wealth of bioinformatic data, but do not yield virions for the wet-lab analyses and further development required for phage therapy. The viability of phages for clinical use faces a number of challenges, including potential adverse reactions in patients, inactivation by the immune system, mode of delivery and even gaining access to purified active phages in a timely manner. Ultimately, these hurdles can be overcome with detailed understanding and characterisation of prospective phage genomics, structure and function.

Recent work has shown that specific strains of *Klebsiella pneumoniae* correlate with inflammatory bowel disease [20] and cardiovascular disease [21] increasing the need for prophylactic control of *Klebsiella* outside of life-threatening infections. However, the therapeutic use of phages demands an understanding of their potential side-effects through characterisation. We need to be aware of any negative aspects of phage biology that can cause problems during phage therapy, such as toxigenic conversion, which occurs when a temperate phage encodes toxin genes [22].

In this paper we describe a selection of bacteriophage isolated against *Klebsiella* spp. from culture collections, clinical, and environmental isolates. Phages were isolated from a number of international environmental samples including rivers, ponds, estuaries, canals, slurry, and sewage. The newly isolated phages were characterised using a combination of traditional and genomic methodologies to understand their infection cycle, host range, and susceptibility to heat and chloroform. We present bacteriophages with siphovirus, myovirus, and podovirus morphologies, spanning six different phage families and nine genera, of which the majority are lytic. A number of these phages have potential use in phage therapy, biotechnology or synthetic biology applications.

Materials and Methods

Bacterial Strains and Culture Conditions

The *Klebsiella* strains used in this work are listed in table 1. All culturing in liquid media was performed with shaking (150 rpm) at 37 °C. All culturing was carried out in Nutrient broth or Lysogeny broth as indicated, with the addition of 5 mM $CaCl_2$ and 5 mM $MgCl_2$ when culturing phages.

Phage Isolation

Phages were isolated from water samples from various sources, listed in table 2. Phages were named using the ICTV binomial system of viral nomenclature [23]. The water samples were filtered through 0.2 µm syringe filters to remove debris and bacteria. Phages were isolated by enrichment: 2.5 mL of filtered water sample was added to 2.5 ml nutrient broth, containing 5 mM $CaCl_2$ and 5 mM $MgCl_2$, and inoculated with 50 µL of *Klebsiella* grown overnight. The enrichment culture was then incubated overnight at 37 °C. This overnight enrichment was centrifuged and the supernatant filtered through a 0.2 µm filter to remove cells. This filtrate was serially diluted down to 10^{-11} in nutrient broth and used in an overlay agar plaque assay. Briefly, plaque assays involved mixing 50 µl of each serial dilution with 0.5 mL of a single *Klebsiella* strain in the logarithmic growth phase (OD_{600} 0.2) and incubated at room temperature for 5 min. To each serial dilution/cell mix, 2.5 mL of cooled, molten Nutrient agar (0.4 % weight/volume) was added and mixed by swirling. The molten agar mix was poured onto 1 % Nutrient agar plates. All overlay agar plates were allowed to set, then inverted and incubated overnight at 37 °C. From the plaque assay plates single plaques were identified, picked using a pipette tip, mixed with 50 µL of nutrient broth, and filtered through a 0.22 µm spin filter. The filtrate underwent two further rounds of plaque assay to ensure that plaque isolates were the result of a single clonal phage.

DNA Extraction

DNA was extracted using the Phenol Chloroform Method [24]. Briefly, phage stocks were concentrated using a protein column with a 30 kDa upper size limit. Following concentration, 750 µL of phage was added to 15 µL DNase I and 80 µL 10X DNase Buffer. This was then incubated at 37 °C for 20 min, before addition of 20 mg/mL Proteinase K which was then incubated at 60°C for 30 min and left at room temperature overnight. Following enzyme treatments, an equal volume of phenol was added, mixed by inversion and centrifuged at 13,000 xg, at 4 °C for 10 min. The upper aqueous layer was then removed and an equal volume of phenol-chloroform (1:1) added, and the centrifugation was repeated. The upper aqueous layer was again removed and added to an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) before repeating the centrifugation. The upper aqueous layer was retained and mixed with 0.1 volume of 7.5 M Ammonium acetate and 2 volumes of ice-cold ethanol. The samples were then incubated at -20 °C overnight to allow the DNA to precipitate. After precipitation, the DNA was pelleted by centrifugation (16,000 xg, 10 min, room temperature). The supernatant was removed and the pellet twice rinsed with 1 mL 70 % ethanol and then centrifuged again to pellet after rinsing. All ethanol was removed and the DNA pellet was allowed to air-dry to remove all ethanol. The DNA was finally suspended in 50 µL of molecular grade water.

For bacteriophage which had a high background level of protein contamination from the host strain, Norgen Phage DNA Isolation Kit was used following the manufacturer's instructions. Briefly, the phage sample is mixed with lysis buffer and heated to 65 °C for 15 min. The sample is mixed with isopropanol and spun through a column to bind the DNA to the column membrane. Three wash steps are then followed using the provided Wash Solution, followed by a spin step to remove ethanol from the spin column. The DNA is then eluted from the spin column in Elution Buffer. To assess the quantity and quality of the isolated DNA for sequencing, both a spectrophotometer-based method and Qubit were used.

Sequencing

Sequencing was performed by MicrobesNG (Birmingham, UK), briefly; genomic DNA libraries were prepared using Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with these modifications: 2 ng of DNA were used as the input, and a PCR elongation time of 1 min. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system. Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina, on a Roche light cycler 96 qPCR machine. Libraries were sequenced on the Illumina HiSeq using a 250 bp paired end protocol.

Bioinformatics

Contig and genome assembly was also carried out by MicrobesNG; reads were trimmed with Trimmomatic 0.30, sliding window quality cutoff of Q15 [25], SPAdes version 3.7 was used for *de novo* assembly [26]. Host and phage genomes were annotated using Prokka [27], for the phages a custom database including all available phage genomes extracted from Genbank was used. The capsule types of the *Klebsiella* strain genomes were predicted using Kaptive [28].

To determine the taxonomy of our *Klebsiella* phage isolates, the genomes were added to VIPtree, an on-line tool to place them on a genome-wide proteomic tree of viral sequences, based on tBLASTx similarities [29], to identify the closest sequenced phage genomes in the database. Phage isolate genomes were then subject to the BLASTn and tBLASTn against all NCBI geneomes. The average nucleotide identity (ANI) of the closest genomes identified by VIPtree and BLAST were compared to our phage genomes, using orthoANI (https://www.ezbiocloud.net/tools/ani) [30]. Genomes with an ANI >95% were designated as the same species [31]. To determine genus-level clustering of phage genomes, a shared protein network analysis was performed using vConTACT2 [32] with the custom phage database (http://millardlab.org/bioinformatics/bacteriophage-genomes/phage-genomes-may2020/). The resulting network graph was visualised and annotated within Cytoscape (version 3.8.0) [33]. Finally, multiple sequence alignments were performed using MAFFT [34] on the DNA polymerase, large terminase subunit and major capsid proteins of our *Klebsiella* phage isolates lacking an assigned genera and the most closely related phages, identified using the above methods. Phylogenetic trees were drawn with RaxML [35] using the GAMMA model of heterogeneity and the maximum-likelihood method based on the JTT substitution matrix. Bootstrap support values were calculated from 1,000 replicates. Trees were visualised with Figtree (version 1.4.4) (http://tree.bio.ed.ac.uk/software/figtree/).

Putative structural genes from each of the characterised phages in this study were analysed for the presence of predicted depolymerase or enzymatic domains or for features common to previously characterised depolymerase proteins. Each putative open reading frame was analysed by BLASTP (v2.10.0; https://blast.ncbi.nlm.nih.gov/Blast.cgi), Pfam HMMER (v3.3; https://www.ebi.ac.uk/Tools/hmmer/) and HHpred (v33.1; https://toolkit.tuebingen.mpg.de/) using the default settings. Sequences from biochemically characterised depolymerase proteins that target *Klebsiella* spp. (table S1) or the putative depolymerases from our curated collection of phages (table S2) were used for analysis. Sequences were aligned with Muscle (v3.8.31) [36] using SeaView (v4) [37]. Phylogenetic tree construction was performed with MegaX [38] with 500 bootstrap calculations using the LG model. Tree topology searches were performed using a combination of NNI and NJ/BioNJ. The tree was subsequently visualised and annotated using iTOL(v4) [39].

Host Range Testing

Host range was tested by plating 4 dilutions of phage stock on to bacterial lawn in 0.4 % Nutrient agar. Any zone of lysis was noted with the following notations: Forms plaques, clearing with high phage titres, reduced lawn with high phage titres, turbid lawn with neat phage, or no effect.

Plaque Formation and Morphology

Once pure phage stocks had been established, phages were plated using the overlay agar plaque assay above on their isolation host. Plates were incubated overnight at 37 °C to allow plaques to form. Plaque appearance was noted and photographs taken.

Transmission Electron Microscopy

To prepare formvar/carbon-coated copper grids (Agar Scientific Ltd, UK) for transmission electron microscope (TEM), the grids were first glow-discharged for 1 min under vacuum. Following this, 5 µL of phage stock was applied to the grid and allowed to incubate for 1.5 min. The grid was then blotted to remove excess liquid. A drop of 2 % uranyl acetate was then applied to the grid and incubated for 1 min, then blotted off. This was repeated four times. The grid was allowed to air dry after the final blot. Phages were then viewed under a TEM. Images were procured using the JEOL 2100Plus TEM.

The morphology of the phage particles, including size of capsid and tail, were calculated using the ImageJ measure function. A range of three to thirty phage particles were used to calculate phage measurements.

Latency Period

Klebsiella cultures in the exponential growth phase were adjusted using a spectrophotometer to an OD_{600nm} of 0.2 and phage were diluted 1:4. The OD_{600nm} was measured every 5 min for 16 hr. Growth was compared to a positive control culture without phage. The latency period was calculated by measuring the time between phage addition and the culture reducing in optical density relative to the positive control, indicating bacterial cell lysis.

Virulence Index

The virulence Index was calculated based on the protocol described by Storms and others [40]. Briefly, bacterial cultures were grown to exponential phase and then adjusted (as above) to an optical density equivalent to 1×10^8 cfu/mL. In a 96-well plate, phages were serially diluted from 1×10^8 pfu/mL to 10 pfu/mL in 100 µL volumes. The bacterial culture was then added in equal volume to the phage dilution, resulting in multiplicity of infections (MOIs) from 1 to 10^{-7}. The optical density of the 96-well plate was read at 600 nm at 5 min intervals for 18 hours. To calculate virulence indices, the area under the curve was calculated for both the bacterial only control and at each phage MOI, from time of initial infection until the culture exited exponential growth stage. The virulence index at each MOI was calculated following the method described in the paper, using RStudio (version 1.1.463) [40]. The MV50 was also calculated, which indicated the MOI at which the phage reached a virulence index of 0.5 (half the theoretical maximum virulence: instantaneous complete killing).

Heat and Chloroform Sensitivity

To investigate heat sensitivity, phage stocks were incubated at 60 °C for 10 min. To calculate chloroform sensitivity, phage stocks were mixed 1:1 with chloroform and incubated for 30 min whilst shaking. Phage stocks were then diluted and enumerated for both heat shock and chloroform sensitivity using the agar overlay method (see *Plaque Formation and Morphology*, above), compared to a positive control to calculate the reduction in plaque forming units.

Results

Host Strains

Klebsiella spp. genomes were sequenced and their capsule types identified. These are listed alongside the source of the strain and used as an isolation strain in table 2. We used 24 strains for host testing, of these, 11 were used as isolation strains. Strains represented six species: *K. pneumoniae*, *K. oxytoca, K. quasipneumoniae, K. variicola*, *K. michagenesis* and *K. aerogenes*.

Klebsiella Capsule Types

Kaptive Web capsule typing analysis revealed that the 24 *Klebsiella* strains belonged to 18 different capsule types, *Klebsiella aerogenes* 30053 could not be typed (table 1). Only three of the *Klebsiella* strains had previously been capsule typed, *Klebsiella pneumoniae* strains DSM 30104, DSM 16358 and ATCC 13882. Their capsule types are 3, 4 and 64 respectively, Kaptive Web designated them correctly as KL3, KL4 and KL64. Three *Klebsiella pneumoniae* strains were identified as capsule type KL2, while two *Klebsiella* spp. each typed as O1v1, OL104 and KL14. All other capsule types were unique.

Genome Sequences of Phages

Genome sequencing of the 30 *Klebsiella* phage isolates revealed that these phages had genomes ranging in length from 16,548 to 268,500 bp. Sequence analysis of the smallest phage, KppS-Ant and the sole phage in *Siphoviridae* unclassified genus, contain annotatable phage genes and showed 100 % identity to a small section of the genome of *Klebsiella pneumoniae pneumoniae* DSM 30104. The raw KppS-Ant sequence data contains incomplete fragments of a second phage genome, with high similarity to the *Slopekvirus* genus phages. PROKKA identified core phage structural genes in all the phages such as: capsid, tail tube genes, tail fibres and nucleic acid machinery.

Phage taxonomy

The *Klebsiella* phage genomes represented 9 diverse, distinct genera, as determined by VIPtree (fig. 1) and vConTACT2 (fig. 2), referred to by their genera; *Nongavirus, Siphoviridae* unclassified genera, *Tunavirinae* unclassified, *Iapetusvirus*, *Drulisvirus*, *Sugarlandvirus, Taipeivirus, Slopekvirus* and *Jiaodavirus*. Genome similarities were compared to known phages identified through vConTACT2 (table 3). Phage groups genera *Tunavirinae* unclassified, *Iapetusvirus*, *Drulisvirus*, *Sugarlandvirus* and *Taipeivirus* showed high similarities to previously identified phages and were grouped into existing phage genera (ANI > 95% same species). However, phage groups *Nongavirus* and *Siphoviridae* unclassified genera were highly dissimilar to characterised phage isolates. A cloned DNA sequence of a phage showed > 90% ANI to the *Siphoviridae* unclassified genera.

PhoH was a common feature in 18 of the 30 phages sequenced, phages from the genera *Iapetusvirus*, *Sugarlandvirus, Taipeivirus,* and *Slopekvirus*. Holin and lysin pairs were identified in groups *Nongavirus*, *Tunavirinae* unclassified and *Taipeivirus*, while endolysin and Rz1 spanin complex genes were identified in *Drulisvirus* and *Jiaodavirus*.

Alignments of our *Klebsiella* isolates and the closest identified phages constructed in VIPtree [29] showed varying levels of amino acid sequence identity but high gene synteny between our isolates and known phages (fig. S3-10). There was low identity, but high gene synteny between our isolates from the genera *Tunavirinae* unclassified, *Iapetusvirus, Drulisvirus*, then *Nongavirus* and the least identity in our *Siphoviridae* unclassified genus phage with previously sequenced phages (fig. S3-7). The highest identity and synteny was seen for the genera *Sugarlandvirus, Taipeivirus, Slopekvirus* and *Jiaodavirus* with previously sequenced phages (fig. S8-10). The phylogenetic trees of DNA polymerase (fig. S11), Major capsid protein (fig. S12) and Terminase large subunit (fig. S13) show conserved branching patterns between all our phage isolates and previous sequenced phages, indicating they are closely related and can be classified in the same genera as the closest previously sequenced phages in the trees.

Host Range Testing

Most phages had a host range which extended past their original isolation host. The number of strains infected by each phage is displayed in figure 3, further information on host range including strain and capsule type specificity is found in figure S1. There were two isolated phage genera that only infected their isolation host, these are both comprised of lysogenic phages; *Nongavirus* and *Siphoviridae* unclassified genera. The lytic phage genera *Tunavirinae* unclassified, *Iapetusvirus*, *Drulisvirus*, *Sugarlandvirus* and *Taipeivirus* infected 4-6 *Klebsiella* strains in addition to their isolation hosts. The closely clustered phage genera *Slopekvirus* and *Jiaodavirus* were also lytic and demonstrated the broadest host range for the given bacterial panel, they were able to produce plaques on up to 9 non-host strains and cause clearance or reduction of the lawn of between 3-22 other *Klebsiella* spp.

Phage encoded depolymerases

Sequence analysis revealed that many of our isolated phages encode at least one putative tail-fibre or tail-spike protein that has predicted features found in other characterised depolymerase proteins. These are listed and described in table S2. Homology detection and structural predictions suggested that these proteins may adopt beta-helical structures which is a common protein architecture of tail-spike proteins or depolymerases and often form trimeric appendages at the basal end of the phage tail. Many of these proteins also contained predicted enzymatic domains such as Pectate_lyase_3 and Peptidase_S74 which can also be characteristic of phage encoded depolymerases. Some of these enzymatic domains can also be found within other structural proteins of the virion including neck and baseplate proteins. For the analysed phages that did not have a predicted tail-fiber depolymerase protein, the potential enzymatic role of other structural proteins was unclear. Typically, phages with the ability to degrade bacterial surface polysaccharides express one depolymerase-like protein specific for a particular polysaccharide. The phylogeny of the depolymerase-like proteins of our phage isolates is shown in figure 4. Phages vB_KppS-RAW, vB_KppS-Eggy and vB_KppS-Pokey, vB_KpP-Yoda, vB_KqP-Goliath and vB_KpP-Screen appear to encode one predicted depolymerase protein. The putative depolymerases from phages vB_KpP-Yoda, vB_KqP-Goliath and vB_KpP-Screen share high sequence conservation (at least ~95 % identity, 100 % query) to the characterised depolymerase Kpv74_56 from *Klebsiella pneumoniae* phage KpV74, which also infects K2 producing strains (fig. 4). Interestingly, vB_KqM-Eowyn, vB_KqvM-LilBean, vB_KqvM-Bilbo and vB_KqvM-Westerburg each contain multiple putative depolymerase-like proteins (7, 4, 4, 5 respectively; fig. 4). The putative depolymerase-like proteins of vB_KqM-Eowyn show low homology to previously biochemically characterised depolymerase (fig. 4). From our infection assays these phages showed activity towards strains that produced a small subset of K-antigens (vB_KqM-Eowyn – KL16, KL110; vB_KqvM-LilBean – KL35; vB_KqvM-Bilbo – KL35, K2 and vB_KqvM-Westerburg - KL35, KL2, KL3).

Morphology of Phages

The morphology of phage plaques was determined for the *Klebsiella* isolation host, using the agar overlay method. Plaque morphology varied in size and also in the presence or absence of halo. A summary of each group is found in figure 5 and further information on each individual phage can be found in supplementary figure S2. It can be seen that 21 of the 30 phage isolates showed a diffuse halo around their plaques (fig. S1 and S2).

The morphology of phage virions was established using TEM. Images of viral particles from TEM were measured using ImageJ to establish the length of the tail and the width of the capsid. All results are displayed in supplementary table S3, and a summary of these including one image for each isolated phage genera (ongoing) is displayed in figure S2. Of the phages images, 13 are myoviruses, 13 are siphoviruses, and 3 are podoviruses (fig. S2). The largest phage in this study was the *Iapetusvirus* phage KvM-Eowyn, which had a capsid width of 140 nm and tail length of 140 nm, this also had the largest genome at 269 Kbp. The smallest phage was KqP-Goliath, a podovirus, which had a capsid width of 41 nm and tail length of 10 nm and belongs to the *Drulisvirus* genus which had the smallest genomes at 44 Kbp excluding the unimaged prophage KppP-Ant. KppS-Raw, a siphovirus, of the *Nongavirus* genus had a comparable capsid size of 46 nm, but a significantly longer

tail (153 nm) and larger genome of 61 Kbp. The smallest phages in the genus *Drulisvirus* produced the largest plaques and halos, hence the name KqP-Goliath for the smallest phage (table S1, fig. S2).

Phage latency and virulence in Host Strains

The latency period, along with the virulence index, was calculated for each phage in the relevant *Klebsiella* isolation host strain. All data for the two metrics is displayed in Figure 6 along with the latency period of the phage. These virulence assays capture different aspects of infection including the virulence index (VP), which is a quantified measure of the virulence of a phage against a bacterial host on a scale of 0 to 1, with 1 being the most virulent, and the MV50, which is the MOI at which the phage achieves 50 % of its maximum theoretical virulence [40].

For six phages, including prophage KppS-Ant a latency period was not achieved, this is indicative of temperate phages, where the growth of the bacteria is dampened, but there is not the characteristic culture crash. These phages also had a low virulence index, close to 0 (fig. 6). Of the phages that did have a latency period, the median time of latency was ~70 minutes, which ranged from approximately 15 minutes to 210 minutes. There was no correlation between latency and the virulence measures (data not shown), implying that latency period is not the driving factor of virulence in phage infection.

Sensitivity to Heat and Chloroform

Figure 7 displays the reduction in phage titre (pfu/mL) after heat or chloroform treatment.

The majority of phage within this study were extremely sensitive to chloroform, however there were a few exceptions to this. Phage KaS-Ahsoka, KppS-Eggy, KoM-Liquor, KpM-Mild, KpP-Yoda (2-15 % reduction in titre) were very slightly reduced by chloroform treatment, whereas phage KpP-Screen, KpM-Wobble, KpM-Milk (32-48 % reduction respectively) were medially affected by chloroform. A number of phages were resistant to or the titre was increased by chloroform treatment (presumably from host cells bursting and releasing extra phage). This included phage KaS-Gatomon, KqM-LilBean, KpM-KalD, KpM-SoFaint KqP-Goliath and KoM-Pickle. The observed level of chloroform sensitivity by our phage isolates did not correlate to phage taxonomy.

Sensitivity to heat was also common amongst the described phage, however there were also exceptions to this rule. Phages KppS-Raw, KqM-Westerburg, SoFaint, and KaS-Gatomon (22, 23, 56, 70 % reduction respectively) were more resistant to heat compared to the rest of the phages within this work.

Discussion

We successfully isolated and characterised 30 *Klebsiella*-infecting phages, belonging to nine phylogenetically distinct lineages, using multiple water samples from different environments and a number of different *Klebsiella* spp. Phages were isolated against a panel of *Klebsiella* species, including clinical isolates, culture collection strains from clinical settings and an environmental strain. The clinical strains belong to the species *Klebsiella pnuemoniae, K. oxytoca, K. quasipneumoniae* and *K. aerogenes*, and the environmental species used is *K. variicola.* The *Klebsiella* spp. used span 18 different capsule types.

The phylogenetic analysis revealed that four of the distinct *Klebsiella* phage genera; *Nongavirus, Siphoviridae* unclassified genera, *Tunavirinae* unclassified and *Iapetusvirus*, did not cluster with other *Klebsiella* phages, but phages infecting *Enterobacteria*, unknown hosts, *Escherichia* and *Serratia.* The *Tunavirinae* isolates clustered closely with the recently proposed genus *pSFunavirus* [41]. This demonstrates that our approach has captured previously unseen phage isolate diversity. Isolates identified in four of the remaining phage genera; *Sugarlandvirus, Taipeivirus, Slopekvirus* and *Jiaodavirus* were classified as the same species as previously isolated phages. There was a single exception in *Sugarlandvirus*, the phage KaS-Veronica, which clustered separately in vConTACT2, had an ANI < 95% to our other *Sugarlandvirus* isolates, as well as the published phages Sugarland and vB_Kpn_IME260. Within these phage genera, there are still genetically diverse phages belonging to multiple species. Within the Sugarlandvirus genus phages, the greatest variation appears to be in the tail fibre genes and we identified 6 distinct new species. However, three of the isolated phages isolated on two different *Klebsiella* species are highly similar, belonging to the same species with only a few SNPs difference. Tail fibres genes are frequently rearranged in phages and allow phages to adhere to their bacterial hosts Tail fibres may consist of enzymes to degrade the bacterial cell walls, membranes and capsules [42].

A number of our phage isolates have been identified as previously described species of *Klebsiella* phages; vB_Kpn_IME260, *Demerecviridae, Sugarlandvirus*; *Klebsiella* phage Sugarland, *Demerecviridae, Sugarlandvirus*; *Klebsiella* virus 0507KN21, *Ackermannviridae, Taipeivirus*; *Klebsiella* phage KP15 *Myoviridae, Tevenvirinae, Slopekvirus*; and *Klebsiella* phage JD18, *Myoviridae*, *Tevenvirinae*, *Jiaodavirus*. Within the genus *Sugarlandvirus*, of our phage isolates six are the classified as the same species as previous isolates, while one phage, vB_KaS-Veronica is a new species in the genus *Sugarlandvirus.* Interestingly in the genus *Iapetusvirus*, vB_KvM-Eowyn is the same species as the unverified NCBI entry, Serratia phage KpHz_2. Our phage isolate vB_KvM-Eowyn was isolated from an estuary, while KpHz_2 was isolated from seawater, however very little information is available on KpHz_2, making it difficult to draw further conclusions. Despite many of the phages belonging to the same species they show divergent characteristics in terms of host range and virulence. The alignments of these genera showed high conservation over a large portion of their genomes, and variability in only a few genes (fig. S3-10).

The phage KppS-Ant has a >99 % sequence similarity of to a section of the *Klebsiella pneumoniae pneumoniae* DSM 30104 genome. In addition to the main contig, the raw sequence data contains small fragments of a second lytic phage of the *Jiaodavirus* genus. Prophages can be induced by host bacterial DNA damage [43], our sequence data indicates that the lytic phage may have induced the *K. pneumoniae* prophage.

During our phage isolations multiple different *Klebsiella* strains were combined in the phage enrichment step to increase the chance of isolating broad host range phages [44]. The subsequent phage groups isolated varied in host range, from host strain specific to broader host range, infecting multiple capsule types. The temperate phages in *Nongavirus* and *Siphoviridae* unclassified genera were host specific, while the lytic phages had broader host ranges, but no phages were able to produce plaques on all 24 *Klebsiella* strains in our panel. The broadest host-range phage isolated,

vB_KoM-MeTiny, degraded 79 % *Klebsiella* and produced plaques on 42 % of the *Klebsiella* tested, which included 9 different capsule types. However, host range testing in a single rich media may not be a true representation of host range, our analysis of a smaller subset of these *Klebsiella* phage isolates in different media indicates that *Klebsiella* show variable phage susceptibility in different media [45].

Klebsiella have a protective polysaccharide capsule that provides resistance to phages, antibiotics and protists [46, 47]. This capsule is the outermost layer of the *Klebsiella* cell and acts an important virulence factor [48]. There are currently at least 77 different *Klebsiella* capsule types that are serologically defined [49, 50]. Serological typing is a very specialised technique, therefore we used the whole genome method of capsule typing [28]. The *Klebsiella* panel covered 18 different capsule types, including three *Klebsiella pneumoniae* strains identified as KL2, an important capsule type in clinical infection and therefore of interest to develop effective therapies against. *Klebsiella* phages have repeatedly shown to be specific to host capsule types [51-53]. This specificity is often linked to phage sugar-degrading enzymes or depolymerases, that target a specific capsule type [54-56]. Given the broad host ranges observed in our collection of phages (fig. 3) and indicative halos [57] recorded in 21 of our 30 phage isolates, we sought out to identify putative depolymerases produced by these phages. Depolymerases are often highly diverse and not all of our phage isolates contained identifiable depolymerase genes, interestingly this included the broadest host-range phages belonging to the genera *Slopekvirus* and *Jiaodavirus*. Characterisation of the putative depolymerases will be important to further investigate the potential extended host range of these phages, beyond the *Klebsiella* strains included in this analysis. Unfortunately, there is limited sequence conservation between many of the putative tail-fiber/tail-spike depolymerase proteins from our collection of phages to those that have been biochemically validated and that further characterisation of these proteins will be critical for future optimisation of phage cocktails for therapeutic uses.

Our phage isolates only suppressed *Klebsiella* growth for up to 7 hours. This suggests that in order to provide universal, effective phage therapy against *Klebsiella* infections, a phage cocktail composed of multiple diverse phages should be developed. The cocktail would prevent growth of *Klebsiella* cells resistant to a single phage or phage genera. The isolates within each of these phage genera were classified as one species, yet they showed differing host ranges and virulence indexes. This demonstrates the importance of isolating a large number of phages for phage therapy and ensuring they are well characterised.

For phage therapy it is important to exclude phages that encode harmful gene products, such as toxins or antimicrobial resistance genes, furthermore temperate phages are also excluded as they are more likely to encode harmful genes and may allow the persistence of their hosts rather than killing them [58]. None of our *Klebsiella* phages contained identifiable toxins or antimicrobial resistance cassettes. KppS-Ant was the only phage that had an identifiable integrase and was identified as a *Klebsiella pneumoniae* DSM 30104 prophage, therefore it is temperate. Additionally, the host infection dynamics of the *Nongavirus* genus phages and low virulence index in combination with the fact that the most closely related phages have been identified as temperate, led us to conclude that the *Nongavirus* genus phages are also temperate and should be excluded from phage therapy. *Jiaodavirus* phages encode a Hoc-like protein, which in T4 phages have been demonstrated to be highly immunogenic, therefore is should be established if these phages cause an immune response before using them for phage therapy [59]. The genomic information for genera *Tunavirinae* unclassified, *Iapetusvirus*, *Sugarlandvirus, Taipeivirus* and *Slopekvirus* indicated that they are lysogenic and suitable for phage therapy.

Phage cocktails are frequently used to improve the efficacy of phage therapy [60, 61]. A few broad host range phages may make a more amenable cocktail by targeting a wider host range of bacteria whilst reducing the chance of phage resistance arising [62]. None of the characterised phages were able to

suppress *Klebsiella* growth in rich media for more than 7 hours suggesting that cocktail of phage may be needed to overcome phage resistant mechanisms in the host. After selection of promising phages, testing of phage combinations is imperative to ensure no unforeseen adverse interactions will occur in a cocktail, such antagonistic phage interactions could result in bacterial stress responses or biofilm formation as seen with sub-lethal antibiotic use [63].

Conclusions

A diverse range of *Klebsiella* phages were isolated from environmental sources. The two broadest host range phage genera *Sugarlandvirus* and *Slopekvirus*, were deemed most suitable for phage therapy, due to their broad host range, high virulence index, low MV50, short latency period and lack of potentially harmful genes. The isolates from *Slopekvirus* clustered into a single species each, with the previously isolated phages *Klebsiella* phage KP15. Despite this, our phage isolates from *Slopekvirus* show variations in both host range and virulence, that may have important impacts on the efficacy of phage therapy. This demonstrates the necessity to microbiologically characterise phages, before selecting phage for therapeutic use rather than selecting based on species level identification alone.

Acknowledgments

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Figure 1. Phylogenetic tree, generated by VIPtree. *Klebsiella* phage isolates (). A-I denote phage genera: A. *Siphoviridae* unclassified A; B. *Siphoviridae* unclassified B; C. *Tunavirinae* unclassified; D. *Lapetusvirus;* E. *Drulisvirus;* F. *Sugarlandvirus;* G. *Taipeivirus*; H. *Slopekvirus* and I. *Jiaodavirus*. Icons indicate phage morphology.

Figure 2. Network analysis of phage core proteins calculated with vConTACT2. Phage nodes are grouped based on clustering similarity. Each coloured, numbered node is a one of our Klebsiella phage isolates, coloured according to the genera to which the phage belongs. The numbers are the lab identification numbers (see table 2).

Figure 3. Number of *Klebsiella* strains infected by each bacteriophage out of a possible 24. Plaques, indicates the number of strains a phage replicated in and resulted in plaques on overlay agar; Clearance, indicates the additional number of strains the phage had some effect to clear or partially clear the bacterial lawn. Totals include the isolation host. Bars marked with # denote an incomplete data set. Coloured outlines related to the phage genus, as identified in figure 1

Figure 4. Phylogenetic tree of previously biochemically characterised depolymerase proteins that target *Klebsiella* spp. and putative depolymerase proteins in our phage isolates. Sequences were aligned with Muscle using SeaView. Phylogenetic tree construction was performed with MegaX with 500 bootstrap calculations using the LG model. Tree topology searches were performed using a combination of NNI and NJ/BioNJ. The tree was subsequently visualised and annotated using iTOL (v4). Depolymerases highlighted in colour blocks have overlapping potential target K antigens.

Figure 5. Representative TEM and plaque images for each phage group. TEM and plaque assays were performed as described in the methods. Scale bars on the TEM images represent 200 nm. Further images of all phages can be found in the supplementary materials, figure S2.

Figure 6. Latency and virulence indices are not correlated, but temperate phages are less virulent. Panel (A) displays the virulence index of the phages. Virulence index is a quantified measure of the phage, in this case in their isolation host. Panel (B) displays the MV50, the MOI at which each phage achieves 50% of their maximal theoretical virulence. Both of these virulence measures are described in more detail by Storms [40]. Panel (C) displays the latency period of the phage. Where this is left blank, a latency period could not be established usually indicating temperate lifestyle. All phage groups are colour coded to their genus, as identified in figure 1.

Figure 7. Phage heat and chloroform sensitivity. The majority of phage were sensitive to heat treatment, but the response to chloroform was more varied. Bars display the reduction in plaque forming units (pfu/mL) as a percentage of the untreated control. The majority of phage were sensitive to heat, with notable exception being KppS-Raw and KqM-Westerburg. Phages demonstrated variable responses to chloroform treatment, those phages indicated with a dash had an increase in phage titre.

Table 1. Details of *Klebsiella* species and strains used in this study. Capsule types are given where applicable, alongside the origin of the strain and indication of use as an isolation host.

Table 2. Phage isolate details. Lab ID refers to the laboratory identification number, source of isolation, indicates where the water sample was collected for phage enrichment and isolation and the strain of isolation indicates *Klebsiella* sp. strain on which 3 rounds of plaque assay isolation were performed.

Table 3. Phage taxonomy and similarity to closest sequenced phage. Family, Subfamily and Genus are assigned based on the closest Comparison phage identified by vConTACT2 analysis, and a similar clustering pattern observed in the core gene phylogenetic trees (Fig. S11-13). ANI refers to Average Nucleotide Identity calculated with orthoANI (https://www.ezbiocloud.net/tools/ani).

Figure S1. Phage host range matrix, determined by serial dilution spot testing.

Figure S2. Phage morphology. Representative phage particle TEM image and plaque morphology. All scale bars represent 200 nm.

Figure S3. *Siphoviridae*, *Nonagvirus* amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S4. *Siphoviridae*, unclassified amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S5. *Tunavirinae*, unclassified amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S6. *Myoviridae, Iapetusvirus* amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S7. *Autographiviridae, Slopekvirinae, Drulisvirus* amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S8. *Demerecviridae, Sugarlandvirus* amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S9. *Ackermannviridae, Taipeivirus* amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S10. Combined *Myoviridae, Tevenvirinae, Slopekvirus* and *Myoviridae, Tevenvirinae, Jiaodavirus* amino acid alignment of our phage isolates and reference genomes identified in vConTACT2 analysis, drawn in VIPtree.

Figure S11 Phylogenetic tree of the core phage gene DNA polymerase. Drawn with RaxML using the GAMMA model of heterogeneity and the maximum-likelihood method based on the JTT substitution matrix. The tree contains reference phages identified by vConTACT2 and our phage isolates (coloured nodes). The coloured phage node groups refer to the genera: **A.** *Nongavirus,* **B.** *Siphoviridae* unclassified, **C.** *Tunavirinae* unclassified, **D.** *Iapetusvirus*, **E.** *Drulisvirus*, **F.** *Sugarlandvirus*, **G.** *Taipeivirus*, **H.** *Slopekvirus* and **I.** *Jiaodavirus.*

Figure S12 Phylogenetic tree of the core phage gene the major capsid protein. Drawn with RaxML using the GAMMA model of heterogeneity and the maximum-likelihood method based on the JTT substitution matrix. The tree contains reference phages identified by vConTACT2 and our phage isolates (coloured nodes). The coloured phage node groups refer to the genera: **A.** *Nongavirus,* **B.** *Siphoviridae* unclassified, **C.** *Tunavirinae* unclassified, **D.** *Iapetusvirus*, **E.** *Drulisvirus*, **F.** *Sugarlandvirus*, **G.** *Taipeivirus*, **H.** *Slopekvirus* and **I.** *Jiaodavirus.*

Figure S13 Phylogenetic tree of the core phage gene terminase large subunit. Drawn with RaxML using the GAMMA model of heterogeneity and the maximum-likelihood method based on the JTT substitution matrix. The tree contains reference phages identified by vConTACT2 and our phage isolates (coloured nodes). The coloured phage node groups refer to the genera: **A.** *Nongavirus,* **B.** *Siphoviridae* unclassified, **C.** *Tunavirinae* unclassified, **D.** *Iapetusvirus*, **E.** *Drulisvirus*, **F.** *Sugarlandvirus*, **G.** *Taipeivirus*, **H.** *Slopekvirus* and **I.** *Jiaodavirus.*

Table S1. List of characterised *Klebsiella* targeting depolymerase tail-fibre proteins used to construct the phylogenetic tree (Figure 4).

Table S2 – List of putative depolymerase tail-fibre proteins from novel phages characterised in this study. Each sequence was analysed by BLASTP, HMMer and HHpred using the default parameters. The top HHpred hits are described for each protein. These sequences in combination with the sequences of characterised depolymerases from phages described in Table S1 were used to construct the phylogenetic tree (Figure 4).

Table S3. Phage measurements; phage particle measurement calculated from TEM imaging and measured with imageJ, and genome size in nucleotide base pairs.

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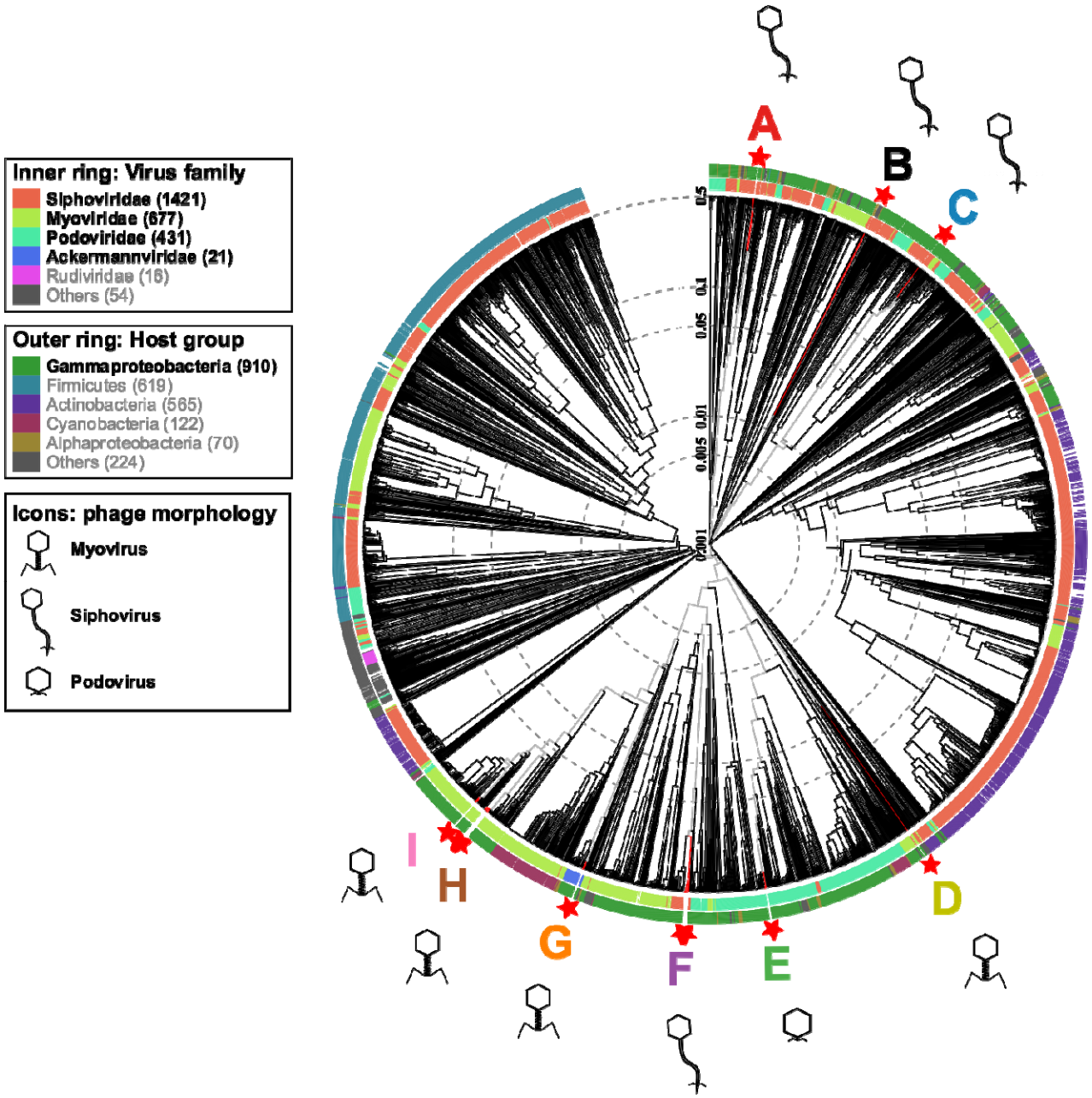
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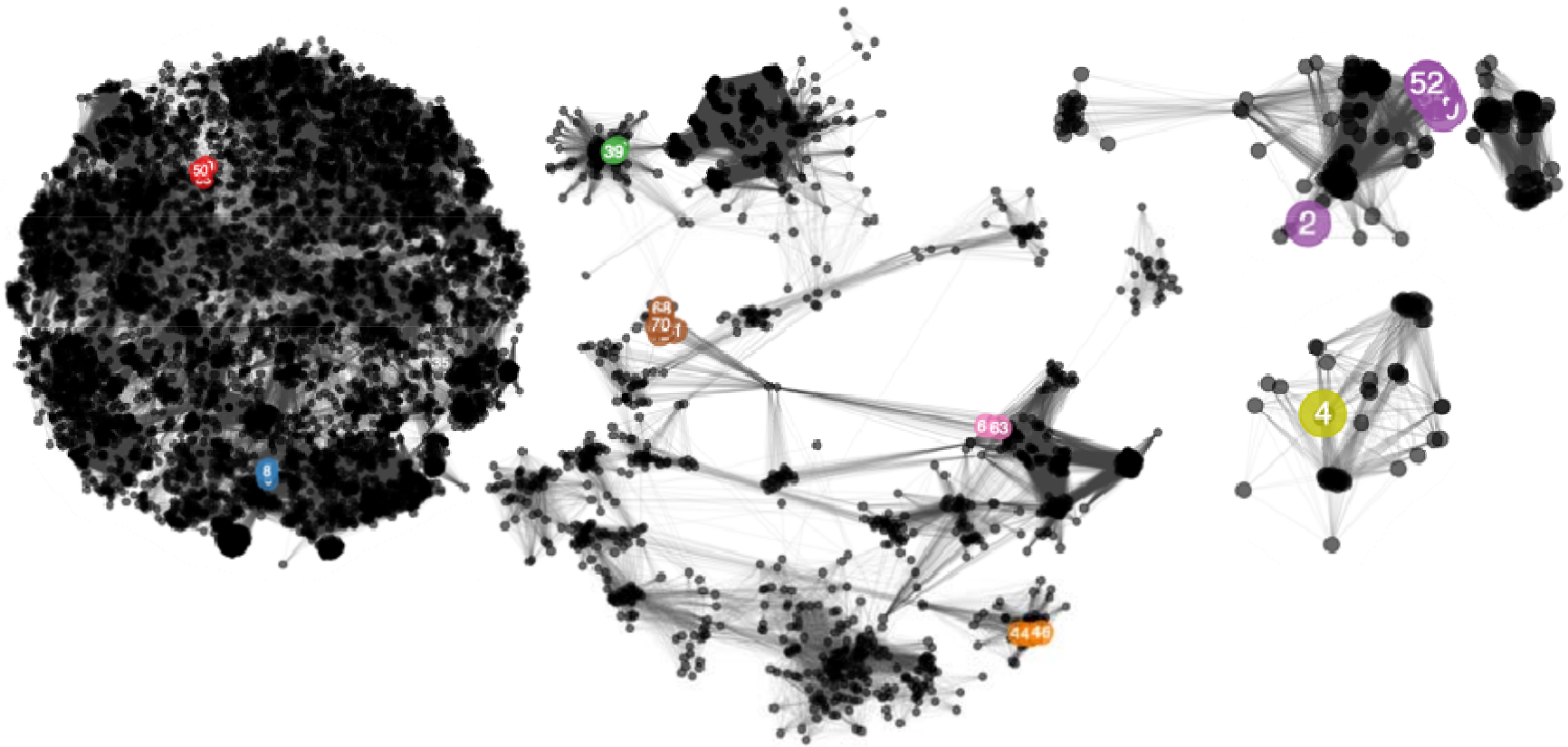
Myoviridae (677)
Podoviridae (431)
Ackermannviridae (21)
Rudiviridae (16)
Others (54)
Outer ring: Host group
Gammaproteobacteria (910)
Firmicutes (619)
Actinobacteria (565)
Cyanobacteria (122)
Alphaproteobacteria (70)
Others (224)
Icons: phage morphology
Myovirus
Siphovirus
Podovirus
A
B
C
D
E
F
G
H
I
0.5
0.1
0.05
0.01
0.005
0.001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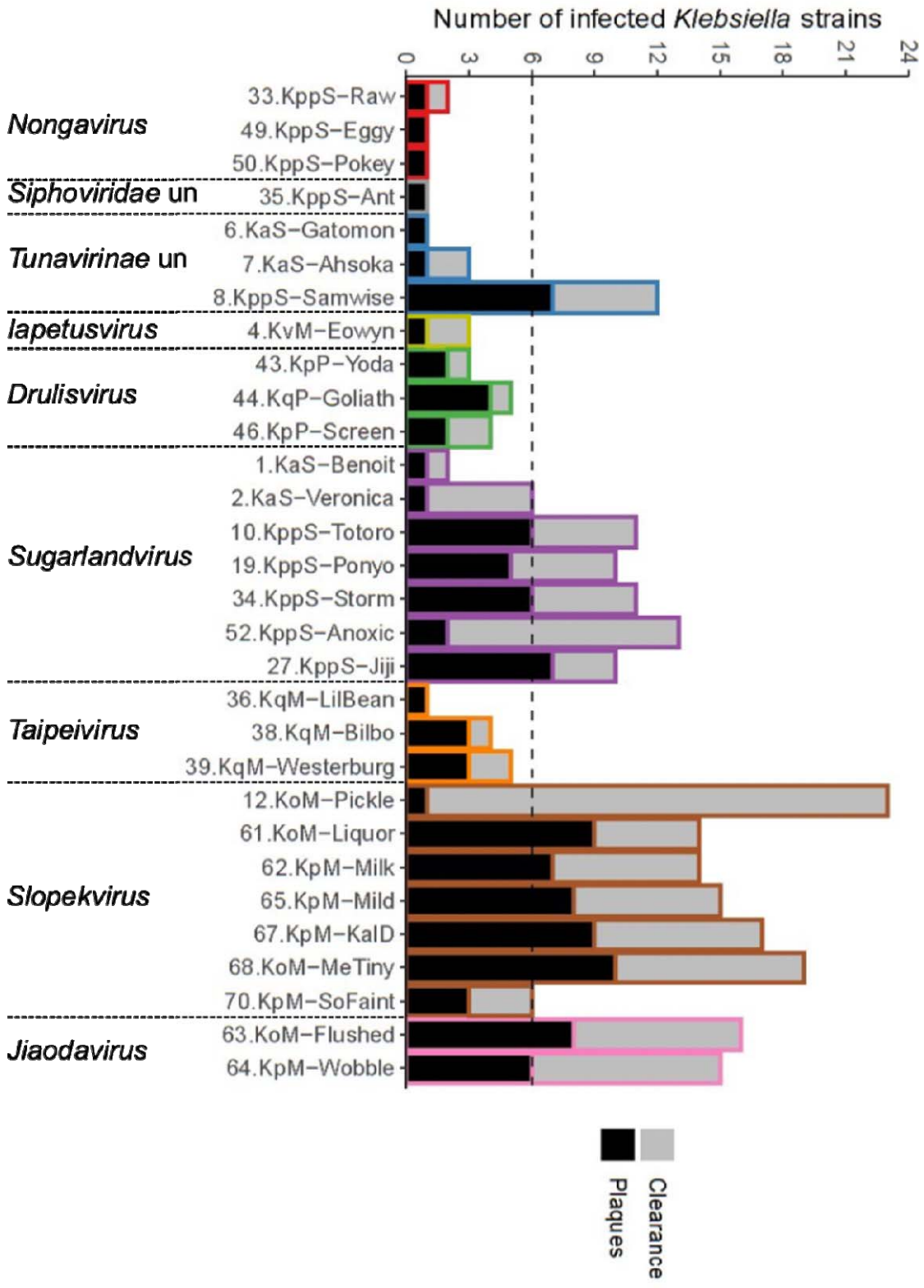
Number of infected Klebsiella strains
0
3
6
9
12
15
18
21
24
Nongavirus
33.KppS−Raw
49.KppS−Eggy
50.KppS−Pokey
Siphoviridae un
35.KppS−Ant
Tunavirinae un
6.KaS−Gatomon
7.KaS−Ahsoka
8.KppS−Samwise
Iapetusvirus
4.KvM−Eowyn
Drulisvirus
43.KpP−Yoda
44.KqP−Goliath
46.KpP−Screen
Sugarlandvirus
1.KaS−Benoit
2.KaS−Veronica
10.KppS−Totoro
19.KppS−Ponyo
34.KppS−Storm
52.KppS−Anoxic
27.KppS−Jiji
Taipeivirus
36.KqM−LilBean
38.KqM−Bilbo
39.KqM−Westerburg
Slopekvirus
12.KoM−Pickle
61.KoM−Liquor
62.KpM−Milk
65.KpM−Mild
67.KpM−KalD
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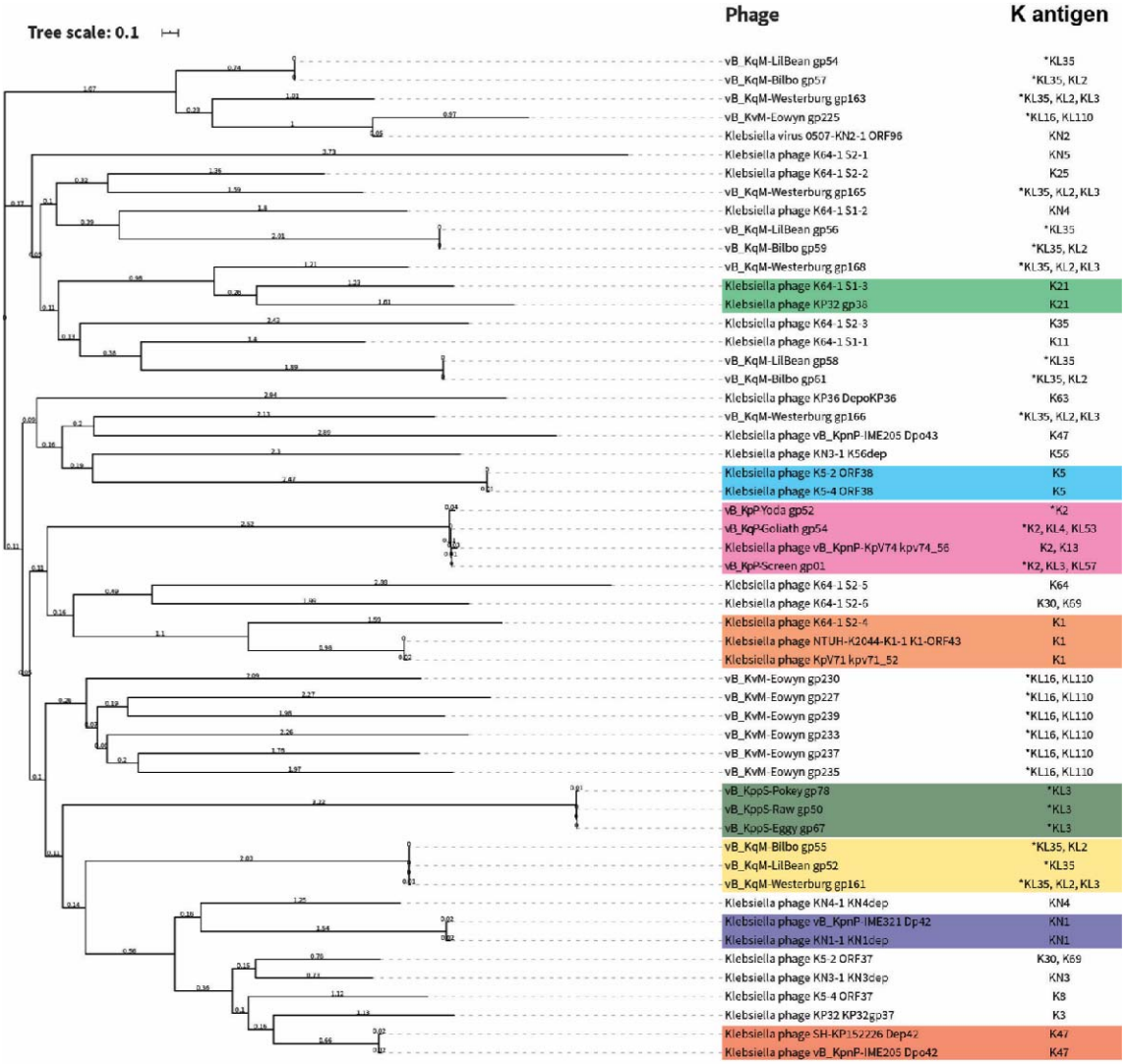
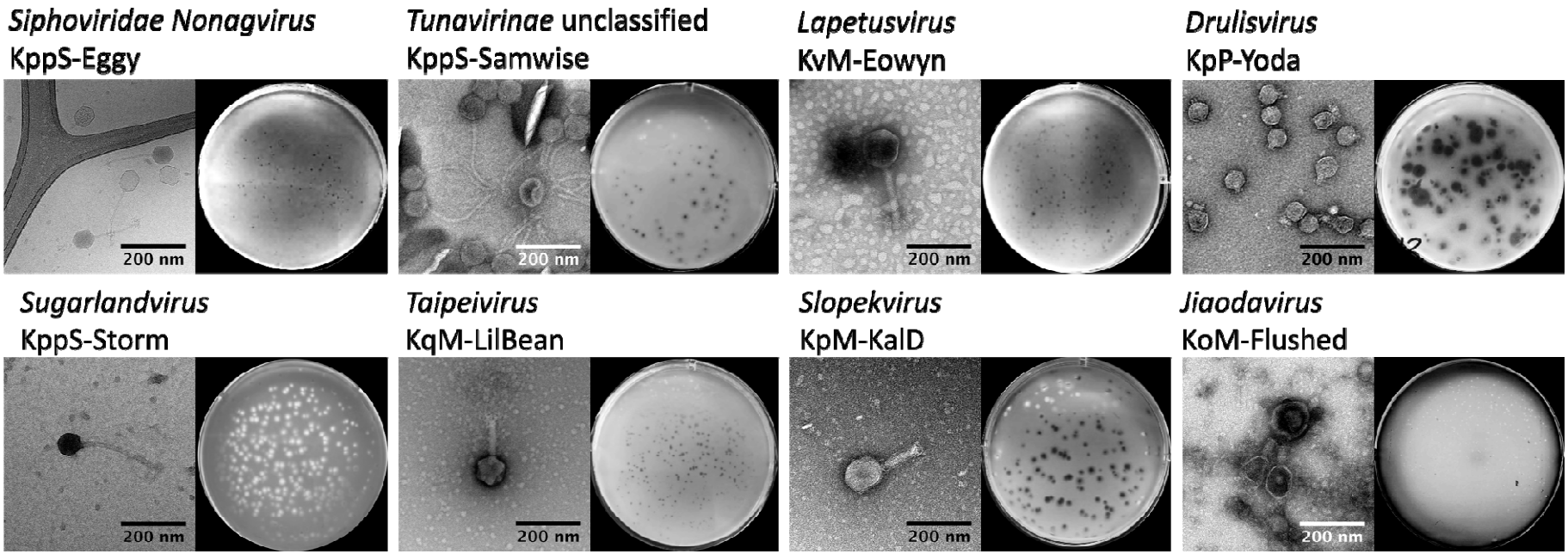
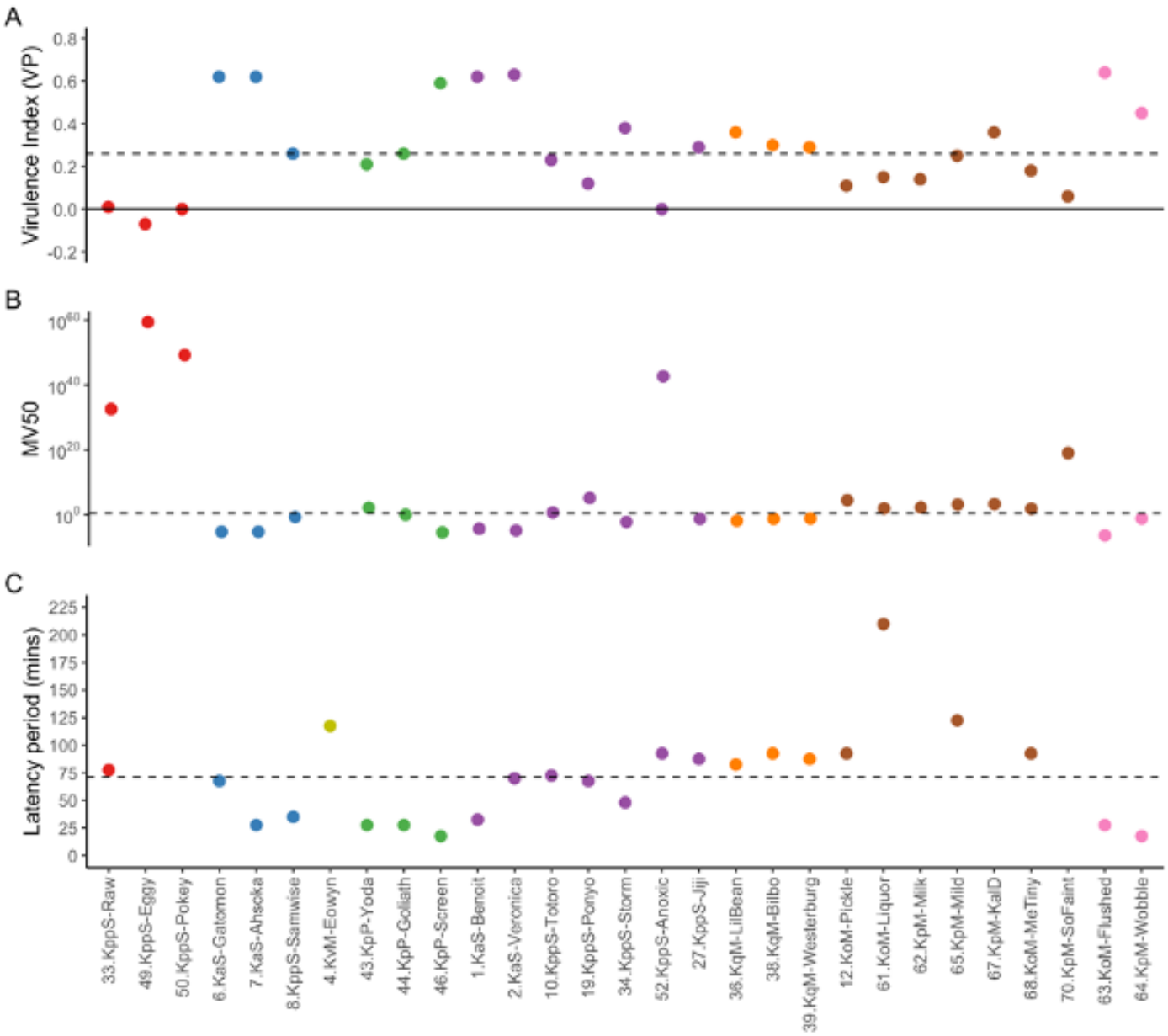
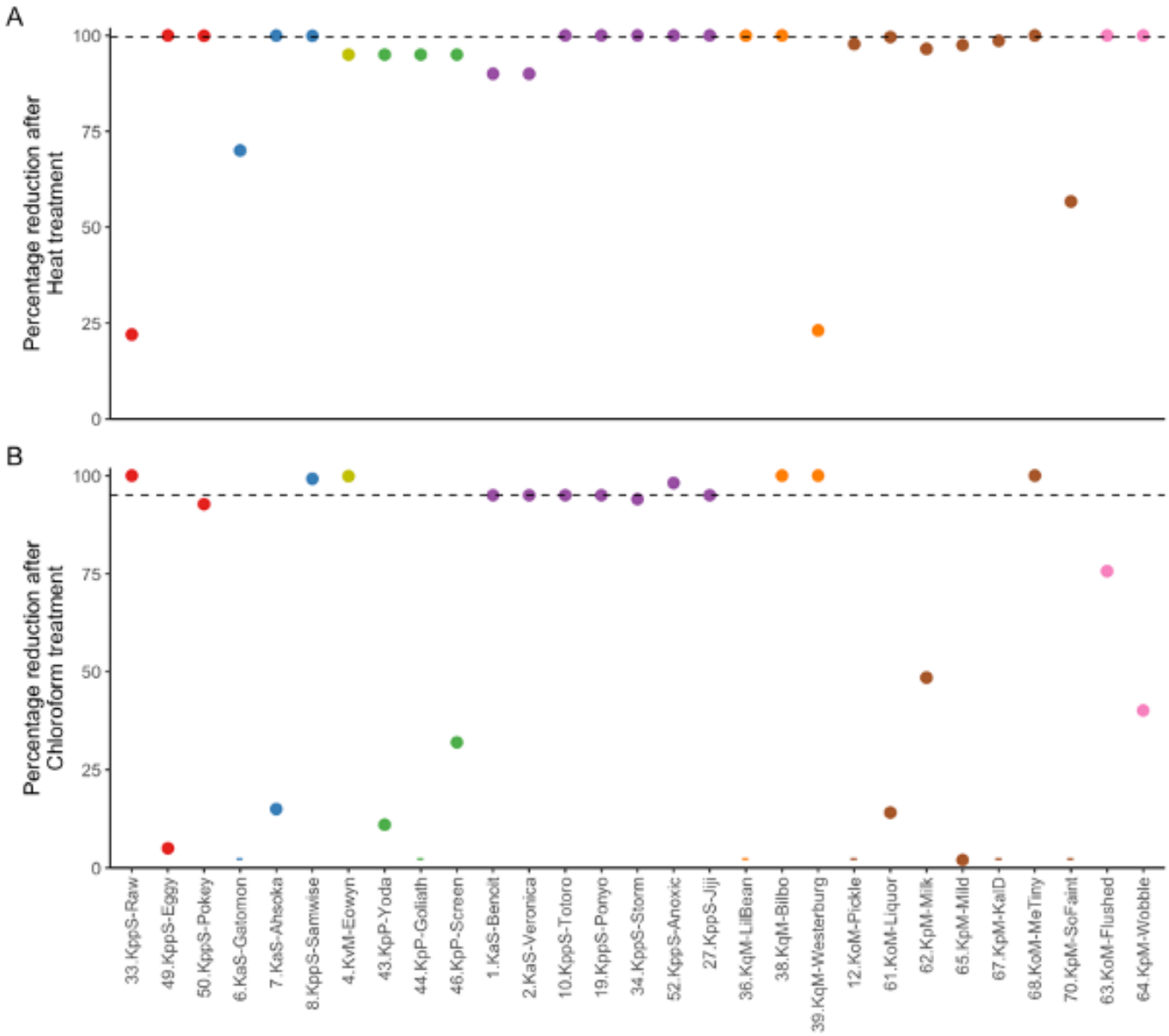
Clearance
Plaques



* Indicates the K antigens produced by the tested strains that showed sensitivity to the phages characterised in this study.


Siphoviridae Nonagvirus
KppS-Eggy
200 nm
Tunavirinae unclassified
KppS-Samwise
200 nm
Lapetusvirus
KvM-Eowyn
200 nm
Drulisvirus
KpP-Yoda
200 nm
Sugarlandvirus
KppS-Storm
200 nm
Taipeivirus
KqM-LilBean
200 nm
Slopekvirus
KpM-KalD
200 nm
Jiaodavirus
KoM-Flushed
200 nm


A
Virulence Index (VP)
0.8
0.6
0.4
0.2
0.0
-0.2
B
MV50
10^{60}
10^{40}
10^{20}
10^{0}
C
Latency period (mins)
225
200
175
150
125
100
75
50
25
0
33.KppS-Raw
49.KppS-Eggy
50.KppS-Pokey
6.KaS-Gatomon
7.KaS-Ahsoka
8.KppS-Samwise
4.KvM-Eowyn
43.KpP-Yoda
44.KpP-Goliath
46.KpP-Screen
1.KaS-Benoit
2.KaS-Veronica
10.KppS-Totoro
19.KppS-Ponyo
34.KppS-Storm
52.KppS-Anoxic
27.KppS-Jiji
36.KqM-LilBean
38.KqM-Bilbo
39.KqM-Westerburg
12.KoM-Pickle
61.KoM-Liquor
62.KpM-Milk
65.KpM-Mild
67.KpM-KalD
68.KoM-MeTiny
70.KpM-SoFaint
63.KoM-Flushed
64.KpM-Wobble


A
Percentage reduction after Heat treatment
100
75
50
25
0
B
Percentage reduction after Chloroform treatment
100
75
50
25
0
33.KppS-Raw
49.KppS-Eggy
50.KppS-Pokey
6.KaS-Gatomon
7.KaS-Ahsoka
8.KppS-Samwise
4.KvM-Eowyn
43.KpP-Yoda
44.KpP-Goliath
46.KpP-Screen
1.KaS-Benolt
2.KaS-Veronica
10.KppS-Totoro
19.KppS-Ponyo
34.KppS-Storm
52.KppS-Anoxic
27.KppS-Jiji
36.KqM-LilBean
38.KqM-Bilbo
39.KqM-Westerburg
12.KoM-Pickle
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62.KpM-Milk
65.KpM-Mild
67.KpM-KalD
68.KoM-MeTiny
70.KpM-SoFaint
63.KoM-Flushed
64.KpM-Wobble

Species		Strain	Capsule Type	Isolation Host?	Source
Klebsiella	*aerogenes*	30053	-	✓	DSMZ Culture Collection
	michiganensis	25444	O1v1	×	DSMZ Culture Collection
	oxytoca	170748	O1v1	×	Clinical Isolate
		5175	KL29	×	DSMZ Culture Collection
		25736	KL74	✓	DSMZ Culture Collection
		170821	OL104	✓	Clinical Isolate
		171266	OL104	×	Clinical Isolate
	pneumoniae	170958	KL28	✓	Clinical Isolate
		171304	KL144	×	Clinical Isolate
		13440	KL38	✓	NCTC Culture Collection
		13442	KL110	×	NCTC Culture Collection
		30104	KL3	✓	DSMZ Culture Collection
		13465	KL57	×	NCTC Culture Collection
		170820	KL158	×	Clinical Isolate
		16358	KL4	×	DSMZ Culture Collection
		170723	KL2	✓	Clinical Isolate
		171167	KL2	×	Clinical Isolate
		13443	KL2	×	NCTC Culture Collection
		13882	KL64	×	ATCC Culture Collection
		13439	KL14	✓	NCTC Culture Collection
	variicola	W12	KL14	×	Environmental Isolate
		15968	KL16	✓	DSMZ Culture Collection
	quasipneumoniae	28211	KL35	✓	DSMZ Culture Collection
		700603	KL53	✓	ATCC Culture Collection

Phage Name	Lab ID	Source of isolation	Strain of isolation
Klebsiella phage vB_KaS-Benoit	1	estuary	*Klebsiella aerogenes* DSM 30053
Klebsiella phage vB_KaS-Veronica	2	marine canal	*Klebsiella aerogenes* DSM 30053
Klebsiella phage vB_KvM-Eowyn	4	estuary	*Klebsiella variicola* DSM 15968
Klebsiella phage vB_KaS-Gatomon	6	marine canal	*Klebsiella aerogenes* DSM 30053
Klebsiella phage vB_KaS-Ahsoka	7	slurry	*Klebsiella aerogenes* DSM 30053
Klebsiella phage vB_KppS-Samwise	8	slurry	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KppS-Totoro	10	estuary	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KoM-Pickle	12	estuary	*Klebsiella oxytoca* DSM 25736
Klebsiella phage vB_KppS-Ponyo	19	river	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KppS-Jiji	27	pond	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KppS-Raw	33	Raw sewage	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KppS-Storm	34	Sewage storm tank	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KppS-Ant	35	Anoxic - sludge	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KqM-LilBean	36	sewage	*Klebsiella quasipneumoniae* DSM 28211
Klebsiella phage vB_KqM-Bilbo	38	sewage	*Klebsiella quasipneumoniae* DSM 28212
Klebsiella phage vB_KqM-Westerburg	39	sewage	*Klebsiella quasipneumoniae* DSM 28213
Klebsiella phage vB_KpP-Yoda	43	Storm tank	*Klebsiella pneumoniae* 170723
Klebsiella phage vB_KqP-Goliath	44	sewage	*Klebsiella quasipneumoniae* DSM 700603
Klebsiella phage vB_KpP-Screen	46	Sewage seive	*Klebsiella pneumoniae* 170723
Klebsiella phage vB_KppS-Eggy	49	Sewage anoxic sludge	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KppS-Pokey	50	Sewage anoxic sludge	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KppS-Anoxic	52	Sewage anoxic sludge	*Klebsiella pneumoniae* DSM 30104
Klebsiella phage vB_KoM-Liquor	61	Sewage - Mixed liquor	*Klebsiella oxytoca* 170821
Klebsiella phage vB_KpM-Milk	62	Polluted river	*Klebsiella oxytoca* 170821
Klebsiella phage vB_KoM-Flushed	63	Sewage - Mixed liquor	*Klebsiella pneumoniae* 170958
Klebsiella phage vB_KpM-Wobble	64	Polluted river	*Klebsiella pneumoniae* 170958
Klebsiella phage vB_KpM-Mild	65	Sewage - Mixed liquor	*Klebsiella pneumoniae* DSM 13439
Klebsiella phage vB_KpM-KalD	67	Polluted river	*Klebsiella pneumoniae* DSM 13439
Klebsiella phage vB_KoM-MeTiny	68	Sewage - Mixed liquor	*Klebsiella oxytoca* DSM 25736
Klebsiella phage vB_KpM-SoFaint	70	Polluted river	*Klebsiella pneumoniae* DSM 13440

Phage	Lab ID	Family	Subfamily	Genus	Comparison phage	Accession	ANI
vB_KppS-Raw	33	*Siphoviridae*		*Nonagvirus*	Enterobacteria phage JenP2	KP719132	66.77
vB_KppS-Eggy	49	*Siphoviridae*		*Nonagvirus*	Enterobacteria phage JenP2	KP719132	65.86
vB_KppS-Pokey	50	*Siphoviridae*		*Nonagvirus*	Enterobacteria phage JenP2	KP719132	66.69
vB_KppS-Ant	35	*Siphoviridae*		*Vieuvirus*	Caudovirales_phage_clone_3F_1	MF417951	90.14
vB_KaS-Gatomon	6	*Drexlerviridae*	*Tunavirinae*	unclassified	Escherichia phage Henu7	MN019128	93.55
vB_KaS-Ahsoka	7	*Drexlerviridae*	*Tunavirinae*	unclassified	Escherichia phage Henu7	MN019128	92.09
vB_KppS-Samwise	8	*Drexlerviridae*	*Tunavirinae*	unclassified	Escherichia phage Henu7	MN019128	93.55
vB_KvM-Eowyn	4	*Myoviridae*		*Iapetusvirus*	Serratia_phage_KpHz_2	KF806589	95.02
vB_KpP-Yoda	43	*Autographiviridae*	*Slopekvirinae*	*Drulisvirus*	Klebsiella phage vB_KpnP_SU552A	KP708986	87.03
vB_KqP-Goliath	44	*Autographiviridae*	*Slopekvirinae*	*Drulisvirus*	Klebsiella phage vB_KpnP_SU552A	KP708986	85.82
vB_KpP-Screen	46	*Autographiviridae*	*Slopekvirinae*	*Drulisvirus*	Klebsiella phage vB_KpnP_SU552A	KP708986	86.58
vB_KaS-Benoit	1	*Demerecviridae*		*Sugarlandvirus*	ref vB_Kpn_IME260	NC_041899	94.29
vB_KaS-Veronica	2	*Demerecviridae*		*Sugarlandvirus*	Klebsiella phage Sugarland	NC_042093	93.46
vB_KppS-Totoro	10	*Demerecviridae*		*Sugarlandvirus*	ref vB_Kpn_IME260	NC_041899	94.29
vB_KppS-Ponyo	19	*Demerecviridae*		*Sugarlandvirus*	Klebsiella phage Sugarland	NC_042093	94.15
vB_KppS-Jiji	27	*Demerecviridae*		*Sugarlandvirus*	Klebsiella phage Sugarland	NC_042093	93.26
vB_KppS-Storm	34	*Demerecviridae*		*Sugarlandvirus*	Klebsiella phage Sugarland	NC_042093	95.02
vB_KppS-Anoxic	52	*Demerecviridae*		*Sugarlandvirus*	ref vB_Kpn_IME260	NC_041899	96.11
vB_KqM-LilBean	36	*Ackermannviridae*		*Taipeivirus*	Klebsiella virus 0507KN21	NC_022343	97.83
vB_KqM-Bilbo	38	*Ackermannviridae*		*Taipeivirus*	Klebsiella virus 0507KN21	NC_022343	97.22
vB_KqM-Westerburg	39	*Ackermannviridae*		*Taipeivirus*	Klebsiella virus 0507KN21	NC_022343	96.93
vB_KoM-Pickle	12	*Myoviridae*	*Tevenvirinae*	*Slopekvirus*	Klebsiella phage KP15	GU295964	97.93
vB_KoM-Liquor	61	*Myoviridae*	*Tevenvirinae*	*Slopekvirus*	Klebsiella phage KP15	GU295964	97.83
vB_KpM-Milk	62	*Myoviridae*	*Tevenvirinae*	*Slopekvirus*	Klebsiella phage KP15	GU295964	98.38
vB_KpM-Mild	65	*Myoviridae*	*Tevenvirinae*	*Slopekvirus*	Klebsiella phage KP15	GU295964	97.65
vB_KpM-KalD	67	*Myoviridae*	*Tevenvirinae*	*Slopekvirus*	Klebsiella phage KP15	GU295964	97.42
vB_KoM-MeTiny	68	*Myoviridae*	*Tevenvirinae*	*Slopekvirus*	Klebsiella phage KP15	GU295964	97.92
vB_KpM-SoFaint	70	*Myoviridae*	*Tevenvirinae*	*Slopekvirus*	Klebsiella phage KP15	GU295964	97.72
vB_KoM-Flushed	63	*Myoviridae*	*Tevenvirinae*	*Jiaodavirus*	Klebsiella phage JD18	KT239446	96.19
vB_KpM-Wobble	64	*Myoviridae*	*Tevenvirinae*	*Jiaodavirus*	Klebsiella phage JD18	KT239446	97.01