BMC Microbiology

RESEARCH **Open Access**

"Sichuanvirus", a novel bacteriophage viral genus, able to lyse carbapenem-resistant *Klebsiella pneumoniae*

Juan Li[1,2], Qingqing Fang[1,3], Huan Luo[4], Yan Feng[1,2], Yu Feng[4] and Zhiyong Zong[1,2,4,5*]

Abstract

Background Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a severe threat for human health and urgently needs new therapeutic approaches. Lytic bacteriophages (phages) are promising clinically viable therapeutic options against CRKP. We attempted to isolate lytic phages against CRKP of sequence type 11 and capsular type 64 (ST11-KL64), the predominant type in China.

Results We recovered a lytic phage from sewage collected at a wastewater treatment station in Sichuan province, China. We obtained the genome of this phage and found that it is distinct from all known phages with the highest overall DNA similarity (12.5%, 16% coverage and 78.4% identity) with phage vB_EcoM_PHB05 (accession no. NC_052652) in ICTV. This phage represents a novel viral genus of the subfamily *Stephanstirmvirinae*, for which we proposed "Sichuanvirus" as the genus name. This phage has a narrow host range lyse specific for KL64 *Klebsiella*. This phage has no genes referring to antimicrobial resistance, virulence, and lysogen and is stable to a wide range of pH and temperatures. We also obtained three bacterial mutants resistant to the phage and performed genome sequencing for them. We therefore discovered that the interruption of a capsular polysaccharide biosynthesis-related gene *wcaJ* by insertion sequences mediated the resistance to this phage.

Conclusion We recovered and characterized a phage of "Sichuanvirus", a novel viral genus of subfamily *Stephanstirmvirinae*, which is suitable for phage therapy. The discovery of this phage expands the arsenal against CRKP.

Keywords Antimicrobial resistance, Phage therapy, *Klebsiella pneumoniae*, *Stephanstirmvirinae*, Microbiology, Bacteriophages, Phage biology

*Correspondence:
Zhiyong Zong
zongzhiy@scu.edu.cn
[1] Center of Infectious Diseases, West China Hospital, Sichuan University, Guoxuexiang 37, Chengdu 610041, China
[2] Division of Infectious Diseases, State Key Laboratory of Biotherapy, Chengdu, China
[3] Department of General Practice, General Practice Medical Center, West China Hospital, Sichuan University, Chengdu, China
[4] Laboratory of Pathogen Research, West China Hospital, Sichuan University, Chengdu, China
[5] State Key Laboratory of Respiratory Health and Multimorbidity, Chengdu, China

Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is an emerging several clinical problem and a threat to public health in the whole world with very limited options for treatment and therefore has been listed as a "critical priority" pathogen by the World Health Organization in urgent need of alternative therapies [1]. CRKP often causes a variety of infections such as bacteremia, pneumonia, and urinary tract infections, and is typically associated with a high mortality. Systematic reviews have unveiled 22.9% to 37.2% pooled mortalities of patients with CRKP infections [2, 3]. Bacteriophages (phages) are bacterial viruses, some of which

© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

can lyse bacteria and therefore may be used to treat bacterial infections. Phage therapy has gained worldwide attention with being carried out as an alternative treatment for refractory infections including those due to *K. pneumonia* [4]. In addition to good safety, phage therapy has achieved positive outcomes in some cases with complicated UTI, fracture-related infection, or periprosthetic joint infection [4–6]. As such, phages represent a viable therapeutic option against CRKP. For phage therapy, typically a few lytic phages against the target bacteria are needed to provide the flexibility and adaptability to constitute cocktails for countering the potential bacterial resistance to phages after exposure. In China, CRKP of sequence type 11 and capsular type 64 (ST11-KL64) is the predominant type [7] and may therefore be a major target of phage therapy. Previous studies including ours have isolated phages targeting KL64 CRKP, primarily from the genus *Przondovirus* [8, 9]. However, phage-resistant mutants can emerge rapidly in vivo and in vitro, highlighting the necessity to expand the biobank of phages available for therapy. Genus *Przondovirus* belongs to family *Autographiviridae* within the class *Caudoviricetes* (formerly belonging to *Caudovirales* order, which was abolished in 2021 [10]). Notably, the majority of phages described to date have a tailed morphology with a double-stranded DNA genome and belong to the class *Caudoviricetes*. Phages of class *Caudoviricetes* typically exhibit a complex structure, characterized by the presence of an icosahedral or elongated capsid with the axis of the tail [11]. The capsid size of *Caudoviricetes* phages can vary remarkably, ranging from 45 to 185 nm in diameters. The tails of *Caudoviricetes* phages may be in any of the three forms, namely contractile, long non-contractile, and short [11, 12]. In this study, we recovered a lytic phage against ST11-KL64 CRKP and uncovered that this phage represents a novel non-*Autographiviridae* viral genus within the class *Caudoviricetes*.

Methods

Genotypic and phenotypic characterization of bacterial host strain

We used a ST11-KL64 CRKP clinical strain, strain 135080, for phage isolation. This strain was assigned to ST11-KL64 by examining its genome sequence (accession no. JANHBP000000000) using Kleborate v2.2.0 [13] and its carbapenem resistance (MIC of meropenem, 256 mg/L) was verified using the Clinical Institute Standard Institute (CLSI) microdilution method [14]. In addition, the strain has $bla_{KPC\text{-}2}$, a carbapenemase-encoding gene, identified using AMRFinderPlus v3.10.23 [15].

Phage isolation

On March 16, 2022, we obtained a 200 ml sewage sample from a wastewater treatment station of Mianning County, Sichuan Province. The water sample was passed through a 0.22 μm membrane and 17 ml of filtered sewage was mixed with 2 ml 10×LB broth and 1 ml of an overnight culture of strain 135080. After incubation at 37°C for 4 h, the mixture was centrifuged at 12,000×g at 4 °C for 2 min. The supernatant was filtered through a 0.22 μm membrane and was diluted with Tris–HCl-$MgSO_4$ (TM buffer). The double-layer agar method [16, 17] was used to obtain individual plaques with diluted supernatant against strain 135080. The isolated phage plaque was further purified clonally for five times to ensure to obtain one single phage. A phage able to lyse 135080 was therefore isolated and named it 175008.

Phage genome sequencing and taxonomic assignation

We sequenced the genome of 175008 using the HiSeq X10 platform (Illumina, San Diego, CA, USA) with phage DNA being prepared using a phage DNA isolation kit (Norgen Biotek; Thorold, Canada). Given the large quantity of sequencing data obtained (see Results), we randomly subsampled 500,000 read pairs to minimize host genome contamination. This subsampling provided approximately 1,000×coverage of the 149,294-bp phage 175008 for genome assembly using Unicycler v0.5.1 [18]. The phage genome was annotated using pharokka v1.7.2 [19] and phold v0.1.4 (https://github.com/gbouras13/phold). The lifestyle of 175008 was predicted using PhaTYP [20]. tRNA genes were also screened using tRNAscan-SE [21]. The genome general overview of 175008 was generated using PHASTEST (https://phastest.ca/). 175008 was searched for its most closely-related phage with genome sequence available in the International Committee on Taxonomy of Viruses (ICTV) by BlastN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The taxonomy of 175008 was determined according to the rule defined by ICTV for the subfamily *Stephanstirmvirinae* (https://ictv.global/taxonomy/taxondetails?taxnode_id=202313647&taxon_name=Stephanstirmvirinae). Specifically, the cutoff for genus demarcation was 70% nucleotide identity of the genome length, which is determined by the mutual intergenomic similarity calculated using VIRIDIC [22]. Further, the cutoff for species assignation was 95% DNA nucleotide identity determined using BlastN within this subfamily. A heatmap of mutual intergenomic similarities between 175008 and phages belonging to the subfamily *Stephanstirmvirinae* (6 of genus *Justusliebigvirus* and 13 of genus *Phapecoctavirus*) in ICTV was created using VIRIDIC [22]. A maximum-likelihood phylogenetic tree based on the amino acid

sequence of the terminase large subunit of the 19 phages belonging to the subfamily *Stephanstirmvirinae* was inferred with phylogeny.fr in "one click" mode according to ICTV recommendation. We performed BlastN searches to investigate whether there are phages closely related to 175008 in addition to those available in ICTV. A synteny plot was obtained using clinker (https://github.com/gamcil/clinker) to compare 175008 with phages sharing a ≥ 70% coverage for the genome sequence.

Morphological observation and phenotypic characterization of phage 175008

For its morphology, 50 μl of phage 175008 solutions containing 10^{10} plaque-forming units (PFU) per ml were dropped on carbon film-coated copper grids (Carbon Type-B 100 mesh; Zhongjingkeyi Technology; Beijing, China) for 10 min and then a drop of 2.0% (w/v) uranyl acetate was added for negative staining. A JEM-1400PLUS transmission electron microscope (JEOL; Tokyo, Japan) was used to observe the morphology at an accelerating voltage of 80 kV. The phenotypic profiles of 175008 comprising the phage titer, optimal multiplicity of infection (MOI), stability at varied pH values and temperatures, in vitro phage bacteriolytic assay, the host range, phage adsorption assay and one-step growth were characterized using methods as described previously [8, 23]. Briefly, 175008 was tested for the thermal stability by one-hour incubation of 10^8 PFU/ml in TM Buffer at 4, 25, 37, 50, 60, or 70°C followed by checking the phage viability using the double-layer agar method. 175008 was also examined for its stability at pH 2 to 13 by one-hour incubation of 10^8 PFU/ml in TM Buffer at 37°C. 175008 of 10^4, 10^5, 10^6, 10^7, or 10^8 PFU/ml was incubated at 37°C for three hours at different MOI, namely 0.01, 0.1, 1, 10, or 100. The optimum MOI was determined when the bacterial solution was completely lysed. A set of *K. pneumoniae* strains of 30 capsular types, determined using Kleborate v2.2.0 [13] for their genome sequences, from our strain collection including 9 ST11-KL64 strains in addition to 135080 (Supplementary Table S1), were used for determining the host range of 175008 by the spot assay [24]. In addition, we examined whether 175008 was able to lyse *E. coli* like many *Stephanstirmvirinae* phages using strains of 11 STs (Supplementary Table S2). 175008 was assayed for its in vitro bacteriolytic activity by adding phages at the optimal MOI (0.01) into the culture of strain 135080 at the pre-logarithmic phase, with a OD_{600} of 0.20, which is equivalent to approximately 10^8 colony forming units (CFU) per ml. LB broth without strain 135080 and the culture of strain 135080 without phages were used as controls.

We assessed the adsorption ability of phage 175008 by mixing 10 ml of the bacterial host strain (10^8 CFU/ml) with 100 μl of phage (10^8 PFU/ml) at a 0.01 MOI, the optimal ratio. The co-cultures (1 ml) were retrieved at 0, 3, 6, 9, 12, 15, 20, and 35 min to count phage titers. To estimate the latent period and burst size of 175008, we conducted one-step growth experiments. Phage 175008 was added at a 0.01 MOI and allowed to adsorb for 35 min at 37°C. We then retrieved 1 ml of the mixture, which was centrifuged at 12,000 × g for 1 min to remove free phage particles. The pellet containing infected cells was then re-suspended in 1 ml LB broth. This mixture was further diluted 100, 1,000, and 10,000 times in tubes, respectively, which were incubated at 37°C. Samples were retrieved from the appropriate tube at various time points up to 110 min to determine phage titers. The number of phage-infected cells was calculated by subtracting the count of free phages in the aliquot filtered through a 0.22 μm membrane from that in the unfiltered sample at the start. Burst size was defined as the average number of phage-infected cells divided by the progeny counts in the stationary phase. All experiments were performed in triplicate.

Genome sequencing of 175,008-resistant bacterial mutants

Three 175008-resistant mutants of the host strain 135080 were randomly selected and subjected to whole genome sequencing using HiSeq X10 system (Illumina). Reads were assembled in contigs using SPAdes v3.15 [25] under the isolate mode. Comparison of the genome sequences of the three 175008-resistant mutants and that of the parental strain 135080 was performed using the BLASTn [26] algorithm with default settings. Insertion sequences were identified using the ISFinder database [27]. The absorption of 175008 to the three phage-resistant mutants was measured as described above.

Results

Identification of a double-stranded DNA lytic phage, 175,008, against ST11-KL64 CRKP

Using ST11-KL64 CRKP clinical strain 135080 as the host, we obtained individual clear pin-like plaques from tenfold dilution of filtered supernatant of the sewage from a wastewater treatment station of Mianning County, Sichuan Province, China. After purification for five times, we obtained a lytic phage, 175008. 175008 was subjected to genome sequencing, which yielded 10,184,334 reads, approximately 1.53 Gb bases. 175008 was identified as a double-stranded DNA phage with a total length of 149,199 bp, 39.71% GC content, and 276 coding sequences (CDS). An overall genome view of this phage is available as Supplementary Fig. S1. The genome of 175008 does not contain any genes related to virulence, lysogeny like, and antimicrobial resistance. This

indicates that 175008 is a strictly virulent phage, which was also identified using PhaTYP [20].

Phage 175008 represents a novel viral genus of the subfamily *Stephanstirmvirinae*

Phage 175008 is distinct from all known phages in ICTV as it shares the highest overall DNA similarity (12.5%, 16% coverage and 78.4% identity) with phage vB_EcoM_PHB05 (accession no. NC_052652). As phage vB_EcoM_PHB05 belongs to the genus *Justusliebigvirus* of the subfamily *Stephanstirmvirinae*, we constructed a phylogenetic tree using the amino acid sequence of the terminase large subunit of all phages belonging to the subfamily *Stephanstirmvirinae* (6 of genus *Justusliebigvirus* and 13 of genus *Phapecoctavirus*) in ICTV according to ICTV recommendation. 175008 locates between the two genera, *Justusliebigvirus* and *Phapecoctavirus*, within the subfamily *Stephanstirmvirinae* (Fig. 1), suggesting that 175008 is a member of this subfamily. We then sought to determine mutual intergenomic similarities between 175008 and those within the subfamily *Stephanstirmvirinae* using VIRIDIC. 175008 had a 31.5% to 36.5% intergenomic similarity with *Stephanstirmvirinae* phages (Fig. 2), indicating 175008 represents a novel genus of the subfamily *Stephanstirmvirinae*. We proposed "Sichuanvirus" as the genus name considering that the phage was recovered from a sewage sample collected in Sichuan Province. Notably, according to ICTV, subfamily is used to contain two or more closely related genera below the family level and no family nor order is assigned to the subfamily *Stephanstirmvirinae*, which instead directly belongs to the class *Caudoviricetes*. In addition to phages available in ICTV, BlastN uncovers that the genome of 175008 shares ≥ 70% coverage (72% to 79%) with four phages in GenBank comprising KP13-26 (accession no. OP617743), KP2025 (accession no. PP919962), FKP3 (accession no. PP895363), and ctAW83 (accession no. BK027261). Using VIRIDIC, 175008 has a ≥ 70% intergenomic similarity with KP13-26 (76.5%), KP2025 (75.9%), and FKP3 (71.9%) (Fig. 2). This indicates that these phages also belong to the genus "Sichuanvirus" but are of different species as their highest mutual intergenomic similarity was 84.9%, which is between KP13-26 and KP2025 and is below the 95% species demarcation cutoff. In contrast, phage ctAW83 has a 61.2% (with FKP3) to 67.5% (with 175008) intergenomic similarity with the abovementioned four phages. Therefore, this phage is likely to represent a new genus within the subfamily *Stephanstirmvirinae*, which warrant further investigation. Consistently, 175008 exhibits more similar synteny plot with KP13-26 and KP2025 (Supplementary Fig. S2).

175008 contains two tail fiber protein genes and 18 tRNA genes

175008 has two tail fiber proteins with 972 and 346 amino acids (aa), respectively (Supplementary Dataset S1). The 972-aa tail fiber protein contains a 58-aa tail

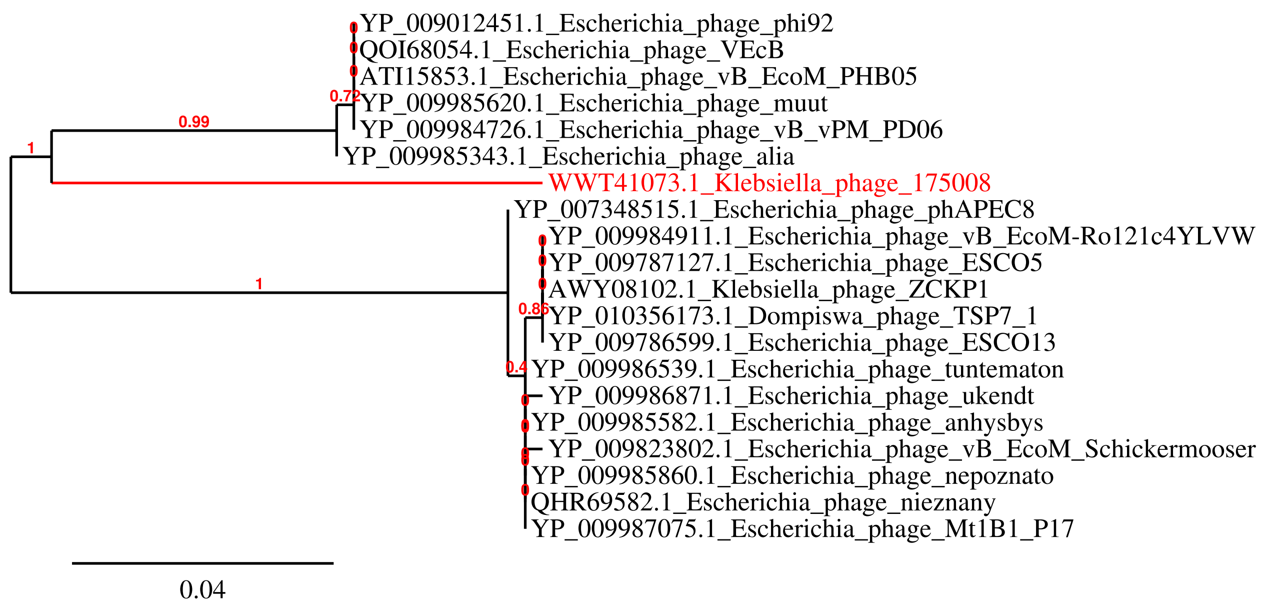

Fig. 1 Phylogenetic tree of 175008 and phages belonging to subfamily *Stephanstirmvirinae*. The tree was inferred based on the amino acid sequence of the terminase large subunit of 175008 and all phages belonging to subfamily *Stephanstirmvirinae* comprising six of genus *Justusliebigvirus* and 13 of genus *Phapecoctavirus* with phylogeny.fr in "one click" mode according to ICTV recommendation

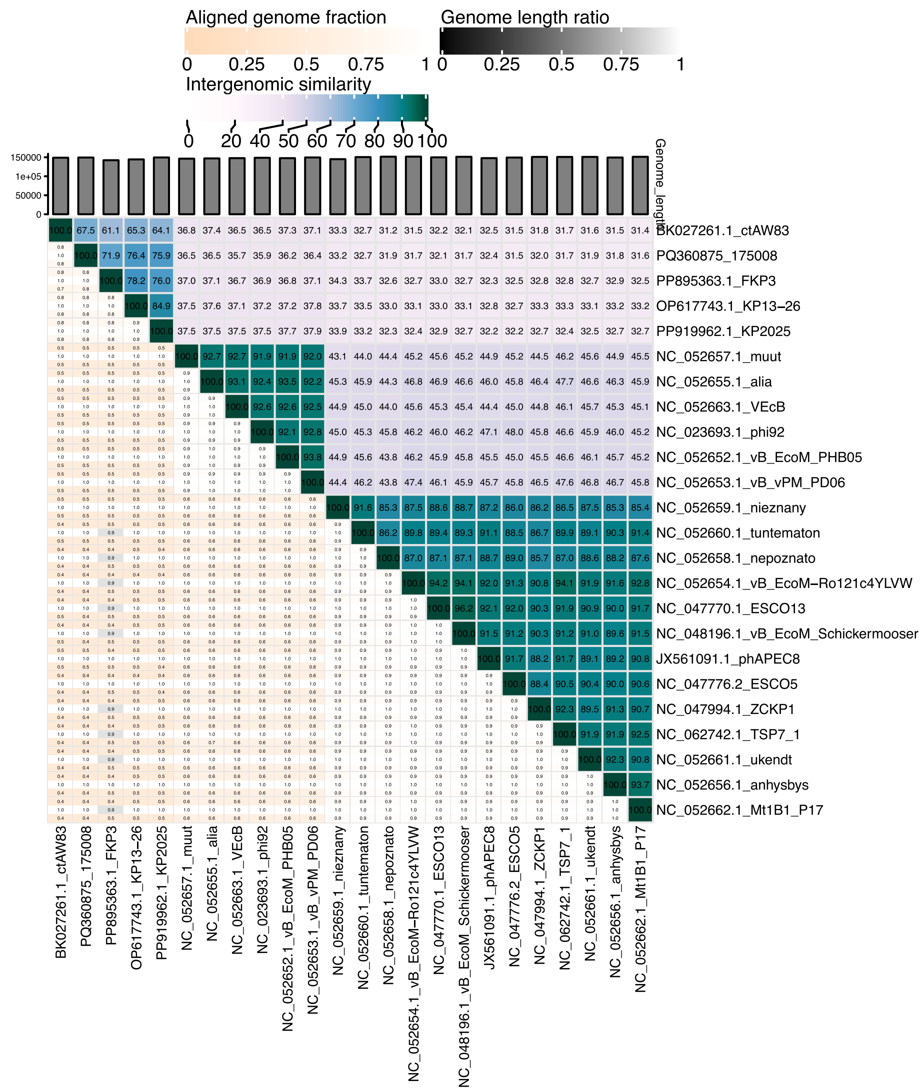

Fig. 2 Mutual intergenomic similarities among 175008, *Stephanstirmvirinae* phages in ICTV, and four related phages in GenBank. Genome sequences of the four related phages, KP13-26, KP2025, FKP3, and ctAW83 shared ≥ 70% coverage with that of 175008. Mutual intergenomic similarities (%) of phages whose names are shown with the accession numbers of their genome sequences are depicted in blue boxes. The alignment genome fractions being present underneath. The six phages of genus *Justusliebigvirus*, and the 13 of genus *Phapecoctavirus* are marked with "#" and "$", respectively. KP13-26, KP2025, FKP3, and 175008 are likely of the same genus and are marked with "*". This heatmap is generated using VIRIDIC [22] was used to generate this heatmap

spike domain (Supplementary Fig. S3), while there is no tail spike domain in the 346-aa tail fiber protein. In addition, a baseplate spike with 232 aa was identified. 175008 also encodes a 171-aa endolysin and a 144-aa tail lysozyme, which is a component of the baseplate central hub and functions as cell puncture [28]. Like those of *Justusliebigvirus* and *Phapecoctavirus* phages within the subfamily *Stephanstirmvirinae*, the genome of 175008 contains multiple tRNA genes, specifically 18 tRNA genes (Dataset S1), which were verified using tRNAscan-SE [21]. Notably, 175008 has genes encoding an 80-aa nicotinamide phosphoribosyltransferase, a 355-aa nicotinamide-nucleotide adenylyltransferase, and a 230-aa nicotinamide mononucleotide transporter (Dataset S1). These compose a functional pyridine nucleotide scavenging pathway for NAD+salvage during the metabolic period of the infection cycle [29].

175008 exhibits myoviruses' morphology, a narrow host range, and suitable features for clinical application

In transmission electron microscopy, 175008 exhibits about a 100 nm long tail attached to an about 46 nm

icosahedral head (Fig. 3), consistent with the appearance of myoviruses phages. 175008 was stable at pH between 3 and 11 and at temperatures between 4 to 50°C, while its titer decreased slightly by 1 log at 60°C but was not detectable at 70°C (Fig. 3). The MOI was 0.01 and with such a MOI, about 90% of 175008 were adsorbed to the host strain 135080 in 35 min (Fig. 3). In double-layer LB agar, 175008 formed a pinpoint-shape lytic plaque against 135080. In in vitro bactericidal assays, 175008 inhibited 135080 after 1 h and the inhibition lasted for about 3 h before bacterial regrowth (Fig. 3). Notably, 175008 only decrease the OD_{600} nm of the culture of 135080 from 0.5 to 0.3 but was unable to render it to become transparent. 175008 had a latent period of 35 to 40 min and a burst size of 25 progeny phages per infected bacterial cell (Fig. 3). As for the host range, 175008 was unable to lyse all 11 *E. coli* strains (Table S2). Against *K. pneumoniae* strains of 30 non-KL64 capsular types, 175008 exhibits a KL64-specific host range without the ability to lyse non-KL64 strains (Table S1).

Interruption of capsular polysaccharide biosynthesis-related gene *wcaJ* was responsible for resistance to 175008

To understand the mechanism mediating resistance to 175008, we obtained three 175008-resistant mutants of 135080, named 135080X3B6, 135080X3B7, and 135080X3B8, respectively, and performed whole genome sequencing for them. We then compared the genome sequences of the three 175008-resistant mutants with that of the parental strain 135080. We therefore identified that the *wcaJ* gene, which encodes a UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase involving in the capsular polysaccharide (CPS) biosynthesis, was interrupted by insertion sequence IS*903B* in 135080X3B6 or IS*Kpn14* in 135080X3B7 and 135080X3B8. The *wcaJ* gene is essential for the formation of the polysaccharide backbone. Previous studies including ours have demonstrated that interruption of *wcaJ* is a known resistance mechanism to phages in *K. pneumoniae* [8, 23, 30, 31]. The absorption of the three phage-resistant mutants at 35 min and 0.01 MOI was 42% (135080X3B6) or 62% (135080X3B7 and 135080X3B8), which was lower than the 90% of the parental strain 135080. This suggests that interruption of *wcaJ* compromises the absorption of phages to bacterial strains.

Discussion

In this study, we recovered and characterized a lytic phage against CRKP of the ST11-KL64 type, which represents a novel viral genus of subfamily *Stephanstirmvirinae*, class *Caudoviricetes*. Currently, only a few lytic phages against ST11-KL64 CRKP have been reported in the literature with most belonging to the genus *Przondovirus* of the family *Autographiviridae* [8, 9, 23, 32, 33] and few of the genus *Taipeivirus* of the family *Ackermannviridae* [34]. Both families belong to the order *Caudovirales* of the class *Caudoviricetes*. The identification of a phage of a new viral genus other than genera of families

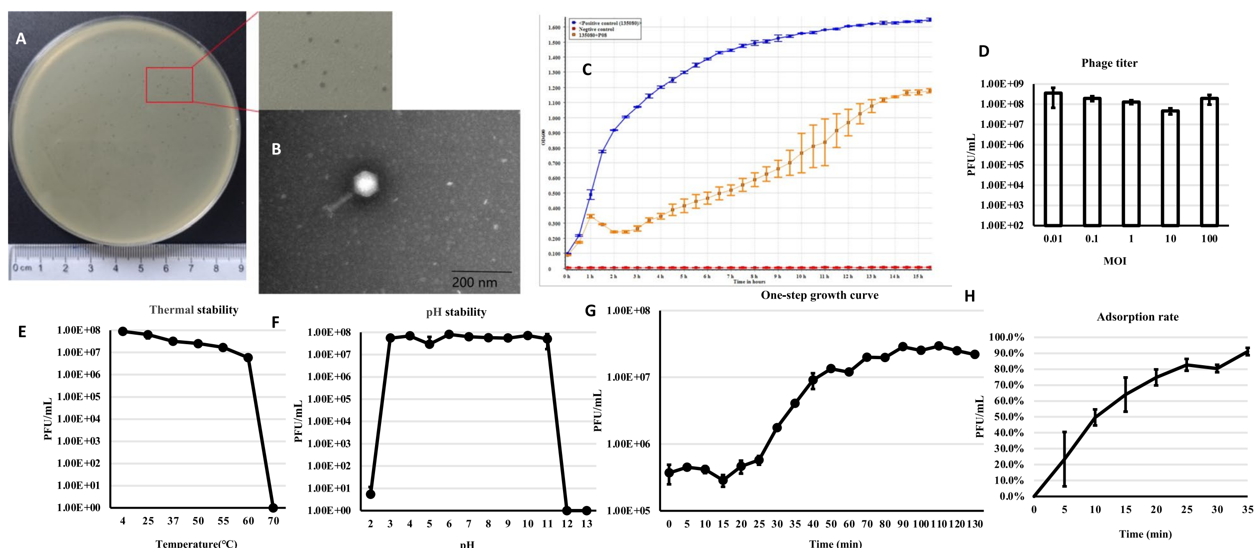

Fig. 3 The morphological and phenotypic features of phage 175008. **A** the pinpoint-shape lytic plaques against strain 135080 on double-layer LB agar. The plagues are enlarged for illustration. **B** the morphology under transmission electron microscopy. Scale, 200 nm. **C** in vitro bactericidal assay against strain 135080. Positive control, strain 135080 alone; negative control, PBS. **D** The optimal multiplicity of infection (MOI). PFU, plague forming units. **E** the stability to pH. **F** the stability to temperatures. **G** one step growth curve. **H** absorption rate

Autographiviridae and *Ackermannviridae* expands the arsenal against CRKP. Study of the resistance mechanisms uncovered that the receptor of 175008 is within CPS, the same as those of *Autographiviridae* and *Ackermannviridae* phages targeting ST11-KL64 CRKP [8, 9, 23, 32–34].

Among members of the subfamily *Stephanstirmvirinae*, all six *Justusliebigvirus* phages and 11 of the 13 phapecoctaviruses were isolated using *Escherichia coli* strains as host (Fig. 1). In contrast, phage TSP7_1 was recovered from a pigeon cloacal swab sample, but its host range remains to be studied [35]. The remaining *Phapecoctavirus* phage, ZCKP1, was isolated using a carbapenem-susceptible *K. pneumoniae* clinical strain from Egypt as the host [36]. Unfortunately, the ST and capsule type of the *K. pneumoniae* strain are unknown [36]. Nevertheless, 175008 is unable to lyse tested *E. coli* strains and exhibits a KL64-specific host range among *Klebsiella*.

A notable feature of 175008's genome is the presence of 18 tRNA genes. The exact function of phage tRNA genes remains to elucidated. However, the emerging evidence has demonstrated that multiple tRNA genes allow the lytic phage to sustain translation along with degradation of the host machinery degrades and therefore dynamically adapt the codon usage during the infection course [37]. In addition, phage-encoded tRNA may evade host tRNA-targeted defenses by becoming insensitive to host anticodon nucleases [38].

In conclusion, we identified a lytic phage representing a novel viral genus with the features suitable for phage therapy. Like previously-identified ST11-KL64-CRKP-targeting phages of different viral families, this phage also binds to the CPS. This highlights that phages with distinct taxonomic positions may act on the same target and more lytic phages against CRKP are waiting for discovery.

Abbreviations

CDS	Coding sequences
CLSI	Clinical Institute Standard Institute
CPS	Capsular polysaccharide
CRKP	Carbapenem-resistant *Klebsiella pneumoniae*
ICTV	International Committee on Taxonomy of Viruses
MOI	Multiplicity of infection
PFU	Plague forming unit
ST	Sequence type
TM	Tris–HCl-$MgSO_4$

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03736-0.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
We acknowledged Ms. Linwan Zhang for helping to manage the project.

Authors' contributions
Z.Z. and J.L. designed the study. J.L. isolated the phage. J.L., H.L., and Yan F. performed experiments. Q.F. and Yu F. analyzed genomic data. Z.Z. wrote the manuscript. All authors approved the final version.

Funding
The work was supported by grants from the National Natural Science Foundation of China (project no. 81861138055 and 82372290), the National Key Research and Development Program of China (grant no. 2022YFC2303900 and 2023YFC2308800), Natural Science Foundation of Sichuan Province (2024NSFSC1753), and grants from the West China Hospital of Sichuan University (1.3.5 project for disciplines of excellence, grant number ZYYC08006 and ZYGD22001).

Data availability
Genome sequence of phage 175008 has been deposited into GenBank under accession no. PQ360875. Genome sequences of the three 175008-resistant mutations of strain 135080 have also been deposited into GenBank under accession no. JBFXMD000000000, JBFXME000000000, and JBFXMF000000000, respectively.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 20 August 2024 Accepted: 30 December 2024
Published online: 13 January 2025

References

1. World Health Organization: WHO updates list of drug-resistant bacteria most threatening to human health. Geneva: World Health Organization 2024, https://www.who.int/news/item/17-05-2024-who-updates-list-of-drug-resistant-bacteria-most-threatening-to-human-health.
2. Agyeman AA, Bergen PJ, Rao GG, Nation RL, Landersdorfer CB. A systematic review and meta-analysis of treatment outcomes following antibiotic therapy among patients with carbapenem-resistant Klebsiella pneumoniae infections. Int J Antimicrob Agents. 2020;55:105833.
3. Hu Y, Yang Y, Feng Y, Fang Q, Wang C, Zhao F, McNally A, Zong Z. Prevalence and clonal diversity of carbapenem-resistant Klebsiella pneumoniae causing neonatal infections: A systematic review of 128 articles across 30 countries. PLoS Med. 2023;20:e1004233.
4. Uyttebroek S, Chen B, Onsea J, Ruythooren F, Debaveye Y, Devolder D, Spriet I, Depypere M, Wagemans J, Lavigne R, et al. Safety and efficacy of phage therapy in difficult-to-treat infections: a systematic review. Lancet Infect Dis. 2022;22:e208–20.
5. Pirnay JP, Djebara S, Steurs G, Griselain J, Cochez C, De Soir S, Glonti T, Spiessens A, VandenBerghe E, Green S, et al. Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study. Nat Microbiol. 2024;9:1434–53.
6. Eskenazi A, Lood C, Wubbolts J, Hites M, Balarjishvili N, Leshkasheli L, Askilashvili L, Kvachadze L, van Noort V, Wagemans J, et al. Combination of pre-adapted bacteriophage therapy and antibiotics for treatment of fracture-related infection due to pandrug-resistant Klebsiella pneumoniae. Nat Commun. 2022;13:302.
7. Zhou K, Xiao T, David S, Wang Q, Zhou Y, Guo L, Aanensen D, Holt KE, Thomson NR, Grundmann H, et al. Novel subclone of carbapenem-resistant Klebsiella pneumoniae sequence type 11 with enhanced virulence and transmissibility. China Emerg Infect Dis. 2020;26:289–97.

8. Fang Q, Feng Y, McNally A, Zong Z. Characterization of phage resistance and phages capable of intestinal decolonization of carbapenem-resistant Klebsiella pneumoniae in mice. Commun Biol. 2022;5:48.
9. Eckstein S, Stender J, Mzoughi S, Vogele K, Kuhn J, Friese D, Bugert C, Handrick S, Ferjani M, Wolfel R, et al. Isolation and characterization of lytic phage TUN1 specific for Klebsiella pneumoniae K64 clinical isolates from Tunisia. BMC Microbiol. 2021;21:186.
10. Turner D, Shkoporov AN, Lood C, Millard AD, Dutilh BE, Alfenas-Zerbini P, van Zyl LJ, Aziz RK, Oksanen HM, Poranen MM, et al. Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. Arch Virol. 2023;168:74.
11. Dion MB, Oechslin F, Moineau S. Phage diversity, genomics and phylogeny. Nat Rev Microbiol. 2020;18:125–38.
12. Gulyaeva A, Garmaeva S, Kurilshikov A, Vich Vila A, Riksen NP, Netea MG, Weersma RK, Fu J, Zhernakova A. Diversity and ecology of caudoviricetes phages with genome terminal repeats in fecal metagenomes from four dutch cohorts. Viruses. 2022;14:2305.
13. Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for Klebsiella pneumoniae and its related species complex. Nat Commun. 2021;12:4188.
14. Zurfluh K, Poirel L, Nordmann P, Klumpp J, Stephan R. First detection of Klebsiella variicola producing OXA-181 carbapenemase in fresh vegetable imported from Asia to Switzerland. Antimicrob Resist Infect Control. 2015;4:38.
15. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann M, Pettengill JB, Prasad AB, Tillman GE, et al. AMRFinderPlus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep. 2021;11:12728.
16. Green M, Sambrook J: Molecular Cloning: A Laboratory Manual, Fourth Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NE 2012, USA 2014.
17. Chen L, Fan J, Yan T, Liu Q, Yuan S, Zhang H, Yang J, Deng D, Huang S, Ma Y. Isolation and characterization of specific phages to prepare a cocktail preventing vibrio sp. Va-F3 Infections in Shrimp (Litopenaeus vannamei). Front Microbiol. 2019;10:02337.
18. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13:e1005595.
19. Bouras G, Nepal R, Houtak G, Psaltis AJ, Wormald PJ, Vreugde S. Pharokka: a fast scalable bacteriophage annotation tool. Bioinformatics. 2023;39:btac776.
20. Shang J, Tang X, Sun Y. PhaTYP: predicting the lifestyle for bacteriophages using BERT. Brief Bioinform. 2023;24:bbac487.
21. Chan PP, Lin BY, Mak AJ, Lowe TM. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. Nucleic Acids Res. 2021;49:9077–96.
22. Moraru C, Varsani A, Kropinski AM. VIRIDIC-a novel tool to calculate the intergenomic similarities of prokaryote-infecting viruses. Viruses. 2020;12:1268.
23. Yin X, Zong Z. Interruption of capsular polysaccharide biosynthesis gene wbaZ by insertion sequence IS903B mediates resistance to a lytic phage against ST11 K64 carbapenem-resistant Klebsiella pneumoniae. msphere. 2022;7:00518–22.
24. Kutter E. Phage host range and efficiency of plating. Methods Mol Biol. 2009;501:141–9.
25. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–77.
26. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421.
27. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. 2006;34:D32-36.
28. Arisaka F, Kanamaru S, Leiman P, Rossmann MG. The tail lysozyme complex of bacteriophage T4. Int J Biochem Cell Biol. 2003;35:16–21.
29. Lee JY, Li Z, Miller ES. Vibrio phage KVP40 encodes a functional NAD(+) salvage pathway. J Bacteriol. 2017;199:00855–816.
30. Tan D, Zhang Y, Qin J, Le S, Gu J. A Frameshift mutation in wcaJ Associated with Phage Resistance in Klebsiella pneumoniae. Microorganisms. 2020;8:378.
31. Cai R, Wang G, Le S, Wu M, Cheng M, Guo Z, Ji Y, Xi H, Zhao C, Wang X, et al. Three capsular polysaccharide synthesis-related glucosyltransferases, GT-1, GT-2 and WcaJ, are associated with virulence and phage sensitivity of Klebsiella pneumoniae. Front Microbiol. 2019;10:1189.
32. Li M, Li P, Chen L, Guo G, Xiao Y, Chen L, Du H, Zhang W. Identification of a phage-derived depolymerase specific for KL64 capsule of Klebsiella pneumoniae and its anti-biofilm effect. Virus Genes. 2021;57:434–42.
33. Li M, Xiao Y, Li P, Wang Z, Qi W, Qi Z, Chen L, Du H, Zhang W. Characterization and genome analysis of Klebsiella phage P509, with lytic activity against clinical carbapenem-resistant Klebsiella pneumoniae of the KL64 capsular type. Arch Virol. 2020;165:2799–806.
34. Feng Y, Fang Q, Luo H, Li J, Yin X, Zong Z. Safety and efficacy of a phage cocktail on murine wound infections caused by carbapenem-resistant Klebsiella pneumoniae. Int J Antimicrob Agents. 2024;63:107088.
35. Khalifeh A, Kraberger S, Dziewulska D, Stenzel T, Varsani A. Complete genome sequence of a phapecoctavirus isolated from a pigeon cloacal swab sample. Microbiol Resour Announc. 2021;10:01471–1420.
36. Taha OA, Connerton PL, Connerton IF, El-Shibiny A. Bacteriophage ZCKP1: a potential treatment for klebsiella pneumoniae isolated from diabetic foot patients. Front Microbiol. 2018;9:2127.
37. Yang JY, Fang W, Miranda-Sanchez F, Brown JM, Kauffman KM, Acevero CM, Bartel DP, Polz MF, Kelly L. Degradation of host translational machinery drives tRNA acquisition in viruses. Cell Syst. 2021;12(771–779):e775.
38. Knecht LE, Veljkovic M, Fieseler L. Diversity and function of phage encoded depolymerases. Front Microbiol. 2019;10:2949.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.