Characterization and Genomic Analysis of Bacteriophage vB_KpnM_IME346 Targeting Clinical Klebsiella pneumoniae Strain of the K63 Capsular Type

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Abstract
A Klebsiella pneumoniae bacteriophage (vB_KpnM_IME346) was isolated from a hospital sewage sample. This bacteriophage specifically infects a clinical K. pneumoniae strain with a K63 capsular polysaccharide structure. The phage genome was evaluated by next-generation sequencing, which revealed a linear double-stranded DNA genome consisting of 49,482 base pairs with a G+C content of 49.1%. The latent period of vB_KpnM_IME346 was shown to be 20 min, and the burst size was 25–30 pfu (plaque-forming units)/infected cell. Transmission electron microscopy and phylogenetic analysis showed that the JD001-like phage belongs to the genus Jedunavirus of the family Myoviridae. The newly isolated vB_KpnM_IME346 shows infectivity in the clinical host K. pneumoniae KP576 strain, indicating that it is a promising alternative to antibacterial agents for removing K. pneumoniae from patients.

Introduction
Klebsiella pneumoniae is a Gram-negative opportunistic pathogenic bacterium and common cause of hospital- and community-acquired urinary tract infections, sepsis, and pneumonia [1]. In recent decades, cases of community-acquired suppurative liver abscess, caused by K. pneumoniae and complicated with metastatic meningitis and endophthalmitis have emerged worldwide, particularly in Asia [2–4], and the clinical picture is rapidly progressing. Uncontrolled application of various antibacterial agents and frequent exposure of various K. pneumoniae isolates to antibacterial agents cause multidrug resistance among the strains [5]. Although antibiotics remain as the first-line treatment for K. pneumoniae infections, alternative treatments are urgently needed because of increasing rates of antibiotic resistance. Infections due to carbapenemase-producing K. pneumoniae (CPKp) have been recognized as an emerging challenge worldwide [6]. One possible strategy for effectively treating K. pneumoniae infections without risking drug-resistant strain development is to exploit the ability of lytic bacteriophages to target pathogenic bacteria.

Bacteriophages, also known as phages, are viruses that specifically recognize their bacterial hosts. Since their discovery 100 years ago phage research has changed basic biology and medicine. With increases in antibiotic resistance, phage therapy has provided a new perspective for treating infections [7]. Recently, an open study using a cocktail of 12 phages to treat patients with burn wound infections of Pseudomonas aeruginosa demonstrated the effectiveness of topical administration of phage therapy. This was the first clinical trial of phage therapy performed in compliance with both good manufacturing practices and good clinical practices [8]. Thus, phage therapy shows potential for clinical treatment. To date, approximately 100 Klebsiella phages have been isolated from different geographic regions, and the
were mixed at a multiplicity of infection of 0.01 (10^7:10^9). Briefly, the phage and bacterial culture in the log phase 
A one-step growth experiment was performed to determine OD
LB culture (\( \text{OD}_{600} = 0.6 \)) and 3 mL 3 × LB. This mixture
500 μL of an exponentially growing K. pneumoniae KP576
membrane. A 3-mL aliquot of the filtrate was mixed with
The supernatant was filtered through a 0.22-μm microporous
from the hospital and centrifuged at 10,000× 
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LB culture (\( \text{OD}_{600} = 0.6 \)) and 3 mL 3 × LB. This mixture
was incubated overnight at 37 °C with shaking, followed by
filtration through a 0.22-μm membrane. The double-layer
agar procedure of phage isolation was repeated four times.

Biological Properties
A one-step growth experiment was performed to determine the lysis curve and phage burst size, as described previously [13]. Briefly, the phage and bacterial culture in the log phase
were mixed at a multiplicity of infection of 0.01 (10^7:10^9
number of phage/number of bacteria ratio), and the phage
was allowed to adsorb to the bacterial cells for 1 min at
37 °C. The mixture was washed with LB medium to remove
unabsorbed phages and avoid secondary adsorption. The
culture was incubated at 37 °C with shaking, and samples
were collected at 5- and 10-min intervals. The phage titers
were then determined using the double-layer agar method.
Phage morphology was further visualized and characterized
by transmission electron microscopy (JEM-1200EX, Jeol,
Tokyo, Japan) at an accelerating voltage of 100 kV.

Host Range Determination
Bacterial strain susceptibility levels were determined as previously described [12]. A total of 12 K. pneumoniae (including capsular type K63 (3/12), K47 (4/12), KN3 (1/12), K64 (2/12), and K81 (2/12)) strains were used for host range evaluation. First, the spotting method was used to evaluate the susceptibility of the bacterial strain to the phage and then the efficiency of plating was determined [14] by the double-layer agar method. The efficiency of plating values
was determined by calculating the ratio of plaque-forming units of each phage-susceptible strain to plaque-forming units of the indicator strain (K. pneumoniae KP576). This experiment was repeated three times.

DNA Extraction, Whole-Genome Sequencing,
and Genomic Analysis
The genomic DNA of the phage was extracted using a standard phenol–chloroform protocol [12]. Briefly, the purified phages were treated by proteinase K (100 mg/mL), SDS
(10%, w/v), and EDTA (0.5 mM, pH 8.0) at 56 °C in water
for 2 h. After that, the sample was washed three times using an equal volume of mixture composed of phenol, chloro-
form, and isooamyl alcohol (25:24:1), followed by centrifu-
gation at 4 °C, 12,000×g for 10 min, to remove the debris.
Then, the supernatant was mixed with isooamyl alcohol kept
at 20 °C overnight. The air-dried precipitate was washed
three times with cold 75% ethanol, and the phages’ genomic
DNA was finally dissolved in TE buffer (10 mM Tris–HCl,
1 mM EDTA [pH 8.0]).

High-throughput sequencing of the phage genomic DNA
was performed using the Illumina MiSeq platform (San
Diego, CA, USA). The whole-genome sequence was assem-
bled using Newbler V3.0 software [15], and annotations
were conducted using the RAST online database (http://
www.rast.nmpdr.org) [16]. Antimicrobial resistance and bac-
terial virulence factors were determined from the following
databases: Virulence Factor Database [17], Comprehensive
Antibiotic Resistance Database [18], Antibiotic Resist-
ance Gene-ANNOtation [19], and ResFinder [20]. Trans-
fer RNAs (tRNAs) were predicted using the trNAscan-SE
software [21]. To identify gene homologs, the genomes of
vB_KpnM_IME346 and vB_KpnM_KpV52 were analyzed
in CoreGenes3.5 for protein identity (http://binf.gmu.edu:
8080/CoreGenes3.5/). The DNA sequence of the polymer-
ase-encoding gene of phage vB_KpnM_IME346 and other
homologous sequences, obtained from the International Committee on Taxonomy of Viruses (https://talk.ictvonline.org/taxonomy/), was used to construct a phylogenetic tree using MEGA 6.0 [22] and neighbor-joining method using 1000 guide repeats. All analyses except those indicated were performed using default parameters.

Results and Discussion

Phage Isolation and Morphology

Using *K. pneumoniae* KP576 as an indicator strain, a previously unidentified phage was isolated and designated as vB_KpnM_IME346. Electron micrography showed that the phage had a typical icosahedral structure and contractile tail, with a head diameter of approximately 53 ± 1 nm and tail length of approximately 83 ± 2 nm, which are characteristic features of phages into the family **Myoviridae** (Fig. 1a).

Biological Properties of Phage vB_KpnM_IME346

The one-step growth curve of the phage showed an incubation period of approximately 20 min. During this period, the number of plaques did not increase, indicating that the phage had not completed replication and assembly. After reaching the plateau phase at 110 min, the burst size was approximately 25–30 plaque-forming unit/cell, indicating that the phage had completely lysed the host cells. The burst size was calculated as the ratio of the final count of phage particles released at the end of the incubation period to the initial count of infected bacterial cells (Fig. 1b).

Host Range Determination

Twelve *K. pneumoniae* (including capsular type K63 (3/12), K47 (4/12), KN3 (1/12), K64 (2/12), and K81 (2/12)) strains were used for phage lysis assays to determine the lytic host range of phage vB_KpnM_IME346. Notably, phage vB_KpnM_IME346 lysed 3 K63 strains but had no effect on strains with capsular type K47, KN3, K64, and K81 (Table 1). The phage specifically targeting *K. pneumoniae* capsular-type K63 strains showed specific host ranges, indicating that it may be useful typing of *K. pneumoniae*.

Genomic Annotation and Analysis

The whole-genome sequence of phage vB_KpnM_IME346 has been deposited in the NCBI database (GenBank accession number: MK685667). The Phage termini of vB_KpnM_IME346 were identified using our proposed “terminus analysis theory” method, without identification of any fixed termini [23]. Therefore, phage vB_KpnM_IME346 genome may adopt the rolling-circle replication mechanism to generate a number of head-to-tail DNA concatemers which serve as substrates for viral DNA packaging [24]. The assembled phage vB_KpnM_IME346 has a linear double-stranded DNA genome with a G+C content of 49.1%. A total of 80 putative open reading frames (ORFs) were predicted in the phage genome, with ATG as the start codon for 77 ORFs and GAT, GTG, and TTG as the start codons for the remaining three ORFs. Putative functions were assigned to 32 products of predicted vB_KpnM_IME346 ORFs, and the remaining 48 ORFs encoded putative hypothetical proteins. No tRNA genes were identified. The entire genome structure of phage vB_KpnM_IME346 is shown in Fig. 2; the arrows represent

Fig. 1  a Transmission electron microscopy of phage vB_KpnM_IME346. Scale bar indicates 100 nm; b one-step growth curve of phage vB_KpnM_IME346. The phage vB_KpnM_IME346 showed a 15-min latent period at 37 °C, with an average burst size of 139 phage particles per infected cell after 100 min. The data are expressed as the means ± SD. The curve represents average results from three independent experiments.
predicted ORFs consisting of genes involved in the phage structure, metabolism, phage structure, DNA replication and regulation, and in the lysis system as well as other predicted functions (Table 1).

We identified three ORFs as potential genes in the replication and regulation modules, including DNA primase (ORF61), DNA helicase (ORF66), and DNA polymerase (ORF80), which play important roles in phage replication. The metabolism cassette module of the genome contains five ORFs; BLASTn analysis of these regions revealed putative glycosyltransferase (ORF46, ORF58), transketolase protein (ORF57), site-specific DNA-methyltransferase (ORF65), and N-acetyltransferase (ORF72), which showed low identity to the corresponding phage sequences in other phages (Fig. 3; Table S1).

Among the 80 predicted ORFs in the phage vB_KpnM_IME346 genome, we identified two putative ORFs encoding proteins associated with lysis function, including endolysin (ORF5) and lytic transglycosylase (ORF18), with a lysozyme-like domain involved in the hydrolysis of beta-1,4-linked polysaccharides. Proteins involved in DNA packaging were encoded by genes in the phage vB_KpnM_IME346 genome, including phage large subunit terminase (ORF53) and a phage small subunit terminase (ORF54), showing 478/482 (99%) and 151/152 (99%) identity to vB_KpnM_ KpV79 (NC_042041.1) and JD001 (JX866719.1), respectively. These genes code for the subunits of the terminase protein which is involved in packaging the DNA into the head shell [25, 26]. Five genes were identified to be involved in forming the phage structure. Baseplate protein (ORF16) plays an important role in the infection by phage by catalyzing local cell wall digestion to facilitate penetration of the tail tube through the cell envelope [27]. ORF19 (tape measure protein) was found to be highly similar to that of Klebsiella phage JD001, with an identity of 415/477 (87%) and E-value of 0. The tape measure protein dictates the tail length and facilitates DNA transit to the cell cytoplasm during infection [28]. Interestingly, a recent study showed that a few Lactococcus lactis phages were highly thermoresistant and these results indicate that the tape measure protein contributes to heat stability [29]. ORF48 encoded head morphogenesis protein that is highly similar to Pectobacterium phage PEAT2 (MG432137.1) with an identity of 97% (245/253), which mainly mediates phage head assembly.

Additionally, 49 genes of unknown function were identified. Furthermore, no toxin-, virulence factor-, or antibiotic resistance-related genes were found, indicating that vB_KpnM_IME346 is a virulent and potential candidate for phage therapy. The genome of phage vB_KpnM_IME346 contains numerous hypothesis proteins of unknown function; therefore, further comprehensive functional analysis is required to determine the safety of using these phages in therapeutic applications.

BLAST alignment (megablast) indicated that the genome of phage vB_KpnM_IME346 showed the greatest nucleotide sequence identity (90.36% identity, 66% coverage) with the genome sequence of Klebsiella phage vB_KpnM_KpV52 (NC_041900.1). CoreGenes 3.5 analysis revealed that 55 genes were shared by vB_KpnM_IME346 and vB_KpnM_ KpV52, whereas 24 genes were unique to vB_KpnM_IME346. Among the unique genes, the main functional
proteins were glycosyltransferase (ORF58), site-specific DNA-methyltransferase (ORF65), N-acetyltransferase (ORF72), and large subunit terminase (ORF53).

To investigate the taxonomy of phage vB_KpnM_IME346, a phylogenetic tree was constructed using the DNA polymerase sequences, including that of the phage and those of other phages in the genus Jedunavirus. The results obtained from the phylogenetic analysis that phage vB_KpnM_IME346 belongs to a JD001-like virus of unclassified genus Jedunavirus. The ORF involved in DNA packaging is shown in yellow. The ORF involved in metabolism is shown in green. The ORF involved in structure is shown in red. (Color figure online)

Fig. 3 Phage vB_KpnM_IME346 genome map. Arrows represent predicted genes, and the direction of each arrow represents the direction of transcription. Different colors denote different phage gene functional groups. The genome starts upstream of ORF1. The ORF involved in DNA replication and regulation is shown in yellow. The ORF involved in DNA packaging is shown in yellow. The ORF involved in metabolism is shown in green. The ORF involved in structure is shown in purple. Red represents the ORF for phage lytic, and ORFs without phage-related functions are shown in dark red.

We investigated the biological properties and genomic organization of virulent bacteriophage vB_KpnM_IME346, which infected the clinical K. pneumoniae KP576 strain. Importantly, toxin-, pathogen-, and drug resistance-related genes were not detected in the vB_KpnM_IME346 genome. Overall, the good lytic ability and unique genomic characteristics of vB_KpnM_IME346 highlight its potential for use as a clinical phage capsule type and drug-resistant treatment for K. pneumoniae.

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Author Contributions GMM isolated the phage IME346. YLX, WY, and GJ characterized their biological characteristics. ZZLL, LHY, and PGQ completed the genome-wide sequencing and genomic analysis. TYG and BCQ conceived and designed the experiments. GMM and BCQ drafted the manuscript. All authors read and agreed to the publication of the manuscript.
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Declarations

Conflict of interest  The authors declare that they have no conflict of interest.

Ethical Approval  The studies involving human participants were reviewed and approved by the Ethics Committee of the Fifth Medical Center of Chinese PLA General Hospital (Former 307th Hospital of PLA), and an exemption of informed consent was obtained (Ethics approval No. ky-2018-10-85).

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References

1. Cai Y, Lo J, Venkatachalam I, Kwa A, Tambyah P, Hsu L, Barnett A, Marimuthu K, Graves N (2020) The impact of healthcare associated infections on mortality and length of stay in Singapore—a time-varying analysis. Infect Control Hosp Epidemiol 41(11):1315–1320
2. Lin Y, Siu L, Lin J, Chen T, Tseng C, Yeh K, Chang F, Fung C (2012) Seroepidemiology of Klebsiella pneumoniae colonizing the intestinal tract of healthy Chinese and overseas Chinese adults in Asian countries. BMC Microbiol 12:13
3. Lok KH, Li KF, Li KK, Szeto ML (2008) Pyogenic liver abscess: clinical profile, microbiological characteristics, and management in a Hong Kong hospital. J Microbiol Immunol Infect 41(6):483–490
4. Yeh KM, Kurup A, Siu LK, Koh YL, Fung CP, Lin JC, Chen TL, Chang FY, Koh TH (2007) Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for Klebsiella pneumoniae liver abscess in Singapore and Taiwan. J Clin Microbiol 45(2):466–471
5. Falcone M, Russo A, Iacovelli A, Restuccia G, Ceccarelli G, Giordano A, Farcomeni A, Morelli A, Venditti M (2016) Predictors of outcome in ICU patients with septic shock caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. Clin Microbiol Infect 22(5):444–450
6. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y (2008) Isolation of imipenem-resistant Enterobacter species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. Antimicrob Agents Chemother 52(4):1413–1418
7. Wittebole X, De Roock S, Opal SM (2014) A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence 5(1):226–235
8. Chen Y, Yang L, Sun E, Song J, Wu B (2019) Characterisation of a newly detected bacteriophage infecting Bordetella bronchiseptica in swine. Arch Virol. 164(1):33–40. https://doi.org/10.1007/s00705-018-4034-0
9. Majkowska-Skrobek G, Łątka A, Berisio R, Maciejewska B, Squeglia F, Romano M, Lavigne R, Struve C, Drulis-Kawa Z (2016) Capsule-targeting depolymerase, derived from Klebsiella KP36 phage, as a tool for the development of anti-virulent strategy. Viruses 8(12):324
10. Volozhantsev NV, Shpirt AM, Borzilov AI, Komisarova EV, Krasilnikova VM, Shashkov AS, Verevkin VV, Kniel A (2020) Characterization and therapeutic potential of bacteriophage-encoded polysaccharide depolymerases with β galactosidase activity against Klebsiella pneumoniae K57 capsular type. Antibiotics 9(11):732
11. Pan YJ, Lin TL, Chen YH, Hsu CR, Hsieh PF, Wu MC, Wang JT (2013) Capsular types of Klebsiella pneumoniae revisited by wcc sequencing. PLoS ONE 8(12):e80670
12. Chen Y, Sun E, Song J, Yang L, Wu B (2018) Complete genome sequence of a novel T7-like bacteriophage from a Pasteurella multocida capsular type A isolate. Curr Microbiol 75(5):574–579
13. Delbruck M (1940) The growth of bacteriophage and lysis of the host. J Gen Physiol 23(5):643–660
14. Khan Mirzaei M, Nilsson AS (2015) Isolation of phages for phage therapy: a comparison of spot tests and efficiency of plating analyses for determination of host range and efficacy. PLoS ONE 10(3):e0118557
15. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jurage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz EM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McCready DE, McKenna PM, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srivinasan M, Tartaro KR, Tomasz A, Vogt KA, Vollmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437(7057):376–380
16. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formisano K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil RK, Paarmann D, Paczian T, Parello B, Pusch GD, Reich C, Stevens R, Vavivasa O, von Stein VA, Wilke A, Zagnitko O (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75
17. Chen L, Zheng D, Liu B, Yang J, Jin Q (2016) VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. Nucleic Acids Res 44(D1):D694–D697
18. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Eijim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O’Brien JS, Pawlowski AC, Piddock LJ, Spanogiannopoulos P, Sutherland AD, Tang I, Tartaro KR, Watanabe J, Weiner MP, Yu BP, Begley RF, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437(7057):376–380
19. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, Rolain JM (2014) ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58(1):212–220
20. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67(11):2640–2644

21. Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25(5):955–964

22. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30(12):2725–2729

23. Li S, Fan H, An X, Fan H, Jiang H, Chen Y, Tong Y (2014) Scrutinizing virus genome termini by high-throughput sequencing. PLoS ONE 9(1):e85806

24. Rao VB, Feiss M (2008) The bacteriophage DNA packaging motor. Annu Rev Genet 42:647–681

25. Sun S, Rao VB, Rossmann MG (2010) Genome packaging in viruses. Curr Opin Struct Biol 20(1):114–120

26. Duffy C, Feiss M (2002) The large subunit of bacteriophage lambda’s terminase plays a role in DNA translocation and packaging termination. J Mol Biol 316(3):547–561

27. Kao SH, McClain WH (1980) Baseplate protein of bacteriophage T4 with both structural and lytic functions. J Virol 34(1):95–103

28. Boulanger P, Jacquot P, Plancon L, Chami M, Engel A, Parquet C, Herbeuval C, Letellier L (2008) Phage T5 straight tail fiber is a multifunctional protein acting as a tape measure and carrying fusogenic and muralytic activities. J Biol Chem 283(20):13556–13564

29. Geagea H, Labrie SJ, Subirade M, Moineau S (2018) The tape measure protein is involved in the heat stability of Lactococcus lactis phages. Appl Environ Microbiol. https://doi.org/10.1128/AEM.02082-17

30. Gabashvili E, Kobakhidze S, Koulouris S, Robinson T, Kotetishvili M (2021) Bi- and multi-directional gene transfer in the natural populations of polyvalent bacteriophages, and their host species spectrum representing foodborne versus other human and/or animal pathogens. Food Environ Virol 13(2):179–202

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