Characterizing the Biology of Lytic Bacteriophage vB_EaeM_ϕEap-3 Infecting Multidrug-Resistant Enterobacter aerogenes

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Carbapenem-resistant Enterobacter aerogenes strains are a major clinical problem because of the lack of effective alternative antibiotics. However, viruses that lyze bacteria, called bacteriophages, have potential therapeutic applications in the control of antibiotic-resistant bacteria. In the present study, a lytic bacteriophage specific for E. aerogenes isolates, designated vB_EaeM_ϕEap-3, was characterized. Based on transmission electron microscopy analysis, phage vB_EaeM_ϕEap-3 was classified as a member of the family Myoviridae (order, Caudovirales). Host range determination revealed that vB_EaeM_ϕEap-3 lyzed 18 of the 28 E. aerogenes strains tested, while a one-step growth curve showed a short latent period and a moderate burst size. The stability of vB_EaeM_ϕEap-3 at various temperatures and pH levels was also examined. Genomic sequencing and bioinformatics analysis revealed that vB_EaeM_ϕEap-3 has a 175,814-bp double-stranded DNA genome that does not contain any genes considered undesirable for the development of therapeutics (e.g., antibiotic resistance genes, toxin-encoding genes, integrase). The phage genome contained 278 putative protein-coding genes and one tRNA gene, tRNA-Met (AUG). Phylogenetic analysis based on large terminase subunit and major capsid protein sequences suggested that vB_EaeM_ϕEap-3 belongs to novel genus “Kp15 virus” within the T4-like virus subfamily. Based on host range, genomic, and physiological parameters, we propose that phage vB_EaeM_ϕEap-3 is a suitable candidate for phage therapy applications.

Keywords: E. aerogenes, bacteriophage, vB_EaeM_ϕEap-3, genome sequencing, Myoviridae

INTRODUCTION

Over the last three decades, Enterobacter aerogenes has increasingly been recognized as an important opportunistic and multidrug-resistant bacterial pathogen associated with nosocomial infections (Davin-Regli and Pages, 2015). The more frequent reports of carbapenem-resistant E. aerogenes are particularly concerning from a public health standpoint. Carbapenems are
first-line drugs for the treatment of severe nosocomial infections caused by multidrug-resistant Enterobacteriaceae (Qin et al., 2014). Owing to the emergence of carbapenem-resistant strains, treatment options for patients suffering from *E. aerogenes* infection are limited, which can have serious consequences. As such, clinicians should be alert to carbapenem-resistant *E. aerogenes* infection to ensure the timely initiation of appropriate therapy (Kuai et al., 2014; Tuon et al., 2015).

Recently, there has been increased interest in the use of obligate lytic phages as a possible alternative or supplement to traditional antibiotics for the treatment of antibiotic-resistant pathogens (Lu and Koeris, 2011). The advantages of phage therapy over currently available antibiotics include rapid self-proliferation, minimal impact on normal flora, ability to control biofilms, and low intrinsic toxicity (Kim et al., 2015). Before clinical application, potential therapeutic phages must be comprehensively examined to ensure safety and efficacy (Lin et al., 2017; Philipson et al., 2018). As yet, *E. aerogenes* bacteriophages have not been extensively investigated. Currently, there are only four reported fully-sequenced *E. aerogenes* phages: F20 (JN672684; Mishra et al., 2012), vB_EaeM_ϕEap-2 (KC028695; Li et al., 2016), vB_EaeM_ϕEap-1 (NC_028772), and UZ1 (unclassified; Verthe et al., 2004). F20 was classified as belonging to the Siphoviridae family of T1-like viruses (Mishra et al., 2012), vB_EaeM_ϕEap-2 also belongs to the family Siphoviridae and is related to *Salmonella* phage FSL SP-031 (KCI39518; Li et al., 2016), and vB_EaeM_ϕEap-1 (NC_028772) is a member of the family Podoviridae. In the current study, we focused on *E. aerogenes* phage vB_EaeM_ϕEap-3, a T4-like bacteriophage belonging to the genus “Kp15 virus” within the family Myoviridae.

**MATERIALS AND METHODS**

**Bacterial Host and Culture Conditions**

*Enterobacter aerogenes* clinical strain 3-SP is a generous gift from Dr. Dongsheng Zhou, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China, which is isolated from a human case of pneumonia at a Chinese teaching hospital (Chen et al., 2015). The isolate was originally obtained as part of routine patient care. Approval was obtained for this original procedure. Informed Oral consent was obtained, and this was sufficient for the ethics committee. Approval was not needed for this retrospective study, as approval had been obtained for the original study. Strain 3-SP was used as a host for the isolation and proliferation of phage vB_EaeM_ϕEap-3 and contains a pNDM-BJ01-like conjugative plasmid named p3SP-NDM that confers carbapenem resistance (Chen et al., 2015).

**Phage Isolation and Purification**

Bacteriophage vB_EaeM_ϕEap-3 was isolated from a sewage wastewater sample from the Navy General Hospital, Beijing, China, using the double-layer overlay technique and *E. aerogenes* 3-SP as the indicator strain, as previously described (Wommack et al., 2009). Briefly, 0.22 μm filtrates of sewage samples were mixed with *E. aerogenes* 3-SP culture to enrich the phage at 37°C. The culture was centrifuged and the supernatant was filtered through a 0.22 μm pore-size membrane to remove the residual bacterial cells. Aliquots of the diluted filtrate were mixed with *E. aerogenes* culture; 3 mL of molten top soft nutrient agar (0.4% agar) was added and mixed, and overlaid on the solidified base nutrient agar (1.5% agar). Following incubation overnight at 37°C, clear phage plaques were picked from the plate. A pure phage suspension was obtained by three rounds of single-plaque purification and reinfection of the exponentially growing 3-SP strain, as reported previously (Kropinski et al., 2009b). Phage titer are expressed in plaque-forming units (PFUs)/mL and were measured using a soft agar overlay method (Kropinski et al., 2009a).

**Transmission Electron Microscopy (TEM)**

The phage particle preparation was centrifuged at 20,000 × g for 2 h and the resulting pellet resuspended in SM buffer (10 mM Tris-HCl, pH 7.5; 100 mM NaCl; 10 mM MgSO₄) to a concentration of ∼10⁹ PFU/mL. Samples were processed by negative staining with 2% (wt/vol) uranyl acetate for 30 s and examined using a Tecnai Spirit 120-kV transmission electron microscope (FEI Company, Hillsboro, OR, United States) at different magnitudes to determine the phage morphology.

**Determination of Bacteriophage Host Range**

The host range of phage vB_EaeM_ϕEap-3 was determined via the spot test method using 28 *E. aerogenes* strains, 16 enterobacterial isolates (*Enterobacter cloacae*, *n* = 2; *Enterobacter sakazakii*, *n* = 2; *Serratia marcescens*, *n* = 5; *Klebsiella pneumoniae*, *n* = 1; *Leclercia adecarboxylata*, *n* = 1; *Raoultella ornithinolytica*, *n* = 1; *Citrobacter freundii*, *n* = 1; *Shigella sonnei*, *n* = 1; *Vibrio parahaemolyticus*, *n* = 1; and *Escherichia coli*, *n* = 1), three Gram-negative non-fermenters (*Pseudomonas aeruginosa*, *n* = 1; *Acinetobacter baumannii*, *n* = 1; and *Achromobacter xylosoxidans*, *n* = 1), and one Gram-positive bacterium (*Stenotrophomonas maltophilia*, *n* = 1). Each strain was grown in 5 mL of Luria–Bertani (LB) broth at 37°C to an optical density at 600 nm (OD₆₀₀) of 0.5 before being centrifuged at 4000 × g for 5 min and resuspended in 3 mL of SM buffer. A 0.2-mL aliquot of bacterial suspension was then mixed with 3 mL of molten soft-agar and poured onto an LB agar plate. After the agar had solidified, 0.01 mL of phage suspension was spotted onto the overlay. Sensitivity of the bacterium to vB_EaeM_ϕEap-3 infection was assessed following overnight incubation as described previously (Kutter, 2009).

**One-Step Growth Curve**

The latency period and burst size of vB_EaeM_ϕEap-3 were determined by monitoring dynamic changes in the number of phage particles during a replicative cycle. Briefly, host strain 3-SP was grown at 37°C to mid-exponential phase (OD₆₀₀ = 0.4–0.5) before being centrifuged at 4000 × g for 10 min at 4°C. The cell pellet was then resuspended in a 0.1-volume of SM buffer. A 0.1-mL aliquot of phage suspension was then added to 0.9 mL of the
bacterial suspension to achieve a multiplicity of infection of 0.01. Phages were allowed to absorb for 5 min at 37°C, and then the mixture was centrifuged twice at 16,000 × g for 2 min to remove the unabsorbed phages. The mixtures were then resuspended in 10 mL of LB broth and incubated at 37°C. Samples were collected at 10-min intervals for 80 min with or without 1% chloroform and immediately diluted and plated for phage titer assays (Pajunen et al., 2000). Results are reported as the average phage titer, while the burst size was calculated by dividing the average PFU/mL of the latent period by the average PFU/mL of the last three time points of the experiment (Buttimer et al., 2018). Results are the mean of three replicates ± standard deviation.

#### TABLE 1 | Host spectrum of vB_EaeM_ϕ Eap-3.

| ID | Lysis |
|----|-------|
| E. aerogenes 3-SP | + |
| E. aerogenes 13208 | − |
| E. aerogenes A29864 | − |
| E. aerogenes A36179 | − |
| E. aerogenes 201316724 | + |
| E. aerogenes 2015-301 | + |
| E. aerogenes AH10 | + |
| E. aerogenes AH12 | + |
| E. aerogenes AH13 | + |
| E. aerogenes AH14 | − |
| E. aerogenes AH15 | + |
| E. aerogenes AH17 | − |
| E. aerogenes AH18 | + |
| E. aerogenes AH2 | − |
| E. aerogenes AH20 | + |
| E. aerogenes AH21 | + |
| E. aerogenes AH22 | − |
| E. aerogenes AH24 | − |
| E. aerogenes AH25 | + |
| E. aerogenes AH28 | − |
| E. aerogenes AH29 | + |
| E. aerogenes AH3 | − |
| E. aerogenes AH30 | − |
| E. aerogenes AH32 | − |
| E. aerogenes AH33 | + |
| E. aerogenes AH34 | + |
| E. aerogenes AH36 | + |
| E. aerogenes ATCC13048 | + |
| E. cloacae T5282 | − |
| E. cloacae Ti3 | − |
| Cronobacter sakazakii 45401 | − |
| C. sakazakii 45402 | − |
| Serratia marcescens wk2050 | − |
| S. marcescens 201315732 | − |
| S. marcescens wj-1 | − |
| S. marcescens wj-2 | − |
| S. marcescens wj-3 | − |
| Escherichia coli ATCC 25922 | − |
| Klebsiella pneumoniae ATCC BAA-1706 | − |
| Achromobacter xylosoxidans A22732 | − |
| Leclercia adcarboxylata P10164 | − |
| Raoultella ornithinolytica YNKp001 | − |
| Stenotrophomonas maltophilia 9665 | − |
| Citrobacter freundii P10159 | − |
| Vibrio paraaerolyticus JS421 | − |
| Pseudomonas aeruginosa PA01 | − |
| Acinetobacter baumannii N1 | − |
| Shigella sonnei #1083 | − |

+, phage-susceptible; −, phage-resistant.

#### Influence of Physical Agents on Phage Viability

The stability of vB_EaeM_ϕ Eap-3 at different pH levels was evaluated by suspending phages at approximately
1.2 × 10⁷ PFU/mL in 1 mL of SM buffer previously adjusted with 1 M NaOH or 1 M HCl to yield a pH range from 1.0–14.0. Phage preparations were incubated at room temperature for 60 min. The stability of vB_EaeM_ϕEap-3 at different temperatures was determined by incubating phage preparations (∼1.2 × 10⁷ PFU/mL) at 4, 25, 37, 50, 60, 70, or 80°C for 15, 30, 45, or 60 min. After treatment, tubes were cooled and serial dilutions of each sample were tested against strain 3-SP in a double-layer agar assay to measure the lytic activity of the phage. Results are expressed as PFU/mL. Each assay was performed in triplicate and the results are the means of the three replicates.

**Extraction of Bacteriophage vB_EaeM_ϕEap-3 DNA**

Cell debris from 500 mL of *E. aerogenes* strain 3-SP culture infected with vB_EaeM_ϕEap-3 was collected by low-speed centrifugation (9000 × g, 10 min, 4°C). Prior to DNA extraction, DNase (1 µg/mL) and RNase (1 µg/mL) were added to the phage lysate, which was then incubated at 37°C for 30 min. Following incubation, phage particles were precipitated with 1 M NaCl and 10% (w/v) polyethylene glycol (PEG) 8000 and 1% Chloroform.

**FIGURE 3** | One-step growth curve of phage vB_EaeM_ϕEap-3. Phage vB_EaeM_ϕEap-3 was grown in an exponential phase culture of Enterobacter aerogenes strain 3-SP. Data points indicate the PFU/mL at different time points. Each data point represents the mean of three independent experiments. Results are expressed as PFU/mL.

**FIGURE 4** | pH stability of phage vB_EaeM_ϕEap-3. Each data point represents the mean of three independent experiments. Standard deviations are shown as vertical lines. Results are expressed as PFU/mL.

**FIGURE 5** | Thermal stability of phage vB_EaeM_ϕEap-3. Each data point represents the mean of three independent experiments. Results were expressed as PFU/mL.

**TABLE 2** | Functional categories of vB_EaeM_ϕEap-3 genes.

| DNA metabolism | Frd (orf60), Td (orf61), NrdA (orf62), NrdB (orf63), DenA (orf65), cd (orf74), NrdC (orf248), NudE (orf181), NrdD (orf250), NrdG (orf258), Tk (orf137), NudH (orf261), PseT (orf70), dCTPase (orf124), DNA methyltransferase (orf125), dNMP kinase (orf191), DNA end protector protein (orf194), and nicotinamide phosphoribosyl transferase (orf245) |
| DNA replication | rIIA (orf1), DNA topoisomerase II medium subunit (orf4), DNA topoisomerase II large subunit (orf5), DexA (orf178), Dda (orf21), DNA primase (orf25), DNA helicase (orf23), DNA polymerase (orf56), sliding clamp (orf42), loader of DNA helicase (orf45), DNA ligase (orf96), helicase (orf224–orf225), rIIB (orf278), RnhB (orf240), EndoVII (orf249), RnhH (orf48), and Ssb (orf44) |
| Replicosome | DNA polymerase (orf56), sliding clamp loader (orf40 and orf41), sliding clamp (orf42), DNA helicase (orf63), DNA primase (orf25), RnhB (orf66), RiaA (orf59), and Ssb (orf44) |
| DNA maturation | The dodecameric portal protein (orf214), the large terminase (orf211), and the small terminase (orf210) |
| Head | Head completion protein (orf195), portal vertex protein (orf214), the small terminase (orf210) |
| Whisker/neck proteins | Neck protein (orf207–208), fibrin neck whiskers (orf206), and whisker protein (orf229) |
| Tail | Tail completion and sheath stabilizer protein (orf192), tail sheath stabilization and completion protein (orf209), tail sheath protein (orf212), and tail tube protein (orf213) |
| Baseplate | The baseplate hub subunit (orf101, orf107), the baseplate distal hub subunit (orf102), the baseplate hub (orf103), the baseplate subunit (orf104–105), baseplate assembly protein (orf106), the baseplate wedge subunit (orf108, orf196, orf199–201), the baseplate hub subunit and tail lysozyme (orf197), baseplate wedge tail fiber connector (orf202), and the baseplate wedge subunit and tail pin (orf203–204) |
| Tail fiber assembly | A chaperone for tail fiber formation (orf190), STFs (orf205), the long tail fiber proximal subunit (orf263), hinge connector of long tail fiber proximal connector (orf264), hinge connector of long tail fiber distal connector (orf265), L-shaped tail fiber protein (orf266), and distal long tail fiber assembly catalyst (orf267) |
| Lysis | Endolysin (orf150), holin (orf268), antiholin (orf135), o-spanin (orf67), and i-spanin (orf88) |
incubated on ice for 1 h. The mixture was then centrifuged at 9000 × g for 10 min at 4°C and the pellet was resuspended in 8 mL of SM buffer. An equal volume of chloroform was added to extract the PEG and cell debris, and then centrifuged at 4000 × g for 15 min. The aqueous phase containing the bacteriophage particles was recovered and transferred to a new tube and DNA was extracted with phenol-chloroform (24:1, vol/vol) and precipitated with 100% ethanol. Finally, DNA samples were dissolved in 0.5 mL of sterile ddH$_2$O and stored at 4°C.

**Genome Sequencing and Bioinformatics Analysis of the Phage Genome**

The genome of vB_EaeM_ϕEap-3 was sequenced using the Illumina HiSeq 2500 system (Illumina, United States). The reads were assembled using SSAKE (v3.8) assembly software. The final assembled sequences were searched against the protein and nucleotide databases available via the National Center for Biotechnology Information website using Basic Local Alignment Search Tool (BLAST) software (Altschul et al., 1997). BLASTP analyses were used to identify putative homologies with predicted phage proteins. Potential open reading frames (ORFs) were identified using PHASTER (Arndt et al., 2016). The annotation was numbered with reference to the “Kp15 virus” genus. Potential tRNAs were identified using tRNAscan-SE Search Server (Lowe and Chan, 2016). Computer-based predictions were checked manually. Multiple sequence alignment of the chromosomes of related bacteriophages was carried out using Mauve software (Darling et al., 2004). Phylogenetic analyses were performed using the large subunit terminase or major capsid protein sequences of bacteriophages reported by the International Committee on Taxonomy of Viruses (ICTV). Analyses were conducted using the neighbor-joining method and 1000 bootstrap replicates by ClustalW. A genome map was generated using the CLC Main Workbench, version 6.1.1 (CLC bio, Aarhus, Denmark).

**RESULTS AND DISCUSSION**

**Phage Isolation and Morphological Characterization**

NDM-1 carbapenemase-producing E. aerogenes strain 3-SP, originally isolated from a human case of pneumonia at a Chinese teaching hospital (Chen et al., 2015), was used as a host to investigate the presence of phages in a wastewater sample from the Navy General Hospital in Beijing. Double-layer overlay plates resulted in a significant number of small plaques (diameter < 1 mm) with a similar morphology, indicating the presence of a single lytic phage (Figure 1). A single plaque was selected and used for phage proliferation and purification. Using TEM, the morphology of the phage was determined. The head of the phage is prolate, with two icosahedral ends and a cylindrical mid-section measuring ∼115 nm. The head is connected by a neck with an apparent collar to a tail tube (∼110 nm long) surrounded by a contractile sheath, a baseplate, and a complex system of tail fibers and spikes (Figure 2). On the basis of morphology and according to Ackermann’s classification (Ackermann, 2009a,b), the phage was classified as belonging to the family *Myoviridae*, which comprises a quarter of tailed bacteriophages and includes the *E. coli* phage T4. The phage was named vB_EaeM_ϕEap-3 according to the proposed naming conventions.
FIGURE 7 | Genome map of phage vB_EaeM_ϕEap-3. The genome map was generated using the CLC Main Workbench, version 6.1.1 (CLC bio, Aarhus, Denmark). Predicted open reading frames are indicated by arrows, with the direction of the arrows representing the direction of transcription.

Phage Host Range
A total of 48 clinical isolates (28 E. aerogenes, 19 non-E. aerogenes Gram-negative bacteria, and one Gram-positive bacterium) were used to evaluate the host range of vB_EaeM_ϕEap-3 (Table 1). Results demonstrated that vB_EaeM_ϕEap-3 had lytic activity specific to E. aerogenes strains (n = 18), with none of the other strains susceptible to infection. vB_EaeM_ϕEap-3 has a broader host range than the previous reported Enterobacter phage vB_EaeM_ϕEap-2 (Li et al., 2016, 2017).

Latency Period and Burst Size Determination
Results from one-step growth experiments showed that vB_EaeM_ϕEap-3 was characterized by a relatively short latent period (approximately 10 min), followed by a rise period of 20 min. A growth plateau was reached within 40 min (Figure 3). The burst size of vB_EaeM_ϕEap-3 was calculated to be approximately 10^9 phage particles per infected bacterial cell.

Sensitivity to Physical Parameters
Results obtained from temperature stability assays demonstrated that vB_EaeM_ϕEap-3 remained stable at temperatures ranging
from 4–37°C. Decreases in infectivity were observed following incubation at 60 or 70°C for 15 min, while the phage was completely inactivated by incubation at 50°C for 60 min or 80°C for 15 min (Figure 4). Results of pH stability testing revealed that phage viability was mainly unaffected following incubation in buffer at pH values ranging from 6–7, while reductions of approximately 30 and 60% were detected at pH 3 and pH 11. vB_EaeM_ϕEap-3 was completely inactivated at pH 1–2 and pH 12–14 (Figure 5).

**Genome Analysis**

The genome of vB_EaeM_ϕEap-3 is composed of a double-stranded DNA molecule of 175,814 bp with a GC content of 42%. A total of 278 putative coding sequences (CDSs) were detected (Supplementary Table S1). A tRNA gene, tRNA-Met (AUG), was detected. Approximately half of the predicted CDSs (n = 149, 53.6%) were present on the same strand. The shortest CDS encodes a putative protein of 26 amino acid residues (orf223), while the longest encodes a putative protein of 1394 residues (orf266). A specific function (e.g., DNA metabolism, structural proteins, enzymes involved in cell lysis) could be assigned to 113 of the 278 predicted proteins (40.6%), with all sequences showing high identity to proteins from phages belonging to the “Kp15 virus” genus of the Tevenvirinae subfamily. No specific function was assigned to the remaining 165 CDSs (59.4%; Table 2). No sequences with significant similarity to known antibiotic resistance, virulence, or toxin proteins, or to elements associated with lysogeny (i.e., integrase), were identified. The presence of the ndd gene (orf276), the deduced amino acid sequence of which shared 100% identity with the nucleoid disruption protein of *Klebsiella* phage KP15, suggested a lytic lifestyle for vB_EaeM_ϕEap-3 (Kesik-Szeloch et al., 2013). Comparative analysis of the whole genome sequence of vB_EaeM_ϕEap-3 against those of phages retrieved from the NCBI databases revealed that vB_EaeM_ϕEap-3 is most closely related to coliphages RB16 (NC_014467; Petrov et al., 2006) and RB43 (NC_007023; Petrov et al., 2006), *Klebsiella* phages KP15 (GU295964; Kesik-Szeloch et al., 2013), KP27 (HQ918180; Kesik-Szeloch et al., 2013), Matisse (KT001918; Provasek et al., 2015), and Miro (KT001919; Mijalis et al., 2015), *Cronobacter*
that vB_EaeM_ Tevenvirinae subfamily. Physiological characterization showed while phylogenetic analysis using previously verified markers that vB_EaeM_ Morphological characterization performed by TEM showed E. aerogenes characterized by its specific lytic activity toward outbreaks and invasive infections such as septicemia (Kuai et al., 2014). In this work, we describe a lytic bacteriophage that vB_EaeM_ϕ E. aerogenes opportunistic pathogen, has become a major clinical challenge. As an important phenotype in Enterobacteriaceae, including E. aerogenes resistance and its growing association with a multidrug-resistant poses a serious problem in a clinical setting because of the spread of antibiotic-resistance among bacterial pathogens could be assigned a metabolic function were devoted to DNA metabolism and, replication (Figure 7). Seven proteins making up the basic replisome, which acts as a biological machine that can move the replication fork through model templates at in vivo speeds (Miller et al., 2003), were also identified in the genome of vB_EaeM_ ϕ Eap-3 (Table 2). The vB_EaeM_ ϕ Eap-3 genome-packaging proteins showed a high degree of similarity to those of KP15 and KP27 viruses. Phage structural proteins identified in vB_EaeM_ϕEap-3 genome included head proteins, whisker/neck proteins, tail proteins, baseplate proteins, and tail fiber proteins (Table 2). As a member of the “Kp15 virus” genus, the lysis system of vB_EaeM_ϕEap-3 is composed of four proteins (endolysin, holin, antiholin, and spanin; Table 2). vB_EaeM_ϕEap-3 holin (orf268), with one transmembrane domain, was identified as a class III holin, and belongs to the holin T superfamily group (Maciejewska et al., 2017). The lysis genes of vB_EaeM_ϕEap-3 show the same organization and >99% predicted amino acid sequence similarity to the same regions of phages KP15 and KP27 (Maciejewska et al., 2017).

Phylogenetic Analysis
A phylogenetic tree based on the predicted large terminase subunit amino acid sequences revealed that vB_EaeM_ϕEap-3 belongs to the genus “Kp15 virus” of the subfamily Tevenvirinae, family Myoviridae (Figure 8A). This classification was confirmed based on the analysis of the major capsid proteins (Figure 8B).

CONCLUSION
The spread of antibiotic-resistance among bacterial pathogens poses a serious problem in a clinical setting because of the lack of available treatment options. In particular, carbapenem resistance and its growing association with a multidrug-resistant phenotype in Enterobacteriaceae, including E. aerogenes, has become a major clinical challenge. As an important opportunistic pathogen, E. aerogenes can cause nosocomial outbreaks and invasive infections such as septicemia (Kuai et al., 2014). In this work, we describe a lytic bacteriophage characterized by its specific lytic activity toward E. aerogenes. Morphological characterization performed by TEM showed that vB_EaeM_ϕEap-3 is a member of the family Myoviridae, while phylogenetic analysis using previously verified markers (Ackermann et al., 2011; Cheepudom et al., 2015) suggested that it belongs to the novel genus “Kp15 virus” of the Tevenvirinae subfamily. Physiological characterization showed that vB_EaeM_ϕEap-3 is characterized by a relatively short latent period and a burst size of 109 phage particles per infected bacterial cell. Results of temperature and pH stability testing also expand our knowledge of this novel phage. These features, together with the host specificity, the close genetic relatedness to the strictly lytic genus “Kp15 virus” phages, and the absence of genes associated with lysogeny, make vB_EaeM_ϕEap-3 an excellent candidate for potential clinical applications, such as decontamination or therapy. Finally, the results confirmed that vB_EaeM_ϕEap-3 is a promising candidate to hinder the colonization of E. aerogenes.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER
The complete genome sequence of phage vB_EaeM_ϕEap-3 is available in GenBank under accession number KT321315.

AUTHOR CONTRIBUTIONS
JZ and ZZ did the experiments and contributed equally to this study as joint first authors. CT, XC, LH, XW, HL, WL, and AJ analyzed the data. RF, ZY, and JY provided the bacterial strains. XZ managed the project and designed the experiments. JZ and ZZ wrote the article.

FUNDING
This work received support from the National Natural Science Foundation of China (Grant No. 31670174).

ACKNOWLEDGMENTS
We would like to thank the Center for Biological Imaging (CBI), Institute of Biophysics, Chinese Academy of Sciences for our electron microscopy work and we would be grateful to Deyin Fan for his help of making EM samples. We also thank Tamsin Sheen, Ph. D., from Liwen Bianji, Edanz Editing China⁷, for editing the English text of a draft of this manuscript.

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.00420/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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