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ORIGINAL ARTICLE

vB-Ea-5: a lytic bacteriophage against multi-drug-resistant *Enterobacter aerogenes*

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ABSTRACT

Background and Objectives: Multi-drug-resistant *Enterobacter aerogenes* is associated with various infectious diseases that cannot be easily treated by antibiotics. However, bacteriophages have potential therapeutic applications in the control of multi-drug-resistant bacteria. In this study, we aimed to isolate and characterize of a lytic bacteriophage that can lyse specifically the multi-drug-resistant (MDR) *E. aerogenes.*

Materials and Methods: Lytic bacteriophage was isolated from Qaem hospital wastewater and characterized morphologically and genetically. Next-generation sequencing was used to complete genome analysis of the isolated bacteriophage.

Results: Based on the transmission electron microscopy feature, the isolated bacteriophage (vB-Ea-5) belongs to the family *Myoviridae*. vB-Ea-5 had a latent period of 25 minutes, a burst size of 13 PFU/ml, and a burst time of 40 min. Genome sequencing revealed that vB-Ea-5 has a 135324 bp genome with 41.41% GC content. The vB-Ea-5 genome codes 212 ORFs 90 of which were categorized into several functional classes such as DNA replication and modification, transcriptional regulation, packaging, structural proteins, and a host lysis protein (Holin). No antibiotic resistance and toxin genes were detected in the genome. SDS-PAGE of vB-Ea-5 proteins exhibited three major and four minor bands with a molecular weight ranging from 18 to 50 kD.

Conclusion: Our study suggests vB-Ea-5 as a potential candidate for phage therapy against MDR *E. aerogenes* infections.

Keywords: Drug resistance; Bacteriophages; Waste water; *Enterobacter aerogenes*; *Myoviridae*

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INTRODUCTION

Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. are named as ESKAPE pathogens. *E. aerogenes*, a Gram-negative rod shape bacterium, a member of the *Enterobacteriaceae* family, is widely found in

the environment such as seawater, river, soil, and sewage (1). ESKAPE pathogens are associated with the highest risk of mortality thereby resulting in increased health care costs (2). Moreover, for therapy broad-spectrum antibiotics is recommended despite many side effects (3). Furthermore, because of the prolonged hospitalization and therapy, the economic burden on the patient is increased (3). The World Health Organization (WHO) has recently listed ESKAPE pathogens in the list of 12 bacteria against which new antibiotics are urgently needed. They describe three categories of pathogens namely critical, high and medium priority, according to the urgency needs for new antibiotics. The *Enterobacter* spp. is one of the pathogens with a critical priority that needs urgent investigation for new efficient treatments.

Antibiotics commonly used against Enterobacter infections include aminoglycosides, fluoroquinolones, carbapenems, cephalosporins and also chloramphenicol, tetracycline, nitrofurantoin, cotrimoxazole, and nalidixic acid. There are incidences of resistance reported against some of newly added antibiotics (4). Therefore, development of highly efficient infection control methods with reduced side effects is an important question.

Recently, phage therapy has attracted much attention (5). After the treatment of Shigellosis by phage therapy in 1917 (6), many researchers started phage isolation and the investigation about the phage application. Nowadays, many countries are starting phage research due to the incidence of antimicrobial drug-resistant agents (7). Bacteriophages are present in various environments such as seawater, river, soil, and sewage and can survive in different conditions. Bacteriophages infect specific host bacteria, then proliferate exponentially, and destroy the host bacteria to be released to infect new host cells; however, it seems that the characterization of new bacteriophages is an essential need for the treatment of human infectious diseases (8). Phage therapy is one of the useful, inexpensive, and specific methods that has attracted increasing attention for the treatment of MDR bacterial infections today. Lytic bacteriophages can destroy host bacteria and phage therapy has been reported in the fight against MDR pathogens (9). As yet, there are a few reported phages against *Enterobacter aerogenes*, for example, a *Siphoviridae* phage F20 of T1-like viruses (10, 11), *Siphoviridae* phage vB_EaeM_0Eap-2 is related to *Salmonella* phage FSL SP-03 (12), a Podoviridae phage vB_EaeM_0Eap-1 (13), unclassified phage UZ1 (10), and a *Myoviridae* phage vB_EaeM_0Eap-3, a T4-like bacteriophage (13). Furthermore, new isolated MDR bacterial strains may be resistant to these phages. Thus, the identification of new phages can be useful for the treatment of these infections (14). This study aimed to isolate and characterize a new phage against multidrug-resistant *E. aerogenes* strains isolated from clinical samples.

MATERIALS AND METHODS

Bacterial strains. *E. aerogenes* strains were isolated from clinical specimens including blood, wounds, trachea, urine of five hospitals and medical centers in the North of Iran (Amol, Mazandaran province, Iran) from January to September 2018. Standard strains, including *Pseudomonas aeruginosa* ATCC 1074, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 35218, *Salmonella* Typhi PTCC 1639, *Enterobacter aerogenes* PTCC 1221, and *Klebsiella pneumonia* PTCC 1290, were obtained from the microbial collection of Pasteur Institute of Iran.

Identification of *E. aerogenes* strains. Isolated strains were identified using biochemical tests and PCR assay according to the American Society for Microbiology (ASM) standard methods (15). The *tonB* was targeted as the diagnostic gene for *E. aerogenes* verification using F-*tonB*: 5'-ACCTCGGTACATCAGGTTATTG-3', and R-tonB: 5'- AGCCGCAGCCTTCTTTATTA-3' primers (designed by AllelID 10, version, company, country). For this, genomic DNA was extracted by QIAamp DNA Mini Kit (QIAGEN, USA) according to the manufacturer instructions. DNA amplification was done in an Applied Biosystems Veriti thermal cycler (USA) using the following steps: initiation-denatured at 94°C for 5 min, 30 cycles of 94°C for 30 sec, 59°C for 30 sec, 72°C for the 30s, and a final-extension at 72°C for 5 min. Finally, The PCRs products were separated by electrophoresis on 2% agarose gel.

Identification of multidrug-resistant strains. The antibiotic susceptibility pattern of the isolates was screened using Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The resistant phenotype of an isolate was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (31st

Edition). Twelve antibiotics including nitrofurantoin (300 µg), chloramphenicol (30 µg), cefotaxime (30 µg), amikacin (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), imipenem (10 µg), norfloxacin (10 µg), gentamycin (10 µg), cefalotin (10 µg), ciprofloxacin (5 µg) and cotrimoxazole (5 µg), were used. All discs were obtained from Padtan Teb, Iran. Multidrug-resistant (MDR) bacteria have been recognized as strain resistant to at least three types of antimicrobial drugs (16).

Phage isolation, purification, and propagation. Wastewater samples (50 ml) was collected from Qaem hospital (Kelardasht, Mazandaran province, Iran) in sterile centrifuge 50 ml bottles in September 2018 and store at 4°C until further processing. The larger particles were removed from the samples by centrifugation at 5000 g for ten minutes. The supernatants were filtered through 0.22 µm syringe filters and stored at 4°C (17). To enrich the isolated bacteriophage, 10 ml of filtered samples was added to 100 ml LB broth (supplemented with 0.1 M of calcium chloride) containing the host strain (EIa2: a clinical isolate No. 2 of *Enterobacter aerogenes* from Imam Ali Hospital) (Table 1) with OD = 0.4-0.6 and incubated in a shaker incubator at 37°C and 40 RPM for 48 h. The suspension (50ml) was centrifuged at 5000 RPM for 15 minutes and the supernatants were filtered through 0.22 µM syringe filters (17).

The double-layer agar method was used to purify the isolated phage (17). In brief, 200 µL of the EIa2 bacterial culture with OD=0.2-0.4 (EIa2 cultivated in LB broth and incubated at 37°C for 5 h) was mixed with 200 µL of the filtrate supernatant and incubated for 15 min at 37°C. Then, 3 ml LB soft agar (containing 0.7% agar) (Merck, Germany) was added and poured on the bottom agar plate, and then swirled to produce a uniform top layer. The plates were incubated for 16 h at 37°C. After incubation, the formation of clear plaques (lysis zones) indicated the presence of the lytic phages (17). There may be several types of lytic phage in a sewage sample so, a clear plaque was picked up and suspended in 1 ml the EIa2 bacterial culture with OD=0.2-0.4 and was incubated 24 h at 37°C then this suspension was cultivated at 10 ml and finally in 100 ml. For better purification, this step performed in triplicate (17). Strain EIa2 was used as a host to isolate phage particles from wastewater, and the isolated phage was named vB-Ea-5.

vB-Ea-5 host range. The host range of the isolated phage vB-Ea-5 was determined against clinically isolated strains of MDR- *E. aerogenes* as well as some other standard strains (Table 1). Spot assay was used to define the host range of the vB-Ea-5 bacteriophage (17). Bacterial strains (Table 1) were cultured on the LB double-layer agar plates. Different dilutions of the purified phages (10^7, 10^5, 10^3, and 10^1 PFU/ml) were prepared. 10 µl of any dilution was dropped two points on the plate. As a control at the opposite point of each drop, 10 µl of LB broth (LB) culture medium was dripped onto the plate and incubated 24 h at 37°C. Then the clear zones of lysis were evaluated. All the experiments were performed in triplicate.

Determination of the optimal multiplicity of infection (MOI). For determination of the optimal multiplicity of infection (MOI), 100 µl of the EIa2 host strain (10^4 CFU/ml) were added in 96 well microplates and mixed with 100 µl several dilutions of phage suspensions (10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 PFU/ml) (18). The turbidity of each well was measured with microplate ELISA reader (ELX800, BioTek Instruments, USA) at 630nm (OD_{630}) at 2 h, 4 h, and 8 h post infection. The wells containing the host strain or phage particles only were used as controls. The experiment was performed in triplicate. Optimal ratio of phage concentration to bacterial concentration adopted for further analysis.

One-step growth curve analysis. 10 ml LB broth containing the host strain (EIa2) with OD600=0.3 was centrifuged for 10 minutes at 5000 g and the pellet was resuspended in 1ml LB broth and mixed with phages with MOI of 0.1. The suspension was incubated at 37°C and sampling was done every 10 min up to 80 min. Samples were centrifuged at 10,000 × g at 4°C for 4 min. The supernatant was diluted and the concentration of phage particles was determined using agar dilution method (17). The time interval between the start of the logarithmic phase and the end of the logarithmic phase determined the burst time. Burst size was calculated by dividing the number of phages at the end of the logarithmic phase by the initial number of phages.

Phage adsorption measurement. Approximately, 10^7 PFU/ml of the isolated phage named vB-Ea-5 in 500 µl was mixed with 500 µl of the overnight cultured Ela2 strain (10^8 CFU/ml), before being incubated at 37°C. Samples were taken at the desired

Table 1. Susceptibilities of clinically isolated strains of MDR *E. aerogenes* to vB-Ea-5

Strain	Source	Sensitivity to vB-Ea-5
EIa1	Imam Ali Hospital	+++
EIa2	Imam Ali Hospital	+++
EIa3	Imam Ali Hospital	+
EIa4	Imam Ali Hospital	-
EIa5	Imam Ali Hospital	-
Em1	Imam Mosabn Jafar Hospital	+
Em2	Imam Mosabn Jafar Hospital	-
Em3	Imam Mosabn Jafar Hospital	+
Em4	Imam Mosabn Jafar Hospital	+
Em5	Imam Mosabn Jafar Hospital	-
Er1	Imam Reza Hospital	++
Er2	Imam Reza Hospital	-
Er3	Imam Reza Hospital	++
Er4	Imam Reza Hospital	-
Er5	Imam Reza Hospital	+
Ep1	Institue Passture of Iran	+
Ep2	Institue Passture of Iran	-
E. aerogenes PTCC1221	Institue Passture of Iran	+
Klebsiella pneumoniae PTCC 1290	Institue Passture of Iran	-
Pseudomonas aeruginosa ATCC 1074	Institue Passture of Iran	-
Staphylococcus aureus ATCC 6538	Institue Passture of Iran	-
E. coli ATCC 35218	Institue Passture of Iran	-
Salmonella typhi PTCC 1639	Institue Passture of Iran	-

+++: lysis at 10^3 PFU/ml, ++: lysis at 10^5 PFU/ml, +: lysis at 10^7 PFU/ml, -: no lysis

intervals (0, 2, 4, 6, 8, 10, and 12 min), centrifuged at 8,000× g for 3 min. The supernatants were then tested by double-layer agar method to calculate the unadsorbed phages. The cultured Ela2 strain without the vB-Ea-5 phage was used as the negative control.

Investigation of phage morphology by transmission electron microscopy (TEM). The vB-Ea-5 phage was prepared according to Brenner and Horne's protocol (19). In brief, the purified phage suspension (10^9 PFU/ml) was centrifuged (Eppendorf centrifuge 5424 R, Germany) at 15000 rpm for 150 minutes and the supernatant was discarded. The pellet was washed with neutral ammonium acetate twice, and then the purified phage particles were deposited on carbon-coated Formvar films on copper grids and stained with 1% uranyl acetate (pH 4.5). The sample was observed with a TEM (ZEISS EM900, Germany) at an acceleration voltage of 50 kV.

SDS-PAGE analysis of the vB-Ea-5 phage structural proteins. The purified phage particles (10^9 PFU) were suspended in a 15 µl loading buffer containing 10% glycerol, 2,5% β-mercaptoethanol, 2% SDS, 0.0025% bromophenol blue in 6.25 mM Tris-HCl (pH= 6.8), boiled at 100°C for 10 min, and loaded onto a 12% polyacrylamide gel in 100-120 MV for 5 h. The SDS-PAGE was stained with silver nitrate (17). The approximate size of the bands created on the polyacrylamide gel was determined using the Paint program and comparing the bands with a marker. After phage genome analysis, open reading frames (ORFS) were predicted. ORFs of structural phage proteins that were similar to protein mass (kDa) of the bands formed on the gel were identified.

Genome sequencing and annotation. The genome of the vB-Ea-5 phage (10^{11} PFU/ml) was extracted by QIAamp MinElute Virus Spin kit (Qiagen, Germany) according to the manufacturer protocol. Next-generation sequencing (NGS) was performed by Novogene Company in China using a Hiseq 2500 sequencer

(Illumina, USA). The qualified sequence reads were assembled using CLC genomic workbench 11. Open reading frames (ORFs) longer than 300 nucleotides were predicted using ORFfinder, GeneMarkS and PHAST (using GLIMMER 3.02) (20, 21). The presence of putative tRNAs was screened using tRNA-scan-SE (22). Predicted promoter regions were identified using neural network promoter prediction (23), and putative terminator structures were identified using Find Term (24). The genomic map was prepared using CGView.

RESULTS

Isolating bacterial strains and determination of antimicrobial susceptibility patterns. Seventeen MDR- *E. aerogenes* strains were isolated from clinical samples (Table 1) and confirmed by PCR assay. All the selected strains were resistant to more than three of the tested antibiotics (Table 2).

Isolating lytic phage. The diameter of its plaques on the host strain was 2 mm. The host range of the isolated phage vB-Ea-5 tested against 17 MDR- *E. aerogenes* is presented in Table 1. vB-Ea-5 infected 11 strains of MDR *E. aerogenes.* It failed to infect standard strains including *P. aeruginosa, S. aureus, E. coli, S.* Typhi and *K. pneumoniae* suggesting this phage has a narrow host range.

The optimal multiplicity of infection (MOI) and one-step growth curve analysis. The optimal MOI of vB-Ea-5 was 10^{-1} and based on the one-step growth curve, the latent time of vB-Ea-5 was 25 min, the burst size was 13 PFU/ml, and the burst time was 40 min (Fig. 1).

Morphology of lytic bacteriophages vB-Ea-5. The transmission electron micrograph showed that vB-Ea-5 has an icosahedral head of 60 nm and a contractile tail of 100 nm length and 15 nm width (Fig. 2). The morphological characteristics of isolated phage as long contracted tail and icosahedral capsid is similar to the *Mayoviridae* family.

SDS-PAGE patterns of structural proteins of vB-Ea-5. Eight protein bands were observed in a silver-stained SDS polyacrylamide gel (Fig. 3). Several bands are more remarkable, which may be related to

Table 2. Results of antibiotic susceptibility testing for MDR *E. aerogenes*

Disk / Bacteria	Cotrimoxazole (5 µg)	Imipenem (10 µg)	Cefalotin (10 µg)	cefotaxime (30 µg)	Gentamycin (10 µg)	Amikacin (30 µg)	ciprofloxacin (5 µg)	Norfloxacin (10 µg)	Nalidixic acid (30 µg)	Nitrofurantoin (300 µg)	Tetracycline (30 µg)	Chloramphenicol (30 µg)
EA1	R	S	I	R	S	S	R	I	S	R	I	S
EA2	I	R	R	I	I	I	R	I	I	R	I	S
EA3	R	R	I	I	S	S	R	R	S	I	I	I
EA4	S	I	R	I	R	I	I	R	I	I	R	I
EA5	R	S	R	I	R	R	I	I	S	I	I	R
EM1	S	R	R	I	R	R	S	S	I	S	I	I
EM2	I	S	R	R	I	I	R	I	R	I	S	S
EM3	R	I	S	S	R	I	R	I	I	R	S	I
EM4	S	I	I	I	R	R	R	I	R	I	I	S
EM5	R	R	S	S	R	I	S	S	R	I	S	S
ER1	I	I	R	R	R	I	S	S	R	I	S	I
ER2	R	I	S	I	I	I	R	I	R	S	I	I
ER3	S	R	I	S	R	R	R	I	S	I	R	I
ER4	I	R	R	I	R	I	I	I	I	R	S	S
ER5	S	I	S	I	R	R	R	I	R	I	S	S
ET1	R	I	I	S	R	I	I	R	R	I	I	S
ET2	S	S	R	I	I	R	S	I	R	I	S	S

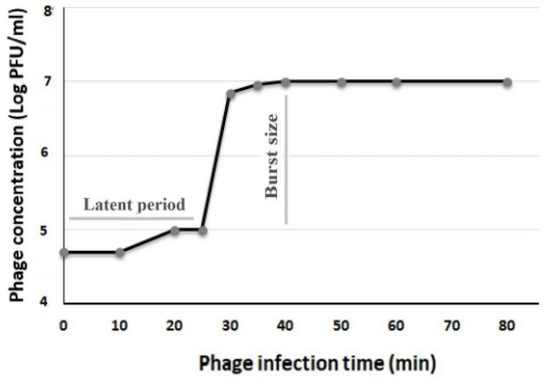

Fig. 1. One-step growth curve of phage vB-Ea-5 in LB broth. Phages were added to cultured host strain (EIa2) at an MOI of 10^{-1} and incubated at 37°C. Samples were taken at various times. The latent time of vB-Ea-5 was 25 min, the burst size was 13 PFU/ml and the burst time was 40 min.

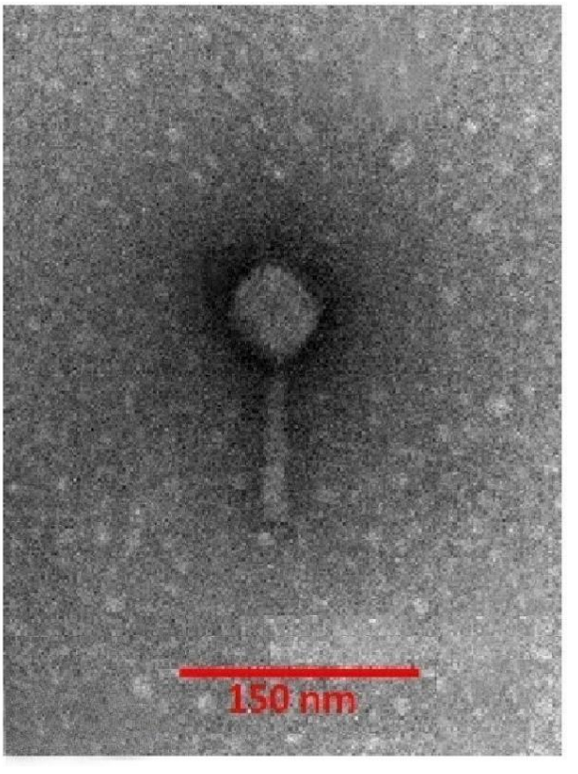

Fig. 2. TEM micrograph of phage vB-Ea-5. The purified bacteriophage was stained with 1% uranyl acetate and observed with a TEM (ZEISS EM900, Germany).

major proteins of the head and tail. Based on protein mass (kDa) similarity in vB-Ea-5 predicted 0RFs, 22 structural proteins were annotated as structural proteins that might be related to the observed band on SDS-PAGE (Table 3).

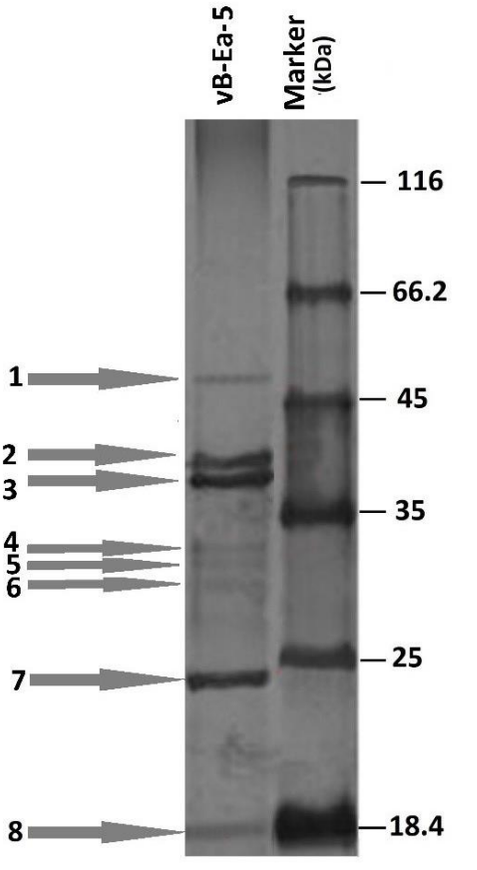

Fig. 3. SDS-PAGE patterns of structural proteins of isolated bacteriophage vB-Ea-5. A protein molecular size marker (Thermo Fisher, Germany) was used to determine the size of bands.

Genome characterization and sequence analysis. According to sequencing data, the complete genome of vB-Ea-5 phage is 135324 bp, with a 41.41% GC content (Fig. 4). The genome comprising of 212 predicted open reading frames (ORFs) and 19 potential tRNA coding genes identified in the vB-Ea-5 genome. A total of 89 ORFs showed similar homology to other known sequences. The vB-Ea-5 ORFs categorized into several functional classes as well as DNA replication, DNA modification, transcriptional regulations (Restriction endonuclease, DNA polymerase, DNA helicase, ribonuclease, DNA methyltransferase, nicking endonuclease, receptor-binding protein....), packaging, and structural (portal (connector) protein, capsid, tail, and head proteins, terminase DNA packaging enzyme, ...), and host lysis protein (Holin). The gene distribution is almost equal in both strands; there are 72 ORFs on the minus and 140 on the plus strand. These terminators have simi-

Table 3. Structural hypothetical proteins of vB-Ea-5 proteins that observed in SDS-PAGE

Name	No. Of Amino acid	Mw (KD)	ORF	Probable or possible gel band
Tail completion and sheath stabilizer protein	196	22.01	ORF70	7
Head completion protein	149	17.89	ORF72	8
Baseplate wedge subunit	336	38.58	ORF79	3
Base plate wedge completion tail fiber socket	302	32.4	ORF80	5
Base plate wedge completion tail pin	220	23.18	ORF82	7
Fibritin	470	50.74	ORF84	1
Neck protein	312	34.71	ORF85	4
Neck protein	266	30.65	ORF86	6
Tail sheath stabilizer and completion protein	276	32.33	ORF87	5
Tail tube protein	163	18.47	ORF92	8
prohead core scaffold protein and protease	213	23.32	ORF97	7
prohead core protein	271	30.26	ORF98	6
head outer capsid protein	185	20.27	ORF106	8
Base plate hub subunit	209	24.3	ORF114	7
Base plate hub assembly protein	250	29.43	ORF115	6
Base plate hub subunit	386	44.25	ORF116	2
Base plate distal hub subunit	175	19.73	ORF117	8
Base plate subunit	349	38.23	ORF119	3
Base plate subunit	320	35.09	ORF120	4
Hinge connector of long-tail fiber proximal connector	382	42.62	ORF174	2
Hinge connector of long-tail fiber distal connector	217	23.31	ORF175	7
Putative major capsid protein	291	32.24	ORF202	5

larities to T4 phage terminators. The BLASTn results showed that vB-Ea-5 was mostly similar to Enterobacteria phage myPSH1140 (GenBank: MG999954.1) with a 99.86% sequence identity.

DISCUSSION

One of the serious problems in the treatment of infections is the extended range of antibiotic-resistance among bacterial strains such as multi drug resistant *E. aerogenes* which is a significant challenge in treatment and has been known as the fourth most regularly bacterium isolated from hospital infections (25).

In this study, a lytic-bacteriophage purified from hospital sewage was isolated and characterized by its specific lytic activity against MDR-*E. aerogenes* clinical isolates. Morphological characterization showed that the novel MDR *E. aerogenes* phage (named vB-Ea-5) is a member of the *Myoviridae* family (26). The *Myoviridae* is a subfamily of Caudovirales (26). The viruses of this subfamily have a DNA genome, an icosahedral large capsid (~150 nm), and have a tail (27) that facilitates the process of phage attachment to the host and transport of genetic material into the bacterial cell (27).

The Host range tests suggested that vB-Ea-5 had lytic activity specific to MDR-*E. aerogenes* strains (n =11). A narrow host range is desirable in bacteriophage therapy. A phage that is limited to a specific species, and even a strain within that species, is sometimes sought because it is likely to minimally interact with other bacteria, and it will therefore not affect the host microbiome. Many studies illustrated that some of the *Myoviridae* family bacteriophages such as vB_EaeM_0Eap-3 (13), myPSH1140 (28) EFDG1, IME_EF3, and VD13 (29) have the potential to the treatment of MDR *Enterobacteriaceae* species.

The one-step growth curve showed multiple parameters of phage vB-Ea-5. The latent time was about 25 min, the burst size was 13 PFU/ml, and the burst time was 40 min, which seems beneficial for phage therapy. Other studies showed the bacteriophages belonging to the *Myoviridae* family have shorter latent

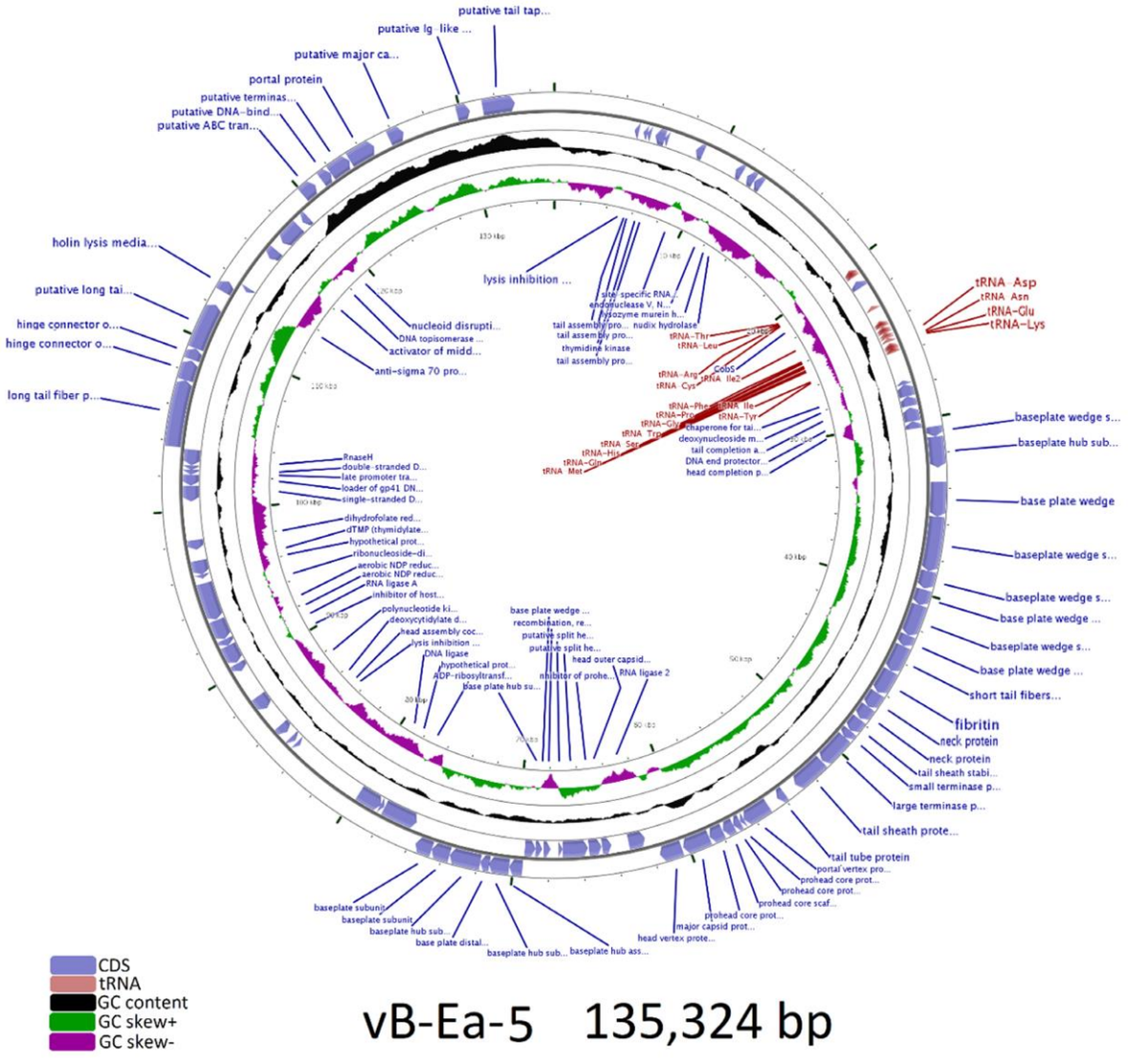

Fig. 4. Genomic map of vB-Ea-5. GC-skews are shown I n green and violet peaks, and the GC contents in black peaks. The outer circle indicates genes on the forward strand, and the inner circle represents genes on the reverse strand. tRNA genes with >60 scores are shown in red.

time and larger burst sizes such as myPSH1140 (latent time: 11 min, burst sizes: 135 PFU/ml) and vB_EaeM_0Eap-3 (latent time: 10 min, burst sizes: 10^9 PFU/ml) (28, 30). It has also been noticed that some earlier study shows the burst size primarily depends on the availability of bacterial host cells (28, 31).

The genome analysis of the vB-Ea-5 illustrated that it is a 135324 bp circular genome, with a 41.41% GC content. 212 open reading frames (ORFs) were predicted in the vB-Ea-5 genome. Eighty-nine ORFs were functionally annotated, and 123 ORFs were annotated as hypothetical proteins. Most genes (66%) were on the plus strand. The shortest CDS encodes a hypothetical protein of 38 amino acid residue, while the longest CDS encodes a long tail fiber proximal subunit contains 1264 amino acid residues. The vB-Ea-5 genome contains some functional proteins such as holin and murein hydrolase that has a role in the host-lysis mechanism (32, 33). For lysing and destroying the host bacteria, most bacteriophages use the holin, but some bacteriophages also have a murein protein that can accelerate bacterial lysis (32). The

mechanism of action of holin is as follows, forming the small pore in the cytoplasmic membrane and allowing the proteins such as endolysins and murein to join to the periplasm and degrade the peptidoglycan (33). Other proteins that play roles in nucleotide metabolism are DNA helicase, RNA ligase 2, RNA polymerase, ADP-ribosylase, and activator of middle period transcription. Blast-n analysis of the whole genome sequence of vB-Ea-5 in NCBI databases revealed that vB-Ea-5 is most closely related to Enterobacteria phage myPSH1140 (GenBank: MG999954.1) with 99.86% sequence identity belonging to the *Myoviridae* family of viruses.

vB-Ea-5 has most of the genes included in gene replication and gene expressions such as DNA helicase, DNA polymerase, DNA ligase, topoisomerase, endonuclease, RNA primase, and RNA polymerase. These genes were also found in other Enterobacter phages, such as CC31, PG7, myPSH1140 (28), and phiEap-2 (12).

The vB-Ea-5 genome, therefore, has early protein genes that typically play several roles in DNA replication and late protein genes that encode structural proteins (34). Also the genome of vB-Ea-5 lacks -associated, toxin, and antibiotic resistance genes.

The SDS-PAGE pattern of structural proteins showed vB-Ea-5 has a protein pattern mass (kDa) similarity of the other *Myoviridae* family member such as CC31 (proteome ID: up000008725), PG7 (proteome ID: up000019300), myPSH1140 (proteome ID: up000244328), and phiEap-2 (proteome ID: up000204002) in the UniProt database. The vB-Ea-5 predicted proteins shown in SDS-PAGE contain structural assembly proteins such as head completion protein, base plate hub subunit, neck protein, hinge connector of long-tail fiber distal connector, tail completion, sheath stabilizer protein, tail tube protein, a pro head core scaffold protein, and protease which facilitate the initial binding of the phage to the bacterial host and may play a role in the specificity of the host (35).

CONCLUSION

Despite the promising results of this study, data might be inadequate to conclude the phages for the successful therapeutic application. Therefore, some additional experiments as well as phage stability analysis, proteomics analysis, and in-vivo studies appear to be necessary for the selection of acceptable vB-Ea-5 for phage therapy. It is suggested that vB-Ea-5 may be a good candidate for anti-MDR-*E. aerogenes* phage therapy research in the future.

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