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PHYLOGENETIC DIVERSITY OF ACIB PHAGES ISOLATED FROM IRAQ BY SEQUENCE ANALYSIS OF THE VIRAL CAPSID ASSEMBLY PROTEIN GENE GP21

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Abstract:

In this study, we conducted the morphological observation, biological and comparison analysis of sequencing by means of detecting a single gene in phages and the phylogenetic tree was constructed using Unweight Pier Group method with Arithmetic Mean (UPGME tree). The novel bacteriophage ϕAcib_6, ϕAcib_A6, ϕAcib_24, ϕAcib_5Al, ϕAcib_A1, ϕAcib_4Al, ϕAcib_A10, ϕAcib_G7, ϕAcib_3Al, ϕAcib_AB2, and ϕAcib_CA1was isolated from the river water (Al_Diwaniyah, Iraq). Host range analysis using a panel of 34 clinical *Acinetobacter* sp. isolates shows successful infection of nine isolates and electron microscopy indicates that ϕAcib_A6, ϕAcib_6 and ϕAcib_5Al showed similar morphological features Myoviruses, while ϕAcib_G7 their classified including pedoviridae family, also ϕAcib_A1and ϕAcib_4Al classified including Siphoviridae family , ϕAcib_A10 and ϕAcib_cA1 their classified including Tectiviridae family; analysis of morphology of ϕAcib_3Al ϕAcib_AB1, and ϕAcib_24, contains e version and unties, which was classified as the family of Plesmoviridae; Inoviridae and unassigned restivel. capsid sequencing annotation unveiled the presence of gene clusters of similarity 98% and the N-terminal region indicates that to most species of *Acinetobacter* bacteriophages it can be adopted as a highly specific diagnostic region.

Keywords: Bacteriophage, Acinetobacter sp., Capsid Gene gp21, Iraq

INTRODUCTION

Acinetobacter sp. is an emerging nosocomial opportunistic human pathogen that poses a significant challenge in hospital settings, causing a spectrum of infections including respiratory, urinary tract, and bloodstream infections. The clinical persistence of nosocomial *Acinetobacter sp.* isolates and their ability to evade a diverse range of antimicrobial agents highlight the urgent need to address this growing public health issue. Regrettably, the resistance of this microbe to dehydration, chemical sanitizers, and detergents renders current remedies inadequate in effectively eradicating *Acinetobacter sp.* from the hospital environment [1]. The employment of lytic agents is one potential resolution for combating the proliferation of antibiotic-resistant strains. These agents, including bacteriophages, bacteriophage-derived antibacterial enzymes, and proteins, hold potential as a promising alternative. Bacteriophages have been utilized for some time in the field of medicine for the identification of specific bacteria, as well as for the treatment of bacterial diseases. In light of the global emergence of antibiotic resistance, phage therapy has garnered significant attention as a supplementary or alternative course of treatment to antibiotic therapy. Notably, phages offer a range of advantages that are not only clinically attractive, but also applicable in various contexts, such as food regulation, agriculture, and industrial practices [3]. The utilization of phages as a preventative disinfectant is an intriguing method, particularly in medical settings and clinical equipment [4]. Genomic characterization is a highly precise method of distinguishing between organisms. The NCBI phage database boasts an impressive 967 phage genome sequences, which exhibit genome sizes spanning from 10 to 500 kbp. As a result, three families contain enveloped bacteriophages. [5]. Bacteriophages are a diverse group with almost all of them containing double-stranded DNA genomes. However, the remaining 4% have genomes consisting of single-stranded DNA or ssRNA according to Hatfull's findings in 2008 [6]. There is currently no one gene found in all phages suitable for use as a universal phylogenetic marker similar to the bacterial 16S ribosomal RNA gene sequences, as noted by Wu et al. in 2013 [7]. PCR primers can be designed from highly conserved genes, including those encoding capsid proteins and DNA polymerases, to investigate the genetic diversity of particular phage groups. By cloning and sequencing PCR products directly from environmental samples, diverse genetic compositions can be assessed the gene in question is accountable for the instigation of the process of head assembly, DNA compaction, as well as the head-tail intersection [8]. Currently, 21 *Acinetobacter sp.* phages have been sequenced and released in databases. AP22, was the first reported *Acinetobacter sp.* phage [9,10]. Unique conserved regions were discovered among the sequences of Strictly lytic *Acinetobacter sp.* phage AP22, which were

found to lyse 68% of the 130 clinical multidrug-resistant *Acinetobacter sp.* isolates tested from Russian clinics. [11,12,13,14]. A series of PCR primers were designed to specifically target the viral capsid. These primers were developed using the conserved regions of the phages. The ultimate goal of these primers was to selectively amplify a 165-bp fragment encoded by gene 21 (gp21). Subsequently, the aforementioned primer set was utilized in PCR-DGGE (denaturing gradient gel electrophoresis) to investigate phages in Iraq. In this study, we provide an investigation on bacteriophages that remain molecularly uncharacterized.

MATERIAL AND METHODS:

During the period spanning from April to July in Iraq, a prospective study was carried out at the College of Biotechnology, Al-Qadisiyah University. Collaborating with the Burn Care Unit at the Burn Specialist Hospital in Al Diwaniyah city, Iraq, the study was conducted from January 2020 to September 2022.

2.1. Isolation of Bacteriophage: A total of thirty-four isolates of *Acinetobacter sp.* were obtained from Al-Diwanyia Teaching and Burn Specialist Hospitals. The strains of *Acinetobacter sp.* mentioned above were identified and their susceptibility rates towards antibiotics were determined in accordance with our previous research [15]. The bacterial strains were all preserved in Luria-Bertani (LB) broth (Oxoid) containing 50% glycerol (v/v) at a temperature of -70°C. Subsequently,a single colonies were cultured in LB broth at 37°C, with shaking at 220 rpm. It is important to note that cultures were incubated at 37°C in all cases, except for the phage infection experiments. The latter were conducted at 30°C, unless otherwise indicated [16]. In addition, LB (10m peptone+10m yeast+5m NaCl + 5mM CaCl2 and $MgSO_4$ as co-facter) was utilized as the culture medium in all phage infection experiments. [17]. Liquid cultures enriched with strains of *Acinetobacter sp.* were utilized as bacterial hosts for the propagation of phages and for the analysis of river water from Al_Diwaniyah, Iraq. The physicochemical parameters were obtained from the Laboratory of Al_Diwaniyah water Treatment Plant, also located in Al_Diwaniyah, Iraq. Each sample underwent centrifugation and the supernatant was filtered through a membrane with a pore size of 0.22 µm. The specimens were subsequently exposed to aeration using either orbital shaking or rotation via roller drum. The expansion of the specimens was then observed by quantifying the optical density (OD) via absorbance at 600nm. For the formulation of the foundational plates, customary Petri dishes were employed, which were brimming with LB or BHI medium comprising of 1.2% agar. In the ensuing step, 4 ml of top LB or THB agar, supplemented with 0.4% agarose, was amalgamated with 0.2 ml of the overnight bacterial cell culture and dispensed onto the bottom agar [18]. The selection of individual plaques from each plate was carried out through the employment of a pipette tip, utilizing the technique of single plaque purification, as delineated in the methods of Goodridge *et al*, [19]. Subsequently, a volume of 1 milliliter of an overnight bacterial culture was amalgamated with a quantity of 99 milliliters of LB broth, which was fortified with a concentration of 0.001M CaCl2, within a 250 milliliter PYREX® screw cap storage vessel. Following incubation at 37 °C with shaking, for a duration of 1 hour, phage lysate was added, at a multiplicity of infection (MOI) of approximately 0.1, and incubated for a further 5-8 hours, or until lysate clears. Subsequent to the lysate, chloroform (1 % v/v) was added to each bottle for a time period of 30 - 60 min, to terminate enrichment. Bacterial debris was separated through the process of centrifugation, specifically at 5000× g for 15 minutes, with the intention of eliminating bacterial debris. The resulting substance was then subjected to filtration sterilization utilizing a 0.22 µm pore size and stored at a temperature of 4 °C [20, 21, 22]. The PEG phage precipitate underwent centrifugation for a duration of 20 minutes at 7000× g and was carried out at a temperature of 4 °C. Following this, the phage was resuspended in TM buffer [23] and underwent ultracentrifugation at 7000 x g for a period of 18 hours. The phage was then extracted several times with chloroform to eliminate any remaining traces of PEG and other contaminants. Further purification of the phage was conducted through centrifugation in CsCl step gradient at 100,000× g for 2 hours. The resulting opalescent band containing the phages was collected, dialyzed against SM buffer, and stored at a temperature of 4 °C [24,27].

2.2. Phenotypic characterization of phages: Transmission electron microscopy micrographs were obtained for purified virus-like particles (VLPs) using negative staining. This technique was carried out according to previously described methods [28]. A portion of the refined phage solution was applied onto a copper grid that was coated with carbon. The grid was subsequently subjected to glow-discharge and treated with negatively stained 1% uranyl acetate for a period of one minute. The grids that were prepared were studied using a JEOL JEM-2100 200 kV transmission electron microscope. Images of the phage particles that were negatively stained were acquired using a Gatan Ultrascan 1000XP CCD camera and Gatan Digital Micrograph software. The measurements of no less than 30 individual particles were averaged to determine the dimensions of the particles.

2.3. Host range determination: The unknown phages were assessed for their lytic activity and host specificity against eleven genotype-varying ESBL of *Acinetobacter sp.* strains by utilizing the double-layer method [29]. The phage suspensions, consisting of approximately 10^9 PFU in 20 µl, were administered onto the surface of double-layered agar plates. These plates were comprised of cells from *Acinetobacter sp.* strains (approximately 200 µl), in addition to other species included in this study, which had been cultivated for 12 hours in LB medium at 37°C to OD600 of 0.3 with 4 mL of soft agar (LB broth supplemented with 0.5% agarose). After the spots had dried, the plates were incubated at 37°C for 18 hours. The investigation delved into the physiology of the phages.

2.4. Phage adsorption and one step growth experiments: Samples of bacteriophages that were carefully selected for the purpose of this study were exposed to infection within *Acinetobacter* sp. strains, with an approximate MOI of 0.01. The incubation process was conducted in LB broth that was fortified with 5% CaCl3 and performed at room temperature. At specific intervals of 20, 30, 35, 40, 45, 50, and 55 minutes, a volume of 100 µL of the samples was

retrieved, subsequently diluted in 0.9 ml of LB broth, and centrifuged at a force of 10,000 × g for a duration of 5 minutes. The supernatants were then subjected to titration in order to determine the number of unabsorbed phages via the plaque assay method. This meticulous procedure was repeated three times. The adsorption constant was calculated over a period of 5 minutes, following the approach described by Adams [30]. For the one-step growth experiments, 20 ml of *Acinetobacter sp.* strains (OD 600 of 0.3) were individually isolated and harvested through centrifugation (4000 × g, 5 min, 4 °C). The resulting pellet was then suspended in 1 ml of LB broth. Bacterial cells were subjected to phage infection with a multiplicity of infection of 0.1. The phage was allowed to adsorb for a duration of 10 minutes at a temperature of 37°C. Subsequently, the mixture underwent centrifugation at a rate of 10,000 × g for a period of 2 minutes, in order to eliminate unabsorbed phage particles. The resulting pellet was then suspended in 20 mL of LB broth. Samples were obtained at intervals of 10 minutes, for a total of 100 minutes of incubation at a temperature of 37°C, and were immediately equilibrated. This process was repeated thrice.

2.5. 1. Phage DNA Isolation and Primer design: The viral DNA was obtained via the concentration of phage particles (109 PFU) from crude lysates using polyethylene glycol 6000 (20%) and subsequent extraction with the utilization of the FavorPrepTM Viral Nucleic Acid Extraction Kit I (FAVORGEN Biotech Corporation - Taiwan). To eliminate bacterial nucleic acids, all DNase- and RNase-treated samples were subjected to heating for 1 h at 37 °C. The quantification of the genome was accomplished using the Nanodrop ND-1000. The phage genome sequencing was conducted through the utilization of the MiSeq platform and Nextera DNA library preparation kit [31]. The position and length of the terminal repeats were identified through examining a region of greater sequencing read coverage compared to the average read depth along the entire genome of the phage. The process of annotating unknown phage genes was conducted through the utilization of GeneMark (http://opal.biology.gatech.edu/genemark/) , which was utilized to predict the protein-coding region. Additionally, PSI-BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi)was employed to conduct a search for similar proteins and conserved domains. Within this study, all primers were designed by utilizing the complete genome sequence of thirty-five *Acinetobacter* sp. lytic bacteriophage isolates. To ensure the identification of perfect and specific conservation among the cluster, two sizable (20bp) regions were scanned. The product sizes were subsequently compared to enable differences to be resolved by gel electrophoresis. This process facilitated consistent primer binding, ultimately resulting in product size. The designed primers were characterized as being between 25-35 nt. in length, with a melting temperature of approximately 62°C. Each primer possessed 18-25 bp homologies flanking the PCR target. Previously designed primer for detection of MCP: F:5'-GCGCACACCCAATGAAGAAG-'3 (MCP for, nucleotide position 482 - 506 of the gene) and R:5'-ACCAACCCAAGCCGTCATAG-'3 (MCP rev, nucleotide position 704 - 682). Amplification procedures were executed utilizing 0.5 millimolar quantities of each primer (Biotez, Berlin, Germany), 2.5 units per 100 milliliters of AmpliTaq Gold DNA polymerase (Applera Italia, Monza, Italy), 1.5 mill molar magnesium chloride, 25 Nano grams of total deoxyribonucleic acid, and 200 mill molar concentrations of each deoxyribonucleoside triphosphate [32]. A Perkin Elmer thermal cycler (mod. 9700; Applied Bio systems) was employed for conducting the DNA amplifications, as per the following conditions: preliminary denaturation at 95 degrees Celsius for 10 minutes; 35 cycles of denaturation at 95 degrees Celsius for 30 seconds, annealing for 30 seconds at 60 degrees Celsius, and extension at 72 degrees Celsius for 1 minute; extension at 72 degrees Celsius for 7 minutes. Polymerase chain reaction products were subsequently scrutinized through electrophoresis on 1.2% (w/v) agarose gels at 10 volts per centimeter for 1 hour in Tris-acetate EDTA buffer (TAE: 40 millimolar Tris acetate, 1 mill molar EDTA, pH 8.0). The DNA fragments were first combined with loading dye, and the GeneRulerTM DNA Ladder Mix was utilized as a size standard, in accordance with the manufacturer's guidelines. The PCR product, which was 544bp and specific to the genes being studied, was then observed under UV light (λ = 312 nm.). In cases where DNA fragment extraction was necessary, EZ-10 Spin Column DNA Gel Extraction Kit was employed, following the manufacturer's instructions. This particular kit was specifically designed for extracting DNA fragments from Gel PCR DNA fragments.

2.5.2. Phage Genome Analysis: The taxonomic composition was investigated through the utilization of MEGAN7, employing the least-common ancestor algorithm and a minimum BLAST-score threshold of 35 to facilitate the mapping of sequencing reads to the NCBI taxonomy. The NR outcomes were implemented at the super-kingdom tier, while viral_refseq findings were applied for lower levels. In order to guarantee the harmonization of taxonomic profiles in MEGAN read counts, standardization was executed through the normalization of 100,000 reads per sample. Subsequently, nucleotide sequences from different regions of AL-Diwanyia governorate were submitted to Genbank, with each clone being assigned its own unique accession number. We confirmed the counting sequences data can easily by arranged the sequences into a FASTA format by saying the consensus as a single test file in the NCBI fasta online. BLAST results were supported with in-silico gene predictions using GeneMark of Mark Brodovosky. The identified genes were compared with the NCBI ORF finder http://www.ncbi.nlmnih.gov/gorf/gorf.htm nucleotide sequences were scanned for homologues using the basic alignment search tool (blastx) https://blast.ncbi.nlm.nih.gov/Blast.cgi Blast.cgi [33] . To search for tRNA genes in the phage sequences the internet tool tRNAscan SE http://lowelab.ucsc.edu/tRNA scan-SE/ was used. Sequence comparison and distance phylograms with the PB1-like phages was conducted using ClustalW2 tool http://www.ebi.ac.uk/Tools/clustalw2/index.hl. The construction of the phylogenetic tree was accomplished through utilization of RAxML through rapid bootstrapping, with a bootstrap of 1000, utilizing the GAMMA LG F protein model.

RESULTS:

3.1. Isolation and properties: Of the phages recorded from an environmental water sample in the city of Al-Diwanyia during the period work, nine of the tested samples exhibited differing degrees of lytic activity towards the bacterial

hosts, as evidenced by variations in both the time required for cell lysis and the overall efficiency of the lysis process. Figure 1; These 9 phages were named according to [34]: ϕAcib_6, ϕAcib_A6, ϕAcib_24, ϕAcib_5Al, ϕAcib_A1, ϕAcib_4Al, ϕAcib_A10, ϕAcib_G7, ϕAcib_3Al, ϕAcib_AB2, and ϕAcib_CA1. Electron microscopy revealed the characteristic a flexible non-contractile tail. Fig. 2. Phages ϕAcib_A6, ϕAcib_6 and ϕAcib_5A1, were judged to belong to the Family Myoviridae family because of the appearance of icasohedral heads diameters varied 84 - 102 nm and thick, contractile tails averaging 90 -112 nm in length. Tail diameters varied 13-22 nm. Shows phage ϕAcib_G7 has icosahedral head 65nm with very short tail, belonging to the Podoviridae family. All four bacteriophages were tailed bacteriophages and identified as members of order Caudovirales. Out of *Acinetobacter* phages isolated ϕAcib_A10, show spherical procapsids and mature phage has hexagonal head 59nm in diameter without non-tailed, it has. ΦAcib_4A1 and ϕAcib_CA1 were not a tailed phage and it has 88.89 nm icasohedral head. According to its morphology, it may be belonging to the Family Corticoviridae according to the taxonomic database of ICTVdB [35,36]; Analysis of morphology of ϕAcib_3A1phage specific to *Acinetobacter sp.* with a TEM contains which was classified as the family of Inoviridae.Host range analysis was conducted for all phages isolated which were screened against different bacterial pathogens included clinically relevant *Acinetobacter sp.* found in Al-Dwinayha/Iraq.: 11 clinical, 3 environmental strains and single control isolate which in total . In addition to choose three different bacterial species that are responsible for the majority of hospital acquired bacterial infections, the bacterial species that were isolated from the farm survey were *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Gemella heamolysans*, as shown in Figure 3. The results of the survey indicated significant differences in terms of the bacterial species and the number of phages that were isolated between each sampling. Despite the high burn values (>400,000 cells.ml-1), a considerable proportion of the samples exhibited zero bacterial growth even after 24 hours of incubation at 37ºC. Out of the total isolates, eleven AB isolates were obtained, of which eight demonstrated susceptibilities to ten phages, while the remaining isolate was susceptible to a variety of phages. The utilization of a one-step growth curve was employed in order to explicate the life cycle of the phages. The findings evinced that the latent period of each phage ranged from 20 to 45 minutes, while both the latent periods and burst sizes displayed considerable variation amongst the phages. The lysis period was discerned to be 45 to 180 minutes, characterized by a substantial escalation in the number of phages, ultimately reaching a plateau. The burst size was determined to be in excess of 350-1200 PFU per infected host cell. Figure. 4.

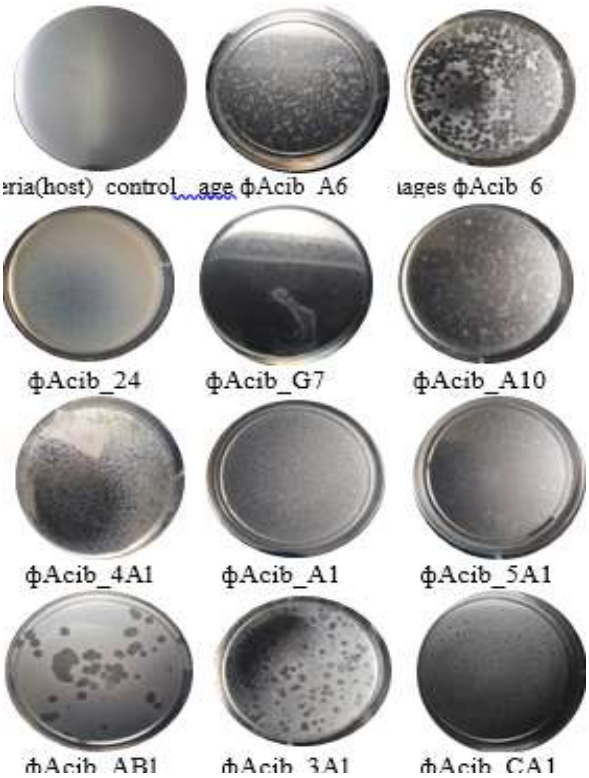


Figure 1. Morphological differences between plaques arise from the initial enrichment lysate and after five rounds of purification

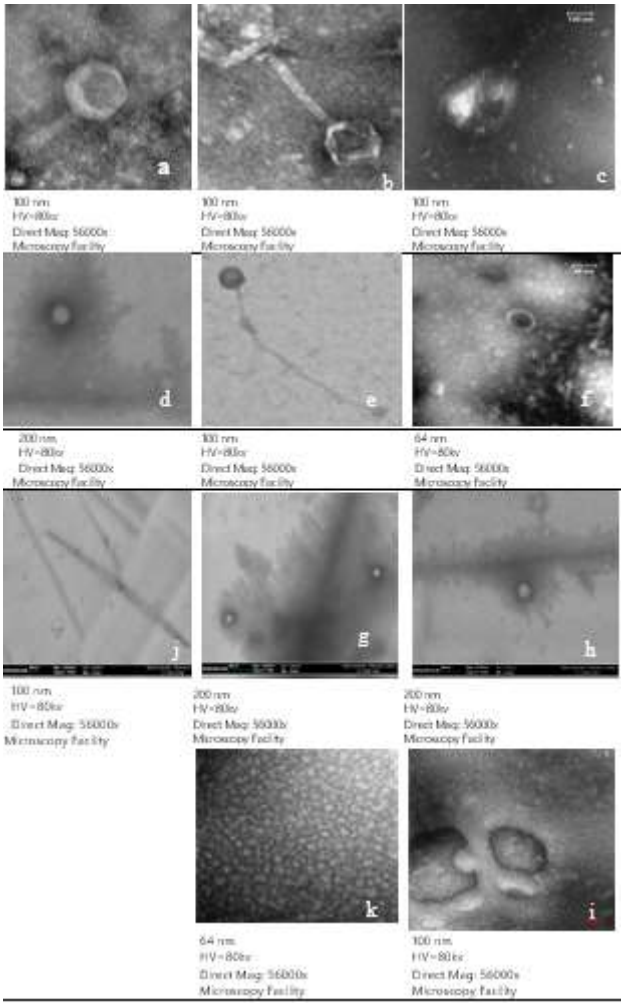


Figure 2: Electron Micrographs of purified *Acinetobacter* phage. [From left] (a) ϕAcib_A6, (b) ϕAcib_6, (c) ϕAcib_5A1, (d) ϕAcib_G7, (e) ϕAcib_A1, [from left] (f) non-tailed ϕAcib_A10, (g) ϕAcib_4A1, (h)ϕAcib_CA1, [From left] (j)filiform ϕAcib_AB1, (k) unassigned ϕAcib_24 and (l) ϕAcib_3A1.

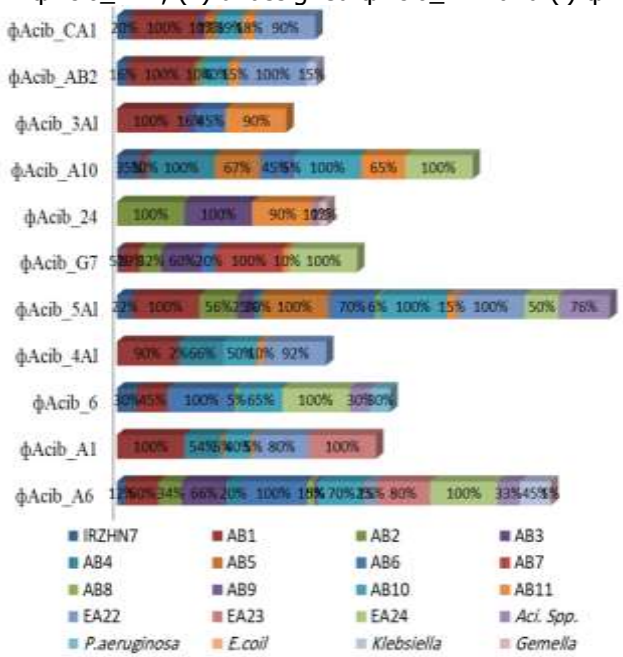


Figure 3: percentage of strains per susceptible to infection by each of the phages

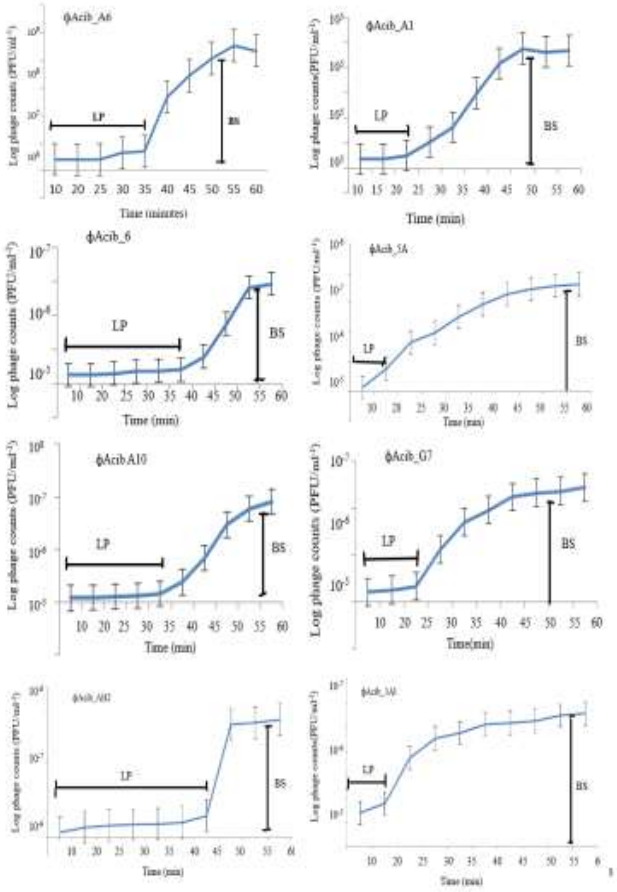


Figure 4:Infection parameters of Phages. Single- step growth curve of ϕAcib_A6, ϕAcib_A1, ϕAcib_6, ϕAcib_5Al, ϕAcib_A10, ϕAcib_G7, ϕAcib_AB2 and ϕAcib_3Al, LP: latent period and BS: burst size.

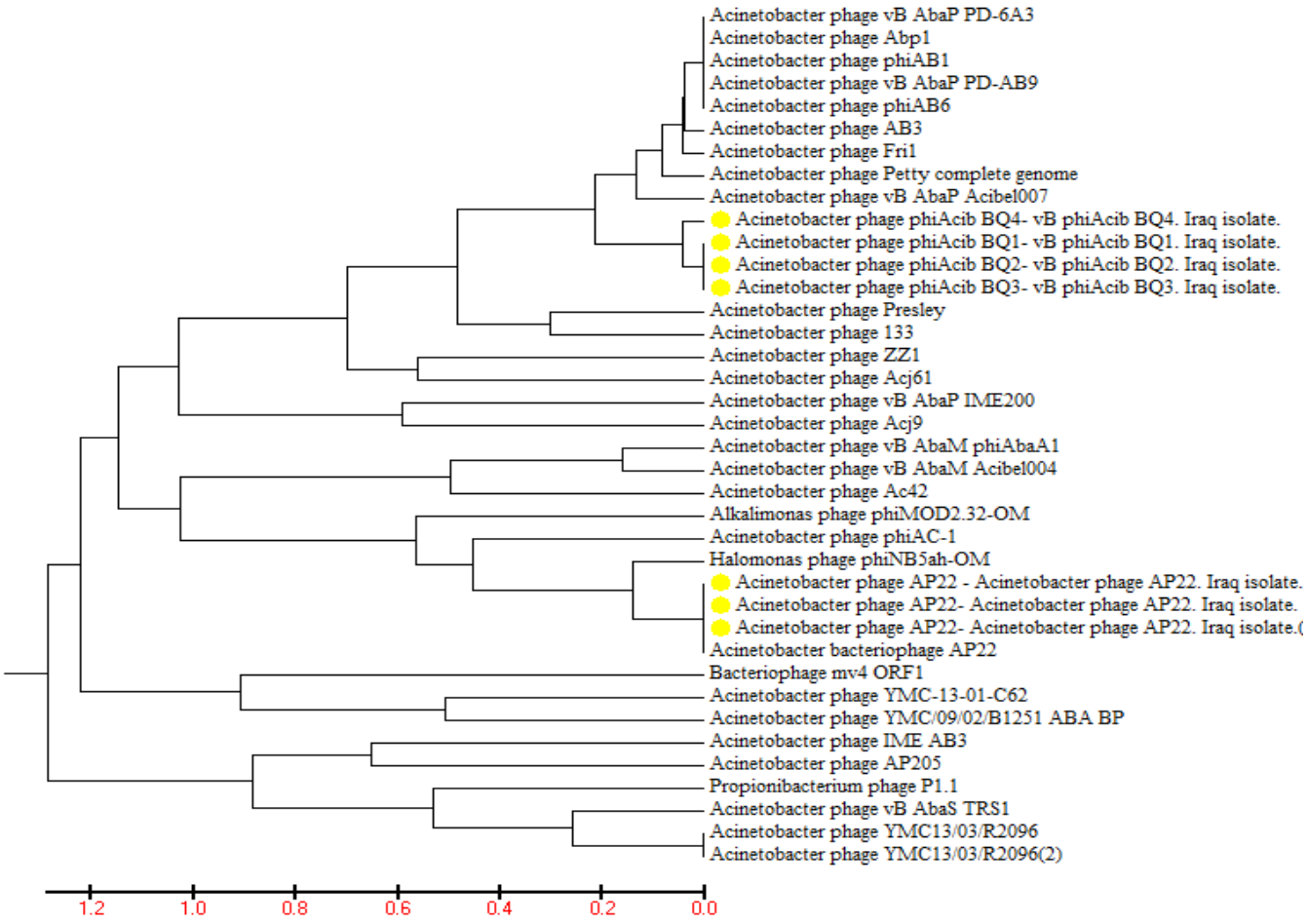
Our investigation into bacteriophages of unknown molecular origin employed two software programs, namely PhiSiGns and GitHub. The capsid gene was denoted by the symbol gp21 and the phages were classified based on various criteria, including the assigned phage name, nucleic acid type, phage family, host domain, host phylum, and host genus. Additionally, a Primer-BLAST sequence similarity search was conducted to ascertain if homologous proteins were present in other selected phage genomes. The results indicated that all observed phages possessed dsDNA genomes as per their phenotypic classification and genome.

Based on the multiple sequence alignment, the major capsid protein was identified as a signature gene with a length of 430 base pairs, utilizing the CLUSTALW multiple sequence alignment program with default settings. The size range was found to be approximately 45-60 kilo base pairs. The nucleotide sets of the present study were verified and confirmed using the National Center for Biotechnology Information (NCBI). The results indicated that (ϕAcib_5A1) exhibited a 100% identity with other world *Acinetobacter sp.* phage AP22 strains, while the analysis confirmed that (ϕAcib_6) displayed a 100% identity with phiAC-1 and with other strains that were subjected to complete *Acinetobacter* bacteriophage genome sequences in relationships with other species of Myoviridae family. One of the most important results we have reached were showed of the (ϕAcib_A6) sequences alignment of uncleocapsid sequences that 100% corresponded with unclassified Acinetobacter-specific bacteriophages. Sequence alignment was carried out utilizing reference strains for the generally recognized Acib_Phages, which had been previously documented in the GenBank database. The resulting outcomes were grouped into families of variation, allowing for the identification of both identity and similarity scores for our sequence. These results were subsequently documented and published within the International Nucleotide Database Sequence Collaboration (INSDC) at the National Center for Biotechnology Information (NCBI) GenBank, European Nucleotide Archive (ENA), and DNA Data Bank of Japan (DDBJ) locations. Furthermore, our published variants were assigned GI version numbers ranging from (GI: 1027910840- GI: 1027910866), as detailed in Table (1). Phylogenetic tree construction was accomplished through BLAST-NCBI-GenBank databases utilizing the distance tree approach, coupled with sequence analysis of the nucleocapsid coding gene, using globally recognized reference strains of the virus of unclassified. The identity score observed within our clones and world reference strains of Acinetobacter bacteriophage exhibited a range of 99%-100% in comparison to other members of the Myoviridae family.

Table 1:Fourteen clones with submission and source, region, accession number.

No.	**Phages Name**	**Submission ID**	**Acce. No.**
1	ϕAcib_5A1(1)	BankIt2111172	MH294478

2	ϕAcib_5A1(2)	BankIt2111172	MH294479
3	ϕAcib_5A1(3)	BankIt2111172	MH294480
4	ϕAcib_6	BankIt2131217	MH593592
5	ϕAcib_A6(1)	BankIt2131217	MH593589
6	ϕAcib_A6(2)	BankIt2131217	MH593590
7	ϕAcib_A6(3)	BankIt2131217	MH593591
8	ϕAcib_ A6(4)	BankIt2100289	MH195145
9	ϕAcib_ A6(5)	BankIt2102015	MH195146



Figuer 5: Results Phylogenetic relationships based on partial nucleotide sequence of capside gene in bacteriophages positive

DISCUSSION

Bacteriophages are obligate parasites of bacteria characterized by the breadth of hosts that they can infect So have potential as biological control agents of *Acinetobacter sp.* host range and their ability to replicate exponentially within their hosts and their specificity make them ideal candidates for more MDR-AB control are depends on the genotypes and morphologies of the phage and the bacterial host, but also on the environment in which they are interacting [36]. Understanding phage host range is critical to predicting the impacts of these parasites in their natural host communities and their utility as therapeutic agents [37]. Lytic phages, specifically, were derived from an environmental water sample in Al-Diwanyia city. The period of work witnessed the implementation of two strategies according to Jurczak-Kurek et al., 2016, with modifications, based on the plaque show and sizes produced. Eleven phages were selected for subsequent screening due to their varying activity, including phage titre, host range analysis, and single-step growth curves. The difference in abundance of host bacteria in sample collection sites could be a potential reason for this scenario, as bacteriophages are obligatory parasites and their proliferation depends on the growth of the host bacteria. Our results align with those of Lachnit *et al.*, 2018. [40] the presence of bacteriophages at contaminated sites was observed to be significantly greater than at pure sites. Sediment characteristic models indicated that the majority of the

environmental stress-related variation in the viral dataset could be explained by the total organic carbon present in the sediments [41]. Our findings differ from those of Sabri et al., 2014 [42], who isolated phages to Escherichia coli from sewage, as well as other previously published studies, such as Jassim et al., 2012 [43], where sewage water was identified as the optimal environmental source for obtaining lytic phages with aggressive infectivity [44]. The observation of various phage types in the water sample was evidenced by the dissimilarity in plaque dimensions. A phage that exhibits a gradual rate of propagation, ultimately yielding a limited number of progeny phage, is inclined to generate a relatively diminutive plaque as opposed to a rapidly proliferating phage [45]. To characterize the life cycle of phages with regards to the infection process, a one-step growth curve was conducted. This particular phage growth kinetic serves as a baseline indicator for its therapeutic potential, and it can effectively be applied to regulate the growth of the host bacterial population. Through a one-step growth curve, the phage burst size, latent period, rise period, and the time required for the phage to cause cell lysis can be determined. Additionally, this method aids in evaluating phenotypic differences that may arise between phage strains on the same host cell. It is worth noting that related phages can exhibit markedly different lytic efficiency on the same host bacteria [46, 47]. Furthermore, these phages show extensive overlap in terms of their physicochemical properties and cannot be differentiated solely based on morphology [48]. When comparing physicochemical parameters of sewage samples used for isolation of bacteriophages with the percentage of isolated phages belonging to different families, it appears that Myoviridae might exhibit higher survivability in poor environment conditions of their hosts than other families [49]. The structural capsid protein is a ~ 11 kDa basic protein with a length of 105 amino acids[50]. Capsid is involved in guiding the virion genomic content into the host cytoplasm after the fusion of viral and host membranes induced by surface proteins [51,52] , analysis revealed that the N- and C-terminal regions of capsid protein are intrinsically disorderedwhere [53] two software programs, PhiSiGns, GitHub and Gene Order 4.0, were utilized in addition to numerous phage sequences to identify closely related phage species and their relevance to other phage for the gene bank. A pair of specific diagnostic primers were designed for one of the common gene structural genes found in most *Acinetobacter* sp. phages, namely the capsid gene, which was referred to with the symbol gp21. The phages were then sorted using various classification criteria such as determented phages name. Subsequently, the phages were selected, The present study employed a program that facilitated the screening of all protein sequences from each phage genome via a Primer-BLAST sequence similarity search. The aim was to determine whether homologous proteins could be found in any of the other selected phage genomes. As a result, genes that were shared amongst multiple phage genomes were successfully integrated into signature gene groups. The identified signature genes were then exported from the web browser as a tab delimited file. Furthermore, compelling evidence was provided to suggest the existence of genes and secondary structure in a previously unmapped region. However, further investigation is required to gain a more comprehensive understanding of the roles this region plays in the fitness and plaque morphology of bacteriophage.

CONCLUSIONS

The identification of nine phage isolates relationships may be achieved via the determination of the nucleotide sequence through the use of the capsid gene, whether or not the entire genome is utilized for comparisons. Consequently, it may be concluded that a single-gene analysis approach for predicting phylogenetic relationships is viable for the majority of highly significant phages**.**

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