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Isolation and characterization of *Acinetobacter* phage vAbaIN10 active against carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates from healthcare-associated infections in Dakar, Senegal

Issa Ndiaye[a,g,*], Laurent Debarbieux[b], Ousmane Sow[a], Bissoume Sambe Ba[c], Moussa Moise Diagne[d], Abdoulaye Cissé[a], Cheikh Fall[a], Yakhya Dieye[a], Ndongo Dia[d], Guillaume Constantin de Magny[e,f], Abdoulaye Seck[a,g]

[a] *Pôle de Microbiologie, Institut Pasteur de Dakar, Dakar, Sénégal*
[b] *Département de Microbiologie, Laboratoire Bactériophage, Bactérie, Hôte, Institut Pasteur, Paris, France*
[c] *World Health Organization WCARO, Dakar, Senegal*
[d] *Département de Virologie, Institut Pasteur de Dakar, Dakar, Sénégal*
[e] *MIVEGEC, Université Montpellier, CNRS, IRD, Montpellier, France*
[f] *MEEDiN, Montpellier Ecology and Evolution of Disease Network, Montpellier, France*
[g] *Faculté de Médecine, Pharmacie et Odontostomatologie, Université Cheikh Anta Diop, Dakar, Sénégal*

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ABSTRACT

Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a critical antimicrobial resistance threat and a WHO-prioritized pathogen. With intrinsic resistance to multiple antibiotics and the emergence of pan-resistant isolates, CRAB infections are challenging to treat, often relying on polymyxins, tigecycline, aminoglycosides, or combinations, though co-resistance is rising globally. Phage therapy is considered as a potential treatment for multidrug-resistant *A. baumannii*. This study focused on isolating and characterizing phages active against CRAB strains from healthcare-associated infections in Dakar, Senegal.

Methods: A lytic phage, *Acinetobacter* vAbaIN10, was isolated from wastewater collected at the Aristide Le Dantec Hospital in Dakar, Senegal. Isolation, host range, efficiency of plating, temperature and pH stability, lysis kinetics, one-step growth test, sequencing, and genomic analysis were performed.

Results: Phage vAbaIN10 belongs to the class *Caudoviricetes* and the genus *Friunavirus*. Its genome is 40,279 bp in size. Phage vAbaIN10 is stable across a wide pH range (3–9) and temperature range (25°C–60°C). The phage's lytic activity was evaluated at different multiplicities of infection (MOI): MOI 10, 1, and 10^{-1}. All MOIs significantly reduced the growth of host bacteria. The one-step growth curve showed that vAbaIN10 had a latency period of 25 min and a burst size of approximately 4.78×10^3 phages per infected bacterial cell. No tRNA, mtRNA, clustered regularly interspaced short palindromic repeat, virulence factors, or antibiotic resistance genes were found in the genome.

Conclusions: The biological and genomic characteristics of vAbaIN10 meet the requirements for its potential use in phage therapy.

1. Introduction

Acinetobacter baumannii is a gram-negative bacterium, nonfermentive, nonmotile, catalase-positive that thrives in diverse environments including soil, water, and sewage, and in healthcare settings. Healthcare-associated Infections (HAIs) caused by *A. baumannii*, ranging from bacteraemia to urinary tract infections, pose sig-

* Corresponding author. Mailing address: Pôle de Microbiologie, Institut Pasteur Dakar, 36, Avenue Pasteur, Dakar, BP 220, Sénégal.
E-mail address: seydina.indiaye14@gmail.com (I. Ndiaye).

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nificant challenges, especially for critically ill or immunocompromised patients [1]. Recently attention has focused on *A. baumannii* due to escalating outbreaks and its robust resistance to antibiotics and other antibacterial treatments [2]. This has led to intensified efforts to understand its virulence, transmission dynamics, environmental persistence, and potential therapeutic interventions [3]. *A. baumannii* is an ESKAPE-E bacterial pathogen (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, Escherichia coli*) and has a higher prevalence of multidrug-resistant (MDR) strains than other pathogens do, which contributes significantly to its clinical problems [4].

The emergence and spread of carbapenem-resistant *A. baumannii* (CRAB) have garnered considerable attention, as carbapenems are among the most efficacious antimicrobial agents against *A. baumannii* infections. CRAB pose significant problems due to its resilience to treatment and are associated with heigh mortality rates from 8% to 40% [5,6]. The designation of CRAB as a critical priority pathogen by the World Health Organization (WHO) underscores the urgent need for novel antibiotics and alternative treatment modalities [7].

Phage therapy (PT), which utilizes bacteriophages (phages), viruses that infect bacteria, represents a promising strategy to combat MDR *A. baumannii* infections. Although they were discovered more than a century ago, phages have exhibited efficacy in the treatment of various bacterial infections. As an alternative or complement to antibiotics, phage therapy holds significant potential in clinical settings [8,9]. In recent years, there have been numerous reports on the isolation and characterization of diverse *A. baumannii* phages [10–12]. In vivo studies have shown the potential of phage therapy in managing MDR *A. baumannii* infections such as pneumonia [5,13,14], wound infections [15,16], and sepsis [17]. Furthermore, several impactful case studies involving human subjects [18–20] have demonstrated the potential efficacy of phage therapy in addressing *A. baumannii* infections. However, limited research in Africa, despite the significant prevalence of *A. baumannii* observed in low and middle-income countries, highlights the need to develop phages research in this region to address antimicrobial resistance (AMR).

In this study, we present the isolation and characterization of a novel *Acinetobacter* phage vAbaIN10, which targets CRAB strains isolated from HAIs in Senegal.

2. Materials and methods

2.1. Bacterial isolates, antibiotic susceptibility testing and growth conditions

Eleven (11) carbapenem-resistant *A. baumannii* strains from HAIs were obtained between April 2018 and July 2021 from two biobanks of routine labs at two tertiary hospitals in Dakar. The samples sources were pus ($n = 4$), vaginal swabs ($n = 1$), bronchial fluid ($n = 3$), or urine ($n = 3$). Antimicrobial susceptibility tests of the isolates were performed following the CA-SFM/EUCAST guidelines (version 2023), and the results are presented in Sup. Table S1. Bacteria were cultured in Luria Bertani (LB) broth or agar (Difco Laboratories, Detroit, MI, USA) at 37°C. K locus types (KLs) were obtained by whole genome sequencing followed by bioinformatic analysis using Kaptive 2.0 [21]. Bacterial growth was monitored turbidimetrically by measuring the optical density at 600 nm (OD_{600}), with an OD unit of 1.0 corresponding to 3×10^8 cells/mL.

2.2. Phage isolation and purification

Wastewater samples were centrifuged (5000 g, 10 min at 4°C), and the supernatants were filtered through a 0.22-µm-pore-size membrane (Millex, Syringe Filter). Phage presence was assessed using the double-layer agar method on the bacterial strains. In this process, 10 µL of the sample was added onto a freshly preformed lawn of the bacterial cell on soft LB agar (0.7%) layered over double-concentrated LB agar (1.4%). A sterile pipette tip was used to select a single plaque, which was then solubilized in phosphate buffer saline solution. Four microliters (4 µL) of the serial dilution of the phage were then spotted onto an agar plate to purify the plaques. This procedure was repeated three times. The purified phages were stored at both −4°C and −80°C in 20% (v/v) glycerol until further experimentation.

2.3. Host range analysis and efficiency of plating (EOP)

The host range of the phages was tested using a spot test on the 11 CRAB isolates. The sequence type, KLs, and O locus were determined via WGS (Sup. Table S2). Briefly, the spot test method in which a bacterial lawn was spread on the top agar, and 4 µL of serially diluted phage lysate were spotted onto the bacterial lawn. After an 18 h incubation, the plates were observed for lysed plaques.

Bacterial strains lysed by *Acinetobacter* phage vAbaIN10 were selected for EOP analysis. The ratio of plaques formed on the test strain to those formed on the host strain lawn was reported as the EOP. The experiments were performed independently in triplicate, with each assay conducted in triplicate.

2.4. Phage thermal and pH stability assay

To assess the thermal stability of phage vAbaIN10, 100 µL of phage (1.38×10^{10} PFU/mL) was incubated for 1 h at 25°C, 40°C, 55°C, 60°C, 65°C, or 70°C, after which 50 µL aliquots were taken to measure the phage titre. After 10-fold dilution, 4 µL of each serially diluted sample was spotted onto LB agar with the host bacterium (AB6) to determine the phage titre. For the pH stability assay, aliquots were collected after 1 h of incubation in SM buffer with various pH values ranging from 3.0 to 12.0. All experiments were performed independently in triplicate, with each assay conducted in triplicate.

2.5. Lytic profile of bacteria after phage infection

An overnight bacterial culture (200 µL) was diluted in 10 mL of fresh LB and incubated at 37°C until the OD_{600} reached 0.2. Bacterial suspensions and phages at different multiplicities of infection (MOIs) (10, 1, and 10^{-1}) were added to microplates and incubated at 37°C with agitation. As a control sample, CRAB isolates were incubated with LB without phage. Bacterial growth was monitored by measuring the OD_{600} every 10 min for 16 h with agitation using a spectrophotometer. The experiments were independently conducted in triplicate, with each assay conducted in triplicate.

2.6. One-step growth assay

One-step growth experiment were conducted as previously described [22]. Briefly, exponential phase *A. baumannii* cultures were adjusted to 10^8 CFU/mL and mixed with phage lysate at an MOI of 10^{-2}. The phages were allowed to absorb onto the bacterial surfaces at room temperature for 5 min. The mixture was subsequently centrifuged at 10,000 g for 30 s, and the supernatant was removed. The pelleted cells were resuspended in 20 mL of preheated (37°C) LB broth and centrifugated at 10,000*g* for 30 s (repeated three times). The final resuspended pellet was incubated at 37°C. Samples were taken at 5 min intervals and the soft agar overlay method was used to determine the phage titre. The time between phage infection and progeny release was defined as the

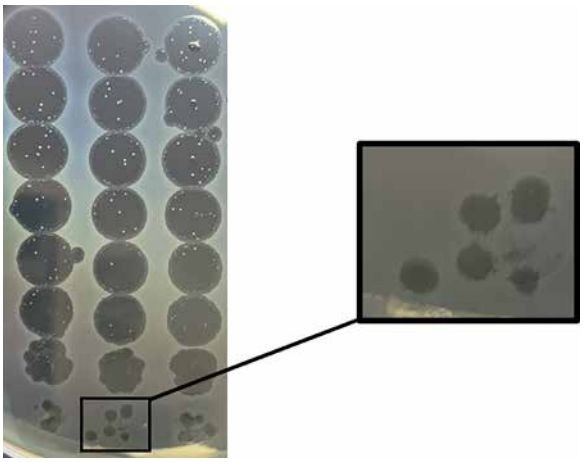

Fig. 1. Plaque morphology of phage vAbaIN10 on isolate AB4. Clear medium-sized plaques (3–4 mm) with a halo.

latent period. The ratio of the phage titre to the number of infected bacterial cells was used to calculate the burst size. The experiments were repeated three times.

2.7. Phage DNA extraction and whole genome sequencing

Genomic DNA was isolated from high-titre phage stocks (>10^{10} PFU/mL). Briefly, 1 mL of phage lysate was treated with 10 µL (20 U) of DNase I and 4 µL of RNase A (20 mg/mL) and incubated for 30 min at 37°C. The mixture was then treated with 20% SDS and 10 mg/mL of proteinase K, followed by an additional 30 min incubation at 37°C. DNA extraction was performed using the phenol-chloroform method and ethanol precipitation [23]. Nucleic acid concentrations were determined using the Qubit 2.0 fluorometer (Thermo Fisher Scientific). Libraries were prepared from 1 ng of DNA using Nextera XT DNA library preparation kits (Illumina, San Diego, CA, USA) following the manufacturer's protocol. WGS was carried out on an Illumina iSeq 100 sequencers utilizing the 300-cycle i1 Reagent V2 Kit (Illumina, San Diego, CA, USA).

2.8. Bioinformatic analysis

Quality control of the reads was performed using FastQC v0.12.1 [24], followed by adaptor trimming with trim-galore v0.6.10 [25]. The trimmed paired-end reads underwent de novo assembly using SPAdes v.3.15.5 [26] in careful mode. The assembled contigs were previsualized using Bandage v 0.9.0 [27]. Coverage per contig and assembly validation were performed using BBMap v 35.85 with default parameters [28], and the host contaminating DNA was manually removed. The input genome was sorted and indexed using Samtools v1.18 [29] followed by assembly error correction via Pilon v1.24 with default parameters [30]. The assembled whole-genome sequences were compared with those of other phages for sequence similarity using the Basic Local Alignment Search Tool (BLASTN) (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch (accessed on 09 August 2024)). In accordance with ICTV recommendations, the phage taxonomy was refined using taxMyphage (https://ptax.ku.dk/, accessed on 17 August 2024) predicted coding sequences (CDSs), transfer RNAs, transfer-messenger RNAs, virulence factors, AMRs genes, clustered regularly interspaced short palindromic repeats, and functional annotations for CDSs were performed using Pharokka v1.3.0 with default parameters [31]. Option 'pharokka_plotter' was used for circular genome visualization.

3. Results

3.1. Phage isolation and purification

Acinetobacter phage vAbaIN10 was isolated from hospital wastewater using AB6 as the host bacterial strain. Through phage purification, concentration, and titration methods, a pure, high-titre stock of phage vAbaIN10 (10^{10} PFUs/mL) was successfully obtained. The plaques produced by phage vAbaIN10 are medium-sized (3–4 mm), clear, and surrounded by a halo on a lawn of three CRAB isolates (AB4, AB6, AB7) (Fig. 1).

3.2. Host range activity determination and EOP assay

The host range activity of phage vAbaIN10 was determined via a spot test and EOP analyses. The spot test results revealed that

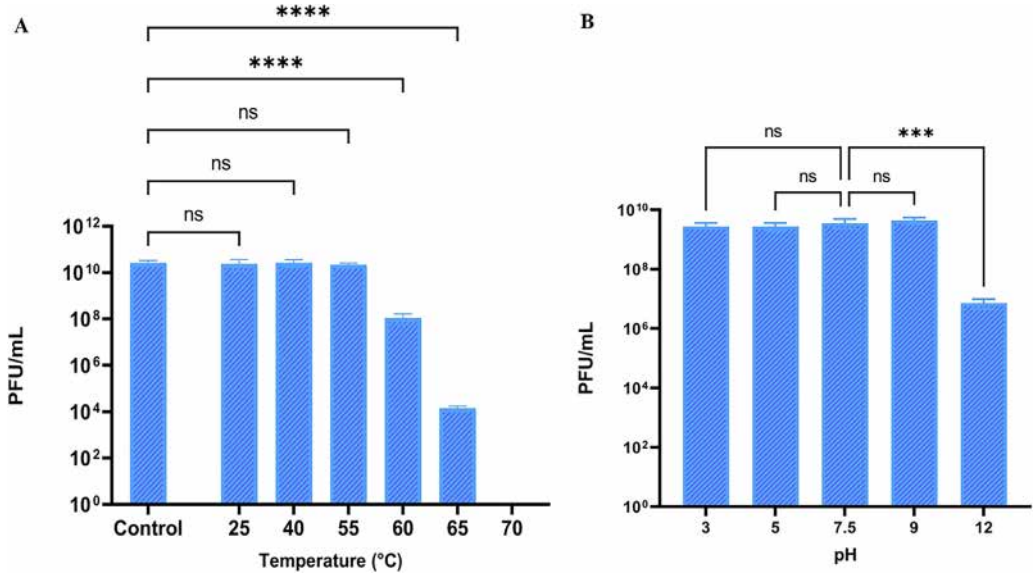

Fig. 2. Stability of phage vAbaIN10 under various conditions. (A) Effect of temperature on the stability of phage vAbaIN10. (B) Effect of pH on the stability of phage vAbaIN10. The experiments were independently performed in triplicate with triplicate assay. The data are presented as the means ± standard errors from three replicates. 'ns' means no significant difference, '*' means $P < 0.05$, '**' means $P < 0.01$, '***' means $P < 0.001$, '****' means $P < 0.0001$.

Table 1
Host range infection and EOP of phage vAbaIN10.

Strain	Resistance	Origin	K locus	Phage vAbaIN10	
				Lytic activity	EOP
Acinetobacter baumannii AB1	XDR	Pus	KL3	–	–
A. baumannii AB2	XDR	Vaginal swab	KL17	–	–
A. baumannii AB3	XDR	Bronchial fluid	KL4	–	–
A. baumannii AB4	XDR	Urine	KL47	+	0.91
A. baumannii AB5	XDR	Urine	KL18	–	–
A. baumannii AB6	XDR	Bronchial fluid	KL47	+	(Host = 1)
A. baumannii AB7	XDR	Urine	KL47	+	0.45
A. baumannii AB8	XDR	Pus	KL17	–	–
A. baumannii AB9	XDR	Bronchial fluid	KL152	–	–
A. baumannii AB10	XDR	Pus	KL17	–	–
A. baumannii AB11	XDR	Pus	KL17	–	–

+ indicates the production of a lytic zone, – indicates the absence of a lytic zone. The experiments were independently performed in triplicate with triplicate assay. The resistance profile was determined according to Magiorakos et al. [53] with phenotypes provided in Sup. Table S1.

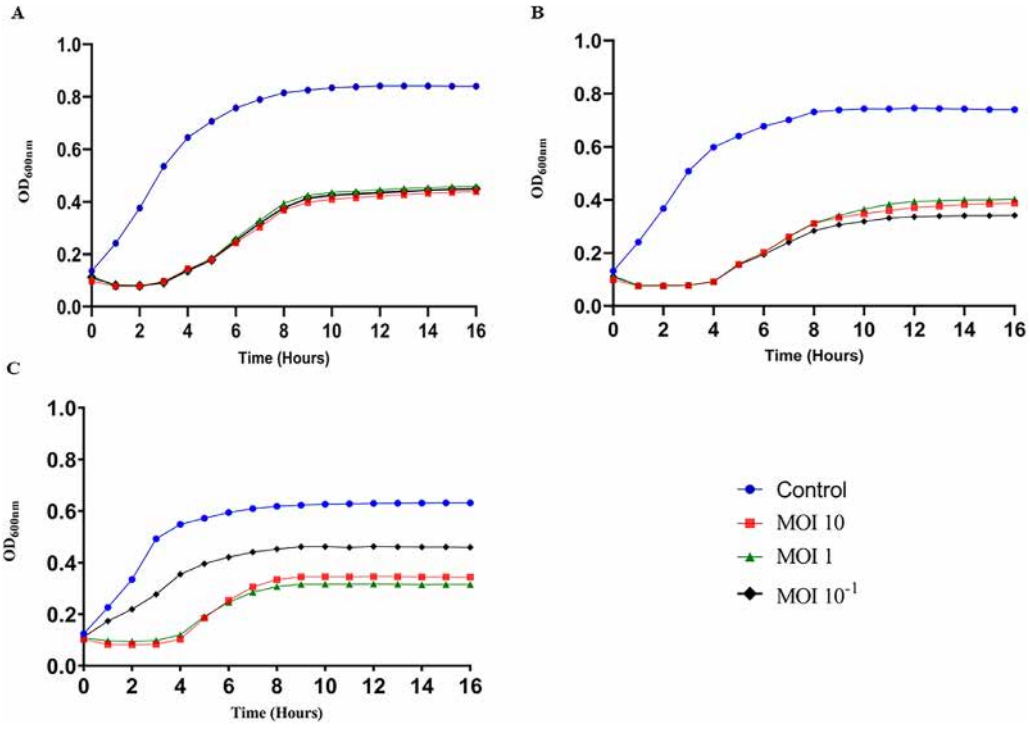

Fig. 3. *In vitro* planktonic cell lysis assay of phage vAbaIN10 against: (A) AB4, (B) AB6, and (C) AB7 at different MOIs. A 1 h timeline was used instead of a 10 min timeline for convenience. The experiments were independently performed in triplicate with triplicate assay.

Acinetobacter phage vAbaIN10 produced lytic zones against 3 of the 11 CRAB isolates tested. The 3 sensitives isolates (AB4, AB6, and AB7) which shared the same KL47 and were further assessed by EOP analysis. The results revealed that phage vAbaIN10 resulted in less productive infection in the CRAB isolates AB4 and AB6 than in the CRAB AB7 isolate (Table 1).

3.3. Thermal and pH stability studies

The stability of phage vAbaIN10 under various thermal and pH conditions was tested (Fig. 2). The thermal stability was evaluated from 25°C to 70°C for 1 hour. The results showed no significant reduction in phage stability after incubation at 25°C (*P*-value = 0.1601), 40°C (*P*-value = 0.6374), or 55°C (*P*-value = 0.9996) (Fig. 2A). A significant reduction in phage activity was observed at 60°C and 65°C (*P*-value <0.0001) and no viable phage were detected after incubation at 70°C. To assess pH sensitivity, the phage was exposed to pH values ranging from 3 to 9 for 1 hour. There was no significant change in phage viability at pH values of 4, 5, or 9 (*P*-value = 0.4137). The stability of the phage was significantly reduced at pH 12 (*P*-value = 0.0044) (Fig. 2B).

3.4. Lysis kinetics

The assessment of a phage's capacity to suppress host strain growth holds pivotal importance for therapeutic applications. Therefore, we investigated the inhibitory potential of phage vAbaIN10 against three strains (AB4, AB6, and AB7) (Fig. 3). Phage infection was initiated at various MOIs (10, 1, and 0.1). The results demonstrated a continuous increase in growth for uninfected CRAB isolates, whereas treatment with phage vAbaIN10 notably reduced bacterial growth (Fig. 3). Moreover, compared with AB4, vAbaIN10 exhibited better infection efficiency against AB6 and AB7 compare to AB4 with 47.23%, 55.52% and 34.33% of reductions, respectively after 16 h of incubation. Notably, no significant differences were observed among the treatments with different MOIs for AB4 and

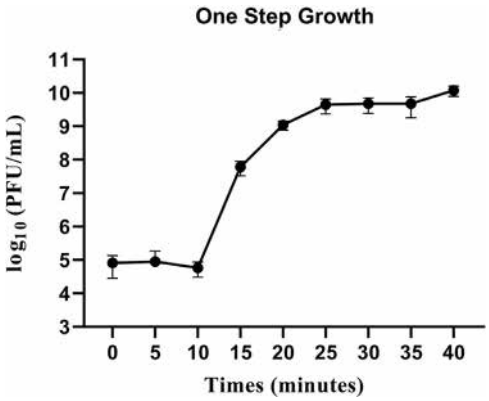

Fig. 4. One-step growth curve of phage vAbaIN10 on AB6. Phages were grown in an exponential phase culture of AB6. The data points indicate the PFUs/mL at different time points. Each data point represents the mean of three independent measurements.

AB6. The detection of bacterial resistance to phage vAbaIN10 occurred at 3 h post-incubation for AB4 and AB6 and at 4 h for AB7. Complete inhibition of bacterial growth was subsequently observed at 8–9 h across all the conditions. Notably, even an MOI as low as 0.1 effectively inhibited bacterial growth.

3.5. One-step growth assay

To identify the different phases of the phage infection process, a one-step growth assay of phage vAbIN10 on AB6 was performed. The latent period (the time between initial infection and the first burst of new phages) was 10 min. After an exponential rise, vAbIN10 reached a plateau after 25 min, with a burst size of approximately 4.78 10^3 phages/infected bacterial cell (Fig. 4).

3.6. Genomic analysis

The whole genome of phage vAbaIN10 was comprehensively characterized. Phylogenetic analysis identified phage vAbaIN10 as a member of the family *Autographiviridae*, subfamily *Beijerinckvirinae*, and genus *Friunavirus*. The genome size is 40,279 bp with a GC content of 39.27% and the CDS density is 95.83%. A total of 58 predicted genes encoding 58 proteins were identified (Fig. 5).

The functional annotation revealed 32 hypothetical proteins and 26 predicted proteins with known putative functions, which were classified into six functional groups. Nine predicted proteins were associated with phage-DNA, RNA, and nucleotide metabolism, including essential enzymes such as RNA polymerase, endonuclease VII, nucleotidyltransferase, exonuclease, DNA polymerase, ATP-dependent DNA ligase, DNA-binding protein, DnaB-like replicative helicase, and DNA primase.

Proteins relevant to the phage head and packaging functions include internal virion proteins with endolysin domains, internal virion lysozyme motifs, internal virion proteins, major head proteins, head scaffolding proteins, and terminase large and small subunits. Tail-related proteins included tail spike proteins, three tail proteins, and a head-tail adaptor acting as a connector. Host lysis-related proteins include endolysin and holin/anti-holin. Additionally, a membrane protein was implicated in moron, auxiliary metabolic genes, and host takeover processes. No transfer RNAs, transfer-messenger RNAs, clustered regularly interspaced short palindromic repeats, virulence factors, or antibiotic resistance genes were found in the genome (Fig. 5). A detailed description of the CDSs can be found in Sup. Tables S3 and S4.

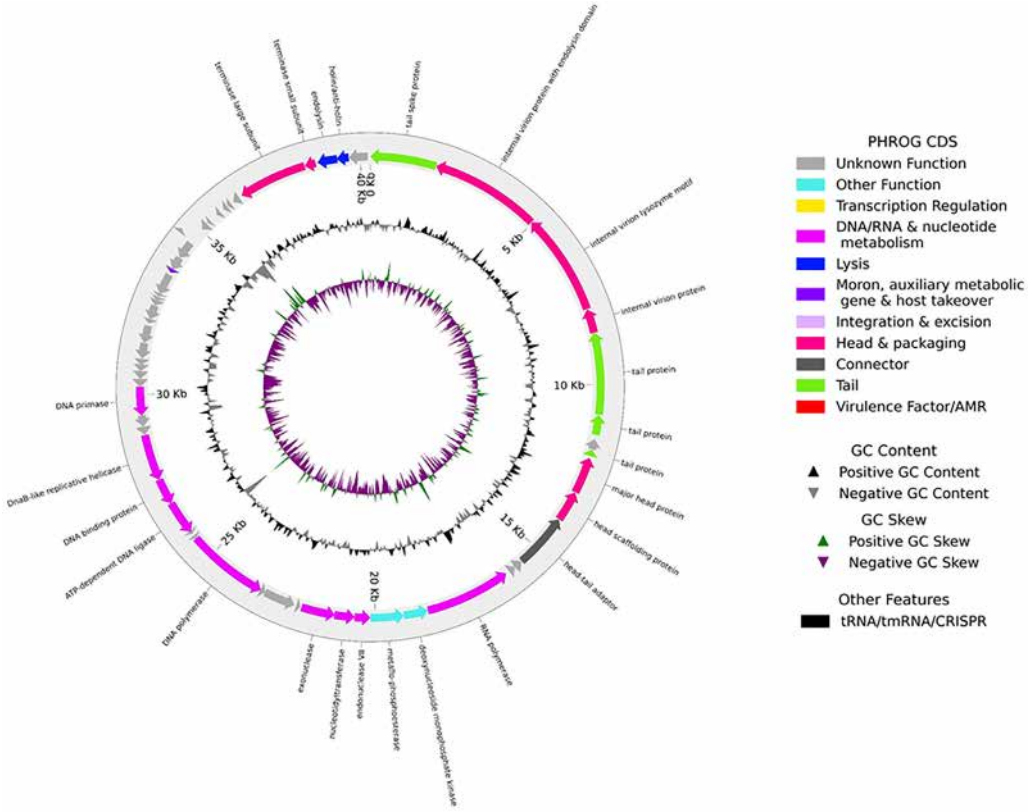

Fig. 5. Predicted CDSs of phage vAbaIN10. Transfer RNAs (tRNAs), transfer-messenger RNAs (tmRNAs), virulence factors (VFs), antimicrobial resistance genes (AMRs), clustered regularly interspaced short palindromic repeats (CRISPRs), and functional annotations of CDSs.

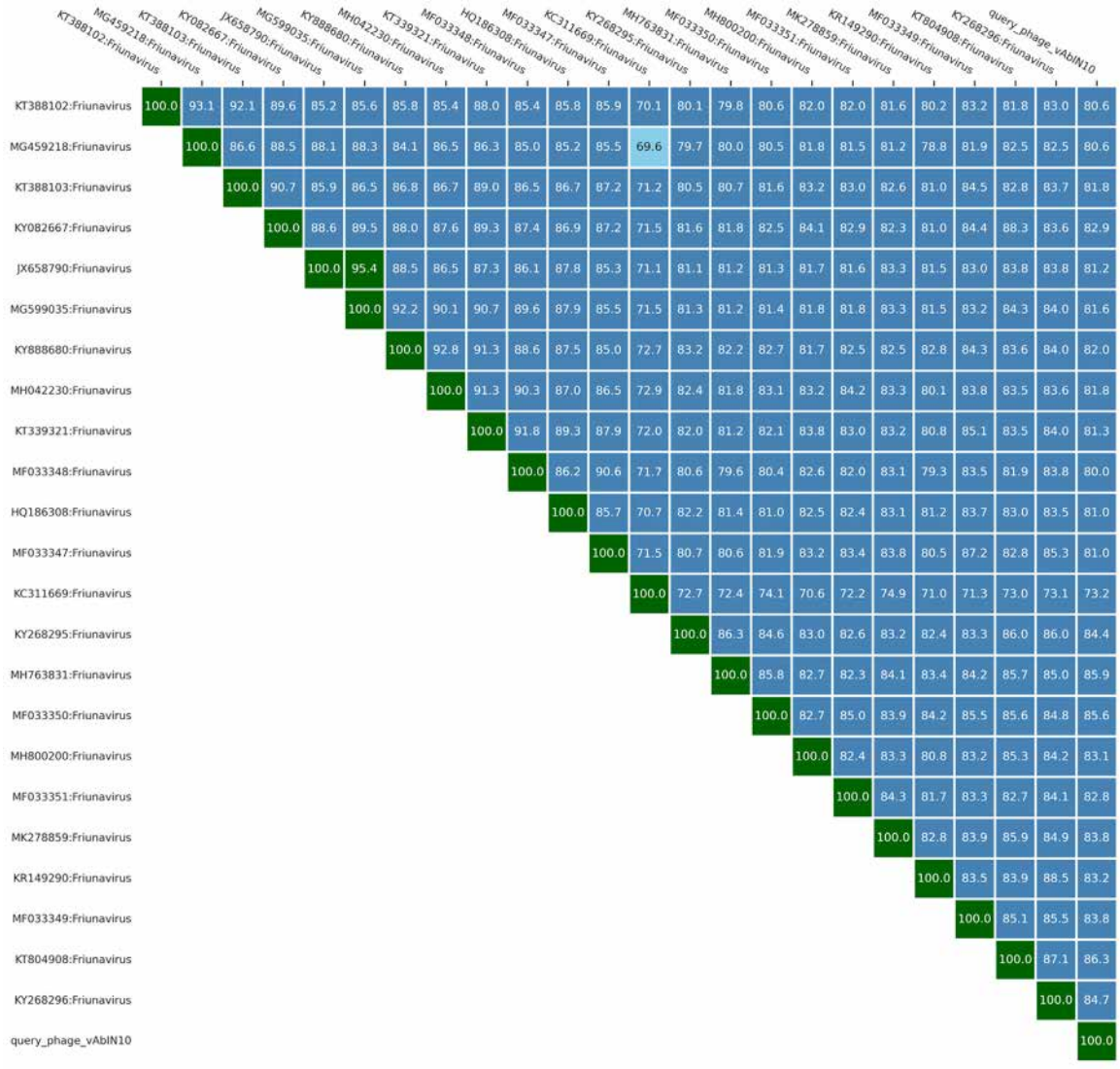

Fig. 6. Percentage sequence similarity between closely related phages calculated using taxMyPhage.

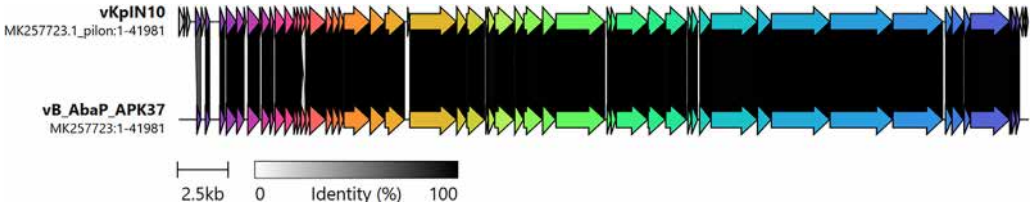

Fig. 7. Clinker gene cluster comparison of the whole genome of phage vAbaIN10 against its closest relative vB_AbaP_APK37 phage, with per cent amino acid identity represented by grayscale links between genomes. Each similarity group is assigned a unique colour.

For comparative genomic analysis, the genome of phage vAbaIN10 was aligned using BlastN against related phages selected on the basis of the sequence coverage range (>80%). ANI analysis of closely related phages revealed that phage vAbaIN10 represents a new species in the genus *Friunavirus* (ANI <95%) and the name '*Friunavirus snin*' was proposed (Fig. 6).

The phage vAbaIN10 presented the highest degree of DNA-level similarity with *Acinetobacter* phage vB_AbaP_APK37 (MK257723.1), with 95.23% identity. The visualization of the phages genome alignments through Clinker (Fig. 7), highlighted gene similarities between the two phages. Interestingly, over half of the CDSs in both phages encode proteins of unknown function, while differences in known CDSs were observed. The phage vAbaIN10 harbours a tail spike protein and a nucleotidyltransferase involved in tail structure and DNA/RNA metabolism, respectively. Conversely, phage vB_AbaP_APK37 contains an HNH endonuclease and a DNA-binding protein.

4. Discussion

A. baumannii, an opportunistic pathogen, has become increasingly important in both community and hospital-acquired infections and is related to various types of infections. It is recognized as a major MDR pathogen on a global scale and has been listed

by the WHO as one of the critical superbugs requiring the urgent development of new antibiotics [7]. Given the pressing need for alternative strategies to treat CRAB infections, phage therapy is currently been re-evaluated, with a few successful compassionate-use cases reported thus far. In this study, we present the characterization of a novel *Acinetobacter* phage, vAbaIN10, isolated from hospital wastewater in Dakar.

Through spot tests and EOP analysis, *Acinetobacter* phage vAbaIN10 demonstrated a narrow host range, specifically targeting KL47 as previously described [32,33]. The capsular polysaccharide (CPS) surrounding *A. baumannii* cells serves as a primary receptor for phages and is crucial in initiating phage-host interactions [34]. The CPS is a high molecular weight polymer consisting of recurring units of oligosaccharides (referred to as K units) connected by a Wzy polymerase [35,36]. The ability of a phage to infect different bacterial hosts at the species or genus level can vary due to the presence of capsule depolymerases [37]. Some phages, such as those effective against *Klebsiella* spp., can infect bacteria from various *Enterobacterales* species [38,39]. In contrast, phages targeting *A. baumannii* are relatively specific *Acinetobacter* spp. [40]. A limited host range can be advantageous, as it helps preserve other bacterial species and minimize disruption of the microbiota [41]. Unlike antibiotics, the narrow host specificity of phages could reduce the development of resistance among bacteria [41]. In this study, phage vAbaIN10 inhibited the growth of 3 out of the 11 strains (AB4, AB6, and AB7). However, its limited host range could pose a challenge in finding specific phages matching the bacteria's KL for each infection.

Environmental factors significantly affect phage stability and therapeutic efficacy [42]. The phage vAbaIN10 displayed notable thermal stability from 25°C to 55°C, with decreased viability at 60°C–65°C and complete inactivation at 70°C. Similarly, it exhibited stability within a pH range of 3–9, with a significant reduction observed at pH 12. These characteristics are consistent with previous findings on *A. baumannii* phages [12,32,43], suggesting their potential applicability in stable storage and as therapeutic agents against CRAB infections.

The phage vAbIN10 has a small latent period (10 min) and a large burst size (4.78 10^3 phages/infected bacterial cell). Studies have reported that phages with long latent periods can be inefficient, while those with short latency may replicate more rapidly and produce more progeny phages [44]. Other *A. baumannii* phages, such as Abp1, 350 phages/cell [45] TAC1, 454 phages/cell [46], AB1, 409 phages/cell [47], and vB_AbaM_IME285, 450 phages/cell [48], have been documented to have lower burst sizes compared to phage vAbIN10 and suggesting that this phage could be a promising candidate for therapeutic use.

Previous reports have shown that the genome size of *Autographiviridae* phages ranges from 39.425 pb to 47.868 pb [12,43,49,50], similar to that of vAbIN10. Other phages from the *Friunavirus* genus, have demonstrated efficacy in human phage therapy trials [51,52]. Among the 58 predicted genes in phage vAbaIN10 genome, 44.83% were identified as having known functions, while more than half of the genes (55.17%) remained hypothetical proteins. Notably, the vAbaIN10 genome lacked genes associated with a temperate life cycle (e.g., integrases), transposable elements, antibiotic resistance, or bacterial virulence, supporting its potential suitability for phage therapy. These findings underscore the potential use of phage vAbIN10 examined in this study for therapeutic purposes. Nevertheless, its limited ability to target only a few hosts could restrict its broader therapeutic applications.

5. Conclusions

AMR poses a growing threat to public health worldwide, with the WHO projecting 10 million annual deaths due to AMR by 2050, 90% of which are expected to occur in Africa and Asia. Addressing AMR in Africa require a multifaceted approach, encompassing improved antibiotic stewardship, surveillance systems, healthcare infrastructure, and the integration of alternative therapies such as phage therapy. Therefore, studies investigating the potential of phage therapy as an alternative or complement to antibiotics are needed. Here we isolated and characterized a lytic phage vAbaIN10 which in term of its characteristics represents a potential candidate for phage therapy against MDR CRAB isolates in Senegal. Therefore, further studies are needed to validate its putative therapeutic value.

Author contributions

Conceptualization, I.N, G.C.M., and A.S.; methodology, I.N. and M.M.D; software, I.N.; validation, G.C.M., and A.S.; formal analysis, I.N.; investigation, I.N.; resources, O.S., A.C, B.S.B., C.F., Y.D., N.D.; data curation, I.N.; writing – original draft preparation, I.N.; writing – review and editing, L.D., M.M.D., O.S., A.C, B.S.B., C.F., Y.D., N.D., G.C.M. and A.S.; visualization, I.N.; supervision, L.D., G.C.M., and A.S.; project administration, G.C.M., and A.S.; funding acquisition, G.C.M.

Data availability statement

The genome assemblies and annotations were deposited in the European Nucleotide Archive (ENA) under project number PRJEB72144 and SRA and assembly accession number ERR12535799.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2024.12.024.

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