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Genomic and proteomic characterization of four novel *Schitoviridae* family phages targeting uropathogenic *Escherichia coli* strain

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Abstract

Background *Escherichia coli*-associated urinary tract infections (UTIs) are among the most prevalent bacterial infections in humans. Typically, antibiotic medication is used to treat UTIs, but over the time, growth of multidrug resistance among these bacteria has created a global public health issue that necessitates other treatment modalities, such as phage therapy.

Methods The UPEC strain PSU-5266 (UE-17) was isolated from human urine samples, while phages were obtained from wastewater. These phages were characterized through host range analysis, stability studies, adsorption assays, and electron microscopy. Additionally, genomic, phylogenetic, and proteomic analyses were conducted to provide further insights.

Results The current study describes the isolation and characterization of four *Escherichia coli* phages designated as UE-S5a, UE-S5b, UE-M3 and UE-M6. Bactericidal assays depicted that all bacteriophages exhibited a strong lytic ability against uropathogenic *E. coli* (UPEC) strain PSU-5266 (UE-17). The phages displayed a broad host range (31–41%) among 104 tested isolates and adsorption rate of 15–20 min. They were stable within pH range of 5–11 and temperature range of 4 to 55 °C. Electron microscopy showed that all phages have icosahedral heads (70–74 nm) and short non-contractile tails, thus exhibiting a podovirus morphology. Sequencing results showed that they have linear double stranded DNA, genome of 73 to 76 kb in length, with GC content of 42% and short direct terminal repeats. Their genomes contain 84–88 predicted genes with putative functions predicted to 42–48% of gene products. The phylogenetic and comparative genomic analysis results depicted that these phages, sharing > 98% sequence similarity, are new members of genus *Gamaleyavirus* of subfamily *Enquatrovirinae*, in the *Schitoviridae* family. Mass spectrometric analysis of purified phage particles identified 44–56 phage particle-associated proteins (PPAPs) belonging to various functional groups such as lysis proteins, structural proteins, DNA packaging related proteins, and proteins involved in replication, metabolism and regulation. In addition, no genes encoding virulence factors, antibiotic resistance or lysogeny factors were identified.

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Conclusion The overall findings suggest that these bacteriophages are potential candidates for phage therapy in treating UTIs caused by UPEC strains.

Keywords Urinary tract infections, Uropathogenic *E. coli*, Phage therapy, *Schitoviridae*, Phage particle-associated proteins (PPAPs)

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases that affected > 400 million people, and caused > 235,000 mortalities worldwide in 2019 [1]. Nearly 50% of women and 12–15% of men experience a urinary tract infection once in their lifetime [2]. Moreover, UTIs rank among the most prevalent infections reported in older people, following closely behind respiratory infections. They contribute to approximately 15.5% of hospitalizations and 6.2% of fatalities in individuals aged over 65 years [3, 4]. This high prevalence rate causes 11 million doctor visits and 1.7 million emergency room visits every year, which has brought a huge economic burden to the health care system, and the global disease treatment and management costs are as high as 6 billion US dollars annually [5]. A European study investigated the healthcare cost of complicated UTIs caused by MDR Gram-negative bacteria in eight high prevalence countries, revealing that the average treatment cost was EUR 5,700 per case [6].

UTIs can be caused by Gram-negative and Gram-positive bacteria as well as by certain fungi but the main etiological agent responsible for up to 80–90% of infections is uropathogenic *Escherichia coli* (UPEC) [7, 8]. UPECs have been classified into four major phylogenetic groups, i.e., A, B1, B2 and D, distinguished by numerous virulence factors and pathogenicity islands (PAI) [9]. These groups express a range of virulence factors, including adhesins, flagella, toxins, iron-acquisition systems, and surface polysaccharides contributing to their ability to cause UTIs [10]. UPECs do not cause only UTIs, but also more severe diseases such as pyelonephritis, often accompanied by sepsis. Generally, antibiotics such as ciprofloxacin, ampicillin, trimethoprim (TMP), sulfamethoxazole (SMX), second or third-generation β-lactam cephalosporins are often used for its treatment [11]. However, even after treatment, patients often suffer from repeated infections of the same or different UPEC strains. In addition, effective long-term treatments are complicated by the increased number of antibiotic resistant UPEC strains [12]. A 2022 study on the economic burden of UTI caused by antibiotic-resistant UPEC strains incurred $426 more in per case treatment costs over a 6-month period compared to those with susceptible strains [13]. According to WHO, Global Antimicrobial Resistance and Use Surveillance System (GLASS) 2022 report, more than 20% of UPEC strains are resistant to first- and second-line antibiotics, such as co-trimoxazole, ampicillin, and fluoroquinolones. Additionally, 41.8% of *E. coli* strains responsible for bloodstream infections exhibit resistance to third-generation cephalosporins, highlighting a concerning trend in antimicrobial resistance [14]. Moreover, the World Health Organization (WHO) has classified carbapenem-resistant *E. coli* as a critical pathogen for new treatment development [15]. Therefore, alternative drugs and therapies are required to cope this problem. Bacteriophage (phage) therapy has received recently growing interest worldwide as a promising alternative to treat antibiotic-resistant bacterial infections [16]. In phage therapy phages are used to treat bacterial infections. The idea of phage therapy was proposed by Felix d'Herelle already in 1917 for the treatment of dysentery caused by strains of *Shigella spp.* [17]. Phages are ubiquitous entities, estimated to reach up to 10^{31} phage particles in the world, and can be found in soil, water, and are part of human normal microflora [18]. Compared with antibiotics, they have several advantages. Phages are very specific and do not disrupt the normal microbiota thus reducing the probability of adverse effects associated with antibiotics, such as dysbiosis and secondary infections. Most phages exhibit bactericidal activity as compared to some antibiotics that have bacteriostatic activity. Additionally, various phages have the ability to disrupt and remove bacterial biofilms [19]. Since phages are only composed of nucleic acids and proteins, they show low toxicity, and unlike broad-spectrum chemical antibiotics, excreted therapeutic phages will only affect a small number of environmental bacteria. Isolation of new phages against pathogenic bacteria is easy and they are usually found from wastewater or sewage [20]. Phages possess a natural ability to co-evolve with bacteria, potentially overcoming existing bacterial resistance mechanisms. Furthermore, phages can be engineered to get desired properties, such as enhanced lytic activity, extended host range, or resistance to host defence mechanisms [21, 22].

Al-Anany et al.., (2023) in a comprehensive review evaluated the efficiency and safety of phage therapy in treating UTIs from 1920 till 2022 [23]. Over 72% of published cases reported positive results on use of phage therapy for UTIs in terms of clinical improvement and eliminating the infection causing bacteria. Phages were also generally considered safe (99%) and were found to produce only mild adverse event (1%) when used in animals or humans [23]. Various studies have been performed on the therapeutic utilization of bacteriophages

on pathogenic bacteria including *E.coli*, *Salmonella spp*, *Staphylococcus aureus*, *K. pneumoniae* [24–27].

Despite the surging interest in bacteriophage therapy for UTIs, significant gaps remain in our knowledge of the genomic and proteomic characteristics of phages targeting UPEC strains. In the current work, we report the isolation, genomic sequencing, and proteomic assessment of four novel bacteriophages targeting UPEC, offering insights into their genomic architecture, protein composition, and therapeutic potential.

Materials and methods

Bacterial strains, media, and growth conditions

The UPEC strain PSU-5266 (UE-17), serotype O25:H4, was isolated from human urine sample, collected from the National Institutes of Health (NIH) Islamabad, Pakistan. PSU-5266 (UE-17) was initially identified for its growth on urine or synthetic urine media [28]. Further identification was done by whole genome sequence analysis (accession number: SAMN27614568). The lysogeny broth (LB) was used for bacterial growth at 37 °C for 16–18 h with constant shaking at 120 rpm. Eosin methylene blue (EMB) agar plates (Oxoid™), or LB agar (LA, LB supplemented with 1.5% agar) were used for bacterial cultures. LB supplemented with 0.4% agar was used to make soft agar plates.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of bacterial strains was performed using the Kirby–Bauer disc-diffusion method, in accordance to the guidelines provided by the clinical and laboratory standards institute (CLSI) [29]. The strains were assessed against a panel of 26 different antibiotics (Table S1). The E. *coli* ATCC-25,922 strain was used as the antibiotic-sensitive control.

Bacteriophages isolation, enrichment, and purification

Bacteriophages isolation was done from wastewater collected from different areas of Pakistan. Wastewater samples were collected in 50 ml sterile polypyrene falcon tubes. After bringing the samples to the laboratory, they were homogenized to break any solid particles, and remaining particulate material was removed by centrifugation at 6000 rpm for 10 min and the obtained supernatant was passed through 0.45 µm syringe filters (HyDocs, London, UK). Enrichment of UPEC phages were performed as reported [30] with few changes. Bacterial strain PSU-5266 (UE-17) was grown in LB at 37 °C overnight. Ten ml of sewage filtrate and 1 ml of overnight bacterial culture were added to 40 ml LB and incubated in a shaking incubator at 37 °C for 16–18 h. The enrichment culture was treated with 1% chloroform, followed by centrifugation at 6000 rpm for 20 min. The obtained supernatant was passed through 0.45 µm filter to remove bacterial cells.

The presence of phages in these lysates was checked by the spot test [31]. Briefly, 100 µl of overnight bacterial culture was mixed with 3 ml of soft agar, vortexed briefly and poured onto LA plates. The plates were incubated at room temperature (RT, 23 °C) for 20 min, after which 5 µl of serial dilutions of phage lysates were spotted onto bacterial lawn and allowed to dry for 30 min. Plates were incubated at 37 °C overnight and were observed for the clear zones of lysis.

Bacteriophage titration and purification

Bacteriophages were enumerated by using the double agar layer method [32, 33]. The phage lysates were serially diluted ten-fold using SM buffer (100 mM NaCl,10 mM $MgSO_4$, 50 mM Tris-HCl, pH 7.5). 100 µl of bacterial culture ($OD_{600} \approx 0.5$) and 50 µl of phage dilution were mixed with 3 ml of molten soft agar, vortexed and poured on LA plates. Plates were incubated at 37 °C overnight. Phage titers were calculated as plaque-forming units per ml (PFU/mL), on the basis of phage dilution and counted plaque numbers. Bacteriophages were plaque-purified by picking single plaques from soft agar [34]. Briefly, a single plaque was picked using a pipette tip or an inoculating loop, mixed with 200 µl SM buffer, and shaken at RT for one hour. The phage concentration was determined by titration, and the plaque-purification was repeated at least three times to ensure the purity of each bacteriophage.

Bacteriophage production from liquid lysates

High titer bacteriophage lysates were prepared using the PEG precipitation method with some modifications [35]. Overnight bacterial culture was diluted 1:10 by adding 5 ml bacterial culture into 45 ml LB broth. Then 50 ml of this diluted bacterial culture, 1 ml of phage lysate (1×10^{10} PFU/ml) and 450 ml of LB were added into 1000 ml Erlenmeyer flask. The cultures were incubated at 37 °C overnight with constant shaking. RNase A and DNase I were added to the flasks, each to final concentration of 1 µg ml^{-1}, followed by incubation at RT for 30 min. Then, solid NaCl (29.2 g) was added, and after incubation on ice for one hr, the lysate was centrifuged at 11000xg for 20 min at 4 °C. The phage particles were precipitated by slowly stirring PEG-8000 at RT into the supernatant until it reached 10% of final concentration. The mixture was then incubated at 4 ∘C for one hr. The precipitated phages were pelleted by centrifugation at 11,000× g for 20 min. The phages pellets were resuspended into 8 ml of TM buffer (50 mM Tris-Cl, 10 mM $MgSO_4$, pH 7.8) and allowed to stand at 4 °C overnight. The obtained suspension was further extracted three times with an equivalent volume of chloroform and then

centrifuged at 3000×g at 4∘C for 15 min to remove any remaining PEG. The glycerol density gradient ultracentrifugation [36] was used to further purify the phages. The phage lysates were centrifuged through 5% and 40% glycerol cushions in TM buffer at 40,000 rpm for 3 h at 4 °C using the BSW55Ti Beckman rotor. The obtained phage pellet was resuspended in SM buffer, and the ultracentrifugation was repeated one more time. Phage titers were determined using the double agar layer technique.

Transmission Electron microscopy

For TEM analysis the high titer phage lysate buffer was changed into neutral 0.1 M ammonium acetate utilizing the Vivaspin 6 (10^5 Da cut-off) ultrafiltration (Minisartfi Sartorius) concentrator. Three µl of phage sample was pipetted on carbon coated 400 mesh copper grids, and after allowing the phages to absorb for 60 s, 3 µl of 3% Uranyl acetate was added, and after 30 s the liquid was removed using filter paper. The prepared grids were observed under 80 kV using a Hitachi HT7800 transmission electron microscope (Institute of Biotechnology, University of Helsinki, Helsinki, Finland). Images were captured via the Gatan Rio 9 (model 1809) bottom-mounted CMOS camera.

Host range and efficacy of plating analysis

Bacteriophages were screened against a total of 104 bacterial strains, comprising of 97 strains of *E. coli*, including four *E. coli* laboratory strains, two *Klebsiella pneumoniae*, two *Bacillus safensis*, two *Streptococcus pneumoniae* strains, and one *Acinetobacter baumannii* strain (Table S2). Bacteriophage sensitivity was tested using the spot test as described above. Plates were observed for zone of lysis after overnight incubation at 37 °C.

To further validate the host range, the efficiency of plating (EOP) assay was performed following a previously described protocol [31]. Briefly, 21 bacterial strains that exhibited clear, turbid, or no spots in the initial spot test were selected and grown under the same conditions. Serial dilutions of the phages (10^8 to 10^2 PFU) were prepared, and 10 µL drops were spotted onto double-layer agar plates. After overnight incubation, the plates were examined for zones of lysis or individual plaques within the spots. The EOP was calculated as the ratio of PFU on the target bacteria to PFU on the host bacteria. Phage infection efficiency was considered high with EOP values ≥ 0.5, medium with EOP values between 0.1 and 0.5, low with EOP values 0.001 and 0.1, and was classify as inefficient with EOP ≤ 0.001.

Rate of phage adsorption

Phage adsorption assay was carried out in accordance with Bleackley et al., (2009) some modification [37]. Phage lysate at a multiplicity of infection (MOI) of 0.1 was added to bacterial culture in LB (OD_{600} ~ 0.5 and/or 1.2×10^8 CFU/ml). 100 µl aliquots of the phage-bacterial suspension were withdrawn from the flask every 3 min for 21 min. The aliquots were diluted 1:10 in ice-chilled SM buffer and centrifuged for 5 min at 4 °C and 12,000 rpm. The supernatant was transferred into a new eppendorf tube and filter sterilized using a 0.22 µm pore size syringe filter. The remaining phage titers in the supernatants were determined using the double layer agar assay as described above. The phage absorption was estimated as the non-adsorbed phage percentage, $N/N_0 \times 100$, where N_0 is pfu ml^{-1} at T = 0 min, and N is pfu ml^{-1} at T = 3,6,9,12,15 min.

Phage stability assays

The stability of phages at various temperatures and pH was studied as described by [38, 39]. The temperature stability of the phages was checked by pre-incubating the phage suspension at different temperatures (4 °C, 25 °C, 35 °C, 45 °C, 55 °C, 60 °C, 65 °C) for 2 h. The phage suspensions were then cooled to RT, diluted 1:10 in TM buffer, and the phage titer determined using the double agar layer method.

For pH stability assay, phage lysates were diluted 1:10 into 1 ml of LB with pH values adjusted to pH 1, 3, 5, 7, 9, 11 and 14 using 1 M NaOH and 1 M HCl solution before autoclaving. After incubation for 60 min at 37 °C, the suspensions were diluted 1:10 in TM buffer, and the phage titers were determined using the double layer agar method.

In vitro phage killing assay

The assay was performed as described previously with some changes [40]. Briefly, overnight culture of the UPEC strain PSU-5266 (UE-17) was diluted in LB to OD_{600} of 0.2. From this, 180 µl aliquots were distributed into 96 microtiter plate wells, to which 20 µl of phage dilutions were added to achieve MOIs of 0.01, 0.1, 1, 10, and 100. 20 µl of LB was pipetted to control wells. The plates were incubated with constant shaking at 37 °C, and the OD_{600} was measured every hr up to 7 h using microplate reader.

Phage DNA isolation

Phage DNAs were extracted utilizing the phenol-chloroform method with few changes [41]. Briefly, 10 µl of RNase A (1 mg/ml) and 3 µl of DNase I (1U/µl) were added to a 1 ml aliquot of the phage lysate, and incubated at 37 °C for one hr. Then 40 µl of EDTA (0.5 M), 3 µl of Proteinase K (20 mg/ml), and 50 µl of 10% SDS, were added to the tube that was gently mixed and incubated at 55 °C for one hr. After allowing the tube to cool for 10–15 min at RT, an equal volume of phenol was added, gently mixed for 10–15 min, and then centrifuged at 13k rpm for 5 min to separate the phases. The

phenol extraction of the aqueous phase was repeated two to three times, followed by extraction with an equal volume of chloroform. To precipitate DNA, 0.1 volume of ice chilled 3 M sodium acetate (pH 7.0) and 2 volumes of absolute EtOH were added to the sample, and centrifuged after 15 min incubation on ice at 13k rpm for 5 min. The obtained pellet was washed with 1 ml of 70% ethanol and air dried at 37 °C for 15–20 min. The pellet was dissolved into 100 µl MiQ water and incubated at 4 °C overnight. Qubit™ 4 Fluorometer device (Invitrogen, Thermo Fisher Scientific) was utilized to determine the DNA concentration.

Phage genome sequencing and bioinformatics analysis

DNA sequencing was performed utilizing the Illumina NovaSeq PE150 platform at Novogene. The quality assessment of the obtained raw sequence reads was conducted via FastQC v.0.12.1 tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). For assembly, subsets comprising 50,000 forward and reverse sequence reads for each phage were prepared using the Chipster platform [42]. The *de-novo* assembly was then performed from these subset sequences using A5 miseq pipeline [43]. To validate the assembly, the original reads were mapped to the assembled contigs using the Geneious Prime 2022.2.2 (https://www.geneious.com/). To identify the phage genome physical ends, the PhageTerm tool was employed [44]. Putative protein-coding open reading frames (ORFs) were identified and annotated through the Rapid Annotation using Subsystem Technology (RAST) [45]. The annotation was further manually verified using Artemis 18.2.0 [46], Pharokka [47], BLASTp (Protein Basic Local Alignment Search Tool) [48] and the MPI bioinformatics toolkit HHpred [49]. The phage life cycle was depicted using the web tool Phage AI (https://phage.ai/) [50] and BACPHLIP [51]. Virulence genes were searched using VirulenceFinder 2.0 (https://bio.tools/virulencefinder) [52], while Antibiotic resistance genes, using ResFinder 4.1 (https://cge.food.dtu.dk/services/ResFinder/) [53]. For the identification of tRNA genes, the tRNAscan-SE tool (http://lowelab.ucsc.edu/tRNAscan-SE/) was used [54].

Restriction endonuclease analysis

To validate genome assembly results, phage DNA digested using restriction enzyme ApaLI (Thermo Fischer Scientific) that was selected as it produced well-separated bands when the phage sequences were subjected to in silico digestion using the NEBcutter tool (https://nc3.neb.com/NEBcutter/). The 20 µl of restriction digestion reactions contained phage DNA (1 µL), restriction enzyme (1 µL), and 10× Fast digest buffer (2 µL) (Thermo Fisher Scientific) and 16 µL water. The tubes were incubated at 37 °C for two hr. The digested DNA fragments were analyzed on 0.7% agarose gel containing Midori green and the bands visualized using the BioRad GelDoc XR + imaging system.

Phage particle proteomics analysis

After the protein concentration of the glycerol-density-gradient-purified phages was determined using Qubit™ 4 Fluorometer (Invitrogen, Thermo Fisher Scientific), the phages ($\sim 10^{10}$ pfu/ml) were sent to the Proteomics Unit, Institute of Biotechnology, University of Helsinki, Finland, for protein identification by liquid chromatography coupled with mass spectrometry (LC-MS/MS).

Phylogeny and genome comparative analysis

The phylogenetic and comparative assessment of the phages was achieved by various approaches. To determine pairwise intergenomic similarities between phages, sixteen bacteriophages exhibiting high similarity to all four phages were identified using BLASTn (Table S3). The analysis was performed using Virus Intergenomic Distance Calculator (VIRDIC) (https://rhea.icbm.uni-oldenburg.de/viridic/) with the default threshold levels for genus (> 70%) and for species (> 95%) discrimination [55]. For the construction of the phylogenetic tree based on terminase large subunit unit amino acid sequences, thirty terminase sequences were identified using BLASTp for phages belonging to different genera of class *Caudoviricetes* (Table S4). The multiple sequence alignment of these sequences was performed in MEGA 11 using Clustal W algorithm, and subsequently the phylogenetic tree was constructed using neighbor-joining phylogenetic tree employing 1,000 bootstrap replicates [56]. The resulting tree was visualized using iTOL [57]. The whole genome sequence comparisons among four phages and their closest relatives were performed utilizing Easyfig v 2.2.5 [58].

Results

Antibiotic susceptibility pattern of the UPEC strain PSU-5266 (UE-17)

The AST results of UPEC strain PSU-5266 (UE-17) showed that strain was resistant to fourteen antibiotics including amoxicillin, ampicillin, tetracycline, gentamicin, streptomycin, ciprofloxacin, norfloxacin, levofloxacin, sulfonamide (SXT), erythromycin, ceftriaxone, cefotaxime, cefepime, ofloxacin, and that it showed intermediate resistance to ceftazidime (Table S1).

Phage isolation and morphology features

The four bacteriophages designated as UE-S5a, UE-S5b, UE-M3 and UE-M6 were isolated from wastewater. The phages produced clear plaques with haloes in the soft agar against their host strain (Fig. 1). The plaques of UE-S5a were 1 ± 0.2 mm in diameter, while those of UE-S5b,

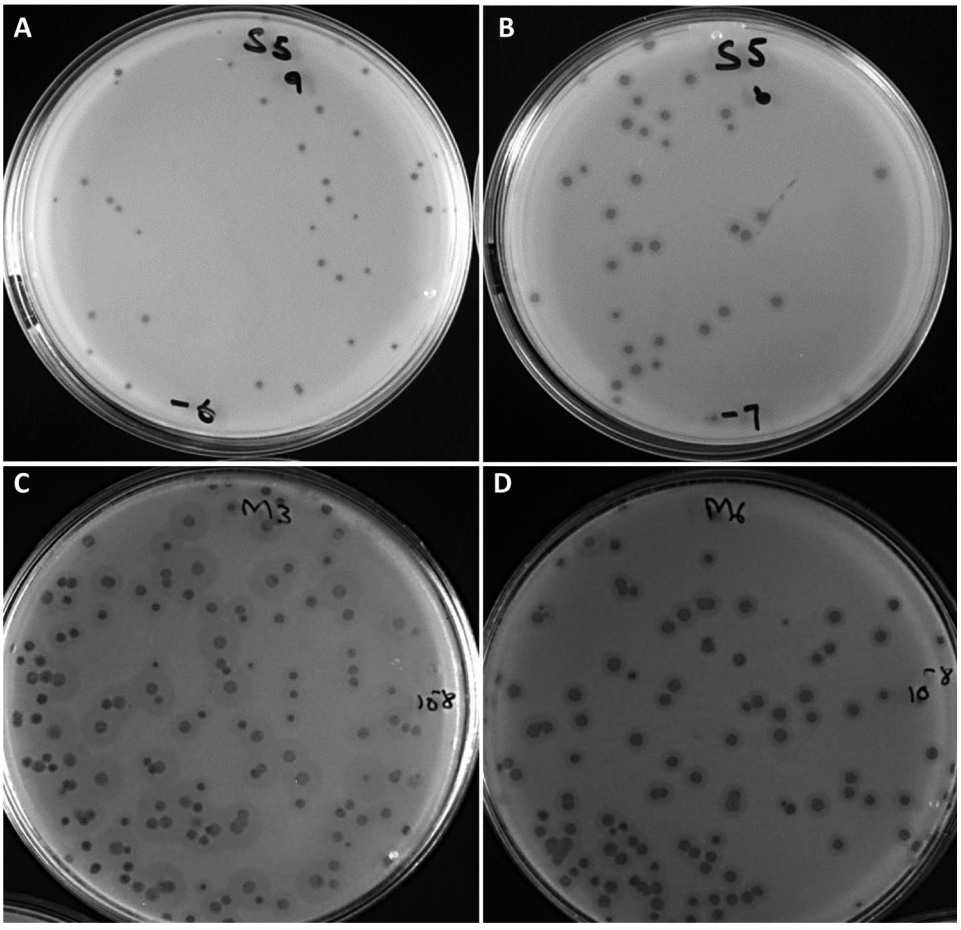

Fig. 1 Plaques formed by phages (**A**) UE-S5a (**B**) UE-S5b (**C**) UE-M3 (**D**) UE-M6, on their UPEC host strain after an overnight incubation at 37°C

Table 1 Dimension of four isolated phages ($n = 10$ phage particles)

Bacteriophages	Capsid (nm)	Tail (length x width nm)
UES5a	74 ± 2	12 ± 3 × 24 ± 3
UE-S5b	70 ± 3	13 ± 2 × 26 ± 3
UE-M3	71 ± 3	19 ± 2 × 16 ± 2
UE-M6	74 ± 3	20 ± 3 × 25 ± 3

UE-M3 and UE-M6 were 2 ± 0.2 mm in diameter. TEM revealed that all phages had icosahedral heads and short non-contractile tails (Table 1; Fig. 2), thus presenting clear podovirus morphology. Based on TEM, all phages were classified into the order *Caudovirales*.

Host range and efficacy of plating analysis

The host range analysis via spot test showed that the phages were able to infect a high number of strains, with clear lysis observed for 31–41% of *E. coli* strains (Table 2). Phage UE-M3 demonstrated the broadest host range infecting 41% of the *E. coli* strains isolated from stool, urine and blood samples. In addition, phage UE-M3 formed turbid spots on 17 UPEC strains. Likewise, phage UE-M6 also showed a broad host range, infecting 33% of the *E. coli* strains. However, phage UE-S5a and UE-S5b displayed relatively narrow host ranges, lysing only 31–32% of the *E. coli* strains. Phages UE-S5a, UE-M3 and UE-M6 effectively lysed the *E. coli* laboratory strains PM191, DH1, and K12 and UE-S5b could infect only the PM191 and K12 strains. In addition, phage UE-M3 and UE-M6 producing turbid plaques on the JM103 laboratory strain. Interestingly, all infected efficiently the Avian pathogenic *E. coli* strain APEC-01 and the mastitis causing *E. coli* strain CME-7. The EOP results revealed distinct infection patterns among the phages. Phages UE-M3 and UE-M6 demonstrated high efficiency (EOP ≥ 0.5) on 4 out of the 22 tested bacterial strains, while exhibiting medium efficiency (EOP ≥ 0.1) on 6 and 5 bacterial strains, respectively. In contrast, phages UE-S5a and UE-S5b showed high efficiency on 2 and 3 bacterial strains, respectively, with medium efficiency observed on 4 and 3 strains, respectively. Additionally, UE-S5a and UE-M6 displayed no efficient infection (EOP ≤ 0.0001) on 7 bacterial strains, whereas UE-S5b and UE-M3 showed no efficiency on 6 bacterial strains.

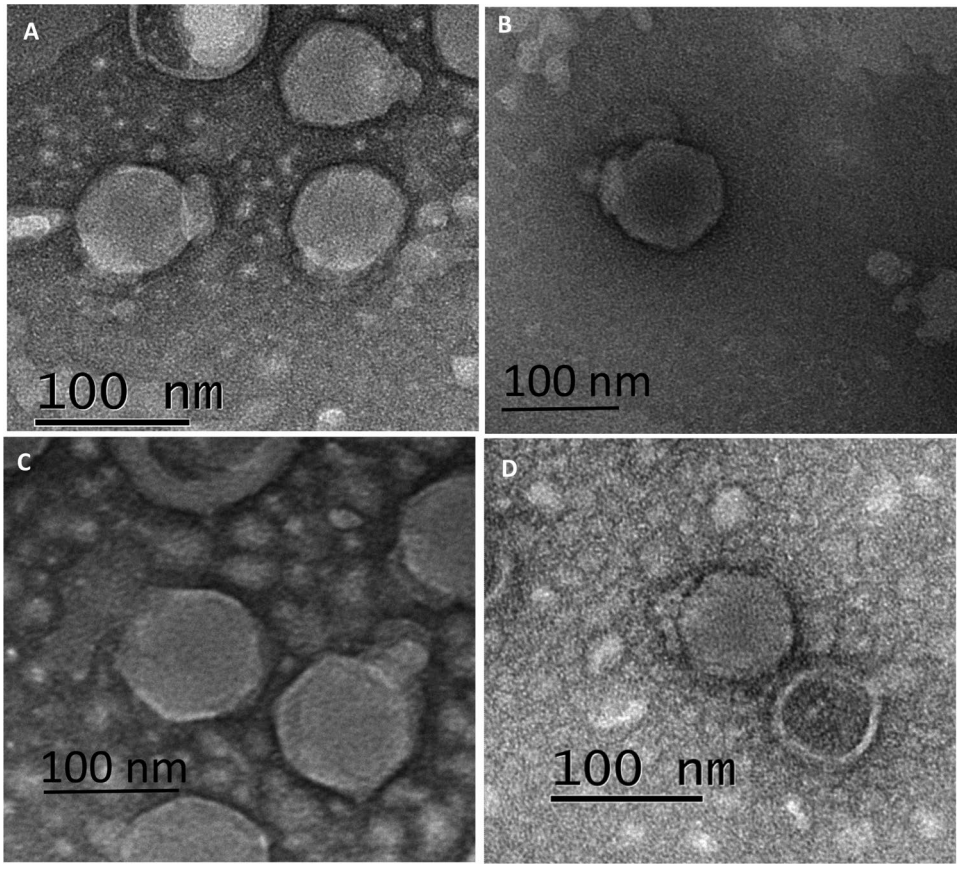

Fig. 2 Transmission electron micrograph of (**A**) UE-S5a (**B**) UE-S5b (**C**) UE-M3 (**D**) UE-M6

Phage adsorption curves

Phage adsorption assay was conducted to evaluate the initial attachment efficiencies of phages to their host bacterial cell. Phage UE- S5a showed efficient adsorption, almost 80% of the phages were absorbed within 3 min and reached 100% adsorption by 15 min. Phage UE-M6 displayed only 50% absorption within 3 min but achieved complete adsorption within 15 min. Phages UE-S5b and UE-M3 exhibited slower adsorption compared to others, with almost 95% adsorption at 20 min (Fig. 3).

Stability of phages at different temperatures and pH

The results of the temperature and pH stability assays of the phages are shown in Fig. 4. The phages, incubated between 4 and 75 °C for 2 h, generally, survived well temperatures up to 45 °C, however, with the exception that phages UE-S5a and UE-M6 displayed a 0.5 to 1 log PFU/ml reduction in their titers at 45 °C (Fig. 4A). Significant declines in the titers were observed for all phages in the temperature range of 55 to 75 °C ($p < 0.0001$). In the pH stability assay, phages tolerated well pH 7, and phages UE-S5a and UE-M3, also pH 9 (Fig. 4B). However, significant reductions in the titers were observed for all phages at pH 3, 5, 11 and 14 ($p < 0.0001$).

Time kill analysis

Bacteriolytic activity of phages UE-S5a, UE-S5b, UE-M3 and UE-M6 was evaluated by infecting UPEC strain PSU-5266 (UE-17) in liquid culture at different MOIs (0.01,0.1,1,10, and 100). Bacterial growth was followed by OD_{600} measurements for 7 h. After the first hr of incubation, all phage treated groups displayed an increase in OD_{600} (Fig. 5). After 2–3 h of incubation, a slight decrease in OD_{600} was noted for groups treated with MOIs 10 and 100. After 7 h of incubation, a noticeable reduction in OD_{600} was observed across all MOIs for all phage treated groups, while a consistent increase in OD_{600} was seen for the control groups. A clear dose related relationship among MOIs and bacterial growth was observed for all four phages. Higher phage concentration (MOIs 10 and 100) significantly restricted the OD_{600} of a bacterial host, however less reduction in OD_{600} was observed at MOIs 0.01 and 0.1. Additionally, throughout the 7-hr experiment, there was no subsequent increase in OD_{600} observed in all phage treated groups.

General genomic characteristics

Phages UE-S5a, UE-S5b, UE-M3 and UE-M6 possess linear double stranded DNA genomes of 73,821, 73,766, 73,728, and 76,110 bp in length, respectively, with a 42%

Table 2 Host range and EOP analysis on different bacterial strains

Bacterial strains	Spot Test				EOP			
	UE-S5a	UE-S5b	UE-M3	UE-M6	UE-S5a	UE-S5b	UE-M3	UE-M6
UPEC-01	++	++	++	++	0.51	0.63	0.66	0.54
UPEC-02	-	-	-	-	-	-	-	-
UPEC-03	-	-	-	-	-	-	-	-
UPEC-04	-	-	-	-	-	-	-	-
UPEC-05	-	-	+	-	-	ND	-	-
UPEC-06	-	-	-	-	-	-	-	-
UPEC-07	-	-	-	-	-	-	-	-
UPEC-08	-	-	++	-	-	ND	-	-
UPEC-09	-	-	++	-	-	ND	-	-
UPEC-10	-	-	-	-	-	-	-	-
UPEC-11	-	-	-	-	-	-	-	-
UPEC-12	-	-	-	-	-	-	-	-
UPEC-13	-	-	-	-	-	-	-	-
UPEC-14	-	-	+	-	-	ND	-	-
UPEC-15	++	++	++	++	0.294	0.412	0.5	0.355
UPEC-16	++	++	++	++	0.382	0.0745	0.303	0.0452
UPEC-18	++	++	++	++	0.471	0.261	0.633	0.355
UPEC-19	-	-	-	-	-	-	-	-
UPEC-20	-	-	-	-	-	-	-	-
UPEC-21	-	-	-	+	-	-	-	ND
UPEC-22	-	-	+	-	-	-	ND	-
UPEC-23	-	-	++	-	-	-	ND	-
UPEC-24	-	-	+	-	-	-	ND	-
UPEC-25	-	-	-	-	-	-	-	-
UPEC-26	++	++	++	++	ND	ND	ND	ND
UPEC-27	-	-	-	-	-	-	-	-
UPEC-28	-	-	+	-	-	-	ND	-
UPEC-29	-	-	-	-	-	-	-	-
UPEC-30	-	-	+	-	-	-	ND	-
UPEC-31	-	-	-	-	-	-	-	-
UPEC-32	++	++	++	-	ND	ND	ND	-
UPEC-33	++	++	++	-	ND	ND	ND	-
UPEC-34	-	-	-	-	-	-	-	-
UPEC-35	-	-	+	-	-	-	ND	-
UPEC-36	++	++	++	++	ND	ND	ND	ND
UPEC-37	-	-	-	-	-	-	-	-
UPEC-38	-	-	+	-	-	-	ND	-
UPEC-39	-	-	+	-	-	-	ND	-
UPEC-40	-	-	+	-	-	-	ND	-
UPEC-41	-	-	-	-	-	-	-	-
UPEC-42	-	-	+	-	-	-	ND	-
UPEC-43	-	-	++	-	-	-	ND	-
UPEC-44	-	-	+	-	-	-	ND	-
UPEC-45	-	-	-	-	-	-	-	-
UPEC-46	-	-	++	-	-	-	ND	-
UPEC-47	-	-	+	-	-	-	ND	-
UPEC-48	-	++	++	++	-	ND	ND	ND
UPEC-49	-	-	-	-	-	-	-	-
UPEC-50	-	-	++	++	-	-	ND	ND
UPEC-51	++	++	++	++	ND	ND	ND	ND
UPEC-52	-	-	-	-	-	-	-	-
UPEC-53	-	-	-	-	-	-	-	-

Table 2 (continued)

Bacterial strains	Spot Test				EOP			
	UE-S5a	UE-S5b	UE-M3	UE-M6	UE-S5a	UE-S5b	UE-M3	UE-M6
UPEC-65	-	-	-	-	-	-	-	-
UPEC-66	++	++	++	++	ND	ND	ND	ND
UPEC-67	++	++	++	++	ND	ND	ND	ND
UPEC-71	++	++	++	++	ND	ND	ND	ND
UPEC-72	++	++	++	++	ND	ND	ND	ND
UPEC-74	+	+	+	-	ND	ND	ND	-
UPEC-75	+	+	-	-	ND	ND	-	-
UPEC-85	++	++	++	++	ND	ND	ND	ND
UPEC-86	-	-	-	-	-	-	-	-
UPEC-87	-	-	-	-	-	-	-	-
UPEC-88	-	-	-	-	-	-	-	-
UPEC-91	+	-	++	+	ND	-	ND	ND
UPEC-92	++	++	++	++	ND	ND	ND	ND
UPEC-96	-	-	-	-	-	-	-	-
UPEC-97	++	++	++	++	ND	ND	ND	ND
UPEC-100	++	++	++	++	ND	ND	ND	ND
UPEC-101	++	++	++	++	ND	ND	ND	ND
UPEC-103	++	++	++	++	ND	ND	ND	ND
UPEC-105	+	+	+	-	ND	ND	ND	-
PE-126	-	-	-	-	-	-	-	-
PE-127	-	-	-	-	-	-	-	-
PE-130	-	-	-	-	-	-	-	-
CME-5	-	-	-	-	-	-	-	-
CME-7	++	++	++	++	ND	ND	ND	ND
BME-2	-	-	-	-	-	-	-	-
BME-10	-	-	-	-	-	-	-	-
APEC-O1	++	++	++	++	ND	ND	ND	ND
EL3622	++	++	++	++	5×10^{-5}	0.392	0.1	0.161
EL3615	++	++	++	++	0.588	1×10^{-2}	0.33	0.645
W6215	+	+	++	+	+	+	1×10^{-5}	+
EL3628	++	++	++	++	5×10^{-2}	1.57	0.367	0.677
UC14886	+	+	++	++	+	+	6×10^{-5}	1×10^{-4}
UD14891	++	++	++	++	5×10^{-7}	1×10^{-6}	6×10^{-3}	6×10^{-6}
UF05451	++	++	++	++	5×10^{-6}	1×10^{-4}	3×10^{-5}	3×10^{-5}
13KP10517	++	++	++	++	8×10^{-3}	7×10^{-2}	0.33	0.258
13KP10501	++	++	++	++	0.294	1.7	1.6	3.8
13MD3432	++	++	++	++	1×10^{-3}	1×10^{-3}	5×10^{-2}	5×10^{-2}
13MD3429	-	++	-	++	-	3×10^{-6}	-	6×10^{-5}
13EL3879	++	++	++	++	2×10^{-6}	5×10^{-5}	1×10^{-2}	4×10^{-2}
UF 05451	-	-	-	-	-	-	-	-
UB 15,026	-	-	+	+	-	-	+	+
JM103	-	-	+	+	-	-	+	+
PM191	++	++	++	++	4×10^{-5}	9×10^{-4}	2×10^{-4}	3×10^{-4}
DH1	++	-	++	++	2×10^{-5}	-	6×10^{-5}	1×10^{-4}
K12	++	++	++	++	1×10^{-4}	3×10^{-4}	5×10^{-4}	2×10^{-4}
Klebsiella 76	-	-	-	-	-	-	-	-
Klebsiella 95	-	-	-	-	-	-	-	-
Bacillus 1G	-	-	-	-	-	-	-	-
Bacillus 4B	-	-	-	-	-	-	-	-
Streptococcus CS44	-	-	-	-	-	-	-	-

Table 2 (continued)

Bacterial strains	Spot Test				EOP			
	UE-S5a	UE-S5b	UE-M3	UE-M6	UE-S5a	UE-S5b	UE-M3	UE-M6
Streptococcus B2B	-	-	-	-	-	-	-	-
Acinetobacter	-	-	-	-	-	-	-	-

++ (clear spot), + (turbid spot), - (no spot), ND (Not done)

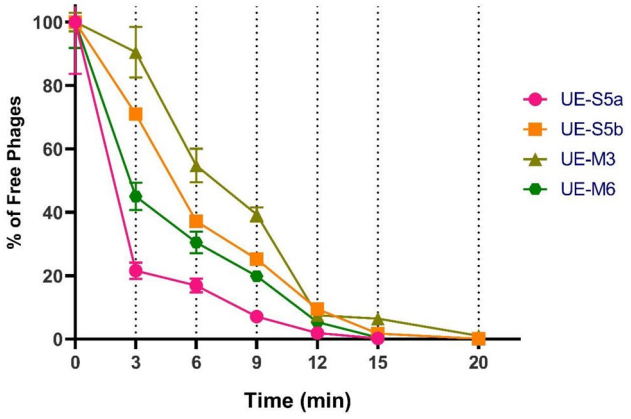

Fig. 3 Adsorption curve of phages UE-S5a, UE-S5b, UE-M3, and UE-M6 against their UPEC host strain. The percentage of unabsorbed/free phages was calculated by $T/T_0 \times 100$. The results presented here are the mean values with SD indicated by error bars from three independent experiments

GC content that is lower than that of the UPEC host strain (50.77%). Approximately 95–98% sequence reads were mapped back to assembled phage contigs, signifying successful genome assemblies for all phages. The experimental restriction digestion results with ApaLI enzyme precisely matched the in-silico digestion fragments, validating the correctness of the assembly for all phages (Figure S1). The PhageTerm analysis identified short direct terminal repeats of 401 bp for all the phages. All four phages harboured three tRNA genes and notably lacked antibiotic resistance and virulence genes. Phage AI analysis classified the phages as virulent with 98% confidence, making these phages promising candidates for therapeutics use (Table 3).

The annotations revealed that the phages UE-S5a, UE-S5b, UE-M3, and UE-M6 have 86, 87, 84, and 88 predicted genes, respectively. While 52–58% of the genes were annotated to encode hypothetical proteins, putative functions were identified for the products of the remaining 43–48% of genes. These gene products were further classified into different functional groups based on database searches, including (I) structural proteins, (II) lysis, (III) DNA packaging, (IV) replication and repair, (V) nucleotide metabolism and (VI) regulatory. In addition, the absence of genes encoding lysogeny associated products such as integrases, excisionases, repressors, or recombinases, further strengthened their potential usefulness for therapeutic purposes.

Phylogenetic and comparative analysis of phages

The pairwise intergenomic similarity comparisons using the VIRDIC software was employed to assess the genetic relatedness of the four phages to sixteen previously identified phages (Fig. 6). The analysis revealed that our four phages were closely related sharing > 98% sequence similarity, suggesting their close evolutionary relationship. Furthermore, the phages exhibited 82% sequence similarity with Escherichia phage PGN829.1 (NC_070871.1), and 81%, with enterobacteria phages Bp4 (NC_024142.2), PD38 (MH669274.1) and vB_EcoS_Uz-1 (OP312987.1). The phylogenetic tree based on the large terminase subunit sequences of thirty phages yielded results largely consistent with the pairwise intergenomic similarity data (Fig. 7). The analysis positioned the four phages in close proximity to established members of the genus *Gamaleyavirus* including *Escherichia* phages U1G (99.62% identity), Caudoviricetes sp. isolate 355 (99.81%), Bp4 (99.43%), PGN829.1 (99.62%), vB_Eco_F22 (98.11%), and *Shigella* virus Moo19 (88.68%).

Based on all these phylogenetic data the four phages can be classified as new members of realm *Duplodnaviria* > kingdom *Heunggongvirae* > phylum *Uroviricota* > class *Caudoviricetes* > family *Schitoviridae* > sub-family *Enquatrovirinae* > genus *Gamaleyavirus*. The whole genome sequence alignments of the four phages with the three most closely related phages PGN829.1, Bp4 and U1G, performed using the Blastn analysis in Easyfig, demonstrated the close similarity between the phage genomes (Fig. 8), with clear difference only between the genomic region encoding for the receptor binding proteins and packaging related.

Phage particle proteomics analysis

LC-MS/MS analysis of tryptic peptides was employed to identify phage particle associated proteins (PPAPs) of phages UE-S5a, UE-S5b, UE-M3, and UE-M6. The LC-MS/MS data provided details on the sequence coverage, molecular mass, protein probability, and the number of unique peptides of the detected proteins. PPAPs were detected by comparing identified tryptic peptide sequences to the tryptic peptides predicted to arise from the predicted gene products of the phages. To ensure accurate identification, only proteins with a high probability score (> 1), at least two identified unique peptides, and/or greater than 5% sequence coverage were considered PPAPs. By applying these criteria, 54, 56, 52 and

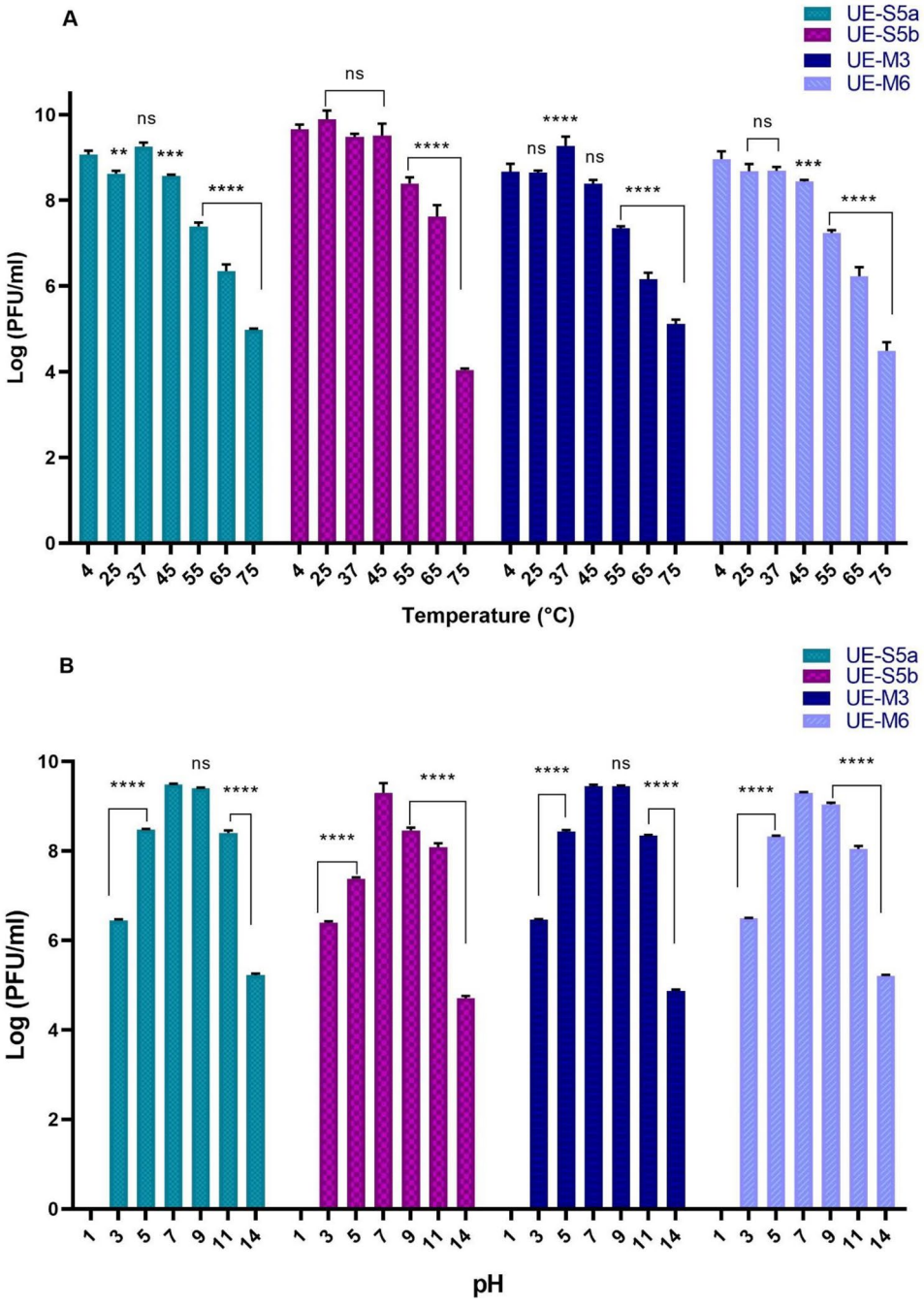

Fig. 4 The stability of phages UE-S5a, UE-S5b, UE-M3, and UE-M6 under various (**A**) temperature and (**B**) pH conditions. Statistical differences were determined by comparing values (**a**) at 4°C and (**b**) at pH 7. Results are the mean values with SD indicated by error bars from three independent experiments. The asterisks **, *** and **** denotes the significant values P<0.01, P<0.001 and P<0.0001, respectively, ns not significant

44 PPAPs were validated for UE-S5a, UE-S5b, UE-M3, and UE-M6 (Tables S5, S6, S7, and S8), respectively. Several PPAPs were identified across all four phages, these included endosialidase, K5 LYASE, G-9 protein, tail needle protein gp26, phage portal (connector) protein, phage tape measure protein and major capsid proteins. Additionally, Hoc head outer capsid protein was identified only in UE-S5a and UE-M6. Five lysis related proteins, holin, endolysin, Rz-like spanin, rIIA lysis inhibitor, and rIIB lysis inhibitor, were also identified as PPAPs. Furthermore, PPAPs that are involved in metabolism, replication and repair were also identified such as RNA polymerase, RUVC, single stranded DNA binding protein, phage-associated DNA primase exonuclease, putative 3'-phosphatase, 5'-polynucleotide kinase, phage-associated DNA polymerase, phage DNA helicase,

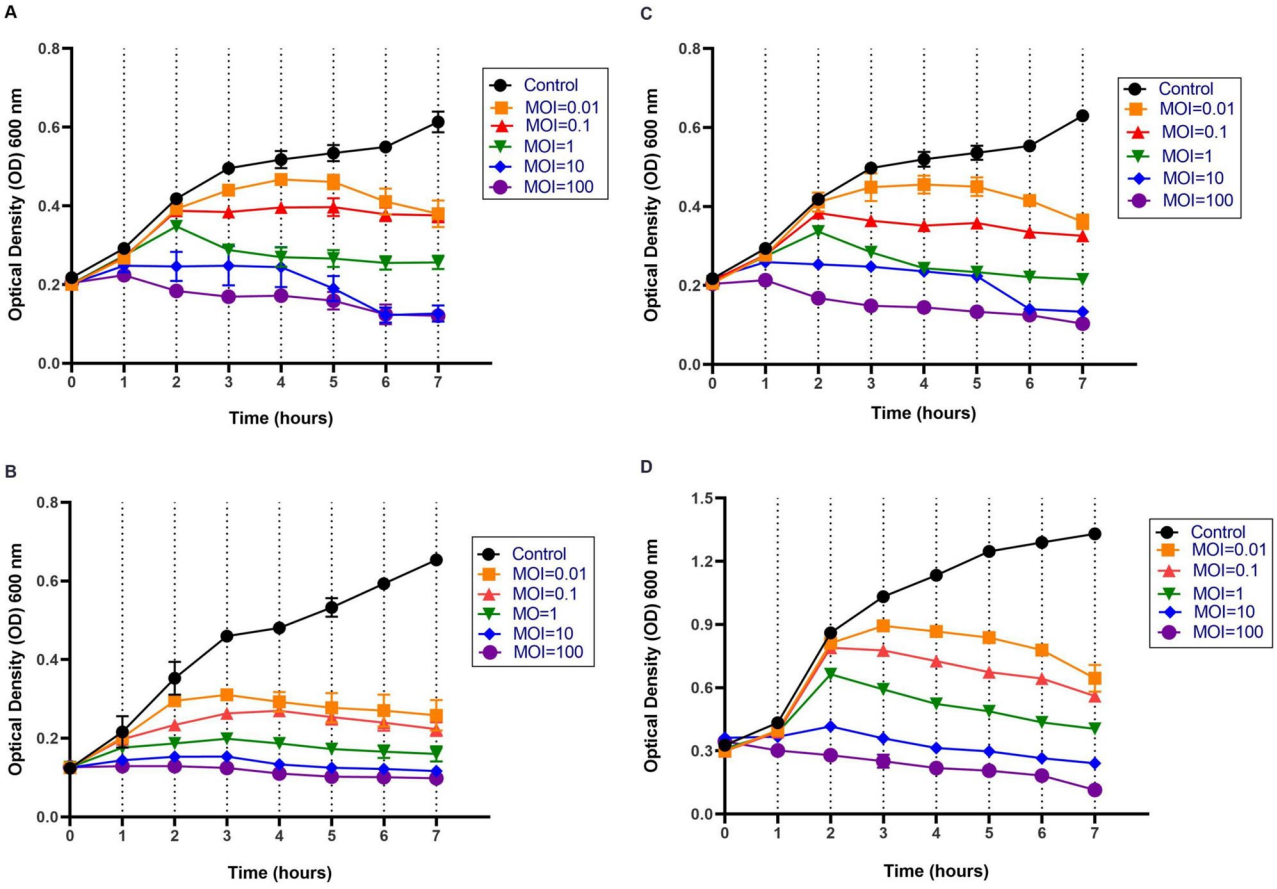

Fig. 5 *In-vitro* time killing curve of bacteriophages (**A**) UE-S5a, (**B**) UE-S5b, (**C**) UE-M3, and (**D**) UE-M6 at various MOIs of 0.01, 0.1, 1, 10 and 100. Control represent UPEC strains PSU-5266 with 20 µl of LB broth. The OD_{600} of the culture was measured for 7 hours. Results are the mean values with SD indicated by error bars from three independent experiments

putative NTP pyro phosphohydrolase, DNA binding protein, FAD-dependent thymidylate synthase, dCTP deaminase, RNAP1, RNAP2, and HNH endonuclease. In addition, few regulatory proteins were also identified.

Discussion

In current study, the four isolated phages designated as UE-S5a, UE-S5b, UE-M3 and UE-M6, were evaluated for their efficacy against the MDR UPEC strain PSU-5266 (UE-17). This strain exhibited resistance to fourteen antibiotics spanning seven distinct antimicrobial classes (Table S1) [59]. TEM micrographs showed that all phages have a podovirus morphology with icosahedral heads (70–74 nm) and short non-contractile tails (Table 1; Fig. 2). The phylogenetic and comparative genomic analysis results indicated that these phages shares > 98% similarity and belong to the family *Schitoviridae* and genus *Gamaleyavirus*. The hallmark features of the *Schitoviridae* family are the occurrence of large (~ 3500 aa) virion-associated RNA polymerase and two small RNA polymerases [60]. Interestingly, all four bacteriophages encode for the large (3456 aa) virion associated RNA polymerase (UES5a_027, UES5b_026, UEM3_026, UEM6_026), and two small ones; RNA Polymerase 1 (UES5b_067, UES5b_070, UEM3_068, UEM6_068) and RNA polymerase 2 (UES5b_069, UES5b_068, UEM3_066, UEM6_066). This further supports their classification under the family *Schitoviridae*. Until December 2024, only 252 members have been classified into this new family [61].

Assessing the ability of bacteriophages to infect different hosts is crucial for selecting therapeutic phages. We screened the phages via spot test against a total of 104 bacterial strains and they demonstrated relatively broad host ranges of 31–41%. Notably, all four phages were also able to infect the avian pathogenic and mastitis causing *E. coli* strains. These findings are in line with a study on UPEC phages MLP2, and MLP3 that were also able to lyse diffusely adherent and enteroaggregative *E. coli*, DAEC and EAggEC, respectively. It was speculated that this might be due to close phylogenetic relation between DAEC and EAggEC with UPEC, or they might share same phage receptors [62]. Similarly, the T4 phage QL01 had a broad host range (52%) and also infected one

Table 3 Genomic features of the isolated phages

Features	UE-S5a	UE-S5b	UE-M3	UE-M6
Genome size (bp)	73,821	73,766	73,728	76,110
GC content (%)	42.99	42.95	42.99	42.89
Predicted genes	86	87	84	88
Hypothetical genes	46 (53.5%)	50 (57.5%)	44 (52%)	51 (58%)
Genes with predicted function	40 (46.5%)	37 (42.5%)	40 (48%)	37 (42%)
tRNA genes	3	3	3	3
Lysogenic genes	none	none	none	none
Antibiotics resistance genes	none	none	none	none
Virulence genes	none	none	none	none
Lifestyle (based on PhageAI)	Virulent (98.71%)	Virulent (98.65%)	Virulent (98.69%)	Virulent (98.91%)
PhageTerm	T7-like short direct terminal repeats (Length 401 bp; position 71,902–72,302 bp)	T7-like short direct terminal repeats (Length 401 bp; position 13,108–13,508 bp)	T7-like short direct terminal repeats (Length 401 bp; position 55,507–55,907 bp)	T7-like short direct terminal repeats (Length 401 bp; position 71,795–72,195 bp)

neonatal meningitis-causing *E. coli* (NMEC) strain [63]. The EOP results further elucidated the infection efficiency of the phages, revealing distinct patterns among them. Phages UE-M3 and UE-M6 demonstrated high efficiency (EOP ≥ 0.5) on 18% bacterial strains, with medium efficiency (EOP ≥ 0.1) in 27% and 22% strains, respectively. This aligns with their broad host range observed in the spot test. In contrast, phages UE-S5a and UE-S5b exhibited narrower host ranges and lower infection efficiencies, with high efficiency limited to 9–13% strains, respectively, and medium efficiency on 13–18% strains. The presence of no productive infections (EOP ≤ 0.0001) in 27–31% strains for all phages suggests that while the phages while the phages could attach to host receptors, they are unable to complete the lytic cycle. This incomplete infection process likely explains the formation of spots on the bacterial lawns without resulting in productive lysis [64]. Although these phages share more than 98% sequence similarity, they exhibit differences in their host range. To investigate the basis for this variation, four receptor binding proteins i.e. endosialidase, K5 lyase, G-9 protein, and tail needle protein gp26—were analysed and compared. The results revealed no significant difference expect single amino acid variation in G-9 protein at position 11 and two amino acid variation in K5 Lyase at position 334 and 385. The phage host range specificity depends mostly on its adsorption onto the bacterial cell that is the key step in the infection process representing the initial point of contact between phage and the host. During adsorption, bacteriophages specific binding proteins recognize complementary receptors on the bacterial surface. Once attached, bacteriophage can eject its DNA into the bacterial cell to initiate phage replication process [65]. In this study, we investigated the adsorption rate of our isolated phages. UE-S5a and UE-M6 phages achieved complete adsorption within 15 min, indicating rapid adsorption. In comparison, phage UE-S5b and UE-M3 exhibited slower adsorption, taking up to 20 min to reach nearly 95% adsorption. This low adsorption rate of phages may be due to the use of low bacterial concentration (10^8 cfu/ml).

The stability of bacteriophages at various temperatures and pH are essential factors for storing and therapeutic applications [66]. Various investigation have conducted on the influence of external factors on phages survivability [67]. Phages UE-S5a, UE-S5b, UE-M3 and UE-M6 were stable up to 45 °C, however a significant decline in titers was observed for all phages within temperature range of 55 to 75 °C. Our results corroborate with previous findings that reported decrease in phage titer with increase in temperature [68, 69]. The phages were stable at pH 7–9, and did not tolerate well lower or higher pH values. These results are consistent with studies on UPEC phage VB_EcoS-Golestan [70], phage vB_Ec_ZCEC14 [71]. The results suggest that our phages can be stored at ambient temperatures and neutral pH without considerable loss of activity.

Understanding of the relationship between multiplicity of infection (MOI) and bacterial counts is an important factor to consider. In our study, a clear dose related relationship among MOIs and bacterial growth was observed for all four phages. Results showed that higher phage concentration (MOIs 1, 10 and 100) significantly restricted the growth of bacterial host, however less reduction in bacterial growth was noted at MOI 0.01 and 0.1. Similar lytic activity results were observed for bacteriophages BF9, BF15, and BF17 against *E. coli* [72], phage vB_SsapS-104 against *Staphylococcus saprophyticus* [73], and phages vB_KpnS_Kp13 against *K. pneumoniae* strain [74]. Additionally, throughout the 7 h experiment, no subsequent increase in bacterial growth was seen in all phage treated groups. This might be due to high phage concentrations likely increased rapid phage adsorption, that leads to decreased bacterial population quickly [75] or possibly due to the absences of phage resistant mutants [76].

The genomic analysis of phages UE-S5a, UE-S5b, UE-M3 and UE-M6 showed that they possess linear double stranded DNA genomes of 73 to 76 kb in length, with GC content of 42% and short 401 bp direct terminal repeats. These features align with previously identified phages of *Schitoviridae* family [77, 78]. All phages harbor three tRNAs i.e., tRNA-Ile-AAT, tRNA-Undet-NNN and

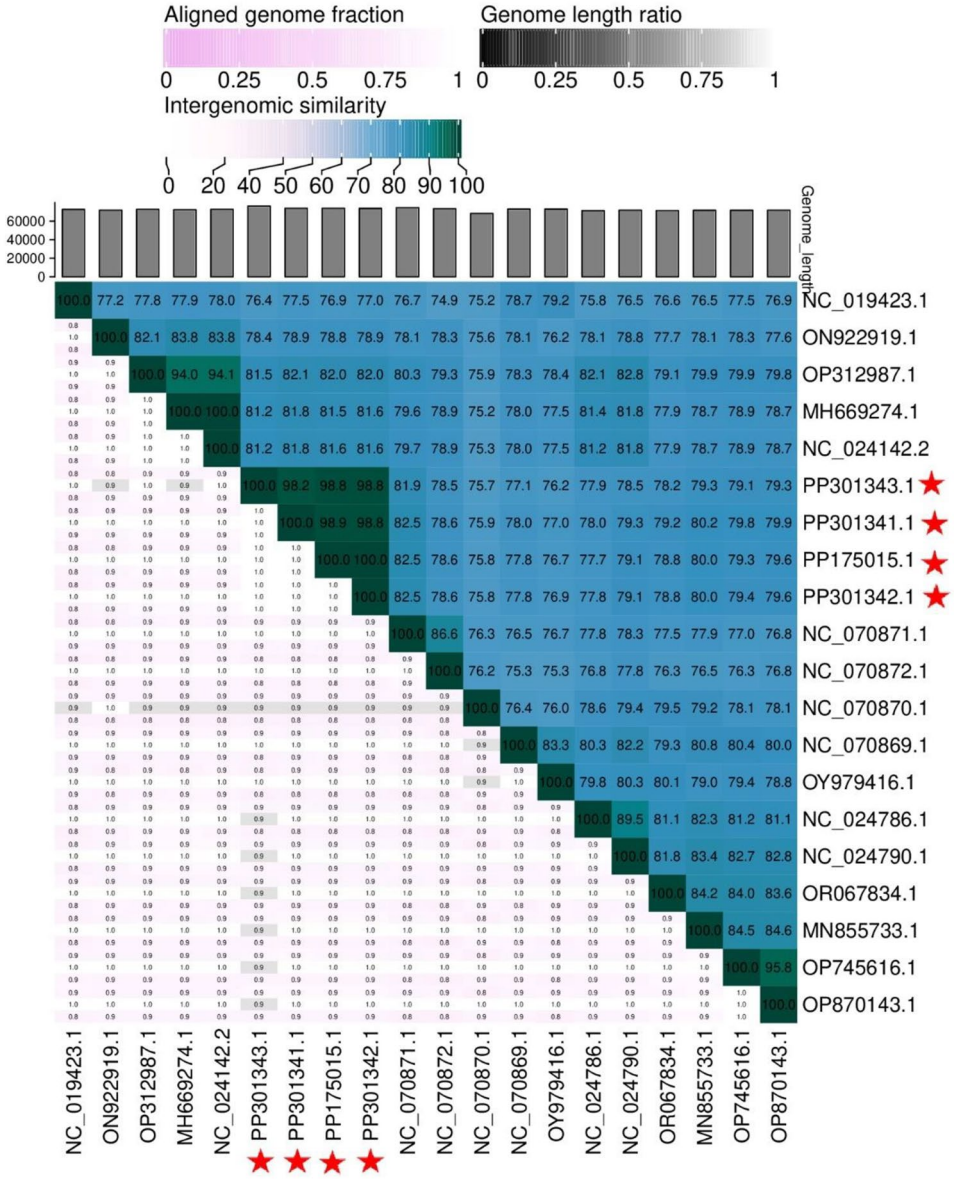

Fig. 6 This heatmap visualizes pairwise comparisons of intergenomic similarity between four isolated and sixteen closely related phages using VIRDIC software. The upper right half shows percentage intergenomic similarity, darker colours indicate higher percentage similarity between genomes. The lower left half shows, three values for each genome pair (top to bottom); top value: Proportion of Genome 1 aligned with its partner, middle value: ratio of the two genomes' lengths, bottom value proportion of Genome 2 aligned with its partner. Darker colours represent lower values, potentially indicating less aligned sequence or significant length differences. Horizontal and vertical axes list corresponding phage GenBank accession numbers. Bacteriophages of current study are marked with a red asterisk (*) next to their accession numbers.

tRNA-Cys-ACA. Several hypotheses have been proposed on the role of these tRNAs, codon compensation being the most established one [79]. Recently Yang et al., 2021, proposed that phages have their own tRNA is to keep their genes translation smooth as the host machinery gets damaged during infection [80]. In addition, safety assessment analysis showed that the bacteriophages did not contain virulence-related or antibiotics resistance genes, or lysogeny-related genes, making them promising candidates for phage therapy [81].

Comparative analysis using VIRDIC, and phylogenetic tree based on phage proteome and large terminase subunit further confirmed that these phages are new members of family *Schitoviridae*, sub-family *Enquatrovirinae* and genus *Gamaleyavirus.* They showed the highest similarity to Escherichia phage PGN829.1 and Enterobacteria phage Bp4 and phage U1G.

We identified altogether 44–56 PPAPs by LC-MS/MS analysis using the accepted criteria [82]. All four phages encode lysis proteins holin, endolysin and spanin. The

Fig. 7 The phylogenetic tree analysis based on the large terminase subunit sequence, illustrating relationship between current study phages and thirty other Caudoviricetes phages (supplementary table S4). UPEC Phages are highlighted in red, while different groups are indicated by different colours

holin form small pores in bacterial cytoplasmic membrane, allowing endolysin to leak into periplasmic space and degrade the peptidoglycan layer, and in the final step spanin degrades the outer membrane [83]. This lytic mechanism is usually employed by phages to infect bacteria cells and release new progeny [84]. Furthermore, genes for head morphogenesis, tail fiber formation, DNA packaging and metabolism were also identified by proteomic analysis.

Conclusion

This study presents four lytic phages UE-S5a, UE-S5b, UE-M3 and UE-M6 that belong to *Gamaleyavirus* genus of *Enquatrovirinae*, a sub-family of *Schitoviridae* family. The phages showed a broad host range, stability at various temperatures and pH values. Moreover, genomic and proteomic results confirmed the absence of genes encoding virulence factors, antibiotics resistance or lysogeny associated proteins. Therefore, we concluded that this study provides significant addition to bacteriophage

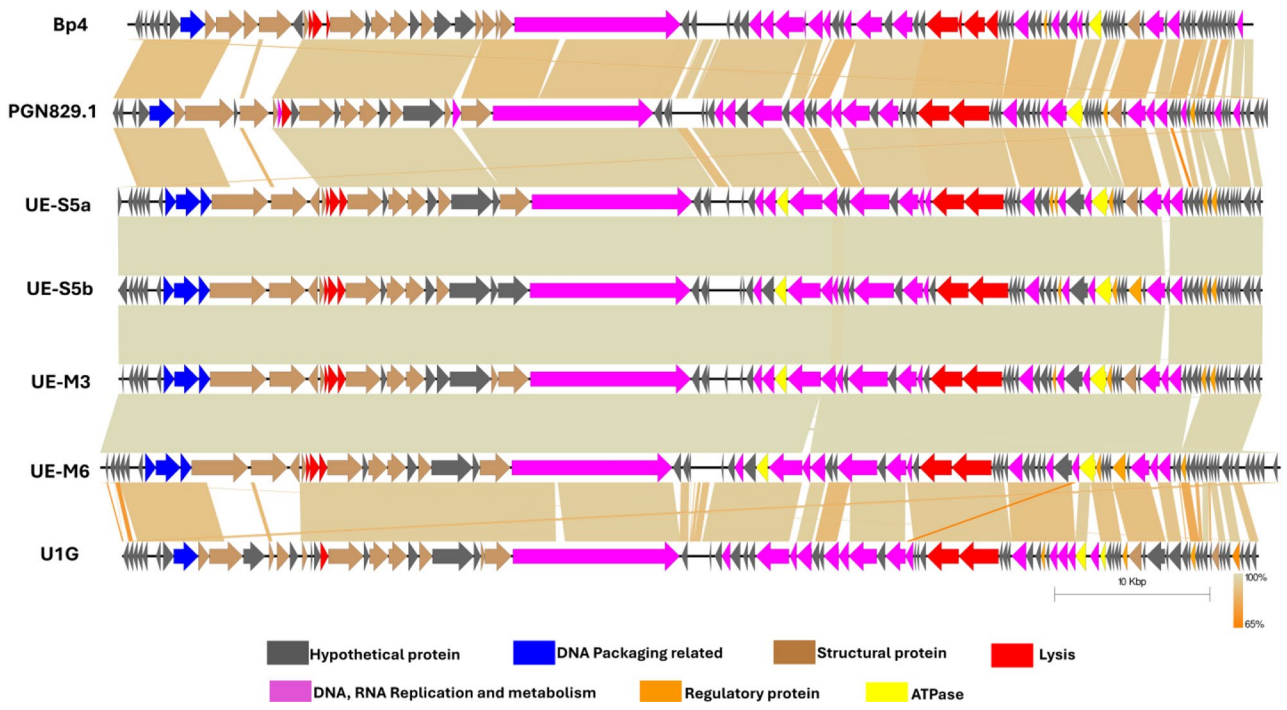

Fig. 8 Comparison of the genome sequences of UE-S5a, UE-S5b, UE-M3, and UE-M6 with closely related members of Escherichia_phage-PGN829.1 (NC_070871.1) and Enterobacteria_phageBp4 (NC_024142.2) and phage_U1G (NC_070872.1). The grey colour between the genome maps indicates level of homology with the scales representing the percentage genome identity between the regions obtained through BLASTn

database and reports these bacteriophages as suitable candidates for phage therapy of UTI caused by UPEC.

Abbreviations

UTIs	Urinary tract infections
MDR	Multiple drug resistance
MOI	Multiplicity of infection
ANI	Average nucleotide identity
OD	Optical density
PFU	Plaque forming unit
WHO	World Health Organization
tRNA	Transfer RNA

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-025-02691-0.

Supplementary Material 1

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Author contributions
HN, MS and FA conceived and designed the study. HN performed the experiments. HN, MS and FA analyzed the data. HN wrote the initial draft and MS and FD edited and commented on the paper. All authors reviewed and approved the final manuscript.

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Data availability
The annotated bacteriophage genome sequences were deposited to GenBank database under the accession numbers PP175015 for phage UE-S5a, PP301341 for phage UES5b, PP301342 for phage UE-M3, and PP301343 for phage UE-M6.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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