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Posted Date: 4 January 2023

doi: 10.20944/preprints202301.0036.v1

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Genome Analysis of Bacteriophage (U1G) of *Schitoviridae*, Host Receptor Prediction Using Machine Learning Tools and Its Evaluation to Mitigate Colistin Resistant Clinical Isolate of *Escherichia coli in vitro* and *in vivo*

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Abstract: The objective of the present study is to isolate phages targeting colistin resistant *E. coli* clinical isolates (U3790 and U1007), sequence and analyze the phage genome and use machine learning tools to predict host cell surface receptor and finally evaluate the efficiency of the phage *in vitro* and *in vivo* in a zebrafish model. Phage targeting colistin resistant U3790 could not be isolated possibly due to presence of capsule and intact prophages in genome of U3790 strain. Phage specific for *E. coli* U1007 was isolated from Ganges River (designated as U1G). The obtained phage was triple purified and enriched. U1G phages had a greater burst size of 195 PFU/cell and a short latent time of 25 min. TEM analysis showed that U1Gphage possessed a capsid of 70 nm in diameter with a shorter tail, which shows that U1G belongs to the family *Podoviridae*. Genome sequencing and analysis revealed that the size of the phage genome is 73275 bp with no tRNA sequence, antibiotic resistant or virulent genes. PHASTER annotation revealed the presence of phage RNA polymerase gene in the genome, which favors the classification of phage under a new family *Schitoviridae*. A machine learning (ML) based multi-class classification model using algorithms such as Random Forest, Logistic Regression, and Decision Tree were employed to predict the host receptor targeted by U1G phage and the best performing two algorithms predicted LPS O antigen as the host receptor targeted by the U1G receptor binding protein (RBP), the tail spike protein.The isolated phage was stable from pH 5.0 to 9.0 and upto 45°C. *In vitro* time kill assay showed an initial 5 log decline of U1007 CFU/ml at 2 h in the presence of U1G followed by regrowth, Addition of colistin with U1G restricted the growth until 6 h, however it also resulted in a regrowth by 24 h. The phage did not pose any toxicity to zebrafish as evidenced by liver/brain enzyme profiles. *In vivo* intramuscular infection study showed that U1G and Col + U1G treatment caused a 0.8 log and 1.4 log decline, respectively underscoring its potential for use in phage cocktail therapy.

Keywords: bacteriophage; colistin resistance; *E. coli*; *Schitoviridae*; zebrafish; machine learning; Host receptor Prediction

1. Introduction

Alternative therapy to tackle antimicrobial resistant pathogens are one of the promising ways in the current multi drug resistant era. Bacteriophages contribute to an essential part of human microbiome and around 10^{30} phages prevail in the biosphere [1]. Owing to these, they qualify to be a tolerable, non-toxic and a reliable tool to serve as adjuvants to antibiotics or for antibiotic sparing

 doi:10.20944/preprints202301.0036.v1

therapy. Phage therapy did not gain therapeutic attention due to the discovery and ease of use of broad spectrum antibiotics in 1930s – 1940s. Nevertheless, the current situation of multidrug resistance has led to resurgence and attention towards phage therapy. However, these are not in active therapeutic practice due to controversies in mode of action and in certain countries like Belgium, phages are used to treat patients only when conventional therapy fails [2]. A previous study reported that lytic bacteriophage belonging to *Myoviridae* from urban sewage, vB_Eco4M-7, was effective against multiple *E. coli* O157 strains, and the phage did not harbor any toxins and virulence factors [3]. Another study showed that a phage PDX, belonging to *Myoviridae*, isolated from wastewater in Portland killed diarrhoeagenic enteroaggregative *E. coli* isolates, leaving the human microbiome undisturbed [4]. Phage therapy has also been widely used as a combination therapy along with antibiotics to tackle MDR pathogens. A recent report showed that overexpression of AmpC in *E. coli* promoted its susceptibility for phage lysis, using OmpA (outer membrane protein) as receptor [5]. In other words, increasing ampicillin resistance aided its susceptibility to phage, which could pave way for use of antibiotics and phage therapy in synergy. Sub lethal doses of ciprofloxacin and ECA2 phage (*Podoviridae*) exhibited synergy against *E. coli*, causing 7.8 log CFU/ml decline in 8 h [6]. In the present study, an attempt to isolate phages targeting colistin resistant *E. coli* clinical isolates were made and the isolated phage was characterized for its morphology and stability. From the genome sequence of phage, the RBP of U1G which is the tail spike protein was identified andby employing machine learning algorithms an attempt to predict host cell surface receptor was undertaken. In addition, ability of the isolated phage to curtail growth of colistin resistant *E. coli* was explored individually and in combination with antibiotics (colistin and meropenem) *in vitro* and *in vivo*.

2. Materials and Methods

2.1. Screening for bacteriophages:

Bacteriophages were screened against the colistin resistant U3790 and U1007 strains from different sources. The Ganges river, The Cauvery river, pond water samples, samples from cowsheds and soil samples from farmland using spot test [7]. Briefly, the samples were incubated with host culture at 37°C and after incubation for 16 – 24 h, the samples were centrifuged at 5000 rpm for 15 min. 3 - 5 µl of the supernatant harbouring phages was spotted on agar plates overlaid with the host culture (U3790/U1007) and the plates were incubated for 18 – 24 h at 37°C. Presence of bacteriophages against a specific host strain can be identified by appearance of clear zones or plaques on the agar plates.

2.2. Isolation and purification of bacteriophages:

In order to isolate the phage, the phage containing supernatant was filtered through 0.45 µm and 0.22 µm syringe filters [8]. The filtered phage lysate was serial diluted in SM buffer (100 mM Sodium Chloride, 8 mM Magnesium sulphate, 50 mM Tris-cl (pH 7.5)) and each dilution was allowed to incubate with mid log cells of the host. After 20 min of incubation, the phage – host mixture was added to 5 ml of soft agar (0.7% Luria Bertani Agar) and overlaid on Nutrient Agar plates. The plates were left undisturbed for 5 – 10 min to solidify, and were incubated at 37°C for 18 – 24 h and observed for plaques. A single plaque was then picked and resuspended in 1 ml of SM buffer, serial diluted, mixed with host cells and overlaid on nutrient agar plates as mentioned earlier. The procedure was repeated thrice to obtain triple purified plaques containing identical morphology. The phage titer was determined at each step and represented as PFU/ml.

2.3. TEM imaging:

The triple purified phages were enriched using the specific host to obtain a high phage titre (>10^{12} PFU/ml). 10 µl of the high titer phage lysate was added to the carbon coated copper grid and was allowed to attach for 2 min [9]. The excess phage lysate was immediately removed carefully using a filter paper and then the phages were stained with 2% uranyl acetate for less than a minute.

 doi:10.20944/preprints202301.0036.v1

The excess stain was removed, the grid was allowed to dry and was then observed under a FEI Tecnai G^2 20 S-Twin Transmission Electron Microscope (TEM) at 200 kV. The TEM images were analysed for the phage morphology to discern the family to which phage belongs.

2.4. One step growth curve:

Burst size and latent period was determined for the isolated phage using one-step growth curve analysis [10]. Mid log cells of the host bacteria were mixed at a multiplicity of infection (MOI) of 1 and incubated at 37°C for 5 – 10 min for adsorption. The cells with adsorbed phages were harvested by centrifugation and resuspended in Nutrient Broth and incubated at 37°C. Phage titer was determined for the samples at different time interval until 60 min. Latent period is the time interval between the phage adsorption to the host and the host cell lysis. Burst size is the number of phages from an infected host cell and is calculated as the ratio of average PFU/ml of latent period to average PFU/ml of last three time points. The experiment was performed in triplicates and the mean represents their average.

2.5. Host specificity:

The host range of the isolated phage was determined by spot assay [11]. 300 µl of the bacterial culture to be tested as host was added to soft agar, overlaid on nutrient agar plates and allowed to solidify. 2 µl of purified phage lysate was spotted on overlaid plates and allowed to dry. The plates were incubated at 37°C for 18 – 24 h and then observed for clear zones. Presence of plaques represent the susceptibility of the bacterial culture to the isolated host.

2.6. Temperature and pH stability:

The temperature sensitivity of the phage was studied by incubating phage at different temperatures 4°C, 16°C, 25°C, 37°C, 45°C, 65°C and 95°C for 1 h [12]. After incubation, the phage lysates were made to adsorb with the host and plated to determine phage titers. Similarly, pH sensitivity of the phage was analysed by incubating the phage at different pH 3.0, 5.0, 7.0, 9.0 and 11.0 for 1 h and PFU/ml was determined post incubation [13]. All experiments were performed in triplicates and represented as survival rate of phage (%).

2.7. In vitro time kill study:

In order to evaluate the efficiency of phage in inhibiting the growth of resistant strains *in vitro*, time kill study [14] was performed with different groups phage, colistin + phage, meropenem + phage, colistin and meropenem only. Mid log cells were subjected to different treatments and at regular time intervals viz., 0, 2, 4, 6 and 24 h, the samples were withdrawn, serial diluted and plated on LA plates. After overnight incubation at 37°C, the CFU/ml was calculated and the difference in colony counts were analysed.

2.8. In vivo toxicity study:

The toxic effect if any of phage on zebrafish was discerned by injecting 10 µl of purified phage lysate (10^4, 10^{10}, and 10^{12} PFU/ml) intramuscularly and enzyme profiles were analysed. Phage injected fish and uninjected fish were monitored for 48 h and then the fish were euthanised and dissected. The brain and liver tissue were isolated, homogenized and the clear supernatant was used for further analysis. Brain and liver enzyme profiles were evaluated using acetylcholine iodide and α/β naphthyl acetate as substrates, respectively [15]. Any significant changes in enzyme levels relative to untreated control was deemed to be toxic to zebrafish.

2.9. In vivo infection:

The efficacy of phage either individually or in combination with antibiotics in preventing growth of MDR strain was evaluated using zebrafish infection [16]. 10 µl of bacterial culture was injected

 doi:10.20944/preprints202301.0036.v1

intramuscularly and 2 h post infection the fish were split to receive different treatment combinations viz., phage alone, phage + colistin, phage + meropenem, colistin alone and meropenem alone. 24 h post treatment, the fish were sacrificed, the infected muscle tissue was dissected, homogenized, serially diluted and plated on LA plates. After incubation for 24-48h at 37°C, the plates were counted for colony counts and represented as mean CFU from triplicate values.

2.10. Genome sequence and analysis:

The phage DNA was extracted using CTAB DNA precipitation method [17]. 1.5 ml of purified high titer phage lysate was incubated at 22°C for 15 min with 10 ng of RNase A and 10 U of DNase I. Post incubation, 80 µl of 0.5 M EDTA and 50 µg of Proteinase K was added and was maintained at 45°C for 15 min, followed by addition of 5% CTAB and incubation in ice for 15 min. The precipitated DNA was harvested at 8000 g and the pellet was resuspended in 1.2 M NaCl. The DNA was further precipitated and washed with ethanol. The pellet was air dried and suspended in 10 mM Tris buffer, which was stored at -20°C until use. The DNA was quantified using Qubit Fluorometer and was then sequenced using Oxford MinIon Nanopore sequencer. Library preparation was performed as per manufacturer's instruction using Ligation sequencing kit (SQK-LSK109) and the sequencing run was performed without live basecalling. Basecalling and demultiplexing were performed using Guppy and de novo assembly of the reads were done using Canu Assembly software .[18] The assembled reads were annotated using Rapid Annotation using Subsystem Technology (RAST) [19]. The assembled genome was searched against nucleotide database available at National Center for Biotechnology Information (NCBI) using Basic local alignment search tool (BLASTn) [20]. Presence or absence of tRNAs were identified using tRNAscan-SE search server [21] and the reads were also fed to PHASTER to determine intactness [22]. The close homologs of U1G genome was identified using BLASTn search and the top phage hits with query coverage above 80 were considered for the analysis. The Average Nucleotide Identity (ANI) between the close homologs of U1G was calculated using the ANI/AAI-Matrix tool (http://enve-omics.ce.gatech.edu/g-matrix/) ([23]. Phylogenetic tree was obtained using ANI-Distance clustering method, UPGMA and visualised using iTOL v6(https://itol.embl.de/) [24]. Antibiotic resistant and virulent genes in the phage genome were analysed using ResFinder and Virulence Finder of CGE [25,26].

2.11. E. coli bacteriophage host receptor prediction using Machine Learning based multi class classifier:

The initial entries of the RBP nucleotide and protein sequences were obtained from the database curated by a study [27] conducted to predict bacterial hosts which consisted of 1232 RBP sequences belonging to nine different bacterial hosts, out of which 400 had *E.coli* as the bacterial host. The bacteriophages which had *E.coli* as host has mapped to the PhRED [28] database which provides information on the corresponding receptor proteins involved in the bacteriophage host interaction out of which only 71 RBP entries had the receptor information. For RBP database entries with PhRED receptor information only, the sequence information was updated by a manual search of annotated proteins and CDS from the genome sequence of bacteriophages. Common search terms used were 'tail fiber protein', 'tail protein', 'tail fiber' along with the bacteriophage name. RBP database entries with sequence information only the receptor information were updated from a literature survey of experimental studies conducted on bacterial host receptor interactions [29,30]. The curated database of RBP and receptor information with 160 entries was used for building the ML model (Table S2). The RBP sequences were presented as a vector of numerical features extracted from both the nucleotide and protein sequences based on the script available with the study (26). A total vector of 218 numerical features (Table S3) was retrieved for each of the RBP dataset entries.

Data Preprocessing involves checking for null values in the dataset, obtaining a balanced dataset, and training it. On performing exploratory data analysis, it was found that the RBP dataset is imbalanced in nature in terms of the target variable distribution with some labels even having a single entry. In order to address this issue, only RBP entries with label count two or more were included and RBP entries with only single-entry outliers were removed, setting the final size of curated dataset to 155. We included SMOTE (Synthetic Minority Oversampling Technique) [31], a

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method of oversampling that creates artificial samples from the class with the lowest count. For training the classifier, SMOTE is utilized to create a training set that artificially balances the class distribution. Three classification algorithms, Random Forest, Multinomial Logistic Regression and Decision Tree were used for the construction of the multiclass classification models and werevalidated using the Nested Cross Validation approach. The range of parameters to be run for the classification models was defined. Multinomial Logistic Regression: C:[0.001, 0.01, 0.1, 1, 10, 100, 1000] and Decision tree Classifier: max_features":[2,4,6],"criterion":["gini","entropy"], Random Forest Classifier: n_estimators":[10,50,100], max_features:[5,10,15], criterion:["gini","entropy"] in order to run the Nested Cross Validation with an Outer Loop Fold of 10 and Inner Loop Fold of 5. In this study, we implemented two feature selection methods, Analysis of Variance (ANOVA) and L2 Regularization, and compared the performance of the trained model with those two datasets to that of the 218 Features incorporated dataset.

The performance evaluation scores like accuracy, precision, F-1 Score and Matthew's Correlation coefficient (MCC) were calculated from the three categories of datasets namely with all features selected dataset with 218 features and ANOVA feature selected dataset with 30 highest scoring features, and L2 -Regularization selected Dataset with 110 features trained with the three classification algorithms. The ROC Curves were generated for each Dataset - Classification algorithm combination. The implementation of Classification algorithms and cross validation was done using the Scikit-learn package (version 1.0.2) available in Python [32].After optimizing the ML model, the RBP sequence of the U1G phage was used to predict the potential host receptor.

3. Results

3.1. Identification of phage against XDR E. coli strains:

Different sources viz., The Ganges River, The Cauvery River, pond samples, soil samples from farmland, samples from cow shed were collected and screened for presence of bacteriophages specific to the extremely drug resistant strains U3790 and U1007. The drug resistance profile of these strains were reported earlier [33]. The different samples incubated with the host bacterium were centrifuged and the supernatant was spotted on plate overlaid with U3790/U1007. We found that none of the samples except the water sample from The Ganges River harbored lytic bacteriophages specific to the XDR strain U1007 (Figure S1A). Unusually, none of the samples tested showed lytic activity against U3790 strain (Figure S1B). Apart from these samples, other sources like local sewage water were also screened for bacteriophages against U3790, but no phages targeting U3790 could be identified. Analysis of U3790 genome sequence (SRA Acc No PRJNA541219) using PHASTER revealed presence of 4 intact prophages which might preclude (super) infections by other related phages, which might account for the inability to isolate lytic phages targeting U3790 strain (Figure S2). This might have prevented superinfection by a new phage. In addition, U3790 forms capsule which might also hinder entry to phages.

3.2. Isolation and purification of U1G:

Spot assay revealed the presence of bacteriophages against U1007 from The Ganges river water sample. In order to isolate the phage, the supernatant was filtered, serial diluted, incubated with U1007 for 20 min and overlaid on nutrient agar plates. Post incubation, the plaques were identified based on clear zone of lysis. The phage morphology was carefully observed and a single plaque morphology was taken up for purification. The phages were triple purified ensuring that similar plaque morphology was repeatedly obtained and a phage stock of high titer (10^{13} PFU/mL) was stored at 4^0C for further use (Figure 1A). The phage specific to U1007 was designated as U1G.

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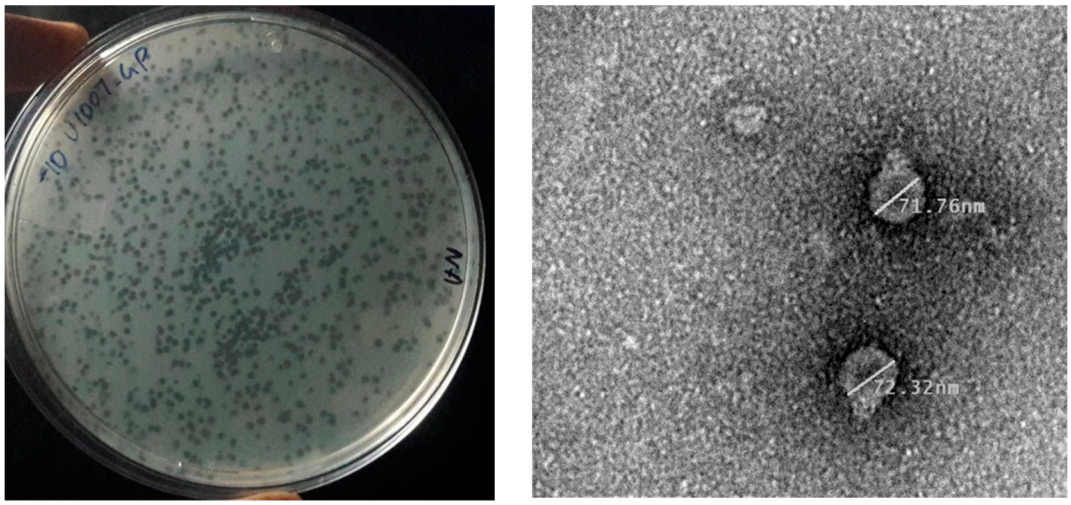

(A) (B)

Figure 1. (**A**) Plaque morphology of U1G (**B**) TEM image of purified U1G (Scale 200 nm).

3.3. TEM imaging:

The morphology of phages were observed by staining the phages with 2% uranyl acetate using Transmission electron microscopy. The TEM image showed that the phage possessed an icosahedral head of mean diameter 71.68 nm and a very short non-contractile tail, thus, U1G can be classified as *Podoviridae* (Figure 1B).

3.4. Genome analysis:

Whole genome sequencing of U1G was performed using Oxford MinIon Nanopore platform. The sequences were trimmed and corrected using Porechop and assembled to contigs using Canu v 1.8. The assembled contigs were annotated using RAST and the genome sequence was submitted in NCBI GenBank (Accession Number: MZ394712). The genome of U1G is 73,275 bp long and it has a GC content of 43%, N50 of 73275 and L50 as 1. RAST annotation revealed the presence of 91 coding sequences, out of which 31 sequences code for phage packaging, replication, and other functions (Table S1). Rest of the proteins were annotated as hypothetical. Annotation of phage genome sequence by PHASTER revealed that the genome was intact. PhagePromoter tool predicted 54 promoters in the genome and absence of tRNAs were revealed by tRNAscan-SE. Genome map of U1G constructed using SnapGene 6.2 showed that the majority of the identified functional modules clustered in the first half of the genome (Figure 2).

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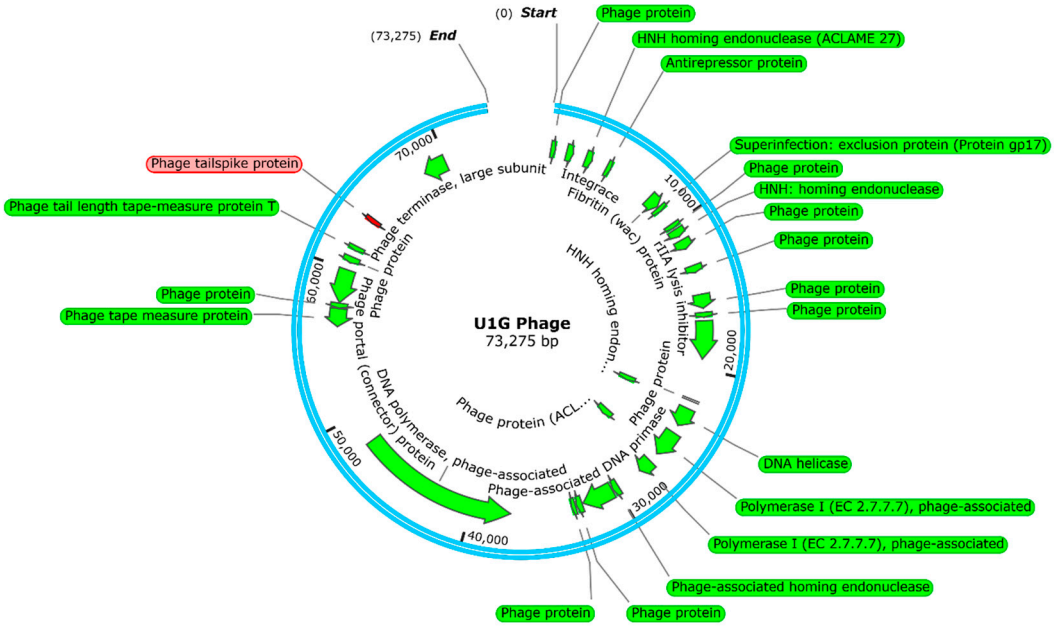

Figure 2. Genome map of Escherichia phage U1G. Putative ORFs of the genome excluding hypothetical proteins annotated using RAST are shown. The image was constructed using SnapGene6.2.

The top nine close homologs of U1G phage genome from BLASTn search were, PGN829, vB_EcoS_Uz-1, vB_EcoM_PD205, PD38, Bp4, St11Ph5, vB_EcoP_PhAPEC7, and vB_EcoP_PhAPEC5 and were used for the ANI matrix calculation and the construction of Phylogram (Figure 3). NCBI BLASTn results showed that the genome matched 95.98% with *Escherichia* phage PGN829.1 with 90% query cover and e value 0. Phylogenetic tree also revealed that U1G is a close relative to *Escherichia* phage PGN829.1 (Figure 3B). However, PGN829.1 was classified under a new family *Schitoviridae* [34], which is highly similar to *Podoviridae* in morphology but harbors virion associated RNA polymerase. Mauve based comparative genome analysis of the close homologs of U1G (identified using ANI matrix) revealed three homologous blocks shared among the genomes. The homologous blocks are syntenic but positional variation for these syntenic regions was observed in different phages (Figure S4) implying that these phages have a common ancestry. PHASTER showed the presence of RNA polymerase subunit in the U1G genome and NCBI blast to detect the presence of virion associated RNA polymerase gene with U1G revealed the presence of homologous region (45314 bp – 48093 bp). Hence U1G was also classified as *Schitoviridae* although the presence of RNA polymerase in the U1G genome was not detected by RAST annotation.

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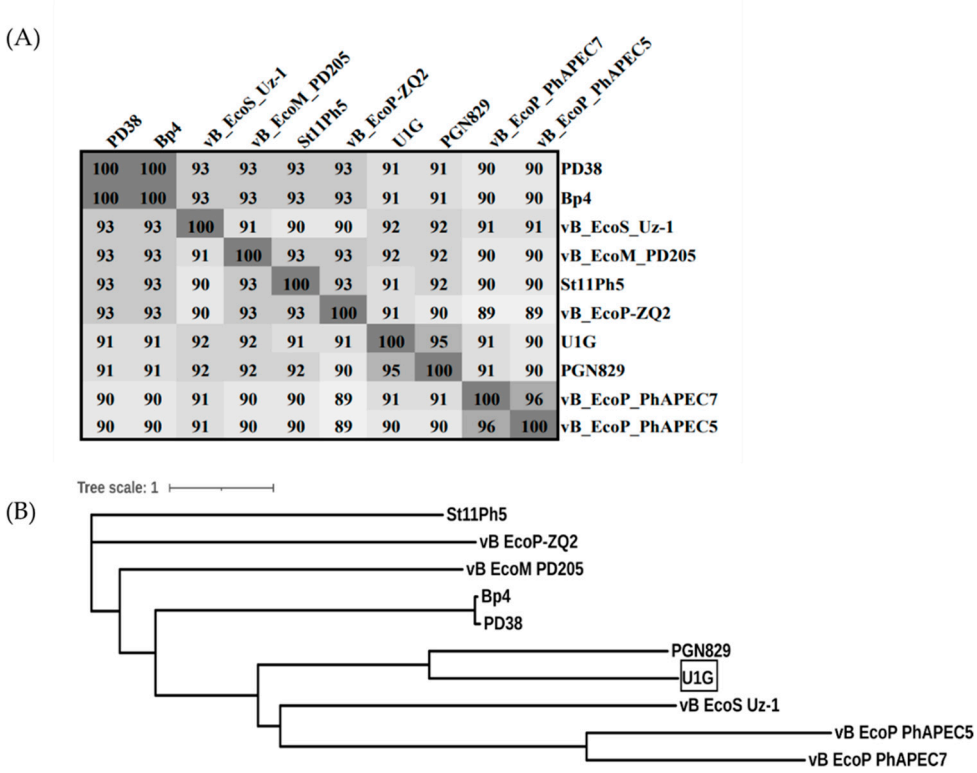

Figure 3. (**A**) ANI matrix calculated for the close homologs of Escherichia phage U1G using ANI/AAI-Matrix tool (http://enve-omics.ce.gatech.edu/g-matrix/). (**B**) Phylogram constructed using the UPGMA clustering method from ANI/AAI-Matrix tool is visualised using iTOL v6.

The phage tail spike protein with 107 amino acid sequence is recognised as the RBP encoded by U1G phage which is used by the phage to attach to bacterial cell surface and is highlighted (with red colour) in Figure2. The homologs of the tailspike protein of U1G from other bacteriophages were obtained using BLASTp search and MEGA11 was used to construct the phylogenetic tree of tailspike protein using UPGMA method with 1000 bootstrap values. The results revealed that tailspike protein of U1G was a close homolog of PGN829 hypothetical protein and is distantly related to tail fiber protein of Salmonella phage SPHG3 (Figure S3). The hypothetical protein of PGN829 with Accession no. AXY82585 is a lengthy one with 628 amino acids. The tail fiber domain-containing protein of Shigella phage pSb-1 (119 amino acids) and the tail spike protein of Dompiswa phage TSP7_1 (102 amino acids) are the RBPs with length comparable to that of tailspike protein of U1G.

3.5. A multiclass-classification model for bacteriophage host-receptor prediction in E. coli:

ML based multi-class classifier using phage RBP sequence was created to predict the potential host receptors for *Escherichia* phage U1G. The RBP host receptor dataset for *E. coli* targeting phage with 160 entries were obtained from the following sources viz., report by Boeckaerts, D. *et al*.[26], PhReD database and literature survey (Table S2). The 218 nucleotide and protein sequence features of the collected RBP sequences were obtained using the script reported earlier [26] and was used for training the ML classification algorithms. The host receptors available in the dataset includes, LPS, Tsx, OmpC, OmpA, LPS core, FhuA, LPS O antigen, LamB, OmpF , FadL, TonB, BtuB, and pili tips and the distribution details are is reported in Figure S8. Three Classification algorithms, Random Forest, Multinomial Logistic Regression and Decision Tree were used for the construction of the multiclass classification models which can predict the host receptors of *E. coli* targeted by phage based on the Receptor Binding Protein (RBP) sequence of the bacteriophage. Performance comparison of the classification algorithms is reported in Table 1 and the model using the Random Forest Classifier Algorithm reported the best Performance in terms of all the metrics followed by the Multinomial Logistic regression Model. We have applied two feature selection methods, ANOVA and L2

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Regularization and the top selected 30 features from first and 110 features from the second method was used for the construction of feature selected ML models and the results are included in Table 1. Overall, the ML model using Random Forest Classifier on the data set consisting of all the 218 features selected produced the highest performance of 93% in terms of precision, 90% Accuracy and an average AUC value of 0.99 in terms of individual class contributions aggregate.

Table 1. Overall Performance Metrics.

	All features			ANOVA Feature Selected Dataset			L2 Regularization Feature Selected Dataset		
Performance Score	**RF**	**DT**	**LR**	**RF**	**DT**	**LR**	**RF**	**DT**	**LR**
Precision	0.93	0.88	0.91	0.86	0.85	0.85	0.84	0.64	0.78
Recall	0.90	0.86	0.91	0.86	0.83	0.83	0.79	0.66	0.77
Accuracy	0.90	0.84	0.90	0.86	0.83	0.83	0.79	0.66	0.77
F-1 Score	0.89	0.85	0.89	0.85	0.83	0.83	0.78	0.63	0.75
MCC	0.89	0.83	0.89	0.85	0.82	0.82	0.78	0.64	0.76

The tailspike protein was identified as the RBP protein for the Escherichia phage U1G and the amino acid sequence of the tail spike protein was used for predicting the host receptor using the multiclass-classification model constructed. The host receptor prediction results from the multi-class classifier are reported in Table 2. Two of the three ML tools predicted LPS O antigen as the host cell surface receptor when all features were selected for analysis. L2 regularization resulted in identification of OmpC /LamB /pili tips. Decision Tree algorithm identified FhuA and TonB as potential host cell surface receptors. The predicted receptors can be validated using knock out strains lacking these cell surface receptors in U1007 background and comparing phage titers with wild type and receptor knocked out strains, which can be pursued as a part of future studies.

Table 2. Predicted Receptors for U1G RBP using ML based multi-class classification methods .

	Predicted Receptors		
	All Features Selected	**ANOVA**	**L2 Regularization**
Random Forest	LPS O antigen	LPS O antigen	OmpC or LamB
Logistic Regression	LPS O antigen	LPS O antigen	pili tips
Decision Tree	FhuA and TonB	LPS	LamB

3.6. Burst Size, Latent Period and Host Specificity:

One-step growth curve showed that U1G possessed a relatively short latent period of 20 min, a rise period of 20 min and a larger burst size of 195 PFU/cell (Figure S5).The host range of U1G was determined by testing the lytic activity of phage against different *E. coli* clinical isolates U3790, U1007, U3176, IDH09733, U2354, U1024, IDH09519 and MG1655 as a reference strain by spot assay. The results showed that U1G showed tropism only towards its host U1007 and not against other isolates (Figure S6). A faint lysis zone was observed against U3790, though it was not significant. This led to the hypothesis that U1G can be specific to colistin resistant *E. coli* clinical isolates, but due to the presence of prophages in U3790 (Figure S2) the lytic activity of phage is limited.

3.7. pH and temperature sensitivity:

Temperature and pH stability of U1G was studied by incubating at different temperatures and by using buffers of different pH, respectively. U1G remained stable in the temperature range of 4°C to 45°C and at 65°C, 50% loss in viability was observed (Figure 4). U1G was unviable a temperature of 95°C.

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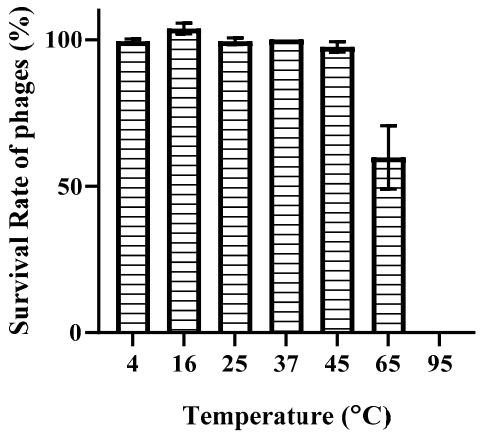

Figure 4. Temperature stability of U1G.

The results of pH sensitivity revealed that U1G was stable at pH 5.0, 7.0 and 9.0 (Figure 5). U1Gphage lost its viabity at extreme pH of 3.0 and 11.0.

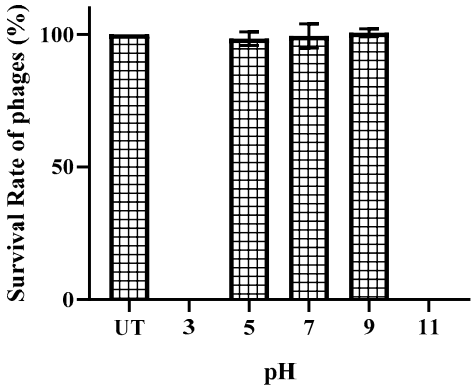

Figure 5. pH sensitivity of U1G revealed that it is unstable at pH 3.0 and 11.0.

3.8. Time kill study:

Time-kill was performed to evaluate the efficacy of U1G against U1007 *in vitro* individually and in combination with antibiotics. The results showed that treatment with only U1G reduced the colony counts by 4 log relative to untreated group within 2 h, but a regrowth was observed at the subsequent time points until 24 h (Figure 6). Upon treatment with only colistin or meropenem, a similar pattern of reduction and regrowth was detected. Combination of colistin/meropenem and U1G caused a 5-6 log decline in colony counts relative to untreated group. Prominently, the regrowth observed in individual treatments was partially prevented by use of combination. Upon comparison between Mero + U1G and Col + U1G groups, the reduction in colony counts was maintained till 6 h in colistin + U1G group and though there was a regrowth, the final cell density in Col +U1G group was 1log lower relative to Mero + U1G group.

 doi:10.20944/preprints202301.0036.v1

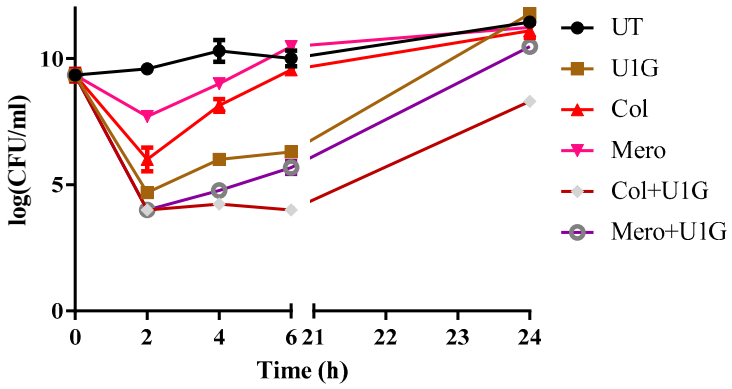

Figure 6. Time kill study. Time kill curve analysis was performed by treatment of early log-phase cells with U1G, Col, Mero, Col + U1G and Mero + U1G and the samples from each group were drawn at specific time points from 0– 24 h. The samples were serially diluted and plated on to LA plates and incubated at 37 °C. The colony count was expressed as log (CFU/ml). The experiment was performed in triplicates and the error bar represents their standard error of the mean.

3.9. In vivo toxicity and infection:

U1G was evaluated for its toxicity in zebrafish by estimating the brain and liver enzyme profiles. Different concentration of phages (10^4, 10^{10} and 10^{12} PFU/ml) were injected intramuscularly and the enzyme profiles were estimated. It was observed that there was a slight increase in α-naphthol release corresponding to control, which was not statistically significant (Figure S7A). The β naphthol (Figure S7B) release and amount of acetylcholine esterase (Figure S7C) was similar to that of untreated control. Hence, U1G did not pose any toxicity to zebrafish even at a relatively high dose. *In vivo* infection study was performed with U1G individually and in combination with antibiotics against U1007 infection. The results showed that treatment with U1G alone resulted in 0.8 log reduction in bioburden (Figure 7). Col + U1G caused a significant reduction of 1.4 log reduction in bacterial bioburden, which was statistically significant relative to U1G group. The other treatments viz., colistin, meropenem and Mero + U1G also did not cause significant reduction in bioburden.

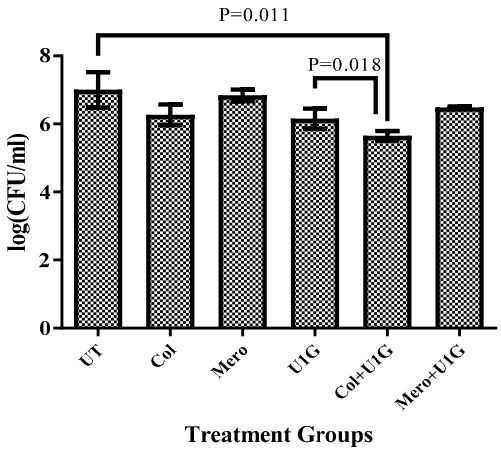

Figure 7. Col + U1G cause significant reduction in bioburden in infected zebrafish.

4. Discussion

The "antibiotic pipeline" had a tremendous growth in the late 20th century and since the beginning of 21st century, the pipeline is experiencing a long lag phase owing to the unpredictable

 doi:10.20944/preprints202301.0036.v1

evolution of antimicrobial resistance leaving drugs like tigecycline, carbapenems and colistin as last resort drugs to treat infections caused by MDR pathogens [35]. Resistance to these drugs have also evolved and hence search for an alternative or adjunct therapy is the need of hour. Desperate need to tackle this jeopardy has reminded the researchers of a century-old bacteriophage therapy. Felix d' Herelle, who identified and termed bacteriophages, was the first to use phage to clinically treat bacterial dysentery in 1919 [36]. Another trial phage therapy was carried out by him on the people of Punjab, India to treat cholera and found a 90% reduction in mortality [37]. Nevertheless, owing to various controversies and rapid progress in development of antibiotics pushed phage therapy behind. However, rising antibiotic resistance has reverted the interests towards bacteriophages. Bacteriophages have been reported against different pathogenic strains of *Pseudomonas aeruginosa, Clostridium difficile, Vibrio parahemolyticus, Staphylococcus aureus, Acinetobacter baumannii, E. coli* and *Klebsiella pneumoniae,* individually or in combination with antibiotics, thus favoring reuse of those antibiotics [35]. In this study, we have isolated phages against *E. coli* clinical isolates (U3790 and U1007) that are resistant to multiple antibiotics, especially to last resort drugs like colistin and carbapenems from different sources. Attempts to identify phages against U3790 was unsuccessful owing to the presence of capsule as reported earlier (Sundarmorrthy et al., 2019a) and intact prophages within its genome. Prophages in bacterial genome can evolve mechanisms like blocking phage genome injection, blocking phage binding and preventing the interaction of phage receptor on the bacterial membrane to evade superinfection by other related phages, though the exact mechanism is still not known [38]. Nevertheless, bacteriophages against XDR *E. coli* U1007 were isolated from The Ganges River (designated as U1G). No phages specific to U1007 were obtained from any other sources tested (Figure S1). We recently reported a phage KpG, belonging to *Podoviridae,* specific to MDR *K. pneumoniae* from Ganges, which was able to curtail the host's planktonic and biofilm mode of growth [39]. There are other numerous reports available on rich diversity of bacteriophages against various pathogens being isolated from The Ganges [40,41]. This is attributed to the origin of Ganges The Himalayan permafrost, which has trapped bacteriophages from a long period and is released gradually while melting and hence it forms a seed source of bacteriophage [42]. The isolated U1G was propagated, purified and its morphology was analyzed using TEM. The TEM image revealed that U1G belongs to family *Podoviridae*, containing an icosahedral head of mean diameter 71.68 nm (Figure 1b) and a short non-contractile tail. However, genome analysis by PHASTER later revealed the presence of RNA polymerase and hence U1G is deemed under *Schitoviridae*. Reports show that there are only 115 members until 2020 that has been classified under this newly proposed family [34]. Interestingly we also observed during whole genome BLAST analysis (Table S4) that a prophage in Enterococcus faecium strain ME3 chromosome displayed 95.94 % identity (with 91% coverage and e-value of 0) with U1G phage genome but the presence and probable expression of antirepressor protein in U1G (Figure 2) might favor its lytic life cycle. Presence of a highly homologous prophage genome in Enterococci imply that U1G might possibly use *Enterococcus faecium* as a host, we tried to infect *Enterococcus faecium* with U1G using spot test, our attempts were unsuccessful probably because genome harbored prophage prevents super infection by a similar (U1G) phage. One step growth data showed that U1G possessed a larger burst size of 195 PFU/cell and a shorter latent period of 25 min, as revealed by the one-step growth curve. The exponential growth of phage and efficiency is majorly dependent on latent period and burst size. Larger burst size are usually resulted from a long latent period and vice versa. However, a phage with shorter latent period and relatively high burst size possess an enhanced capability to lyse host cells faster [43]. Host specificity assay revealed that U1G was highly specific to its host U1007 and a faint lysis zone was observed against colistin resistant U3790 (Figure S6). No clearance or appearance of plaques were seen in other clinical isolates, including the reference strain MG1655. This leads to a hypothesis that the phage U1G might be specific to colistin resistant *E. coli* isolates and does not affect other *E. coli* pathogens or commensals. As colistin resistance confers chemical alteration to LPS [44], it is likely that altered LPS could serve as a receptor for phage entry. As surmised use of machine learning tools random forest and logistic regression identified LPS O antigen as the host receptor capitalized by the phage tail protein for entry into the host. However, this has to be validated using knock out strains of *E. coli* lacking O antigen

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in future studies. Another study showed that a panel of colistin resistant *K. pneumoniae* were more susceptible to lytic phage, isolated from sewage water, than to their respective colistin susceptible strains [45]. the isolated phages were negatively charged and since colistin resistant strains possess reduced negative charge, electrostatic interaction favored the enhanced susceptibility of colistin resistant strains to the phages [45]. U1G was found to be stable at pH 5.0, 7.0, 9.0 and temperature range of 4°C - 45°C and a 50% retention of phage viability at 65°C.

In vitro time kill study with U1G, individually and in combination with colistin/meropenem showed that colistin + U1G combination showed better results relative to either U1G or meropenem + U1G (Figure 5). The enhanced efficacy of combination of antibiotic and phage may be attributed to the pleiotropic changes that occur in bacteria [46]. Regrowth after 2 h in U1G group shows that a population of U1007 is becoming resistant to U1G. As O antigen is predicted as the target, alteration of O antigen is a common phenomenon so bacteria gaining resistance to U1G is possible hence despite short latent time and larger burst size, U1G has better potential for use in phage cocktails. The same occurs in colistin + U1G group, nevertheless, the bacteria during the attempt to develop phage resistance might partially lose resistance to colistin and hence the combination can achieve enhanced killing than when individually treated. Zebrafish model has been used to study the efficiency of bacteriophages in curtailing infections caused by *P. aeruginosa, K. pneumoniae, E. coli* and *E. faecalis* [39,47–49]. Nevertheless, majority of studies has compared the effect of antibiotics and phage therapy and scarce reports are available that has studied the combination of antibiotic and phage therapy. In our earlier study, we found that the combination of Streptomycin and KpG (*Podoviridae* phage specific to *K. pneumoniae*) curtailed the infection by 98% relative to untreated control, whereas KpG alone caused 77% reduction and only streptomycin resulted in 63% reduction in colony counts [39]. In the present study, U1G and colistin + U1G declined the bioburden by 0.8 and 1.4 log, respectively (Figure 7), corresponding to 86% and 95% reduction in bioburden. The efficiency of the combination can be further enhanced by development of a phage cocktail that can reduce the probability of bacterial regrowth *in vitro* and can produce an enhanced effect *in vivo*.

5. Conclusions

Our study revealed that U1G phage from Ganges and colistin combination is effective in curtailing the growth of colistin resistant *E. coli* (U1007) both *in vitro and in vivo.* Machine learning tools predicted LPS O antigen is host cell surface receptor which will be validated by future studies. Although it is non-toxic and effective the decline in bacterial bioburden during in vivo infection has to be enhanced propably by the use of a phage cocktail. Once lytic potential of U1G phage is enhanced, it can be evaluated as a part of cocktail to curtail colistin resistant *E. coli* in higher animal models.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org, Figure S1: Spot assay showing the presence of bacteriophages specific to U1007, Figure S2: Presence of four intact prophages in U3790 genome was revealed by PHASTER, Figure S3: Phylogenetic tree of the tailspike protein, Figure S4: Genome alignment of the close homologs of Escherichia phage U1G with Mauve, Figure S5: One-step growth curve of U1G, Figure S6: U1G is highly specific to U1007, with a slight lysis against U3790, Figure S7: U1G was non-toxic to zebrafish at different concentrations as evidenced by brain and liver enzyme profiles, Figure S8: The receptor label distribution in the dataset with multiple entries, Table S1: Features of U1G genome annotated by RAST and PHASTER, Table S2: The dataset with RBP and host receptor information used for building the ML models, Table S3: Receptor Binding Protein Features, Table S4:Top five hits from BLASTn search of U1G genome

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, S.N. and S.M.; methodology, N.S.S, V.K.U.; software, S.K.S and S.S.; validation, V.K.U. and J.S.R..; formal analysis, S.M..; investigation, N.S.S, S.N & SM; resources, V.K.U.; data curation, V.K.U, S.S and S.K.S; writing—original draft preparation, N.S.S.; writing—review and editing S.N and S.M,; visualization, S.M and N.S.S.; supervision, S.N. and S.M; project administration, S.N..; funding acquisition, S.N. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Funding: This research was funded by ICMR, Govt of India, grant number 67/5/2020-DDI/BMS.

Institutional Review Board Statement: All animal studies were approved by Institutional Animal Ethical Committee, SASTRA Deemed University, Thanjavur, India.

Informed Consent Statement: Not Applicable.

Data Availability Statement: All essential data has been provided either in the main text or as supplementary information. Further data can be shared upon request to sai@scbt.sastra.edu or sumamohan@scbt.sastra.edu.

Acknowledgments: SN & VKU are grateful to the financial support provided by ICMR Adhoc grant to SN (Ref No: 67/5/2020-DDI/BMS). NSS thanks CSIR for financial assistance through SRF program (09/1095(0063)/2020-EMR-1). The authors thank infrastructural support provided by SASTRA Deemed University as Central Research Facility to carry out this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Romero-Calle D, Benevides RG, Góes-Neto A, Billington C (2019) Bacteriophages as alternatives to antibiotics in clinical care. Antibiotics 8
2. Brives C, Pourraz J (2020) Phage therapy as a potential solution in the fight against AMR: obstacles and possible futures. Palgrave Commun 6:1–11. https://doi.org/10.1057/s41599-020-0478-4
3. Necel A, Bloch S, Nejman-Faleńczyk B, et al (2020) Characterization of a bacteriophage, vB_Eco4M-7, that effectively infects many Escherichia coli O157 strains. Sci Rep 10:1–14. https://doi.org/10.1038/s41598-020-60568-4
4. Cepko LCS, Garling EE, Dinsdale MJ, et al (2020) Myoviridae phage PDX kills enteroaggregative Escherichia coli without human microbiome dysbiosis. J Med Microbiol 69:309–323. https://doi.org/10.1099/jmm.0.001162
5. Wang S, Yin B, Yu L, et al (2020) Overexpression of AmpC Promotes Bacteriophage Lysis of Ampicillin-Resistant Escherichia coli. Front Microbiol 10:2973. https://doi.org/10.3389/fmicb.2019.02973
6. Valério N, Oliveira C, Jesus V, et al (2017) Effects of single and combined use of bacteriophages and antibiotics to inactivate Escherichia coli. Virus Res 240:8–17. https://doi.org/10.1016/j.virusres.2017.07.015
7. Champagne CP, Gardner N (1995) The spot test method for the in-plant enumeration of bacteriophages with paired cultures of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus. Int Dairy J 5:417–425. https://doi.org/10.1016/0958-6946(95)00011-Q
8. Adams M (1959) Assay of phages by the agar layer method. In: Bacteriophages. Interscience Publishers, pp 450–451
9. Sulcius S, Staniulis J, Paškauskas R (2011) Morphology and distribution of phage-like particles in a eutrophic boreal lagoon. Oceanologia 53:587–603. https://doi.org/10.5697/oc.53-2.587
10. Pajunen M, Kiljunen S, Skurnik M (2000) Bacteriophage phiYeO3-12, specific for Yersinia enterocolitica serotype O:3, is related to coliphages T3 and T7. J Bacteriol 182:5114–20. https://doi.org/10.1128/jb.182.18.5114-5120.2000
11. Zhao J, Zhang Z, Tian C, et al (2019) Characterizing the Biology of Lytic Bacteriophage vB_EaeM_φEap-3 Infecting Multidrug-Resistant Enterobacter aerogenes. Front Microbiol 10:420. https://doi.org/10.3389/fmicb.2019.00420
12. Kering KK, Zhang X, Nyaruaba R, et al (2020) Application of adaptive evolution to improve the stability of bacteriophages during storage. Viruses 12:423. https://doi.org/10.3390/v12040423
13. Anand T, Virmani N, Kumar S, et al (2020) Phage therapy for treatment of virulent Klebsiella pneumoniae infection in a mouse model. J Glob Antimicrob Resist 21:34–41. https://doi.org/10.1016/j.jgar.2019.09.018
14. Grillon A, Schramm F, Kleinberg M, Jehl F (2016) Comparative activity of ciprofloxacin, levofloxacin and moxifloxacin against Klebsiella pneumoniae, Pseudomonas aeruginosa and Stenotrophomonas maltophilia assessed by minimum inhibitory concentrations and time-kill studies. PLoS One 11:1–10. https://doi.org/10.1371/journal.pone.0156690
15. Ganesan A, Christena LR, Subbarao HMV, et al (2016) Identification of benzochromene derivatives as a highly specific NorA efflux pump inhibitor to mitigate the drug resistant strains of s. aureus†. RSC Adv 6:30258–30267. https://doi.org/10.1039/C6RA01981A
16. Neely M, Pfeifer J, Caparon M (2002) Streptococcus-zebrafish model of bacterial pathogenesis. Infect Immun 70:3904–3914.

17. Boulé J, Sholberg PL, Lehman SM, et al (2011) Isolation and characterization of eight bacteriophages infecting Erwinia amylovora and their potential as biological control agents in British Columbia, Canada. Can J Plant Pathol 33:308–317. https://doi.org/10.1080/07060661.2011.588250
18. Koren S, Walenz BP, Berlin K, et al (2017) Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:gr.215087.116. https://doi.org/10.1101/GR.215087.116
19. Aziz RK, Bartels D, Best AA, et al (2008) The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75
20. M J, I Z, Y R, et al (2008) NCBI BLAST: a better web interface. Nucleic Acids Res 36:. https://doi.org/10.1093/NAR/GKN201
21. PP C, TM L (2019) tRNAscan-SE: Searching for tRNA Genes in Genomic Sequences. Methods Mol Biol 1962:1–14. https://doi.org/10.1007/978-1-4939-9173-0_1
22. Arndt D, Marcu A, Liang Y, Wishart DS (2017) PHAST, PHASTER and PHASTEST: Tools for finding prophage in bacterial genomes. Brief Bioinform. https://doi.org/10.1093/bib/bbx121
23. LM R-R, KT K (2016) The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. https://doi.org/10.7287/PEERJ.PREPRINTS.1900V1
24. Letunic I, Bork P (2021) Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res 49:W293–W296. https://doi.org/10.1093/NAR/GKAB301
25. Zankari E, Hasman H, Cosentino S, et al (2012) Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261
26. Joensen KG, Scheutz F, Lund O, et al (2014) Real-Time Whole-Genome Sequencing for Routine Typing, Surveillance, and Outbreak Detection of Verotoxigenic Escherichia coli. J Clin Microbiol 52:1501–1510. https://doi.org/10.1128/JCM.03617-13
27. Boeckaerts D, Stock M, Criel B, et al (2021) Predicting bacteriophage hosts based on sequences of annotated receptor-binding proteins. Sci Reports 2021 111 11:1–14. https://doi.org/10.1038/s41598-021-81063-4
28. Bertozzi Silva J, Storms Z, Sauvageau D (2016) Host receptors for bacteriophage adsorption. FEMS Microbiol Lett 363:. https://doi.org/10.1093/femsle/fnw002
29. Chatterjee S, Rothenberg E (2012) Interaction of Bacteriophage λ with Its E. coli Receptor, LamB. Viruses 4:3162. https://doi.org/10.3390/V4113162
30. Sandulache R, Prehm P, Kamp D (1984) Cell wall receptor for bacteriophage Mu G(+). J Bacteriol 160:299. https://doi.org/10.1128/JB.160.1.299-303.1984
31. Blagus R, Lusa L (2013) SMOTE for high-dimensional class-imbalanced data. BMC Bioinformatics 14:1–16.
32. Pedregosa FABIANPEDREGOSA F, Michel V, Grisel OLIVIERGRISEL O, et al (2011) Scikit-learn: Machine Learning in Python Gaël Varoquaux Bertrand Thirion Vincent Dubourg Alexandre Passos PEDREGOSA, VAROQUAUX, GRAMFORT ET AL. Matthieu Perrot. J Mach Learn Res 12:2825–2830
33. Sundaramoorthy NS, Mohan HM, Subramaniam S, et al (2019) Ursolic acid inhibits colistin efflux and curtails colistin resistant Enterobacteriaceae. AMB Express 9:27. https://doi.org/10.1186/s13568-019-0750-4
34. J W, D T, AD M, et al (2020) From Orphan Phage to a Proposed New Family-the Diversity of N4-Like Viruses. Antibiot (Basel, Switzerland) 9:1–12. https://doi.org/10.3390/ANTIBIOTICS9100663
35. Lin DM, Koskella B, Lin HC (2017) Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther 8:162. https://doi.org/10.4292/wjgpt.v8.i3.162
36. Dublanchet A, Bourne S (2007) The epic of phage therapy. In: Canadian Journal of Infectious Diseases and Medical Microbiology. Hindawi Limited, pp 15–18
37. Chanishvili N (2012) Phage Therapy-History from Twort and d'Herelle Through Soviet Experience to Current Approaches. In: Advances in Virus Research. Academic Press Inc., pp 3–40
38. Bondy-Denomy J, Qian J, Westra ER, et al (2016) Prophages mediate defense against phage infection through diverse mechanisms. ISME J 10:2854–2866. https://doi.org/10.1038/ismej.2016.79
39. Sundaramoorthy NS, Thothathri S, Bhaskaran M, et al (2021) Phages from Ganges River curtail in vitro biofilms and planktonic growth of drug resistant Klebsiella pneumoniae in a zebrafish infection model. AMB Express 11:. https://doi.org/10.1186/s13568-021-01181-0
40. Dimri A, Awasthi C, Uniyal S, et al (2019) Isolation and Characterization of Coliform Bacteria and Bacteriophages from Ganga River in Northern Himalayan Regions. Int J Curr Microbiol Appl Sci 8:1582–1592. https://doi.org/10.20546/ijcmas.2019.811.183

41. Manohar P, Tamhankar AJ, Lundborg CS, Nachimuthu R (2019) Therapeutic Characterization and Efficacy of Bacteriophage Cocktails Infecting Escherichia coli, Klebsiella pneumoniae, and Enterobacter Species. Front Microbiol 10:574. https://doi.org/10.3389/fmicb.2019.00574
42. Khairnar K (2016) Ganges: Special at its origin. J. Biol. Res. 23:16
43. Abedon ST, Herschler TD, Stopar D (2001) Bacteriophage Latent-Period Evolution as a Response to Resource Availability. Appl Environ Microbiol 67:4233–4241. https://doi.org/10.1128/AEM.67.9.4233-4241.2001
44. Olaitan AO, Morand S, Rolain J-M (2014) Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol 5:643. https://doi.org/10.3389/fmicb.2014.00643
45. Hao G, Chen AI, Liu M, et al (2019) Colistin resistance-mediated bacterial surface modification sensitizes phage infection. Antimicrob Agents Chemother 63:. https://doi.org/10.1128/AAC.01609-19
46. Burmeister AR, Fortier A, Roush C, et al (2020) Pleiotropy complicates a trade-off between phage resistance and antibiotic resistance. Proc Natl Acad Sci 201919888. https://doi.org/10.1073/pnas.1919888117
47. M A-Z, M W, EK C, et al (2019) Identification of Novel Bacteriophages with Therapeutic Potential That Target Enterococcus faecalis. Infect Immun 87:. https://doi.org/10.1128/IAI.00512-19
48. Cafora M, Deflorian G, Forti F, et al (2019) Phage therapy against Pseudomonas aeruginosa infections in a cystic fibrosis zebrafish model. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-018-37636-x
49. M E, M DZ, HJ S (2020) Application of phage therapy: Synergistic effect of phage EcSw (ΦEcSw) and antibiotic combination towards antibiotic-resistant Escherichia coli. Transbound Emerg Dis 67:2809–2817. https://doi.org/10.1111/TBED.13646