Isolation and Characterization of Lytic Bacteriophage Against Multi-drug Resistant *Pseudomonas Aeruginosa*

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen frequently causing healthcare-associated infections. The apocalyptic rise of antimicrobial resistance has rekindled interest in age-old phage therapy that uses phages (viruses that infect bacteria) to kill the targeted pathogenic bacteria. Because of its specificity, phages are often considered as potential personalized therapeutic candidate for treating bacterial infections.

Methods: In this study, we isolated and purified lytic phages against multi-drug resistant *P. aeruginosa* using soft agar overlay technique. Phage characteristics like thermal and pH stability, latent period and burst size were determined using one-step growth assay while multiple host range spectrum was determined by spot assay. The phages were further characterized using protein profiling.

Results: Three Pseudomonas phages (øCDBT-PA31, øCDBT-PA56 and øCDBT-PA58) were isolated from the holy rivers of Kathmandu valley. Among 3 phages, øCDBT-PA31 demonstrated multiple host range and could lyse multi-drug resistant strain of *P. aeruginosa*. Further, øCDBT-PA31 showed latent period of 30 minutes with corresponding burst sizes of 423-525 PFU/cell. Interestingly, øCDBT-PA31 also tolerated a wide range of adverse conditions, such as high temperature (50°C) and pH 3-11. Further, protein profiling revealed that øCDBT-PA31 has 4 and øCDBT-PA11 had 3 distinct bands in the gradient gel ranging from approximately 3.5-29 kilodaltons (kDa) suggesting them to be morphologically distinct from each other.

Conclusions: As multi-drug resistant bacteria are emerging as a global problem, lytic phages can be an alternative treatment strategy when all available antibiotics fail.

Keywords: Antimicrobial resistance (AMR); bacteriophage; *P. aeruginosa*; phage therapy; SDS-PAGE.

INTRODUCTION

Antimicrobial resistance [AMR, also often called multi-drug resistance (MDR)] is a global problem and thus considered a burning issue all over the world with no concrete solution/alternative at sight. *Pseudomonas aeruginosa* - a common cause of hospital acquired infections (HAIs), including pneumonia and bloodstream, urinary tract, and surgical site infections.[1]

Further, it is also one of the major pathogens colonizing cystic fibrosis (CF) lungs promoting an accelerated decline in pulmonary functions ultimately causing morbidity and mortality in CF. Bacteriophage (phage) is a group of viruses that infect bacteria specifically.[2]

Recent reports in application of phage in therapeutics has shown encouraging outcomes against difficult to treat and/or MDR infections. In this study, we isolated three lytic Pseudomonas phages using *P. aeruginosa* clinical isolates (CIs) as a host from the water sample collected from various holy rivers of Kathmandu valley. We further investigated the phage dynamics (burst size, latent period), stability (temperature, pH) and the host range against wide range of *P. aeruginosa* CIs.

METHODS

P. aeruginosa CIs were collected from Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal and preserved at Central Department of Biotechnology, Kirtipur, Nepal. All bacterial strains were propagated in nutrient agar (NA) (HiMedia, India). Freshly prepared exponentially growing broth culture of bacteria propagated in Luria-Bertani (LB) (HiMedia, India) media was used as host for phage isolation. This was

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achieved by inoculating an isolated colony of bacteria in 15.0 ml LB broth followed by incubation at 37°C with constant agitation (200 rpm) until mid-log phase (OD_{600} = 1.0 corresponding to 2.04 × 10^8 CFU/ml) was reached. Tryptic soy agar (TSA) (HiMedia, India) with varying agar concentration was used for isolation of phage using soft agar overlay technique.

Antibiotic susceptibility of collected CIs was confirmed by Kirby-Bauer disk diffusion susceptibility test protocol following Clinical & Laboratory Standards Institute (CLSI) guidelines. Different classes of antibiotics like beta-lactams, carbapenems, macrolide, fluoroquinolones, aminoglycosides were used for determining multi-drug resistant phenotype. The list of antibiotics tested, and corresponding CIs are listed in Table 1.

Isolation: Water samples were collected from rivers of Kathmandu valley [collection location: Balkhu (Bagmati river), and Kalanki (Balkhu river)] in a sterile 50.0 ml Falcon tube. Before collection, the water was mixed thoroughly, and the sediments were collected with the overlying water from collection sites. Phage isolation was performed by soft agar overlay technique as described previously.[3]

Purification: A completely isolated clear plaques were picked by using pipette tip and dissolved in 1.0 ml sodium chloride-magnesium sulfate (SM) buffer (5.8 g/L NaCl, 2 g/L $MgSO_4.7H_2O$, 50 ml 1.0 M Tris, pH 7.5, 2% gelatin). The mixture was filtered through 0.2 µm syringe filter (Axiva Sichem, Haryana, India) to remove the bacterial contamination. The filtrate was further used for soft agar overlay assay as mentioned before and next day, an isolated plaque was picked. The process was repeated 3 times and the pure phage strain was collected from the plates of last round. For this, the plates from third round containing plaques were flooded with 10.0 ml of SM buffer and 2 drops of chloroform was added into it. The plates were sealed and incubated at rotating shaker (80 rpm for 30 minutes) for phage elution/diffusion from the plaques. The SM buffer was collected in a Falcon tube and centrifuged at 4000 rpm for 20 mins. Then, the supernatant was filtered through 0.2 µm syringe filter (Axiva Sichem, Haryana, India) to obtain high titer of pure phage strain. Finally, the phage lysates were stored at 4°C until further use.

Host range spectrum of purified phage was determined by spot assay on all available CIs. Briefly, 10.0 µl of serially diluted phage preparations (10^{-8}, 10^{-9} and 10^{-10} PFU/ml) was spotted on the double layered lawn cultures of the bacterial strains and allowed to absorb completely. SM buffer was used as negative control. The plates were incubated at 37°C for 24 hours and the next day, clear lysis spots were checked. A positive spot appears as complete obliteration of the entire drop area, whereas a negative spot test will result in the bacterial lawn growing normally in the region of the spots.

The pH of the SM buffer was adjusted with either 1.0 M HCl or 0.5 M NaOH to obtain a pH range of 1-14. A total of 100 µl of known phage suspension (5 × 10^8 PFU/ml) was inoculated into 1.0 ml of pH-adjusted SM buffers. After incubation for 1 hour at 37°C, the surviving phage particles were enumerated immediately spotting 10.0 µl of serially diluted phage suspension on previously prepared double layer agar with host bacteria on top agar. Similarly, thermal stability of all phages at different temperatures (50, 60, 70, 80 and 90°C) was determined by incubating the known concentration of phage (10^7 PFU/ml) at indicated temperatures for 30 mins and 60 mins at pH 7.0 in SM buffer. The surviving phages were enumerated by spot assay as described earlier.

Overnight cultures of *P. aeruginosa* was adjusted to optical density (OD_{600}) of 1.0 (2.04 × 10^8 CFU/ml) in fresh TSB media. A single phage stock was added to give multiplicity of infection (MOI) of 100 and then the mixtures were incubated at 37°C for 1, 2, 3, 4 and 5 hrs respectively with gentle shaking. Phage-free culture (only bacteria) and bacteria-free culture (only phage) were also included as controls. Bacterial cell densities were determined at 0, 1, 2, 3, 4 and 5 hrs by spectrophotometry at 600 nm wavelength.

Phage growth cycle parameters (latent period and burst size) were determined from the dynamic change of the number of free and total phages using one-step growth assay. Briefly, 10.0 ml of a mid-exponential phase culture was harvested by centrifugation (7,000 x g, 5 min, 4°C) and resuspended in 5.0 ml of LB broth and adjusted to 1.0 OD_{600} (approximately 2.04 × 10^8 CFU/ml) using spectrophotometer. To this suspension, appropriate volume (5.0 ml) of phage stock solution was added to have a MOI of 0.001 (2.04 ×10^5 PFU/ml) and left at room temperature for 5 min without snaking for phage adsorption. The mixture was then centrifuged as described above and the pellet was resuspended in 10.0 ml of fresh TSB medium. Samples were taken at every 10 min interval over a period of 2 hrs. The sample was plated immediately using soft agar overlay technique and incubated at 37°C for 24 hrs. Next day plates were monitored for plaques.

Protein profiling of two phages (øCDBT-PA31 and øCDBT-

PA11) was carried out using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 2 different methods: acetone precipitation and direct heating. For acetone precipitation, 500 µl of purified phage solution was precipitated with 4 volumes of ice-cold acetone for 90 minutes. Supernatant was decanted and pellet was air dried, resuspended in 100 µl PBS (8.0 g/L NaCl, 0.2 g/L KCl, 0.2 g/KH_2PO_4 1.44 g/L Na_2HPO_4 × $2H_2O$, pH 7.5). SDS-PAGE was carried out according to Laemmli. Briefly, 25.0 µl of phage sample was added to 25.0 µl of 2 × Laemmli buffer and boiled for 10 mins. Samples were then loaded to 10% PAGEr™ Precast Gels (Lonza Inc., Rockland, USA) and electrophoresed with tris-glycine buffer. Five microliters of protein marker with 1.0 µl of loading dye was also loaded after boiling for 5-10 mins. After electrophoresis, the gels were stained with coomassie brilliant blue R-250 (CBB) (Bio-Rad Laboratories, India) overnight and then the bands were visualized after de-staining. For direct heating, 25.0 µl of purified *Pseudomonas* phages were mixed with equal volume of 2x sample buffer (0.125 M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 2% mercaptoethanol, 0.02% bromophenol blue) and heated in a boiling water bath for 3-5 minutes. Protein profiles were then estimated as described earlier using the precast gel.

For transmission electron microscopy (TEM), the purified high titer phage lysate of øCDBT-PA31 was fixed with 2% paraformaldehyde and 2.5 % glutaraldehyde. Two microliters of the fixed sample were spread on a carbon-coated copper grid and negatively stained with 2.0 µl of 2% (w/v) uranyl acetate (pH 4.5). The copper grid was dried and examined under JEM-2100F transmission electron microscope (JEOL, Japan) at 200 kV field emission.

RESULTS

Three lytic Pseudomonas phages were isolated from the waters of Bagmati river and Balkhu river using different clinical isolates (CIs) of *P. aeruginosa* as primary host. The isolated phages were named according to the bacterial host used for phage isolation. As such, Pseudomonas phage CDBT-PA31 (hereafter øCDBT-PA31) was isolated using PA31 as a host. Likewise, Pseudomonas phage CDBT-PA56 (hereafter øCDBT-PA56) and Pseudomonas phage CDBT-PA58 (hereafter øCDBT-PA58) were isolated using PA56 and PA58 as host respectively (Figure 1). øCDBT-PA31 produced a well-defined sharp edged pinhead plaques of 0.1 mm (diameter) without halo whereas øCDBT-PA56 produced plaques of 0.4 mm (diameter) with halo and øCDBT-PA58 also produced plaques of 0.4

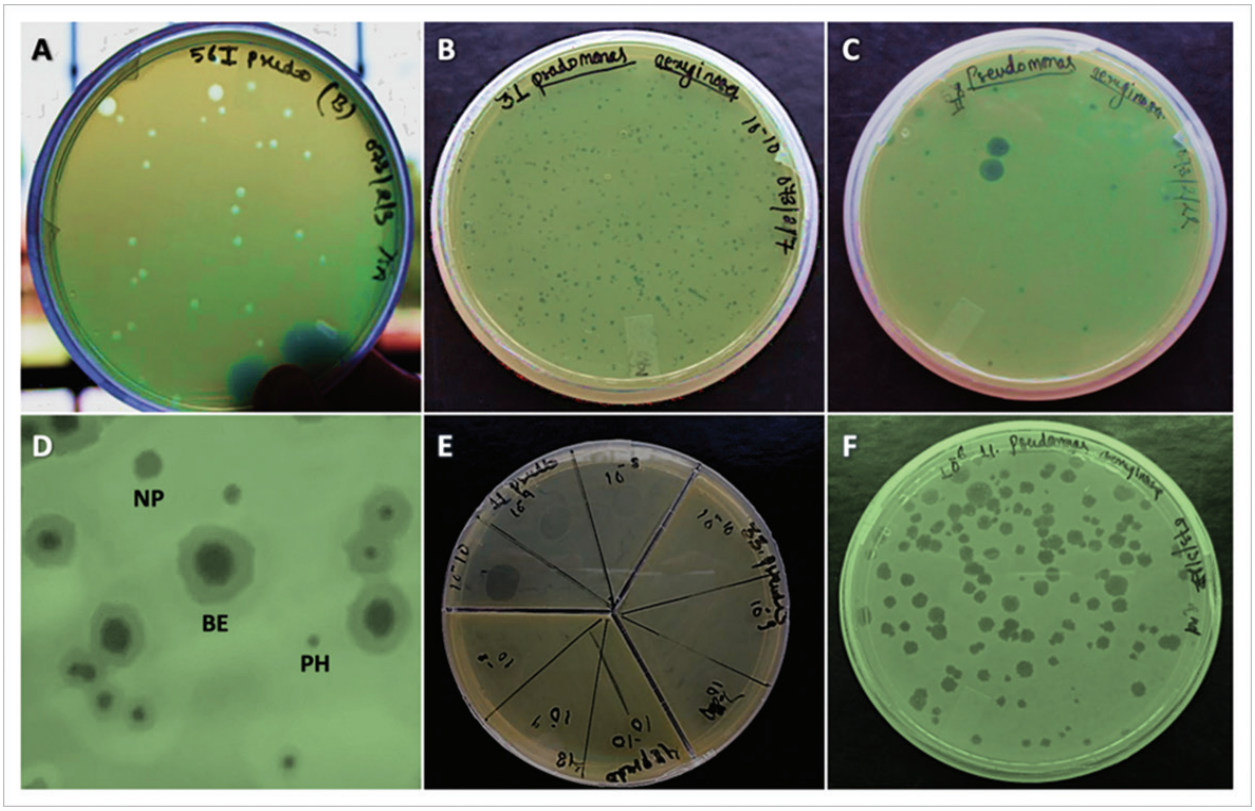

Figure 1. Isolation of lytic phages against clinical isolates of *P. aeruginosa*.

mm (diameter) with bull's eye morphology. No phages were isolated against *P. vulgaris* and other *Pseudomonas* spp. (Table 1).

Plaque morphology of isolated phages, øCDBT-PA56 **(A)**, øCDBT-PA31 **(B)** and øCDBT-PA58 **(C)**. **(D)** Different types of plaque morphologies: NP = normal plaque without Bull's eye, BE = plaque with Bull's eye, PH = pin-head plaques. **(E)** Spot assay showing intraspecies host lysis against other *P. aeruginosa* (PA11) by øCDBT-PA31 in different dilutions. **(F)** Intraspecies host range of øCDBT-PA31 confirmed on PA11 by DLAA method with 10^{-6} dilution.

The intra-species multi host range (MHR) spectrum of all 3 purified phages was assessed by spot assay. All 3 phages were able to lyse multiple strains other than their own host implying broad host range of isolated phages. Among them, øCDBT-PA31 showed broadest host range among the tested CIs, showed lytic activity against MDR *P. aeruginosa* (PA11) and thus was selected for further characterization. The lysis spots produced by high titer phage were larger and clearer that spots produce by lower titer indicating phage mediated lysis being dose dependent.

Table 1. Clinical isolates (CIs) of P. aeruginosa used as host for isolation of phage.

Bacterial strain	Strain code	Antibiotic resistance*	Phage characteristics
P. aeruginosa	PA11	ofloxacin, amoxyclav, bacitracin, teicoplanin, amikacin, cefotaxime, gentamycin, meropenem, penicillin G, piperacillin/tazobactam, piperacillin, methicillin, vancomycin, cloxacillin, cefoxitin, ceftazidime	Yes (clear)
	PA31	amoxyclav, bacitracin, teicoplanin, amikacin, cefotaxime, penicillin G, methicillin, cloxacillin, cefoxitin	Yes (clear, small pin-head shaped)
	PA35	ofloxacin, amoxyclav, bacitracin, teicoplanin, cefotaxime, meropenem, piperacillin/tazobactam, piperacillin, methicillin, vancomycin, cloxacillin, cefoxitin, ceftazidime	No
	PA37	ofloxacin, amoxyclav, bacitracin, teicoplanin, cefotaxime, piperacillin/tazobactam, piperacillin, methicillin, vancomycin, cloxacillin, cefoxitin, ceftazidime	No
	PA56	amoxyclav, bacitracin, teicoplanin, cefotaxime, penicillin G, piperacillin, methicillin, vancomycin, cloxacillin, cefoxitin, ceftazidime	Yes (turbid)
	PA57	ofloxacin, amoxyclav, bacitracin, teicoplanin, cefotaxime, penicillin G, methicillin, vancomycin, cloxacillin, cefoxitin, ceftazidime.	No
	PA58	amoxyclav, bacitracin, teicoplanin, cefotaxime, penicillin G, methicillin, vancomycin, cloxacillin, cefoxitin, ceftazidime	Yes (clear with bull's eye)
	PA36	ampicillin, cefotaxime, vancomycin, nalidixic acid	No
	PA27	ampicillin	No
Pseudomonas spp.	Pse13	cefotaxime, nalidixic acid, piperacillin, ampicillin	No
	Pse14	ofloxacin, cefotaxime, meropenem, piperacillin/tazobactam, piperacillin, methicillin, vancomycin, nalidixic acid, ampicillin	No
P. vulgaris	PV35	ampicillin, vancomycin, piperacillin	No

* Antibiotic resistance was determined by Kirby-Bauer disc diffusion method following Clinical & Laboratory Standards Institute (CLSI) guidelines. Please refer to Figure 2 for zone diameters of individual strains expressed in millimeters (mm).

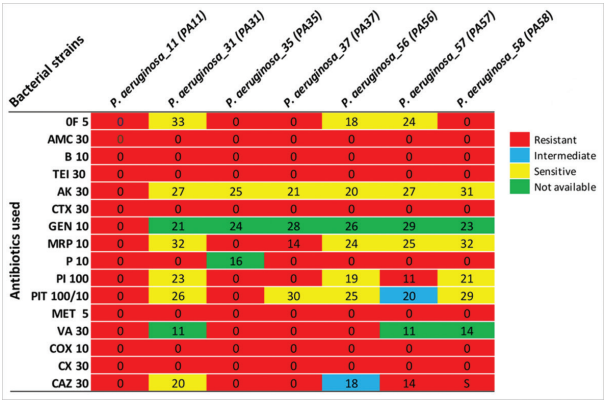

Figure 2. Antibiotic susceptibility testing (AST) of various clinical isolates (CIs).

Sixteen different antibiotics were tested (y-axis) against 7 different CIs and among them *P. aeruginosa_11* (PA11) was resistant to all the antibiotics tested. The numbers inside the colored box represent lysis zone in millimeters (mm).

The effect of pH on øCDBT-PA31 activity was observed by incubating known concentration (5.0×10^8 PFU/ml) of phage at different pH levels ranging from 1 to 12 for 1.0 hr. øCDBT-PA31 did not lose its viability within pH 3-10 while it significantly lost its viability/activity at pH 11 (Figure 3A). The phages completely lost their activity at pH higher than 11 and lower than 3. Although, øCDBT-PA31 was viable at pH 3-10, the phage titer decreased by ~4 $\log_{10}$ fold (initial = 5.0×10^8 PFU/ml, observed = ~$5.0 \pm 0.5 \times 10^4$ PFU/ml). Further, maximum stability of øCDBT-PA31 was observed at pH 6 (6.51×10^4 PFU/ml). The phage titer at pH 11 was significantly decreased to 4.35×10^2 PFU/ml after an hour of incubation at 37°C.

Similarly, thermal stability of øCDBT-PA31 at pH 7.0 was also determined by spot assay after incubating known concentration (5.0×10^8 PFU/ml) of phage at different temperature. The results showed that øCDBT-PA31 was viable at 50°C for 30 min and 60 min (Figure 3B). However, the viral titer significantly decreased to 6.15×10^3 PFU/ml (30 min) and to 5.63×10^3 PFU/ml (60 min). Further, the number of viable phage decreased to 3.0×10^2 PFU/ml after 30 min incubation at 60°C and to 1.0×10^2 PFU/ml after 30 min incubation at 70°C. No phage viability was observed at 80°C and higher.

In vitro phage mediated lysis of host bacteria (PA31) by øCDBT-PA31 was estimated using spectrophotometry. øCDBT-PA31 achieved a reduction of 1.13, 1.38, 2.05, 2.23 and 2.4 log CFU/ml after 1h, 2h, 3h, 4h and 5h respectively (Figure 3C). The reduction of bacterial cells in percentage were also calculated and is depicted in Table 2. The number of viable *P. aeruginosa* (PA31) was reduced by about 2.4 log fold (26.11%) when treated with phage at MOI of 100 compared to the phage-free control after 5.0 h incubation.

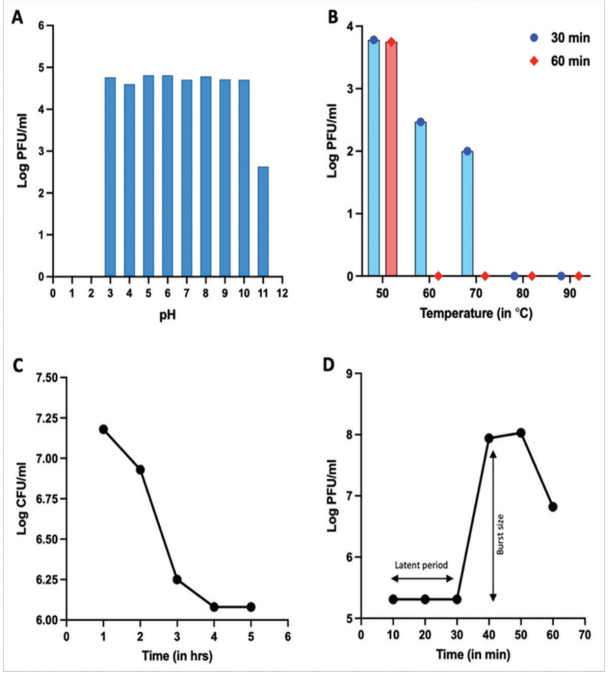

Figure 3. Stability and characterization of øCDBT-PA31.

Table 2. Spectrophotometer reading of bacterial cells.

Time (hrs)	OD	CFU/ml	Log CFU/ml	Log reduction in bacterial density	Percentage reduction in bacterial density (%)
1	0.075	1.53×10^7	7.18	1.13	12.29
2	0.042	8.56×10^6	6.93	1.38	15.01
3	0.009	1.83×10^6	6.26	2.05	22.30
4	0.006	1.22×10^6	6.08	2.23	24.26
5	0.004	8.16×10^5	5.91	2.40	26.11

OD = optical density at 600 nm of wavelength, CFU = colony forming unit

(A) pH stability of øCDBT-PA31: øCDBT-PA31 was viable within pH range of 3-11 after 1 hr of incubation while it completely lost its viability at pH lower than 3 and higher than 11. **(B)** Temperature stability of øCDBT-PA31: øCDBT-PA31 was viable at 50°C when incubated for 30 mins and 60 mins while it gradually lost it viability at higher temperature. **(C)** *In vitro* phage mediated lysis by øCDBT-PA31 on its parent host *P. aeruginosa_31* (PA31): Bacterial concentration of PA31 was significantly reduced by about 2.29 log-fold (expressed in CFU/ml) when treated with øCDBT-PA31 at MOI of 100. **(D)** One-step growth curve of øCDBT-PA31 on PA31 strain: The latent period of øCDBT-PA31 was 30 minutes and yielded a burst size of 423-525 PFU per infected cell.

We further characterized the growth cycle of øCDBT-PA31 using one-step growth assay to identify different phases of a phage infection process. The latent period of øCDBT-PA31 was 30 minutes and yielded a burst size of 423-525 PFU per infected cell (Figure 3D).

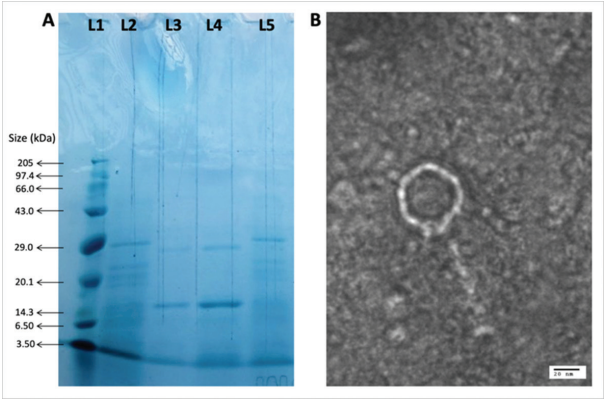

Figure 4. Phage characterization based on protein profiling and transmission electron micrograph. (A)

Protein profiling of øCDBT-PA31 and øCDBT-PA11. Lane 1 = Protein molecular weight marker (Genei, size = 3.5-205 kilodaltons (kDa) Lane 2 = øCDBT-PA31 (acetone precipitation), Lane 3 = øCDBT-P11 (direct heating), Lane 4 = øCDBT-PA11 (acetone precipitation), Lane 5 = øCDBT-PA-31 (direct heating). The difference in protein profiling between two phages implies that they belong to different phage family. **(B)** Transmission electron microscopy of øCDBT-PA31. The scale bar at the bottom right corresponds to 20 nanometres (nm).

To further characterize the phages, protein profiling of øCDBT-PA31 and øCDBT-PA11 was carried out using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Four distinct bands of øCDBT-PA31 and 3 distinct bands of øCDBT-PA11 was observed in the gradient gel ranging from approximately 3.5 to 29.0 kilodaltons (kDa) (Figure 4A). For øCDBT-PA31, the most predominant polypeptide appeared at a size of approximately 29.0 kDa and could be assigned to the major capsid protein. Similarly, based on size, other three protein bands could be correlated with structural proteins: head-tail connector protein, capsid assembly protein, internal virion protein. Likewise, the major structural protein (capsid) for øCDBT-PA11 appeared to be of 14.3 kDa (smaller than øCDBT-PA31). The remaining two bands could be assumed as minor structural proteins. The size difference in major structural protein (capsid) implies that the two phages are not identical and possibly belongs to different families.

Transmission electron microscopy revealed that øCDBT-PA31 had icosahedral capsid (54 nm) with relatively long tail (length = 95 nm, width = 8 nm) (Figure 4B. Thus, according to the ICTV classification guidelines, it belonged to *Siphoviridae* family of Caudovirales order.

DISCUSSION

Natural environment is a reservoir for variety of phages. As phages require bacteria for their multiplication, bacteria rich habitat is considered fertile niche for co-evolution of phages. Because of an unregulated antibiotic use (which accelerate emergence of AMR strains) and direct disposal of sewages, hospital waste, industrial effluents into rivers in south Asian countries like Nepal and India, possibility of finding phages against MDR bacteria is high in these regions. Previously, we've shown that phages against carbapenem-resistant *Klebsiella pneumoniae* are abundant in river waters of Kathmandu valley.[3] These phages improved survival of mice without eliciting detrimental inflammatory responses.[4] This led us to search for more therapeutic phages in rivers of Kathmandu, Nepal. Three phages potentially having strictly virulent lifestyle were isolated from the river water flowing through Balkhu (Bagmati river) and Kalanki (Balkhu river) area. Since the phages were able to effectively lyse different CIs in addition to their primary host, they can be considered as phages with wide-host-range (WHR) akin to wide-spectrum antibiotics which is a desirable character for therapeutic phages. Further, observation of bull's eye plaque in øCDBT-PA58 clearly indicates that the phage has additional depolymerase activity that can further inhibit the growth of surrounding bacterial cells possibly by depolymerizing the alginic acid capsule of *P. aeruginosa*.[5] The alginase produced by such phages may be used alone or in combination with other drug/phage to increase the well-being of CF patients by facilitating the expectoration of sputum, accelerating phagocytic

uptake of bacteria and perturbing bacterial growth in biofilms.[5] Similar results were also observed with phage derived depolymerase enzyme alginase by Chegini, Khoshbayan[6] and Latino, Midoux.[7] Further, compared to bacteria, phages are four times more abundant in mucus layers because the protein shell of a phage can effectively bind mucins. This protects the underlying cells from potential bacterial pathogens, providing additional layer of non-specific immunity.[8] As anti-*Pseudomonas* phages has been in trail against chronic otitis, ear infection, CF already, biocontrol of bacterial infection using phages is gaining traction. Also, a French team showed that a specific cocktail of ten phages was able to significantly reduce bacterial growth *P. aeruginosa*.[9] Further, The studies on virulent phages against biofilms has shown decrease of the biomass in a biofilm but couldn't eradicate it.[10] To overcome this, combination therapy (phage + antibiotics) has been proposed as phage-antibiotic synergy (PAS) has shown encouraging results. Recently, polyvalent phages were also found to conjugate with magnetic colloidal nanoparticle clusters enhancing biofilm penetration for microbial control.[11] In a lethal mouse model of pneumonia using an MDR *P. aeruginosa* strain from CF patients, researchers reported that a single intranasal administration of anti-*Pseudomonas* phage(s) resulted in 90%-100% survival rate and reduced pathological damage when given within 2 hrs post infection .[9]

Further, phage parameters like pH and temperature stability, latent period and burst size are cardinal in application of phage as therapeutics. øCDBT-PA31 demonstrated extended tolerance to varying pH (3-11) and to high temperature (50°C) for 60 min making it more fit for application in diverse environment and wide range of animals including human. One-step growth assay of øCDBT-PA31 showed latent period of 30 min with corresponding burst size of 423-525 PFU/infected cell. As phages with small latent period and high burst size are desirable, our phage has potential to be a therapeutic phage. Also, in *in vitro* phage mediated lysis assay, øCDBT-PA31 was able to significantly decrease bacterial population within 3.0 hrs of phage treatment with MOI 100. Protein profiling using SDS-PAGE of two phages (øCDBT-PA11 and øCDBT-PA31) clearly indicated that they had different protein components in their structure implying they are morphologically distinct from each other. Further, TEM analysis of øCDBT-PA31 confirmed that the phage belonged to *Siphoviridae* family that harbors multiple members of virulent *Pseudomonas* phage. In conclusion, we isolated and purified 3 lytic phages against 3 different MDR CIs of *P. aeruginosa*. Among them, øCDBT-PA31 showed lytic activity against two *P. aeruginosa* CIs (PA31 and PA11) that were resistant to all tested classes of antibiotics. As the lysis spots were completely clear, we assumed øCDBT-PA31 to be virulent.

CONCLUSIONS

Based on these findings, we conclude that øCDBT-PA31 isolated from Bagmati river could be a therapeutic phage. However, genomic studies are necessary to rule out any lysogeny module and absence of other harmful (AMR, toxic, virulent) genes in the phage.

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COMPETING INTEREST

We declare no competing interests.

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Competing interests: None declared

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