Morphology Characterization of Ten Lytic Bacteriophages from Different Family Targeting *Klebsiella pneumoniae*

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Abstract

Phage therapy is the therapeutic use of bacteriophages to treat highly drug resistant bacterial infections. *Klebsiella pneumonia* (KP) has emerged as an increasingly important cause of community-acquired nosocomial infections and many of these strains are highly virulent with multidrug resistance (MDR) ability and exhibit a strong propensity to spread making infection cause by it difficult to treat. Treatment utilizing bacteriophage specific to KP (KP phage) can be used as an alternative counter measure. Characterization of bacteriophage is utmost important in assisting the application of bacteriophage in phage therapy. In the present study, samples from sewage water and cockles were screened for KP phage. Enrichment detection methods followed by isolation of phage by double agar layers method was done using 9 strains of MDR-KP as host system. KP phages were characterized on the basis of plaque morphology, host range and transmission electron microscope (TEM). A total of 58 lytic KP phages were isolated from sewage (42) and cockle (16). Investigation done on host range showed that 52 were KP phage and 6 have broad host range. Further identification by transmission electron microscopy (TEM) was done to 10 selected phages that showed potent lytic activity against MDR-KP. Image from TEM show selected phages were from subfamily caudovirales (7) with icosahedral head and pentagonal structure with short non-contractile tail suggesting it belongs to the family of *Podoviridae*. Two phages belonged to the family *Myoviridae with isometric head and contractile tail* and one from the family *Siphoviridae* with icosahedral and a non-contractile tail. Therefore, this study has partially characterized and revealed 10 potential lytic bacteriophages as therapeutic agent against MDR-KP which mostly resembles *Podoviridae* family.

Keywords: *Klebsiella pneumoniae*, cockle, sewage, phage therapy, TEM.

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Introduction

Klebsiella pneumoniae (KP) was first described in 1882 by Carl Friedlander as an encapsulated bacillus after isolating the bacterium from the lungs of those who had died from pneumonia. The bacterium typically colonizes human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract. Once the bacterium enters the body, it can display high degrees of virulence and antibiotic resistance. KP is ubiquitous in nature and predominately causes nosocomial infections especially in immunocompromised patients [1]. Today, KP pneumonia is considered the most common cause of hospital-acquired pneumonia in the United States and the organism accounts for 3% to 8% of all nosocomial bacterial infections [2, 3].

Antibiotics should be the ideal treatment for bacterial infection. Unfortunately, extensive usage of broad-spectrum antibiotics in hospitalized patients has led to its increased carriage as well as development of multidrug resistance (MDR) due to the production of extended-spectrum β-lactamases [4]. At present, a number of antibiotics currently in use are becoming ineffective to control bacterial pathogens because many bacteria have conferred multi-drug resistance.

WHO reports show that drug resistance in bacteria has been found in all regions of the world, accounting for approximately 50% of *Escherichia coli*, *K. pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections that are resistant to most potent antibiotics, such as cephalosporin which is a third-generation drug, resulted in a high mortality rate [5]. Clinicians and scientists working in this area have demonstrated renewed interest in phage therapy against MDR-bacteria. This has rekindled the interest in phage therapy which involves the use of bacteriophages as a safe and effective modality in dealing with MDR pathogens [6, 7].

The efficacy of phages to control bacterial infections had been extensively reviewed [8-10]. The ability of phages to rapidly kill or lyse bacteria, and their specificity makes them effective self-replicating antibacterial drugs of the near future [11]. One of the major reasons for failure of phage therapy during pre-antibiotic era was the application of uncharacterized phage preparations that led to inconsistent outcome of the therapy. The use of well-characterized bacteriophages, such as phages that target single species host or with narrower host range should be used as therapeutic agents. Well defined spectrum and virulence characterization will produce consistency, stability and standardization of therapy [10, 12, 13]. In light to the character mentioned, this study aims to characterize bacteriophages targeting MDR KP through observation of morphological characteristics and their host range spectrum.

Materials and Methods

Bacterial strain and growth media

K. pneumoniae strains and other 22 pathogenic bacteria, used for host range were obtained from Culture Collection Centre of Biological Sciences and Biotechnology, Faculty of Science and Technology, University Kebangsaan Malaysia (UKM). *K. pneumoniae* strains were previously tested for antibiotic sensitivity. All strain used were resistant to at least two antibiotics (unpublished data). Culture was maintained in the laboratory by using nutrient agar slants at 4 °C. For liquid preparations, bacterial culture was grown in Luria Bertani (LB; Oxoid) at 37°C with agitation at 200 rpm.

Bacteriophage isolation, detection and titration

Bacteriophages were isolated by using four cockle samples and 16 raw sewage samples obtained from UKM and Indah water sewage unit according to previously described methods [14, 15]. In brief, 200 mL of fresh sewage was mixed with 20 mL of bacteriophage broth, 20 mL of *K. pneumoniae* strains in broth culture (OD600 = 0.5) with BHI broth aseptically added to a 1 L flask and incubated at 37 ºC for 24 h with shaking at 55 rpm. Next, the mixture was centrifuged at 4500 rpm and the supernatant was filtered through a sterile membrane filter (0.22 µm, Chm Spain). Cockle samples were purchased from various fresh markets, including Giant market and NSK Food market and used for the isolation of bacteriophages by following the protocol outlined by Sangseedum [16]. The phage titer was determined by two-fold serial dilution of host and incubated at 37 ºC for 20 min before the addition of top LB soft agar. Mixture was poured onto the surface of a LB agar and was allowed to harden and then incubated at 37 ºC for 24 h. Host only and LB soft agar without filtrate was used as negative control. Three rounds of infection and picking of isolated plaques were performed to obtain pure bacteriophage suspensions**.** The phage was propagated and purified following the methods of Sambrook and Russel [15].

Host range determination

The ability of isolated phages to target different strains of *K. pneumoniae* was assessed by the spot test technique using 15 strains of *K. pneumoniae* beside range of Gram-positive and Gram-negative clinically isolated (Table 1). To test the susceptibility of bacterial isolates, a spot test was used [17]. In brief, bacterial isolates were grown in LB broth. After the cultures reached the early log phase (OD at 600 nm, 0.5±0.7), 200 µL of bacteria was mixed with 5 mL soft agar and poured onto the bottom agar to solidify. Then, 10 µL of phage filtrate was collected and spotted on the soft agar at a titter of 10^9 pfu/mL on LB agar. The plates were allowed to dry and examined for lytic zones or plaque formation against an uninfected negative control after overnight incubation at 37°C.

Transsmison electron microscopy (TEM)

The morphology of the phages was determined using a 100-kV transmission electron microscope (Thermofisher) with the same protocol previously described [18]. In brief five microliters of (10^{10}) pfu/mL polyethylene glycol (PEG) precipitated, and phage filtrate were drop onto a grid surface and stained negatively with 2% uranyl acetate, the excess stain was removed immediately. For bacteria phage combination phage was added for bacterial culture in 100 multiplicity of infection (MOI) and subjected for TEM after incubation for designated period. Ten isolated phages were classified according to the International Committee on Taxonomy of Viruses ICTV [19].

Results and Discussion

Bacteriophage isolation and host range determination

A total of 58 lytic bacteriophages (42 from sewage and 16 from cockle) were isolated and collected by using nine strains of KP as the host. Ten phages were chosen for further characterization based on their phage morphology findings and their specificity towards virulent MDR-KP. As for the phage morphology observation, these phages were detected as

clear round plaque center (0.5-6) mm in diameter surrounded by a large halo (Figure 1; Table 1), with huge increase in halo size after incubation for three days. The haloes surrounding the plaque indicate the presence of phage depolymerase capable of degrading capsular polysaccharide produced by *K. pneumoniae* cells [20]. Depolymerase and related enzymes have been found to enhance the biofilm-eradicating activity of bacteriophages compared with non-depolymerase-producing bacteriophages [21].

Host range analysis was conducted for the purified bacteriophages (n= 58) to identify bacteriophage that can target more than 1 type of *Klebsiella pneumoniae* capsules (broad host range). Bacteriophages with broad host range are considered suitable candidates for phage therapy trials since the bacteriophage can be used against a wide range of species strains [17]. In spot test assay, bacteriophages were challenged against a collection of 15 clinical isolates of MDR *K. pneumoniae* in addition to a diverse panel of 22 gram negative and positive clinical strains including *S. aureus, E. coli, P. aeruginosa, salmonella* and others.

In this study, all non MDR-KP selected pathogens are the most common human pathogens and they are included as host for the determination of the characteristics of the phage if they have broad or narrow specific host range. Results shows a total of six bacteriophages were observed to have polyvalent activity against MDR-KP and other bacterial strains (Supplementary data S1 & S2), k7w9 has the broadest host range that included 4 MDR-KP strains besides MRSA, *S. aureus and Bacillus subtillis.* The rest 52 bacteriophages were specific towards MDR-KP only and have a narrow range of lytic activity against 15 *K. pneumoniae* strains tested. From 52 phages, 9 were selected due to its specificity towards *Klebsiella* that have virulent capsular type 20 (K20) (kpukmcc 1126) (Supplementary data Table S1) that is associated with hypermucoid (result not shown). K20 is one of the *K. pneumoniae* isolates that showed the highest rate of virulent [22]. From this group of KP phage, three phages k9coc, k8coc and k9w6 showed the broadest host range against four different MDR-KP strain while phages k2w5, kp99 and k9w5 showed lytic spectrum against three MDR-KP strains used. In addition, k2b, k2a and k2w6 had the narrowest host range against MDR-KP strains by exhibiting lytic activity on only two strains MDR-KP. The specificity of interaction between phage tail structures and host receptor defines the host range of these viruses [23]. Some phages have specificity at the strain level whereas some have broader host ranges and can infect multiple bacterial strains within a single species or even multiple related species [24].

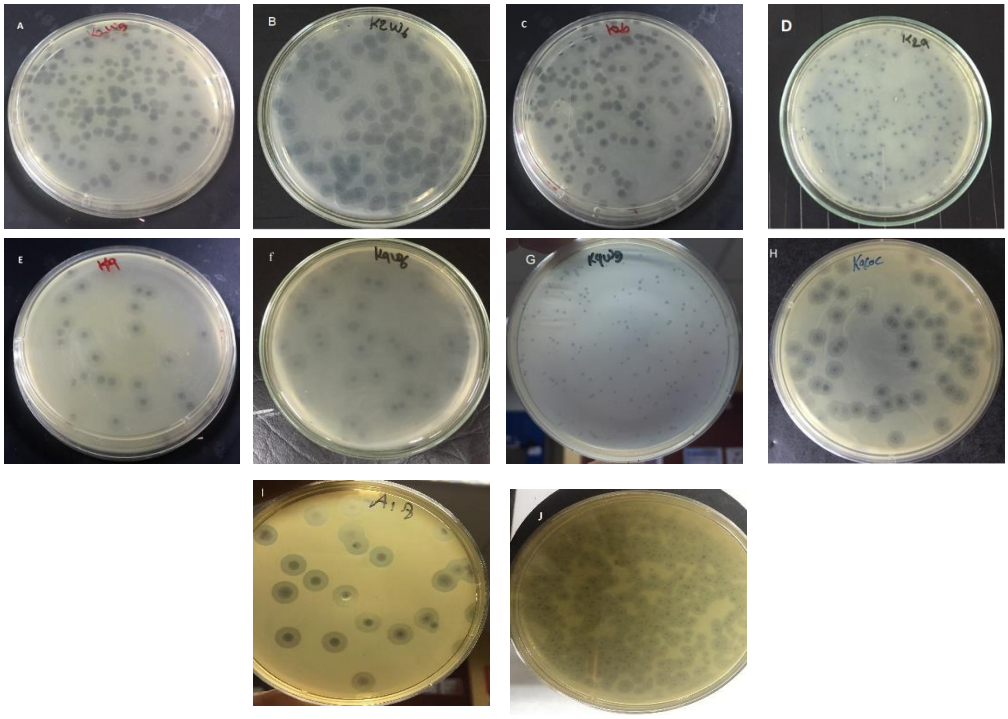

Figure 1. Plaque morphology of phage show different size of plaque k2w5 (A), k2w6 (B), k2b (C), k2a (D), kP99 (E), k9w6b (F), k9w5 (G), k9coc (H), k8coc (I) and k7w9 (J).

Table 1. Morphological properties and family classification of ten phages

Phage	k2a	k2b	k2w5	k2w6	kP99	k9w5	k9w6	k9coc	k8coc	k7w9
Clear zone (mm)	1.5	1.5	4	6	1	0.5	1	1	3	1.5
Halo (mm)	0.5	6.5	4	5	5	0.5	6	7	6	1
Family	*Podoviridae*	*Podoviridae*	*Podoviridae*	*Podoviridae*	*Podoviridae*	*Myoviridae*	*Podoviridae*	*Podoviridae*	*Myoviridae*	*Siphoviridae*
Capsid diameter (mm)	50	54	60	52	57	100	52	58	60	70
Tail length (mm)	-	-	-	-	-	100	-	-	79	140

Phage morphology using TEM

Out of 52 bacteriophages that targeted MDR-KP, 9 bacteriophages that targeted more than 1 MDR-KP strains and specific towards capsule type 20 (k9coc, k8coc, k9w6, k2w5, k9w5, k2b, k2a, k2w6 and kp99) along with one broad spectrum polyvalent phage (k7w9),

seven phages were observed to have icosahedral head and pentagonal structure approximately 52 - 60 nm with short non-contractile tail suggesting it belongs to the family of *Podoviridae* (Figure 2). According to previous research *Podoviridae* were the most frequently isolated KP bacteriophage [25, 26]. Another two phages, k9w5 and k8coc phage were classified into the family *Myoviridae* due to the presence of icosahedral heads and contractile sheaths produced, phage particles of k8coc were smaller than those of k9w5 and had an isometric head 60 nm in diameter and a complex contractile tail 79 nm in length. On the other hand, k9w5 phage particles consisting of an elongated isometric head of 100 nm and a complex contractile tail 100 nm in length. These two phages were also observed to be smaller than the previously isolated *Myoviridae* phage [27]. Bacteriophage from *Myoviridae* family has a wide host range and potential applications in phage therapy [28]. Only one phage, k7w9 had an icosahedral head of length 70 nm and a flexible non-contractile tail 140 nm long and belongs to the family *Siphoviridae (*Figure2). Based on our observation in TEM analysis, one of MDR-KP strains (KPukm1133) is flagellated. This new evolution of KP was also observed in clinical strain isolated in Spain [29]. The presence of flagella increases the virulence of the bacteria because it facilitates colonization of target host tissues in several pathogenic bacteria [30].

Next, adsorption for bacteriophage k2w5 and k9w5 were monitored under TEM toward two host KP2 and KP9 , TEM showed that adsorption of the phages takes place all around the cell surface at an early stage of infection in a rapid and similar fashion especially for k2w5 that could indicate predominate of *Podoviridae* phage receptors around the bacterial cell, which is in accordance with *K. pneumoniae*-infecting phages [31], before the phages injected their DNA into the cell, and empty capsids could be observed as being attached to the cell wall (black arrows in ,Figure 3).

Bacteriophages recognize capsule with a receptor binding protein (RBP) that contains a depolymerase domain. This domain degrades the capsule to initiate phage infection [32]. Post-replication nascent phages were formed inside the infected cell (Figure 3, Red arrow) until rapture of the bacterial cell wall released multiple phages from the lysed bacteria

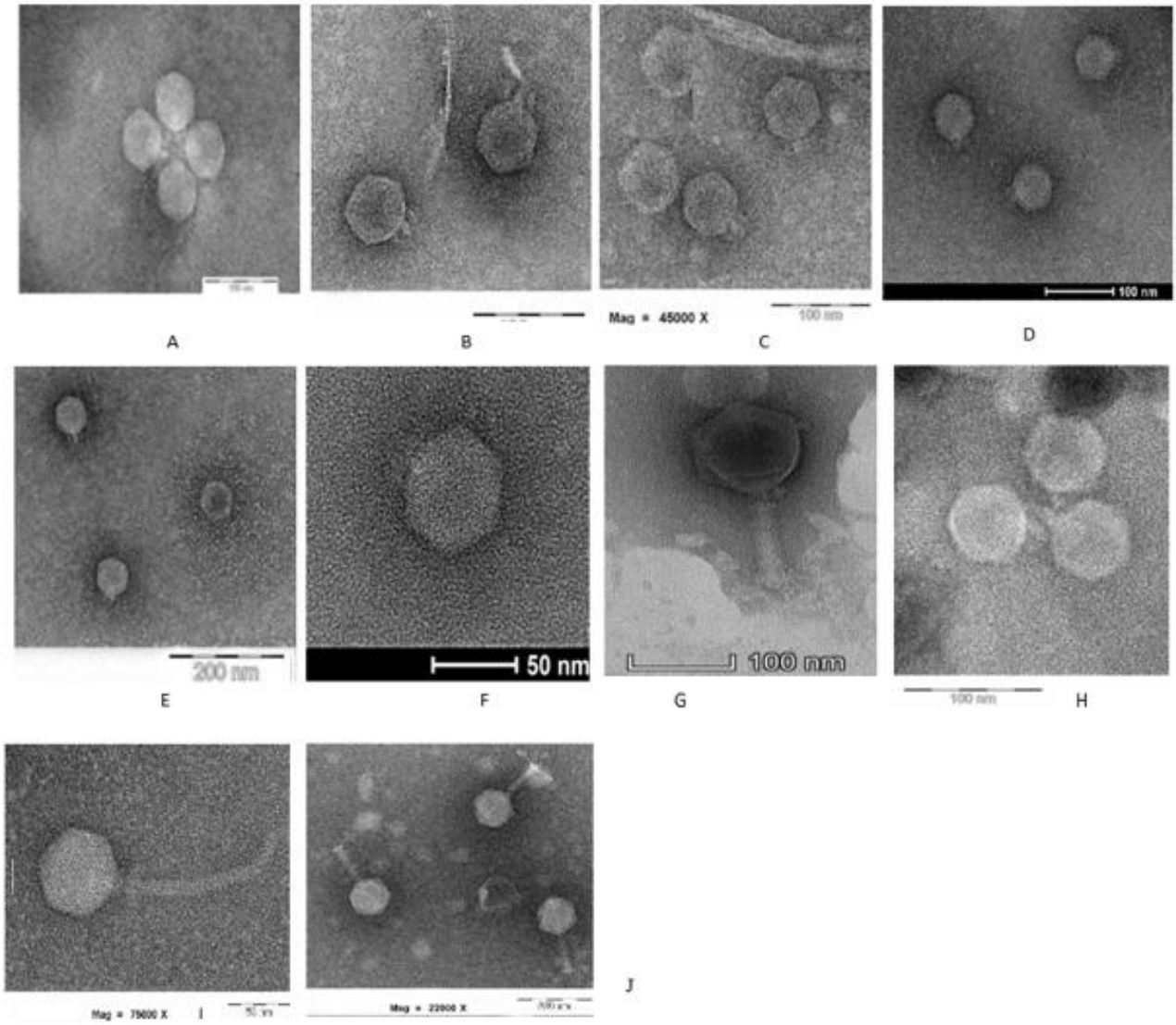

Figure 2. TEM images of Negative staining of phages k9w6b (A), k2w6 (B), kP99 (C), k2a (D), k2w5 (E), k2b (F), k9w5 (G), k9coc (H), k7w9 (I) and k8coc (J) staining with 2% uranyl acetate.

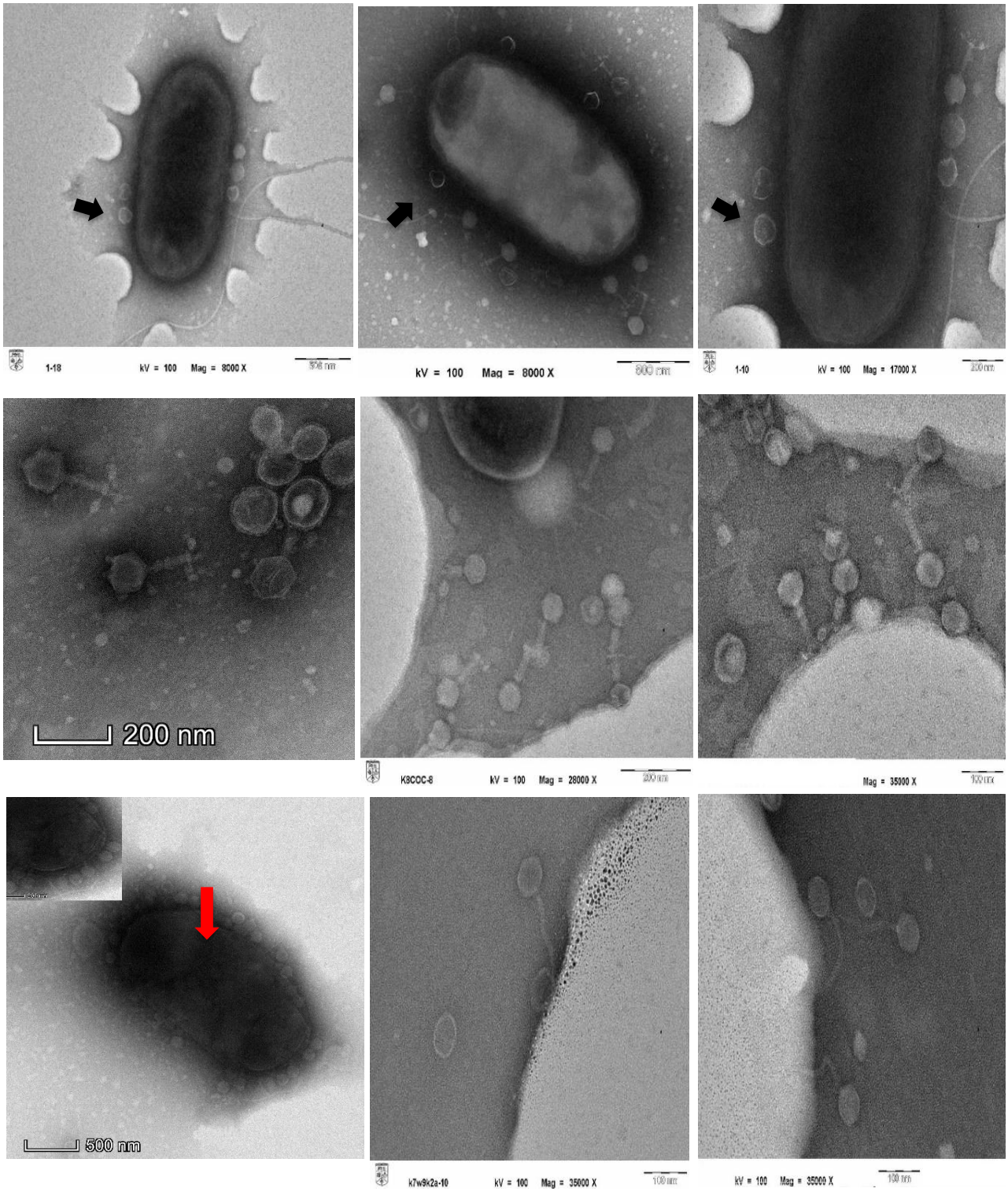

Figure 3. TEM image of phage particles on the cell surface of *K. pneumoniae* with phage the first line k9w5 with KPukm1133 (showing flagella expressed by *K. pneumoniae* one polar flagellum with the characteristic wave-like morphology. Photographs are representative of independent experiments in which several fields were analyzed, the second line show k8coc binding on KPukm1132 and the last line (right) show k7w9 and (left) k2w5 as example of Podoviridae phage adsorption.

Conclusion

In conclusion, a ten highly lytic bacteriophages were isolated from cockle and sewage samples and partially characterized have therapeutic potential against CR-*K. pneumoniae*. Analysis of TEM studies revealed that most of isolated phages resembles *Podoviridae* family and has a strong lytic activity against *K. pneumoniae*. Thus, the isolated phages will be effectively proposed to treat pneumonia or others MDR-KP infections in near future. However, further investigation about biological, molecular and antibacterial characteristic of these phages are required to expand the use of phages.

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Author Contributions

All authors contributed toward data analysis, drafting, and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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Supplementary data

Table S1 *K. pneumoniae* strains used in host range and their susceptibility to the phages

	Phage	Isolation host	KPukmcc 1125	KPukmcc 1126	KPukmcc 1127	KPukmcc 1128	KPukmcc 1129	KPukmcc 1130	KPukmcc 1131	KPukmcc 1132	KPukmcc 1133	KPukmcc 1134	KPukmcc 1135	KPukmcc 1136	KPukmcc 1137	KPukmcc 1138	KPukmcc 1139
1	Kph1	KPukmcc 1125	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
2	Kph3	KPukmcc 1127	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
3	Kph4	KPukmcc 1128	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
4	Kph5a	KPukmcc 1129	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
5	Kph5c	KPukmcc 1129	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
6	Kph6	KPukmcc 1130	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
7	Kpf8	KPukmcc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
8	Kpa8	KPukmcc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
9	K8Ws1	KPukmcc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
10	K8Ws2	KPukmcc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
11	K8Ws3	KPukmcc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

12	K8Cs4	KPukm cc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
13	K8w5	KPukm cc 1132	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
14	K8w6	KPukm cc 1132	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-
15	K8ukm	KPukm cc 1132	-	-	-	-	-	-	-	+	+	-	-		-	-	-
16	K2a	KPukm cc 1126	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
17	K2b	KPukm cc 1126	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
18	K2w5	KPukm cc 1126	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-
19	K2w6	KPukm cc 1126	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
20	*Kp99*	KPukm cc 1133	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-
21	K9w5	KPukm cc 1133	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-
22	K9w6b	KPukm cc 1133	-	+	-	-	-	-	-	+	+	-	-	+	-	-	-
23	K8cs7	KPukm cc 1132	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
24	K9cs7a	KPukm cc 1133	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
25	K9cs7b	KPukm cc 1133	-		-	-	-	-	-	+	+	-	-	-	-	-	-
26	K9w7a	KPukm cc 1133	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
27	K9w7b	KPukm cc 1133	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-

28	K5w8a	KPukm cc 1129	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-
29	K5w8b	KPukm cc 1129	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
30	K2w8a	KPukm cc 1126	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
31	K2w8b	KPukm cc 1126	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
32	K8w8	KPukm cc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
33	K9w8a	KPukm cc 1133	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
34	K9w8b	KPukm cc 1133	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
35	K9w6a	KPukm cc 1133	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-
36	K9ws1	KPukm cc 1133	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
37	K2w7	KPukm cc 1126	-	+	-	-	-	-	-	+	+	-	-	+	-	-	-
38	K1coc	KPukm cc 1125	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
39	K1w9	KPukm cc 1126	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
40	K2coc	KPukm cc 1126	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
41	K2w9	KPukm cc 1126	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-
42	K3coc	KPukm cc 1127	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
43	K3w9	KPukm cc 1127	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-

44	K4coc	KPukm cc 1128	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
45	K4w9	KPukm cc 1128	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
46	K5coc	KPukm cc 1129	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
47	K5w9	KPukm cc 1129	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
48	K6coc	KPukm cc 1130	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-
49	K6w9a	KPukm cc 1130	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
50	K6w9b	KPukm cc 1130	+	-	-	+	-	+	-	-	-	-	-	+	-	-	-
51	K7coc	KPukm cc 1131	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
52	K7w9	KPukm cc 1131	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-
53	K8coc	KPukm cc 1132	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-
54	K8w9	KPukm cc 1132	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
55	K9coc	KPukm cc 1133	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-
56	K9w9a	KPukm cc 1133	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
57	K9w9b	KPukm cc 1133	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
58	K9w9c	KPukm cc 1133	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

Table S2 Bacterial strains used in host range and their susceptibility to the phages

Bacteria	Bacteriophage																			
	K2a	K2b	Kp99	K9w1	Ukm8	K8w1	K8w2	K8w3	K8Cs4	Kpf8	kpa	K2w5	K2w6	K8w5	K8w6	K9w5	K9w6a	K9w6b	K9w9 B	K9w9 C
***S. aureus* 99**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***S. aureus* 4**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA/pa	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA 3	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA 2908	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA 2192	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 11035**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 1**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* ATCC 25922**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 4**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 2891**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* R1707903**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* B 7546**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* P6171**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* 2909**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* P6168**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Salmonella typhi	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Salmonella typhi* 2356**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Bacillus subtillis	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Shigella	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

Serratia marcescens	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Enterococcus faecalis	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

Continuation of Table S2

Bacteria	**Bacteriophage**																
	K5w8a	K5w8b	K2w8a	K2w8b	K8w8	K9w8a	K9w8b	K8cs7	K9cs7a	K9cs7b	K9w7a	K9w7b	K2w7	Kph4	Kph5a	Kph5c	Kph6
***S. aureus* 99**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***S. aureus* 4**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA/pa	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA 3	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA 2908	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MRSA 2192	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 11035**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 1**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* ATCC 25922**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 4**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 2891**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* R1707903**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* B 7546**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* P6171**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

***Pseudomonas aeruginosa* 2909**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* P6168**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Salmonella typhi	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Salmonella typhi* 2356**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Bacillus subtillis	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Shigella	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Serratia marcescens	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Enterococcus faecalis	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

Continuation of Table S2

Bacteria	**Bacteriophage**																				
	K1coc	**K1w9**	**K2coc**	**K2w9**	**K3coc**	**K3w9**	**K4coc**	**K4w9**	**K5coc**	**K5w9**	**K6coc**	**K6w9b**	**K6w9c**	**K7coc**	**K7w9**	**K8coc**	**K8w9**	**K9coc**	**K9w9A**	Kph1	Kph3
***S. aureus* 99**	_	_	_	+	_	_	+	+	_	_	_	_	+	_	+	-	_	_	_	_	_
MRSA 2908	_	_	_	+	_	_	+	+	_	_	_	_	+	_	+	_	_	_	_	_	_
MRSA 2192	_	_	_	+	_	_	+	_	_	_	_	_	+	_	+	_	_	_	_	_	_
***E. coli* 5688**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 2616**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* E11**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* ATCC 25922**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 11035**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* N**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 4109**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* 2891**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* E3**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***E. coli* E6**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* R1707903**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* P6171**	_	_	_	+	_	_	+	_	_	_	_	_	_	_	_	_	_	_	_	_	_
***Pseudomonas aeruginosa* 2909**	_	_	+	+	_	_	+	+	_	_	_	_	+	_	+	_	_	_	_	_	_
***Pseudomonas aeruginosa* P6168**	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

Salmonella typhi	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bacillus subtillis	–	–	–	+	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Shigella	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Enterococcus faecalis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–