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Isolation and characterization of a novel Jumbo phage active against the emerging ST307 carbapenemase-producing *K. pneumoniae* clone

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PHAGE*in*LYON

INTRODUCTION

The recent and fast emergence of carbapenem-resistant *Klebsiella pneumoniae* strains calls for urgent development of alternatives to antibiotics. This major burden has been linked to the dissemination of few successful high-risk clones, such as the ST307 lineage which has spread in different parts of the world, and currently exhibits various carbapenemase-production patterns.

Bacteriophage therapy is a promising strategy to face multidrug resistance. Here, we report the description of the V1KP1 Jumbo phage and the evaluation of its activity against a panel of *K. pneumoniae* strains belonging to various ST including ST307 and producing various types of carbapenemases. We also assessed the impact of capsule type on V1KP1 activity as it has previously been associated to phage specificity.[1]

METHODS

- **Phages**

V1KP1 was isolated from a sewage sample on a ST307 clinical strain.
A: Phage morphology was characterized using transmission electronic microscopy (Jeol 1400 JEM, Tokyo, Japan).

B: Phage genome was sequenced using Illumina/Miseq technology and annotated using PATRIC (v 3.6.9)

C: Jumbo phage activity was evaluated through the **spot test assay** and the determination of the **Efficiency Of Plating** ratio (n=3):

$$\mathrm{EOP} = \frac{\text{phage titer on a test strain}}{\text{phage titer on a reference strain}}$$

D: Capsule type was determined using *wzi* gene sequencing[1] (Brisse *et al*, J. Clin. Microbiol. 2013, 51(12):4073).

- **Bacterial strains**

The panel included 83 clinical strains of *K. pneumoniae* belonging to different strain types. ST307 was over-represented (n=30) on purpose to evaluate phage activity on this prevalent sequence type.

A - B TEM analysis of V1KP1. Phage suspension was studied and showed *Myoviridae* particles with an average diameter of 95 and 125 nm for the length of the phage capsid and the tail respectively (Fig 1).
Phage V1KP1 possesses a 346 057 bp genome and thus belongs to a unique group of phages, known as Jumbo phages. Sequence analysis showed that it belonged to the *Myoviridae* family and the *Alcyoneusvirus* genus. V1KP1 best blast hit (NCBI) is vb_KleM_RaK2 (91% cov ; 98.46% id). We identified 626 predicted protein-coding genes, covering 94.5% of the genome (Fig 2).

Figure 1. V1KP1 morphology. TEM observation

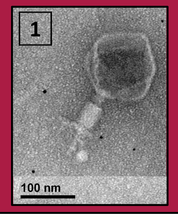
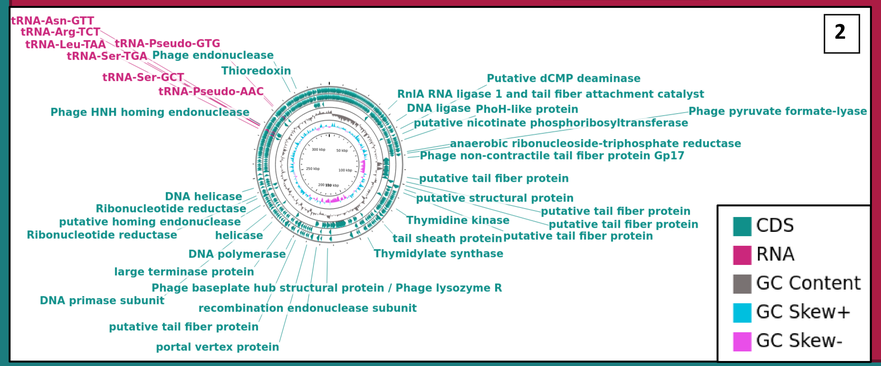


Figure 2. V1KP1 annotation. Circular genomic map of V1KP1 genome



RESULTS

C - D

- V1KP1 presented a narrow host range. It was active against 80% (24/30) of ST307 strains and 9,4% (5/53) of non-ST307 strains, including all ST16 (3/3) and ST20 (1/1), ST327 (1/1) isolates. Of note, it was not active against strains belonging to STs closely related to ST307.
- V1KP1 activity on ST307 strains **did not depend** on carbapenemase production (Table 1).
- Capsular type was not predictive of phage activity. If *wzi* alleles were well conserved among ST307 strains (allele type 173), ST11, ST16 and ST45 shared the same capsule type but V1KP1 was active only against ST16 strains. V1KP1 was also active against 2 other strains (ST327, ST20) with distant allele types (Fig 3).

Carbapenemase	V1KP1 activity
None	1/1
OXA-48	12/14
OXA-181	2/2
OXA-1, OXA-48	1/2
NDM-1	1/2
NDM-7	3/4
KPC-3	4/4
VIM-1	0/1

Table 1. Phage V1KP1 activity according to carbabenemase production of ST307 strains.
x/y: x=sensitive strains; y=number of tested strains

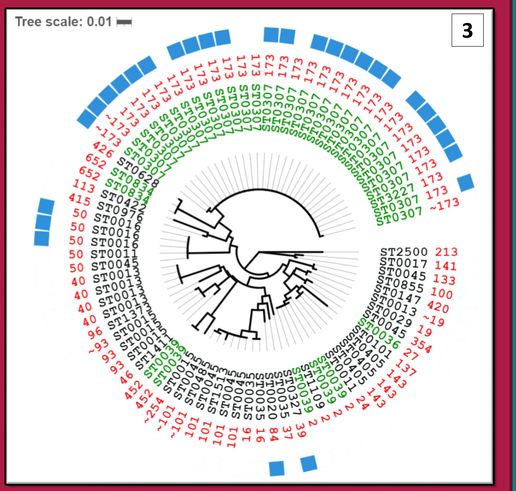


Figure 3. Phage V1KP1 host range according to ST and *wzi* allele sequence.
Phylogenetic tree built based on *wzi* gene sequences.
First layer: green = ST307 strains; Black = others
Middle layer: WZI allele type; ~ = closest *wzi* allele
External layer: V1KP1 activity (blue square=EOP>0; empty=no or weak lysis)

CONCLUSIONS

Phage V1KP1 genome analysis revealed a novel phage belonging to *Alcyoneusvirus* genus. The phage V1KP1 is active on a large panel of ST307 isolates, regardless of the type of carbapenemase produced. We noticed that it is active on several *K. pneumoniae* STs and this activity is not related to capsule types.

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