

## Introduction

*Klebsiella pneumoniae* is on the “critical priority” list for the discovery of new control methods due to its antimicrobial resistance. Responsible of some urinary tract infections (UTIs), the bacteriophage therapy represents a promising alternative against the bacteria. The aim of this study was to isolate and characterize new bacteriophages against a *K. pneumoniae* isolated from UTI and to assess their efficacy *in vitro* and *in vivo* in a *Galleria (G.) mellonella* larvae model.

## Material, method and results

### 2. Antibigram of the *K. pneumoniae* QAMH130326/0185 strain evaluated by disk diffusion method or Minimal Inhibitory Concentration (MIC)

Method	Antibiotics	Results (in mm)	Interpretation
Disk diffusion	Amoxicillin + clavulanic acid	17	R
	Ampicillin	6	R
	Aztreonam	34	S
	Cefotaxim	31	S
	Cefotaxim + clavulanic acid	32	Not a ESBL
	Cefoxitin	25	S
	Cefuroxim	16	R
	Fosfomycine	16	R
	Gentamycine	24	S
	Meropenem	31	S
	Nitrofurantoin	19	S
	Norfloxacin	39	S
	Trimethoprim + sulfamethoxazole	21	S
	Ceftiofur	28	S
	Ceftazidim	21	I
Colistin	0.5	S	

References EUCAST 2021 for: Amoxicillin + clavulanic acid, Ampicillin, Aztreonam, Cefotaxim, Cefotaxim + clavulanic acid, Cefoxitin, Cefuroxim, Fosfomycine, Gentamycine, Meropenem, Nitrofurantoin, Norfloxacin, Trimethoprim + sulfamethoxazole and Colistin  
References CASM VET 2019 for: Ceftiofur and Ceftazidim  
S: sensible to the antibiotic; R: resistant to the antibiotic; I: intermediate sensitive to the antibiotic; MIC: minimal inhibitory concentration

### 3. Bacteriophages' isolation

**Results:**  
**vB\_KpnA\_K3-ULINTkp1** in North Station of Brussel sewage water  
**vB\_KpnA\_K3-ULINTkp2** in Oupeye sewage water

### 4. Bacteriophages' morphology

Transmission electron microscopy (Tecnai Spirit microscope)

**Results:**  
- Podoviridae  
- Non-enveloped icosahedral head  
- Diameter of approximately 55 nm  
- Very short tail

Plaques of lysis on Petri dishes

### 5. Phages genome sequencing

**Results:**  
**vB\_KpnA\_K3-ULINTkp1**: 44290bp  
**vB\_KpnA\_K3-ULINTkp2**: 43685bp  
ID shared with phiKpS2 and vB\_KpnA\_SU503  
Caudovirales order, Autographiviridae family, Slopekvirinae subfamily, Drulivirus genus

### 1. Genome sequencing of *K. pneumoniae* QAMH130326/0185 (isolated from on UTI)

DNA extraction → Sequencing → Assembling → Annotation

**Results:**  
• Sequence type : **ST13**  
• *wzi* gene : *wzi-40* → **K3** capsular type  
• Virulence factors : ***clb-3*, *ybt-17*** on **ICE-Kp10**  
• Acquires resistance genes : ***bla<sub>OXA-1</sub>*, *sul-1*** and ***aadA1***  
• Chromosomal resistance genes : ***fosA*, *OqxAB*** and ***SHV-1***  
• Structural proteins : **FimA, B, D, F, G** and **H**

### 8. Adsorption times and low MOI kinetic curves

**ADSORPTION TIMES:** Mix of the phage + *K. pneumoniae* QAMH 130326/0185 at MOI 0,1 for 10' → each 2', the unabsorbed phages were titrated to measure the adsorption times (when P/P0 < 0,1).  
**Results:**  
**vB\_KpnA\_K3-ULINTkp1:** 4'  
**vB\_KpnA\_K3-ULINTkp2:** 6'

**LOW MOI KINETIC CURVES:** Mix of the phage + *K. pneumoniae* QAMH 130326/0185 at MOI 0,1 as long as the adsorption times → free phages were removed → each 5' during 100', the new produced phages were titrated to measure the latent period and the beginning of phage release) and the steady states.  
**Results:**  
**vB\_KpnA\_K3-ULINTkp1:** 5-10' for the latent period and steady state after 40'  
**vB\_KpnA\_K3-ULINTkp2:** 10-15' for the latent period and steady state after 45'

### 6. pH and temperature stability of the phages

**Results:**

pH stability after 1h incubation

Temperatures stability after 1h incubation

### 9. *Galleria (G.) mellonella* survival rates

Phages efficacy was tested in this *in vivo* model using the **MOI 10** and **100** (and **1000** if 100 was not enough). The larvae were **2 times inoculated** (1h between both injections) with different treatment combinations (see table below). The survival rates were evaluated each 24h for 72h (via **Kaplan-Meier curves** and **Logrank tests**):

**Results:**  
- Both phages were **safe**  
- **vB\_KpnA\_K3-ULINTkp1:** ↑ survival with MOI 1000  
- **vB\_KpnA\_K3-ULINTkp2:** ↑ survival with MOI 10 and 100

### 7. Host range and efficiency of plating (EOP)

**HOST RANGES:** Spot-tests on overlays:  
**31 Klebsiella strains**  
**8 UPEC Escherichia coli**  
The test is positive if there is lysis.

**EOP:**  

$$EOP = \frac{[\text{phage}]_{\text{target bacteria}}}{[\text{phage}]_{\text{host bacteria}}}$$

**Results:**  
For **vB\_KpnA\_K3-ULINTkp1:**  
- *K. pneumoniae* SB5904 = **0,0072**  
For **vB\_KpnA\_K3-ULINTkp2:**  
- *K. pneumoniae* SB5904 = **0,0455**  
- *K. oxytoca* QAMH 121209/0028 = **0,0007**  
- *K. pneumoniae* QAMH 130528/0682 = **0,0001**  
- *K. pneumoniae* SB5890 = **0,0009**  
- *K. pneumoniae* ATCC27736: **K30**  
- *K. pneumoniae* QAMH 130528/0682 : **K81**  
- *K. pneumoniae* SB5890: **ST17**

### 10. Bacterial load and phage replication in *G. mellonella*

For each phage, 120 larvae were divided into 6 groups and inoculated following the same protocol as for the survival experiment (using a **MOI of 100**). The larvae were crushed at 24h and 72h and the **phage** and **bacteria** were titrated in the larvae juice.

**Results:**  
- After 24 or 72h : **[bacteria] in positive group > treated-infected groups, [phage] in safety group < treated-infected groups**  
- In all the infected groups (treated or not) the **[bacteria]** or **[phage]** decreased over time  
- **Lower [bacteria]** with **vB\_KpnA\_K3-ULINTkp2**  
- **Higher [phage]** with **vB\_KpnA\_K3-ULINTkp1**

## Conclusion

In conclusion, these two newly isolated *Klebsiella* phages demonstrated their efficacy *in vitro* and *in vivo* by increasing the survival of *G. mellonella* larvae infected with a *K. pneumoniae* ST13 K3, even if this did not result in a complete elimination of the inoculated bacteria. Although these phages are genomically closely related, vB\_KpnA\_K3-ULINTkp2 showed a better efficacy in the *in vivo* *G. mellonella* model and a broader host spectrum than vB\_KpnA\_K3-ULINTkp1.