

Isolation and characterisation of bacteriophages active against *Pseudomonas aeruginosa* strains isolated from ocular infections

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Background



Microbial keratitis

is the infection of the cornea caused by bacteria, fungi, Acanthamoeba and some viruses¹.



Pseudomonas aeruginosa

is the leading cause of contact lens-related MK, around 70% of cases¹.



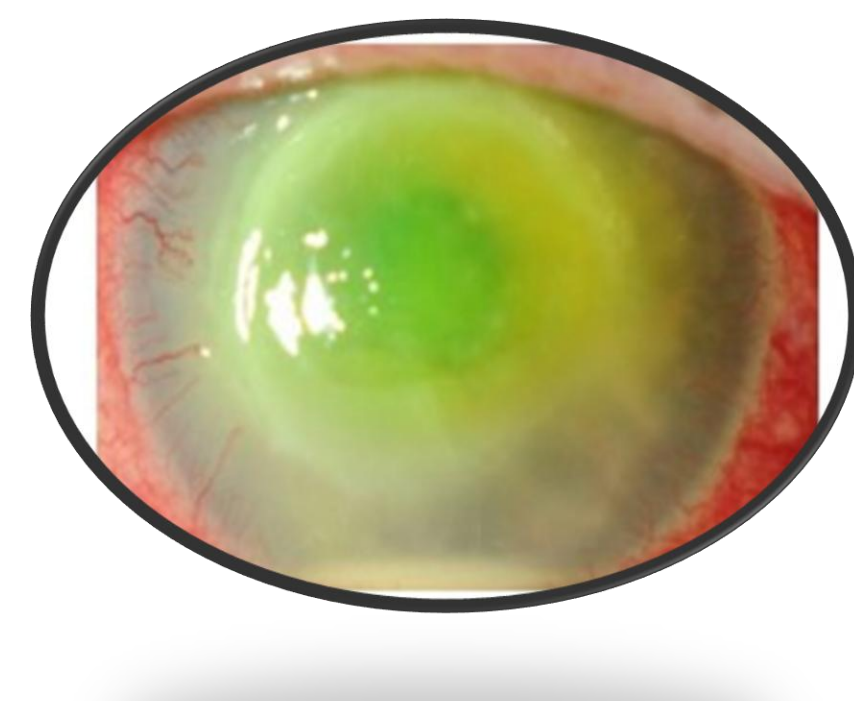
Antimicrobial resistance

is increasing, making this pathogen challenging to treat.



Alternative treatment needed:

Bacteriophages (viruses that kill bacteria) to limit ocular infections caused by MDR pathogens².



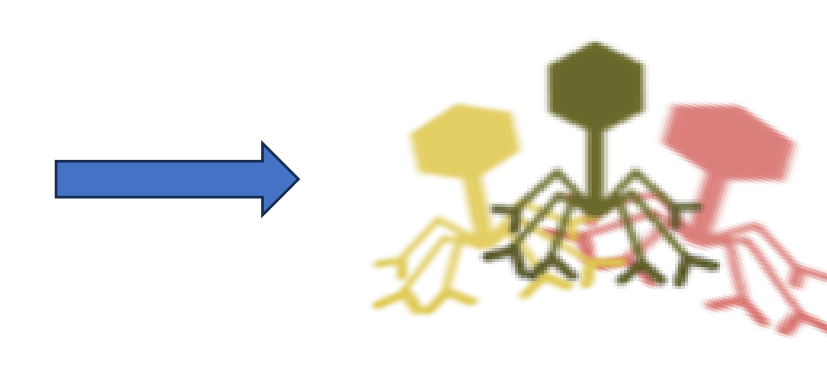
Aims of Research

- To isolate, propagate and purify phages
- To improve and characterize our preliminary phage cocktail
- To assess the ability of this phage cocktail to reduce pathology during *P. aeruginosa* keratitis in an animal model

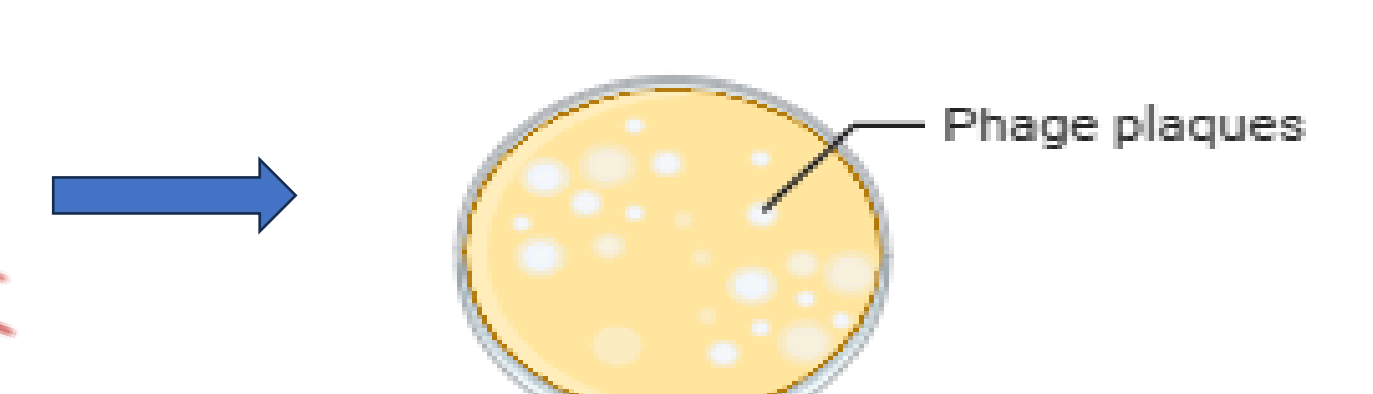
Materials and Methods



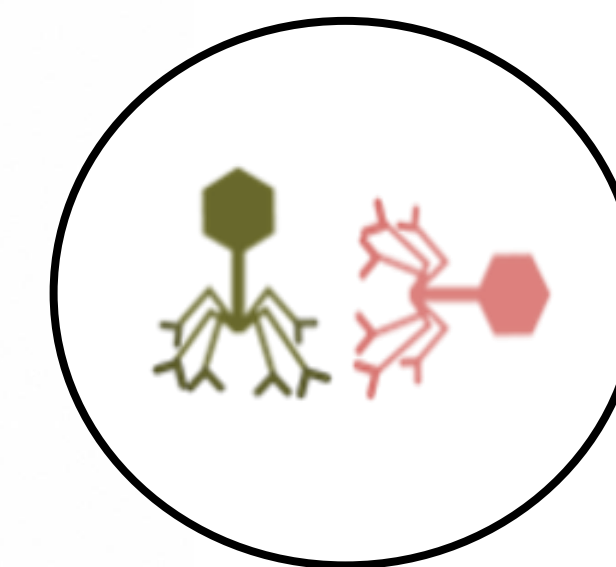
Sewage water from Sydney water, Sydney



Isolation of phages



Phage confirmation and quantification



Optimised phage library



Phage characterization by TEM, lab assays and WGS



Results

Figure 1: Host determination of Phages

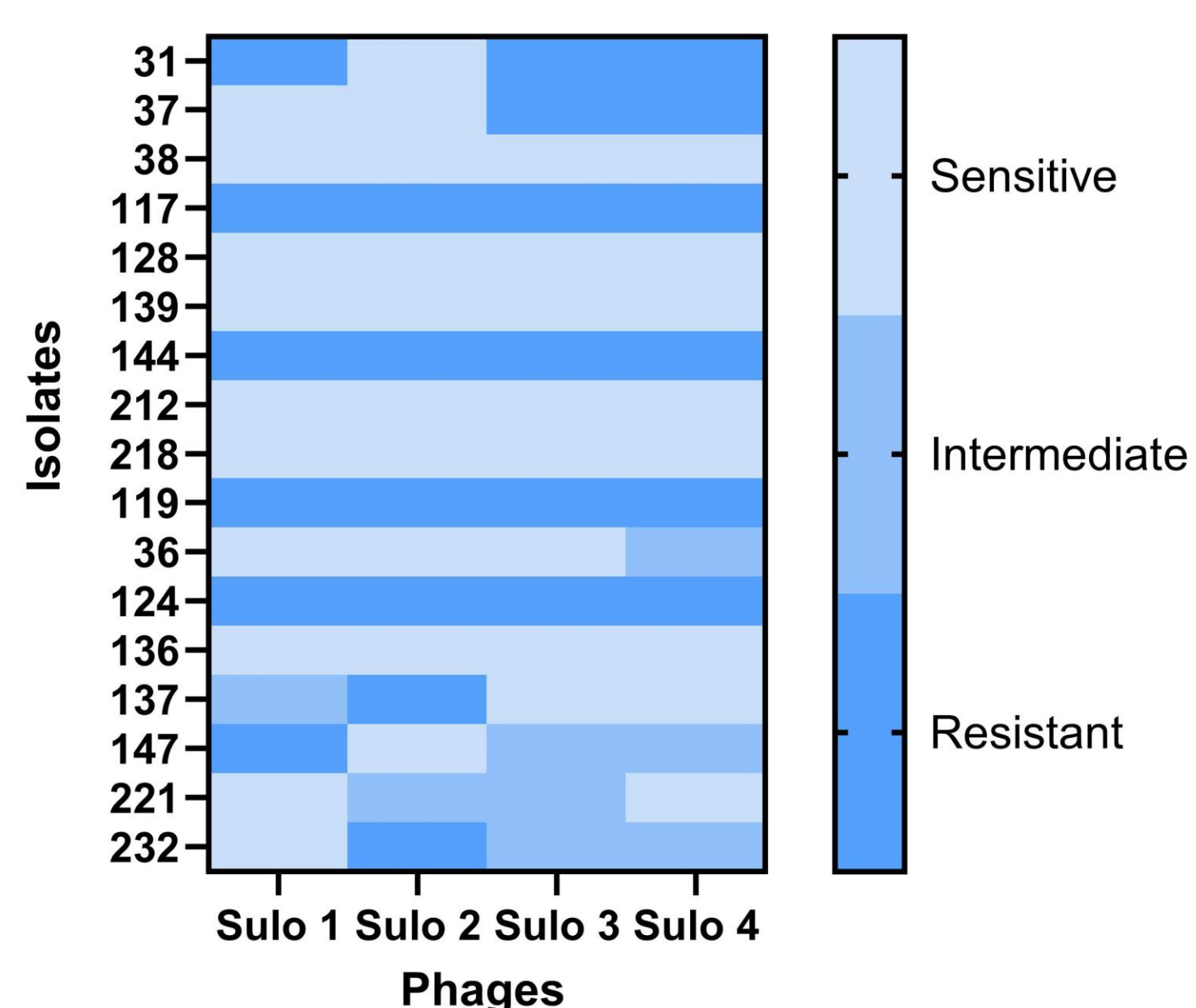


Table 1: Phages with their susceptibilities against isolates (n=11)

Phages	Susceptibility Rates (%)
Sulo 1	59
Sulo 2	59
Sulo 3	47
Sulo 4	47

Figure 2: Thermal stability of phages

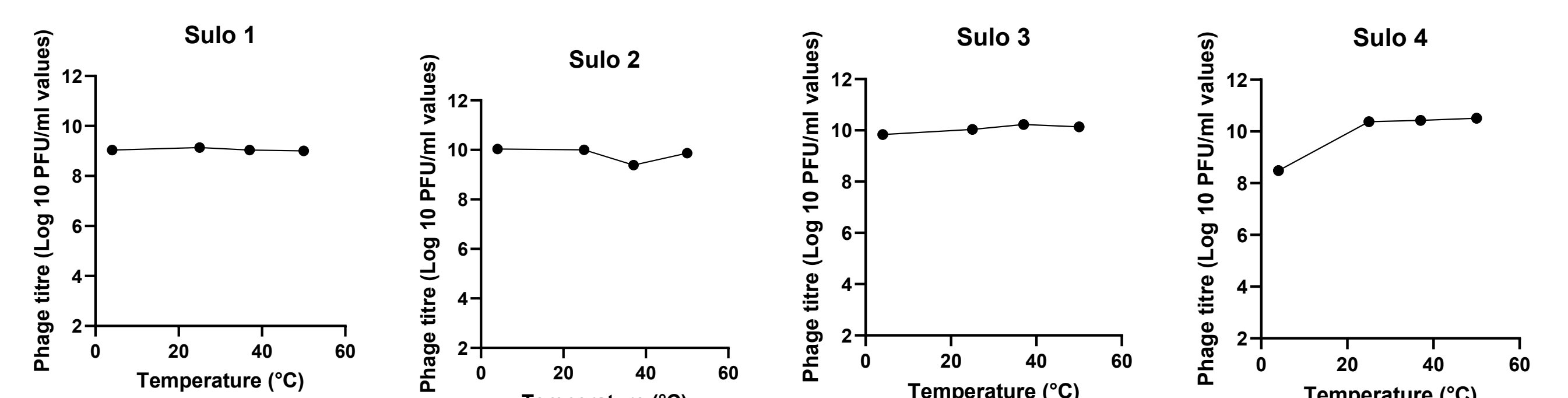
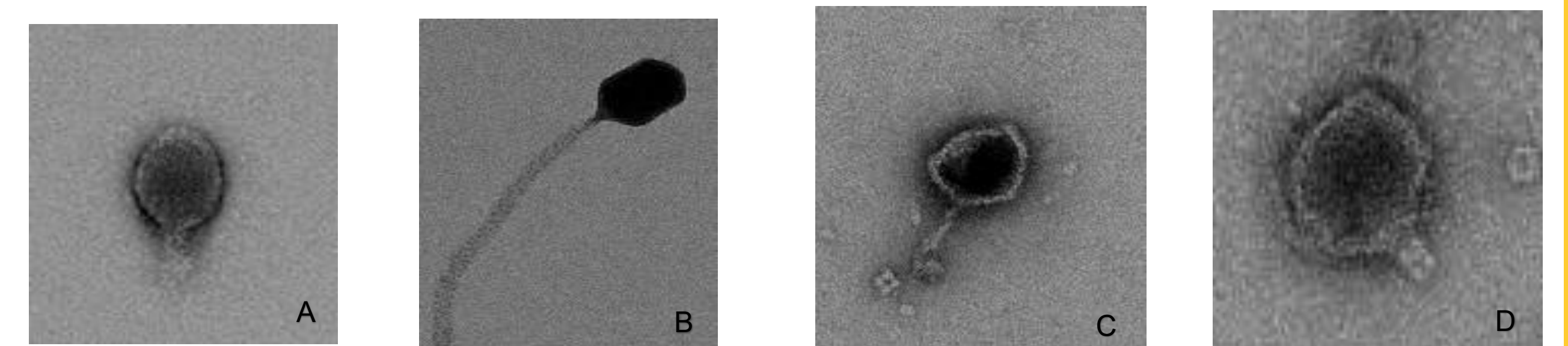


Figure 4: TEM analysis of phages



Identification of phages was done by TEM (100 nm resolution) (A) Sulo 1 – Podoviridae, short stubby tails, (B) Sulo 2 – Myoviridae, long contractile tails, (C) Sulo 3 – Siphoviridae, thin long non contractile tails, (D) Sulo 4 – Podoviridae short stubby tails

Table 3 : Whole genome sequencing of phages

Phages	Scientific name BLAST (Phage)	Percentage identification	Accession number	Genome size (kb)	GC Content (%)
Sulo 1	PJN P053	100	OR941788.1	177.3	56.6
	Vb_Pae_AM.P2	100	MT416090.1		
Sulo 2	<i>Pseudomonas</i> phage phikF77	98	NC_012418.1	103	58.8
	<i>Pseudomonas</i> phage BrSP08	98	PQ493644.1		
Sulo 3	<i>Pseudomonas</i> phage PA_CQ9	99	OQ828464.1	88	57.1
	<i>Pseudomonas</i> phage Arefeen 1	99	OR472559.1		

Figure 3: One Step Growth Curve

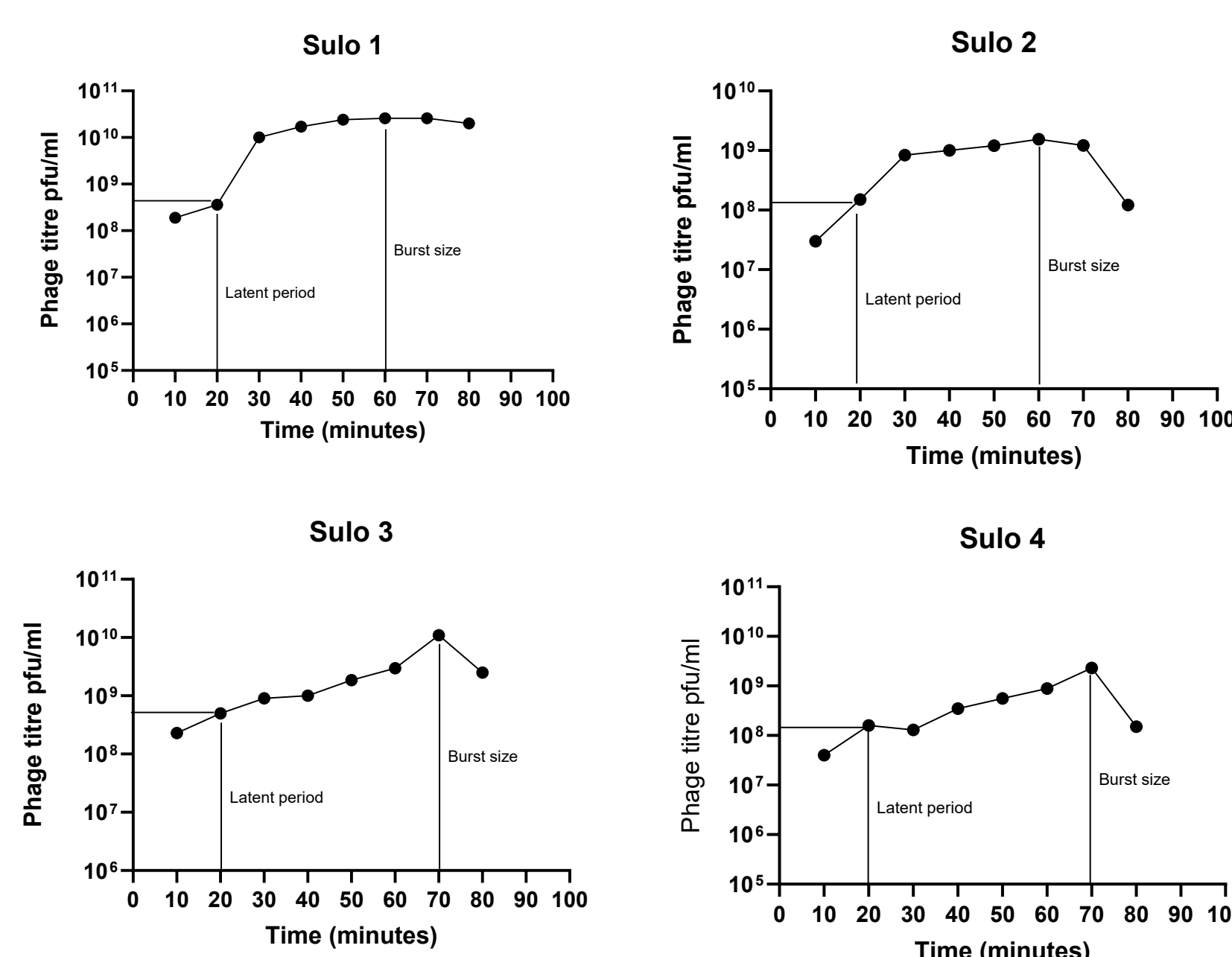


Table 2: Latent period and Burst size of phages

Phages	Latency (mins.)	Burst size (pfu/infected cell)
Sulo 1	20	260
Sulo 2	20	122
Sulo 3	20	110
Sulo 4	20	230

One step growth curve is used to measure **latent period** (time between infecting bacterial cell by phage and release of other phages) and **burst size** (average number of phage particles produced by infected bacterial cell)

Discussion and Conclusion

- This study has demonstrated that all phages showed efficient lytic activity against *Pseudomonas aeruginosa* isolates from microbial keratitis with Sulo 1 and Sulo 2 being the most effective.
- The newly isolated phages could be distinguished in terms of morphology, host range, thermal stability, burst size and latent period and TEM identification.
- This study will help us to improve phage therapeutic efficacy via optimized phage cocktails and phage-antibiotic combinations.

References

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