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INTRODUCTION

Bacteriophages, viruses specific of bacteria, are a promising **therapeutic alternative to antibiotics** to treat multi-drug resistant bacteria, such as staphylococci. **Coagulase negative staphylococci (CNS)** are major pathogens responsible for severe, chronic and challenging infections, such as **prosthetic joint infections (PJI)**. Due to their **high level of antimicrobial resistance** and to their **ability to form biofilm**, both favoring the chronic evolution of PJI, **CNS are considered as a major indication for phage therapy**. Previous studies have reported the activity of some *Myoviridae* anti-*Staphylococcus aureus* phages against few coagulase negative staphylococci strains, but more studies describing this activity on large collections of clinical isolates are required to assess their therapeutic potential.

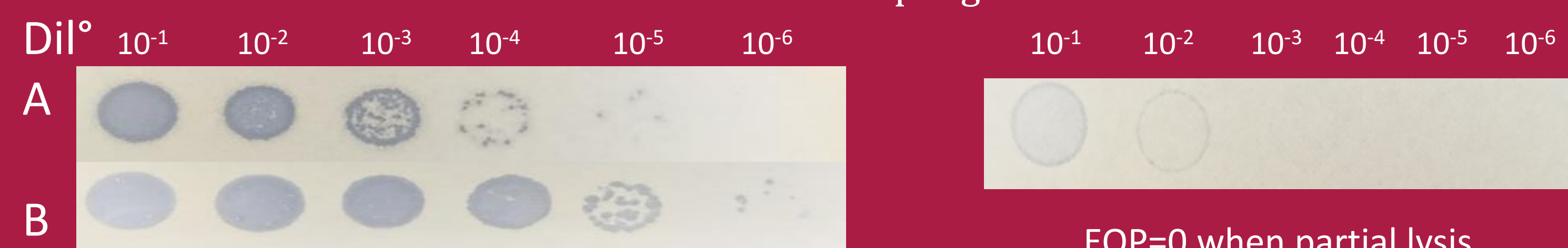
Aim: In this study, we assessed the activity of seventeen recently isolated anti-*Staphylococcus aureus* phages against a collection of CNS strains belonging to 7 species frequently involved in PJI.

METHODS

Phages

Seventeen *Myoviridae* phages belonging to two genera (*Kayvirus*, n=13; *Silviavirus*, n=3) recently isolated from wastewater samples and active on *S. aureus* isolates were included in this study. We used the **spot test assay** to assess their activity spectrum with the determination of the

$$\text{Efficiency Of Plating ratio: EOP} = \frac{A = \text{phage titer on a test strain}}{B = \text{phage titer on a reference strain}}$$



Phage efficacy is maximum if EOP≈1. Phages were classified as “efficient” if EOP≥0.001.

EOP=0 when partial lysis (external lysis without replication of phages) is observed (no individual plaques).

Bacterial strains

The panel included 32 CNS strains isolated from PJI and belonging to 7 different species : *S. epidermidis* (n=10), *S. lugdunensis* (n=5), *S. capitis* (n=5), *S. pseudintermedius* (n=3), *S. haemolyticus* (n=3), *S. caprae* (n=3), *S. warneri* (n=3).

RESULTS

➤ *Kayvirus* phages were more active than *Silviavirus* phages on CNS isolates

We observed an EOP ratio ≥ 0.001 for 17 *versus* 4 strains and a weak lysis for 14 *versus* additional strains with at least one *Kayvirus* or *Silviavirus* phages respectively.

➤ Activity of phages varied depending on bacterial species

We observed an EOP ratio ≥ 0.001 with *Kayvirus* phages for 2/3 or more of *S. capitis*, *S. caprae*, *S. lugdunensis* and *S. warneri* strains and for less than 50% of *S. epidermidis*, *S. haemolyticus* and *S. pseudintermedius* strains.

➤ The activity spectrum varied among *Kayvirus* phages

We observed an EOP ratio ≥ 0.001 for 5 to 15 and a weak lysis for 13 to 24 strains depending on phages. V1 SA9 was the most active phage.

	n	EOP ≥ 0,001		Weak lysis	
		<i>Kayvirus</i>	<i>Silviavirus</i>	<i>Kayvirus</i>	<i>Silviavirus</i>
<i>S. epidermidis</i>	10	4	1	5	1
<i>S. capitis</i>	5	4	0	1	0
<i>S. caprae</i>	3	2	0	1	1
<i>S. haemolyticus</i>	3	1	1	1	0
<i>S. lugdunensis</i>	5	4	2	1	3
<i>S. pseudintermedius</i>	4	0	1	4	0
<i>S. warneri</i>	3	2	0	1	0
Total	33	17	5	14	5

Table 1: Number of strains with EOP ≥ 0,001 or weak lysis for at least one phage of each genus.

	n	<i>Kayvirus</i>													<i>Silviavirus</i>				
		V1SA1	V1SA5	V1SA6	V1SA7	V1SA8	V1SA9	V1SA10	V1SA11	V1SA12	V1SA13	V1SA14	V1SA15	V1SA16	V1SA18	V1SA19	V1SA20	V1SA22	
		X-Y : number of strains with EOP ≥ 0,001 - or with weak lysis																	
<i>S. epidermidis</i>	10	0-6	0-8	0-7	1-5	2-4	4-4	0-7	1-5	0-6	0-8	0-7	0-8	0-8	0-7	1-1	1-0	1-1	
<i>S. capitis</i>	5	4-1	3-1	3-2	4-1	4-1	4-1	4-1	4-1	4-1	3-2	3-2	4-1	3-2	3-2	0-0	0-0	0-0	
<i>S. caprae</i>	3	2-1	0-3	2-1	2-1	2-1	2-1	1-2	1-2	1-2	1-2	2-1	0-3	2-1	2-1	0-1	0-0	0-0	
<i>S. haemolyticus</i>	3	1-1	0-2	0-2	1-1	1-1	1-1	0-2	0-2	0-2	0-2	0-2	1-1	0-2	0-1	1-0	0-0	0-0	
<i>S. lugdunensis</i>	5	2-3	2-3	4-1	4-1	3-2	4-1	3-2	4-1	2-3	1-4	2-3	4-1	4-1	3-2	2-2	1-1	1-0	
<i>S. pseudintermedius</i>	4	2-0	0-3	0-3	0-2	0-2	0-4	0-2	0-2	0-2	0-2	0-3	0-2	0-3	0-0	1-0	0-1	0-1	
<i>S. warneri</i>	3	1-2	0-3	0-3	1-2	0-3	0-3	1-2	0-3	0-3	1-2	0-3	2-1	2-1	0-3	0-0	0-0	0-0	
Total	33	10-16	5-24	9-19	13-13	12-14	15-15	9-18	10-16	7-19	6-22	7-21	11-17	11-18	8-16	5-4	2-2	2-2	

Table 2: Detailed activity spectrum of seventeen anti-*S. aureus* phages against a panel of 33 CNS strains

CONCLUSIONS

We report the activity of anti-*Staphylococcus aureus* polyvalent phages against CNS species causing PJI. ***Kayvirus* phages were more active than *Silviavirus* phages.** Further work should focus on the isolation of phages targeting *Staphylococcus epidermidis*, bacterial species against which the present collection of phages was insufficiently active, while it is a major pathogen in this context.

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