

# Isolation and characterization of bacteriophage against carbapenem resistant *Pseudomonas aeruginosa*

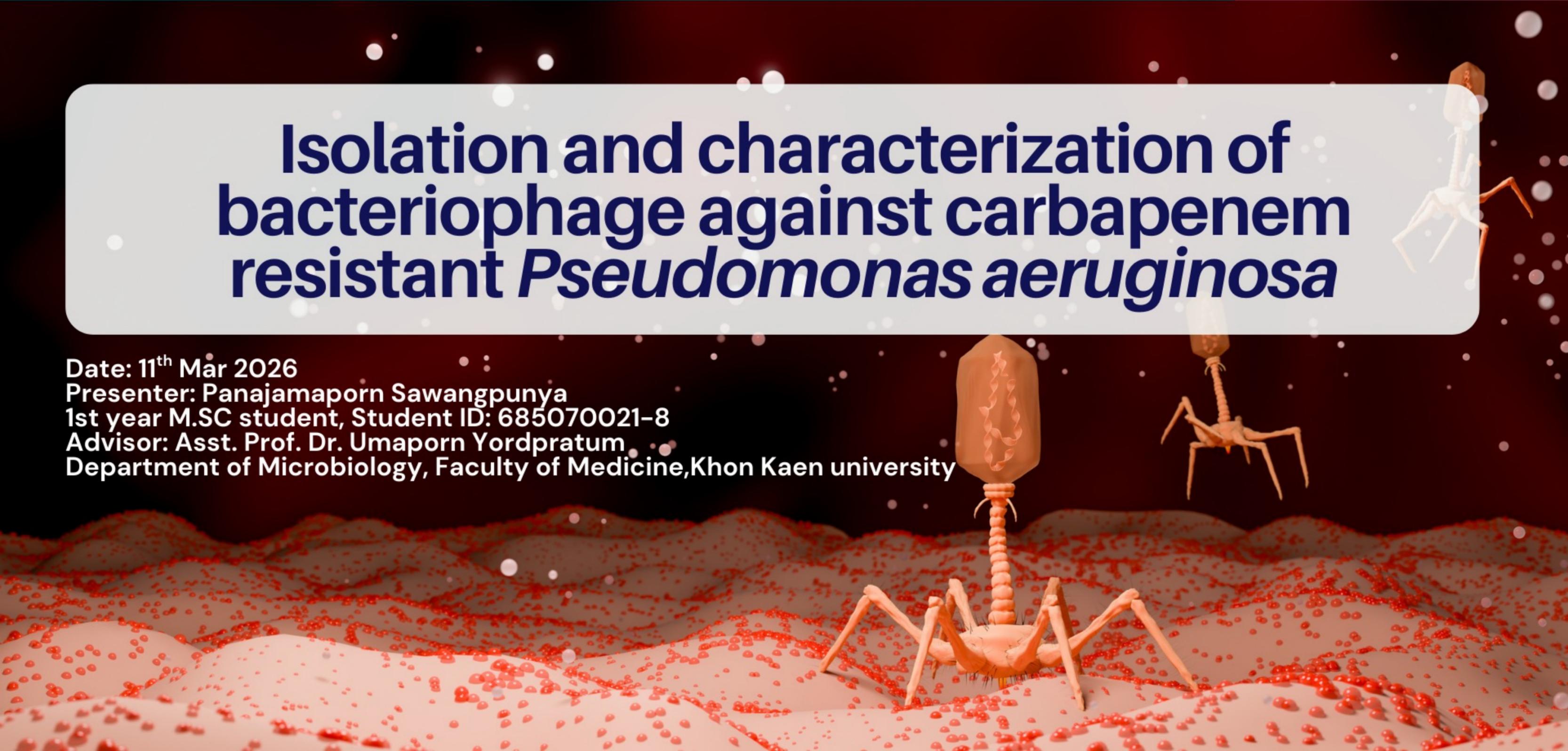
Date: 11<sup>th</sup> Mar 2026

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1st year M.SC student, Student ID: 685070021-8

Advisor: Asst. Prof. Dr. Umaporn Yordpratum

Department of Microbiology, Faculty of Medicine, Khon Kaen university



# Introduction

## *Pseudomonas aeruginosa*



- Gram-negative bacillus
- Widely in natural environment
- Nosocomial infections

### Common infections:

- Pneumonia
- Bloodstream infections
- Urinary tract infections
- Surgical site infections

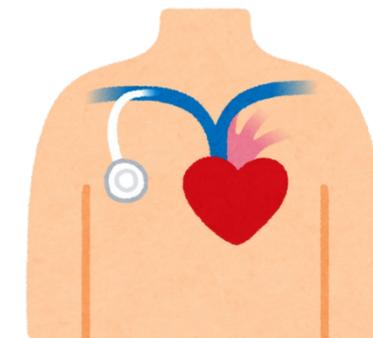
*P. aeruginosa*



biofilm formation

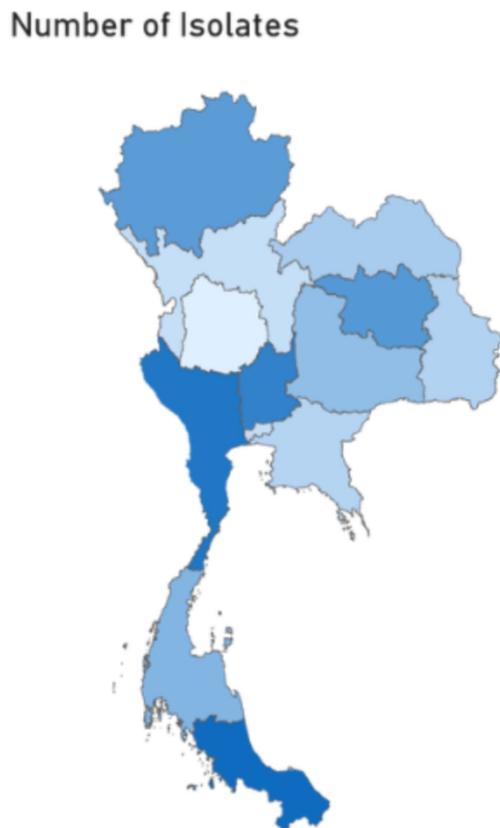
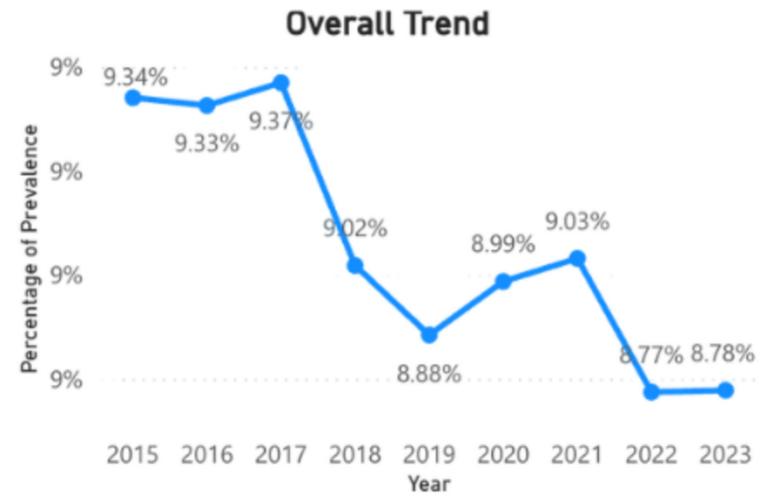
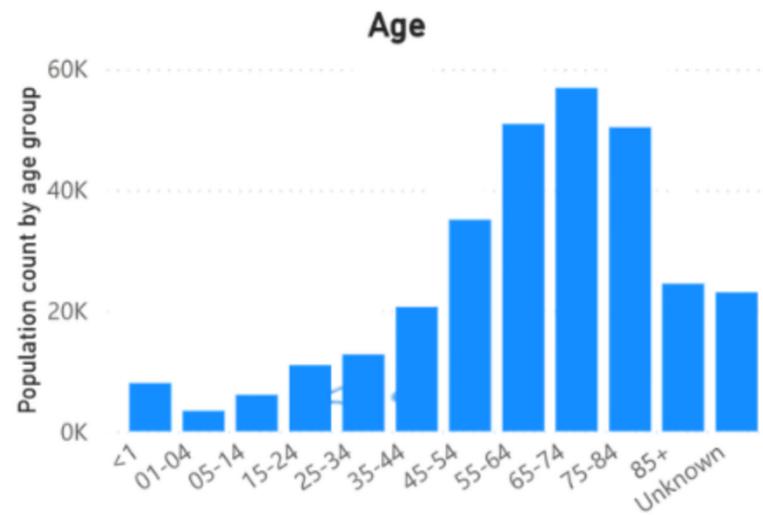


formed on medical devices used for treatment, such as catheters, vascular lines, and tracheal tubes



# Introduction

## National Prevalence of Bacterial Infection Dashboard

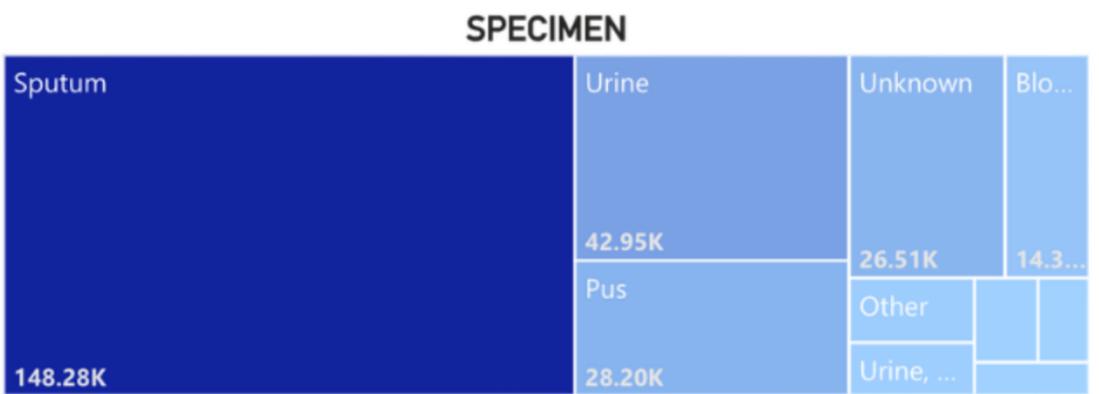
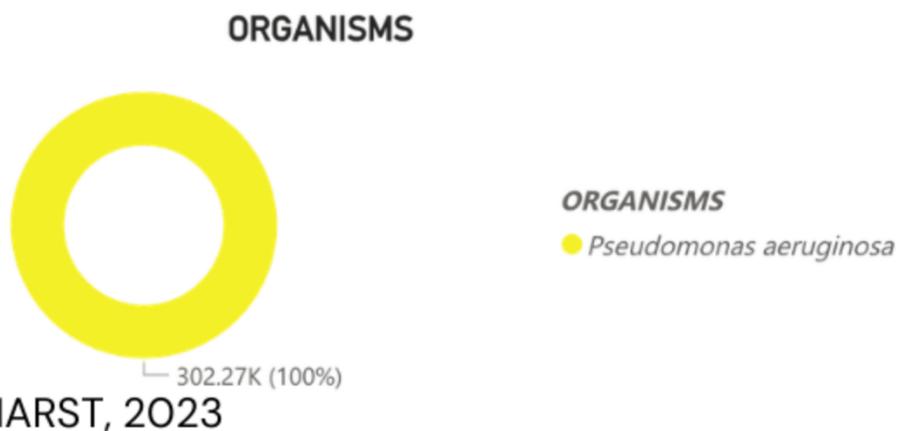
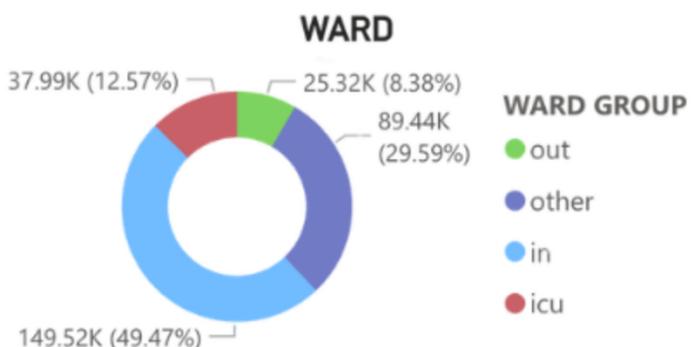
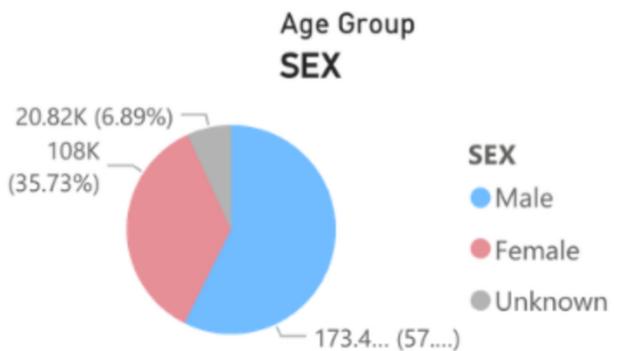
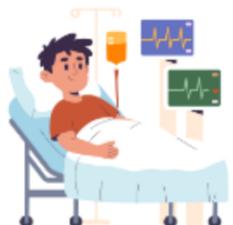


Data from **109 hospitals**  
(~302,000 isolates)



2015-2023  
Prevalence ~9%

Most common specimen:  
Sputum



# Introduction

## Antibiotic-resistant

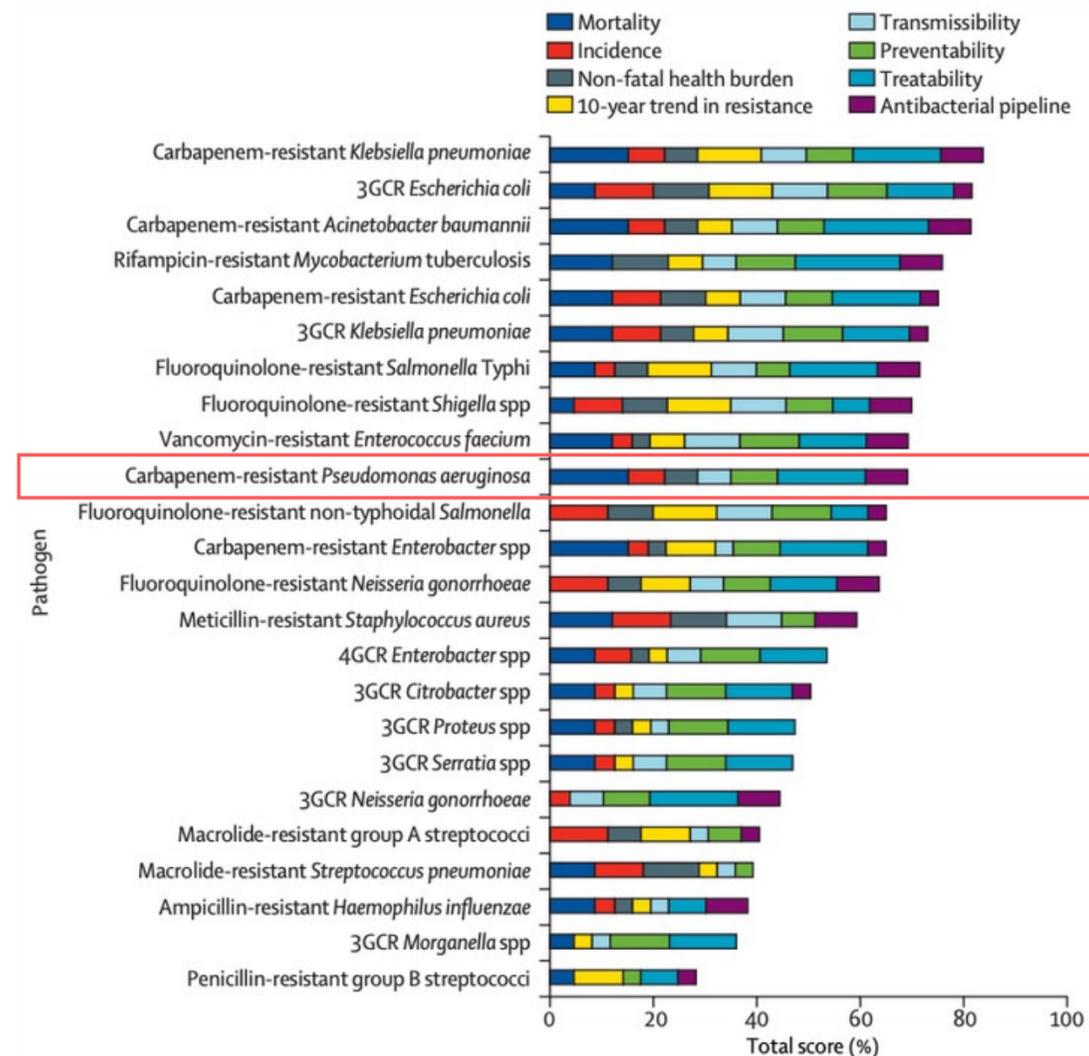


- A major global public health threat
- Resistant bacteria are harder to treat
- Increases healthcare costs & mortality

### IMPACT

- Longer and more complicated treatments
- Higher medical costs
- Increased morbidity and mortality

Antibiotic-resistant *P. aeruginosa* is a significant global problem because it can form biofilms, exhibit multiple resistance mechanisms, and cause treatment failure in chronically ill or hospitalized patients.



In the 2024 WHO BPPL ranking:

**carbapenem-resistant *P. aeruginosa***

*P. aeruginosa* remains a **critical pathogen** due to high disease burden, rising long-term antibiotic resistance, and limited new treatment options

# Introduction

## Bacteriophage



“A type of virus that infects and replicates inside bacteria”



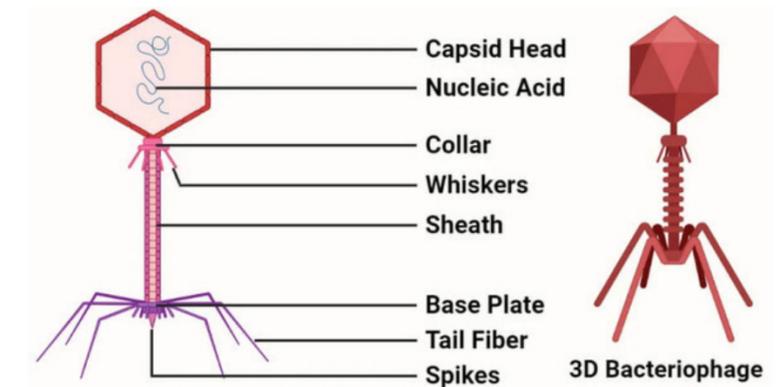
can be found in soil and seawater, oceanic and terrestrial surfaces and extreme environments

- Specific to the target bacteria
- Minor side effect
- Abundance in nature
- Harmless to human

## FUNCTION

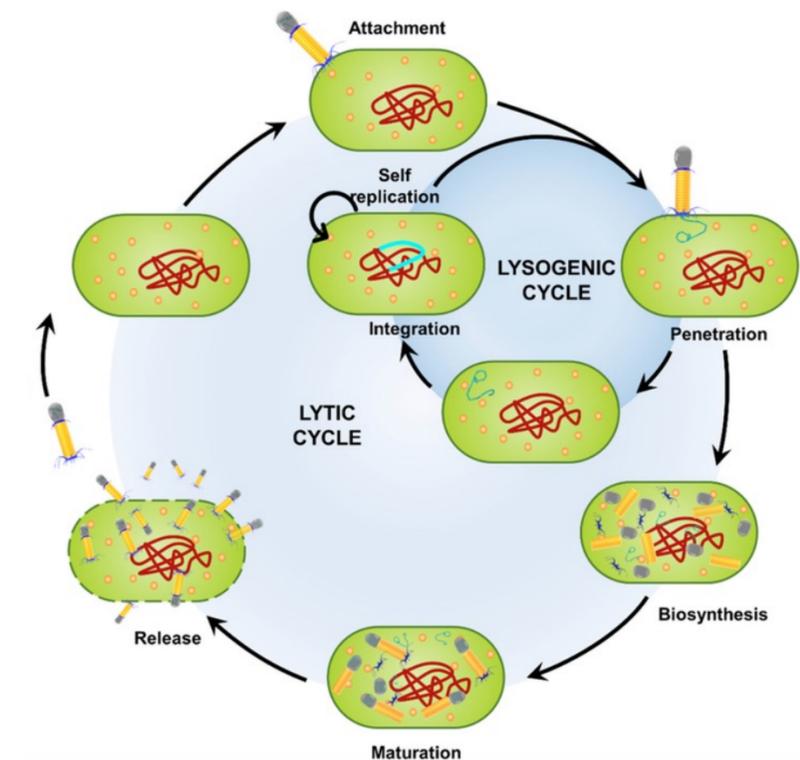
- Control of bacterial populations
- Potential medical applications: Researchers are exploring “**phage therapy**” to treat antibiotic-resistant bacterial infections.

## Phage structure:



[Sapkota.A, 2022](#)

## Life cycle of bacteriophage:



Monish B., Kusum K., Sakshi M., 2024

# Introduction



## Phage therapy:

To assess how effective phage therapy can be in treating bacterial infections, In preclinical infection models

### Efficacy of phage therapy in preclinical models of bacterial infection: a systematic review and meta-analysis

Sergio Alejandro Gómez-Ochoa, Melissa Pitton, Luca G Valente, Cristian David Sosa Vesga, Jorge Largo, Andrea Carolina Quiroga-Centeno, Juliana Alexandra Hernández Vargas, Silvia Juliana Trujillo-Cáceres, Taulant Muka, David R Cameron\*, Yok-Ai Que\*

	Country	Animal	Infection setting	Pathogen	Number of phages; phage name(s)	Phage administration route	Outcomes assessed	Overall results	Included in meta-analysis?	Reason for exclusion
Albac et al (2020) <sup>16</sup>	France	Mice	Skin or burn	<i>Staphylococcus aureus</i>	3; 1493, 1815, and 1957	Subcutaneous	Bacterial load	Phages reduce bacterial load	Yes	NA
Alemayehu et al (2012) <sup>17</sup>	Ireland	Mice	Respiratory	<i>Pseudomonas aeruginosa</i>	2; PHIMR299-2 and PHINH-4	Respiratory*	Bacterial load	Phages reduce bacterial load	No	Insufficient information
Cha et al (2018) <sup>18</sup>	South Korea	Mice	Respiratory	<i>Acinetobacter baumannii</i>	5; PBAB08, PBAB25, PBAB68, PBAB80, and PBAB93	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chadha et al (2016) <sup>19</sup>	India	Mice	Skin or burn	<i>Klebsiella pneumoniae</i>	5; Kpn1, Kpn2, Kpn3, Kpn4, and Kpn5	Topical or superficial	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies
Chadha et al (2017) <sup>20</sup>	India	Mice	Skin or burn	<i>Klebsiella pneumoniae</i>	5; KØ1, KØ, KØ3, KØ4, and KØ5	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chang et al (2018) <sup>21</sup>	Australia	Mice	Respiratory	<i>Pseudomonas aeruginosa</i>	1; PEV20	Respiratory	Bacterial load	Phages reduce bacterial load	Yes	NA
Chen et al (2021) <sup>22</sup>	China	Mice	Respiratory	<i>Pseudomonas aeruginosa</i>	2; MYY9 and HX1	Respiratory	Bacterial load	Phages reduce bacterial load	Yes	NA

- Applicable to multiple infection models and many MDR pathogens
- Very flexible administration route
- Almost all studies report positive outcomes

**➤ strong consistency of efficacy**

The Lancet Microbe, 2022

Preclinical studies demonstrated that phage therapy effectively reduced bacterial burden and mortality across multiple infection models, pathogens, and administration routes

# Bacteriophages for treating *P. aeruginosa* in Thailand

## Characterization of N4-like Pseudomonas Phage vB\_Pae-PA14 Isolated from Seawater Sampled in Thailand

Akkaraphol Srichaisupakit<sup>1</sup>   , Peechanika Chopjitt<sup>2</sup>  and Anusak Kerdsin<sup>2</sup> 

## Phage-resistant *Pseudomonas aeruginosa* against a novel lytic phage JJ01 exhibits hypersensitivity to colistin and reduces biofilm production

[Wichanan Wannasrichan](#)<sup>1</sup>, [Htut Htut Htoo](#)<sup>2</sup>, [Rubsadej Suwansaeng](#)<sup>2</sup>, [Joe Pogliano](#)<sup>3</sup>, [Poochit Nonejuie](#)<sup>2</sup>,  
[Vorrapon Chaikeratisak](#)<sup>1,\*</sup>

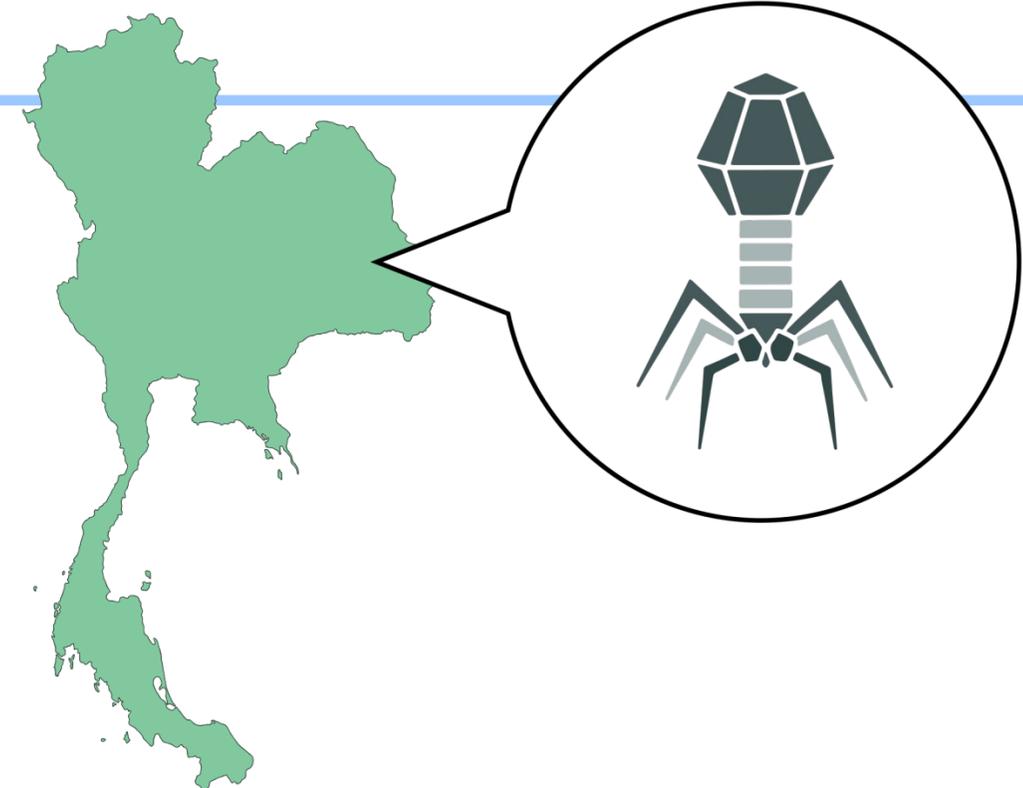
# Bacteriophages for treating *P. aeruginosa* in Thailand

## A novel virulent Litunavirus phage possesses therapeutic value against multidrug resistant *Pseudomonas aeruginosa*

Varintip Lerdsittikul<sup>1</sup>, Metawee Thongdee<sup>2</sup>, Somjit Chaiwattananarungruengpaisan<sup>2</sup>,  
Thassanant Atithev<sup>3</sup>, Sukanya Apiratwarrasakul<sup>1</sup>, Patoowithatanung<sup>4</sup>, Martha R J Clokie<sup>5</sup>,  
Sunee Korbsrisate<sup>6</sup>

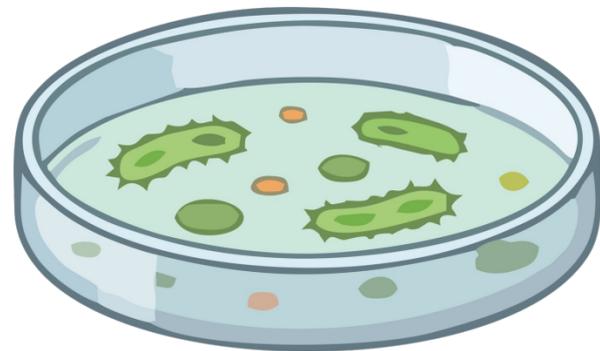
### Gap of research

- Lack of local clinical relevance
- Absence of a systematic phage library



**Hypothesis:** The identified phages, isolated from environmental sources and bacterial hosts, will specifically infect and lyse *P. aeruginosa*, including carbapenem-resistant strains

## Objectives



1. To **isolate bacteriophages** specific to *P. aeruginosa*, including carbapenem-resistant strains, from **environmental samples**

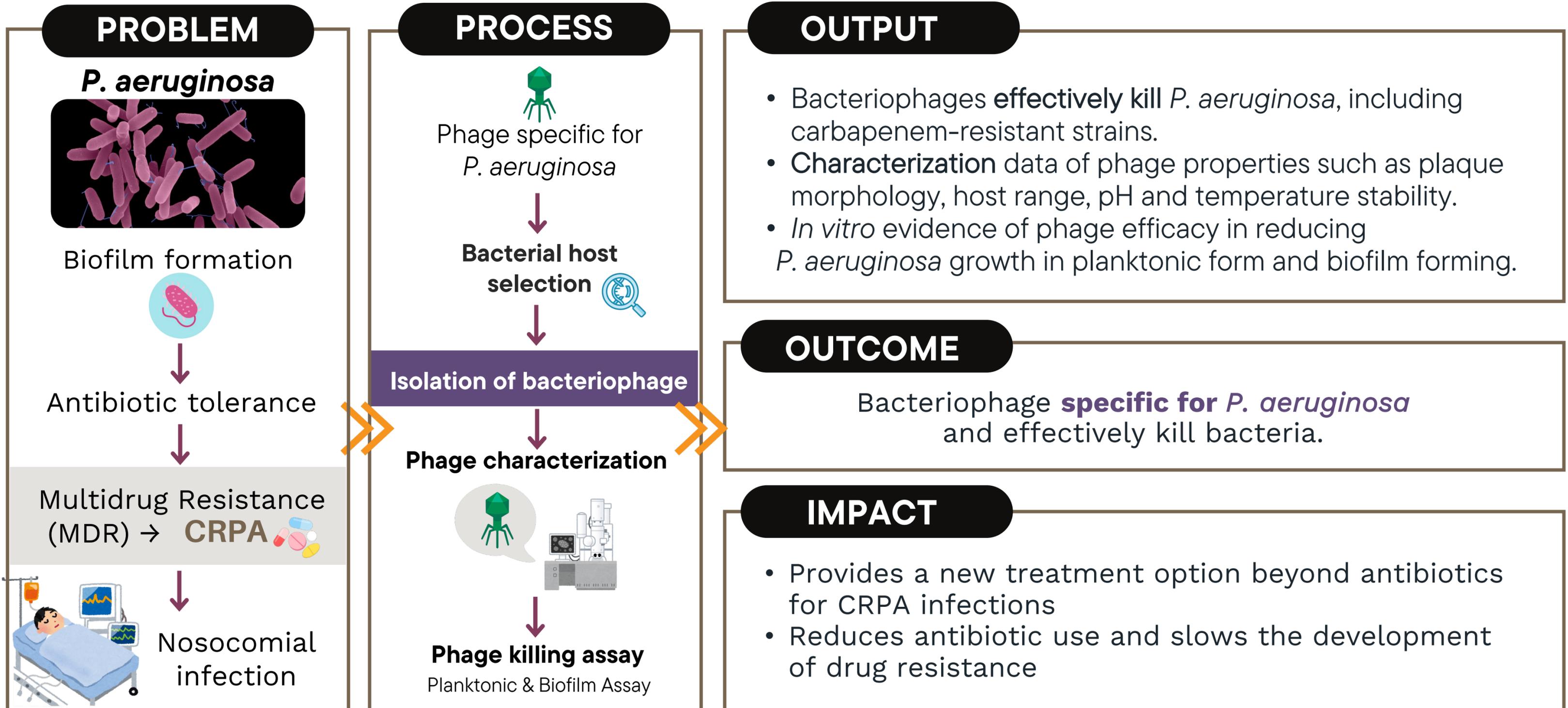


2. To **characterize** the biological properties of the isolated phages, including host range, plaque morphology, and stability test

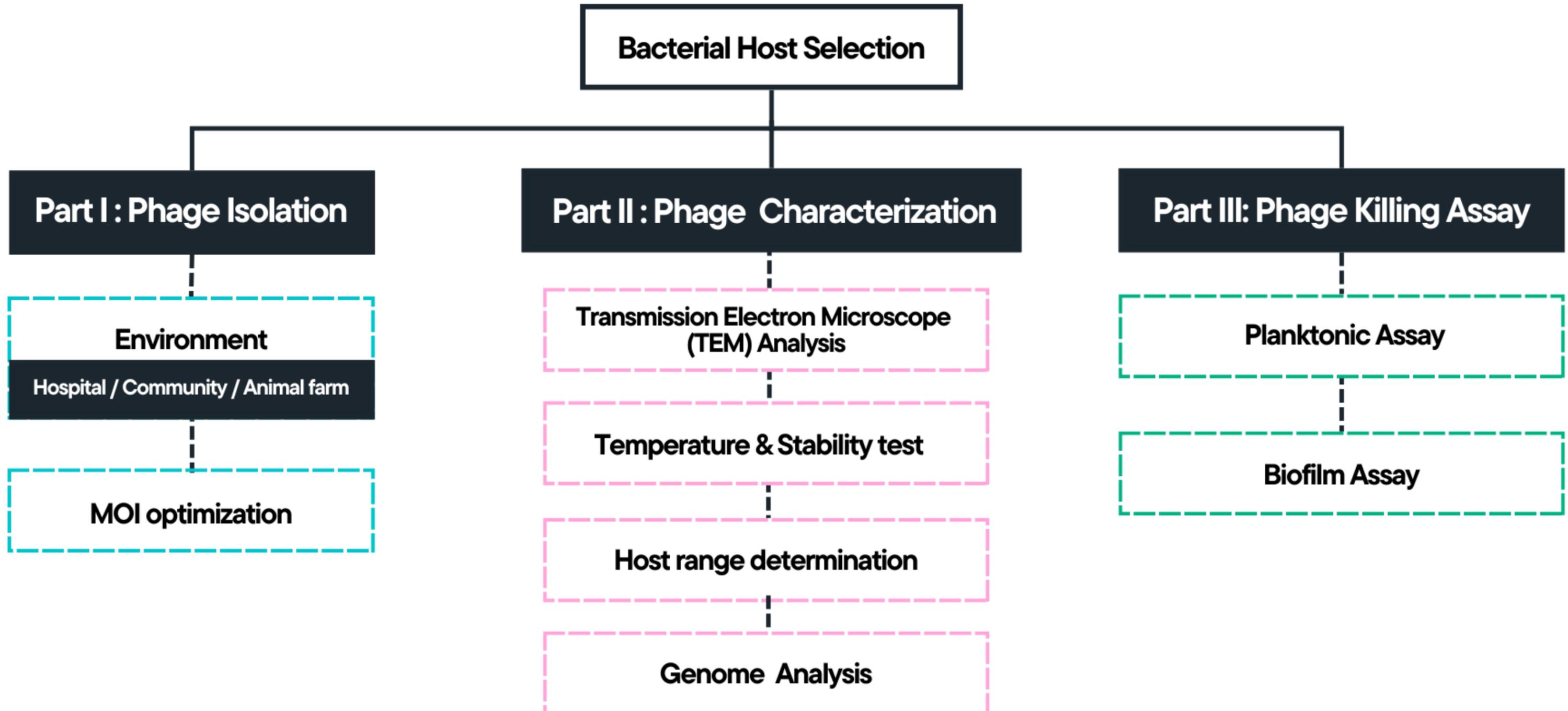


3. To evaluate the *in vitro* efficacy of selected phages in reducing *P. aeruginosa* growth in planktonic cultures and biofilm forms

# Conceptual Framework



# Study Design

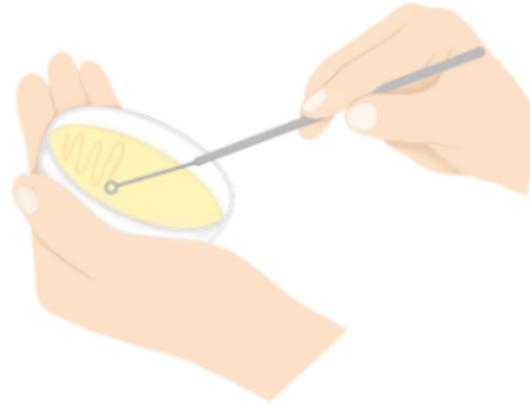


# I: Culture Preparation of *Pseudomonas aeruginosa*

Retrieve from storage



-80°C freezer



Streak on NA agar

## Target strains:

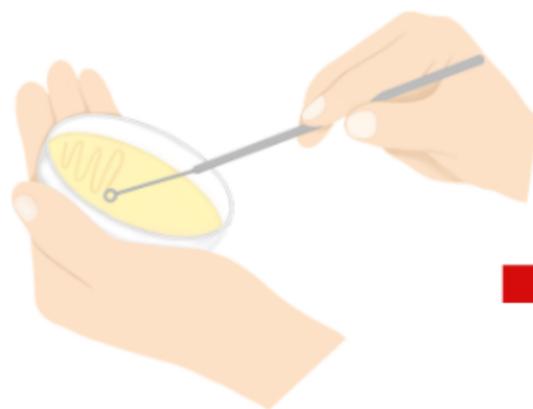
- |              |               |
|--------------|---------------|
| 1) 70-A-14-1 | 6) 70-B-14-1  |
| 2) 70-B-13-1 | 7) 70-B-14-2  |
| 3) 70-B-13-2 | 8) 70-B-14-4  |
| 4) 70-B-13-3 | 9) 70-C-13-1  |
| 5) 70-B-13-4 | 10) 70-D-13-1 |

In strain 1.3 = MDR, 1.4 = XDR

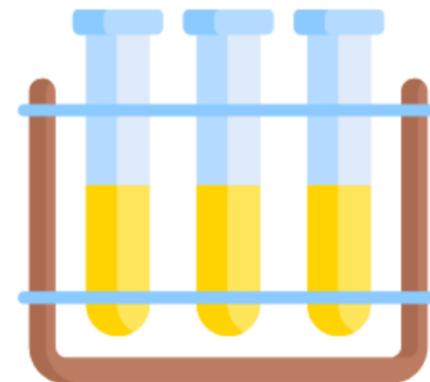
\*MDR: Multidrug resistance

XDR: Extensively drug-resistant

# II: Preparation of Bacterial Host for Host Detection



Collect single colony  
in *P. aeruginosa*



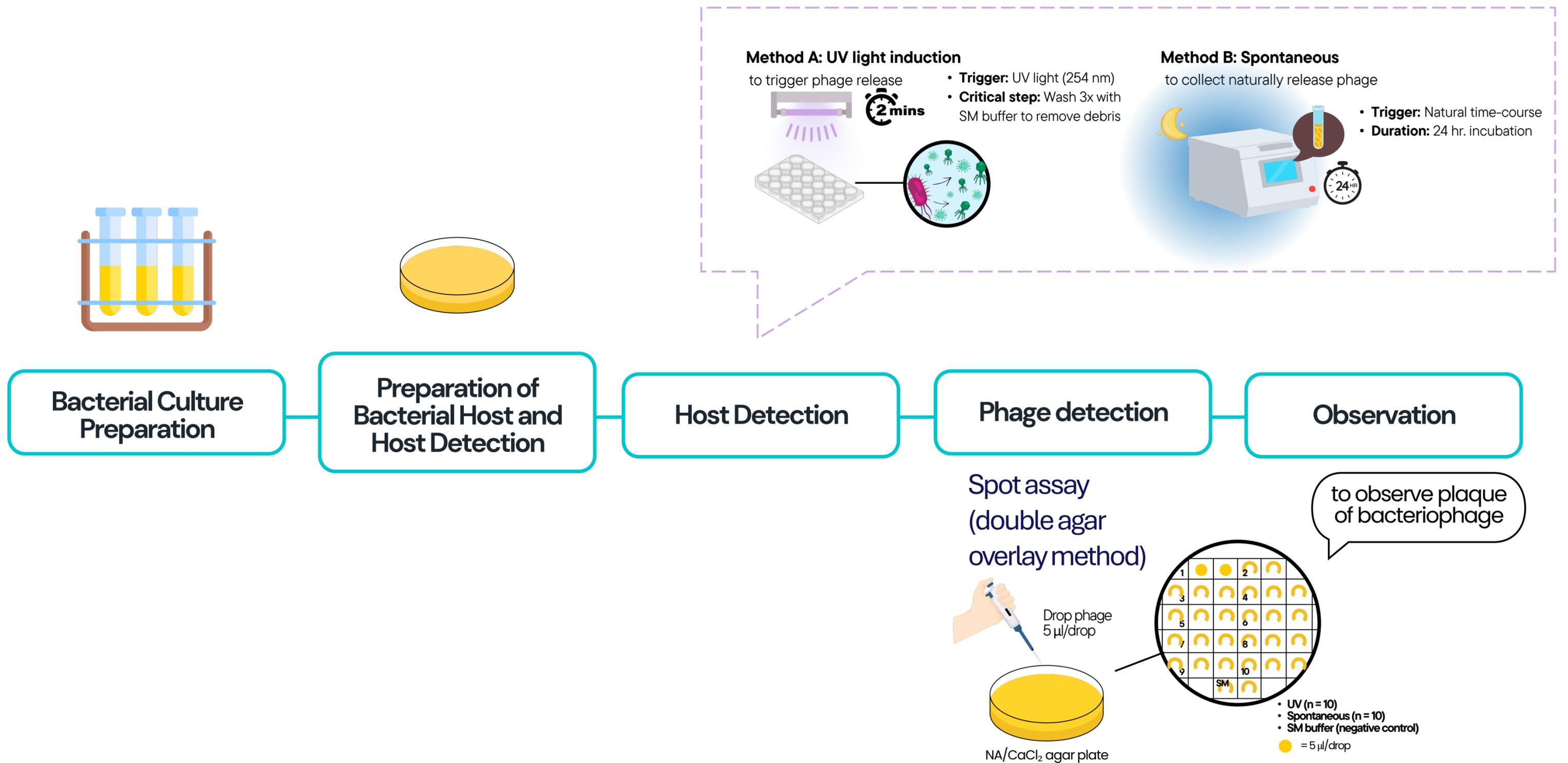
LB broth

Incubate

37 °C, 16-18hr.



# Bacterial Host Selection:



# RESULTS

 Good host for detect bacteriophages  
 Plaque on media

## Method A: UV induction

Phage ID	Host A	Host B	Host C	Host D	Host E	Host F	Host G	Host H	Host I	Host J
1) 70-A-1.4-1										
2) 70-B-1.3-1									+	
3) 70-B-1.3-2										
4) 70-B-1.3-3									+	
5) 70-B-1.3-4										
6) 70-B-1.4-1										
7) 70-B-1.4-2										+
8) 70-B-1.4-4									+	+
9) 70-C-1.3-1										+
10) 70-D-1.3-1									+	

## Method B: Spontaneous (24 hr.)

Phage ID	Host A	Host B	Host C	Host D	Host E	Host F	Host G	Host H	Host I	Host J
1) 70-A-1.4-1		+								+
2) 70-B-1.3-1										
3) 70-B-1.3-2										
4) 70-B-1.3-3		+							+	+
5) 70-B-1.3-4					ND					
6) 70-B-1.4-1										
7) 70-B-1.4-2	+	+							+	+
8) 70-B-1.4-4		+							+	
9) 70-C-1.3-1		+								
10) 70-D-1.3-1	+	+						+	+	

Table 1: Representative Spot Assay results. Clear zones (plaques) indicate susceptibility, while the SM Buffer spot (negative control) remains non-transparent

# RESULTS

 Good host for detect bacteriophages  
 Plaque on media

## Method A: UV induction

<i>P. aeruginosa</i> (UV)	Host A	Host B	Host C	Host D	Host E	Host F	Host G	Host H	Host I	Host J
1) 70-A-1.4-1										
2) 70-B-1.3-1									+	
3) 70-B-1.3-2										
4) 70-B-1.3-3										
5) 70-B-1.3-4										
6) 70-B-1.4-1										
7) 70-B-1.4-2										+
8) 70-B-1.4-4									+	+
9) 70-C-1.3-1										+
10) 70-D-1.3-1									+	

Based on the results, **Bacteria 1, 3, 5, and 6** were not produced phage by UV induction.

## Method B: Spontaneous (24 hr.)

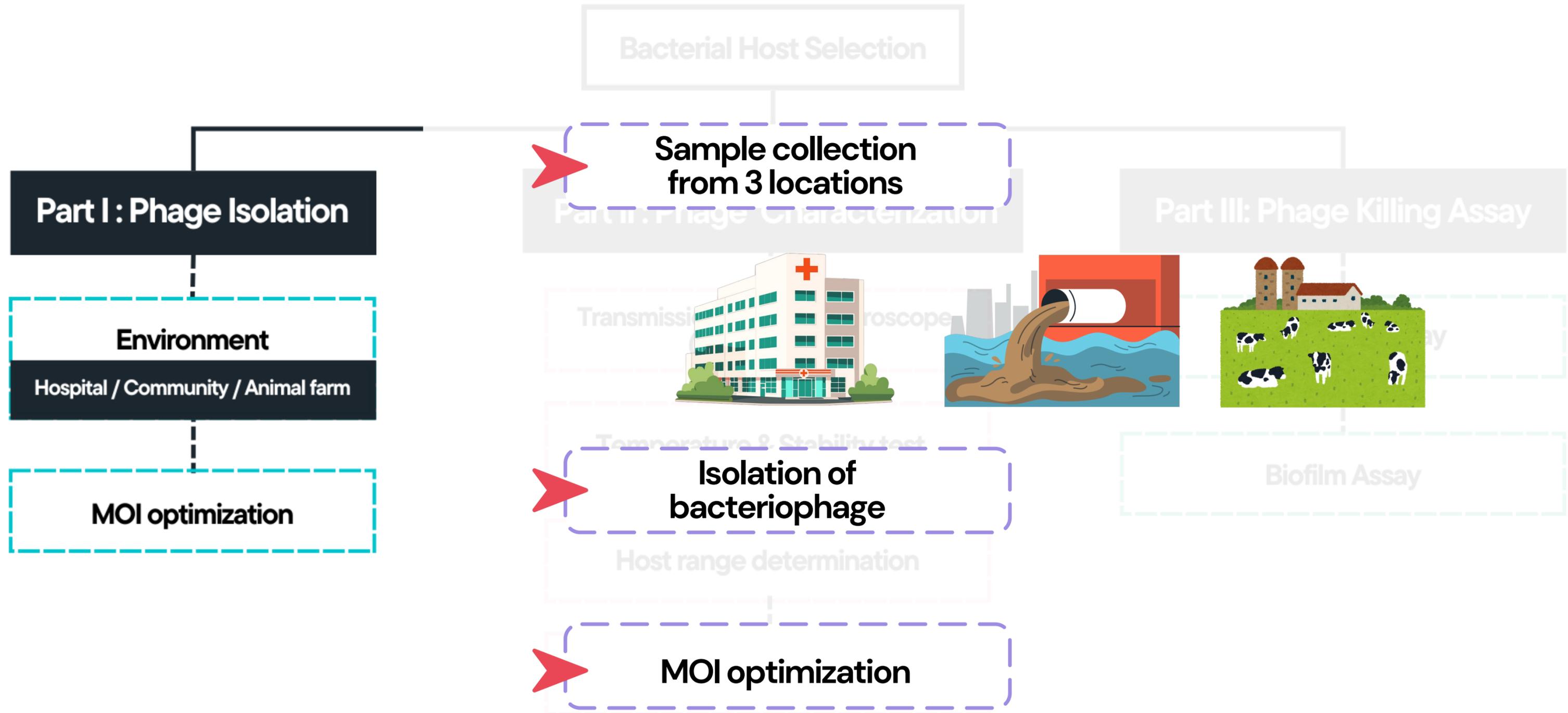
<i>P. aeruginosa</i> (S)	Host A	Host B	Host C	Host D	Host E	Host F	Host G	Host H	Host I	Host J
1) 70-A-1.4-1		+								+
2) 70-B-1.3-1										
3) 70-B-1.3-2										
4) 70-B-1.3-3		+							+	+
5) 70-B-1.3-4					ND					
6) 70-B-1.4-1										
7) 70-B-1.4-2	+	+								
8) 70-B-1.4-4		+								
9) 70-C-1.3-1		+								
10) 70-D-1.3-1	+	+						+	+	

Based on the results, **Bacteria 2, 3, 5 and 6** were not spontaneous produced phage.

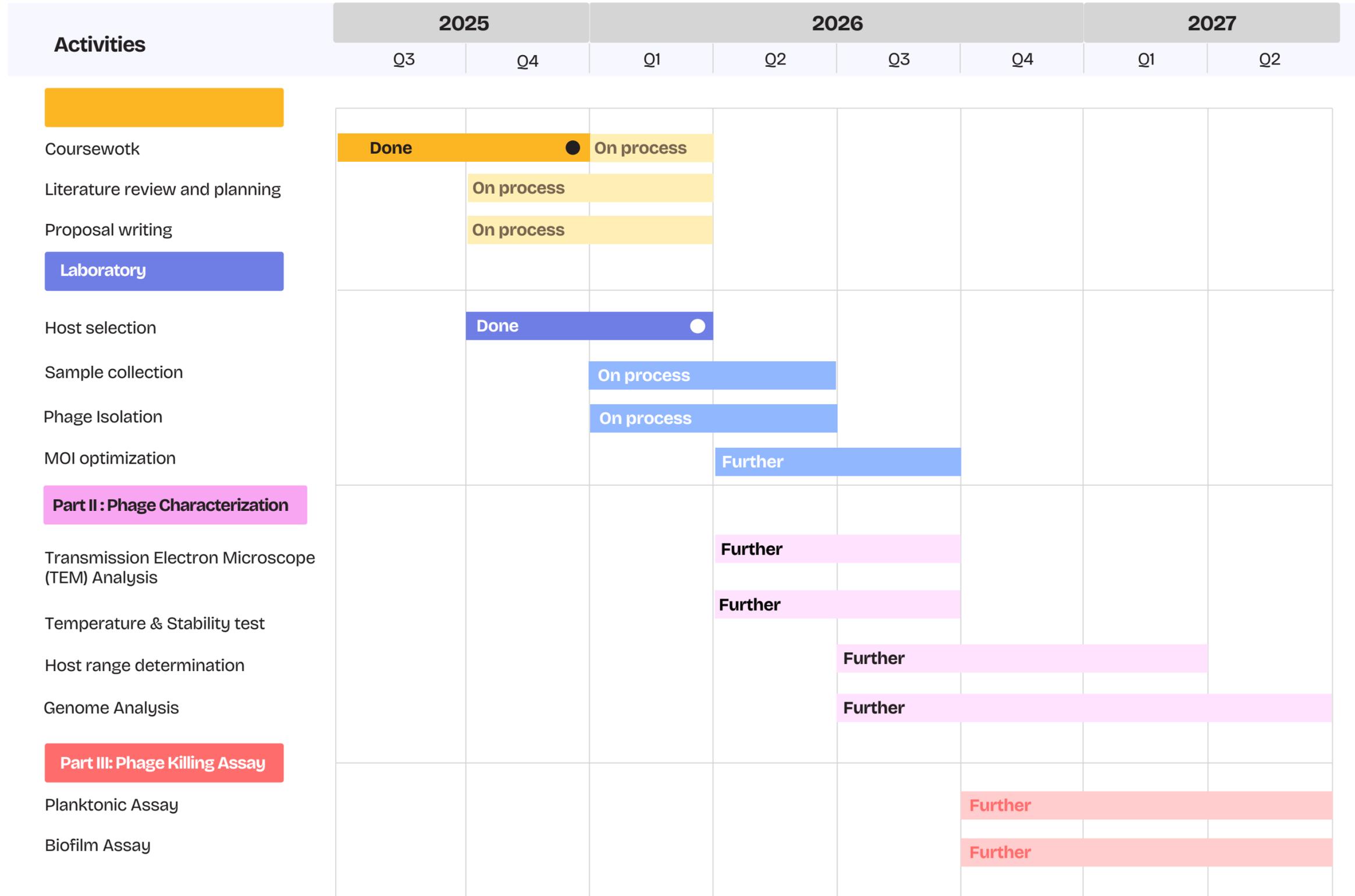
Table 1: Representative Spot Assay results. Clear zones (plaques) indicate susceptibility, while the SM Buffer spot (negative control) remains non-transparent



# Further work



# THESIS PLAN



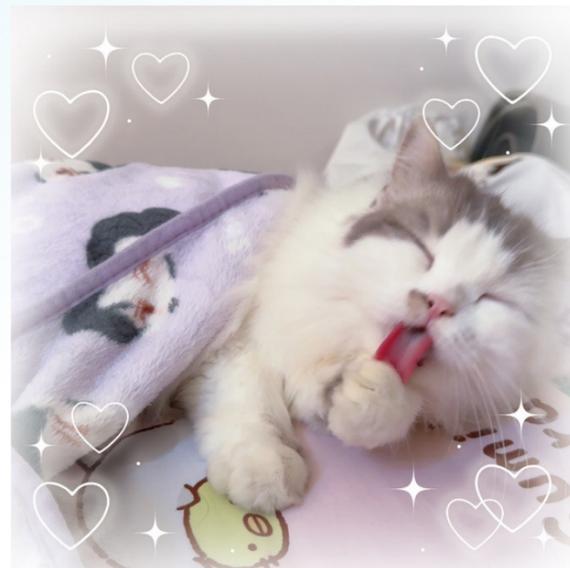
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Emotional support



**Tenlee**  
Emotional support

Thank you for your  
kind attention

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Q&A or Suggestion