



Bacteriophage-vancomycin combination against vancomycin intermediate *Staphylococcus aureus*

3rd THESIS PROGRESSION

The effect of phage and vancomycin
combination against *S. aureus in vitro*

Presented by:

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Advisor : Asst. Prof. Dr. Umaporn Yordpratum

Co-advisor: Dr. Pratsanee Hiangraj



Date: March 11, 2026

INTRODUCTION

Diseases

- **Superficial skin lesions**
: impetigo & cellulitis
- **Life-threatening diseases**
: osteomyelitis, bacteraemia, & endocarditis

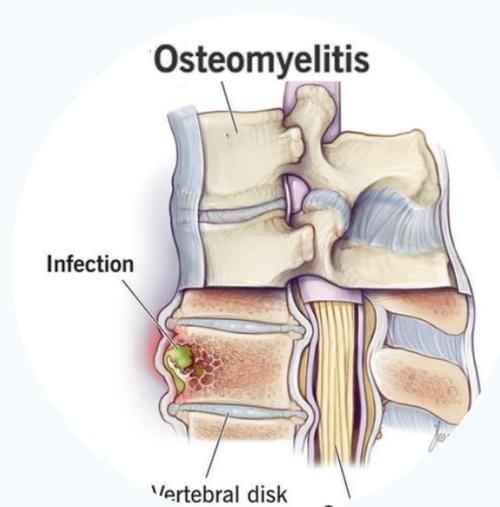
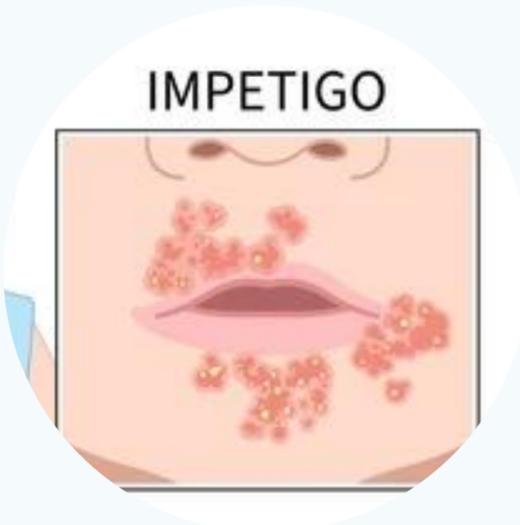
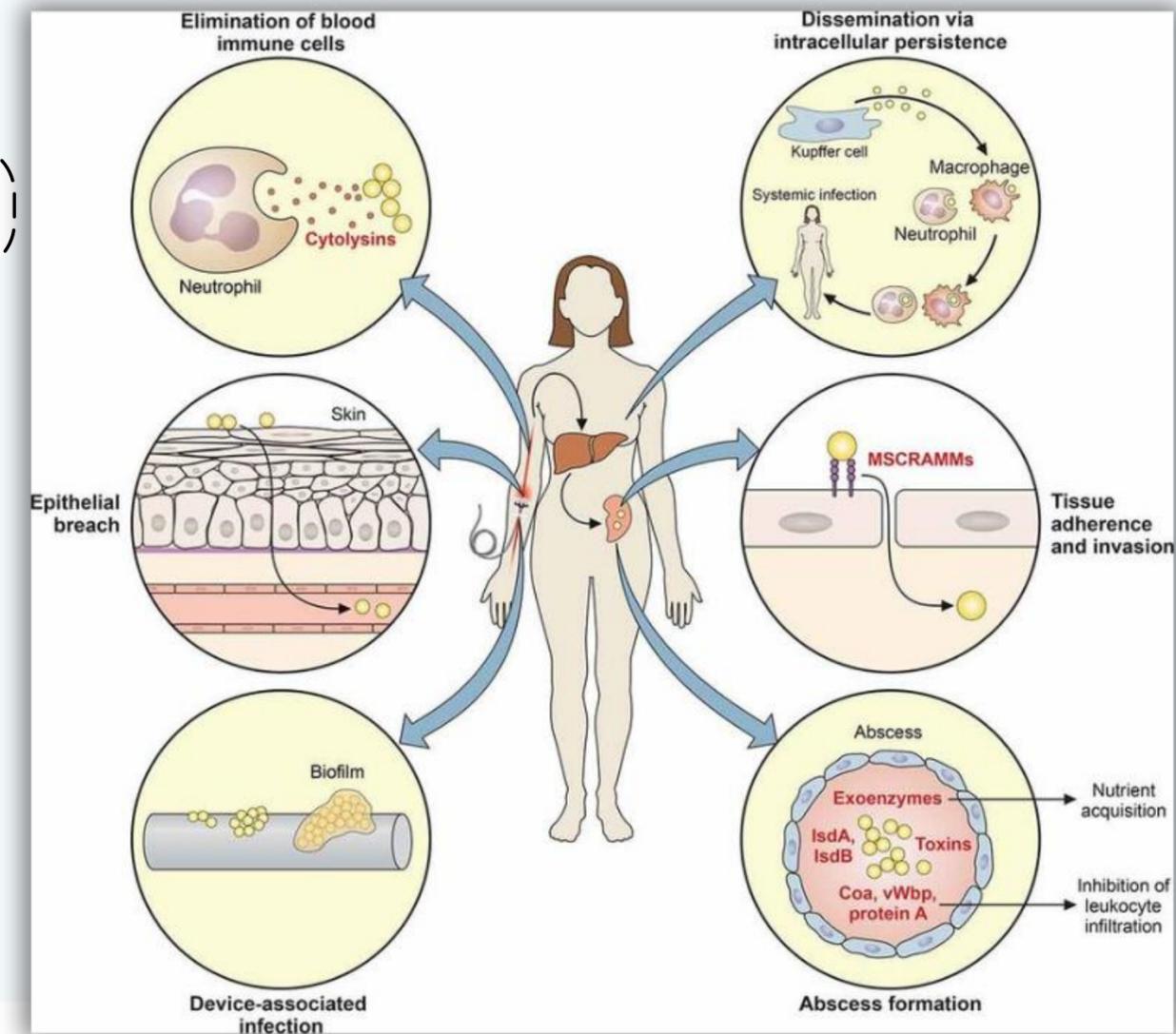
Bacteriology

- Gram positive cocci
- commensal organism
- commonly found on skin & mucous membranes

Staphylococcus aureus



Stages of *S. aureus* systemic infection



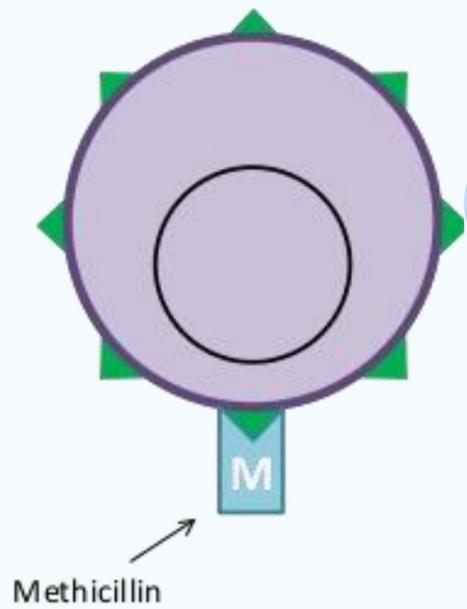
Cleveland Clinic, 2024

(Cheung et al., 2021)

INTRODUCTION



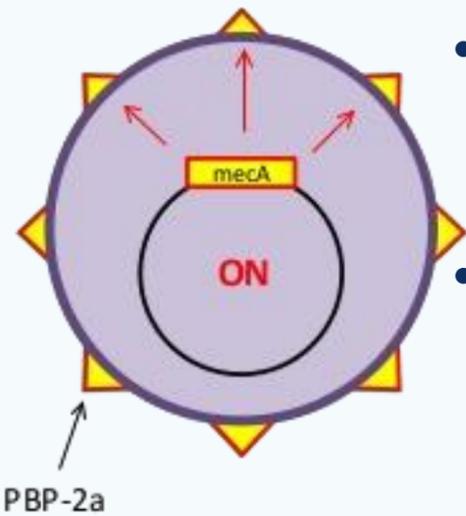
Antibiotics Treatment



MSSA treatment

- **β-Lactam** (Methicillin, Flucloxacillin) —| Cell wall synthesis
- **Macrolides** (Erythromycin) —| Protein synthesis

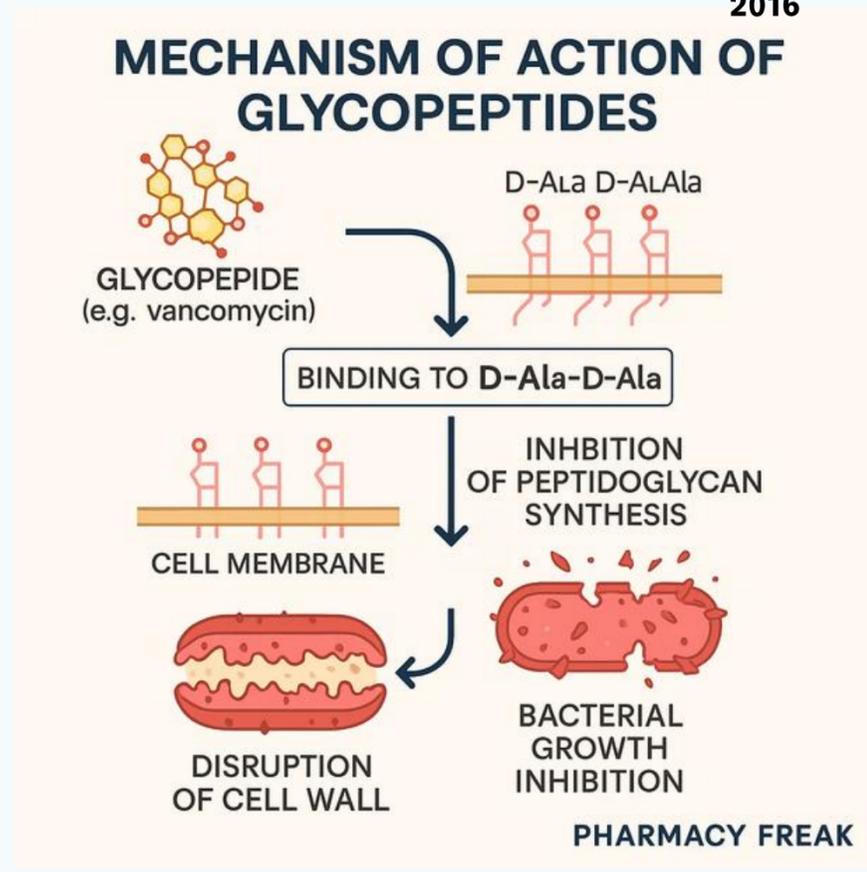
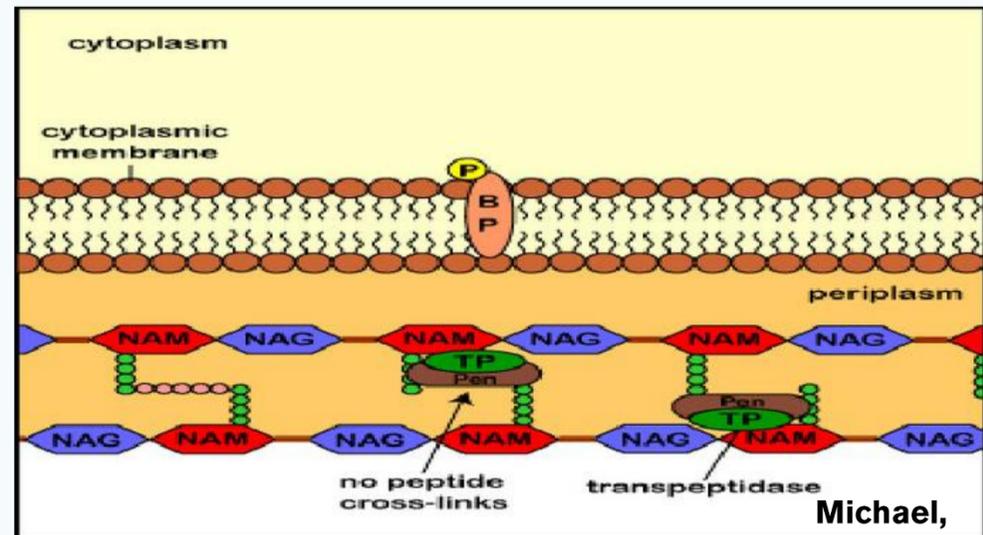
MRSA treatment



- HA-MRSA (multi-antibiotics resistance)
- CA-MRSA (non-multiresistant)
- **Glycopeptides** (Vancomycin) —| Cell wall synthesis
- **Lincosamides** (Clindamycin) —| Protein synthesis

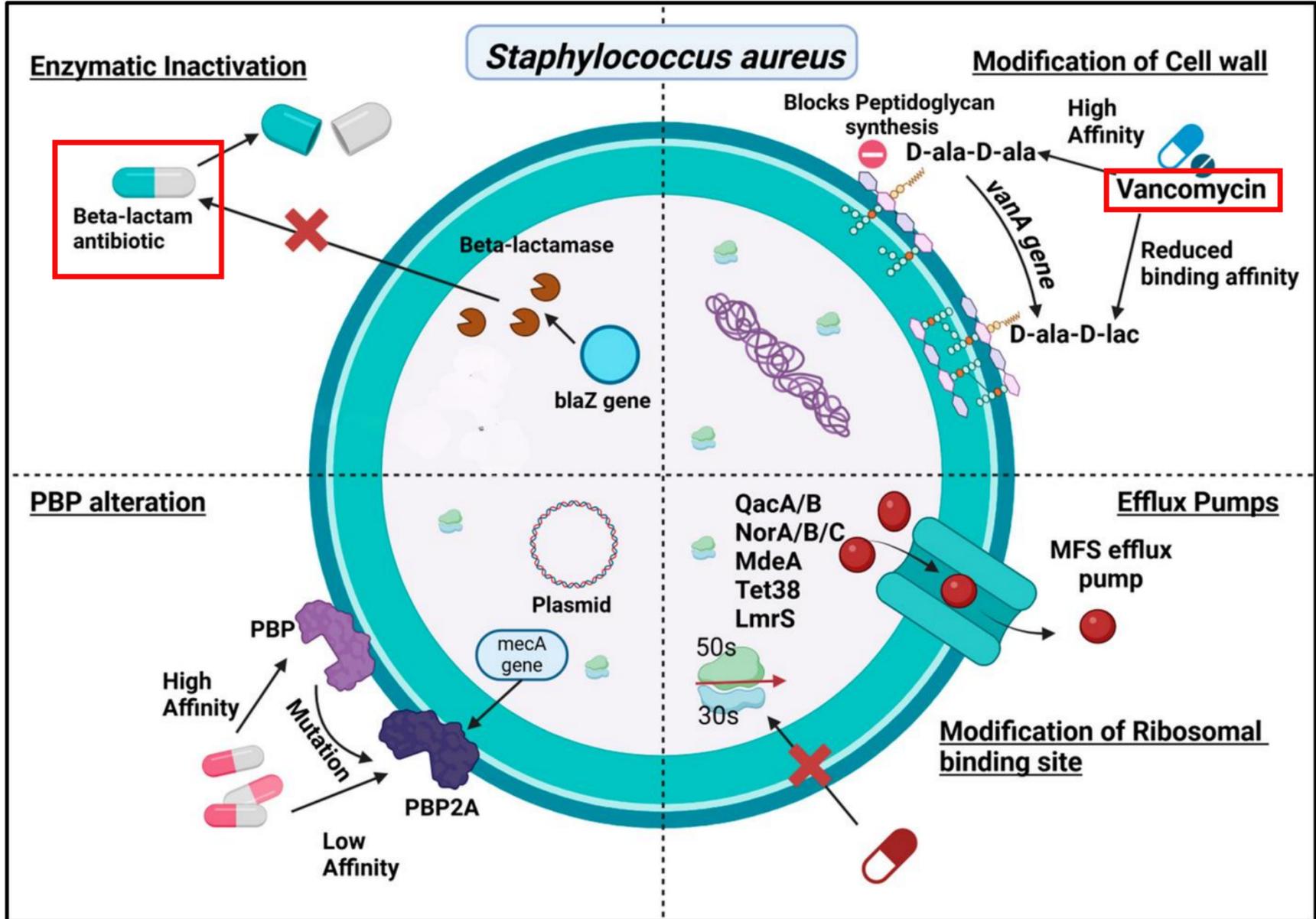
Use vancomycin as first-line treatment of MRSA

Mechanism of action of beta-lactamases

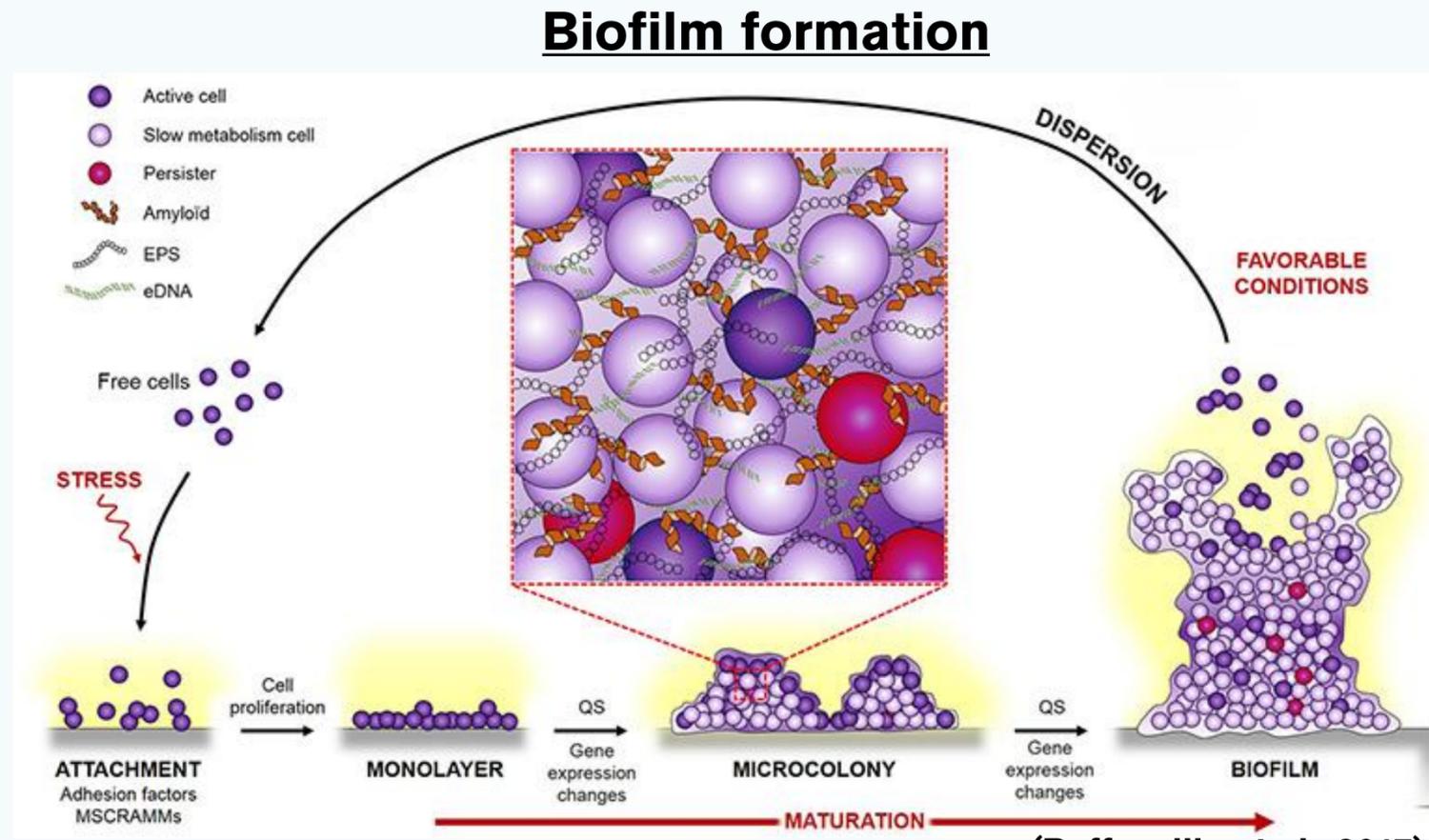


INTRODUCTION

Antibiotics resistance mechanisms



(Rajput et al., 2024)



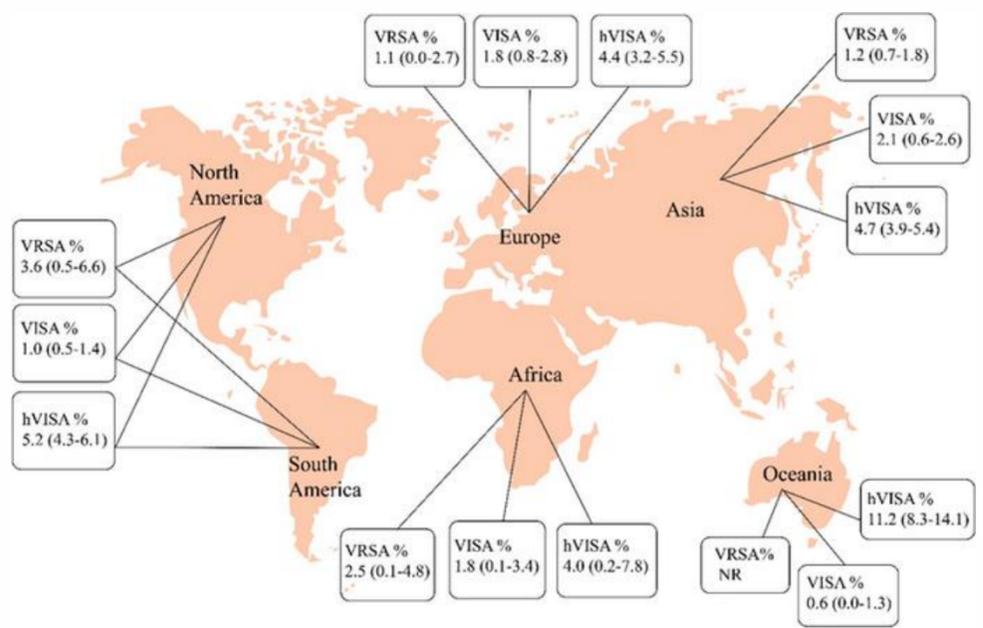
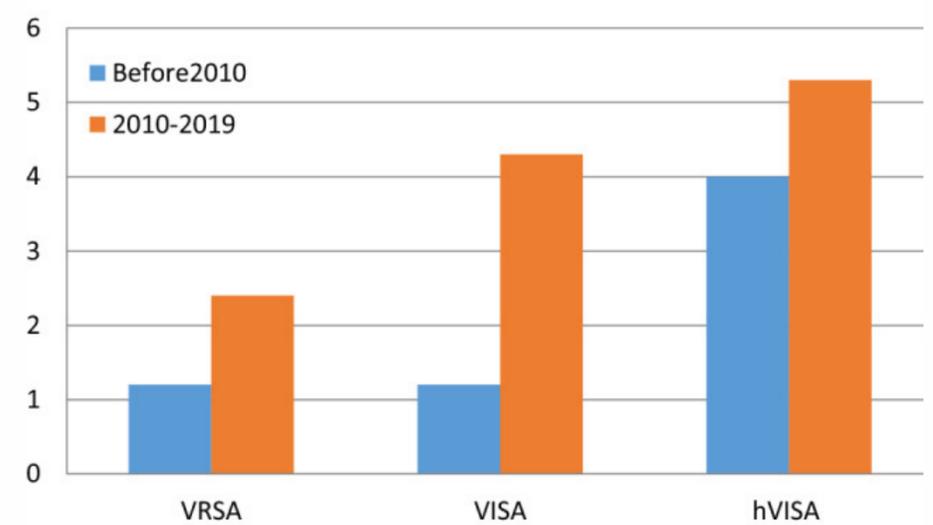
(Reffuveille et al., 2017)

INTRODUCTION

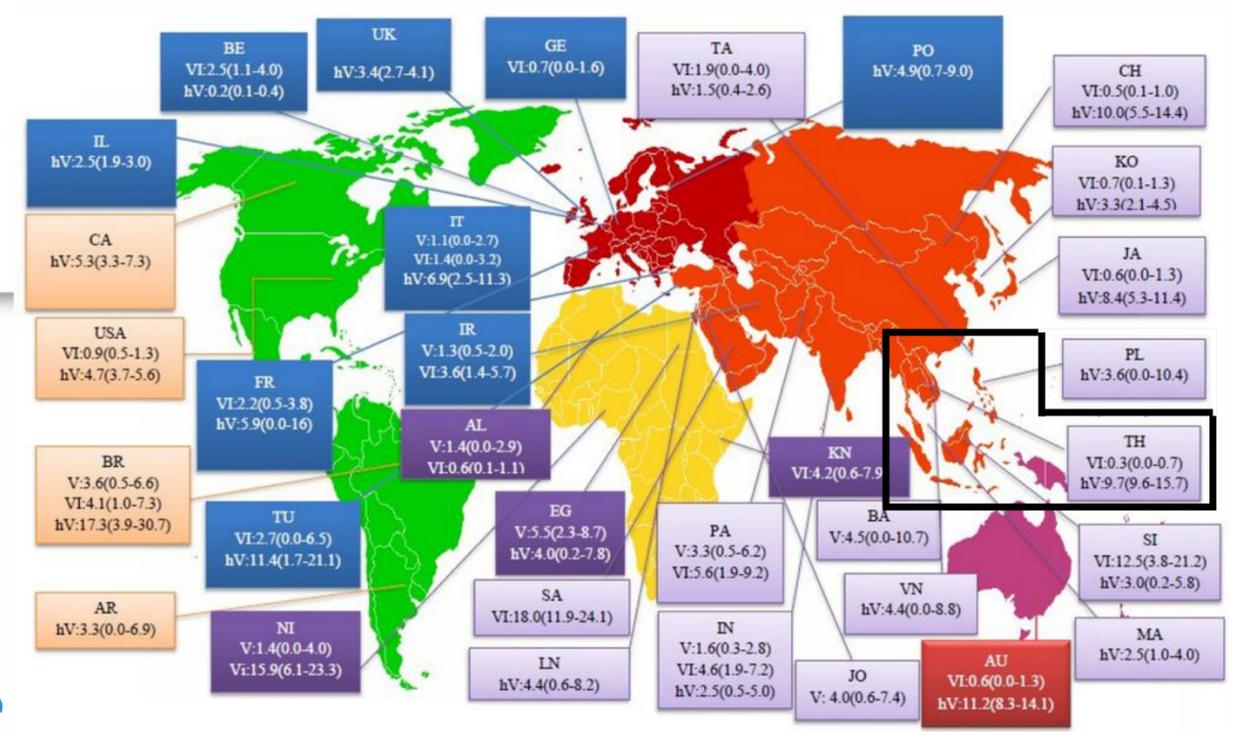
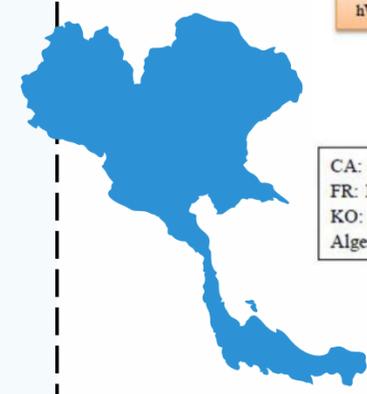
The prevalence of VRSA, VISA & hVISA



Percentage of VRSA, VISA and hVISA during two periods



OPEN Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: a systematic review and meta-analysis
 Aref Shariati^{1,2}, Masoud Dadashi^{3,4,5}, Majid Taati Moghadam^{1,2}, Alex van Belkum⁵,
Shariat et al., 2020



CA: Canada, USA: United States of America, BR: Brazil, AR: Argentina, UK: United Kingdom, BE: Belgium, GE: Germany, PO: Poland, IT: Italy, FR: France, TU: Turkey, IL: Ireland, IR: Iran, IN: India, TA: Taiwan, TH: Thailand, PL: Philippines, MA: Malaysia, VN: Vietnam, CH: China, KO: Korea, JA: Japan, BA: Bangladesh, SI: Singapore, PA: Pakistan, LN: Lebanon, SA: Saudi-Arabia, JO: Jordan, EG: Egypt, NI: Nigeria, AL: Algeria, KN: Kenya, AU: Australia.

Thailand
VISA: a prevalence of 0.3%
hVISA: a prevalence of 9.7%

Global, 2020
 The highest prevalent of VRSA (3.6%), hVISA (5.2%) was encountered in the USA while **VISA** (2.1%) was more prevalent in **Asia**.

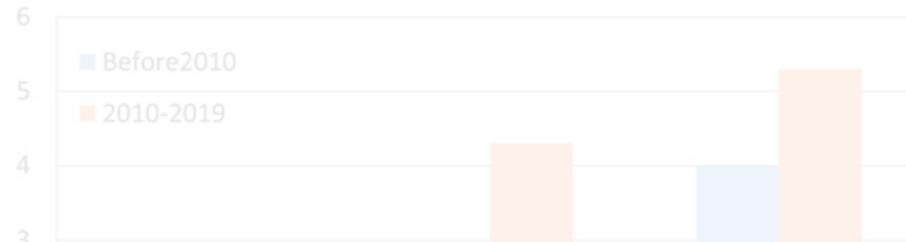
- VRSA= Vancomycin-resistant *S. aureus* (MIC ≥ 16 µg/ml)
- VISA= Vancomycin-intermediate *S. aureus* (MIC 4-8 µg/ml)
- hVISA= heterogeneously VISA (MIC ≤ 2 µg/ml, some = MIC of VISA)

INTRODUCTION



The prevalence of VRSA, VISA & hVISA

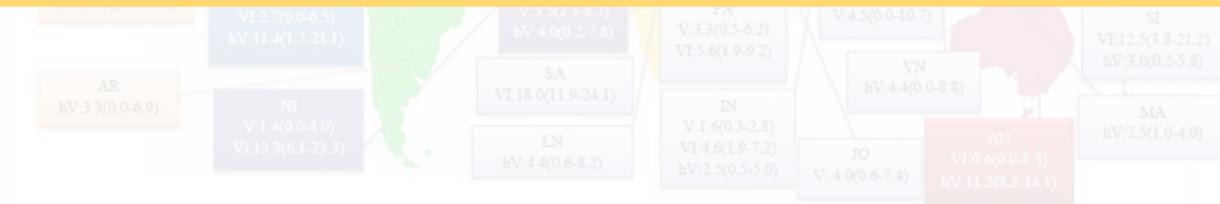
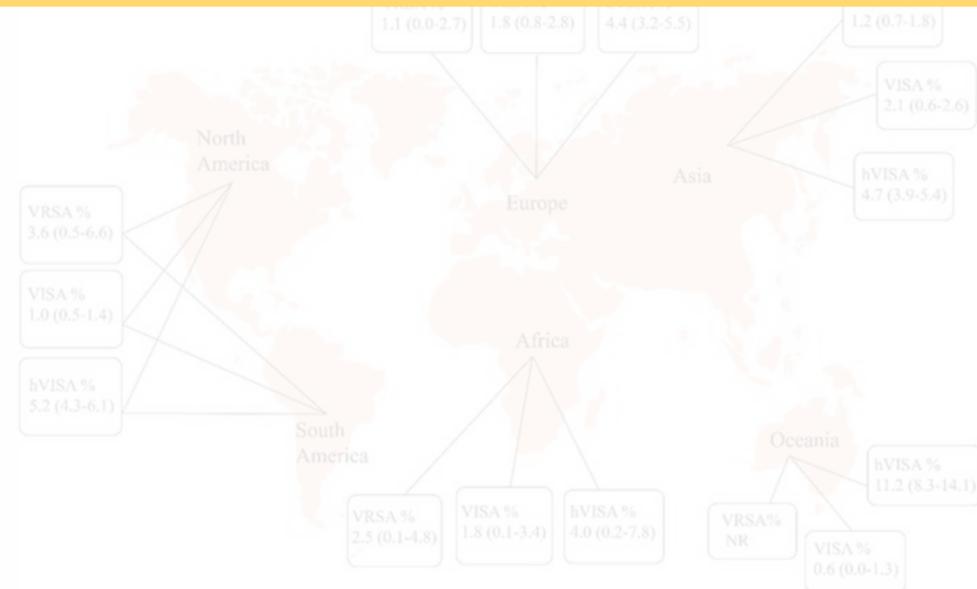
Percentage of VRSA, VISA and hVISA during two periods



OPEN Global prevalence and distribution of vancomycin resistant, vancomycin intermediate and heterogeneously vancomycin intermediate *Staphylococcus aureus* clinical isolates: a systematic review



These prevalences of antibiotic-resistant *S. aureus* highlight the urgent need for alternative therapies



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Thailand, 2020
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Global, 2010-2019

The highest prevalence of VRSA (3.6%), hVISA (5.2%) was encountered in the USA while **VISA** (2.1%) was more prevalent in **Asia**.

Bacteriophage Therapy: A Promising Alternative



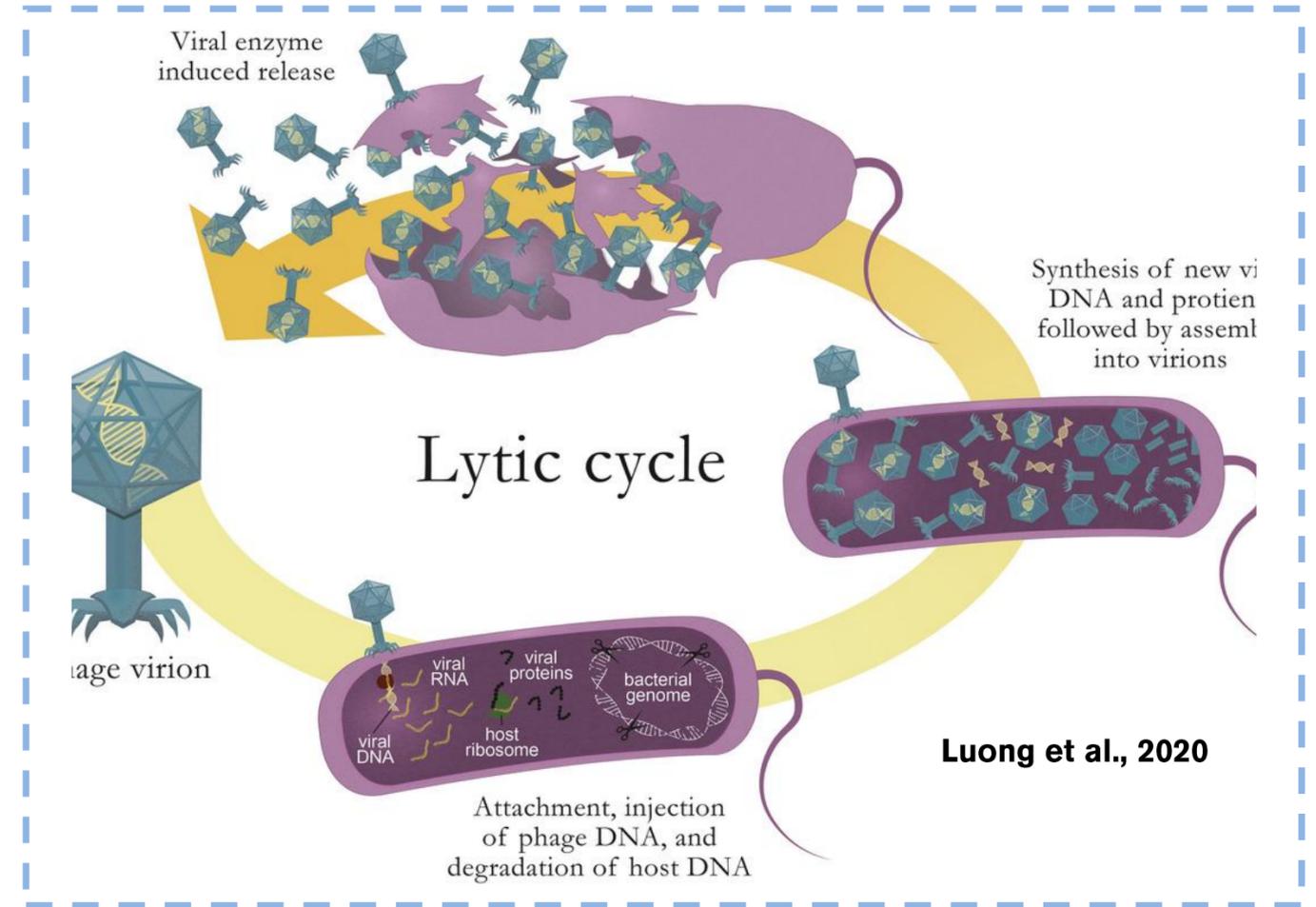
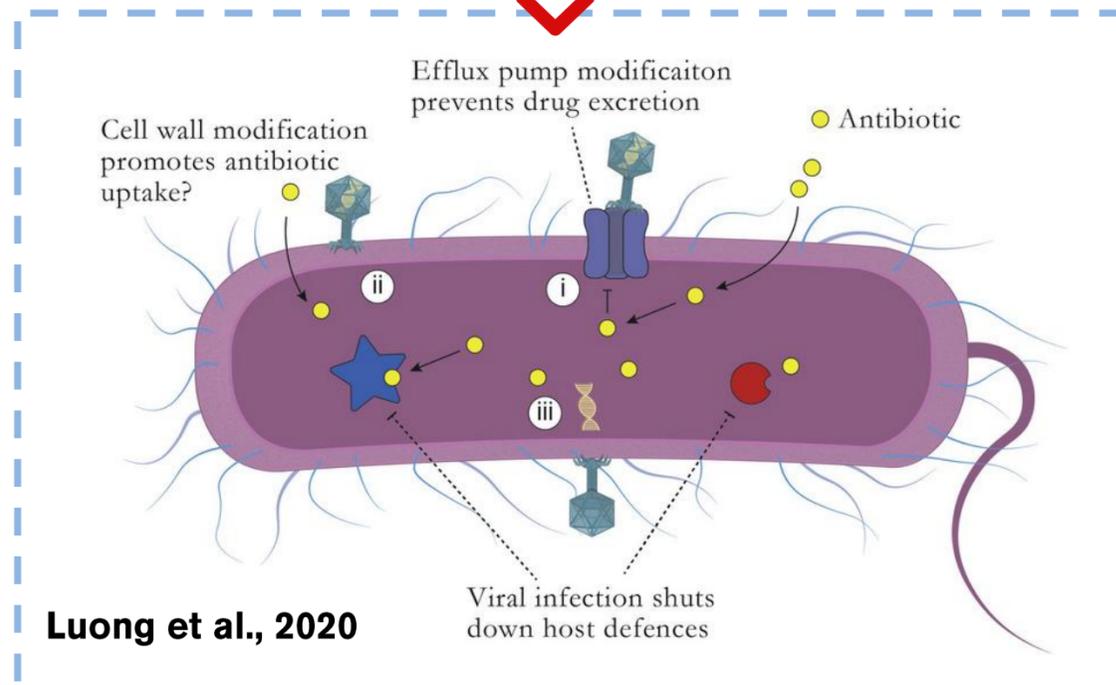
Phages have specifically infected & lysed bacteria.



Self-replicating, highly specific, & potentially safer than antibiotics



Potential to combat biofilms & overcome antibiotics resistance (as potential antibiotic adjuvants)



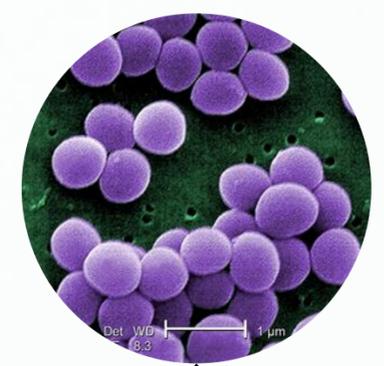
Review of phage-antibiotic combination therapy against antibiotic-resistant *S. aureus*

Phage-Antibiotic	Target bacteria	Model	Result	Reference
Phage SA11 + ciprofloxacin	<i>S. aureus</i> (biofilm)	<i>in vitro</i>	Combination therapy reduced biofilm formation & prevented resistant mutants.	(Jo, Kim, et al., 2016)
Phage Remus + vancomycin	MRSA	<i>ex vivo</i> & <i>in vivo</i>	Remus alone significantly reduced viable MRSA in aggregates & combination therapy had synergistic effect.	(Taha et al., 2023b)
Phage K (local) + systemic vancomycin	MRSA	<i>in vitro</i>	Local phage K plus systemic vancomycin produced a significantly greater reduction of MRSA on prosthesis surfaces	(Herren et al., 2025)
Multi-route phage therapy + antibiotics	PVL-producing MRSA	in human	Phage therapy given via multiple routes (e.g. topical, perhaps systemic) in combination with antibiotics led to full recovery	(Van Nieuwenhuysse et al., 2024)

Rationale: lack of published studies that specifically test phage-antibiotic combination therapy against VISA conducted in Thailand

PROBLEM

VISA



Vancomycin Resistant



Failure to treat

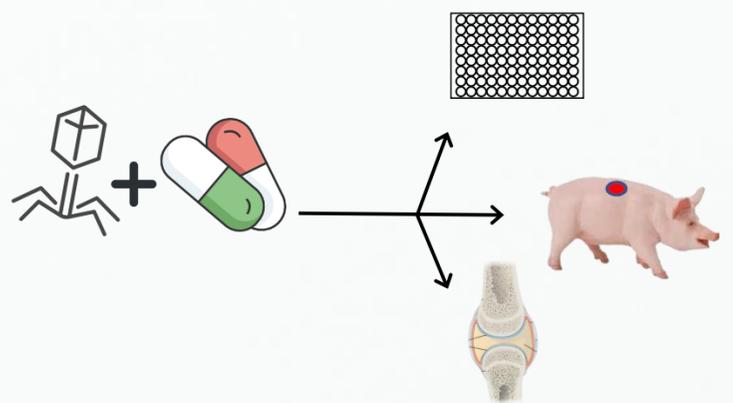
PROCESS



S. aureus phage



Phage characterization



Effect of phage+vancomycin determination *in vitro* & *ex vivo*

OUTPUT

- Killing activity of phage + vancomycin in planktonic and biofilm.

OUTCOME

- A new alternative therapeutic method for vancomycin-intermediate *S. aureus* (VISA) infection

IMPACT

- Phage can be alternative treatment for antibiotic resistant *S. aureus* infection
- Phage therapy can reduce antibiotic dose for treatment

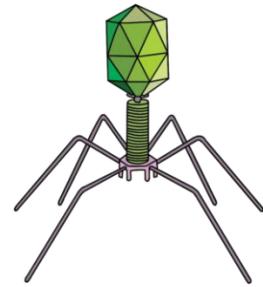
HYPOTHESIS

The isolated *S. aureus*-specific bacteriophage(s) show **lytic morphology & a genome lacking antibiotic-resistant or virulence factor genes** while encoding functions required for infection and bacterial lysis, consistent with suitability for therapeutic use.

The combination of *S. aureus* phage and vancomycin **reduces vancomycin-intermediate *S. aureus* (VISA) *in vitro***, both in planktonic cultures and biofilms, and *ex vivo* in a porcine skin and synovial fluid model.

OBJECTIVES

1. To **characterize the structure and genome** of phages specific to *S. aureus*
2. To evaluate the effect of phage and vancomycin against VISA in both **planktonic cultures** and **biofilms**
3. To evaluate the effect of phage and vancomycin against VISA in **porcine skin**
4. To evaluate the effect of phage and vancomycin against VISA in **human synovial fluid**



Phage Isolation
(Previous Study)

Phage propagation & Titration

Part I: Phage characterization

Transmission Electron Microscopy (TEM) Analysis

Host range determination

Genome Analysis/DNA extraction

Hemolytic activity

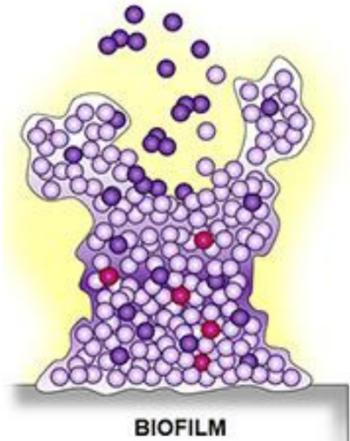
The optimal MOI for phage propagation
Broth vs Soft agar method

Part II: Effect of phage+vancomycin determination *in vitro*

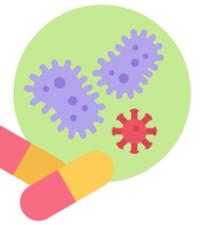
The effect of vancomycin on *S. aureus* phage
S. aureus phage + Vancomycin

Planktonic Assay
Bacterial count (OD600nm) (CFU/ml)

Biofilm Assay
Crystal Violet Staining (Total Biomass)



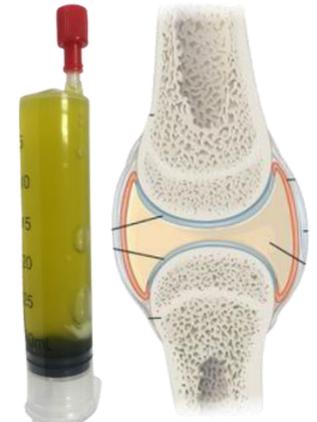
Part III: Effect of phage+vancomycin determination *ex vivo*

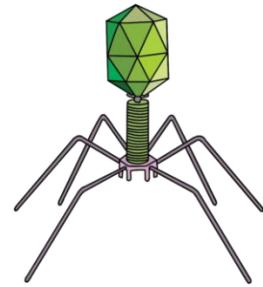


Porcine skin
Bacterial count (CFU/ml)



Human synovial fluid
Bacterial count (CFU/ml)





Phage Isolation
(Previous Study)

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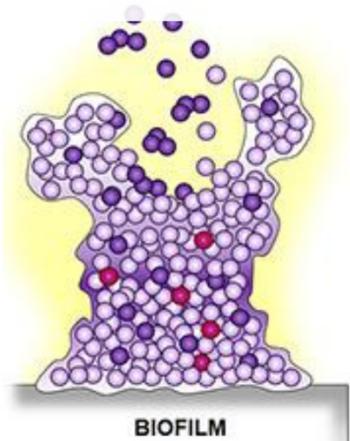
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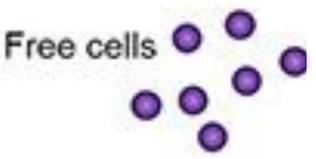
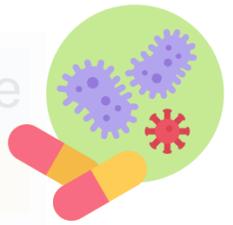
The effect of vancomycin on *S. aureus* phage
S. aureus phage + Vancomycin

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Crystal Violet Staining (Total Biomass)



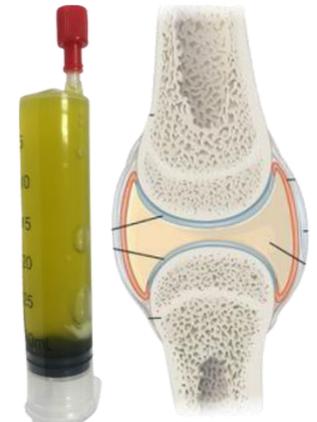
Part III: Effect of phage+vancomycin determination *ex vivo*



Porcine skin
Bacterial count (CFU/ml)



Human synovial fluid
Bacterial count (CFU/ml)



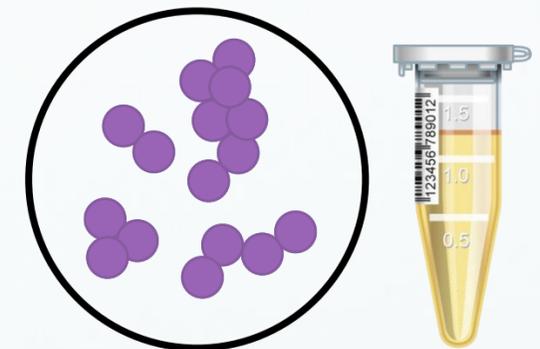
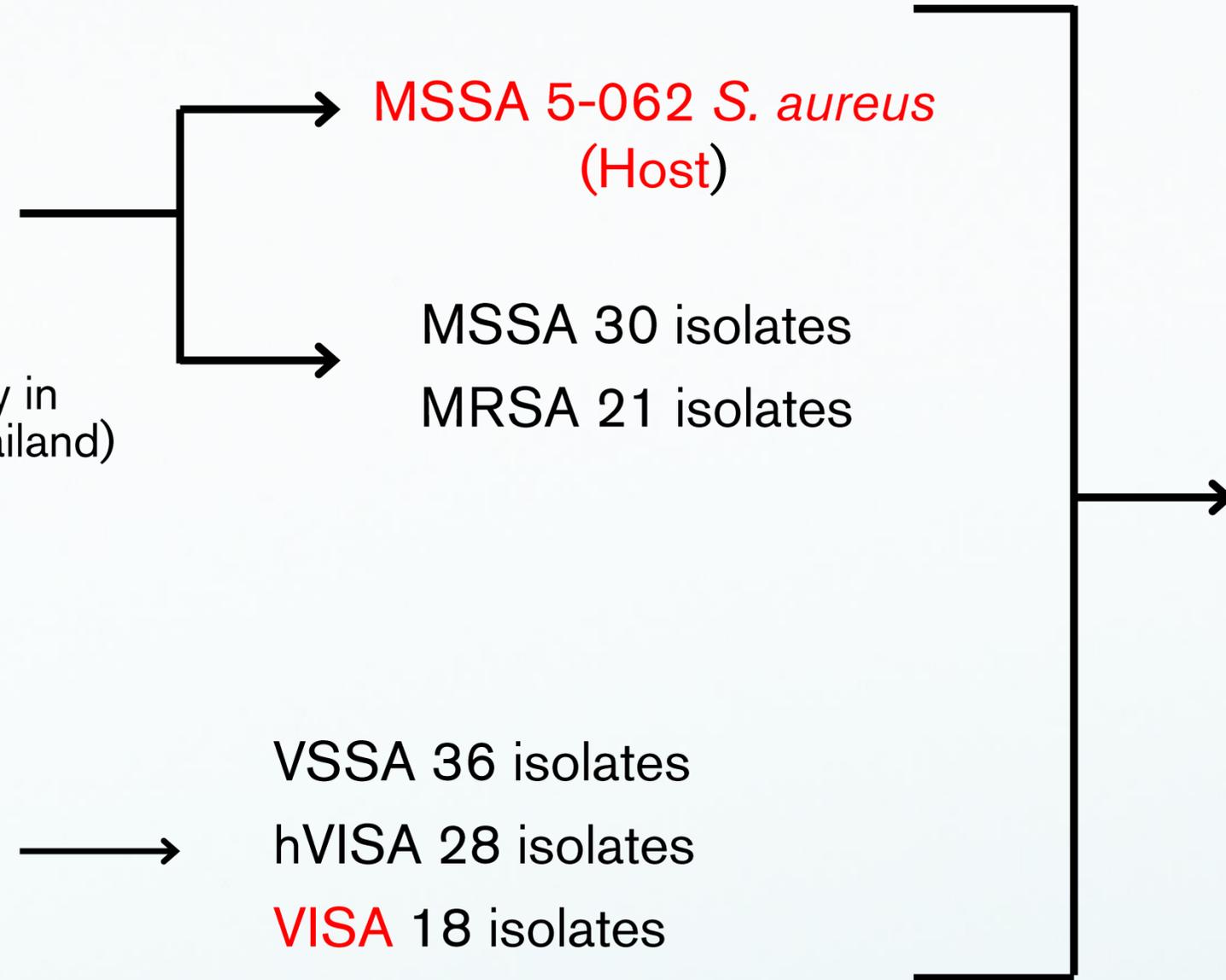
Bacterial isolation



The clinical microbiology laboratory in Srinagarind Hospital (Khon Kaen, Thailand)



Asst. Prof. Auttawit Sirichoat

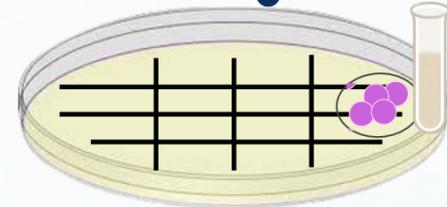
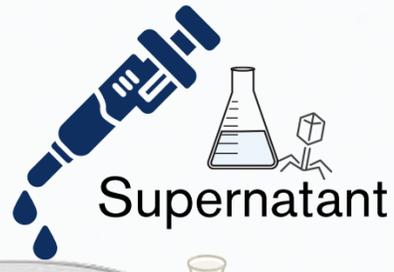


All 134 *S. aureus* strains are stored in 20% glycerol at -80°C .



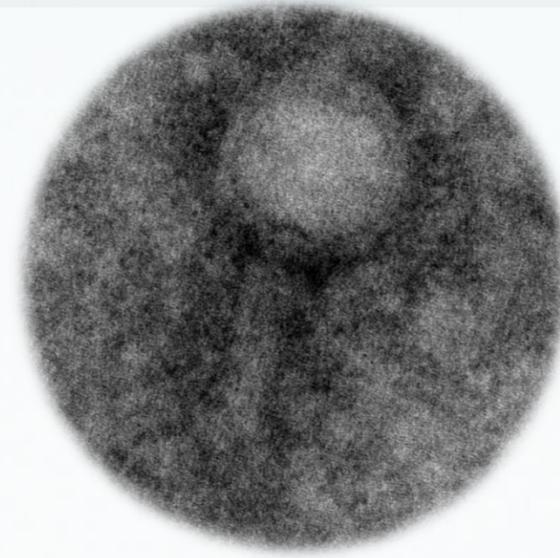
A poultry farm
(AG farms, KhonKean University)

Spot assay

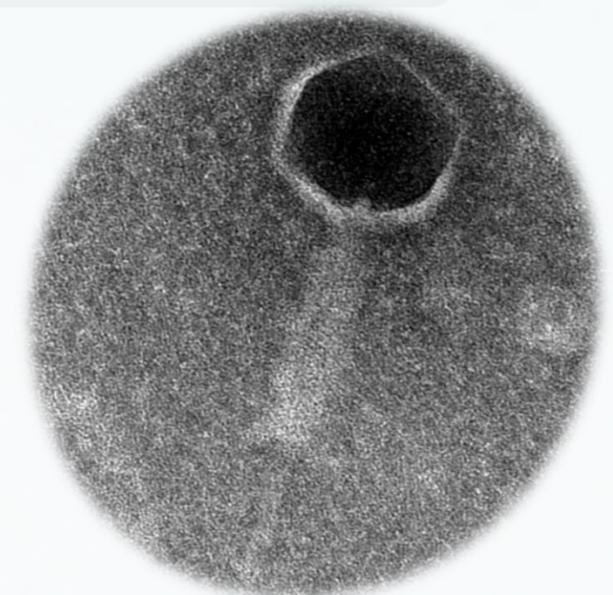


NA with CaCl_2

Isolation

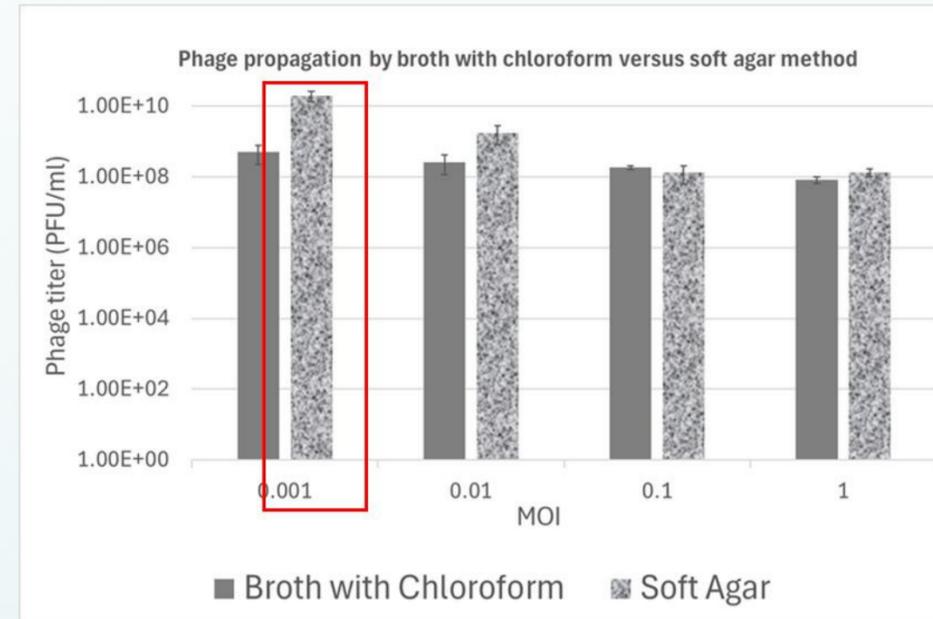


Phage W31



Phage W32

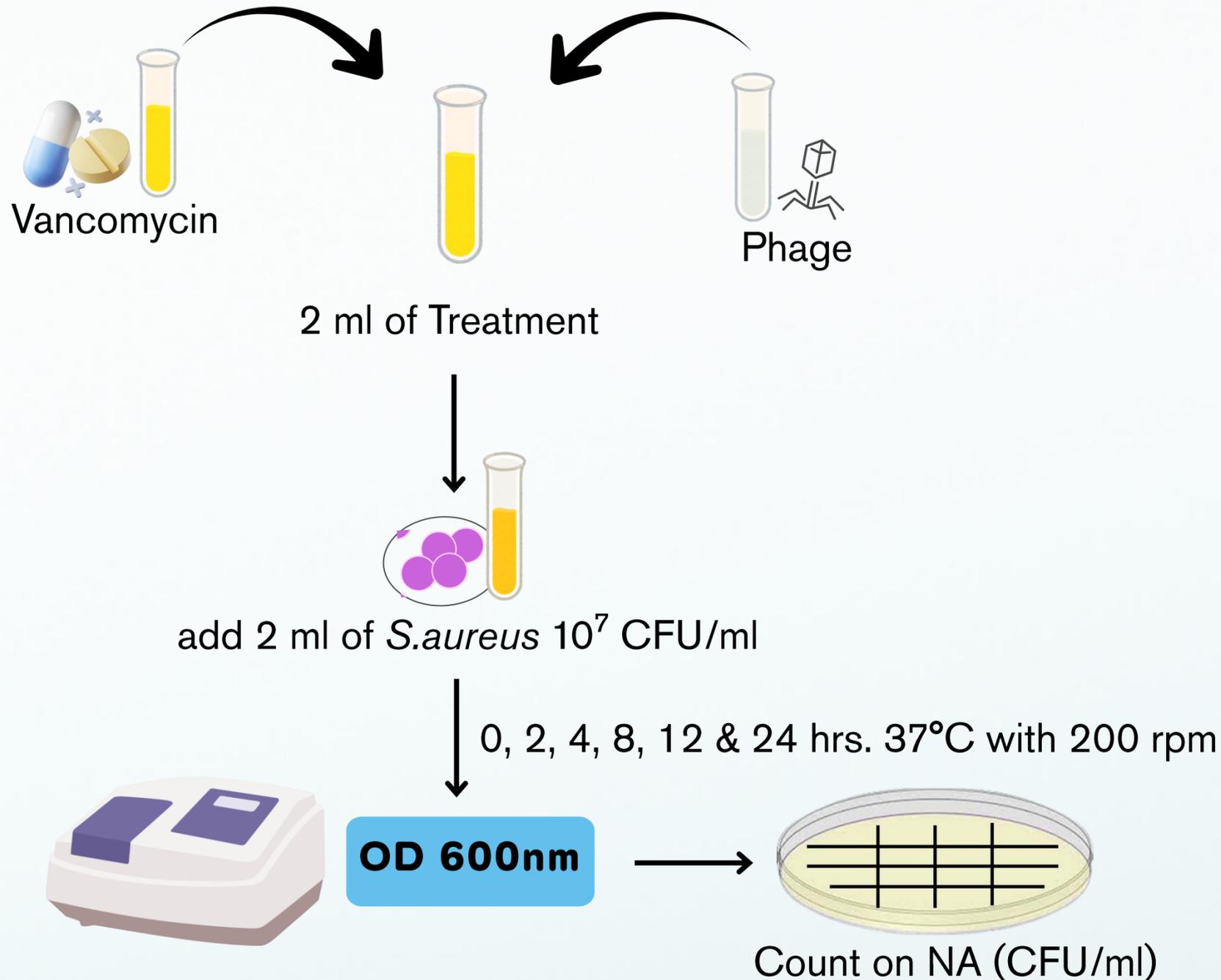
Broth(Chloroform) vs Soft agar method



The optimal MOI for phage W31 & W32 propagation is 0.001 by the Soft agar method

Objective: To evaluate the efficacy of phage and vancomycin against *S. aureus in vitro*

Planktonic killing activity



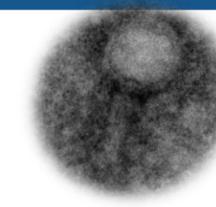
Phage (ϕ): W31 & W32

***S.aureus*:** MSSA 5-062 & VISA 17-97

2 mL of (15 condition/*S.aureus*)

- **Control:** SM buffer
- W31 MOI 1 ($\phi 10^7$ PFU/ml)
- W31 MOI 0.1 ($\phi 10^6$ PFU/ml)
- W32 MOI 1 ($\phi 10^7$ PFU/ml)
- W32 MOI 0.1 ($\phi 10^6$ PFU/ml)
- Vancomycin 1/4 MIC
- Vancomycin 1/2 MIC
- **Combination** : W31 MOI 1 + Vancomycin 1/4 MIC
- : W31 MOI 1 + Vancomycin 1/2 MIC
- : W31 MOI 0.1 + Vancomycin 1/4 MIC
- : W31 MOI 0.1 + Vancomycin 1/2 MIC
- : W32 MOI 1 + Vancomycin 1/4 MIC
- : W32 MOI 1 + Vancomycin 1/2 MIC
- : W32 MOI 0.1 + Vancomycin 1/4 MIC
- : W32 MOI 0.1 + Vancomycin 1/2 MIC

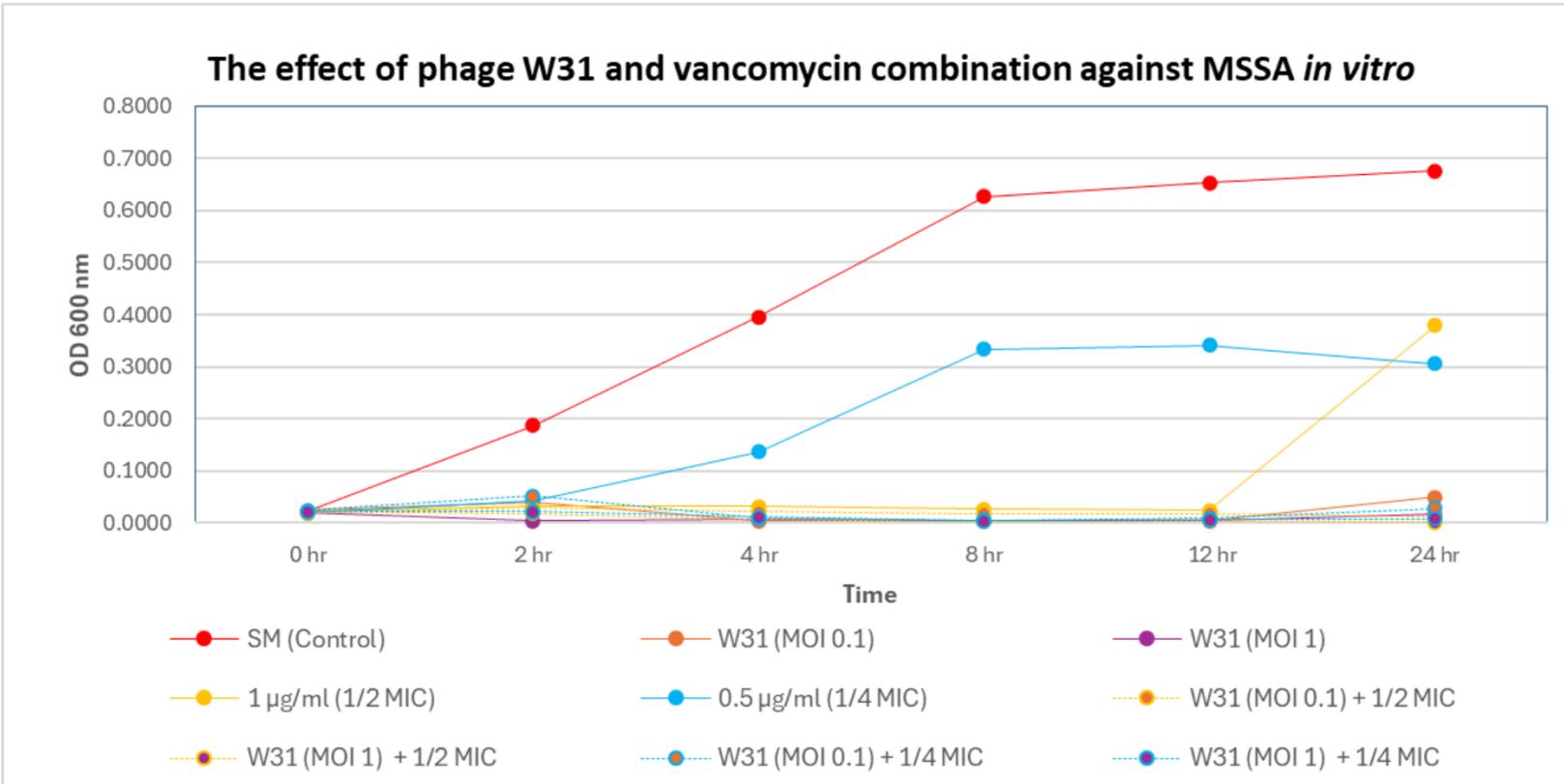
Objective: Evaluating the efficacy of phage & vancomycin



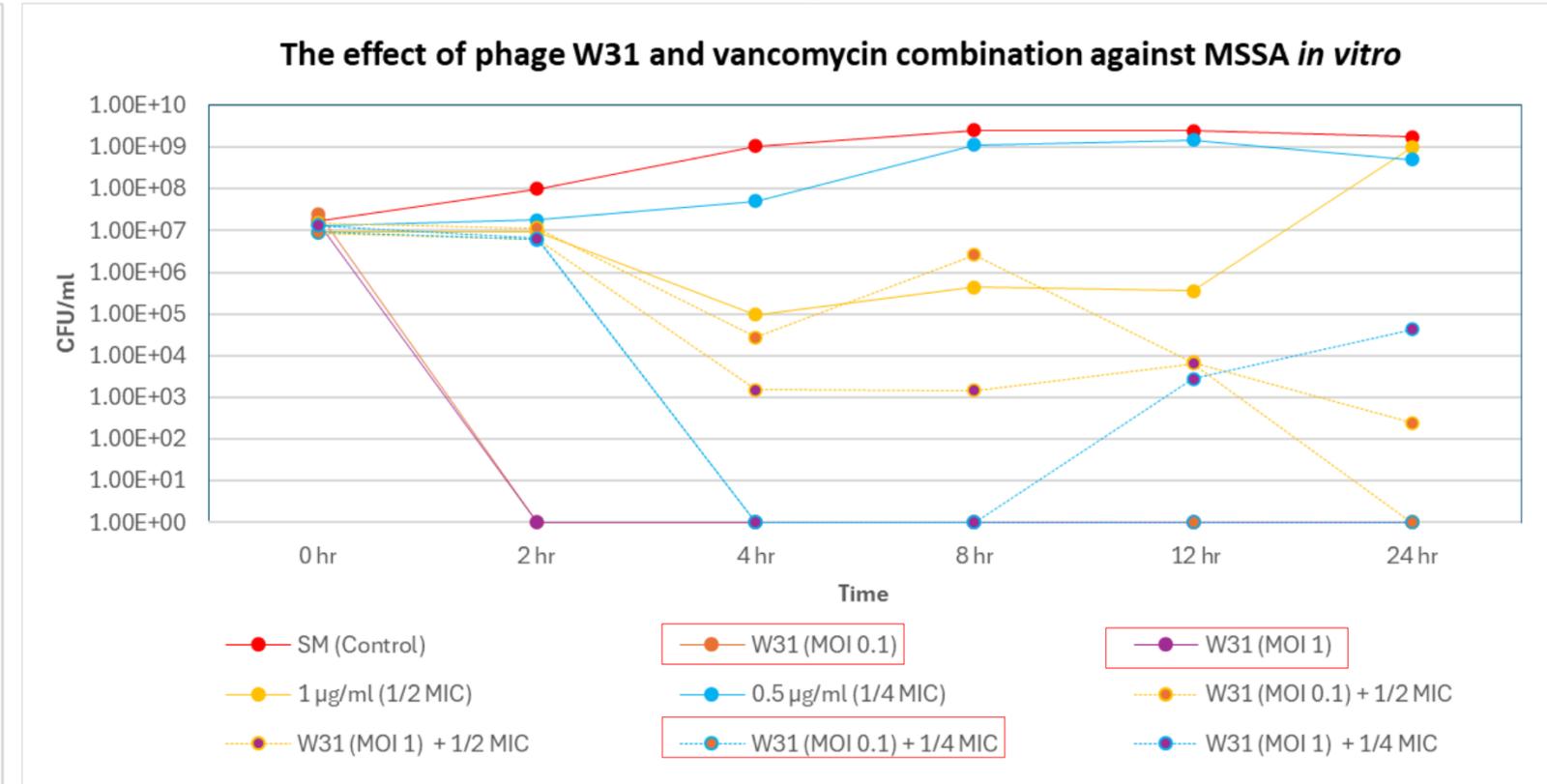
W31



MSSA



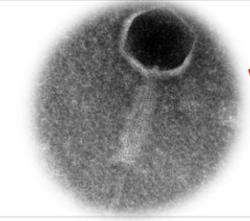
OD 600 nm



CFU/ml

These results show that phage W31 alone (MOI 0.1 & 1) reduces MSSA by 8 log units within 2 hrs.

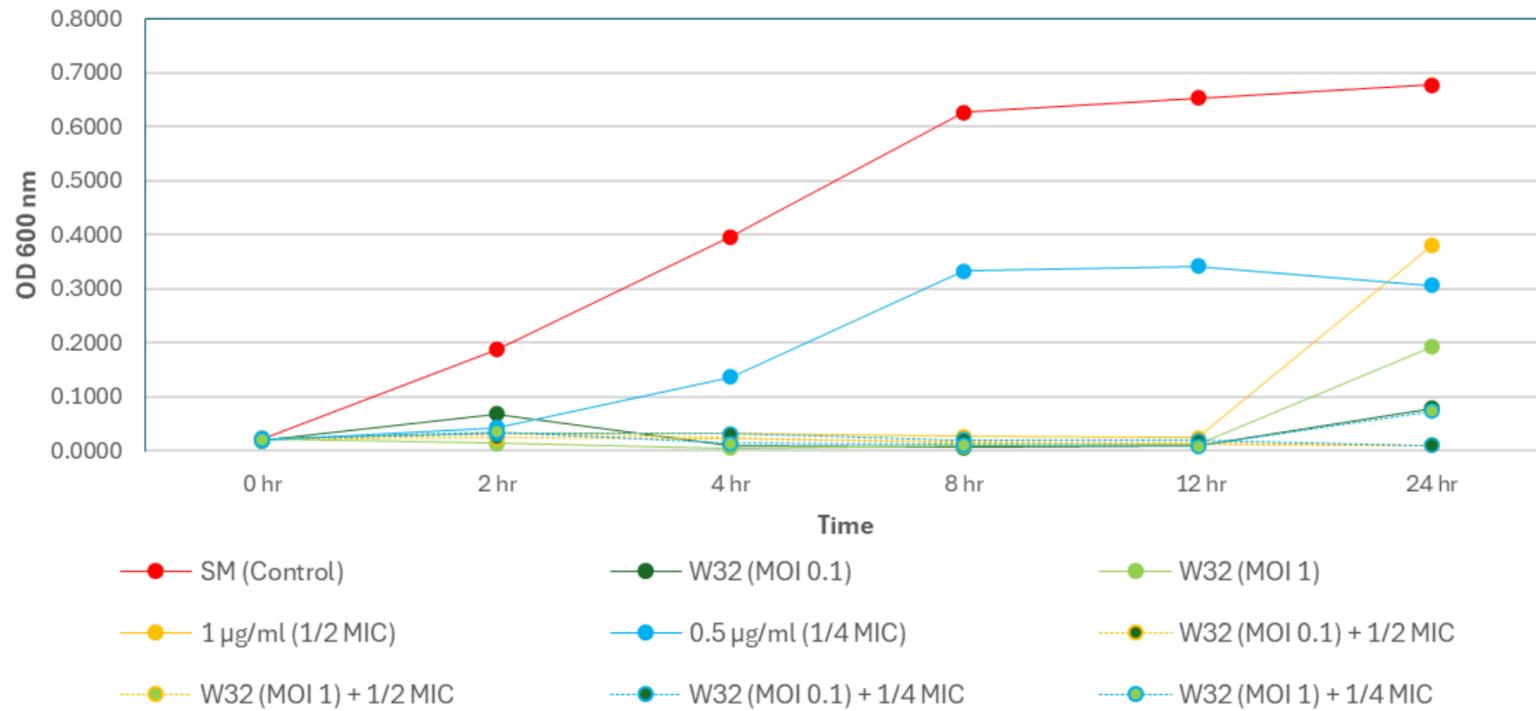
Objective: Evaluating the efficacy of phage & vancomycin



W32

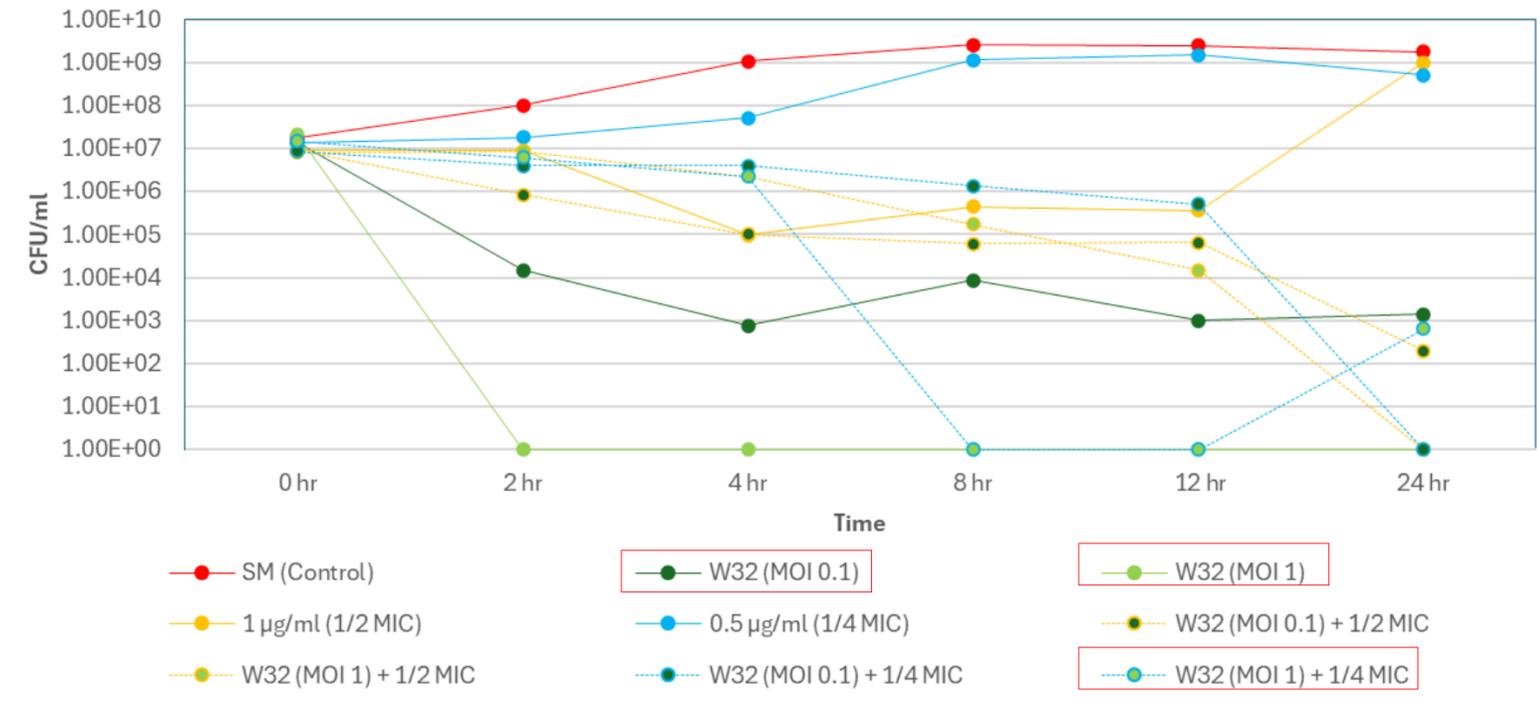
MSSA

The effect of phage W32 and vancomycin combination against MSSA *in vitro*



OD 600 nm

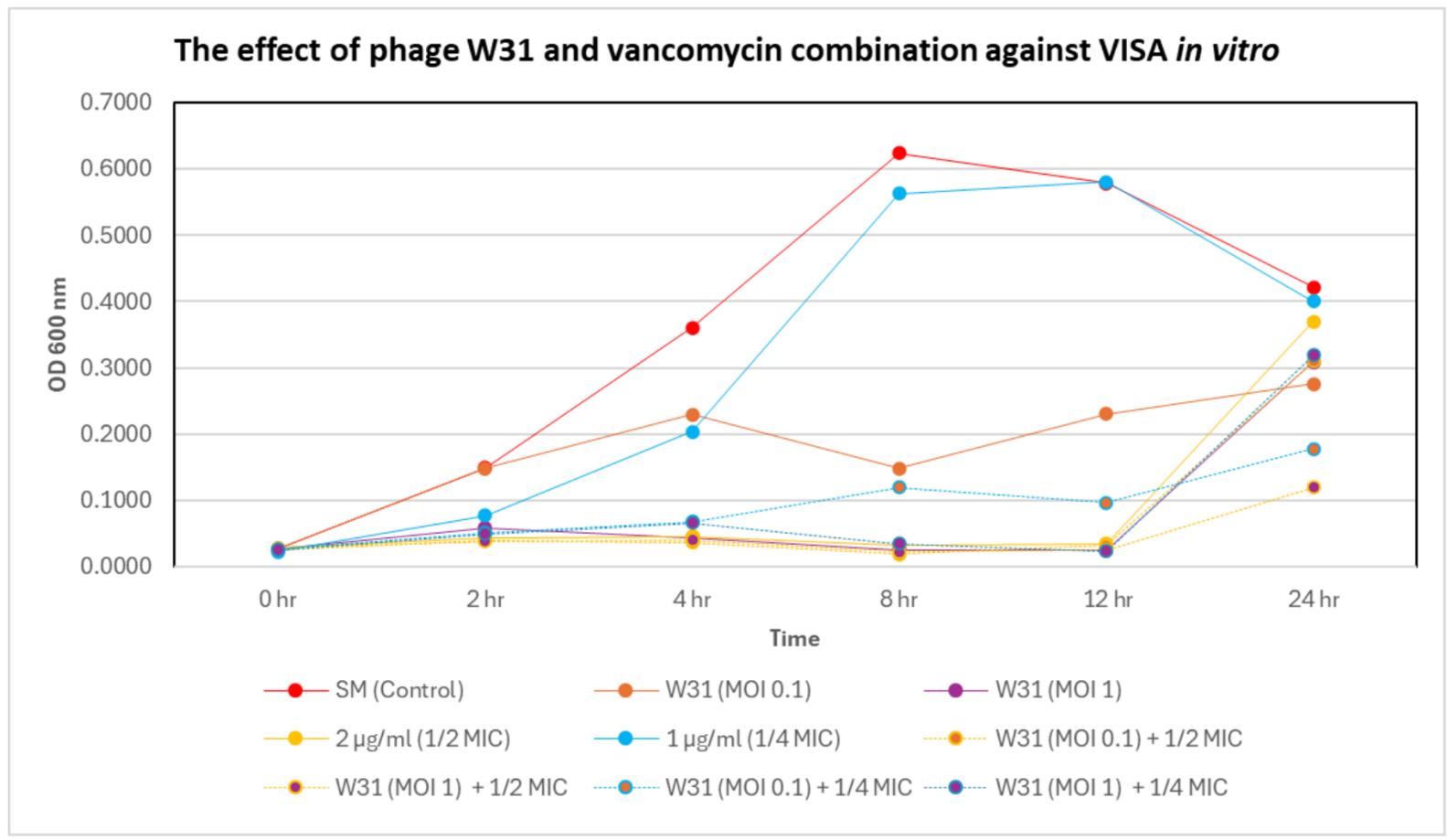
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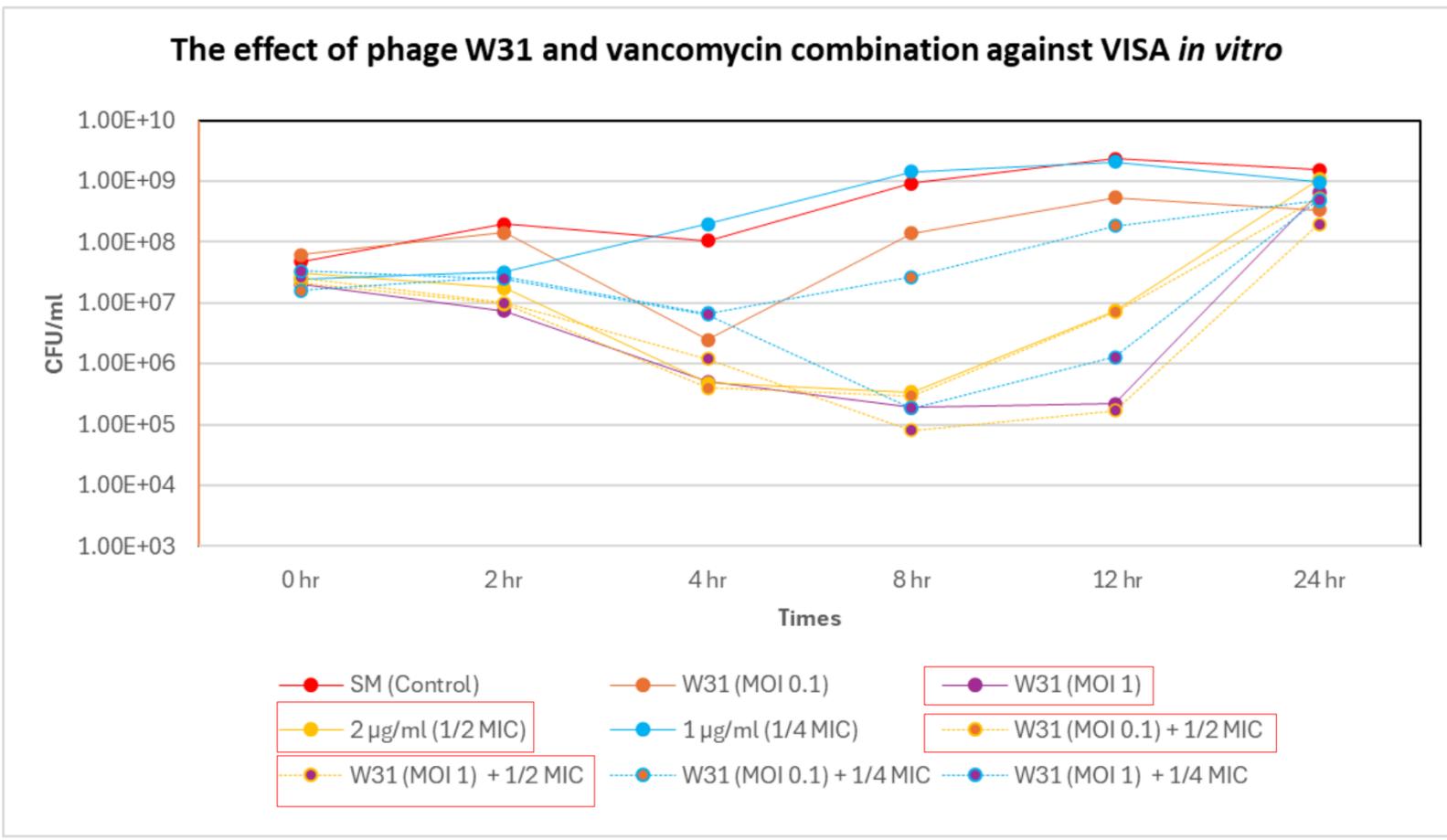
CFU/ml

These results show that phage W31 (MOI 1) alone reduces MSSA by 8 log units within 2 hrs.

Objective: Evaluating the efficacy of phage & vancomycin



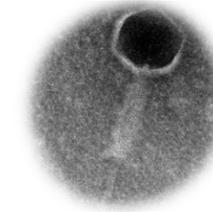
OD 600 nm



CFU/ml

The phage–vancomycin combinations (W31 MOI 0.1 + 1/2 MIC & MOI 1 + 1/2 MIC) showed greater bacterial reduction 2–4 log units during the first 4–8 hours compared with single treatments.

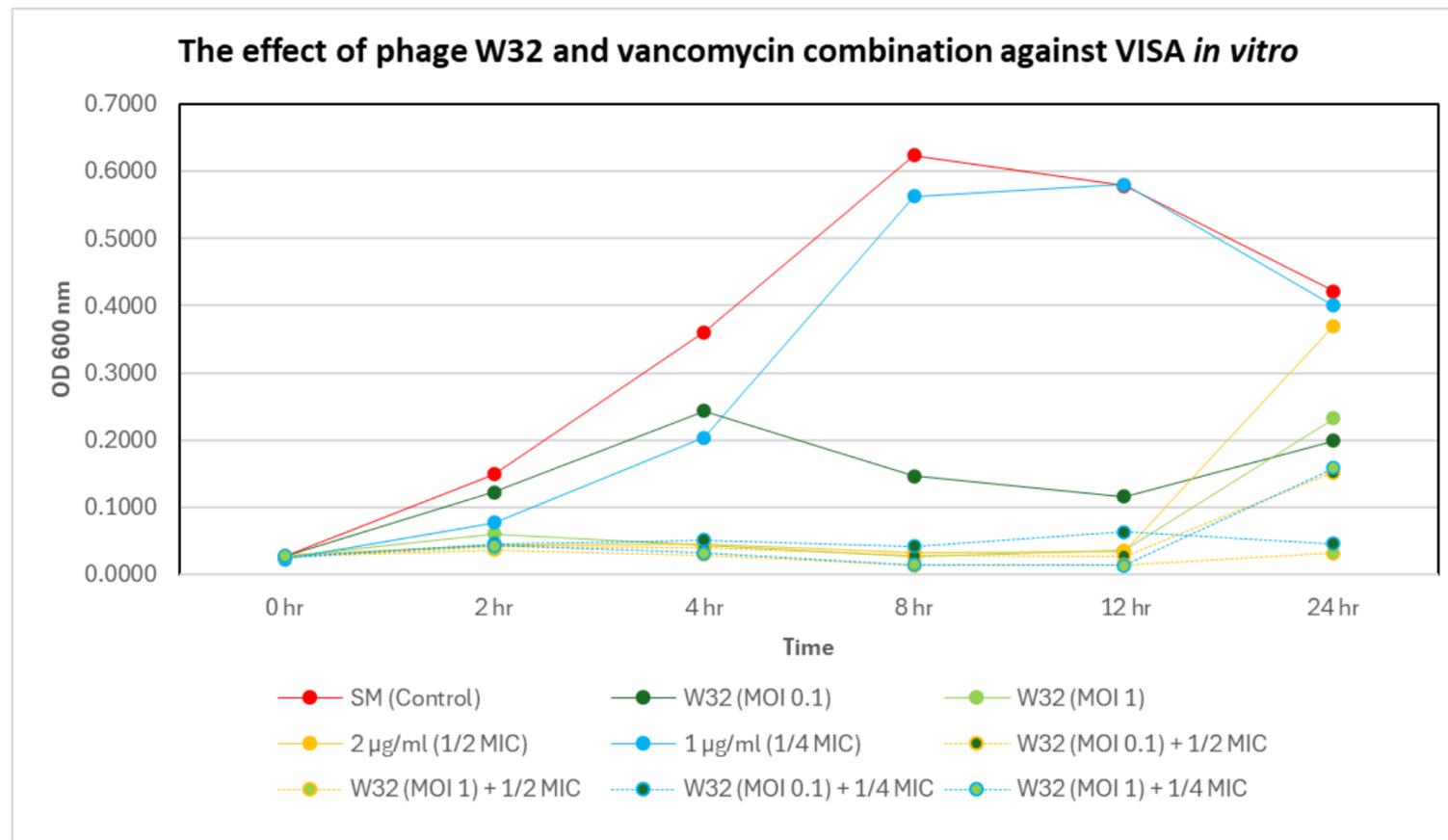
Objective: Evaluating the efficacy of phage & vancomycin



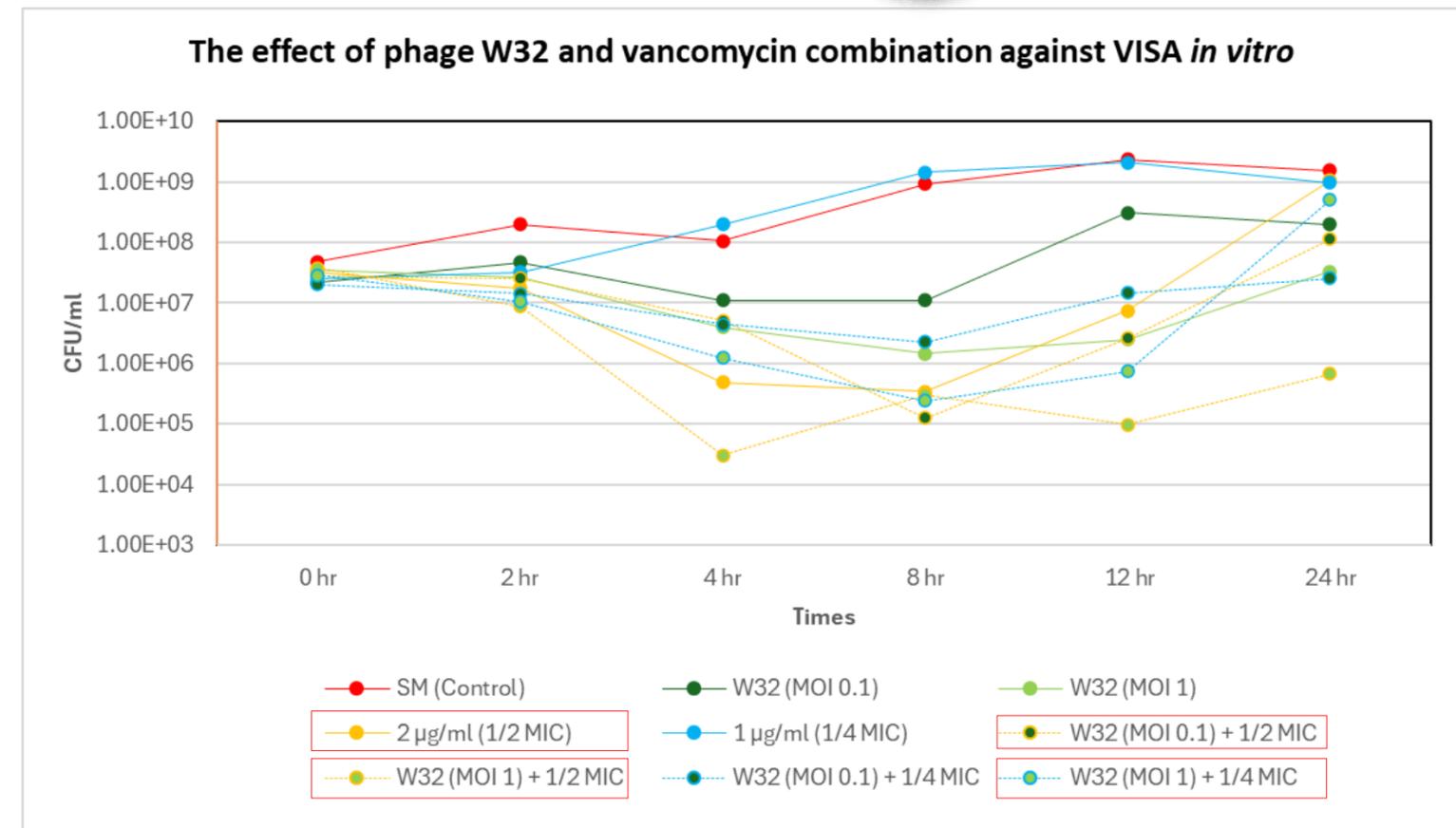
W32



VISA



OD 600 nm



CFU/ml

The phage–vancomycin combinations (W32 MOI 1+ 1/2 MIC) showed greater 3-4 log units bacterial reduction after 4 hr and overtime, indicating enhanced antibacterial activity; except at 8 hr, W32 MOI 0.1+ 1/2 MIC showed slightly greater reduction by 5 log units

- The combination of bacteriophages W31 and W32 with vancomycin enhanced antibacterial activity against both MSSA and VISA.
- Phage treatment was more effective against MSSA, while VISA showed reduced susceptibility and bacterial regrowth.
- The phage–vancomycin combination demonstrated improved bacterial reduction compared with vancomycin alone.

To-Do list	Semester 1 (2025)	Semester 2 (2026)				Finished
		Dec	Jan	Feb	Mar	Pending
The optimal MOI for phage propagation						Future work
Transmission Electron Microscopy (TEM) Analysis						
Host range determination						
Genome Analysis/DNA extraction						
Hemolytic activity						
The effect of vancomycin on <i>S. aureus</i> phage						
Effect of phage+vancomycin combination <i>in vitro</i>						
Effect of phage+vancomycin combination <i>ex vivo</i>						

Acknowledgment



Asst. Prof. Dr. Umaporn Yordpratum
Advisor



Dr. Pratsanee Hiangraj
Co-Advisor



UY Lab



THANK YOU
FOR YOUR KIND ATTENTION

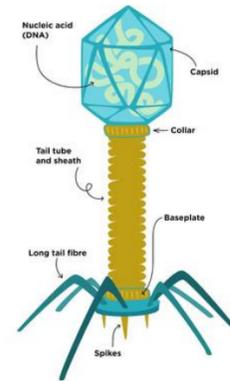


Introduction

Bacteriophage

Advantage

- Safe in human
- High efficiency
- High specificity
- Bactericidal,
- increase in number over the course of treatment
- tend only minimally to disrupt normal flora
- effective against antibiotic-sensitive & antibiotic-resistant bacteria
- easily discovered
- capable of disrupting bacterial biofilms
- low inherent toxicities



Disadvantage

- Not all phages make for good therapeutics
- The narrow spectrum of available host strains
- Phages are not unique pharmaceuticals
- The special pharmacokinetics
harmacodynamics in vivo
- immune responses hurdles
- phage resistance hurdles

Broth Method

✓ Pros:

- **Higher Phage Yield:** Can produce large quantities of phages relatively quickly.
- **Scalable:** Suitable for large-scale phage amplification in industrial or research applications.
- **Easier Sample Processing:** Liquid culture can be easily filtered and centrifuged for phage purification.

✗ Cons:

- **Difficult to Isolate Individual Phage Clones:** Unlike plaques on agar, it is harder to distinguish between different phage variants.
- **Requires Constant Monitoring:** Phage-bacteria interactions in liquid culture are dynamic and need careful optimization (e.g., MOI, incubation time).
- **Risk of Bacterial Overgrowth or Lysis Inhibition:** If phage concentration is too high or too low, bacterial growth may be uncontrolled or prematurely inhibited.

Soft Agar Method

✓ Pros:

- **Easy Plaque Enumeration:** Produces clear, countable plaques, making it ideal for titration and plaque assays.
- **Efficient Isolation of Pure Phage Clones:** Each plaque originates from a single phage, allowing easy isolation of specific variants.
- **Good for Low-Titer Phage Stocks:** Even a small number of phages can be detected due to localized lysis.
- **Prevents Bacterial Overgrowth:** The semi-solid medium limits excessive bacterial growth, making plaque formation clearer.

✗ Cons:

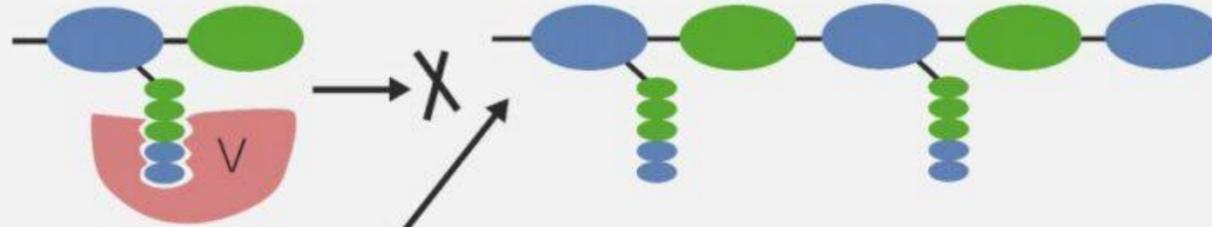
- **Lower Phage Yield:** Because the method is localized on plates, it may not produce high concentrations of phage.
- **More Labor-Intensive:** Requires preparation of plates and careful handling to avoid contamination.
- **Limited Volume:** Since it's plate-based, the method is unsuitable for large-scale phage production.

Vancomycin action

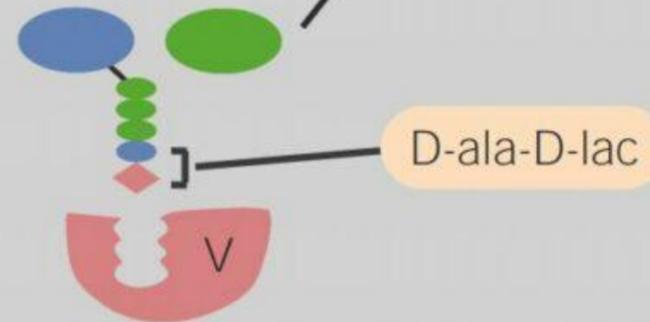
Normal incorporation of precursors



Vancomycin binds to precursors and blocks incorporation (vanco sensitivity)



Synthesis of D-ala-D-lac precursors that cannot bind vancomycin (vanco resistance)



Phage therapy against VISA *in vitro*



MECHANISMS OF RESISTANCE



Antibiotic Exposure Leads to Reduced Phage Susceptibility in Vancomycin Intermediate *Staphylococcus aureus* (VISA)

Shawna McCallin,^{a,b} Carmen Menzi,^a Swenja Lassen,^b Jean Daraspe,^c Frank Oechslin,^a Philippe Moreillon^a

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VISA/hVISA development reduced susceptibility to certain phages (phage DNA replication was blocked in some VISA strains)

A broad lysis screen (“StaphLyse™” phage cocktail) included VISA strains: Some phage cocktails can infect VISA *in vitro*, though not necessarily *in vivo*.

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Bacteriophage therapy for drug-resistant *Staphylococcus aureus* infections

Kaixin Liu^{1,2†}, Chao Wang^{2†}, Xudong Zhou^{2,3}, Xudong Guo², Yi Yang², Wanying Liu², Rongtao Zhao^{2*} and Hongbin Song^{1,2,3*}

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Phage therapy (systemic) against MRSA



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Systemic + intranasal
phage + antibiotics

Refractory chronic
rhinosinusitis with MRSA

Adult patient(s) with chronic
rhinosinusitis, failing surgeries &
antibiotic regimens

Combination of systemic and topical/intranasal phage therapy plus
antibiotics resulted in resolution of infection, improvement of
symptoms; phage therapy appeared safe; more dramatic
improvement when high local phage concentration achieved.

[pubmed.ncbi.nlm.nih.gov/38111111/](#) +1

Case Report: successful use of phage therapy in refractory MRSA chronic rhinosinusitis



J. Martin Rodriguez^{a,*}, Bradford A Woodworth^a, Bri'Anna Horne^b, Joseph Fackler^b,
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Article



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Combination of bacteriophages and vancomycin in a co-delivery hydrogel for localized treatment of fracture-related infections



Baixing Chen^{1,2,3}, Luis Ponce Benavente⁴, Marco Chitto³, Virginia Post², Caroline Constant²,
Stephan Zeiter³, Pamela Nylund³, Matteo D'Este³, Mercedes González Moreno⁴, Andrej Trampuz⁴,
Jeroen Wagemans⁵, Rob Lavigne⁵, Jolien Onsea^{1,2}, R. Geoff Richards³,
Willem-Jan Metsemakers^{1,2} & T. Fintan Moriarty³ ✉

Phage(s) co-delivered
in CMC hydrogel +
systemic vancomycin

Methicillin-
resistant
S. aureus
(MRSA)

In vivo

Local phage+antibiotic co-
delivery improved local
bacterial control when
compared to systemic antibiotic
alone

(Chen et al.,
2024)