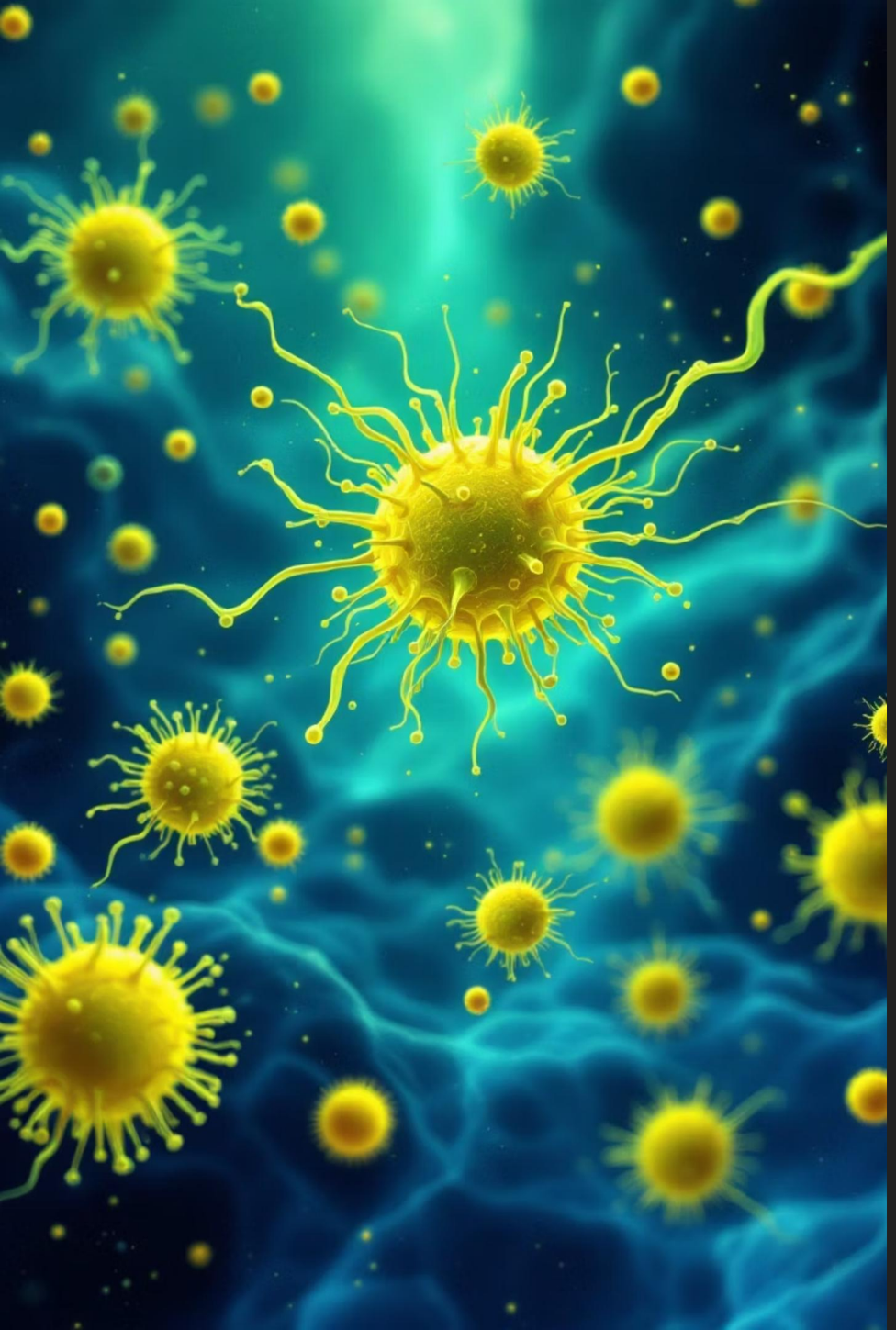


Quantifying Phage-Bacteria Dynamics In Vitro

Rapid Emergence of Phage-Resistant Klebsiella pneumoniae

Research Seminar | August 2025

Presented by Maxymia

Executive Summary

Problem

Multidrug-resistant *Klebsiella pneumoniae* represents a critical public health threat, necessitating alternatives to conventional antibiotics

Method

Combined in vitro experimentation with mathematical modeling to quantify population dynamics between bacteria and phage

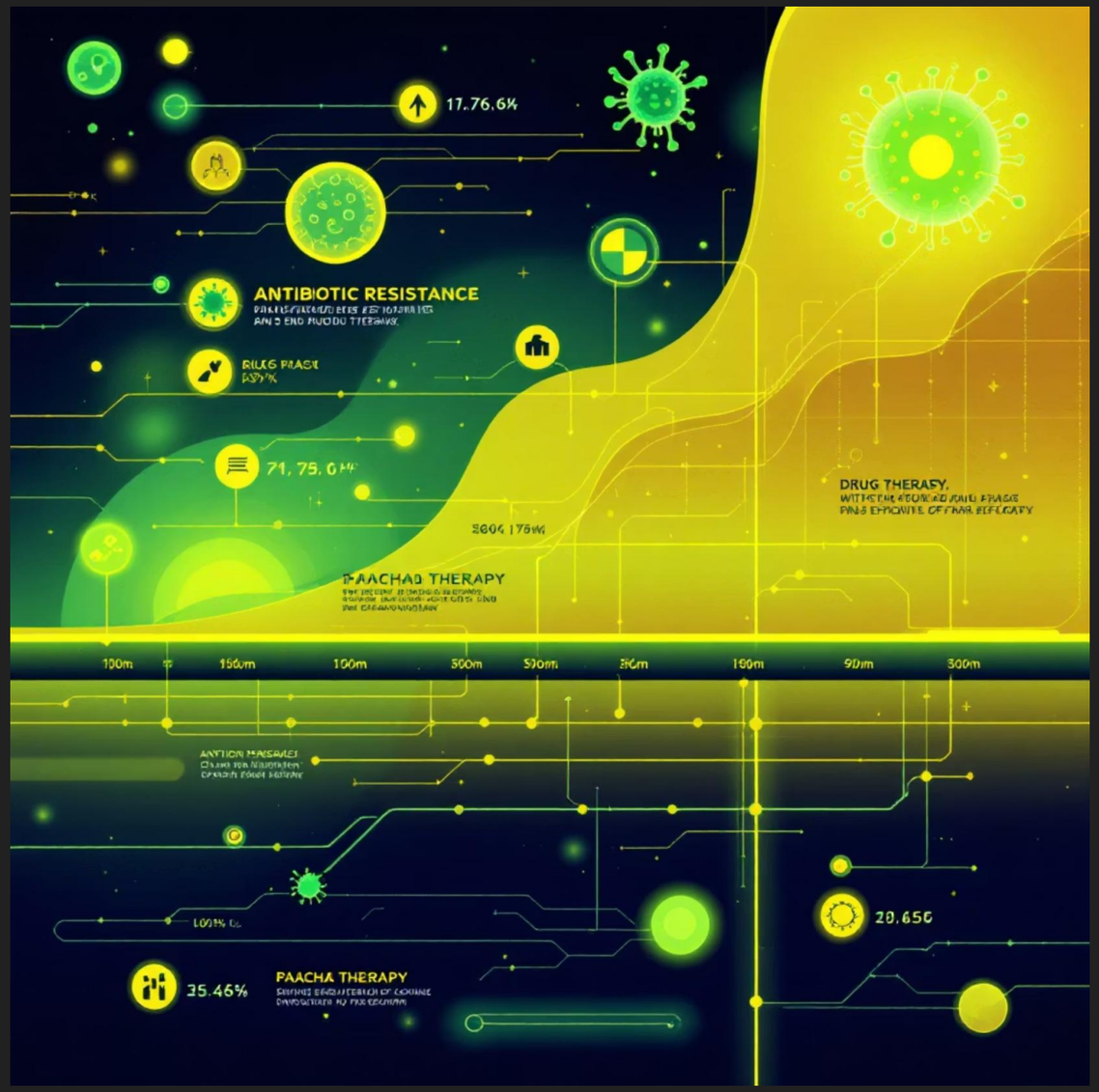
Key Results

Phage-resistant mutants emerge rapidly and show no fitness cost ($\gamma \approx 0.58\ h^{-1}$), comparable to susceptible strains

Significance

Provides critical parameters for designing effective phage therapies and highlights the challenge of bacterial resistance

Background & Motivation



The Antibiotic Crisis

Rising antimicrobial resistance threatens our ability to treat common infections, with phage therapy emerging as a promising alternative

The Knowledge Gap

Effective phage therapy requires understanding interaction kinetics, yet critical parameters—particularly resistance emergence rates—remain poorly quantified

Our Focus

Determining the key parameters governing *K. pneumoniae* interaction with bacteriophage vB_Kpn_2-P4 to inform therapeutic strategies

Research Questions & Objectives

1

Bacterial Growth Characteristics

Measure the intrinsic growth rate (k) and carrying capacity (C) for *K. pneumoniae* Kpn63 in vitro

2

Phage-Bacteria Interaction Parameters

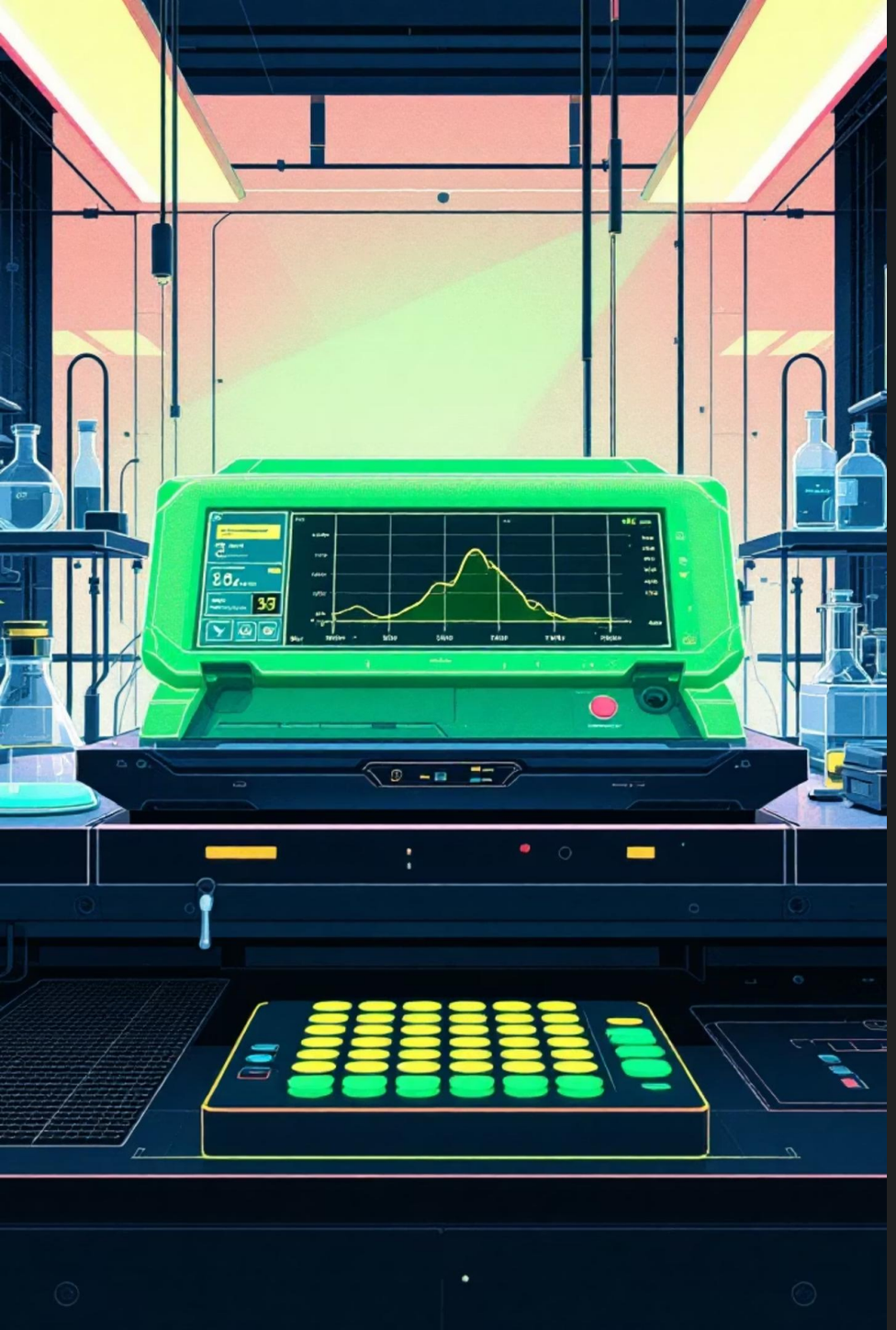
Estimate phage infection rates (ρ), burst sizes (β), and probability of phage-resistant mutant emergence (μ)

3

Resistance Fitness Cost

Determine if resistant mutants exhibit reduced growth rates compared to susceptible bacteria

Primary Question: Can we quantify the key parameters of phage-bacteria co-evolution in a clinically relevant strain?



Materials & Experimental Design

Bacterial Strain

Klebsiella pneumoniae Kpn63

- Clinical, carbapenem-resistant isolate
- High-risk clone (KL-64, ST-147)

Bacteriophage

vB_Kpn_2-P4

- Lytic phage with broad host range
- Selected for activity against Kpn63

Experimental Protocol

- 96-well plate format
- OD_{600} measurements every 10 minutes
- 16.5-hour monitoring period

Experimental Sets

- Bacteria-only: 8 replicates × 4 initial ODs
- Phage-bacteria co-culture: 21 replicates

Methodology

Experimental Data

Time-series OD_{600} measurements of bacterial growth with and without phage

Mathematical Models

Bacterial Growth: Logistic model (k, C)

Phage-Bacteria: 4-component ODE system tracking susceptible, infected, resistant bacteria, and phages

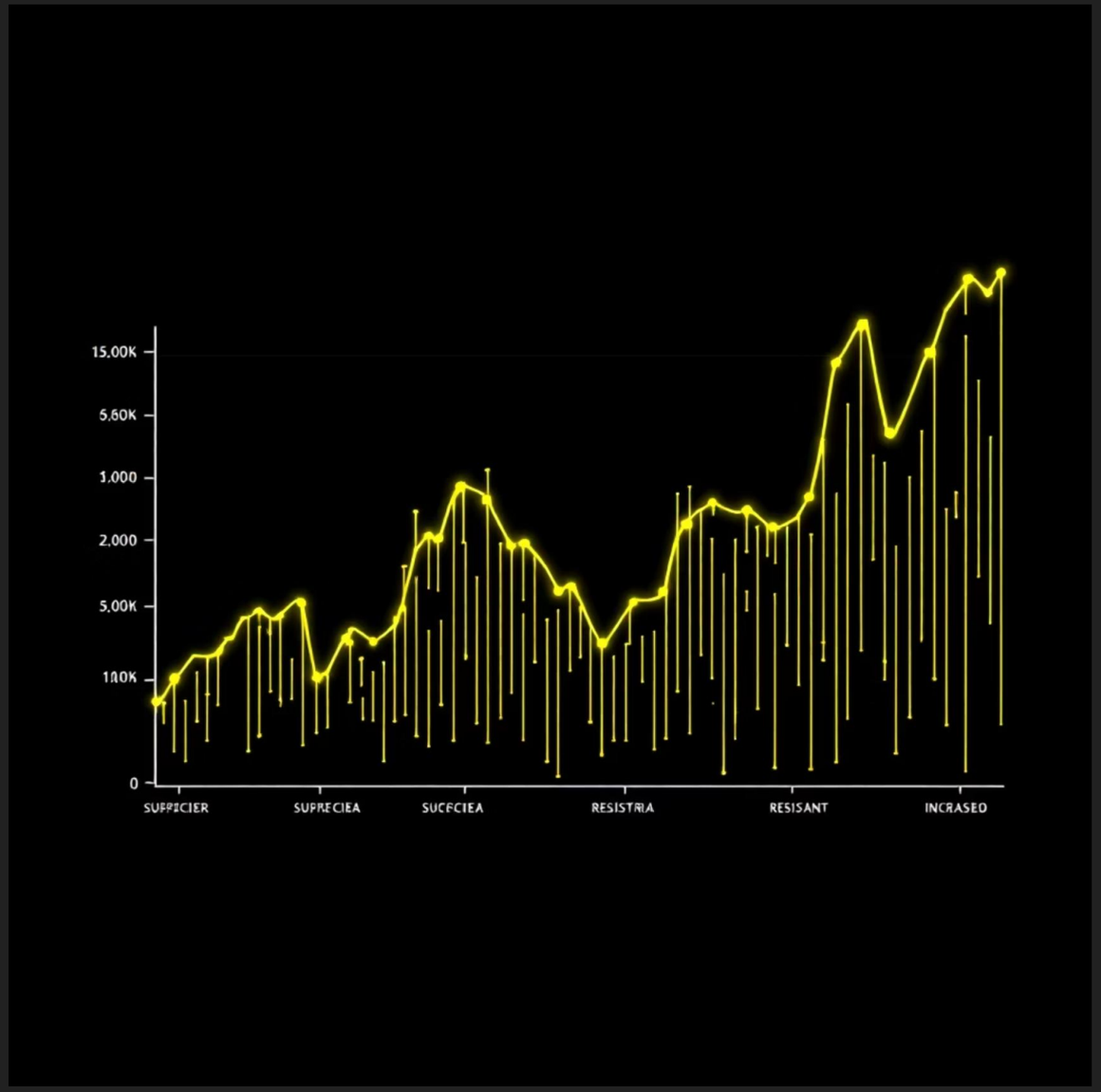
Parameter Estimation

- Levenberg-Marquardt algorithm
- Bayesian Inference

Parameter Validation

Cross-validation between methods to ensure robust estimation

Results: Rapid Resistance Emergence



0.58 h^{-1}

Resistant Growth Rate

Nearly identical to susceptible strain (0.54 h^{-1})

0.45-0.55

Resistance Emergence

High probability of de novo resistant mutants

170-226

Phage Burst Size

New virions released per lysed cell

0.96

Infection Rate

High phage effectiveness against susceptible cells

Comparison to Published Data

Parameter	This Study	Literature Values	Reference
Growth Rate (k)	0.54 h^{-1}	0.75 h^{-1} (P. aeruginosa)	Rodriguez-Gonzalez et al.
Burst Size (β)	170-226	32-303	Various Kpn phage studies
Infection Rate (ρ)	0.96	0.85-0.99	Phage-bacteria models
Resistance Rate (μ)	0.45-0.55	Highly variable	Limited comparable data

Unique Contribution: Complete integrated parameter set for a clinically relevant carbapenem-resistant *K. pneumoniae* strain and its phage

Error Analysis & Limitations

In Vitro vs. In Vivo Translation

Parameters quantified under laboratory conditions may differ from those in actual infection environments

- Absence of immune system factors
- Homogeneous environment vs. tissue heterogeneity

Model Simplifications

Mathematical framework necessarily omits some biological complexities

- Spatial heterogeneity not accounted for
- Assumes uniformity in phage-bacteria interactions

Statistical Uncertainty

Parameter variations between estimation methods

- Burst size: $\beta = 226$ (LM) vs. 170 (BI)
- Increasing replicate variability over time

Implications for Phage Therapy

Monotherapy Limitations

Rapid emergence of "no-cost" resistance suggests single-phage approaches likely to fail quickly

Parameterized Models

Quantified rates enable sophisticated computational models to test therapeutic strategies *in silico*

Strategic Approaches

- Phage cocktails targeting multiple receptors
- Phage-antibiotic synergy to suppress resistance
- Sequential phage administration strategies