Appendix A: Biological Questions and Model Overview

A1. Biological Questions

Antimicrobial resistance is often treated as a binary property, cells are either resistant or sensitive. However, resistance can also emerge dynamically as a time-dependent intracellular phenotype, shaped by feedback between antimicrobial exposure, cellular growth, and expression of resistance determinants.

The central question of this project is:

> *Can weak intrinsic host efflux activity sustain sufficient growth under antimicrobial pressure to allow plasmid copy number amplification and delayed expression of plasmid-encoded efflux pumps, ultimately leading to full resistance, even when plasmid-encoded resistance alone is initially insufficient?*

More specifically:

- Under what conditions does a resistance plasmid invade or go extinct within a single cell?
- How do intracellular drug accumulation, growth inhibition, and plasmid burden interact to determine this outcome?
- Does intrinsic host efflux activity lower intracellular drug concentration enough to permit plasmid amplification and delayed resistance expression?

This work focuses on intracellular dynamics within a single cell, treating resistance emergence as a race between antimicrobial toxicity and plasmid-driven efflux expression.

A2. Model Assumptions

To address this question, a mechanistic intracellular model is constructed with the following assumptions:

1. The cell is treated as a well-mixed compartment.
2. The extracellular antimicrobial concentration is constant in time.
3. Antimicrobial action is bacteriostatic, acting by reducing growth rate.
4. Plasmid replication is coupled to host growth and incurs a fitness cost.
5. Efflux pump expression is proportional to plasmid copy-number and introduces a delay between plasmid amplification and resistance.
6. Plasmid copy-number is treated as a continuous variable in the deterministic model, but stochastic effects at low copy-numbers are introduced later.

Under these assumptions, resistance is an emergent property of the intracellular dynamical system, rather than a fixed trait.

A3. State Variables

The intracellular system is described by the following state variables:

Variables	Description
$A_{(t)}$	Intracellular antimicrobial concentration
$P_{(t)}$	Plasmid copy-number
$M_{(t)}$	Efflux pump mRNA
$Q_{(t)}$	Efflux pump protein

A4. Full ODE System

The intracellular dynamics are governed by the following system of ordinary differential equations:

Antimicrobial dynamics:

$$\frac{dA}{dt} = k_{in}A_{ext} - k_{out}E(Q)A$$

where total efflux activity is

$$E(Q) = E_h + \beta Q$$

Growth inhibition

$$G(A) = G_{max}\frac{1}{1 + \left(\frac{A}{IC_{50}}\right)^h}$$

Plasmid copy-number dynamics

$$\frac{dP}{dt} = r_P G(A)P - c_P P - \gamma P^2$$

Efflux pump gene expression

$$\frac{dM}{dt} = k_m P - \delta_m M$$

$$\frac{dQ}{dt} = k_q M - \delta_q Q$$

A5. Parameter Definition

Antimicrobial transport and efflux:

Parameter	Description
k_{in}	Antimicrobial influx rate constant
k_{out}	Efflux rate per unit efflux activity

E_h	Intrinsic host efflux activity
β	Efflux activity per efflux protein molecule
A_{ext}	Extracellular antimicrobial concentration

Growth inhibition

Parameter	Description
G_{max}	Maximum growth rate in absence of antimicrobial
IC_{50}	Intracellular antimicrobial concentration causing 50% growth inhibition
h	Hill coefficient for growth inhibition

Plasmid replication and cost

Parameter	Description
r_P	Plasmid replication rate per unit host growth
c_P	Linear plasmid-associated fitness cost
γ	Nonlinear copy-number control parameter

Gene expression dynamics

Parameter	Description
k_m	Transcription rate per plasmid copy
δ_m	mRNA degradation rate
k_q	Translation rate
δ_q	Protein degradation or dilution rate

A6. Experimental Scenarios

To explore the emergence of resistance, control parameters (systematically varied) and fixed parameters (held constant) are distinguished.

Primary control parameters:

- Intrinsic host influx activity, E_h
- Intracellular antimicrobial concentration, A_{ext}

Experimental scenarios considered:

- No antimicrobial ($A_{ext} = 0$), plasmid present
- Antimicrobial present ($A_{ext} > 0$) with plasmid, no intrinsic efflux ($E_h = 0$)
- Antimicrobial present ($A_{ext} > 0$) with plasmid and intrinsic efflux ($E_h > 0$)
