Predictive biology: understanding and reversing the evolution of antibiotic resistance

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Predictive technologies are everywhere
Good predictions depend on context

- Good recommendation
  - The Lord of the Rings
  - The Hobbit
  - Bachelorette

- Or worse...
  - Before You Die, You See
  - The Ring
Predicting microbial community dynamics is still a big challenge!!!
Paradigm of predictive microbiology

1. Well-designed, thoughtful, and precise experimental measurements

2. Mechanistic models describing how bacteria change over time

3. Predictive machine learning algorithms to tease out underlying relationship
A prime case for predictive biology: antibiotic resistance

Superbugs Don’t Respect Borders
How NDM-1 spread around the world

Year 2006

Human microbiome
Animal microbiome
Environmental microbiome
How do we prevent the emergence of antibiotic resistance?

1. Develop new antimicrobial strategies

2. Better utilize existing drugs to prolong shelf life

To do so requires better understanding of antibiotic mechanism!
Growth rate correlates with antibiotic efficacy

Conventional wisdom:

- Bacterial growth rate
- Antibiotic effectiveness

Antibiotic

Primary target: disrupted cellular processes

Cell death

Most active during growth
Metabolism also plays a role in antibiotic efficacy.

Primary target: disrupted cellular processes

Secondary effects: metabolic processes

Survival

Cell death
ATP predicts survival better than growth rate

How does metabolism contribute to emergence of resistance?
Modes of resistance acquisition

1. Spontaneous genetic mutations
   - Antibiotic sensitive bacteria
   - Antibiotic resistant bacteria

2. Horizontal gene transfer (HGT)
   - Resistance gene
   - DNA
   - Primary mode for resistance dissemination

How does metabolism contribute to emergence of resistance?
Predicting resistance by incorporating metabolism

1. Model → experiment
   
   Do cells evolve metabolic-specific resistance mutations?

2. Experiment → model
   
   How does metabolism impact resistance dissemination?
Dynamic model of metabolic-dependent antibiotic lethality

A: Antibiotic
N: Cell density
Why is metabolism not a prominent mechanism of resistance?

1. Altered import/export
2. Target modification
3. Antibiotic inactivation/degradation

Antibiotic

Enzyme

Target
Classic protocol: growth-dependent selection
Alternative evolution: metabolic adaptation

Classic protocol:
growth-dependent selection

Updated protocol:
metabolic-dependent selection

Evolutionary time

Antibiotic
Metabolism
Evolution protocol comparison

Classic protocol: growth-dependent selection

Updated protocol: metabolic-dependent selection

OD

MIC (minimum inhibitory concentration)

Days

OD

Days

WT Strep Cipro Carb
Both evolutions lead to acquired resistance

- **Streptomycin**
- **Ciprofloxacin**
- **Carbenicillin**

**OD**

**WT**  •  **Classic evolution**  •  **Metabolic evolution**

**MIC**
Alternative evolution reveals novel metabolic mutations.
Metabolic mutations are clinically and environmentally relevant.
Metabolic mutations independently confer resistance

Model predicts mutant dynamics

Simulation

Normalized survival

Y (A.U.)

Paraquat (µg/ml)

Experimental data

Normalized survival

WT

gldt

sucAN

M → Y → A → M

*
A "White-Box" Approach for Interpretable Machine Learning

**NETWORK MODELING:**
Metabolic Simulations

**BIOCHEMICAL SCREEN:**
Systematic Perturbations

**MACHINE LEARNING:**
Regression Analyses

**EXPERIMENTAL VALIDATION:**
Causal Pathway Mechanisms

Yang JH, Cell 2019
Purine Biosynthesis Contributes to Antibiotic Lethality

AMPICILLIN

CIPROFLOXACIN

Yang JH, Cell 2019
ML identifies metabolic mutants that confer resistance

Random forest classifier

Input: Genome
Features: Unique SNP
Response: Resistant

Output: Unique SNP
Feature importance

Same mutant from evolution!

Pos1755 Pos1461 Pos2439 Pos1170 Pos1062 Pos978 Pos642 Pos1261
1. Model → experiment

- Predictive models inform experimental design to identify metabolic mutants
- Metabolic mutations highly prevalent in pathogens
- Mutations independently confer resistance

2. Experiment → model

How does metabolism impact resistance dissemination?
Conjugation: primary mode for resistance dissemination

**Resistant**
- Antibiotic resistance gene

**Sensitive**
- Plasmid
  - Circular, self-replicating DNA
  - High prevalence
  - Carry antibiotic resistance and catabolic genes
  - Stable in diverse species

**Conjugation: transfer of genetic material via cell-cell contact**

1. **Donor**
2. **Recipient**
3. **Transconjugant**
Conjugation: primary mode for resistance dissemination

Conjugation efficiency: $\eta_C$

Population | Resistance | Fluorescence
--- | --- | ---
D | Kan | GFP
R | Cm | mCherry
T | Kan + Cm | GFP + mCherry

4 Hours pre-growth: selection with Kanamycin (Kan) + Chloramphenicol (Cm)

Conjugation dynamics are governed by two processes:

1. **Conjugation efficiency**
   - Kinetic rate of plasmid transfer from donor to recipient
   - $\eta_c$

2. **Growth dynamics**
   - Relative growth rates of plasmid-free cells compared to plasmid-carrying cells
Metabolism impacts on conjugation efficiency

Nutrient composition

Physiological state

Genetic background

Values for different nutrient compositions and physiological states are shown in the graph. The normalized conjugation efficiency $n_c$ is plotted against the percentage of glucose. The genetic background includes metabolic mutants (incP) and target mutants (incF), with distinctions made between metabolic mutant (sucA) and target mutant (gyrA).
Intrinsic plasmid burden

Environment-mediated burden

Genetic background

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cost</th>
<th>Growth rate, $\mu$ (hr$^{-1}$)</th>
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<tbody>
<tr>
<td>incF</td>
<td>0.1</td>
<td>0.3</td>
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<tr>
<td>incP</td>
<td>0.2</td>
<td>0.4</td>
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Cost = $\mu/\mu_{\text{plasmid}}$

Microbial communities are “hot spots” for HGT!


How do these parameters contribute to resistance dissemination?
Mathematical model for conjugation dynamics

Plasmid-free cells
growth rate: $\mu$

Conjugation: $\eta_c$

Plasmid loss: $\kappa$

Plasmid cost: $\alpha$

How do these parameters contribute to resistance dissemination?
Plasmid persistence in simple bacterial communities

Plasmid persistence in complex bacterial communities

Analytical solution of plasmid persistence: sufficiently high $\eta_C$

**Plasmid stability criterion**

$\eta_{crit} = f(\text{plasmid cost, plasmid loss rate})$

**Plasmid persistence** ($\eta_C > \eta_{crit}$)

**Plasmid elimination** ($\eta_C < \eta_{crit}$)

Model predicts persistence of native plasmids

Quantitative prediction

$\Delta n > 0 \rightarrow \text{Persistence}$

$\Delta n < 0 \rightarrow \text{Reversal}$

Validation

Model insights: reversing resistance

Inhibiting conjugation (Lin)

Leveraging metabolic burden (Pheno)

$$\eta_C \approx \eta_{Crit}$$
...and it works!

Quantitative prediction

- Lin and Pheno
- +Lin and Pheno

Building ML model of metabolic genes on conjugative plasmids

<table>
<thead>
<tr>
<th>AMINO GLYCOSIDE</th>
<th>BETA-LACTAM</th>
<th>BLEOMYCIN</th>
<th>CARBAPENEM</th>
<th>CEPHALOSPORIN</th>
<th>FOSFOMICIN</th>
<th>MACROLIDE</th>
<th>MULTIDRUG</th>
<th>PEPTIDE</th>
<th>PHENICOL</th>
<th>QUINOLONE</th>
<th>RIFAMYCIN</th>
<th>SULFONAMIDE</th>
<th>TETRACYCLINE</th>
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**Predictive likelihood**

- **XENOBOTICS**
- **TERPENOIDS**
- **SECONDARY**
- **OTHER AMINO ACIDS**
- **NUCLEOTIDE**
- **LIPID**
- **GLYCAN BIOSYNTHESIS**
- **ENERGY**
- **COFACTOR**
- **CARB**
- **AMINO ACID**
Integrating HGT model with QMRA for environmental applications

Case study: ESBL E. coli recreational water exposure to treated wastewater effluent

Team:
• Ashley Heida, ASU
• Julia Gambino, Seaford High School, NY
• Kaylee Sanderson, Seaford High School, NY
• Mary E. Schoen, Soller Environmental
• Michael A. Jahne, USEPA
• Jay Garland, USEPA
• Lucia Ramirez, ASU
• Allison J. Lopatkin, Barnard College/ Columbia U.
• Kerry Hamilton, ASU
Metabolism and antibiotic resistance

1. Model → experiment
   - Predictive models inform experimental design to identify metabolic mutants
   - Metabolic mutations highly prevalent in pathogens
   - Mutations independently confer resistance

2. Experiment → model
   - Conjugation efficiency and growth dynamics depend on bacterial metabolism
   - Modeling accurately predicts plasmid persistence
   - Interfering in conjugation dynamics can predictably modulate plasmid outcome

Metabolic-dependent selection

[Diagram showing metabolic-dependent selection]
Paradigm of predictive microbiology

Thank you!

Lopatkin Lab members:
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Alana Palomino
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Shuqiang Huang, PhD
Hannah Meredith, PhD
Tatyana Sysoeva, PhD

Check out the Lopatkin lab at:
www.lopatkinlab.com