Model of antibiotic action on bacterial growth

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Collaborators:
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Matt Scott (Waterloo, Ontario), Rosalind Allen (Edinburgh)
Plan: Model of Antibiotic Action on Bacterial Growth

Plan

I  Antibiotics: the challenges
II  Simple model of dynamics in bacterial cell
III  Comparison with experiments
IV  Conclusions and thoughts

Reference:
Antibiotics in the 20th Century

- Alexander Fleming (1928):
  "Fleming was a Scottish bacteriologist and Nobel Prize winner (Medicine, 1945), best known for his discovery of penicillin"

- By 1940s penicillin was being mass-produced by the American drugs industry.

- Leading causes of US deaths:
  1900: pneumonia, tuberculosis, diarrhoea
  1997: heart disease, cancer, stroke

- Major contributors to global health in the past century
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- To combat resistance need good models for how antibiotics work
How do antibiotics work?

Target processes that differ between bacteria and human cells

- Inhibition of Cell Wall Synthesis
  - beta-lactams, vancomycin
- Inhibition of Protein Synthesis
  - aminoglycosides, tetracyclines, chloramphenicol, macrolides
- Alteration of Cell Membranes
  - polymyxins
- Inhibition of Nucleic Acid Synthesis
  - quinolones, metronidazole, rifampicin
- Interference with metabolism
  - sulfonamides, trimethoprim

Rough Classification: Bacteriostatic and Bactericidal
Unicellular prokaryotic organism (no nucleus or organelles)

Active unit - spherocylinder often with flagella acting as propellor

Lots of them!

“There are approximately ten times as many bacterial cells in the human flora as there are human cells in the body, with the largest number of the human flora being in the gut flora, and a large number on the skin”.- wikipedia

Form dense aggregates such as biofilms

From statistical physics perspective ideal candidate for ‘active matter’ microconstituent: driven, noisy, profuse
Exponential growth of bacteria: \( N(t) = N_0 2^{t/t_d} = N_0 e^{\lambda_0 t} \)

- \( \lambda_0 \) is the growth rate; \( t_d \) is the doubling time
- Growth-inhibition curve is \( \lambda(a) \)
- \( IC_{50} \) is antibiotic dose required to half the growth rate
- Standard wisdom has it that slower growing bacteria (e.g. biofilms) are more resilient to antibiotics.
Our Experiments (Matt Scott)

Grow bacteria under different nutrient conditions
E. coli MG1655, MOPS media with 6 different nutrients and different growth rates.

Glucose (glu) 0.64; Glucose + amino acids 1.09; Glucose + amino acids + nucleotides 1.68
Glycerol (gly) 0.40; Glycerol + amino acids 0.85; Glycerol + amino acids + nucleotides 1.35

Measure the efficacy (IC50) of 4 different antibiotics as a function of growth rate
Chloramphenicol: bacteriostatic
Tetracycline: bacteriostatic
Streptomycin: bactericidal
Kanamycin: bactericidal
Growth inhibition for ‘bacteriostatic’ antibiotics

Model of antibiotic action on bacterial growth

[Graphs showing concentration vs. relative growth rate and IC50 for Tetracycline and Chloramphenicol]
Growth inhibition for ‘bactericidal’ antibiotics

M. R. Evans

Model of antibiotic action on bacterial growth
Summary of Experimental Results

Bacteriostatic antibiotics (chlor, tet)
- Fast-growing bacteria are more susceptible
- Growth inhibition curve shows gradual decrease

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Why do different antibiotics behave differently?
A simple model

Antibiotic crosses membrane; net inflow rate $J$.

Antibiotic binds ribosomes at rate $k_{on}$, unbinds at rate $k_{off}$.

Cell grows at rate $\lambda$, diluting cell contents (division not explicitly included).

New ribosomes (unbound) are synthesized at rate $s$. 
Three variables $a, r_u, r_b$ describe the state of the cell.

**Dynamics:**

$$\frac{da}{dt} = -\lambda(?)a + f(r_u, r_b, a) + J(a_{ex}, a)$$

$$\frac{dr_u}{dt} = -\lambda(?)r_u + f(r_u, r_b, a) + s(?)$$

$$\frac{dr_b}{dt} = -\lambda(?)r_b - f(r_u, r_b, a)$$

where unbinding/binding rate

$$f(r_u, r_b, a) = k_{off}r_b - k_{on}a(r_u - r_{min})$$

and influx

$$J(a_{ex}, a) = P_{in}a_{ex} - P_{out}a$$
Three variables \( a, r_u, r_b \) describe the state of the cell.

**Dynamics:**

\[
\frac{da}{dt} = -\lambda(?)a + f(r_u, r_b, a) + J(a_{ex}, a)
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\]

and influx

\[
J(a_{ex}, a) = P_{\text{in}}a_{ex} - P_{\text{out}}a
\]

How do we model synthesis rate \( s \) and growth rate \( \lambda \)?
Empirical Growth Laws

H. Bremer and P. Dennis, in *E. coli and Salmonella* (1996)

M. Scott, C. W. Gunderson, E. M. Mateescu, Z. Zhang and T. Hwa
*Science* (2010) 330, 1099:

1. measured ribosome concentration as function of growth rate (in absence of antibiotic) and found linear relation

   \[ r_u = \frac{\lambda_0}{\kappa t} + r_{\text{min}} \]

   i.e. growth rate increases linearly with unbound ribosome concentration

2. measured total ribosome concentration as function of growth rate, in presence of antibiotic and found another linear relation

   \[ r_u + r_b = r_{\text{max}} - \lambda C \]

   i.e. The cell upregulates ribosome synthesis to compensate for antibiotic challenge
Mathematical model II: steady state

(1) \[ 0 = \frac{da}{dt} = -\lambda a + f(r_u, r_b, a) + J(a_{ex}, a) \]

(2) \[ 0 = \frac{dr_u}{dt} = -\lambda r_u + f(r_u, r_b, a) + s \]

(3) \[ 0 = \frac{dr_b}{dt} = -\lambda r_b - f(r_u, r_b, a) \]

In steady state (2) + (3)

\[ s = \lambda (r_b + r_u) = \lambda (r_{max} - \lambda C) \Rightarrow s(\lambda) \]

using second growth law

Then solve (1–3) plus first growth law

\[ r_u = \frac{\lambda}{\kappa_t} + r_{min} \]

for the four variables \( \lambda, a, r_u, r_b \) in terms of parameters of cell and \( a_{ex} \)
Mathematical model III: predictions

\[
\left( \frac{\lambda}{\lambda_0} \right)^3 - \left( \frac{\lambda}{\lambda_0} \right)^2 + \left( \frac{\lambda}{\lambda_0} \right) \left[ \frac{a_{ex}}{2 \ IC_50^* \ \frac{\lambda_0^*}{\lambda_0}} + \frac{1}{4} \left( \frac{\lambda_0^*}{\lambda_0} \right)^2 \right] - \frac{1}{4} \left( \frac{\lambda_0^*}{\lambda_0} \right)^2 = 0
\]

where \( \lambda_0^* = 2\sqrt{P_{out} \kappa_t k_{off} / k_{on}} \) is the ‘reversibility parameter’

and \( IC_50^* = \frac{\lambda_0^* (r_{max} - r_{min})}{2P_{in}} \) is another parameter combination

Set \( \frac{\lambda}{\lambda_0} = \frac{1}{2} \) and solve for \( a_{ex} \):

\[
\frac{IC_50}{IC_50^*} = \frac{1}{2} \left[ \left( \frac{\lambda_0}{\lambda_0^*} \right) + \left( \frac{\lambda_0^*}{\lambda_0} \right) \right]
\]
Predictions for inhibition curves

For $\frac{\lambda^*_0}{\lambda_0} < 0.3$ get hysteresis curve $\rightarrow$ discontinuity

Limit $\frac{\lambda^*_0}{\lambda_0} \ll 1$

$$\frac{\lambda}{\lambda_0} = \frac{1}{2} \left[ 1 - \sqrt{1 - \frac{a_{ex}}{IC_{50}}} \right]$$

For $\frac{\lambda^*_0}{\lambda_0} > 0.3$ get gradual decrease

Limit $\frac{\lambda^*_0}{\lambda_0} \gg 1$

$$\frac{\lambda}{\lambda_0} = \left[ 1 + \frac{a_{ex}}{IC_{50}} \right]^{-1} \text{ langmuir-like}$$
Comparison with experiment I

Collapse of data onto limiting growth-inhibition curves - no free parameters

\[
\frac{\lambda}{\lambda_0} = \frac{1}{2} \left[ 1 - \sqrt{1 - \frac{a_{ex}}{IC_{50}}} \right]
\]

\[
\frac{\lambda}{\lambda_0} = \left[ 1 + \frac{a_{ex}}{IC_{50}} \right]^{-1}
\]

Streptomycin and Kanamycin

Tetracycline and Chloramphenicol

<table>
<thead>
<tr>
<th>Relative drug concentration (a_{ex}/IC_{50})</th>
<th>0</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative growth rate (\lambda/\lambda_0)</td>
<td>1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

\(\lambda_0\) (/h) 0.40 0.64 0.85 1.09 1.35 1.68
Comparison with experiment II

Fit three glycerol curves with two parameters $\lambda^*_0$ and $IC_{50}^*$

**Streptomycin**

**Kanamycin**

**Tetracycline**

**Chloramphenicol**
Universal Curve

\[ \frac{IC_{50}}{IC_{50}^*} = \frac{1}{2} \left[ \left( \frac{\lambda_0}{\lambda_0^*} \right) + \left( \frac{\lambda_0^*}{\lambda_0} \right) \right] \]
Conclusions

- Complex relation between susceptibility and growth environment captured by simple model in which growth and ribosome synthesis rates depend on ribosome concentration.

- ‘reversibility rate’ $\lambda^*_0$ provides natural spectrum classification of ribosome-targetting antibiotic action.

- Chloramphenicol and tetracycline (bacteriostatic) -> Fast-growing cells are more susceptible.

- Streptomycin and kanamycin (bactericidal) -> Fast-growing cells are less susceptible.

- Further tests of theory by using mutants to vary $\kappa_t$ and hence the reduced parameters.
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