Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics – Supplementary Information

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1 Supplementary Text and Figures

1.1 Model structure and components

1.1.1 Empirical constraints representing cell physiology

In our model, the state variables are $a$, the intracellular antibiotic concentration, $r_u$, the concentration of free (unbound) ribosomes, and $r_b$, the concentration of antibiotic-bound ribosomes. The dynamics of these variables are governed by Eqs. 1-3 in the main text. These equations are placed within a physiological context by imposing empirical relations between the growth rate and ribosome content, as observed in recent experiments by Scott et al. (Scott et al., 2010). These relations are given by Eqs. 4 and 6 in the main text. The first constraint, Eq. 4 in the main text, states that the growth rate is linearly related to the free ribosome concentration $r_u$ (solid line in Fig. 2B), with an “offset” $r_{\text{min}}$ which corresponds to a minimal concentration of free ribosomes needed for growth (Scott et al., 2010). These “inactive” ribosomes are assumed not to bind antibiotic. The second constraint, Eqs. 5-6 in the main text, states that when the growth rate is decreased by imposing translational inhibition (starting from a drug-free growth rate $\lambda_0$), the cell responds by upregulating its total ribosome content, such that total ribosome concentration $r_{\text{tot}} = r_u + r_b$ increases linearly with decreasing growth rate, reaching a universal maximum $r_{\text{max}}$ as the growth rate tends to zero (dashed lines in Fig. 2B). The expression for the total ribosome concentration as a function of growth rate (Eq. 5 of the main text; dashed lines in Fig. 2B) can be derived by applying simple geometry to the diagram in Fig. 2B. Because the maximal possible ribosome concentration $r_{\text{max}}$ is universal, cells which are initially growing faster (large $\lambda_0$) have less capacity to upregulate their ribosome content (shallower gradient of the dashed line), whereas cells that initially grow slower (small $\lambda_0$) can increase their ribosome content by a larger factor in response to translational inhibition.

It is important to note that these two empirical constraints are not contradictory, because the first concerns the free ribosome concentration $r_u$, while the second concerns the total ribosome concentration $r_{\text{tot}} = r_u + r_b$. Maintaining a given growth rate $\lambda$ requires the same free ribosome concentration $r_u$ in the presence or absence of antibiotic, but in the presence of antibiotic the total ribosome concentration will be higher due to the antibiotic-bound ribosomes $r_b$ which do not contribute to growth.

The upregulation of the total ribosome pool which is encapsulated in the second empirical relation is crucial to our model; without it the model is unable to reproduce the negative correlation between IC$_{50}$ and growth rate $\lambda_0$ which we observe for our bacteriostatic antibiotics. For example, the model of Ref (Elf et al., 2006), which includes positive correlation between free ribosome concentration and growth rate, but not upregulation of total ribosome concentration upon translational inhibition, predicts only a positive relation between IC$_{50}$ and drug-free growth rate $\lambda_0$. This is discussed in more detail in section 1.4.
1.1.2 Expressing the empirical constraints in terms of concentrations (Fig S1)

The state variables in our model are concentrations of free and bound ribosomes and of antibiotic, whereas in previous work (Scott et al., 2010) the empirical relations linking ribosome content and growth rate were expressed in terms of ribosome mass fraction (i.e. the mass fraction of the total protein pool that is ribosomal protein). Here we describe how to obtain the empirical relations in terms of ribosome concentration.

![Exponential growth rate vs ribosome concentration](image)

**Figure S1:** Empirical relation between ribosome concentration and growth rate. Data from Bremer and Dennis (Bremer & Dennis, 1996), is converted into units of ribosome concentration and plotted as a function of exponential growth rate $\lambda$. The minimal ribosome concentration compatible with growth, $r_{\text{min}}$, can be directly read off the graph. To obtain the maximal ribosome concentration $r_{\text{max}}$, we use the observation of Scott et al. (Scott et al., 2010) that the maximal ribosome fraction corresponds to a drug-free growth rate of $\lambda_{\text{max}} = 2.85\text{h}^{-1}$.

For the first empirical relation (Eq. 4 of the main text; positive linear correlation between ribosome content and growth rate in the absence of antibiotic), we use the data of Bremer and Dennis, who have tabulated the number of ribosomes per cell, and the dry mass per cell, as functions of growth rate for *E. coli* B/r (Bremer & Dennis, 1996). The ratio of these numbers gives the number of ribosomes per unit dry mass, which we denote $N_R/M_{\text{cell,dry}}$, as a function of the growth rate. To convert $N_R/M_{\text{cell,dry}}$ to ribosome concentration, we note that the ribosome concentration $r = N_R/V_{\text{cell}}$ (where $V_{\text{cell}}$ is the cell volume) can be written as $r = N_R/V_{\text{cell}} = (N_R/M_{\text{cell,dry}}) \times (M_{\text{cell,dry}}/M_{\text{cell,wet}}) \times (M_{\text{cell,wet}}/V_{\text{cell}})$. The cell density, $M_{\text{cell,wet}}/V_{\text{cell}}$, has been measured by Kubitschek et al. to be 1.09 g/mL, independent of growth rate (Kubitschek et al., 1984). We can relate the wet and dry cell masses, $M_{\text{cell,wet}}$ and $M_{\text{cell,dry}}$, by noting that Cayley et al. have measured the water-accessible cytoplasmic volume for *E. coli* K-12 as 2.1 $\mu\text{l}$ / mg dry weight under conditions of optimal osmolarity, corresponding to the MOPS medium.
used in our experiments (Cayley et al., 1991). Taking the density of water to be 1 g / mL, this implies that, in wet cells, for every unit of dry weight there are 2.1 mass units of water – and thus that the ratio of dry cell mass to wet cell mass, $M_{\text{cell,dry}}/M_{\text{cell,wet}}$, is 1 / 3.1. This allows us to convert the data of Bremer and Dennis into ribosome concentration as a function of growth rate. The results of this conversion are shown in Fig. S1. As observed by Scott et al. (Scott et al., 2010), the relation is approximately linear, with an intercept $r_{\text{min}} = 19.3 \mu M$ and inverse slope $\kappa_t = 6.1 \times 10^{-2} \mu M^{-1} h^{-1}$. In these experiments, no antibiotic is present, so that the measured ribosome concentration corresponds to the free ribosome concentration, $r_u$ in our model. Thus we can write Eq. 4 of the main text:

$$r_u = r_{\text{min}} + \frac{\lambda}{\kappa_t}. \tag{S1}$$

The parameters $\kappa_t$ and $r_{\text{min}}$ can be directly measured from the slope and intercept of the graph in Fig. S1; $\kappa_t = 6.1 \times 10^{-2} \mu M h^{-1}$ is in good agreement with the result of Scott et al. (Scott et al., 2010).

For the second empirical relation (Eq. 5 of the main text; negative linear correlation between total ribosome content and growth rate under translation-inhibition), we assume that the linear relationship between ribosome mass fraction and growth rate observed by Scott et al. (Scott et al., 2010) also holds for the ribosome concentration - i.e. we assume that we can write $r_{\text{tot}} = r_{\text{max}} - b\lambda$. The constant $b$ can be determined by noting that in the absence of antibiotic, $\lambda = \lambda_0$ and $r_{\text{tot}} = r_u = r_{\text{min}} + \lambda_0/\kappa_t$; this implies Eq. 5 of the main text:

$$r_{\text{tot}} = r_{\text{max}} - \Delta r \lambda \left( \frac{1}{\lambda_0} - \frac{1}{(\kappa_t \Delta r)} \right) \tag{S2}$$

where $\Delta r = r_{\text{max}} - r_{\text{min}}$. To obtain a numerical value for $r_{\text{max}}$, we note that in the experiments of Scott et al. (Scott et al., 2010), the maximal ribosome fraction corresponds to a drug-free growth rate of $\lambda_{\text{max}} = 2.85 h^{-1}$. Reading off from Fig. S1 the ribosome concentration corresponding to $\lambda_0 = 2.85 h^{-1}$ (red arrow), we find that $r_{\text{max}} = 65.8 \mu M$.

The second empirical relation allows us to determine the ribosome synthesis rate $s$. At steady state the rate of ribosome synthesis must match the rate of ribosome removal by dilution: i.e. we require $s = \lambda r_{\text{tot}}$ (this can also be seen by adding together Eqs. 2 and 3 of the main text and setting the time derivatives to zero). This leads to the expression for the synthesis rate; Eq. 6 of the main text:

$$s(\lambda) = \lambda r_{\text{tot}} = \lambda \left[ r_{\text{max}} - \lambda \Delta r \left( \frac{1}{\lambda_0} - \frac{1}{(\kappa_t \Delta r)} \right) \right]. \tag{S3}$$

1.1.3 Antibiotic influx rate

We assume that the antibiotic influx rate, $J$ in Eq. 1 of the main text, is given by

$$J = P_{\text{in}} a_{\text{ex}} - P_{\text{out}} a, \tag{S4}$$
where \(a_{\text{ex}}\) is the extracellular antibiotic concentration. Here we assume that the membrane permeabilities \(P_{\text{in}}\) and \(P_{\text{out}}\) are constants. It is important to note, however, that, for aminoglycoside antibiotics at much higher concentrations than considered in this work, \((\sim 10 \times \text{IC}_{50})\), misfolded membrane proteins can disrupt the cell membrane, which may lead to changes in the permeability (Kohanski et al., 2008). This could be included in the model by making \(P_{\text{in}}\) and/or \(P_{\text{out}}\) dependent on the state variables \((a\) or \(r_b\)).

### 1.1.4 Accounting for dilution due to cell growth

In our model, cell division is not represented explicitly, because a cell division event does not affect the intracellular concentrations (assuming equi-partition of the cell contents between daughter cells). Instead our model tracks the intracellular concentrations in time within a lineage of cells. The dilution of material due to cell division is represented by “sink” terms \(-\lambda a, -\lambda r_u, -\lambda r_b\) in Eqs. 1-3 of the main text. To see how these terms arise, consider a generic intracellular component whose number of molecules is \(N\) and whose concentration is \(\frac{N}{V}\) where \(V\) is the cell volume. We suppose that the component is generated at a rate \(g\) per unit volume and that the cell increases its volume (i.e. grows) exponentially at rate \(\lambda\). The dynamical equations for \(N\) and \(V\) in a growing cell are \(\frac{dN}{dt} = gV\) and \(\frac{dV}{dt} = \lambda V\). Combining these relations gives us a dynamical equation for the concentration \(n\):

\[
\frac{dn}{dt} = \frac{1}{V}(\frac{dN}{dt}) - (\frac{N}{V^2})(\frac{dV}{dt}) = g - \lambda n,
\]

in which the sink term arises naturally.

### 1.2 Theoretical predictions of the model

#### 1.2.1 Steady-state solution of the model; assumptions and prediction of growth inhibition curves (Fig. S2)

We now discuss the solution of equations Eqs. 1–3 of the main text for exponentially growing cells. In steady state, these equations read:

\[
0 = -k_{\text{on}} a (r_u - r_{\text{min}}) + k_{\text{off}} r_b - \lambda a + P_{\text{in}} a_{\text{ex}} - P_{\text{out}} a,
\]

\(\text{(S5)}\)

\[
0 = -k_{\text{on}} a (r_u - r_{\text{min}}) + k_{\text{off}} r_b - \lambda r_u + s(\lambda),
\]

\(\text{(S6)}\)

\[
0 = k_{\text{on}} a (r_u - r_{\text{min}}) - k_{\text{off}} r_b - \lambda r_b.
\]

\(\text{(S7)}\)

We wish to obtain from these equations a prediction for the growth rate \(\lambda\) as a function of the extracellular antibiotic concentration \(a_{\text{ex}}\) and the drug-free growth rate \(\lambda_0\). To this end we solve the equations subject to the constraints given by the empirical relations, Eqs. S1 and S3 (Eqs. 4 and 6 of the main text).

We first use the positive correlation between unbound ribosome concentration and growth rate, Eq. S1, to eliminate \(r_u\) in favour of \(\lambda\). Rearranging Eq. S7 then gives an expression for the bound ribosome concentration \(r_b\)

\[
r_b = \frac{k_{\text{on}} a \lambda}{\kappa_t (\lambda + k_{\text{off}})}.
\]

\(\text{(S8)}\)
Substituting this into the steady-state condition on the intracellular antibiotic concentration $a$, Eq. S5, yields

$$0 = P_{\text{in}} a_{\text{ex}} - a \left[P_{\text{out}} + \frac{k_{\text{on}} \lambda^2}{\kappa_t (\lambda + k_{\text{off}})} + \lambda\right]$$

(S9)

which in turn yields an expression for the steady-state concentration $a$. Similarly, eliminating the bound ribosome concentration $r_b$ from Eq. S6 yields,

$$0 = -\lambda \left[r_{\text{min}} + \frac{\lambda}{\kappa_t}\right] - \frac{k_{\text{on}} a \lambda^2}{\kappa_t (\lambda + k_{\text{off}})} + s(\lambda).$$

(S10)

Substituting the expression for the ribosome synthesis rate, Eq S3, into Eq. S10, leads to

$$0 = \left(1 - \frac{\lambda}{\lambda_0}\right) \Delta r - \frac{a \lambda k_{\text{on}}}{\kappa_t (\lambda + k_{\text{off}})} \left[\frac{P_{\text{in}} a_{\text{ex}}}{k_{\text{on}} \lambda_0^2} + \lambda\right].$$

(S11)

Combining Eqs. S9 and S11 to eliminate the intracellular antibiotic concentration $a$ generates an expression for the growth rate $\lambda$ as a function of the extracellular antibiotic concentration $a_{\text{ex}}$,

$$0 = \left(1 - \frac{\lambda}{\lambda_0}\right) \Delta r - \frac{\lambda k_{\text{on}}}{\kappa_t (\lambda + k_{\text{off}})} \left[\frac{P_{\text{in}} a_{\text{ex}}}{k_{\text{on}} \lambda_0^2} + \lambda\right].$$

(S12)

Eq. S12 can be rearranged to give a cubic equation for the growth rate $\lambda$ scaled relative to the antibiotic-free growth rate $\lambda_0$:

$$0 = -\left(\frac{\lambda}{\lambda_0}\right)^3 \left[(k_{\text{on}} + \kappa_t) \lambda_0^2\right] + \left(\frac{\lambda}{\lambda_0}\right)^2 \left[(k_{\text{on}} + \kappa_t) \lambda_0^2 - (P_{\text{out}} + k_{\text{off}}) \kappa_t \lambda_0\right]
$$

$$+ \left(\frac{\lambda}{\lambda_0}\right) \left[(P_{\text{out}} + k_{\text{off}}) \kappa_t \lambda_0 - \frac{k_{\text{on}} P_{\text{in}} a_{\text{ex}}}{\Delta r} \lambda_0 - P_{\text{out}} k_{\text{off}} \kappa_t\right] + P_{\text{out}} k_{\text{off}} \kappa_t.$$

(S13)

Dividing through by $k_{\text{on}}$ we find that

$$0 = -\left(\frac{\lambda}{\lambda_0}\right)^3 \left[(1 + \frac{\kappa_t}{k_{\text{on}}}) \lambda_0^2\right] + \left(\frac{\lambda}{\lambda_0}\right)^2 \left[(1 + \frac{\kappa_t}{k_{\text{on}}}) \lambda_0^2 - (P_{\text{out}} + k_{\text{off}}) \frac{\kappa_t}{k_{\text{on}}} \lambda_0\right]
$$

$$+ \left(\frac{\lambda}{\lambda_0}\right) \left[(P_{\text{out}} + k_{\text{off}}) \kappa_t \lambda_0 - \frac{P_{\text{in}} a_{\text{ex}}}{\Delta r} \lambda_0 - P_{\text{out}} K_D \kappa_t\right] + P_{\text{out}} K_D \kappa_t,$$

(S14)

where $K_D = k_{\text{off}}/k_{\text{on}}$. Defining the parameter combinations $\lambda_0^* = 2\sqrt{P_{\text{out}} \kappa_t K_D}$ and $IC_{50}^* = \lambda_0^* \Delta r/(2P_{\text{in}})$, as in the main text, and dividing through by $(\lambda_0^*)^2$, we can rewrite Eq. S14 as

$$0 = -\left(\frac{\lambda}{\lambda_0}\right)^3 \left(\frac{\lambda_0}{\lambda_0^*}\right)^2 \left[(1 + \frac{\kappa_t}{k_{\text{on}}})\right] + \left(\frac{\lambda}{\lambda_0}\right)^2 \left[(1 + \frac{\kappa_t}{k_{\text{on}}}) \left(\frac{\lambda_0}{\lambda_0^*}\right)^2 - \frac{P_{\text{out}} + k_{\text{off}}}{2P_{\text{out}} k_{\text{off}}} \left(\sqrt{\frac{\kappa_t}{k_{\text{on}}}}\right) \left(\frac{\lambda_0}{\lambda_0^*}\right)\right].$$
\[
+ \left( \frac{\lambda}{\lambda_0} \right) \left[ \frac{(P_{\text{out}} + k_{\text{off}})}{2\sqrt{P_{\text{out}}k_{\text{off}}}} \left( \sqrt{\frac{k_{\text{i}}}{k_{\text{on}}}} \left( \frac{\lambda_0}{\lambda_0} - \frac{a_{\text{ex}}}{2IC_{50}^* \lambda_0^*} \left( \frac{\lambda_0}{\lambda_0} - 1 \right) \right) \right) + \frac{1}{4} \right] \right].
\] (S15)

If we assume that \(k_{\text{on}} \gg k_{\text{i}}\) and also that \((P_{\text{out}} + k_{\text{off}})/\sqrt{P_{\text{out}}k_{\text{off}}}\) does not become very large, then Eq. S15 simplifies to Eq. 7 of the main text:

\[
0 = \left( \frac{\lambda}{\lambda_0} \right)^3 - \left( \frac{\lambda}{\lambda_0} \right)^2 + \left( \frac{\lambda}{\lambda_0} \right) \left[ \frac{a_{\text{ex}}}{2IC_{50}^*} \left( \frac{\lambda_0}{\lambda_0} - \frac{1}{4} \right) \right] - \frac{1}{4} \left( \frac{\lambda_0}{\lambda_0} \right)^2.
\] (S16)

Figure S2: The effect of the reversibility parameter \(\lambda_0^*\) on the shape of the inhibition curve \(\lambda(a_{\text{ex}})\). The inhibition curve \(\lambda(a_{\text{ex}})\) is obtained from the fixed points of the model dynamics, Eq. S15, for different choices of the critical parameter \(\lambda_0^* = 2\sqrt{P_{\text{out}}k_{\text{i}}K_D}\) that quantifies the reversibility in binding and transport. **A and B:** For near-irreversible binding \(\lambda_0^* \ll \lambda_0\), [**A:** \(\lambda_0^*/\lambda_0 = 0.083\) \((K_D = 0.01 \mu M)\) and **B:** \(\lambda_0^*/\lambda_0 = 0.3\) \((K_D = 0.13 \mu M)\)], the inhibition curve exhibits an abrupt transition from growth to no growth close to the half-inhibition concentration. Furthermore, we predict the existence of a second, slow-growing subpopulation in the region of antibiotic concentration where the model has three fixed points (gray band). **C and D:** For reversible binding \(\lambda_0^* \gg \lambda_0\) [**C:** \(\lambda_0^*/\lambda_0 = 0.45\) \((K_D = 0.3 \mu M)\) and **D:** \(\lambda_0^*/\lambda_0 = 0.83\) \((K_D = 1 \mu M)\)], the inhibition curve decreases smoothly and a single fixed point is evident. In the figure, \(\lambda_0^*\) is varied by changing the dissociation constant \(K_D = k_{\text{off}}/k_{\text{on}}\), with the remaining parameters fixed \(P_{\text{out}} = P_{\text{in}} = k_{\text{i}}\Delta t = 2.85 h^{-1}\) and \(\lambda_0 = 1 h^{-1}\). The full lines show the stable fixed points; dashed lines show unstable fixed points.

The roots of this cubic equation for \(\lambda\) give the steady growth rate as a function of the model parameters and of the drug-free growth rate \(\lambda_0\). Fig. S2 shows predictions for \(\lambda(a_{\text{ex}})\) obtained from Eq. S13, for increasing values of \(\lambda_0^*\) (with \(\lambda_0 = 1 h^{-1}\)). For \(\lambda_0^* \ll \lambda_0\) (Fig. S2A and B), Eq. S13 has three solutions. The stability of these solutions can be determined by performing a linear stability analysis of the dynamical equations; this reveals that two of the solutions are dynamically stable (full lines in Fig. S2) and one is unstable (dashed line). In our experiments, we expect to observe the upper stable solution, since we begin with the initial condition \(\lambda/\lambda_0 = 1\), which is closer to the upper fixed point than the lower one. The observation that we have two stable states does, however, suggest that measurements of individual cell growth rates might
reveal population-level heterogeneity. Such measurements have recently been performed for antibiotic-resistant strains (Deris et al., 2013) but to our knowledge have not been carried out for antibiotic-sensitive strains such as those used in our work. For $\lambda^*_0 \gg \lambda_0$ (Fig. S2C and D), the bistable regime vanishes and a unique steady state prevails.

1.2.2 Growth-rate dependence of the IC$_{50}$

A prediction for the IC$_{50}$ is obtained by setting $a_{ex} = IC_{50}$ and $\lambda = \lambda_0/2$ in Eq. S15. This gives

$$0 = 1 + \frac{\kappa_t}{k_{on}} + \left( \frac{P_{out} + k_{off}}{\sqrt{P_{out} k_{off}}} \right) \left( \sqrt{\frac{\kappa_t}{k_{on}}} \left( \frac{\lambda^*_0}{\lambda_0} \right) - 2 \frac{IC_{50}}{IC^*_0} \left( \frac{\lambda^*_0}{\lambda_0} \right) + \left( \frac{\lambda^*_0}{\lambda_0} \right)^2 \right)$$

(S17)

This equation can be solved to give an expression for the IC$_{50}$ as a function of the antibiotic-free growth rate $\lambda_0$:

$$\frac{IC_{50}}{IC^*_0} = \frac{1}{2} \left( 1 + \frac{\kappa_t}{k_{on}} \right) \left( \frac{\lambda_0}{\lambda^*_0} \right) + \left( \frac{P_{out} + k_{off}}{\sqrt{P_{out} k_{off}}} \right) \left( \sqrt{\frac{\kappa_t}{k_{on}}} \right) + \left( \frac{\lambda^*_0}{\lambda_0} \right)$$

(S18)

Making the same assumptions mentioned above, namely that $k_{on} \gg \kappa_t$ and that $(P_{out} + k_{off})/\sqrt{P_{out} k_{off}}$ does not diverge, Eq. S18 reduces to

$$\frac{IC_{50}}{IC^*_0} = \frac{1}{2} \left[ \left( \frac{\lambda_0}{\lambda^*_0} \right) + \left( \frac{\lambda^*_0}{\lambda_0} \right) \right]$$

(S19)

which corresponds to Eq. 10 in the main text.

In Eq. S19, if $\lambda_0 < \lambda^*_0$, we expect the second term to dominate, so that the IC$_{50}$ decreases with increasing $\lambda_0$; i.e. fast-growing cells are more sensitive. However if $\lambda_0 > \lambda^*_0$, the first term dominates, the IC$_{50}$ increases with increasing $\lambda_0$ and fast-growing cells are less sensitive to antibiotic.

Parameters extracted from literature data suggest that the assumption $k_{on} \gg \kappa_t$ is satisfied for all the antibiotics considered in this work (see Section 1.3.3); the largest value of $\kappa_t/k_{on}$ is obtained for chloramphenicol, for which $\kappa_t/k_{on} \lesssim 1/18$.

1.2.3 Limit of the model for small $\lambda^*_0$

If the critical parameter $\lambda^*_0$ is small, corresponding to very slow antibiotic efflux ($P_{out}$), or very slow antibiotic-ribosome dissociation ($k_{off}$), the predictions of the model can be simplified. For $\lambda^*_0/\lambda_0 \ll 1$, the cubic Eq. S16 (Eq. 7 in the main text) reduces to a quadratic:

$$0 = \left( \frac{\lambda}{\lambda_0} \right)^2 - \left( \frac{\lambda}{\lambda_0} \right) + \frac{a_{ex}}{2IC^*_0} \left( \frac{\lambda^*_0}{\lambda_0} \right)$$

(S20)
which can be solved to give the prediction for the form of the inhibition curve:

\[
\frac{\lambda}{\lambda_0} = \frac{1}{2} \left[ 1 + \sqrt{1 - \frac{2a_{\text{ex}}}{\text{IC}_{50}^*} \frac{\lambda_0^*}{\lambda_0}} \right],
\]

(S21)

for \(a_{\text{ex}} < \text{IC}_{50}/2\); and \(\lambda/\lambda_0 = 0\) otherwise. In this limit Eq. S19 for the \(\text{IC}_{50}\) reduces to

\[
\text{IC}_{50} = \frac{\lambda_0 \text{IC}_{50}^*}{2\lambda_0^*} = \frac{\Delta r \lambda_0}{4P_{\text{in}}}. 
\]

(S22)

Because \(\Delta r = r_{\text{max}} - r_{\text{min}} = 46.5\mu M\) is a universal constant it should, in principle, be possible to estimate the permeability constant \(P_{\text{in}}\) from the slope of \(\text{IC}_{50}(\lambda_0)\). For streptomycin and kanamycin, however, our fits suggest that we are not quite in the small \(\lambda_0^*\) limit (a better fit to the \(\text{IC}_{50}\) data is obtained using the full expression, Eq. S19 than the linear approximation, as shown in Fig. S4).

Using Eq. S22 for the \(\text{IC}_{50}\) allows us to express the inhibition curve, Eq. S21 as

\[
\frac{\lambda}{\lambda_0} = \frac{1}{2} \left[ 1 + \sqrt{1 - \frac{a_{\text{ex}}}{\text{IC}_{50}}} \right],
\]

(S23)

for \(a_{\text{ex}} \leq \text{IC}_{50}\), and \(\lambda/\lambda_0 = 0\) otherwise. This limiting form is compared to our data for streptomycin and kanamycin in Fig. 5 of the main text, and Fig. S4.

1.2.4 Limit of the model for large \(\lambda_0^*\)

The predictions of the model also simplify in the limit that \(\lambda_0^*\) is large, corresponding to rapid antibiotic efflux (\(P_{\text{out}}\)) and antibiotic-ribosome dissociation (\(k_{\text{off}}\)). In this case the cubic Eq. S16 reduces to

\[
0 = \left( \frac{\lambda}{\lambda_0} \right) \left[ \frac{a_{\text{ex}}}{\text{IC}_{50}^*} + \frac{1}{2} \left( \frac{\lambda_0^*}{\lambda_0} \right) \right] - \frac{1}{2} \left( \frac{\lambda_0^*}{\lambda_0} \right),
\]

(S24)

which can be solved to give a Langmuir-like expression for the relative growth rate

\[
\frac{\lambda}{\lambda_0} = \frac{1}{1 + \frac{2a_{\text{ex}}}{\text{IC}_{50}}} \left( \frac{\lambda_0^*}{\lambda_0} \right).
\]

(S25)

In this case the \(\text{IC}_{50}\) (the antibiotic concentration to achieve half-inhibition \(\lambda/\lambda_0 = 1/2\)) is simply

\[
\text{IC}_{50} = \frac{\text{IC}_{50}^* \lambda_0^*}{2\lambda_0} = \left( \frac{k_{\text{off}}}{k_{\text{on}}} \right) \left( \frac{P_{\text{out}}}{P_{\text{in}}} \right) \left( \frac{\kappa_t \Delta r}{\lambda_0} \right),
\]

(S26)

and the inhibition curve reduces to the simple form

\[
\frac{\lambda}{\lambda_0} = \frac{1}{1 + \frac{a_{\text{ex}}}{\text{IC}_{50}}}.
\]

(S27)
Note that in this case the IC\textsubscript{50} is inversely proportional to the drug-free growth rate $\lambda_0$. These predictions are compared to our data for tetracycline and chloramphenicol in Fig. 5 of the main text and Fig. S4.

Interestingly, this form of the inhibition curve can be understood as a modified form of a simple Langmuir-like binding curve for the antibiotic-ribosome equilibrium. Writing the inhibition curve Eq. S27 in terms of the free ribosome concentration using Eq. S1, we obtain $(r_u - r_{\text{min}})/(r_0 - r_{\text{min}}) = (1 + a_{\text{ex}}/K_{D,\text{eff}})^{-1}$, where $r_0$ is the drug-free ribosome concentration $r_0 = \lambda_0/\kappa + r_{\text{min}}$, and $K_{D,\text{eff}}$ is an effective dissociation constant $K_{D,\text{eff}} = K_D \times (P_{\text{out}}/P_{\text{in}}) \times (\kappa_t \Delta r/\lambda_0)$. Thus the effect of cell physiology is to rescale the \textit{in vitro} dissociation constant $K_D$ by a factor that depends both on the membrane permeability and, crucially, on the drug-free growth rate $\lambda_0$.

1.3 Model fits to experimental data and parameter extraction

1.3.1 Model fits to growth inhibition curves on glucose-based media (Fig. S3)

Fig. S3 shows model fits to our experimental growth inhibition curves on the three glucose-based media. These plots are analogous to those shown in Fig. 3 of the main text for the glycerol-based media.

1.3.2 Residuals for fits to growth inhibition curves

The sums of squares of the residuals (RSS values) for the fits of our model to our experimental growth inhibition curves (Fig. 3 of the main text and Fig. S3) were:

- Streptomycin, glycerol media: RSS = 0.18, 21 data points.
- Streptomycin, glucose media: RSS = 0.63, 21 data points.
- Kanamycin, glycerol media: RSS = 1.07, 18 data points.
- Kanamycin, glucose media: RSS = 0.40, 19 data points.
- Tetracycline, glycerol media: RSS = 0.013, 22 data points.
- Tetracycline, glucose media: RSS = 0.015, 18 data points.
- Chloramphenicol, glycerol media: RSS = 0.023, 22 data points.
- Chloramphenicol, glucose media: RSS = 0.145, 18 data points.

1.3.3 Model predictions for $\lambda_0^*$ and IC\textsubscript{50}$^*$ and comparison to literature values

Fitting our data for the nutrient-dependent growth inhibition curves to the prediction of the model (Eq. 7 of the main text) allows us to extract values for the critical parameters $\lambda_0^*$ and IC\textsubscript{50}$^*$. Tables S3 and S4 list these values. In many cases, estimates for biochemical parameters for membrane transport and ribosome binding are available in the literature; these can be used to obtain estimated ranges for $\lambda_0^* = 2\sqrt{P_{\text{out}}\kappa_t K_D}$ and IC\textsubscript{50}$^* = \lambda_0^* \Delta r/(2P_{\text{in}})$, which are compared
Figure S3: Model fits to growth inhibition curve data for glucose-based media. The parameters $\lambda^*_0$ and $IC^*_{50}$ are obtained by numerical fitting of the solution of the cubic equation, Eq. 7, to our experimental growth inhibition curves. Data sets for different drug-free growth rates (i.e. the different curves in each panel) were fitted simultaneously with the same values of $\lambda^*_0$ and $IC^*_{50}$, but separate fits were obtained for glycerol-based and glucose-based media. For each fit, the bold line shows the best fit to the data while the narrow lines represent 95% confidence intervals on the value of the parameter $\lambda^*_0$. To obtain these intervals (as well as the error bars on the fits for $\lambda^*_0$ and $IC^*_{50}$), we performed fits on 1000 randomised datasets generated by sampling within the experimental error ranges on the measured growth-inhibition data. The parameters obtained by our fitting procedure are listed and compared to literature data in Table S4.

to the results of our fits in Table S4 [using $\kappa_t = 6.1 \times 10^{-2}\mu\text{Mh}^{-1}$ and $\Delta r = 46.5\mu\text{M}$ (Scott et al., 2010)]. In most cases, the values given by our fits are within or close to the range of the literature results.

Literature values for the membrane transport parameters $P_{\text{out}}$ and $P_{\text{in}}$ were obtained from literature data that tracks the accumulation of intracellular antibiotic over time, upon exposure of cells to high concentrations of extracellular antibiotic. To extract $P_{\text{out}}$ and $P_{\text{in}}$ from these data, we assumed that the intracellular antibiotic concentration $a$ obeys $da/dt = P_{\text{in}}a_{\text{ex}} - P_{\text{out}}a$ (neglecting the fraction of antibiotic that is bound to ribosomes, since typically $a \gg IC_{50}$). This equation has the solution $a(t) = (P_{\text{in}}a_{\text{ex}}/P_{\text{out}})[1 - \exp(-P_{\text{out}}t)]$. Hence, $P_{\text{out}}$ can be found from the rate of increase of intracellular antibiotic and $P_{\text{in}}$ can be found from the saturation level of intracellular antibiotic, $a_{\text{sat}} = P_{\text{in}}a_{\text{ex}}/P_{\text{out}}$. 

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Tetracycline
For tetracycline, the dissociation constant $K_D$ has been reported as 0.5-20μM (Epe & Woolley, 1984; Tritton, 1977; Berens, 2001) [note that in Ref. (Tritton, 1977) $K_D$ can be obtained as the inverse of the quasi-first order effective association constant]. Reported experiments that track the inflow of tetracycline into cells allow one to obtain estimates for $P_{out} = 80-120h^{-1}$ (Argast & Beck, 1985) and $P_{in} \approx 17 	imes P_{out} = 1360-2040h^{-1}$ (Argast & Beck, 1985). This leads to values of $\lambda_0^* = 2\sqrt{P_{out}}k_{in}K_D$ in the range 3.1-24h$^{-1}$ and $IC_{50}^* = \lambda_0^*\Delta r/(2P_{in})$ in the range 0.04-0.4μM. $k_{on}$ for tetracycline has also been measured as $0.285\mu M^{-1}s^{-1} = 1026\mu M^{-1}h^{-1}$ (Tritton, 1977). This gives the ratio $k_{on}/\kappa_t$ as $1.7 \times 10^4$, so that we are well within the range where the approximation $k_{on}/\kappa_t \gg 1$, used in our calculations, is valid.

Chloramphenicol
For chloramphenicol, the dissociation constant $K_D$ has been reported as 0.5-5μM (Harvey & Koch, 1980; Pongs et al., 1973; Contreras & Vazquez, 1977; Goldberg et al., 1977) [these experiments were not all carried out at the same temperature as our experiments, but $K_D$ has been found not to vary significantly between 0 and 30°C (Harvey & Koch, 1980)]. Estimated values for the membrane transport parameters are $P_{out} = 15-30h^{-1}$ (Abdel-Sayed, 1987; George & Hall, 2002) and $P_{in} = 75-4000h^{-1}$ (Abdel-Sayed, 1987; George & Hall, 2002). We note that the latter range is very large; this is due to two very different values being reported for the ratio of intra- to extra-cellular chloramphenicol in Refs. (Abdel-Sayed, 1987) and (George & Hall, 2002). Taking the range of values spanning the results of both papers, this leads to values of $\lambda_0^*$ in the range 1.35-6.0h$^{-1}$ and $IC_{50}^*$ in the range 0.008-1.86μM. The binding constant $k_{on}$ for chloramphenicol has also been estimated as $3 \times 10^{-4} - 3.6 \times 10^{-3} \mu M^{-1}s^{-1} = 1.08-13\mu M^{-1}h^{-1}$ (Harvey & Koch, 1980). This gives the ratio $k_{on}/\kappa_t$ as $18 - 213$, so that $k_{on}/\kappa_t \gg 1$ remains a reasonable approximation.

Streptomycin
For the aminoglycosides streptomycin and kanamycin, reports differ as to the reversibility of antibiotic-ribosome binding. A common view is that the effect of aminoglycoside binding is irreversible (Faraji et al., 2006; Davis, 1987; Wishart et al., 2006; Davies, 1991). However, other reports suggest that dihydrostreptomycin binds reversibly to ribosomes with $K_D = 0.1\mu M$ (Chang & Flaks, 1972; Franklin & Snow, 2006). We therefore assume that $K_D = 0 - 0.1\mu M$.

In experiments tracking the accumulation of streptomycin inside cells, no saturation of the intracellular antibiotic was observed after 30 minutes (Bryan & Van Den Elzen, 1976). This suggests that $P_{out} < 1/30\, \text{min}^{-1}$. In the same experiments, $P_{in}$ can be determined by the slope of the curve for accumulation of intracellular antibiotic, giving $P_{in} = 0.9 - 1.5h^{-1}$ (Bryan & Van Den Elzen, 1976) [it is important to note, however, that aminoglycosides promote the synthesis of mis-translated proteins that disrupt the membrane, so that for streptomycin and kanamycin, influx may not be a linear process as assumed here]. Taking these ranges of parameters, we obtain $\lambda_0^*$ in the range 0.22h$^{-1}$ and $IC_{50}^*$ in the range 0.5-6.8μM. $k_{on}$ for streptomycin has also
been measured as $0.16 - 0.56\mu M^{-1}s^{-1} = 576 - 2016\mu M^{-1}h^{-1}$ (Chang & Flaks, 1972). This gives the ratio $k_{on}/\kappa_t$ as $9 \times 10^2 - 33 \times 10^4$, so that $k_{on}/\kappa_t \gg 1$ is a valid approximation.

**Kanamycin**

For kanamycin, it is difficult to obtain literature predictions for $\lambda_0^*$ and $IC_{50}^*$. As for streptomycin, a conflicting picture appears as to the reversibility of ribosome binding; while this is generally accepted to be irreversible for aminoglycosides (Faraji et al., 2006; Davis, 1987; Wishart et al., 2006; Davies, 1991), non-zero dissociation constants for kanamycin have been reported ($K_D = 1.8\mu M$ for binding to the small ribosomal subunit and $K_D = 2.5\mu M$ for binding to the large subunit (Misumi et al., 1978)). The existence of two different ribosome binding sites for kanamycin, not considered in our model, is an additional complicating factor (Misumi et al., 1978; Franklin & Snow, 2006). Moreover, no data on membrane transport properties appear to be available for kanamycin.

### 1.3.4 Fitting the model to growth-dependent susceptibility data [IC$_{50}$(\lambda_0)], rather than growth inhibition curves (Fig. S4)

In Figs. 3 and 4 of the main text, and Tables S3 and S4, we obtain values for the critical parameters $\lambda_0^*$ and $IC_{50}^*$ by fitting our data for nutrient-dependent growth inhibition curves to the predictions of the model (solution of Eq. 7 of the main text). This requires us to solve the cubic equation (Eq. 7 of the main text). A mathematically simpler, but less well-constrained, alternative approach would be to obtain $\lambda_0^*$ and $IC_{50}^*$ by instead fitting the data for the growth-dependent susceptibility ($IC_{50}(\lambda_0)$) to the model prediction (Eq. 10 of the main text).

To test the robustness of our conclusions to the fitting procedure, we also implemented this alternative approach. To constrain the fits as much as possible, we fit the data for $IC_{50}(\lambda_0)$ using a global fit with shared parameters, such that, for a given antibiotic, $\lambda_0^*$ is assumed to be common for both glycerol-based media and glucose-based media, but $IC_{50}^*$ can differ between these media classes. This amounts to allowing $P_{in}$ but not $P_{out}$ to depend on the carbon source. In these fits, the total number of parameters was 3 per antibiotic ($\lambda_0^*$ plus $2 \times IC_{50}^*$) and the total number of data points was 6 per antibiotic (3 glucose-based media plus 3 glycerol-based media) \(^1\). To be sure that the global minimum was found, we systematically searched the space of the three parameters using a grid-based procedure, for each antibiotic.

The results of fitting $IC_{50}(\lambda_0)$ to our data are shown in Fig S4. Fits for $IC_{50}(\lambda_0)$ are shown in the top panels (black lines; note that for tetracycline and chloramphenicol we have plotted $1/\lambda_0$ on the horizontal axis). The resulting values of $\lambda_0^*$ and $IC_{50}^*$ are given in the caption, and are consistent with the values obtained by fitting the growth inhibition curves (compare to Tables S3 and S4). The bottom panels (solid lines) in Fig S4 show predictions for the growth inhibi-

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\(^1\)This compares to 4 parameters per approximately 36 data points for our fits to the growth inhibition curves in Fig. 3.
tion curves, obtained by inputting these values of $\lambda^*_0$ and IC$_{50}$ into the cubic equation, Eq. 7 of the main text. The agreement with the data remains good, even though these fits are less well constrained than those of the main text.

Fig. S4 also shows the predictions of the limiting cases of the model for small $\lambda^*_0$ (for streptomycin and kanamycin) and large $\lambda^*_0$ (for tetracycline and chloramphenicol), using the same values of $\lambda^*_0$ and IC$_{50}$. In the top panels, the brown lines show model predictions for the IC$_{50}$ in the “bactericidal” and “bacteriostatic” limits, Eqs. S22 and S26 respectively. In the bottom panels, the dashed lines show predictions for the growth inhibition curves in the same limits, Eqs. S23 and S27. The bactericidal (small $\lambda^*_0$) limit of the model is in reasonable but not excellent agreement with the data for both streptomycin and kanamycin, consistent with the fact that the fitted values of $\lambda^*_0 = 0.45$ for streptomycin and $\lambda^*_0 = 0.4$ for kanamycin are comparable with our slowest experimental growth rates. The bacteriostatic (large $\lambda^*_0$) limit of the model is in good agreement with the data for tetracycline, consistent with the fact that here our fitted value of $\lambda^*_0 = 3.9$ is much larger than any of our experimental growth rates. In contrast, for chloramphenicol, bacteriostatic limit of the model is in poorer agreement with the data, consistent with the fact that the fitted value of $\lambda^*_0 = 1.35$ is within the range of our experimental growth rates.

### 1.4 Importance of up-regulation of ribosome synthesis in the model

In previous work, Elf et al. (Elf et al., 2006) proposed a similar mathematical model, which accounts very generally for the inhibition of growth by intracellular antibiotic and predicts a bistability in growth rate, due to a positive feedback in which antibiotic inhibits growth, hence slowing dilution and allowing more antibiotic to build up in the cell. Elf et al. consider the specific case of ribosome-targeting antibiotics, taking into account (equilibrated) binding of antibiotic to ribosomes and a linear relation between free ribosome concentration and growth rate, as well as dilution of intracellular antibiotic due to growth – but not accounting for up-regulation of ribosome synthesis upon translation inhibition. This model predicts that the IC$_{50}$ should be a universally increasing (non-linear) function of the drug-free growth rate $\lambda_0$, and thus cannot explain our experimental data for tetracycline and chloramphenicol, where the IC$_{50}$ decreases with $\lambda_0$. In fact upregulation of ribosome production is essential to explain this behaviour. Solving our model as in section 1.2.1 above, but using a constant ribosome synthesis rate $s$ (i.e. assuming that the total ribosome concentration remains fixed at its drug-free value $r_{\text{tot}} = r_{\text{min}} + \lambda_0/\kappa_t$, and using the steady-state condition $s = \lambda r_{\text{tot}}$), gives the result

$$\text{IC}_{50} = \frac{\lambda_0^2 \left(1 + \frac{k_{\text{on}}}{\kappa_t}\right) + 2\lambda_0 \left(P_{\text{out}} + k_{\text{off}}\right) + 4P_{\text{out}}k_{\text{off}}}{4k_{\text{on}}P_{\text{in}}}$$

which is an increasing function of $\lambda_0$ for all parameter values. Thus the results that we see for the bacteriostatic antibiotics cannot be explained without including ribosome upregulation upon translational inhibition.
Figure S4: Comparison between the model predictions and our experimental data, when we obtain $\lambda_0^*$ and $IC_{50}^*$ by fitting $IC_{50}(\lambda_0)$ rather than $\lambda(\alpha_{ex})/\lambda_0$. A global fit with shared parameters is used such that for each antibiotic, $\lambda_0^*$ is assumed to be common to all media but $IC_{50}^*$ is allowed to differ between glucose-based and glycerol-based media. A - D: Dependence of the half-inhibition concentration $IC_{50}$ on the drug-free growth rate $\lambda_0$. Black lines show the universal curve (Eq. 10 in the main text); brown lines show its linear limits. Solid and dashed lines are for glycerol-based and glucose-based media. Note that the data for tetracycline and chloramphenicol (C and D) are plotted versus inverse drug-free growth rate ($1/\lambda_0$). Symbols are as in Fig. 1 of the main text; brown lines show its linear limits. Solid and dashed lines are for glycerol-based and glucose-based media. Note that the data for tetracycline and chloramphenicol (C and D) are plotted versus inverse drug-free growth rate ($1/\lambda_0$). Symbols are as in Fig. 1. of the main text. The fit parameters are as follows. Streptomycin: $\lambda_0^* = 0.45h^{-1}, IC_{50}^* = 0.3\mu g/ml$ (glucose), $IC_{50}^* = 0.2\mu g/ml$ (glucose), $IC_{50}^* = 0.1\mu g/ml$ (glycerol), Tetracycline: $\lambda_0^* = 3.9h^{-1}, IC_{50}^* = 0.6\mu M$ (glucose), $IC_{50}^* = 0.3\mu M$ (glycerol), Chloramphenicol: $\lambda_0^* = 1.35h^{-1}, IC_{50}^* = 4.5\mu M$ (glucose), $IC_{50}^* = 2.9\mu M$ (glycerol). E - H: Growth inhibition curves for glycerol-based media, compared with the prediction of the full model (Eq. 7 of the main text) (solid lines), and with the theoretically-predicted forms in the limits of large or small $\lambda_0^*$ (dashed lines), using the same parameters as in panels A - D.

1.5 Susceptibility to kanamycin for the translation mutant (Fig. S5)

In the main text, we show that a mutant strain with impaired translation shows growth-dependent susceptibility to tetracycline that is in quantitative agreement with the predictions of the model for a reversible antibiotic (Fig. 6). The situation is more complex for our irreversible antibiotics, because the mutant is partially resistant to both streptomycin and kanamycin, implying
that molecular binding and transport parameters for these drugs are likely to be altered as well as the translation rate. Nevertheless, growth medium-dependent inhibition curves for the mutant on all 6 media are well-fitted by the model (Fig. S4, using numerical solution of the cubic equation, Eq. 7 of the main text; raw data is given in Table S6). The values for $\lambda^*_0$ and $IC_{50}^*$ for the mutant that emerge from these fits are: glucose: $\lambda^*_0 = 0.29h^{-1}$; $IC_{50}^* = 0.81\mu g/ml$, glycerol: $\lambda^*_0 = 0.21h^{-1}$; $IC_{50}^* = 0.37\mu g/ml$. Comparing these to the results obtained from equivalent data fits for the wild-type strain (Fig. 3; glucose: $\lambda^*_0 = 0.47h^{-1}$; $IC_{50}^* = 0.26\mu g/ml$, glycerol: $\lambda^*_0 = 0.17h^{-1}$; $IC_{50}^* = 0.05\mu g/ml), we see that the fold-changes of $\lambda^*_0$ and $IC_{50}^*$ in the mutant are different. This suggests that, as expected, molecular parameters for transport and/or binding of kanamycin are affected by the mutation as well as the translation rate $\kappa_t$.

Figure S5: Growth-medium dependent growth-inhibition curves for the translation mutant in the presence of kanamycin. The symbols show experimental data for glycerol-based media A and glucose-based media B; symbols are as in Fig. 1. of the main text. The solid lines show fits of the model to the data, using numerical solution of the cubic equation (Eq. 7 of the main text). Separate fits were performed for glycerol and glucose-based media, but for each media type all 3 inhibition curves were fit simultaneously. The resulting fit parameters were: glycerol: $\lambda^*_0 = 0.21h^{-1}$; $IC_{50}^* = 0.37\mu g/ml$, glucose: $\lambda^*_0 = 0.29h^{-1}$; $IC_{50}^* = 0.81\mu g/ml$.

1.6 Model predictions with growth-state dependent transport parameters

Here we explore the effects of including a growth-rate dependence for either the antibiotic influx rate $P_{in}$ or the efflux rate $P_{out}$. In our study $P_{in}$ and $P_{out}$ have been assumed to be constants, but it is possible that as the cell becomes inhibited by antibiotic, it will become either more permeable to antibiotic ($P_{in}$ increases as $\lambda$ decreases), or less able to expel antibiotic ($P_{out}$ decreases as $\lambda$ decreases). For simplicity we assume these dependences to be linear, and we also assume that in the absence of antibiotic the transport rates take fixed values $P_{in}^0$ and $P_{out}^0$ which are growth-medium independent.
Growth-rate dependent influx

Let us first assume a linear increase in the influx rate $P_{\text{in}}$ as the growth rate decreases under antibiotic treatment. This can be described by the functional form

$$P_{\text{in}}(\lambda) = P_{\text{in}}^0 + \Delta P_{\text{in}} \left(1 - \frac{\lambda}{\lambda_0}\right)$$

where $\Delta P_{\text{in}} = P_{\text{in}}^{\text{max}} - P_{\text{in}}^0$ with $P_{\text{in}}^{\text{max}}$ being the influx rate at zero growth rate. Inserting this into the generic cubic equation Eq. (S14), we obtain

$$0 = -\left(\frac{\lambda}{\lambda_0}\right)^3 \left[1 + \frac{\kappa_t}{k_{\text{on}}}\right] \lambda_0^2 + \left(\frac{\lambda}{\lambda_0}\right)^2 \left[1 + \frac{\kappa_t}{k_{\text{on}}}\right] \lambda_0^2 \left(\frac{P_{\text{out}} + k_{\text{off}}}{k_{\text{on}}}\right)^2 - \left(\frac{\lambda}{\lambda_0}\right)^2 \left(\frac{\kappa_t}{k_{\text{on}}}\right) \lambda_0^2\right] + P_{\text{out}} K_{Dk_t}$$

(S29)

We now define the parameter combinations $\lambda_0^* = 2\sqrt{P_{\text{out}} k_t K_D}$ (as before) and $\text{IC}_{50}^{**} = \lambda_0^*/(2P_{\text{in}}^{\text{max}})$ (note that this is slightly different to our previous definition of $\text{IC}_{50}^{**}$). We also, as before, divide through by $(\lambda_0^*)^2$, and assume that $k_{\text{on}} \gg \kappa_t$ and that $(P_{\text{out}} + k_{\text{off}})/\sqrt{P_{\text{out}} k_{\text{off}}}$ does not become very large. This results in a modified form of the cubic equation, Eq. 7 of the main text:

$$0 = -\left(\frac{\lambda}{\lambda_0}\right)^3 - \left(\frac{\lambda}{\lambda_0}\right)^2 \left[1 + \frac{\alpha_{\text{ex}}}{2\text{IC}_{50}^{**}} \left(\frac{\lambda_0^*}{\lambda_0}\right) \left(\frac{\Delta P_{\text{in}}}{P_{\text{in}}^{\text{max}}}\right)\right] + \left(\frac{\lambda}{\lambda_0}\right)^2 \left[\alpha_{\text{ex}} \left(\frac{\lambda_0^*}{\lambda_0}\right) \left(\frac{P_{\text{in}}^{\text{max}}}{\lambda_0}\right) + \frac{1}{4} \left(\frac{\lambda_0^*}{\lambda_0}\right)\right] - \frac{1}{4} \left(\frac{\lambda_0^*}{\lambda_0}\right)^2$$

(S30)

To determine how the susceptibility varies with drug-free growth rate $\lambda_0$, we set $\alpha_{\text{ex}} = 1\text{IC}_{50}$ and $\lambda = \lambda_0/2$. This gives

$$\frac{\text{IC}_{50}}{\text{IC}_{50}^{**}} = \frac{1}{2} \left(\frac{P_{\text{in}}^{\text{max}}}{P_{\text{in}}^0 + P_{\text{in}}^{\text{max}}}\right) \left[\left(\frac{\lambda_0}{\lambda_0^*}\right) + \left(\frac{\lambda_0^*}{\lambda_0}\right)\right]$$

(S31)

Thus the modified model behaves in the same way as our “basic” model; the IC$_{50}$ is simply scaled by a constant.

Growth-rate dependent efflux

Next let us suppose instead that the efflux rate changes with growth rate under antibiotic challenge. This can be described by the functional form

$$P_{\text{out}}(\lambda) = P_{\text{out}}^{\text{min}} + \Delta P_{\text{out}} \left(\frac{\lambda}{\lambda_0}\right)$$

where $P_{\text{out}}^{\text{min}}$ is the efflux rate when cell growth is zero and $\Delta P_{\text{out}} = P_{\text{out}}^0 - P_{\text{out}}^{\text{min}}$, with $P_{\text{out}}^0$ being the drug-free efflux rate.
Substituting this into the generic cubic equation, Eq. S14, and defining the parameter combinations $\lambda^{**} = 2\sqrt{P_{\text{min}}^{\text{out}} K_D}$ and $\text{IC}_{50}^{**} = \lambda_0^{**} \Delta r/(2P_{\text{in}})$, and following the same procedure as in the “basic” model (including assuming that $k_{\text{on}} \gg \kappa_t$ and that $(P_{\text{min}}^{\text{out}} + k_{\text{off}})/\sqrt{P_{\text{min}}^{\text{out}} k_{\text{off}}}$ does not become very large), we eventually obtain the following cubic equation:

$$0 = -\left(\frac{\lambda}{\lambda_0}\right)^3 \left[ \left(\frac{\lambda_0}{\lambda^{**}}\right)^2 + \frac{1}{4} \frac{\Delta P_{\text{out}}}{\lambda_0} \right] + \left(\frac{\lambda}{\lambda_0}\right)^2 \left[ \left(\frac{\lambda}{\lambda^{**}}\right)^2 + \frac{1}{4} \frac{\Delta P_{\text{out}}}{\lambda_0} \right] + \left(\frac{\lambda}{\lambda_0}\right) \left[ -\frac{a_{\text{ex}}}{2\text{IC}_{50}^{**}} \lambda_0 - \frac{1}{4} \frac{\Delta P_{\text{out}}}{\lambda_0} \right] + \frac{1}{4} \left(\frac{\lambda}{\lambda_0}\right)^2 \left[ 1 + \frac{1}{2} \frac{\Delta P_{\text{out}}}{\lambda_0} \right] \left(\frac{\lambda}{\lambda_0}\right)^2 - \frac{1}{4} \left(\frac{\lambda}{\lambda_0}\right)^2 \right] \right]$$

(S32)

We also note that $\frac{1}{4} \frac{\Delta P_{\text{out}}}{\lambda_0} \approx 0$ under the same assumptions as above. This then leads to

$$0 = \left(\frac{\lambda}{\lambda_0}\right)^3 \left[ 1 - \frac{1}{4} \frac{\Delta P_{\text{out}}}{\lambda_0} \right] \left(\frac{\lambda^{**}}{\lambda_0}\right)^2 + \left(\frac{\lambda}{\lambda_0}\right) \left[ \frac{a_{\text{ex}}}{2\text{IC}_{50}^{**}} \lambda_0 + \frac{1}{4} \left[ 1 - \frac{\Delta P_{\text{out}}}{\lambda_0} \right] \left(\frac{\lambda^{**}}{\lambda_0}\right)^2 \right] \left(\frac{\lambda}{\lambda_0}\right)^2 - \frac{1}{4} \left(\frac{\lambda}{\lambda_0}\right)^2$$

(S33)

which looks very similar to Eq. 7 of the main text, with some extra terms in $\Delta P_{\text{out}}^{\text{in}}$.

To determine how the IC$_{50}$ depends on the drug-free growth rate $\lambda_0$, we set $a_{\text{ex}} = \text{IC}_{50}$ and $\lambda = \lambda_0/2$. This gives

$$\frac{\text{IC}_{50}}{\text{IC}_{50}^{**}} = \frac{1}{2} \left[ \frac{\lambda_0}{\lambda^{**}} + \frac{\lambda^{**}}{\lambda_0} \left[ 1 + \frac{1}{2} \frac{\Delta P_{\text{out}}^{\text{in}}}{\lambda_0} \right] \right]$$

(S34)

Once again, this result has the same structure as the key result of our “basic” model, Eq. 10 of the main text. Here, however, the turning point between “reversible” and “irreversible” behaviour is shifted by a constant.
1.7 Sample growth curves (Fig. S6)

Fig. S6 shows typical growth curves for our experiments, in the presence and absence of antibiotic. Here we show data for growth of our wild-type strain *E. coli* MG1655 on glucose with casamino acids, in the absence of antibiotic and in the presence of increasing concentrations of chloramphenicol. Growth curves for the other drugs are qualitatively similar. The vertical axis shows the number of cell doublings, as computed from measurements of the optical density at 600nm (OD$_{600}$). In all our experiments, we were careful to maintain cell cultures in the exponential phase of growth by appropriate dilution of the growth medium.

![Chloramphenicol in glucose casamino acids](image)

Figure S6: Sample growth curves. Different symbols correspond to replicates done on different days. Light scattering at 600 nm (OD600) was measured through a 1 cm quartz cuvette. Samples were taken from test tube cultures adapted to exponential growth in a waterbath shaker at 37C. Tubes were removed from the shaker less than 10 seconds during sampling. To ease comparison, data has been normalized relative to the OD600 at t=0. The data shown is from cells grown in glucose casamino acids medium, with 0 µM, 2 µM, 4 µM, 8 µM, 12 µM and 16 µM chloramphenicol as indicated; similar growth curves are obtained in other growth media and antibiotic concentrations.
2 Supporting Tables

Table S1: Growth rate in the absence of antibiotics

| Medium          | Doubling Rate (dbl/h\(^{-1}\)) | Error  | Growth Rate \(\lambda_0\) (h\(^{-1}\)) | Repeats |
|-----------------|-------------------------------|--------|----------------------------------------|---------|
| Glucose RDM     | 2.42                          | 0.06   | 1.68                                   | 2       |
| Glycerol RDM    | 1.95                          | 0.00   | 1.35                                   | 2       |
| Glucose cAA     | 1.58                          | 0.00   | 1.09                                   | 2       |
| Glycerol cAA    | 1.22                          | 0.02   | 0.85                                   | 2       |
| Glucose MIN     | 0.92                          | 0.01   | 0.64                                   | 2       |
| Glycerol MIN    | 0.58                          | 0.02   | 0.40                                   | 6       |

Table S1: Growth rate in the absence of antibiotics (referred to in the text as “drug-free growth rate”). Abbreviations used in the table: Glucose RDM - Neidhardt’s rich defined MOPS medium (Teknova) with 0.2% (w/v) glucose; Glycerol RDM - Neidhardt’s rich defined MOPS medium (Teknova) with 0.2% (v/v) glycerol; Glucose cAA - Neidhardt’s minimal MOPS medium (Teknova) with 0.2% (w/v) glucose and 0.2% (w/v) casamino acids; Glycerol cAA - Neidhardt’s minimal MOPS medium (Teknova) with 0.2% (v/v) glycerol and 0.2% (w/v) casamino acids; Glucose MIN - Neidhardt’s minimal MOPS medium (Teknova) with 0.2% (w/v) glucose; Glycerol MIN - Neidhardt’s minimal MOPS medium (Teknova) with 0.2% (v/v) glycerol. Errors are expressed as standard deviation among repeats done on different days. Growth Rate = 0.693 \times\) Doubling Rate.
Table S2: Experimental data and error estimates for the plots of Fig. 1
| Glucose RDM (Rich MOPS medium with 0.2% glucose) | \( \lambda_0 = 1.68 \text{h}^{-1} \) |
|---|---|
| **Kanamycin** | **Streptomycin** |
| | |
| **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** | **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** |
| (\( \mu g/mL \)) | (dbl/h) | (/h) | | (\( \mu g/mL \)) | (dbl/h) | (/h) | |
| 0.2 | 2.31 | 0.07 | 1.60 | 3 | 0.3 | 2.26 | 0.02 | 1.57 | 2 |
| 0.3 | 2.21 | 0.03 | 1.53 | 3 | 0.4 | 2.06 | 0.02 | 1.42 | 2 |
| 0.4 | 1.26 | 0.04 | 0.87 | 3 | 0.5 | 1.56 | 0.07 | 1.08 | 2 |
| 0.5 | 0.54 | 0.02 | 0.37 | 2 | 0.6 | 0.82 | 0.09 | 0.57 | 2 |
| 0.6 | 0.33 | 0.04 | 0.23 | 3 | 0.7 | 0.00 | 0.00 | 0.00 | 2 |
| 0.7 | 0.08 | 0.06 | 0.05 | 2 | | | | | |

| Chloramphenicol | Tetracycline |
|---|---|
| **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** | **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** |
| (\( \mu M \)) | (dbl/h) | (/h) | | (\( \mu M \)) | (dbl/h) | (/h) | |
| 2 | 1.85 | 0.05 | 1.28 | 2 | 0.4 | 1.53 | 0.06 | 1.06 | 2 |
| 4 | 1.38 | 0.03 | 0.96 | 2 | 0.8 | 1.21 | 0.03 | 0.84 | 2 |
| 8 | 0.70 | 0.01 | 0.49 | 2 | 1.2 | 1.02 | 0.00 | 0.71 | 2 |
| 12 | 0.38 | 0.00 | 0.26 | 2 | 1.6 | 0.83 | 0.04 | 0.58 | 2 |
| 16 | 0.23 | 0.03 | 0.16 | 2 | 2 | 0.61 | 0.01 | 0.43 | 2 |

| Glycerol RDM (Rich MOPS medium with 0.2% glycerol) | \( \lambda_0 = 1.35 \text{h}^{-1} \) |
|---|---|
| **Kanamycin** | **Streptomycin** |
| | |
| **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** | **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** |
| (\( \mu g/mL \)) | (dbl/h) | (/h) | | (\( \mu g/mL \)) | (dbl/h) | (/h) | |
| 0.2 | 1.59 | 0.01 | 1.10 | 2 | 0.2 | 1.72 | 0.02 | 1.19 | 2 |
| 0.25 | 0.92 | 0.01 | 0.64 | 2 | 0.3 | 1.58 | 0.01 | 1.10 | 2 |
| 0.3 | 0.59 | 0.04 | 0.41 | 2 | 0.4 | 1.03 | 0.03 | 0.71 | 2 |
| 0.35 | 0.16 | 0.00 | 0.11 | 2 | 0.5 | 0.44 | 0.01 | 0.30 | 2 |
| | | | | 0.6 | 0.08 | 0.11 | 0.06 | 0.2 |
| | | | | 0.7 | 0.00 | n/a | 0.00 | 1 |

| Chloramphenicol | Tetracycline |
|---|---|
| **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** | **Conc.** | **Doubling Rate** | **Growth Rate** | **Repeat** |
| (\( \mu M \)) | (dbl/h) | (/h) | | (\( \mu M \)) | (dbl/h) | (/h) | |
| 2 | 1.19 | 0.05 | 0.83 | 2 | 0.2 | 1.36 | 0.04 | 0.94 | 2 |
| 2.5 | 1.04 | 0.00 | 0.72 | 2 | 0.4 | 1.11 | 0.06 | 0.77 | 2 |
| 3 | 0.94 | 0.04 | 0.65 | 2 | 0.6 | 0.87 | n/a | 0.60 | 1 |
| 6 | 0.50 | 0.04 | 0.34 | 2 | 0.8 | 0.73 | 0.04 | 0.50 | 2 |
| 8 | 0.36 | 0.03 | 0.25 | 2 | 1.2 | 0.44 | 0.00 | 0.31 | 2 |
| 12 | 0.27 | 0.03 | 0.19 | 2 | 1.6 | 0.39 | n/a | 0.27 | 1 |
| | | | | 2 | 0.35 | 0.04 | 0.24 | 2 |
Glucose cAA (MOPS medium with 0.2% glucose and 0.2% casamino acids) \( \lambda_0 = 1.09\text{h}^{-1} \)

| Kanamycin | Streptomycin |
|-----------|--------------|
| Conc. (\(\mu\text{g/mL}\)) | Doubling Rate (dbl/h) | Error | Growth Rate (h) | Repeats | Conc. (\(\mu\text{g/mL}\)) | Doubling Rate (dbl/h) | Error | Growth Rate (h) | Repeats |
| 0.2 | 1.55 | 0.02 | 1.08 | 2 | 0.1 | 1.52 | 0.08 | 1.05 | 2 |
| 0.3 | 1.41 | 0.03 | 0.98 | 2 | 0.3 | 1.36 | 0.04 | 0.94 | 2 |
| 0.35 | 1.02 | 0.02 | 0.71 | 2 | 0.4 | 1.07 | 0.08 | 0.74 | 3 |
| 0.4 | 0.58 | 0.00 | 0.40 | 2 | 0.5 | 0.40 | 0.14 | 0.27 | 2 |
| 0.45 | 0.15 | 0.01 | 0.10 | 2 | 0.6 | 0.03 | 0.04 | 0.02 | 2 |

Chloramphenicol

| Tetracycline |
|--------------|
| Conc. (\(\mu\text{M}\)) | Doubling Rate (dbl/h) | Error | Growth Rate (h) | Repeats |
| 2 | 1.06 | 0.01 | 0.73 | 4 |
| 4 | 0.85 | 0.01 | 0.59 | 2 |
| 8 | 0.51 | 0.02 | 0.36 | 4 |
| 12 | 0.36 | 0.02 | 0.25 | 4 |
| 16 | 0.27 | 0.01 | 0.19 | 4 |

Glycerol cAA (MOPS medium with 0.2% glycerol and 0.2% casamino acids) \( \lambda_0 = 0.85\text{h}^{-1} \)

| Kanamycin | Streptomycin |
|-----------|--------------|
| Conc. (\(\mu\text{g/mL}\)) | Doubling Rate (dbl/h) | Error | Growth Rate (h) | Repeats | Conc. (\(\mu\text{g/mL}\)) | Doubling Rate (dbl/h) | Error | Growth Rate (h) | Repeats |
| 0.05 | 1.25 | 0.01 | 0.86 | 2 | 0.2 | 1.07 | 0.09 | 0.74 | 2 |
| 0.07 | 1.21 | 0.03 | 0.84 | 2 | 0.3 | 0.49 | 0.06 | 0.34 | 2 |
| 0.08 | 1.03 | 0.07 | 0.72 | 2 | 0.35 | 0.20 | 0.05 | 0.14 | 2 |
| 0.09 | 0.69 | n/a | 0.48 | 1 | 0.4 | 0.08 | 0.01 | 0.05 | 2 |
| 0.1 | 0.40 | 0.00 | 0.28 | 2 | 0.5 | 0.07 | 0.09 | 0.05 | 2 |
| 0.11 | 0.11 | n/a | 0.08 | 1 | 0.6 | 0.05 | 0.07 | 0.03 | 2 |

Chloramphenicol

| Tetracycline |
|--------------|
| Conc. (\(\mu\text{M}\)) | Doubling Rate (dbl/h) | Error | Growth Rate (h) | Repeats |
| 2 | 0.68 | 0.04 | 0.47 | 4 |
| 3 | 0.58 | 0.01 | 0.40 | 2 |
| 4 | 0.51 | 0.02 | 0.35 | 3 |
| 6 | 0.42 | 0.04 | 0.29 | 3 |
| 8 | 0.33 | 0.01 | 0.23 | 3 |
| 12 | 0.24 | 0.02 | 0.17 | 2 |
| 16 | 0.22 | 0.07 | 0.15 | 2 |
| Glucose MIN (MOPS medium with 0.2% glucose) $\lambda_0 = 0.64h^{-1}$ |
|---|
| **Kanamycin** | **Streptomycin** |
| **Conc.** ($\mu g/mL$) | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** | **Conc.** ($\mu g/mL$) | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** |
| 0.22 | 0.83 | 0.07 | 0.57 | 2 | 0.1 | 0.96 | 0.66 | 0.04 | 3 |
| 0.24 | 0.55 | 0.01 | 0.38 | 2 | 0.15 | 0.92 | 0.64 | 0.05 | 3 |
| 0.25 | 0.46 | 0.13 | 0.32 | 2 | 0.2 | 0.92 | 0.64 | 0.02 | 3 |
| 0.26 | 0.41 | 0.01 | 0.29 | 2 | 0.25 | 0.81 | 0.56 | 0.07 | 3 |
| 0.28 | 0.13 | 0.03 | 0.09 | 2 | 0.3 | 0.68 | 0.47 | 0.08 | 3 |
| | | | | | | 0.35 | 0.54 | 0.37 | 0.09 | 2 |
| | | | | | | 0.38 | 0.03 | 0.02 | 0.00 | 2 |
| | | | | | | 0.42 | 0.00 | 0.00 | 0.02 | 2 |

| Glycerol MIN (MOPS medium with 0.2% glycerol) $\lambda_0 = 0.40h^{-1}$ |
|---|
| **Kanamycin** | **Streptomycin** |
| **Conc.** ($\mu g/mL$) | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** | **Conc.** ($\mu g/mL$) | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** |
| 0.04 | 0.55 | 0.01 | 0.38 | 2 | 0.05 | 0.58 | 0.01 | 0.40 | 2 |
| 0.05 | 0.55 | 0.06 | 0.38 | 2 | 0.125 | 0.55 | 0.02 | 0.38 | 2 |
| 0.06 | 0.36 | 0.01 | 0.25 | 2 | 0.15 | 0.48 | 0.04 | 0.33 | 2 |
| 0.07 | 0.21 | 0.03 | 0.14 | 2 | 0.2 | 0.28 | 0.01 | 0.19 | 2 |
| 0.08 | 0.12 | 0.04 | 0.08 | 2 | 0.25 | 0.13 | 0.00 | 0.09 | 2 |
| | | | | | | 0.3 | 0.09 | 0.00 | 0.06 | 2 |

| Chloramphenicol | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** |
|---|
| **Conc.** ($\mu g/mL$) | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** |
| 0.04 | 0.55 | 0.01 | 0.38 | 2 |
| 0.05 | 0.55 | 0.06 | 0.38 | 2 |
| 0.06 | 0.36 | 0.01 | 0.25 | 2 |
| 0.07 | 0.21 | 0.03 | 0.14 | 2 |
| 0.08 | 0.12 | 0.04 | 0.08 | 2 |

| Tetracycline | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** |
|---|
| **Conc.** ($\mu$M) | **Doubling Rate** (dbl/h) | **Error** | **Growth Rate** (/h) | **Repeats** |
| 2 | 0.71 | 0.05 | 0.49 | 3 |
| 4 | 0.55 | 0.01 | 0.38 | 2 |
| 8 | 0.35 | 0.01 | 0.24 | 2 |
| 12 | 0.24 | 0.03 | 0.17 | 2 |
| 16 | 0.18 | 0.02 | 0.13 | 2 |
| 2 | 0.48 | 0.02 | 0.33 | 3 |
| 4 | 0.37 | 0.01 | 0.25 | 3 |
| 6 | 0.28 | 0.01 | 0.19 | 2 |
| 8 | 0.23 | 0.01 | 0.16 | 2 |
| 12 | 0.18 | 0.00 | 0.12 | 2 |
| 16 | 0.15 | 0.00 | 0.11 | 2 |
Table S3: Half-inhibition concentration $IC_{50}$ and fitted values of $IC_{50}^*$ and $\lambda_0^*$

| Medium    | Streptomycin | Kanamycin |
|-----------|--------------|-----------|
|           | $IC_{50}$ ($\mu$g/ml) | $IC_{50}^*$ ($\mu$g/ml) | $\lambda_0^*$ (h$^{-1}$) | $IC_{50}$ ($\mu$g/ml) | $IC_{50}^*$ ($\mu$g/ml) | $\lambda_0^*$ (h$^{-1}$) |
| Glu. RDM  | 0.55 ± 0.01  | 0.36 ± 0.01 | 0.57 ± 0.4 | 0.407 ± 0.005 |
| Glu. cAA | 0.44 ± 0.015 | 0.36 ± 0.003 | 0.31 ± 0.01 | 0.094 ± 0.004 |
| Gly. MIN  | 0.354 ± 0.02 | 0.25 ± 0.01  | 0.246 ± 0.003 |
| Gly. cAA | 0.28 ± 0.015 | 0.189 ± 0.003 | 0.31 ± 0.01 | 0.094 ± 0.004 |
| Gly. MIN  | 0.196 ± 0.01 | 0.065 ± 0.004 |

| Medium    | Tetracycline | Chloramphenicol |
|-----------|--------------|-----------------|
|           | $IC_{50}$ ($\mu$M) | $IC_{50}^*$ ($\mu$M) | $\lambda_0^*$ (h$^{-1}$) | $IC_{50}$ ($\mu$M) | $IC_{50}^*$ ($\mu$M) | $\lambda_0^*$ (h$^{-1}$) |
| Glu. RDM  | 0.8 ± 0.05   | 0.359 ± 0.008   | 6.3 ± 0.4 | 4.8 ± 0.3 |
| Glu. cAA | 1.0 ± 0.1    | 0.229 ± 0.002   | 5.24 ± 0.09 | 2.65 ± 0.4 |
| Gly. MIN  | 1.45 ± 0.1   | 5.7 ± 0.6 |

Table S3: Half-inhibition concentration inferred from inhibition curves. The half inhibition concentration, $IC_{50}$, was obtained from the growth inhibition curves (Table S2); its error was estimated by-eye, taking into account the errors on the inhibition curves. The parameters $IC_{50}^*$ and $\lambda_0^*$ are obtained by fitting the nutrient-dependent growth inhibition curves to the prediction of the model, obtained by solving the cubic equation, Eq. 9 in the main text. Media abbreviations are as in Table S1.
Table S4: Comparison of fitted parameters to literature values.

| Antibiotic   | This Study                      | Literature                                |
|--------------|---------------------------------|-------------------------------------------|
|              | $\text{IC}_{50}^*$ | $\lambda_0^*$ (h$^{-1}$) | $\text{IC}_{50}^*$ | $\lambda_0^*$ (h$^{-1}$) |
| Streptomycin | 0.36µg/ml (Glucose) | 0.57 (Glucose) | 0 - 5.7µg/ml | 0 - 0.22 |
|              | 0.19µg/ml (Glycerol) | 0.31 (Glycerol) |
| Kanamycin    | 0.26µg/ml (Glucose) | 0.47 (Glucose) | Unknown | Unknown |
|              | 0.05µg/ml (Glycerol) | 0.17 (Glycerol) |
| Tetracycline | 0.36µM (Glucose) | 6.3 (Glucose) | 0.04 - 0.4µM | 3.1 - 24 |
|              | 0.23µM (Glycerol) | 5.2 (Glycerol) |
| Chloramphenicol | 4.5µM (Glucose) | 1.3 (Glucose) | 0.008 - 1.86µM | 1.35 - 6.0 |
|              | 2.5µM (Glycerol) | 1.8 (Glycerol) |
Table S5: RNA/protein ratio for the translation mutant

| Growth medium | Growth rate $\lambda_0$ (/h) | Error | RNA/Protein ($\mu$g/$\mu$g) | Error |
|---------------|-------------------------------|-------|-------------------------------|-------|
| Glucose RDM  • | 1.36                          | 0.02  | 0.68                          | 0.01  |
| Glycerol RDM ■ | 1.08                          | 0.01  | 0.56                          | 0.01  |
| Glucose cAA  • | 0.86                          | 0.01  | 0.42                          | 0.03  |
| Glycerol cAA ■ | 0.67                          | 0.01  | 0.39                          | 0.01  |
| Glucose MIN  • | 0.47                          | 0.01  | 0.35                          | 0.01  |
| Glycerol MIN ■ | 0.38                          | 0.01  | 0.29                          | 0.01  |

Table S5: Error is the standard deviation between two experiments done on different days. From a linear least-squares fit, the slope of the mutant data as plotted in Fig. 6 of the main text is $0.387 \mu$g RNA/ $\mu$g Protein · h. From a corresponding linear least-squares fit to the data in Scott et al. (Scott et al., 2010), the slope of the wildtype data is $0.250 \mu$g RNA/ $\mu$g Protein · h. The translational capacity $\kappa_t$ is directly proportional to the inverse slope. As a result, the ratio of the translational capacity of the mutant to that of the wildtype is $\kappa_t^{\text{MUT}} = (0.250)/(0.387) \kappa_t^{\text{WT}} = 0.65 \kappa_t^{\text{WT}}$. 
Table S6: Antibiotic growth-inhibition data for the translation mutant

**Glucose RDM $\lambda_0 = 1.36h^{-1}$**  
**Glycerol RDM $\lambda_0 = 1.08h^{-1}$**

| Kanamycin | Kanamycin |
|-----------|-----------|
| Concentration ($\mu g/mL$) | Doubling Rate (dbl/h) | Growth Rate (h) | Concentration ($\mu g/mL$) | Doubling Rate (dbl/h) | Growth Rate (h) |
|-----------|-------------|----------------|-----------|-------------|----------------|----------------|
| 0.5       | 1.96        | 1.35           | 0.8       | 0.97        | 0.67           |
| 1.0       | 0.92        | 0.63           | 0.9       | 0.74        | 0.51           |
| 1.2       | 0.88        | 0.61           | 1.0       | 0.54        | 0.37           |
| 1.4       | 0.79        | 0.55           | 1.1       | 0.33        | 0.23           |
| 1.6       | 0.75        | 0.52           | 1.2       | 0.16        | 0.11           |
| 1.8       | 0.66        | 0.46           | 1.3       | 0.10        | 0.07           |
| 2.0       | 0.18        | 0.13           |           |             |                |

**Glucose cAA $\lambda_0 = 0.86h^{-1}$**  
**Glycerol cAA $\lambda_0 = 0.67h^{-1}$**

| Kanamycin | Kanamycin |
|-----------|-----------|
| Concentration ($\mu g/mL$) | Doubling Rate (dbl/h) | Growth Rate (h) | Concentration ($\mu g/mL$) | Doubling Rate (dbl/h) | Growth Rate (h) |
|-----------|-------------|----------------|-----------|-------------|----------------|----------------|
| 0.8       | 1.03        | 0.71           | 0.4       | 1.0         | 0.69           |
| 0.9       | 0.95        | 0.66           | 0.5       | 1.0         | 0.69           |
| 1.0       | 0.85        | 0.59           | 0.6       | 0.89        | 0.62           |
| 1.15      | 0.92        | 0.64           | 0.65      | 0.38        | 0.26           |
| 1.2       | 0.71        | 0.49           | 0.7       | 0.29        | 0.20           |
|           |             |                | 0.75      | 0           | 0              |

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| Glucose MIN | $\lambda_0 = 0.47h^{-1}$ |
|-------------|-------------------------|
| **Tetracycline** | **Kanamycin** |
| Conc. ($\mu$M) | Doubling Rate (dbl/h) | Growth Rate (/h) | Conc. ($\mu$g/mL) | Doubling Rate (dbl/h) | Growth Rate (/h) |
| 0.4 | 0.56 | 0.39 | 0.8 | 0.54 | 0.38 |
| 0.8 | 0.47 | 0.33 | 0.9 | 0.44 | 0.31 |
| 1.0 | 0.44 | 0.30 | 1.0 | 0.33 | 0.23 |
| 1.2 | 0.38 | 0.26 | 1.1 | 0.26 | 0.18 |
| 1.4 | 0.35 | 0.24 | 1.15 | 0.21 | 0.15 |
| 1.8 | 0.31 | 0.21 | 1.2 | 0.03 | 0.02 |

| Glycerol MIN | $\lambda_0 = 0.38h^{-1}$ |
|-------------|-------------------------|
| **Tetracycline** | **Kanamycin** |
| Conc. ($\mu$M) | Doubling Rate (dbl/h) | Growth Rate (/h) | Conc. ($\mu$g/mL) | Doubling Rate (dbl/h) | Growth Rate (/h) |
| 0.4 | 0.42 | 0.29 | 0.16 | 0.54 | 0.38 |
| 0.8 | 0.31 | 0.22 | 0.2 | 0.42 | 0.29 |
| 1.0 | 0.29 | 0.20 | 0.24 | 0.29 | 0.20 |
| 1.2 | 0.26 | 0.18 | 0.28 | 0.20 | 0.14 |
| 1.4 | 0.24 | 0.16 | 0.32 | 0.0 | 0.0 |
| 1.8 | 0.20 | 0.14 | | | |
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