Modeling the emergence of antibiotic resistance in the environment: An analytical solution for the minimum selection concentration

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Supplemental material (23 pages)

1. Supplemental text on model definitions, derivation, and assumptions

1.1. Selection coefficient

Relative fitness between strains is a key determining factor governing bacterial competition (1). Relative fitness is often conceptualized as a fitness cost (2–4), or as a selection coefficient (4–9). These parameters indicate the intrinsic fitness difference between bacteria strains (e.g., sensitive vs. resistant), as a result of a specific antibiotic resistance mechanism. To achieve mathematical consistency among the different terms, we now summarize how the selection coefficient is measured in the laboratory as a rate (the absolute selection coefficient (σ [h^{-1}]) or in relative terms (sc, adimensional) and then relate it to the fitness cost (fc).

1.1.1. Absolute selection coefficient (σ)

The selection coefficient is a measurement of the impact of a particular heritable trait on intrinsic growth rate, and consequent rate of selection for or against that trait in competition experiments. In evaluating resistance mechanisms, selection can favor sensitive strains (sensitive more fit than resistant), resistant strains (resistant more fit than sensitive), or exhibit no difference between strains. We focus on the common case where selection favors sensitive strains.

Selection experiments calculate relative fitness based on differences in the ratio of measured biomass density (or cell count) over time (B_t) between strains(1, 8, 10):

$$\sigma = [\ln(B_{r,t}/B_{s,t}) - \ln(B_{r,t=0}/B_{s,t=0})]/t \quad \text{(S1)}$$

Here, the subscript t = 0 indicates biomass measured at the beginning of the experiment. The following calculations demonstrate that σ is in units of t^{-1} (e.g., h^{-1}):

$$B_{r,t} = B_{r,t=0} \times \left(e^{N_{int,r}t}\right); B_{s,t} = B_{s,t=0} \times \left(e^{N_{int,s}t}\right) \quad \text{(S2)}$$

$$\sigma = \frac{\ln\left(\frac{B_{r,t=0} \times e^{N_{int,r}t}}{(B_{s,t=0} \times e^{N_{int,s}t}}\right) - \ln\left(\frac{B_{r,t=0}}{B_{s,t=0}}\right)}{t} = \frac{\ln\left(\frac{e^{N_{int,r}t}}{e^{N_{int,s}t}}\right)}{t} = \frac{\ln\left(e^{N_{int,r}t}\right) - \ln\left(e^{N_{int,s}t}\right)}{t} = N_{int,r} - N_{int,s} \quad \text{(S3)}$$

where $N_{int,s}$ and $N_{int,r}$ are the intrinsic net growth rate in the absence of antibiotic (of sensitive (s) and resistant (r) bacteria), represented as:

$$N_{int} = R_{int} - D_{int} \quad \text{(S4)}$$

and R_{int} is intrinsic growth rate, and D_{int} is loss due to mortality or, in continuous cultures, dilution.

We see that σ is therefore equal to the difference in net growth rate between the two strains and has units of $[h^{-1}]$ as mentioned above. This is the experimental selection coefficient (σ) determined by Gullberg et al. and elsewhere (5, 6, 8, 11).

1.1.2. Dimensionless selection coefficient (sc)

It is also possible to define a dimensionless selection coefficient (sc) obtained by dividing the inverse of the selection coefficient (−σ) by the net growth rate of the sensitive strain (Eq. 9 in the text):

$$sc = -\frac{\sigma}{N_{int,s}} = \frac{N_{int,s} - N_{int,r}}{N_{int,s}} = 1 - \frac{N_{int,r}}{N_{int,s}} \quad \text{(S5)}$$

1.2. Converting MIC to EC_{50}

To convert the MIC value of a strain to its corresponding EC_{50} value, we first substitute a = MIC into the equation for death due to antibiotic (Eq. 3) to obtain $D_{ab}(\mathrm{MIC}) = k_{max} \frac{\mathrm{MIC}^{\kappa}}{\mathrm{MIC}^{\kappa} + (EC_{50})^{\kappa}}$. We will demonstrate this for the sensitive strain. We first recognize that:

$$D_{ab,s}(\mathrm{MIC}) = k_{max,s} \frac{(MIC_s)^{\kappa}}{(MIC_s)^{\kappa} + (EC_{50,s})^{\kappa}} = (R_{int} - D_{int} - N_{min}) \frac{(MIC_s)^{\kappa}}{(MIC_s)^{\kappa} + (EC_{50,s})^{\kappa}} = R_{int} - D_{int} \quad \text{(S6)}$$

based on $k_{max} = R_{int} - D_{int} - N_{min}$ (Eq. 4), and the observation of zero net growth rate at the MIC: i.e., $D_{ab,s}(MIC_s) = N_{int} = R_{int} - D_{int}$. Algebraically solving for the $EC_{50,s}$ term, we find that:

$$EC_{50,s} = MIC_s \left(\frac{-N_{min}}{R_{int} - D_{int}} \right)^{\frac{1}{\kappa}} \quad \text{(S7)}$$

Thus, for a given set of conditions (κ, R_{int}, D_{int}, N_{min},), $EC_{50,s}$ is simply a constant multiple of the sensitive strain MIC. For the resistant strain, the $EC_{50,r}$ is obtained by the same solution, substituting MIC_r and replacing R_{int} by (R_{int} + σ) per Eq. 2, such that:

$$EC_{50,r} = MIC_r \left(\frac{-N_{min}}{R_{int} + \sigma - D_{int}} \right)^{\frac{1}{\kappa}} \quad \text{(S8)}$$

These relationships can be employed to convert the generalized Hill equation formulation of $D_{ab}(a) = k_{max} \frac{a^{\kappa}}{a^{\kappa} + (EC_{50})^{\kappa}}$ (Eq. 3) to an MIC based formulation, by substituting the MIC equation

for EC_{50} (employing Eqs. S7 and S8, above). Recalling the net growth rates without antibiotic (i.e., $N_{int,s} = R_{int} - D_{int}$):

$$D_{ab,s}(a) = (N_{int,s} - N_{min}) \frac{a^{\kappa}}{a^{\kappa} + \frac{(-N_{min})}{N_{int,s}}(MIC_s)^{\kappa}} \text{ and } D_{ab,r}(a) = (N_{int,r} - N_{min}) \frac{a^{\kappa}}{a^{\kappa} + \frac{(-N_{min})}{N_{int,r}}(MIC_r)^{\kappa}} .$$

These are Eqs. 5 and 6, respectively in the text.

1.3. Derivation of MSC/MIC$_s$ ratio

To obtain MSC as a function of MIC_s, we begin by noting that at the MSC, the difference in net growth rate is equal to zero as defined in Eq. 8 in the text:

$$\Delta N(a = MSC) = N_{int,r} - N_{int,s} + D_{ab,s}(a = MSC) - D_{ab,r}(a = MSC) = 0$$

Incorporating Eqs. 5 and 6:

$$\Delta N(MSC) = N_{int,r} - N_{int,s} + (N_{int,s} - N_{min}) \frac{(MSC)^{\kappa}}{(MSC)^{\kappa} + \frac{(-N_{min})}{N_{int,s}}(MIC_s)^{\kappa}} - (N_{int,r} - N_{min}) \frac{(MSC)^{\kappa}}{(MSC)^{\kappa} + \frac{(-N_{min})}{N_{int,r}}(MIC_r)^{\kappa}} = 0 \quad \text{(S9)}$$

To obtain the ratio MSC/MIC_s, we divide numerator and denominator by MIC_s^{κ}:

$$\Delta N(MSC) = N_{int,r} - N_{int,s} + (N_{int,s} - N_{min}) \frac{(MSC/MIC_s)^{\kappa}}{(MSC/MIC_s)^{\kappa} + \frac{(-N_{min})}{N_{int,s}}} - (N_{int,r} - N_{min}) \frac{(MSC/MIC_s)^{\kappa}}{(MSC/MIC_s)^{\kappa} + \frac{(-N_{min})}{N_{int,s}}(MIC_r/MIC_s)^{\kappa}} \quad \text{(S10)}$$

$$= 0$$

From Eq. S10, the algebraic solution was obtained for $(MSC/MIC_s)^{\kappa}$ employing the Equations and Systems Solver ("solve" function) in MATLAB (Symbolic Math Toolbox, R2013a, MathWorks, Natick, MA, USA). The general solution satisfying this equation is:

$$(MSC/MIC_s)^{\kappa} = \frac{N_{min}(MIC_r/MIC_s)^{\kappa}(N_{int,r} - N_{int,s})}{(MIC_r/MIC_s)^{\kappa} N_{int,s}(N_{int,r} - N_{min}) + N_{int,r}(N_{min} - N_{int,s})} \quad \text{(S11)}$$

$$(MSC/MIC_s)^{\kappa} = \frac{(-N_{int,r} + N_{int,s})}{N_{int,s}\left(1 + \frac{N_{int,r}}{-N_{min}}\right) + \frac{N_{int,r}(N_{min} - N_{int,s})}{(MIC_r/MIC_s)^{\kappa}(-N_{min})}} = \frac{(N_{int,s} - N_{int,r})/N_{int,s}}{\left(1 + \frac{N_{int,r}}{-N_{min}}\right) - \frac{N_{int,r}(N_{int,s} - N_{min})}{N_{int,s}(MIC_r/MIC_s)^{\kappa}(-N_{min})}} \quad \text{(S12)}$$

In order to make the selection coefficient explicit, Eq. S11 can be transformed employing the dimensionless selection coefficient (sc) by introducing Eq. 9; i.e., $N_{int,s} - N_{int,r} = (sc\ N_{int,s})$, and $N_{int,r} = N_{int,s}(1 - sc)$, obtaining:

$$(MSC/MIC_s)^{\kappa} = \frac{-N_{min} N_{int,s}(MIC_r/MIC_s)^{\kappa} sc}{(MIC_r/MIC_s)^{\kappa} N_{int,s}(N_{int,r} - N_{min}) + N_{int,s}(1 - sc)(N_{min} - N_{int,s})} \quad \text{(S13)}$$

Dividing the numerator and denominator by $-N_{min}N_{int,s}(MIC_r/MIC_s)^{\kappa}$ obtains:

$$(MSC/MIC_s)^{\kappa}=\frac{sc}{\frac{(N_{int,r}-N_{min})}{-N_{min}}+\frac{(1-sc)(N_{min}-N_{int,s})}{-N_{min}(MIC_r/MIC_s)^{\kappa}}} \quad \text{(S14)}$$

and elevating both sides of the equation to power κ^{-1} and rearranging:

$$MSC/MIC_s=\left(\frac{sc}{1-\frac{N_{int,r}}{N_{min}}-\frac{(1-sc)\left(1-\frac{N_{int,s}}{N_{min}}\right)}{\left(\frac{MIC_r}{MIC_s}\right)^{\kappa}}}\right)^{\frac{1}{\kappa}} \quad \text{(S15)}$$

Finally, this is depicted with N_{min} as a negative term since the minimal growth rate is often negative, giving us Eqn. 10 in the text:

$$MSC/MIC_s=\left(\frac{sc}{1+\frac{N_{int,r}}{-N_{min}}-\frac{(1-sc)\left(1+\frac{N_{int,s}}{-N_{min}}\right)}{\left(\frac{MIC_r}{MIC_s}\right)^{\kappa}}}\right)^{\frac{1}{\kappa}}$$

To represent this in terms of the experimentally derived selection parameters (σ) in Gullberg et al. (8, 11), we simply note that $sc = -\sigma / N_{int,s}$ (Eq. 9):

$$MSC/MIC_s=\left(\frac{-\frac{\sigma}{N_{int,s}}}{1+\frac{N_{int,r}}{-N_{min}}-\frac{(1+\frac{\sigma}{N_{int,s}})\left(1+\frac{N_{int,s}}{-N_{min}}\right)}{\left(\frac{MIC_r}{MIC_s}\right)^{\kappa}}}\right)^{\frac{1}{\kappa}} \quad \text{(S16)}$$

1.4. Effect of assuming same κ and N_{min} for sensitive versus resistant strains

To arrive at an analytical solution for MSC/MIC_s (Eq. 10), it was necessary to assume identical N_{min} and κ for sensitive versus resistant strains. A simple Monte Carlo Simulation-based sensitivity analysis was employed to evaluate the importance of sensitive versus resistant strain N_{min} and κ, and consequently which growth parameter in which strain is most important, for predicting MSC. In the simulation, the MSC was directly calculated by solving for $\Delta N = 0$ in Eq. 8, while relaxing the assumptions of identical N_{min} and κ in Eqs. 5 and 6. Separate parameters

were established for sensitive ($N_{min,s}$, κ_s) versus resistant strains ($N_{min,r}$, κ_r), resulting in the following formulations of antibiotic dependent growth reduction to be substituted into Eq. 8:

$$D_{ab,s}(a) = (N_{int,s} - N_{min,s}) \frac{(a)^{\kappa_s}}{(a)^{\kappa_s} + \frac{(-N_{min,s})}{N_{int,s}} (MIC_s)^{\kappa_s}} \quad \text{(S17)}$$

$$D_{ab,r}(a) = (N_{int,r} - N_{min,r}) \frac{(a)^{\kappa_r}}{(a)^{\kappa_r} + \frac{(-N_{min,r})}{N_{int,r}} (MIC_r)^{\kappa_r}} \quad \text{(S18)}$$

Two scenarios were simulated, each including 20,000 parameter sets. In both scenarios, MIC_s was set at 20. In order to examine the influence of varying growth rate parameters in the presence of either small or large increases in resistance, MIC_r was set at 30 and 200 in the first and second scenarios, respectively. $N_{min,s}$, $N_{min,r}$, κ_s, and κ_r were separately selected from uniform distributions with fixed ranges, listed in Table S1. Intrinsic growth rates were fixed at $N_{int,s} = 2$, and $N_{int,r} = 1.8$. Sensitivity was estimated by comparing spearman rank correlation coefficients (ρ) between each of the four parameters and MSC.

In both scenarios, the predicted MSC was most sensitive to κ_s, and was not sensitive to either N_{min} value (Table S1). In Scenario 1 (MIC_s close to MIC_r), the MSC was moderately sensitive to κ_r, but in Scenario 2, where MIC_r was 10 times MIC_s, the MSC was only sensitive to κ_s. These results indicate that κ_s is the most important parameter to estimate empirically in order to predict MSC, and that κ_r only contributes to understanding MSC when MIC_r is quite close to MIC_s. As a result, the assumptions that $\kappa = \kappa_s = \kappa_r$ and $N_{min} = N_{min,s} = N_{min,r}$ will not impede prediction of MSC, provided that effort is made to determine κ_s, empirically.

2. Supplemental text on model evaluation methods

2.1. PRESS statistic and other model diagnostic statistics

For the convenience of the reader, we provide standard statistical definitions in this section of the Supplemental Material. Total and residual sum of squares (SSY and SSE, respectively) are the standard terms. The total sum of squares (SSY) is the calculation of sum of squared differences between each individual response (y_i) and the average response, $\bar{y}$:

$$SSY = \sum_{i=1}^{n} (y_i - \bar{y})^2.$$

In this study, y represents ΔN, the growth rate difference between sensitive and resistant strain. The residual sum of squares (SSE) is:

$$\mathrm{SSE} = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$$

where $\hat{y}_i$ = fitted model prediction for observation i. As always, model $R^2 = 1 - (\mathrm{SSE}/\mathrm{SSY})$. Analogous to SSE, the PRESS (predictive residual error sum of squares) statistic is a residual sum of squares between each empirical observation and model fit to the data set with that individual observation removed:

$$\mathrm{PRESS} = \sum_{i=1}^{n} (y_i - \hat{y}_{i,-i})^2$$

Here $\hat{y}_{i,-i}$ = prediction for observation i based on a model fitted using the data set with observation i removed. PRESS is therefore a jackknife estimate of dependence of model fit on individual observations; i.e., a leave-one-out cross-validation technique. Useful results for evaluating model fit include Q^2, the cross validated R^2:

$Q^2 = 1 - (\mathrm{PRESS}/\mathrm{TSS})$.

Q^2, and also PRESS/SSY are validation techniques that provide information regarding the predictive ability of the model for observations not employed in the estimation of the model. Q^2 is a direct measure of model predictive power, analogous to R^2, but for out-of-model observations (and therefore less susceptible to overfitting). When PRESS/SSY < 1, the model predicts the data better than chance, and further decreases of PRESS/SSY down towards 0 represent progressive improvements in model predictive ability (12). R^2 Q^2, and PRESS/SSY were calculated in MATLAB for all fitted models.

Table S1. Monte Carlo Simulation evaluate sensitivity of MSC to differing values of κ and N_{min} for susceptible (s) versus resistant (r) strains.

Parameter	Range (min, max)	Spearman ρ	
		Scenario 1 $MIC_s = 20$, $MIC_r = 30$	Scenario 2 $MIC_s = 20$, $MIC_r = 200$
κ_s	0.5, 10	+0.83	+0.97
κ_r	0.5, 10	−0.39	−0.09
$N_{min,s}$	−10, −1	−0.09	−0.11
$N_{min,r}$	−10, −1	+0.03	+0.003

Table S2. Complete results from all model fitting. Fitted: which parameters were varied to allow fitting to observed data. Averaged: whether data were averaged from each antibiotic concentration and strain, or raw data from each experiment. n: sample size. Initial κ: starting κ value in nonlinear estimation. CV κ range: range of κ results in leave one out cross validation. Q^2 = cross validated $R^2 = 1 - (PRESS/TSS)$.

Scenario:						Results:								
Compound	Taxa	Fitted	Averaged	Strains	n	Initial κ	κ	CV κ range	N_{min}	Q^2	R^2	PRESS/ SSY	PRESS/ SSE	Data Source
As	*E. coli*	K	No	2	20	1	0.7	0.7	−2	0.81	0.84	0.30	1.14	(11)
As	*E. coli*	K	Yes	2	5	1	0.7	0.7 − 0.8	−2	0.80	0.92	0.29	2.44	(11)
As	*E. coli*	κ, N_{min}	No	2	20	1	1.2	1.1 − 1.2	−0.2	0.88	0.91	0.12	1.30	(11)
As	*E. coli*	κ, N_{min}	Yes	2	5	1	1.2	1.1 − 1.2	−0.2	0.99	1.00	0.01	24.1	(11)
CIP	*E. coli*	κ	No	5	144	2	2.0	2.0	−2	0.77	0.78	0.31	1.03	(8)
CIP	*E. coli*	κ	Yes	5	24	2	2.0	1.9 − 2.0	−2	0.78	0.81	0.29	1.20	(8)
CIP [a]	*E. coli*	κ	Yes	4	18 [a]	2	2.1	2.1	−2	0.97	0.97	0.04	1.23	(8)
CIP	*E. coli*	κ, N_{min}	No	5	144	2	1.6	1.6 − 1.7	−5.2e+8 [b]	0.78	0.79	0.32	1.03	(8)
CIP	*E. coli*	κ, N_{min}	Yes	5	24	2	1.6	1.6 − 1.9	−3.8e+9 [b]	0.77	0.83	0.32	1.30	(8)
CIP [a]	*E. coli*	κ, N_{min}	Yes	4	18 [a]	2	2.4	2.3 − 2.4	−0.8	0.98	0.98	0.02	1.40	(8)
Cu	*E. coli*	κ	No	2	8	2	1.9	1.8 − 2.1	−2	0.43	0.73	0.80	2.13	(11)
Cu	*E. coli*	κ	Yes	2	4	2	1.9	1.8 − 3.1	−2	c	0.88	c	c	(11)
Cu	*E. coli*	κ, N_{min}	No	2	8	2	5.7	3.5 − 6.3	−0.0003	0.40	0.82	0.68	3.36	(11)
Cu	*E. coli*	κ, N_{min}	Yes	2	4	2	5.7	2.9 − 5.7	−0.0003	c	0.98	c	c	(11)
ERY	*E. coli*	κ	No	3	64	2	3.5	3.4 − 3.5	−2	0.93	0.94	0.07	1.06	(11)
ERY	*E. coli*	κ	Yes	3	11	2	3.4	3.3 − 3.5	−2	0.92	0.95	0.09	1.64	(11)
ERY	*E. coli*	κ, N_{min}	No	3	64	2	2.6	2.6	−4.0e+8 [b]	0.70	0.73	0.18	1.11	(11)
ERY	*E. coli*	κ, N_{min}	Yes	3	11	2	2.7	2.6	−3.4e+8 [b]	0.75	0.87	0.17	1.88	(11)
KAN	*E. coli*	κ	No	2	72	2	10.5	10.4 − 10.6	−2	−0.48	−0.47	0.43	1.01	(11)
KAN	*E. coli*	κ	Yes	2	5	2	10.5	7.1 − 10.8	−2	−19.5	−0.76	3.67	11.6	(11)
KAN	*E. coli*	κ, N_{min}	No	2	72	2	6.0	6.0 − 6.1	−1.2e+11 [b]	−7.31	−6.76	0.86	1.07	(11)
KAN	*E. coli*	κ, N_{min}	Yes	2	5	2	6.0	5.7 − 6.7	−1.5e+11 [b]	−16.2	−11.4	1.06	1.39	(11)
STR	Salmonella [d]	κ	No	2	87	2	5.0	5.0	−2	0.66	0.67	0.25	1.02	(8)
STR	Salmonella	κ	Yes	2	5	2	5.0	4.1 − 5.2	−2	−1.05	0.70	1.47	6.87	(8)
STR	Salmonella	κ, N_{min}	No	2	87	2	3.4	3.4	−8.1e+11 [b]	−0.22	−0.16	0.30	1.05	(8)
STR	Salmonella	κ, N_{min}	Yes	2	5	2	3.4	2.9	−1.0e+12 [b]	−2.35	−0.26	0.77	2.66	(8)
TET	*E. coli*	κ	No	3	60	2	1.6	1.6	−2	0.89	0.89	0.09	1.03	(11)
TET	*E. coli*	κ	Yes	3	10	2	1.6	1.6	−2	0.94	0.95	0.05	1.19	(11)
TET	*E. coli*	κ, N_{min}	No	3	60	2	2.1	2.1 − 2.2	−0.4	0.90	0.91	0.07	1.08	(11)
TET	*E. coli*	κ, N_{min}	Yes	3	10	2	2.0	1.8 − 2.2	−0.5	0.93	0.96	0.05	1.70	(11)

TET	Salmonella [d]	κ	No	2	154	1	1.2	1.2	−2	0.93	0.93	0.04	1.02	(8)
TET	Salmonella	κ	Yes	2	5	1	1.2	1.2	−2	0.97	0.99	0.01	2.14	(8)
TET	Salmonella	κ, N_{min}	No	2	154	1	1.2	1.2	−1.8	0.93	0.93	0.04	1.04	(8)
TET	Salmonella	κ, N_{min}	Yes	2	5	1	1.2	1.0 − 1.5	−2.1	0.91	0.99	0.04	7.70	(8)
TMP	*E. coli*	κ	No	2	118	2	2.5	2.5	−2	0.87	0.88	0.07	1.03	(11)
TMP	*E. coli*	κ	Yes	2	5	2	2.5	2.5	−2	0.99	0.99	0.01	1.19	(11)
TMP	*E. coli*	κ, N_{min}	No	2	118	2	2.2	2.1 − 2.2	−10.9	0.87	0.88	0.07	1.06	(11)
TMP	*E. coli*	κ, N_{min}	Yes	2	5	2	2.2	2.0 − 2.5	−10.3	0.96	0.99	0.02	4.17	(11)

a. Simulation with *gyrA1(S83L)* removed. b. Model fitting insensitive to N_{min}. c. Insufficient n for cross validation statistics (n = 4).
d. *Salmonella enterica* serovar Typhimurium LT2

Table S3. Comparison of MSC/MIC_s ratios experimentally observed (8, 11) versus predicted by fitted model (Eq. 10) for different resistance mechanisms and compounds. Fitted: which parameters (κ, N_{min}) were varied to allow fitting to observed data. Data are plotted in Fig. 3.

Compound	Taxa	Resistance gene	MSC observed (ng ml^{-1})	MIC_s (ng ml^{-1})	Ref.	MSC/MIC_s observed	MSC/MIC_s fitted κ	MSC/MIC_s fitted κ, N_{min}
Arsenite	*E. coli*	pUUH239.2	90 [a]	12,500 [a]	(11)	0.0072	0.0064	0.0077
Trimethoprim	*E. coli*	pUUH239.2	33	190	(11)	0.174	0.180	0.178
Tetracycline	*E. coli*	pUUH239.2	45	750	(11)	0.060	0.063	0.070
Tetracycline	*E. coli*	*tetRA*	30	750	(11)	0.040	0.014	0.021
Erythromycin	*E. coli*	pUUH239.2	3000	12,000	(11)	0.250	0.266	0.229
Erythromycin	*E. coli*	*mph*	< 200	12,000	(11)	< 0.017	0.074	0.044
Kanamycin	*E. coli*	pUUH239.2	470	750	(11)	0.627	0.656	0.532
Cu(II) sulfate	*E. coli*	pUUH239.2	90	1,300	(11)	0.069	0.035	0.079
Ciprofloxacin	*E. coli*	*GyrA1(S83L)*	b	23	(8)	0.0043	0.024	0.017
Ciprofloxacin	*E. coli*	*GyrA2(D87N)*	b	23	(8)	0.10	0.088	0.080
Ciprofloxacin	*E. coli*	*ΔmarR*	b	23	(8)	0.10	0.097	0.094
Ciprofloxacin	*E. coli*	*ΔacrR*	b	23	(8)	0.10	0.091	0.087
Streptomycin	Salmonella [c]	*rpsL105(K42R)*	b	4,000	(8)	0.25	0.383	0.290
Tetracycline	Salmonella	*cobA367::Tn10dtet*	b	1,500	(8)	0.01	0.0077	0.0077

a. Units are μM. b. Not reported in study. c. *Salmonella enterica* serovar Typhimurium LT2

Table S4. Laboratory growth parameters employed to illustrate ranges in MSC/MIC_s ratios. For all simulations, MICr was set at 10*MICs.

Compound	κ	MIC_s [μg ml^{-1}]	N_{int} [h^{-1}]	N_{min} [h^{-1}]	Reference
Ciprofloxacin #1	1.42	0.7	1.59	−15.7	Ankomah et al. (13)
Ampicillin #1	4.53	3.47	1.57	−1.16	Ankomah et al. (13)
Tetracycline #1	1.46	0.92	1.30	−8.32	Ankomah et al. (13)
Tobramycin	2.67	1.2	1.08	−16.6	Ankomah et al. (13)
Ciprofloxacin #2	1.1	0.03	0.88	−6.5	Regoes et al. (14)
Ampicillin #2	0.75	8	0.75	−4.0	Regoes et al. (14)
Rifampin	2.5	8	0.70	−4.3	Regoes et al. (14)
Streptomycin	1.9	32	0.89	−8.8	Regoes et al. (14)
Tetracycline #2	0.61	1	0.81	−8.1	Regoes et al. (14)

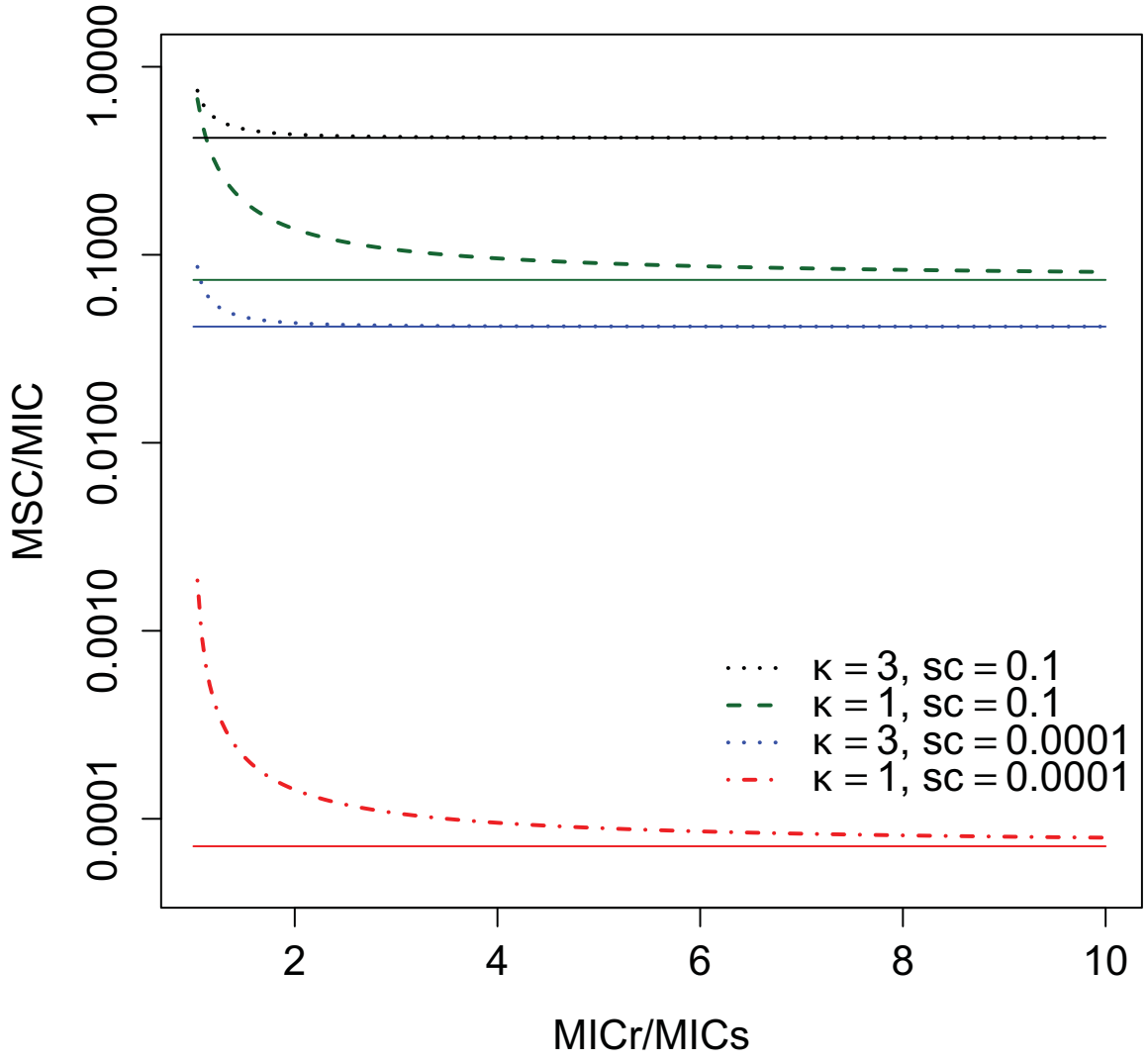

Fig. S1. Calculated MSC/MIC ratio as a function of MIC_r/MIC_s for different κ and sc values. Dotted lines are from the full form of the analytical solution (Eq. 10) and solid horizontal lines are from the simplified solution, which does not include MIC_r or MIC_s (Eq. 11). MSC/MIC is most sensitive to MIC_r/MIC_s when $MIC_r < 2 \times MIC_s$ and the solutions converge for high MIC_r. Other parameter values: $N_{min} = -5$; $N_{int,s} = 2$; $MIC_s = 25$. Note log scale y-axis.

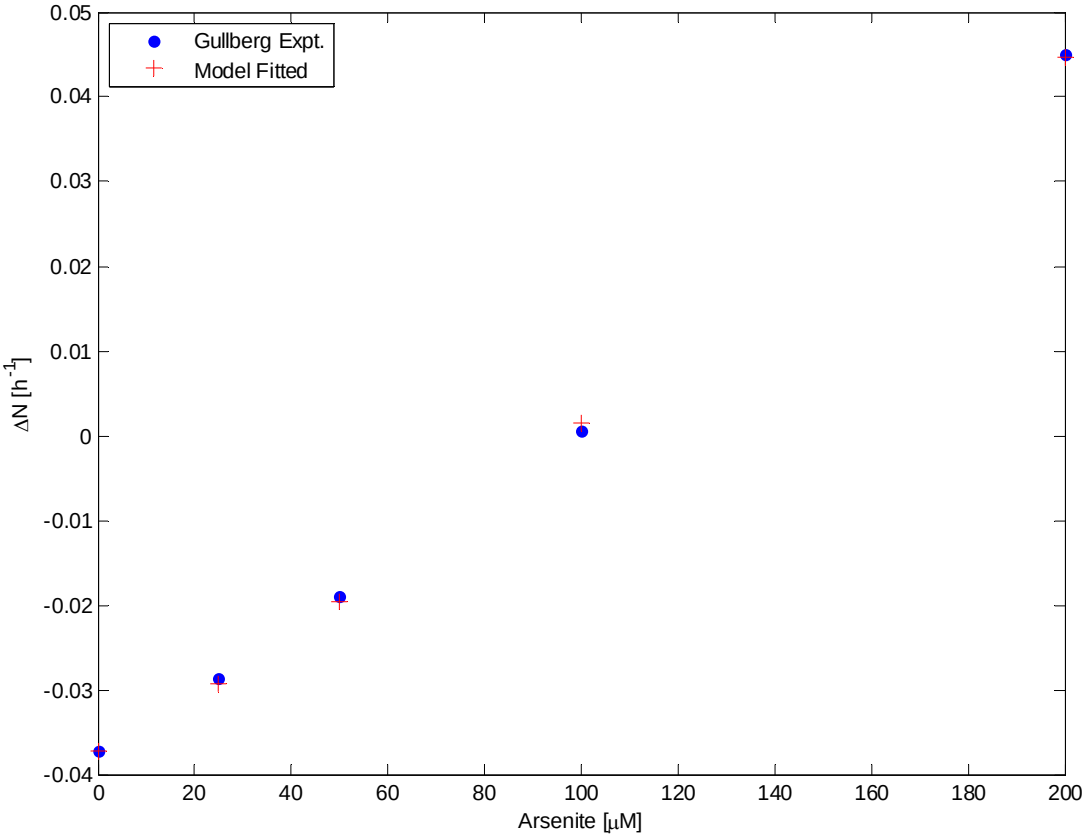

Fig. S2. Model fit to published (11) average growth rate differences (ΔN) for arsenite [μM], employing *Escherechia coli* (data from , 11), fitting both κ and N_{min}.

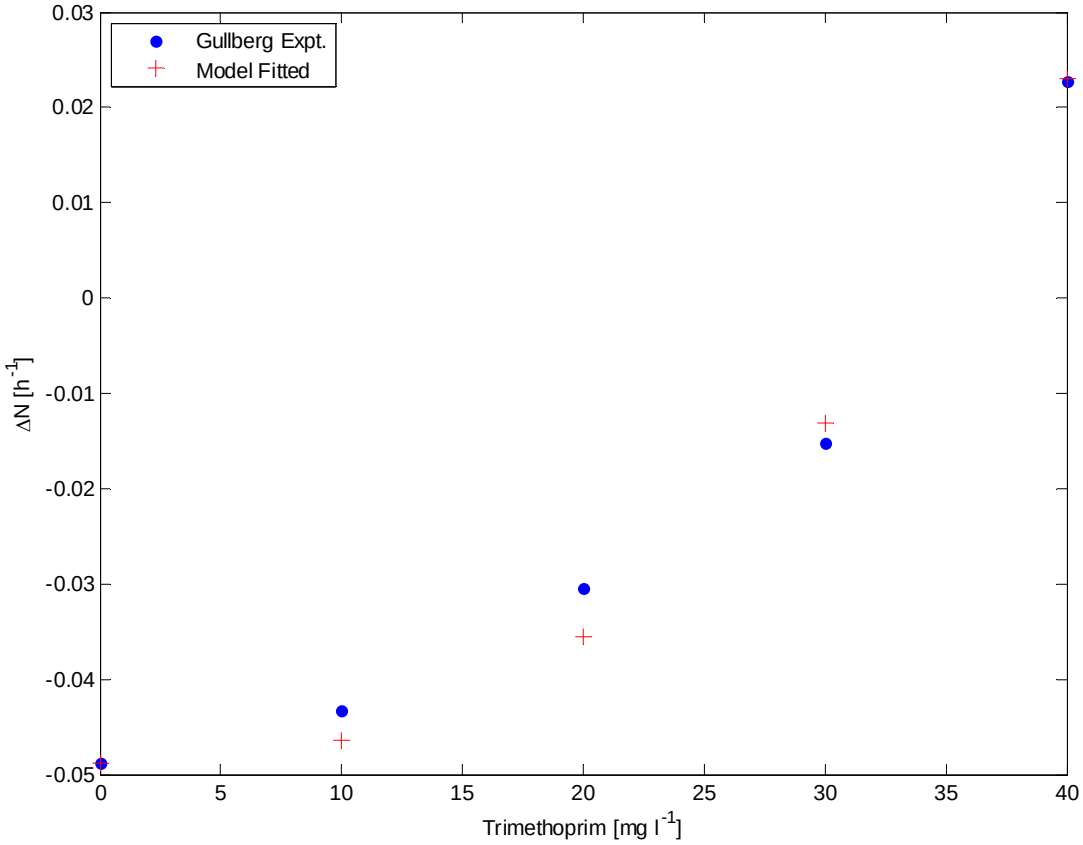

Fig. S3. Model fit to published (11) average growth rate differences (ΔN) for trimethoprim [mg l^{-1}], employing *Escherechia coli* (data from , 11), fitting κ only (N_{min} = −2).

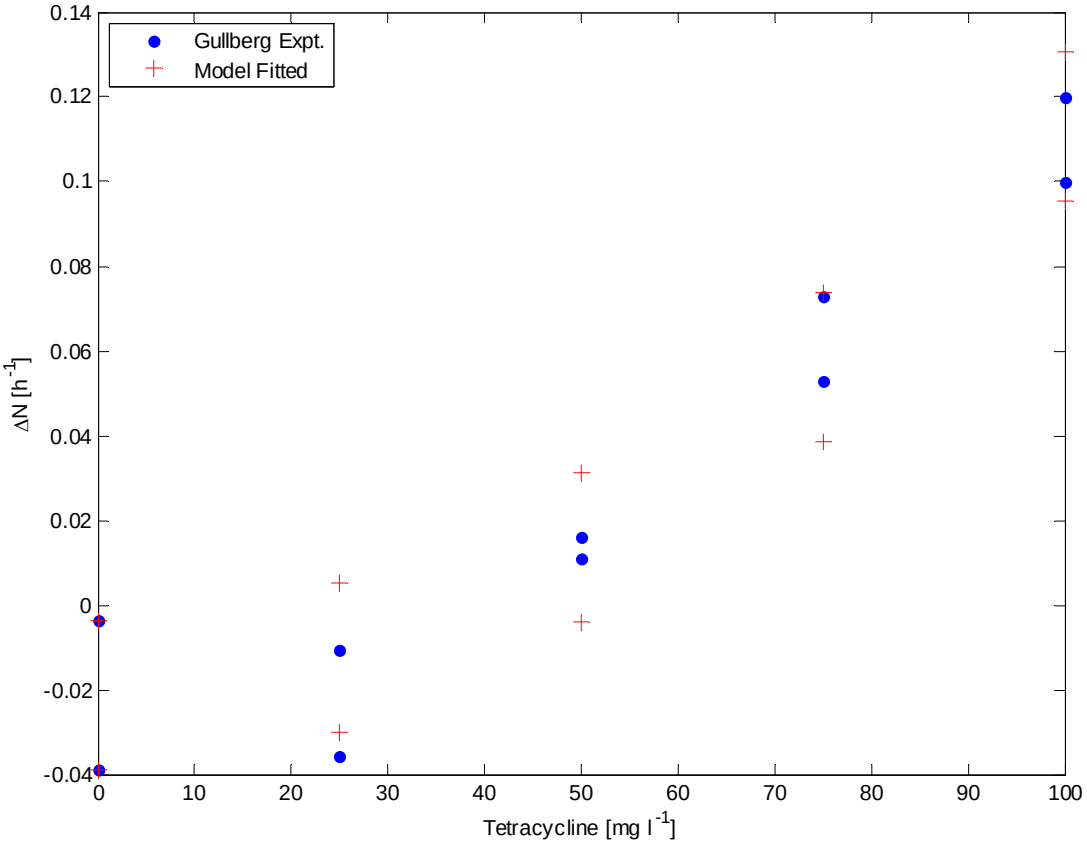

Fig. S4. Model fit to published (11) average growth rate differences (ΔN) for tetracycline, employing *Escherechia coli* (data from , 11), fitting both κ and N_{min}.

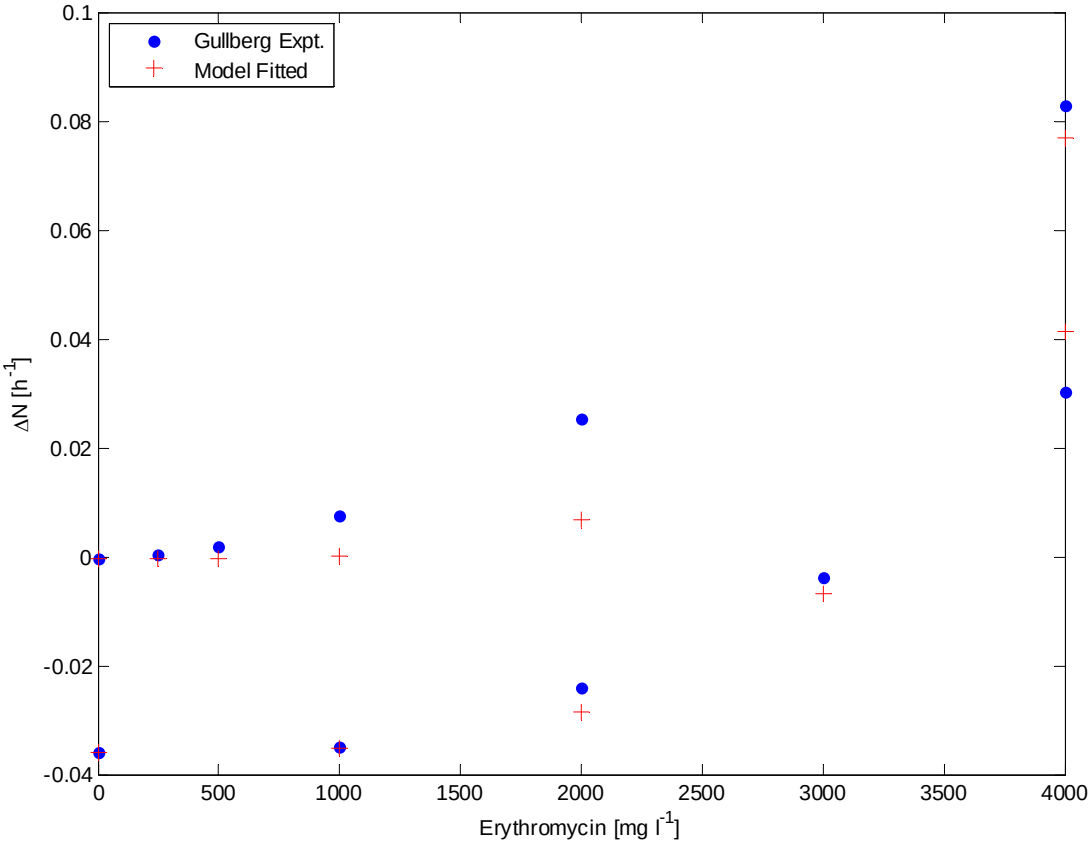

Fig. S5. Model fit to published (11) average growth rate differences (ΔN) for erythromycin [mg l^{-1}], employing *Escherechia coli* (data from , 11), fitting κ only (N_{min} = −2).

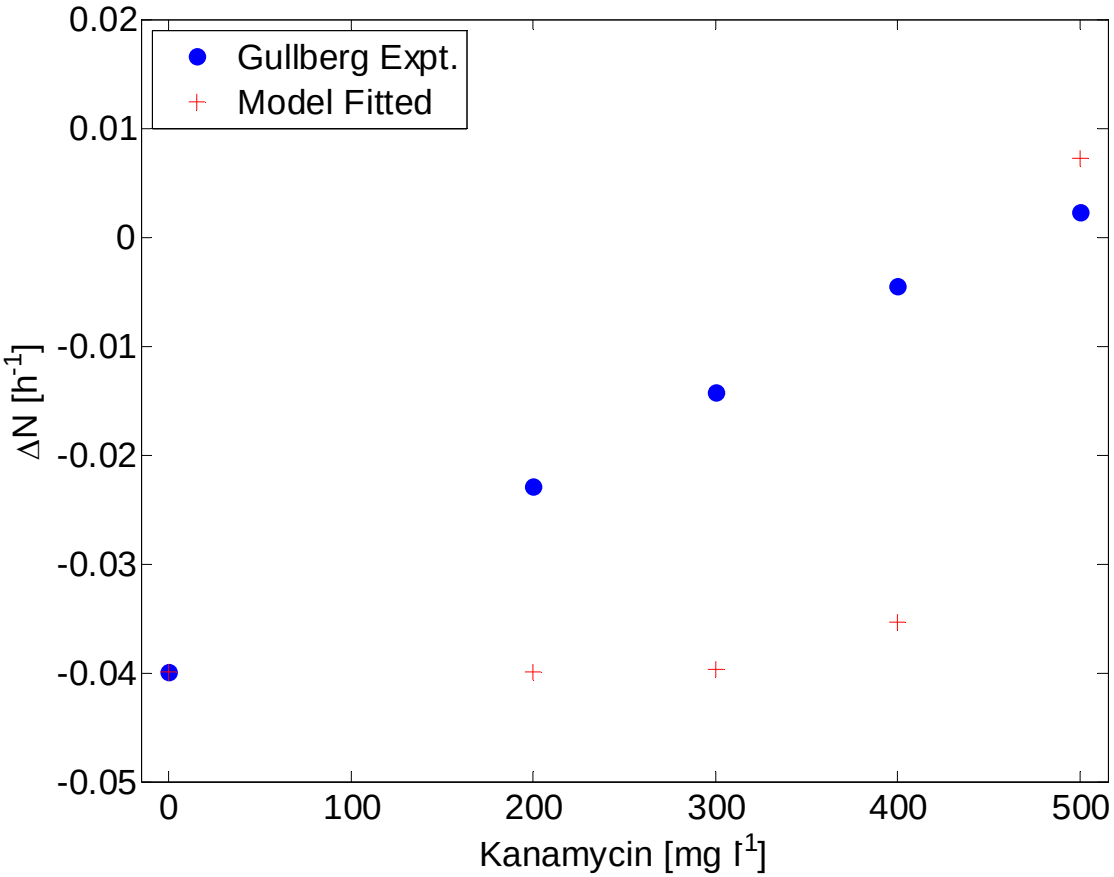

Fig. S6. Model fit to published (11) average growth rate differences (ΔN) for kanamycin [mg l^{-1}], employing *Escherechia coli* (data from , 11), fitting κ only (N_{min} = −2).

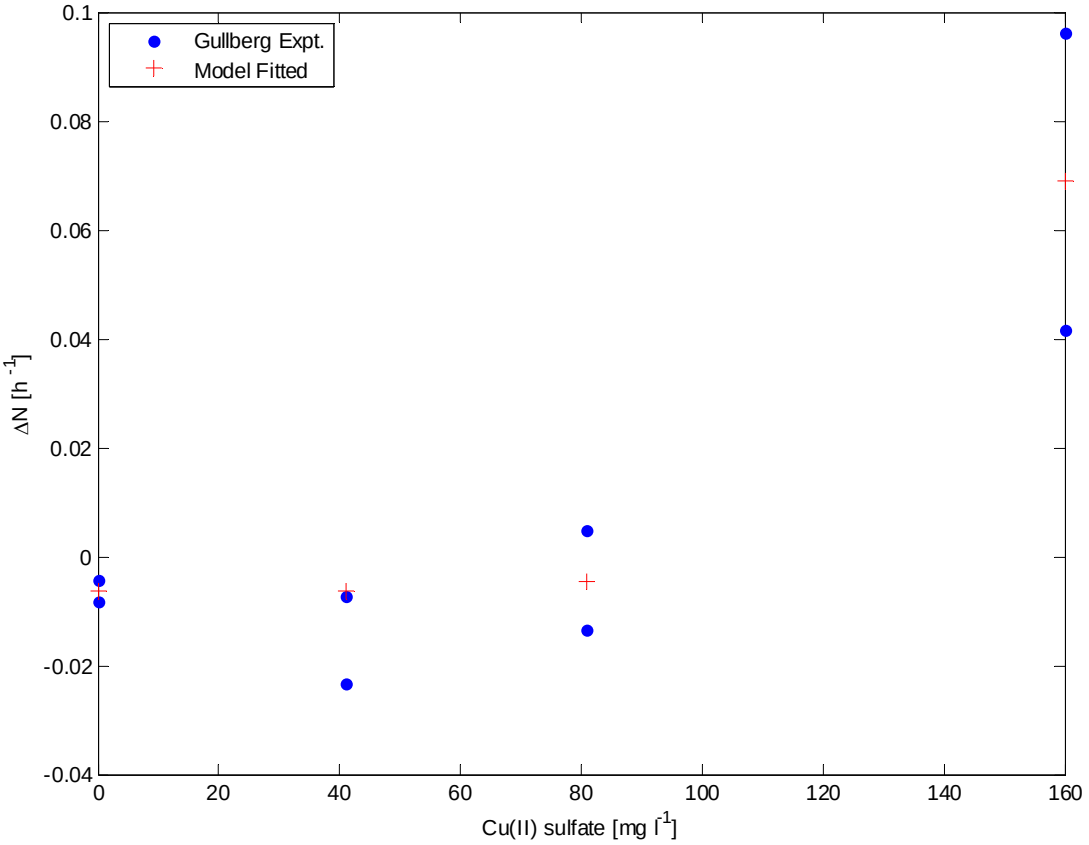

Fig. S7. Model fit to published (11) individual experimental growth rate differences (ΔN) for Cu(II) sulfate [mg l^{-1}], employing *Escherechia coli* (data from , 11), fitting both κ and N_{min}. Individual experimental results (n = 8) were plotted instead of average results for each concentration (n = 4) to achieve a sufficient sample size for the nonlinear model fit.

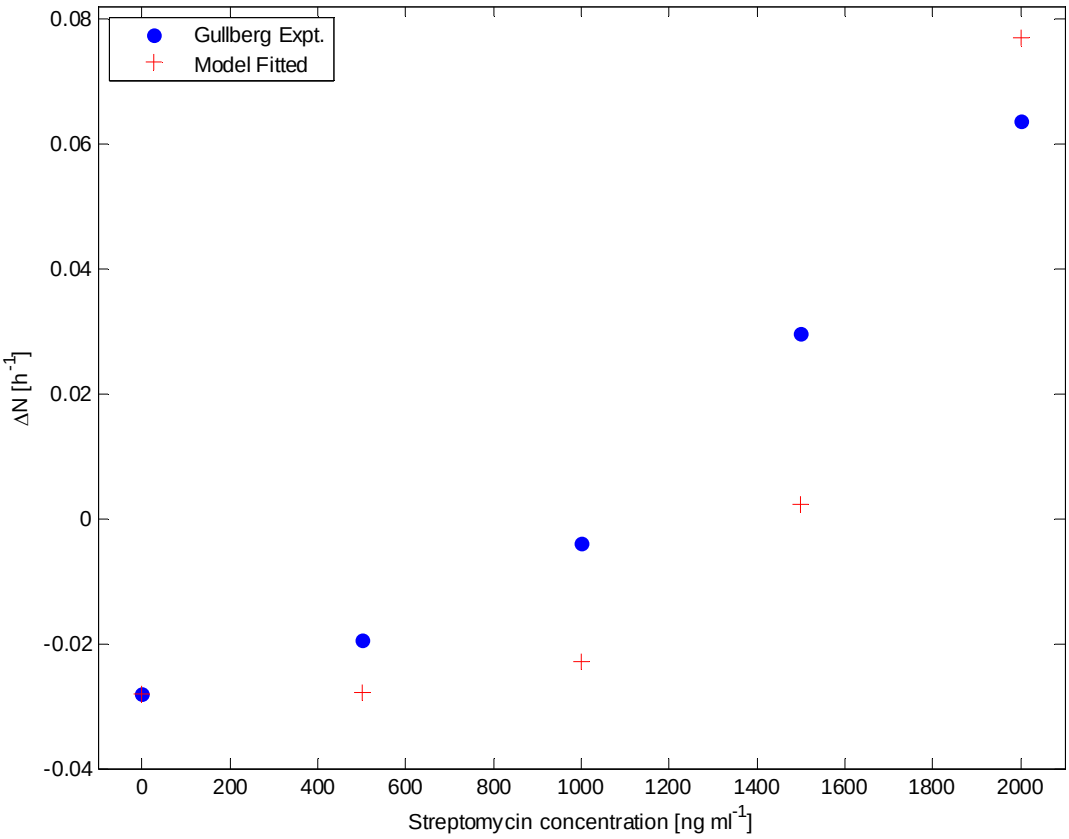

Fig. S8. Model fit to published (8) average growth rate differences (ΔN) for streptomycin, employing *Salmonella enterica* serovar Typhimurium (data from , 8), fitting κ only (N_{min} = −2).

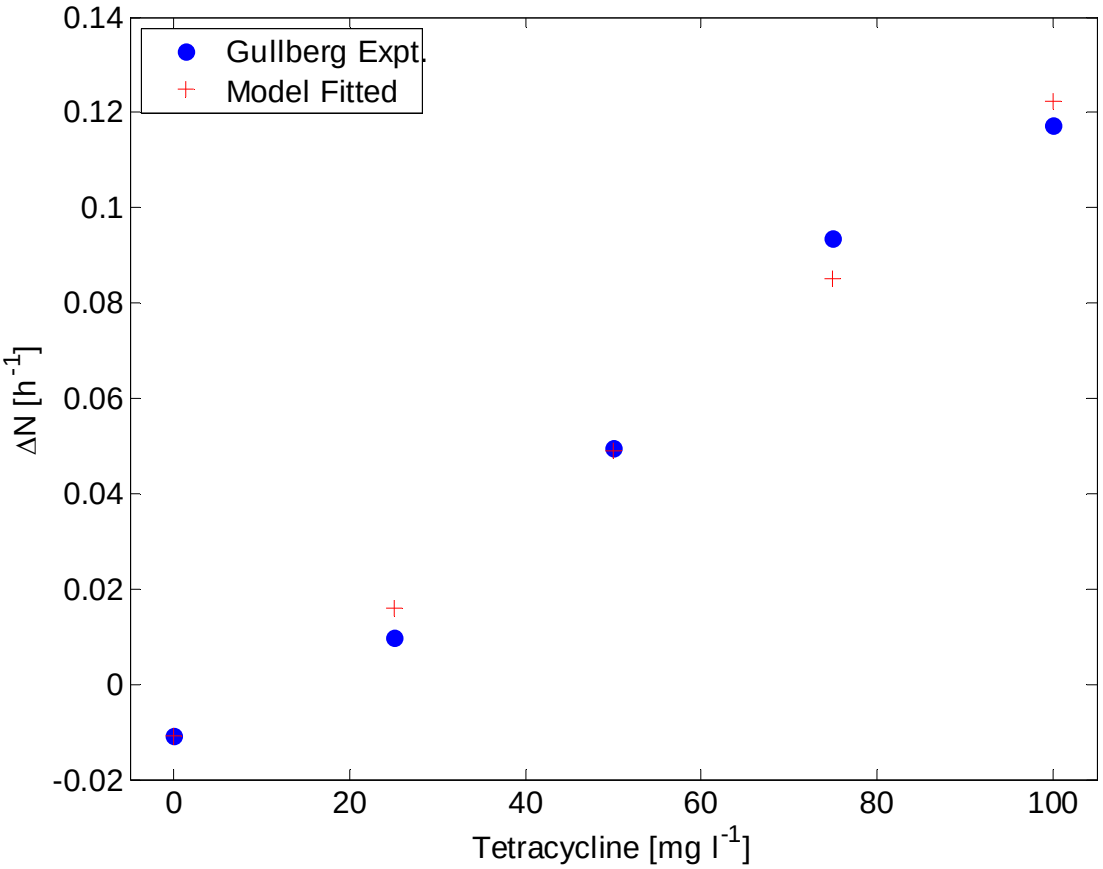

Fig. S9. Model fit to published (8) average growth rate differences (ΔN) for tetracycline, employing *Salmonella enterica* serovar Typhimurium (data from , 8), fitting κ only (N_{min} = −2).

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