Impact of the intracellular ribosomal concentration on *in vitro* bacterial growth kinetics

—the antibacterial effect of linezolid on *S. aureus* in time-kill assays—

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Objective

Typical time-kill curve models neglect or over-simplify bacterial physiology. Our aim was i) to develop a framework which allows for mechanistic integration of drug effects on the single cell level and ii) to predict the impact of (multiple) antibiotic perturbations on bacterial population growth.

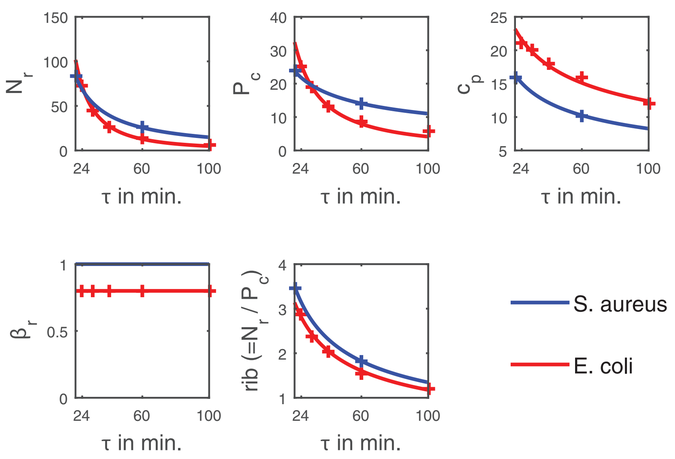
Methods

Cell state is linked to bacterial population growth and *vica versa*

Cell state $S = (N_\mathrm{r}, c_\mathrm{p}, \beta_\mathrm{r}, P_\mathrm{c})$ captured key biological processes that determine growth

In nutritional favorable media, as used in time-kill experiments, the cell state is primarily defined by doubling time $\tau = 60\log(2)/k_\mathrm{g}$, where k_g denotes the exponential growth rate constant in $1/\mathrm{h}$ (data from [1, 3, 4])

S_i	Description and units
N_r	number of 1×10^3 ribosomes per cell
P_c	protein mass per cell in 1×10^8 amino acid (aa) residues
c_p	peptide chain elongation rate per active ribosome in aa/s
β_r	fraction of active ribosomes



Based on the paradigms of translation, the exponential growth rate constant was expressed in terms of the cell state [1]

$$k_\mathrm{g}(t) = 0.036 \cdot \overbrace{\mathrm{rib}(t)}^{\text{ribosomal 'concentration'}} \cdot \overbrace{c_\mathrm{p} \cdot \beta_\mathrm{r}}^{\text{ribosomal efficiency}} \cdot \left(1 - \frac{N(t)}{N_\mathrm{max}}\right)$$

Implementation of drug effects: exemplified for linezolid

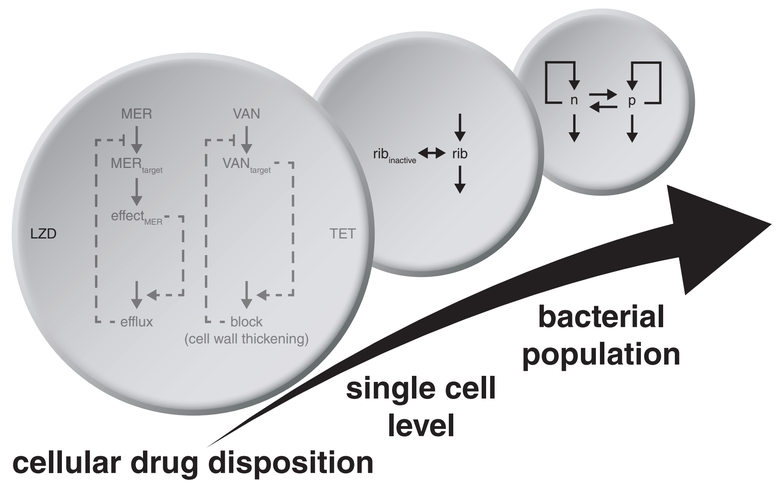
Linezolid (concentration denoted by LZD) is a protein biosynthesis inhibitor for which we did not observe development of resistance over the experimental time course $\tau_\mathrm{E} = 24\,\mathrm{h}$

The mechanism of growth inhibition was a reduced fraction of active ribosomes compared to growth control $\beta_\mathrm{r} = \beta_\mathrm{r,control} \cdot (1 - E_\mathrm{g})$

$$E_\mathrm{g} = \frac{\mathrm{LZD}^{\gamma_\mathrm{g}}}{(\frac{\mathrm{rib}_\mathrm{control}}{\mathrm{rib}} \cdot \mathrm{EC50_g})^{\gamma_\mathrm{g}} + \mathrm{LZD}^{\gamma_\mathrm{g}}}$$

At higher concentrations, linezolid also induced death

$$k_\mathrm{d} = \frac{\mathrm{Emax} \cdot \mathrm{LZD}^{\gamma_\mathrm{d}}}{(\frac{\mathrm{rib}_\mathrm{control}}{\mathrm{rib}} \cdot \mathrm{EC50_d})^{\gamma_\mathrm{d}} + \mathrm{LZD}^{\gamma_\mathrm{d}}}$$



Exploitation of the balanced growth condition ($\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{rib} = 0$, $\mathrm{rib} = \mathrm{rib_{control}}(k_\mathrm{g,control})$ and $k_\mathrm{g} = k_\mathrm{g,control}$) lead to a system of ODEs describing linezolid drug action

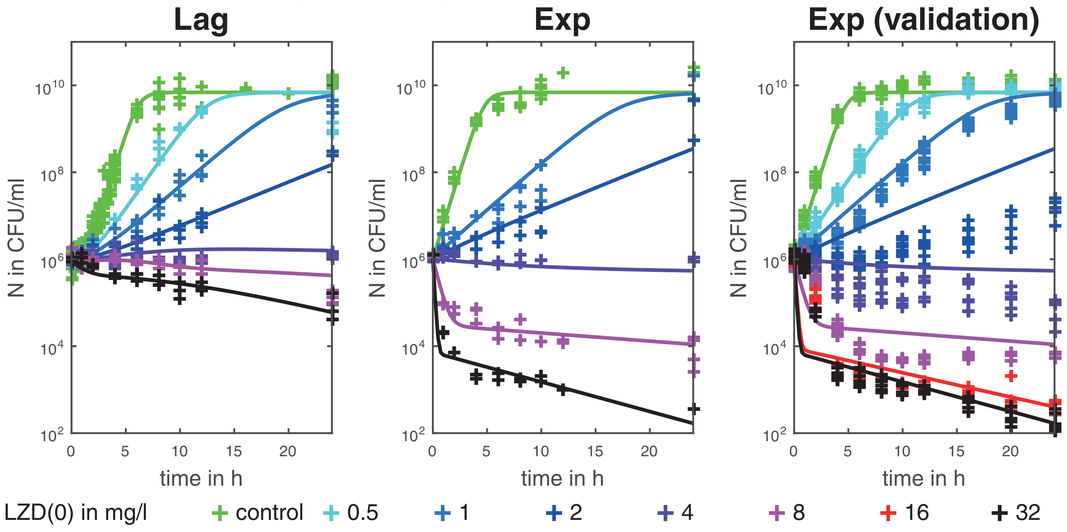
$$\begin{aligned}
\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{LZD} &= -k_\mathrm{deg,LZD} \cdot \mathrm{LZD} \\
\frac{\mathrm{d}}{\mathrm{d}t}\mathrm{rib} &= k_\mathrm{syn,rib} - k_\mathrm{g} \cdot \mathrm{rib} = k_\mathrm{g} \cdot (\mathrm{rib_{control}} - \mathrm{rib}) \\
\frac{\mathrm{d}}{\mathrm{d}t}n &= (k_\mathrm{g} - k_\mathrm{d}) \cdot n - k_\mathrm{np} \cdot n + k_\mathrm{pn} \cdot p \\
\frac{\mathrm{d}}{\mathrm{d}t}p &= \alpha_\mathrm{per} \cdot (k_\mathrm{g} - k_\mathrm{d}) \cdot p + k_\mathrm{np} \cdot n - k_\mathrm{pn} \cdot p
\end{aligned}$$

Persisting cells p suffer from growth impairment and benefit from death protection in comparison to normal cells n—the sum represents the total population $N(t) = n(t) + p(t)$—initialized as $n(0) = (1 - f_\mathrm{p}) \cdot N(0)$ and $p(0) = f_\mathrm{p} \cdot N(0)$

Results

Intracellular ribosomal concentration impacts linezolid potency

S. aureus ATCC 29213 starting either in lag or exponential phase (Exp), data from [5, 6, 7]

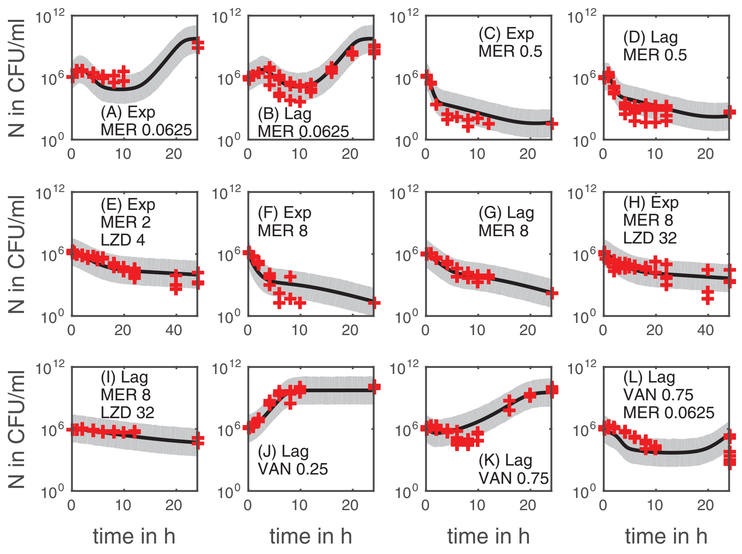


Predictions with median of the posterior (flat prior; MCMC[2] implemented in Matlab; 3 chains each 100 000 samples from overdispersed initials were pooled; Gelman-Rubin convergence $\hat{R} < 1.2$)

Parameter	Unit	Description	Estimated 50th (5th–95th) percentile of posterior Exp	Lag
N_0	CFU/ml	initial inoculum	8.76×10^5 (7.67×10^5–1.04×10^6)	1.05×10^6 (9.53×10^5–1.15×10^6)
rib_0	$\frac{1 \times 10^{-5}}{\mathrm{aa}}$	initial ribosomal concentration	$\mathrm{rib_{control}}$	0.57 (0.29–1.65)
f_p	-	initial persister fraction	0	0.46 (0.16–0.78)
$k_\mathrm{g,control}$	1/h	control growth rate constant	2.06 (1.88–2.26)	
N_max	CFU/ml	carrying capacity	6.9×10^9 (5.5×10^9–8.8×10^9)	
k_np	1/h	switching rate constant ($n \to p$)	0.06 (0.03–0.14)	
k_pn	1/h	switching rate constant ($p \to n$)	0.01	
α_per	-	persistence factor	0.015 (0.009–0.022)	
$k_\mathrm{deg,LZD}$	1/h	degradation rate constant	0.000101	
$\mathrm{EC50_g}$	mg/l	drug potency	0.44 (0.36–0.55)	
γ_g	-	slope factor	1.06 (0.88–1.26)	
$\mathrm{EC50_d}$	mg/l	drug potency	11.03 (9.11–16.40)	
γ_d	-	slope factor	3.71 (2.56–4.82)	
Emax	1/h	maximum effect	10	

Extension to cell wall antibiotics and drug combinations

Meropenem (MER) and vancomycin (VAN) initial concentrations in mg/l; full model has 10 ODEs, 23 estimated parameters and is not shown; data from [6, 7]; prediction intervals in gray (5th to 95th percentile of 10 000 simulations using sampled parameters from posterior distribution and including residual variability)



- **Adaptive resistance:** change in cellular drug disposition impairs target engagement for meropenem and vancomycin (A,B,J,K,L)
- **Delayed drug effects:** effector species for meropenem induces growth dependent death (A,B,L)
- **Eagle effect:** bell shaped dose–response relation for meropenem (D,G) and (C,F)
- **Antagonism:** growth depended killing effect of meropenem is reduced by simultaneous exposure to linezolid (G,I)

Expected additivity

$E_\mathrm{Bliss}(A, B) = E(A) + E(B) - E(A) \cdot E(B)$

Deviation from expected additivity

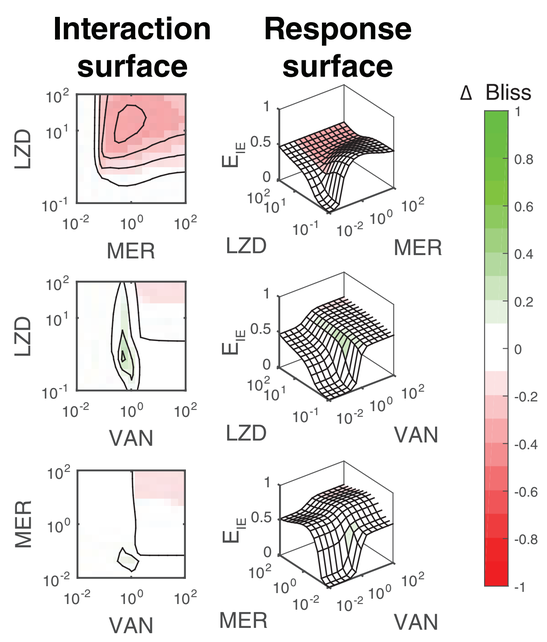
$\Delta_\mathrm{Bliss} = E_\mathrm{Model} - E_\mathrm{Bliss}$

From bacterial population dynamics **pharmacodynamic summary endpoints** are derived:

$$\mathrm{AUTKC} = \int_0^{\tau_\mathrm{E}} \log_{10}(N)\mathrm{d}t$$

$$E_\mathrm{IE} = 1 - \frac{\mathrm{AUTKC_{drug}}}{\mathrm{AUTKC_{control}}}$$

Exemplary defining the intensity of the killing effect as summary endpoint $E = E_\mathrm{IE}$ (simulations shown for lag phase ATCC 29213 cultures)



Classification

Total synergy

$\Delta_\mathrm{Bliss} = 1$

Additivity

$|\Delta_\mathrm{Bliss}| \approx 0$

Total antagonism

$\Delta_\mathrm{Bliss} = -1$

Systematic exploration of different experimental protocols and endpoint definitions results in a one step ordinal variability with respect to interaction classification

Summary

Information on intracellular dynamics offers crucial insight to understand bacterial population growth. Shifts in susceptibility for lag and exponential phase cultures, as well as complex interaction patterns are quantitatively explained by taking into account the cellular level.

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