Quantifying adaptive resistance in bacteria

using well-designed dynamic time-kill curve experiments

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Background

In vitro **time-kill curve experiments** allow to study the concentration-effect relationship of antimicrobial treatments. Mathematical models leverage the experimental data by allowing to simulate microbial growth under clinically relevant concentration-time profiles and under combination therapy.

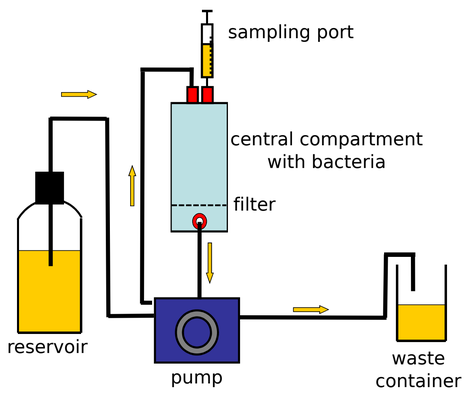
However, some dynamic processes such as **adaptive resistance** and persister formation (dormant state), both stress-triggered phenotypic changes, are **difficult to quantify reliably from these experiments** [1].

Objective

Propose **tailored time-kill curve experiments** to differentiate adaptive resistance and persister formation using **Optimal Design** theory.

Time-kill curve experiments

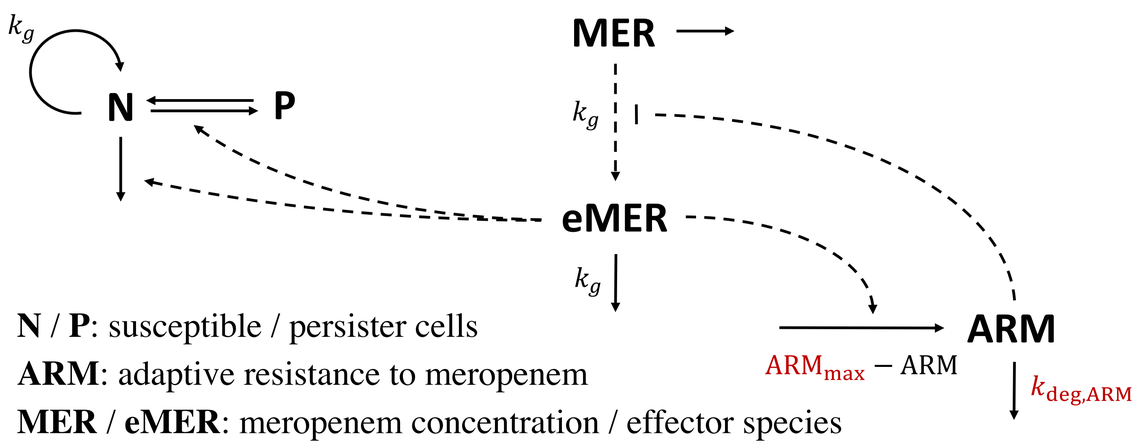
- Bacteria in a culture flask are exposed to antibiotics in an ***in vitro* infection model** (IVIM)
- IVIM can be **static** (no change in drug concentrations; no exchange of culture medium) or **dynamic** (pumping system allows for time-varying drug concentrations; see Figure)
- **Time-kill curves (TKC)**: obtained by repeated sampling from an IVIM and quantifying bacterial concentrations



Sketch of a dynamic *in vitro* infection model (adapted from [2])

Bacterial growth model

Model for antibiotic combination therapy, developed previously from static TKC data and mechanistic understanding [3,4]; only meropenem effect is considered here.



N / **P**: susceptible / persister cells
ARM: adaptive resistance to meropenem
MER / **eMER**: meropenem concentration / effector species
k_g: growth rate function depending on cell state (log- vs. lag-phase)

Assumptions on adaptive resistance during model development:

- Adaptive resistance does not wear off: $\mathrm{k_{deg,ARM}} = 0\ \mathrm{h^{-1}}$
- Adaptive resistance can inhibit meropenem effect completely: $\mathrm{ARM_{max}} = 1$

Could these parameters have been determined from ***dynamic*** *time-kill curve experiments?*

Design of time-kill curve experiments

D_S-optimality

- Maximise information on interesting parameters (I)
- Account for correlations (C) with nuisance parameters (N)
- Robust variant: log D_S averaged over parameters

Here: interesting ≙ $\mathrm{k_{deg,ARM}}$ and $\mathrm{ARM_{max}}$

$$FIM = \begin{pmatrix} I & C \\ C^T & N \end{pmatrix}$$

Optimality criterion [5]:

$$D_S = \det(I - CN^{-1}C^T)$$

Idea for dynamic TKC experiments:

1. Adaptive resistance has to be triggered (high drug concentration),
2. For positive $\mathrm{k_{deg,ARM}}$, adaptive resistance can wear off in a drug-free rest phase
3. Reduction of adaptive resistance manifests in a decrease of bacterial load after a second exposure (possibly lower) to antibiotics

→ we consider **intermittent antibiotic exposure experiments** (and for comparison, static conditions and dynamic 1-compartment kinetics)

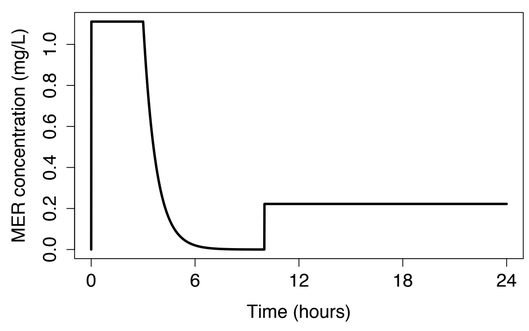
Prior information:

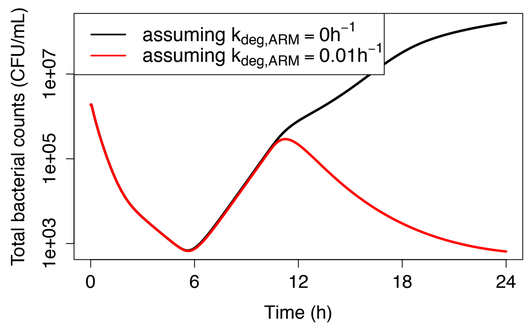
$\mathrm{FIM_{static}}$ (from modelling static TKC data)

$$\mathrm{FIM} = \underbrace{\mathrm{FIM_{static}}}_{\substack{\text{performed} \\ \text{experiment}}} + \underbrace{\mathrm{FIM_{dynamic}}}_{\substack{\text{planned} \\ \text{experiment}}}$$

Results

Intermittent exposure experiment discriminating $\mathrm{k_{deg,ARM}}$ values





Optimal design for different experimental setups

Assumed true parameters		Predicted standard errors (SE) for optimal design					
		SE ($\mathrm{k_{deg,ARM}}$)			SE ($\mathrm{ARM_{max}}$)		
$k_{\mathrm{deg,ARM}}$	$\mathrm{ARM_{max}}$	static	1-cmt	inter-mittent	static	1-cmt	inter-mittent
0	**1**	0.003	0.004	0.003	0.03	0.04	0.03
0.01	**1**	0.02	0.01	0.02	0.2	0.2	0.1
0.1	**1**	1.3	0.2	**0.02**	6.8	1.2	**0.1**
0	**0.9**	0.03	0.02	0.03	0.2	0.2	0.3
0.01	**0.9**	0.04	0.03	0.02	0.3	0.3	0.2
0.1	**0.9**	2.0	0.3	**0.06**	8.7	1.3	**0.2**

good precision with intermittent exposure design

optimality criterion ($\mathbb{E}\log \mathrm{D_S}$) for best **robust** design		
static	1-cmt	intermittent
9.09	11.17	11.47

→ benefit of intermittent exposure design less prominent

Conclusions

- Static TKC data can be leveraged for designing dynamic TKC experiments
- Tailored experiments contribute to elucidating bacterial resistance mechanisms
- Robust simultaneous characterisation of several resistance mechanisms remains challenging

References

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