Novel strategies to tackle bacterial infections: targeting adhesion and persistence

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1st July 2019, Disease Modelling and Public Health, ICTS Bangalore

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AMR and the need for new treatments

Antibiotics

- Antibiotics are widely used to treat bacterial infections

- They act by killing the bacteria, or inhibiting their growth
- First discovered in the early 20th Century

Antimicrobial resistance

- Decades since last new class of antibiotic discovered

[1]The Review on Antimicrobial Resistance, J. O'Neill, 2014

Antimicrobial resistance

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Antimicrobial resistance

- Decades since last new class of antibiotic discovered
- High levels of resistance found in all regions of the world
- Huge problem in developing countries, where antibiotics readily available
- Predicted 10 million deaths p.a. by 2050[1]

[1]The Review on Antimicrobial Resistance, J. O'Neill, 2014

Alternatives to antibiotics: anti-virulence drugs

We need new strategies to combat antimicrobial resistance, e.g.

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- Lots of possible mechanisms to target, e.g.
  - cell adhesion
  - toxin production
  - persister formation
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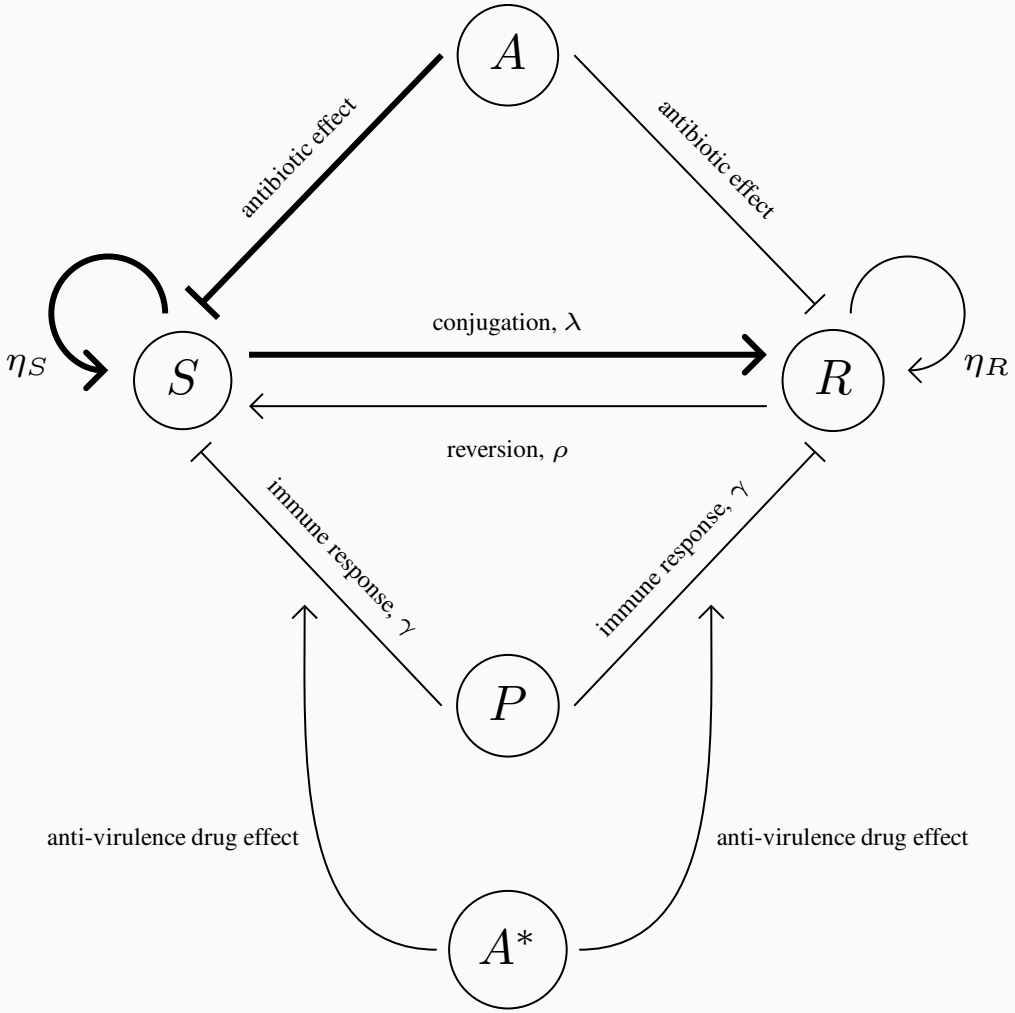
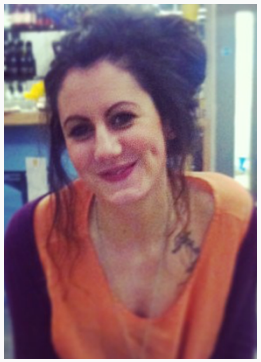
  - cell adhesion
  - toxin production
  - persister formation
  - efflux pumps

Problem: they don't currently clear infections!

A generic anti-virulence drug

Modelling a generic anti-virulence drug





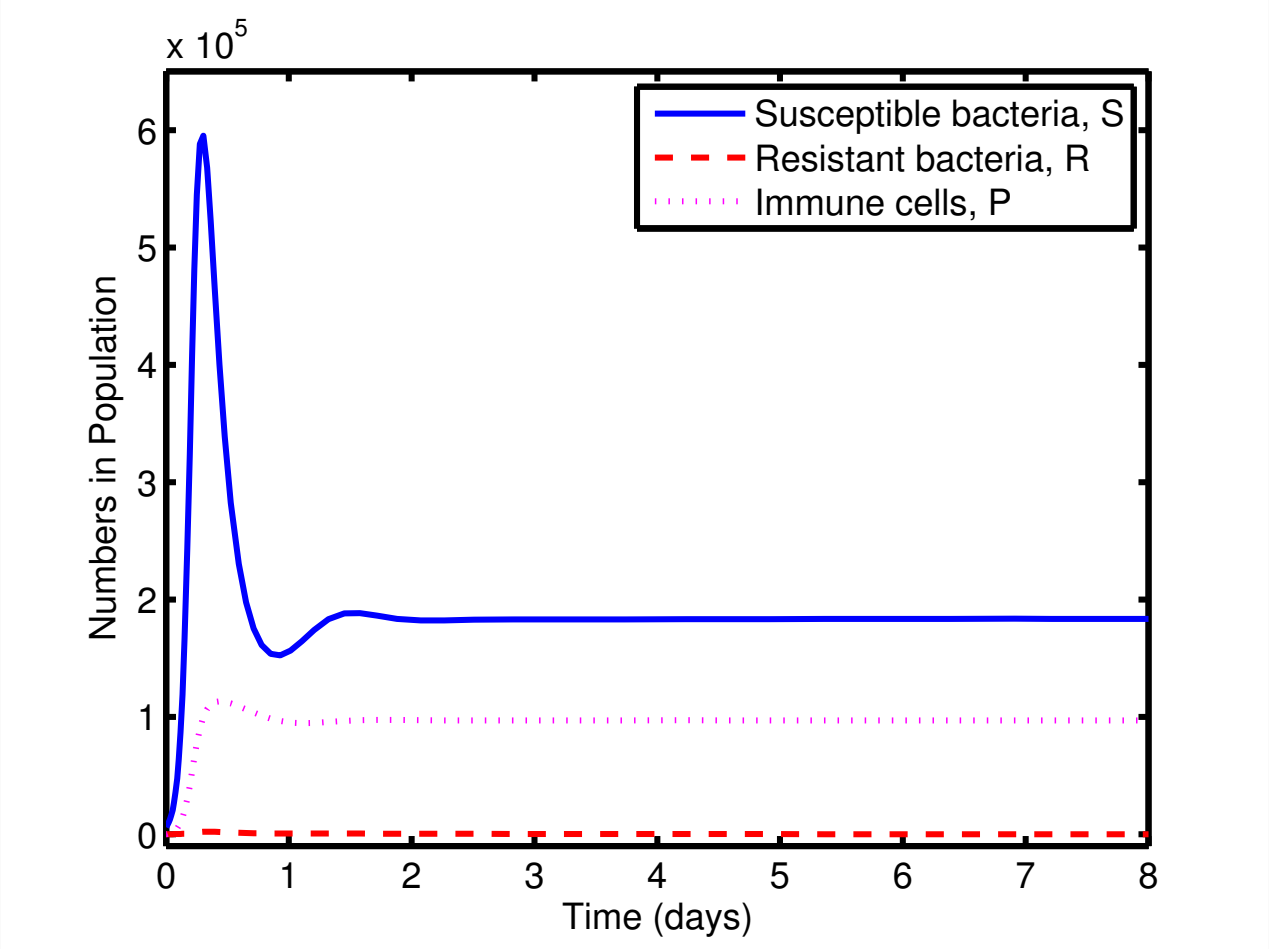
Lucy Ternent

Anti-virulence model

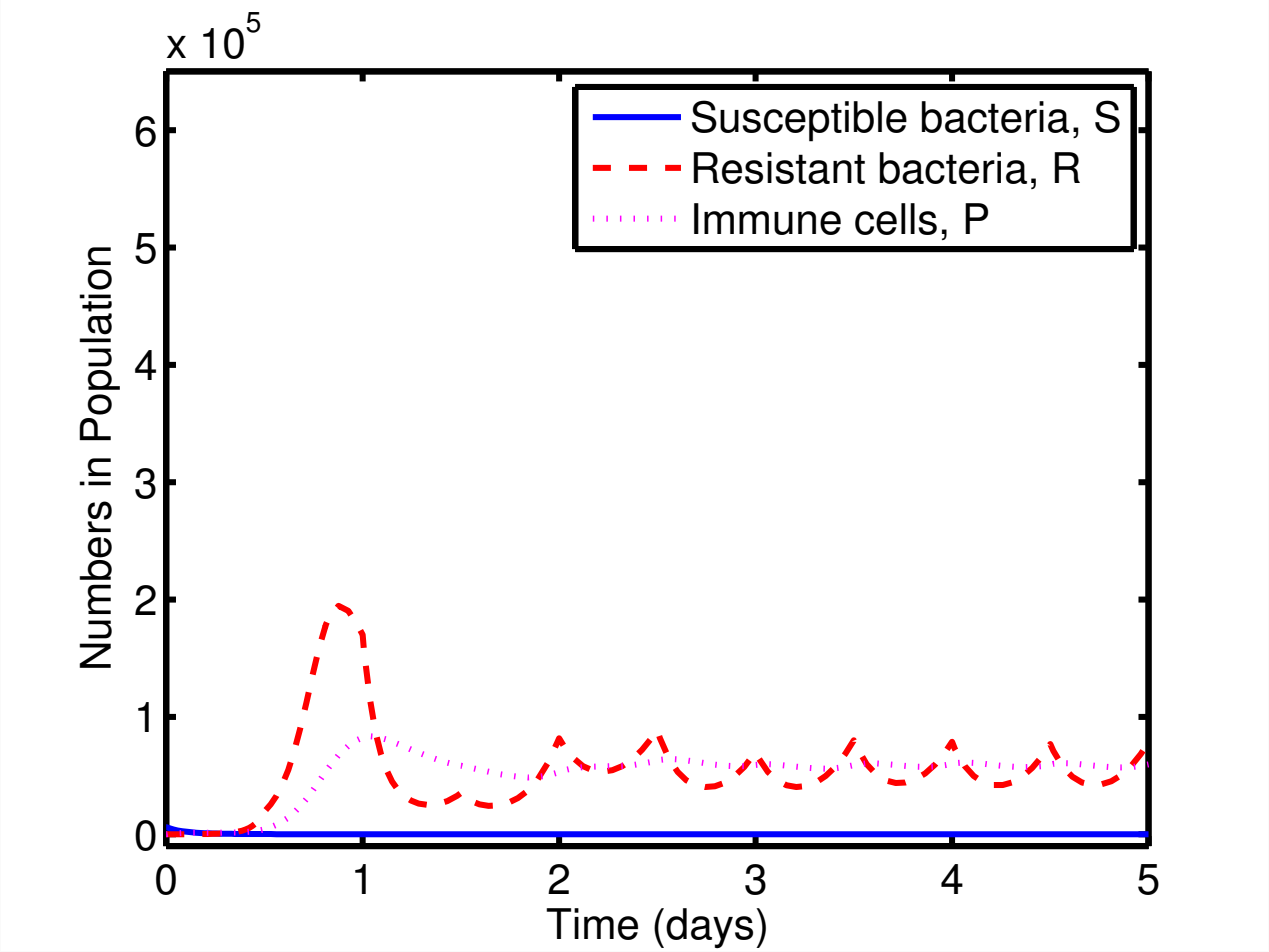
$$\frac{dA}{dt} = -\alpha A,$$
$$\frac{dA^*}{dt} = -\kappa A^*,$$
$$\frac{dP}{dt} = \beta\left(S+R\right)\left(1-\frac{P}{P_{\max}}\right) - \delta(S+R)P - \delta_P P,$$
$$\frac{dS}{dt} = \eta_S S\left(1-\frac{S+R}{K}\right) - \mu_S(A)S - \left(\gamma+\zeta\left(A^*\right)\right)PS - \lambda SR + \rho R - \psi S,$$
$$\frac{dR}{dt} = (1-c)\eta_S R\left(1-\frac{S+R}{K}\right) - \mu_R(A)R - \left(\gamma+\zeta\left(A^*\right)\right)PR + \lambda SR - \rho R - \psi R.$$

$$\mu_i(A) = \frac{E^i_{max}A}{A^i_{50}+A} \qquad \zeta(A^*) = \frac{\gamma_{\max}A^*}{\gamma_{50}+A^*}$$

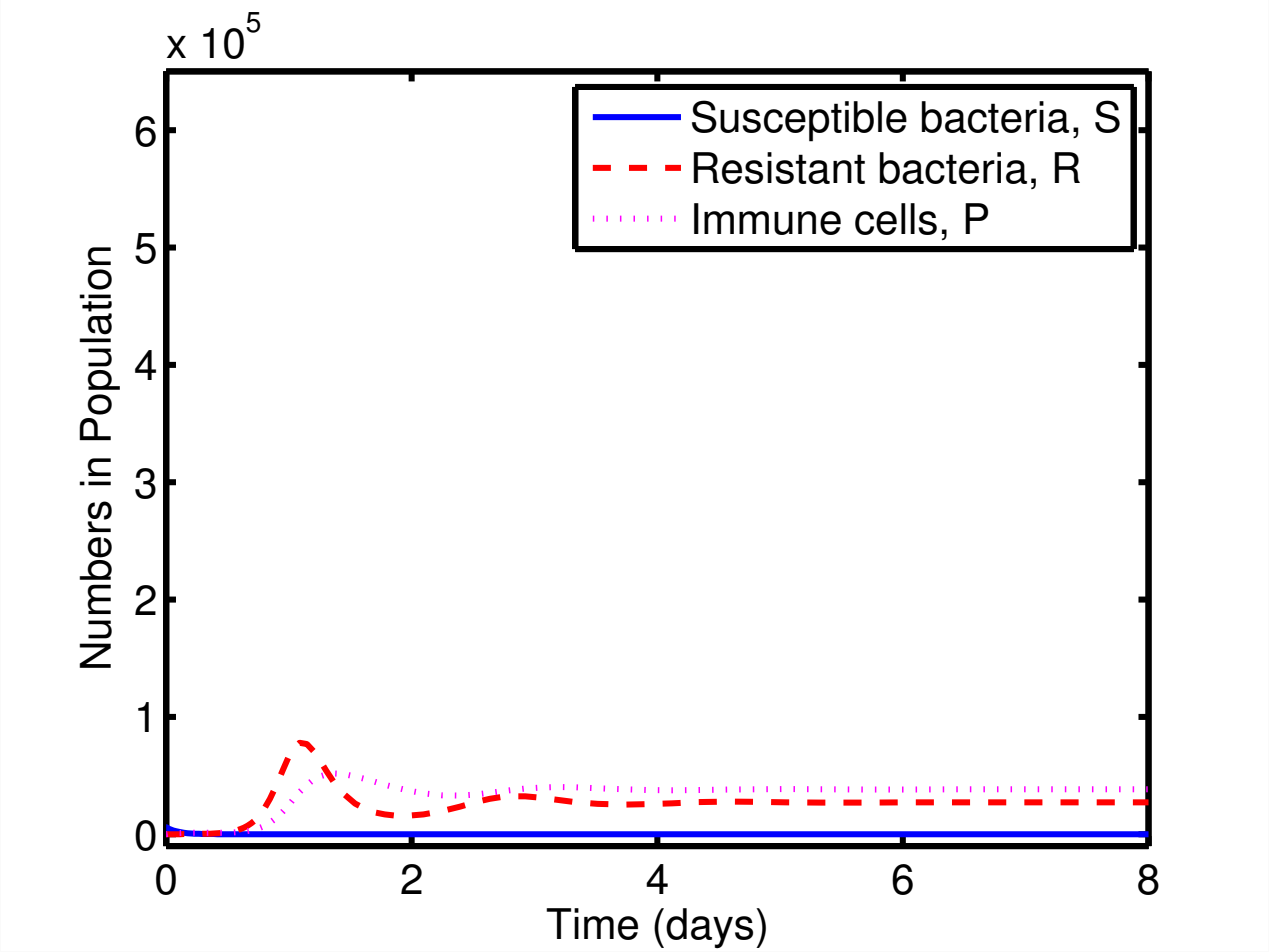
Model Simulation – no treatment (low initial resistance)



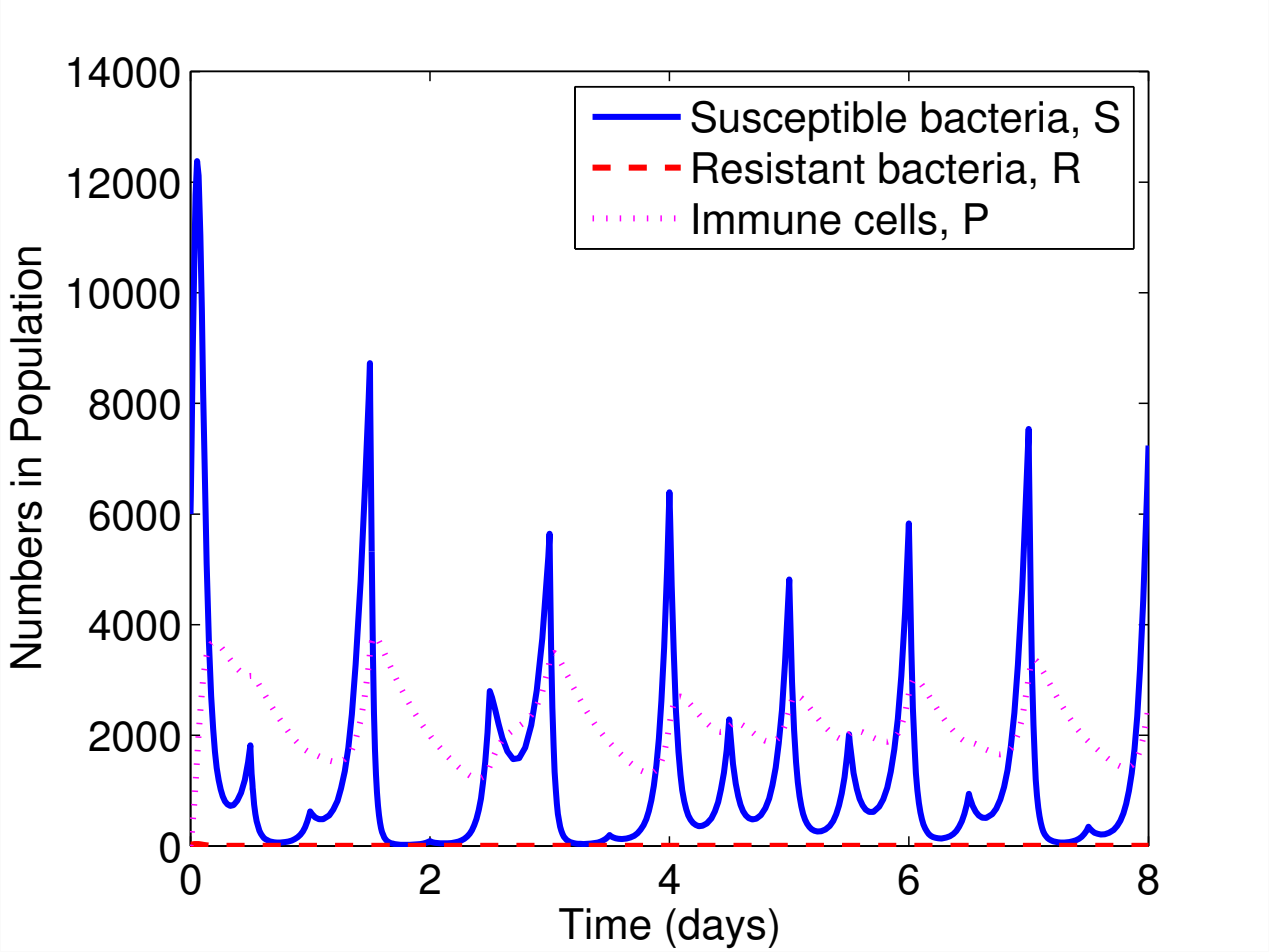
Model Simulation – antibiotic (dosing)



Model Simulation – antibiotic (constant)



Model Simulation – anti-virulence drug



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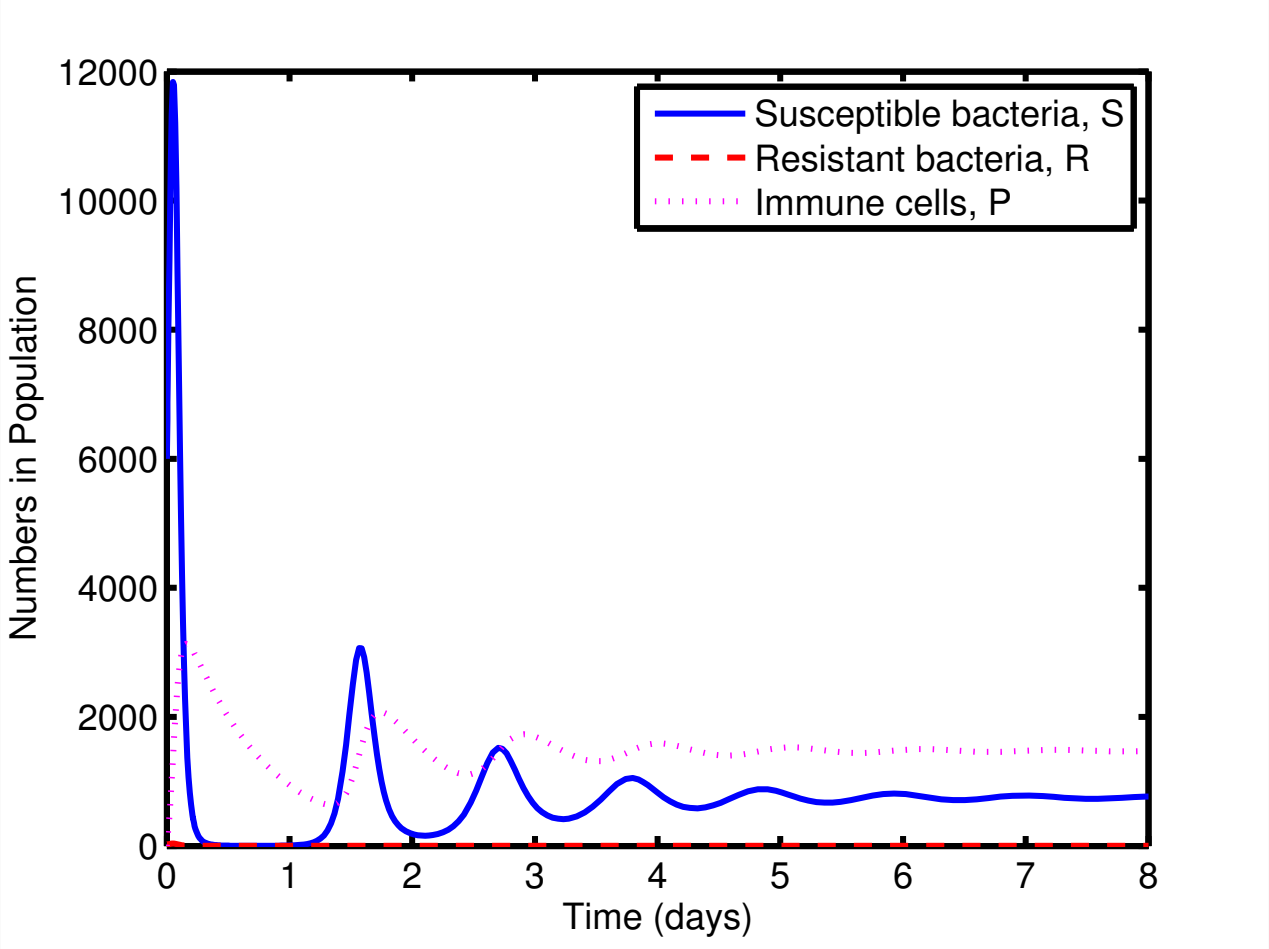

Combining Antibiotics and Anti-Virulence Drugs

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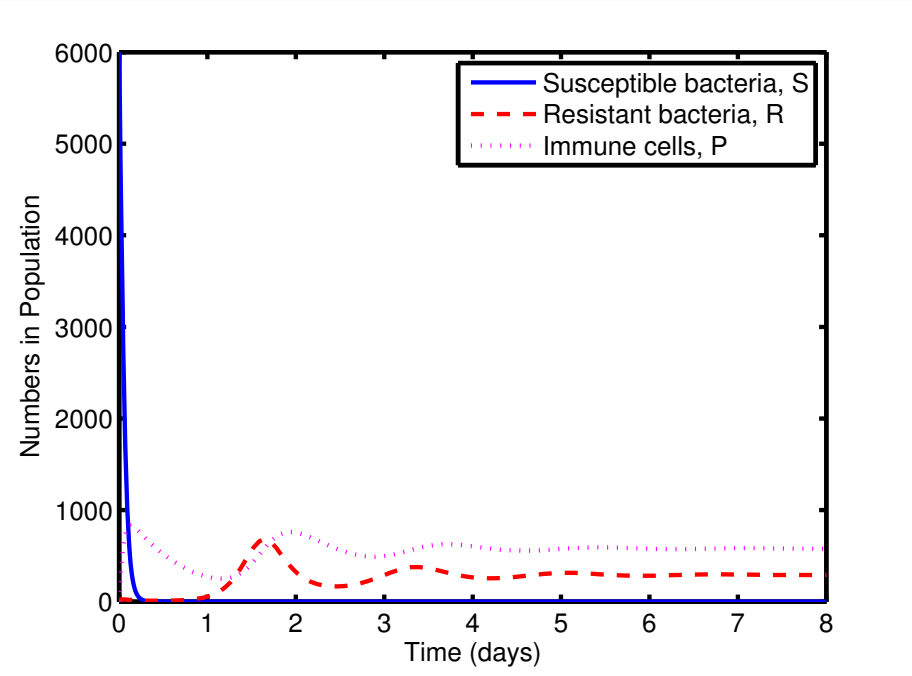
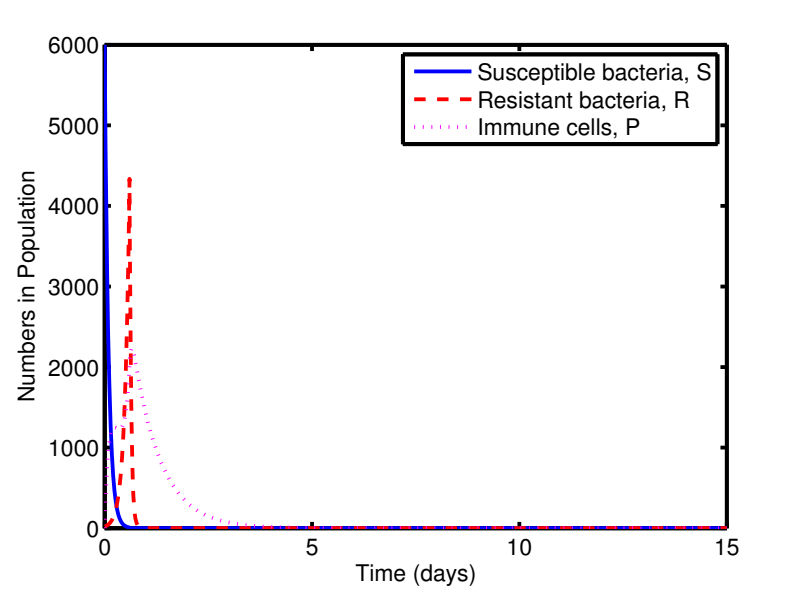


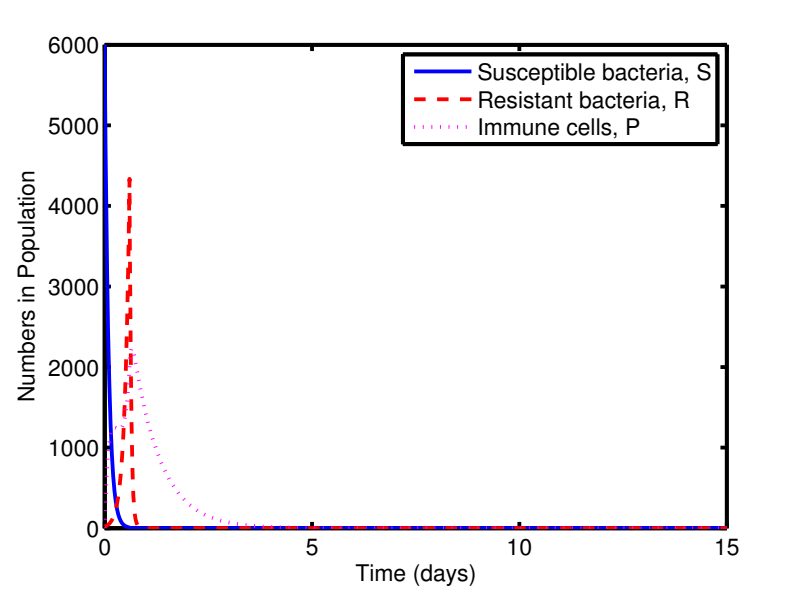
- Susceptible bacteria cleared
- Small population of resistant bacteria remain (unless fitness cost of antibiotic-resistance is sufficiently high)

Combining drugs with a time delay



Antibiotic then anti-virulence drug after $t = 14.4$ hours.

Combining drugs with a time delay



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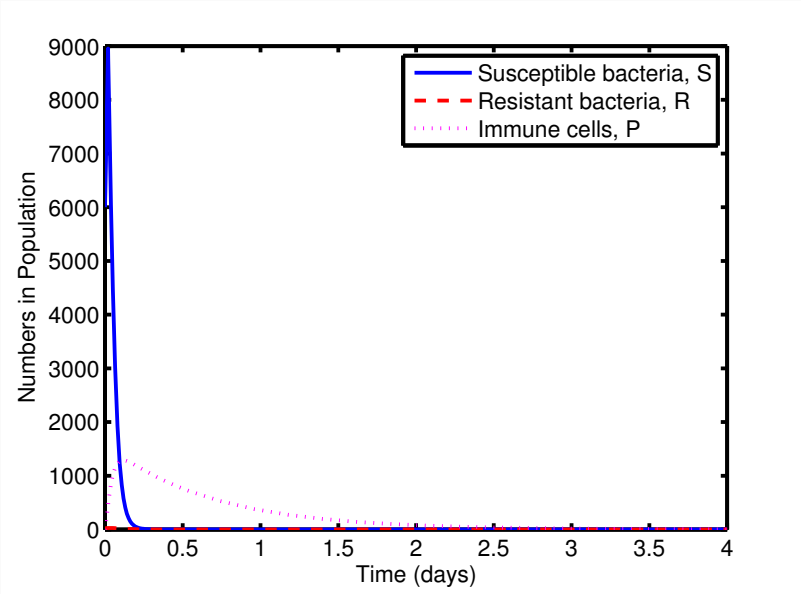
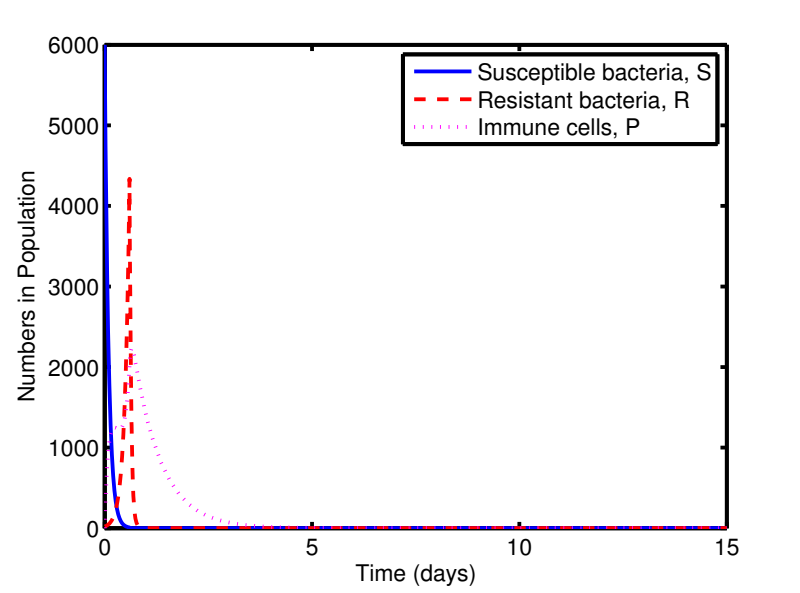
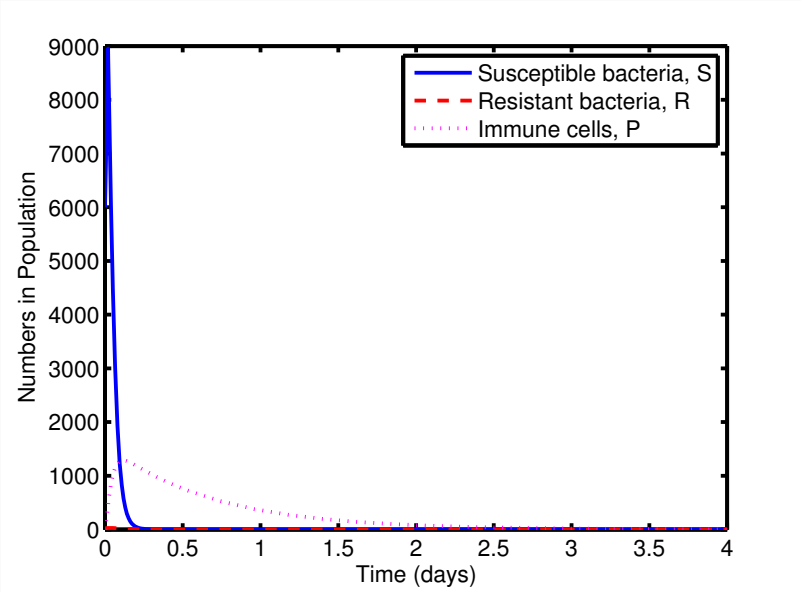


Anti-virulence drug then antibiotic after $t = 0.5$ hours.

Combining drugs with a time delay





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Complete bacterial elimination of a mixed antibiotic-resistant/susceptible infection can be achieved with the right treatment strategy

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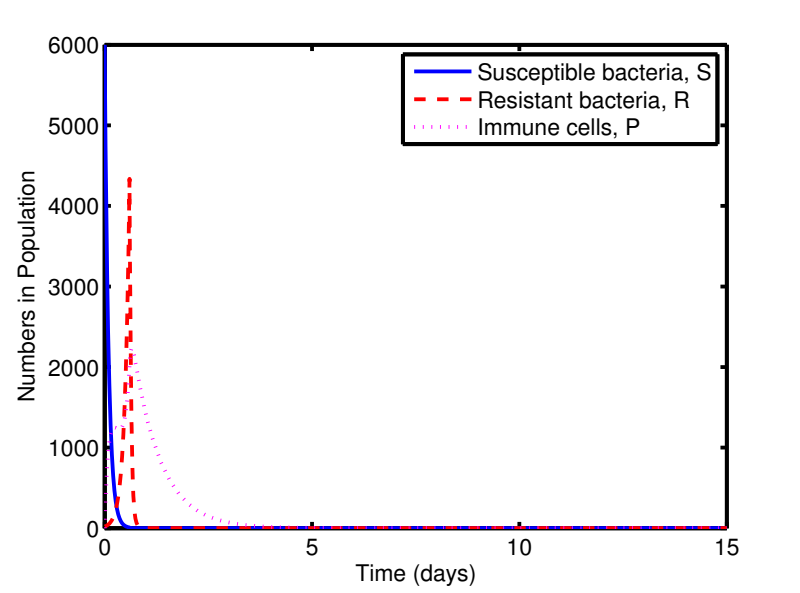

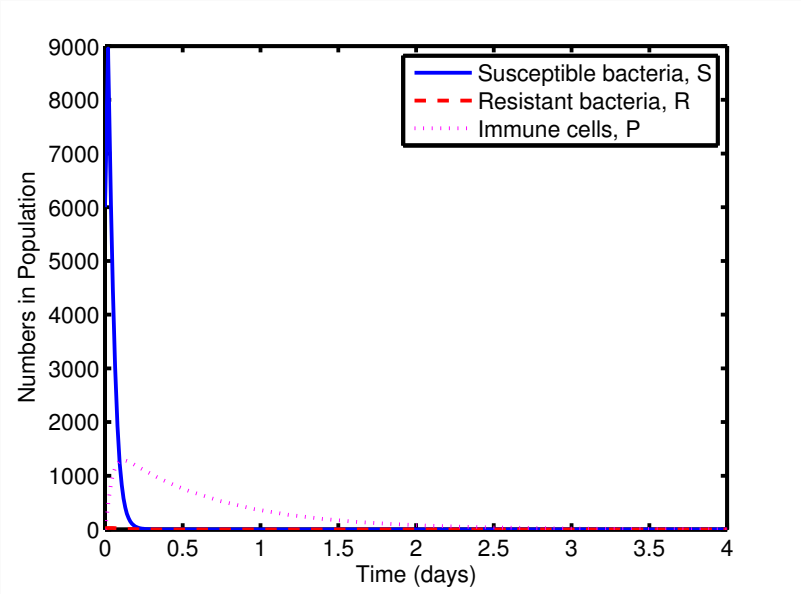

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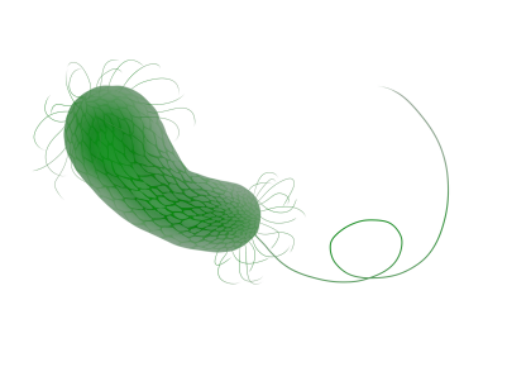
Extend model to be bacteria/treatment specific!

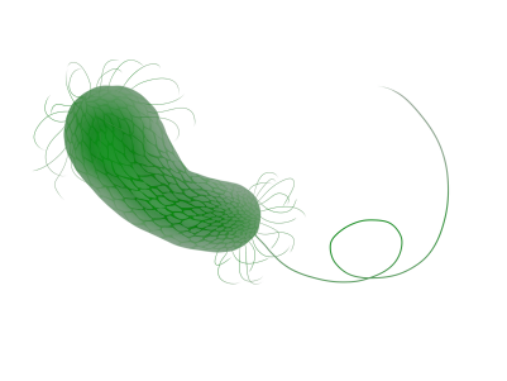
Pseudomonas aeruginosa



By DataBase Center for Life Science (DBCLS) (http://togotv.dbcls.jp/ja/togopic.2017.38.html), via Wikimedia Commons

- Pathogenic, Gram-negative, nosocomial

Pseudomonas aeruginosa



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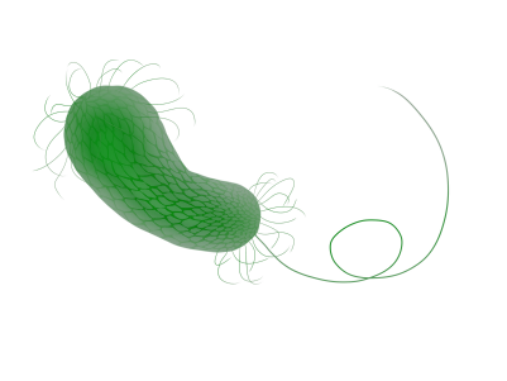

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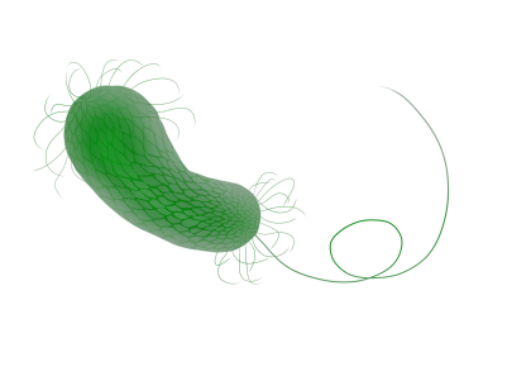

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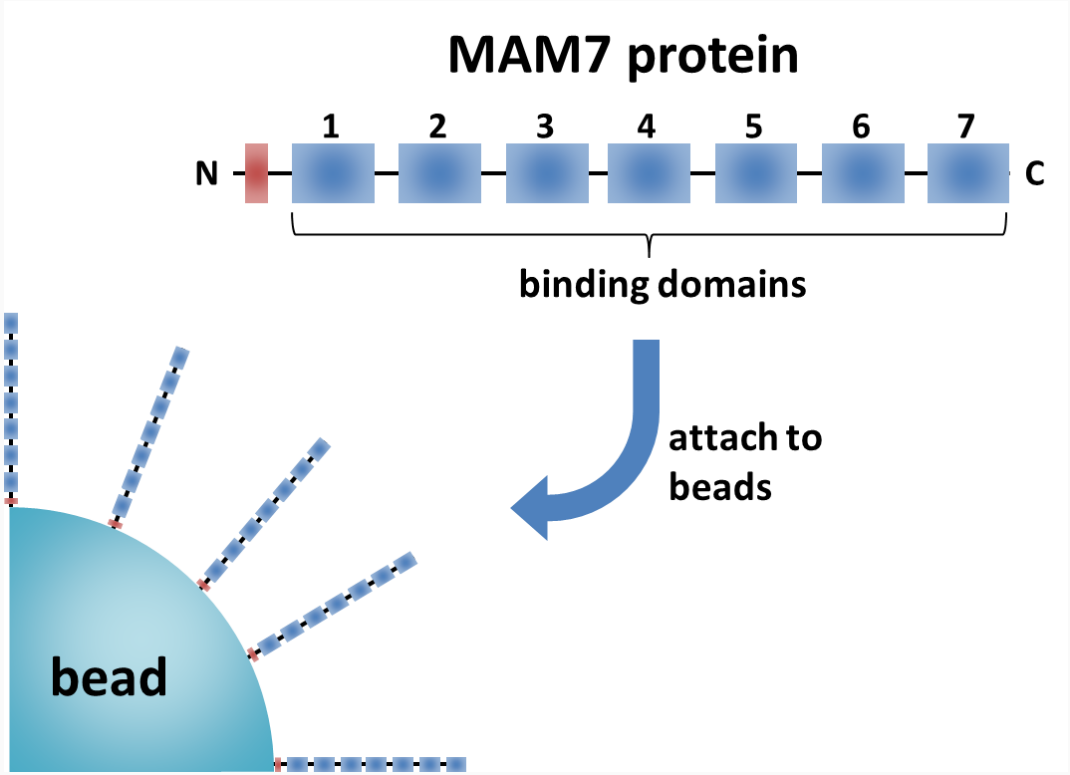
- Pathogenic, Gram-negative, nosocomial
- Particularly dangerous for immunocompromised patients
- Multi-antibiotic-resistant strains occur
- WHO: priority pathogen (critical level)

Targeting adhesion

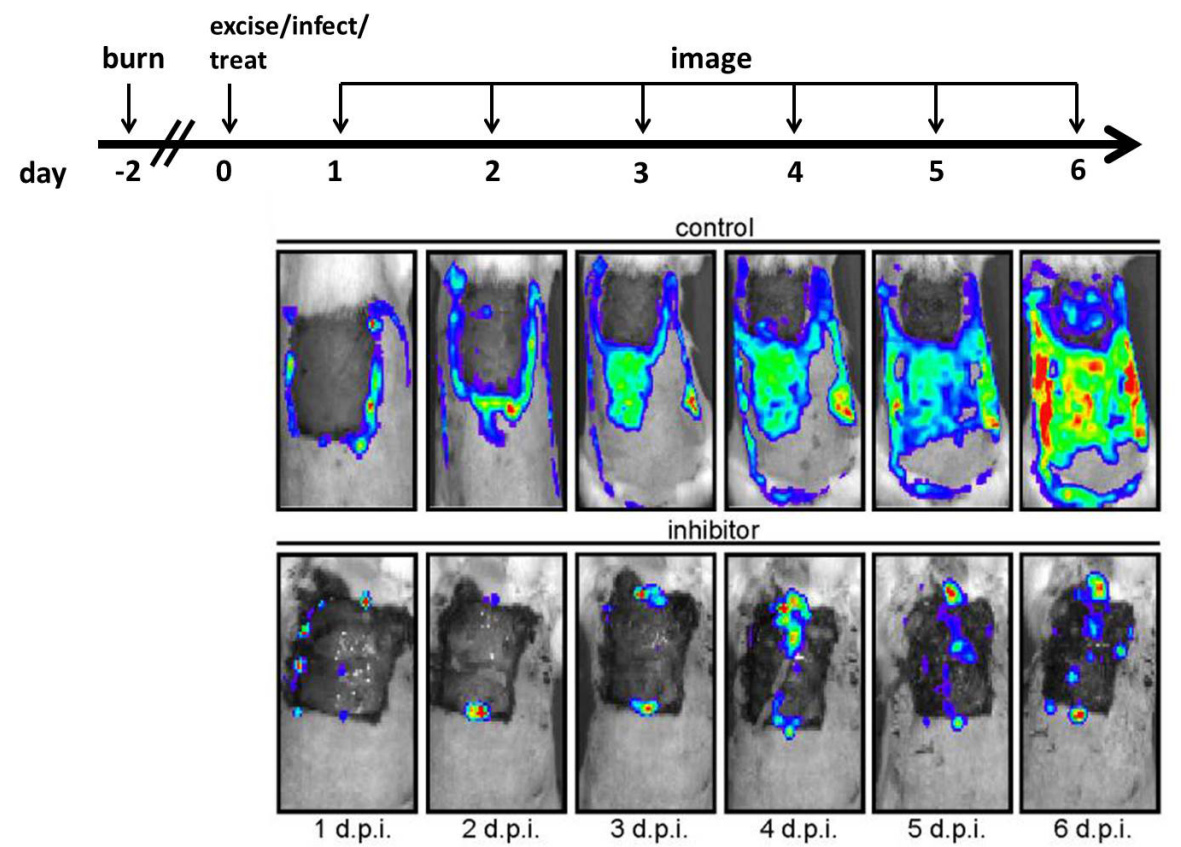
Multivalent Adhesion Molecule 7



Anne-Marie Krachler
U. Texas

- MAM7s are found on the surface of a number of bacterial species, including *P. aeruginosa*
- They mediate initial host attachment
- Treatment consists of polystyrene microbeads coated in MAM7s

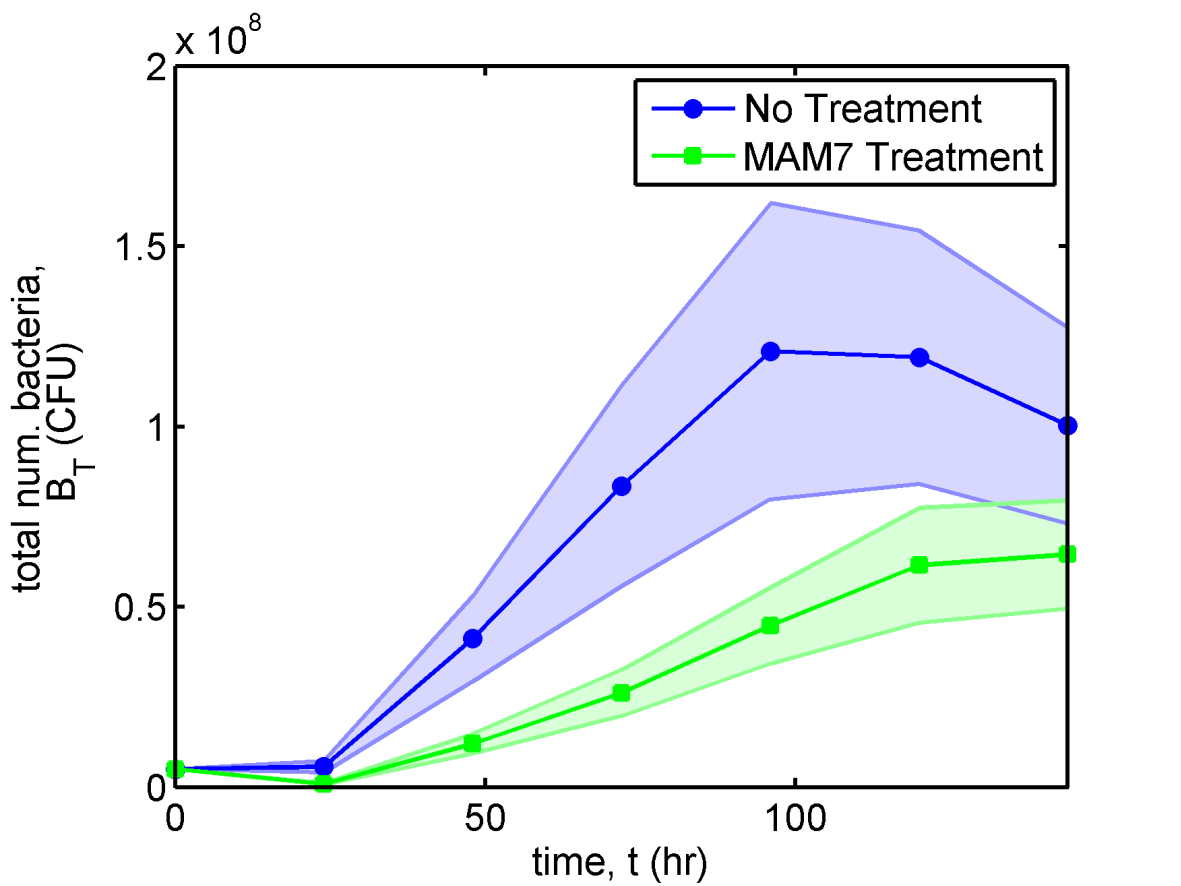
Burn-wound experimental model



Huebinger *et al.* Sci. Rep. 2016

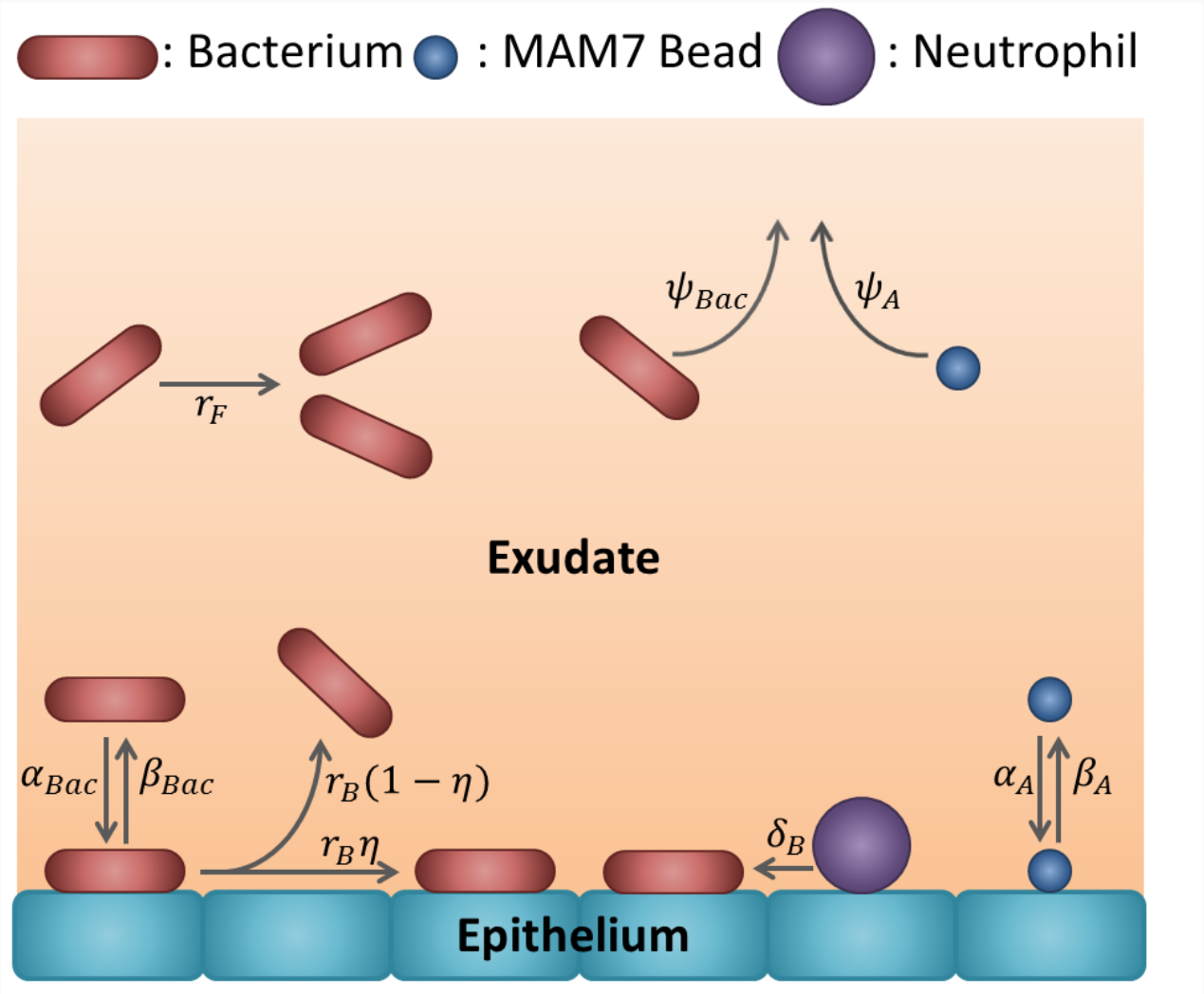
- *P. aeruginosa* bioluminesce
- The bacterial population is inhibited with MAM7 treatment

Burn-wound experimental model



- What happens beyond 7 days?
- How can we improve the treatment?

Model formulation



Paul Roberts
U. Birmingham/
Sussex

Model formulation

B_F : free bacteria density ($B_F(0) = 1.0 \times 10^6$ cells cm^{-3})
B_B : bound bacteria density ($B_B(0) = 0$ cells cm^{-2})
E : free binding site density ($= E_{init} - \phi_{Bac} B_B - \phi_A A_B$ sites cm^{-2})
A_F : free bead density ($A_F(0) = 6.5 \times 10^7$ beads cm^{-3})
A_B : bound bead density ($A_B(0) = 0$ beads cm^{-2})
t : time (hours)

$$\frac{\mathrm{d}B_F}{\mathrm{d}t} = r_F B_F \left(1 - \frac{B_F}{K_F}\right) + (1 - \eta(E)) H(K_B - B_B) \frac{r_B}{h} B_B \left(1 - \frac{B_B}{K_B}\right) - \alpha_{Bac} A B_F E + \frac{\beta_{Bac}}{h} B_B - \psi_{Bac}(t) B_F$$

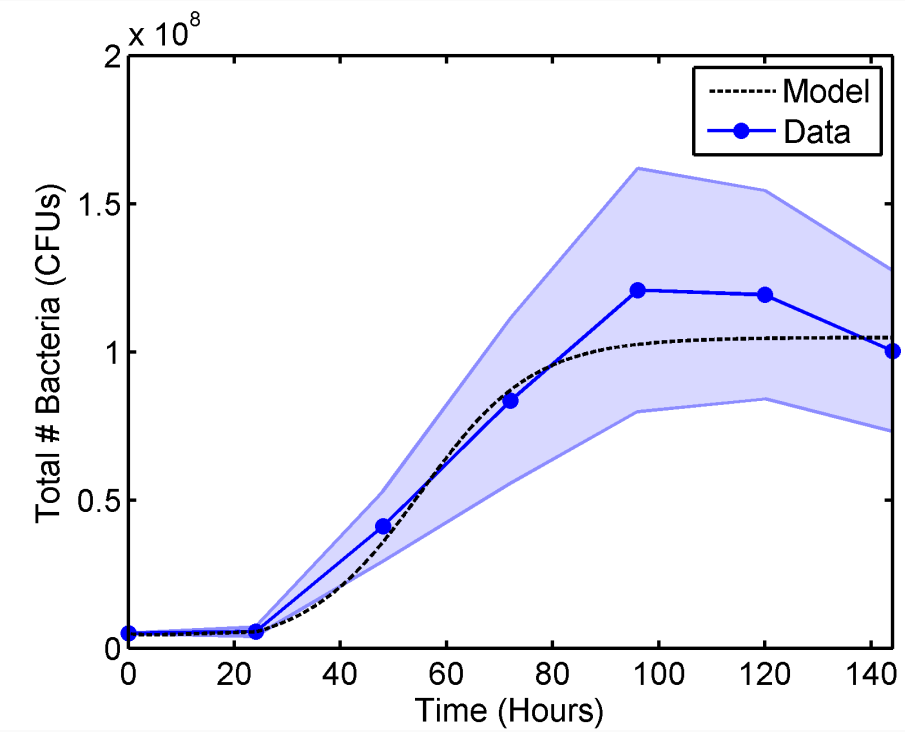
$$\frac{\mathrm{d}B_B}{\mathrm{d}t} = (1 + (\eta(E) - 1) H(K_B - B_B)) r_B B_B \left(1 - \frac{B_B}{K_B}\right) + \alpha_{Bac} V B_F E - \beta_{Bac} B_B - \delta_B B_B$$

$$\frac{\mathrm{d}A_F}{\mathrm{d}t} = -\alpha_A A A_F E + \frac{\beta_A}{h} A_B - \psi_A(t) A_F$$

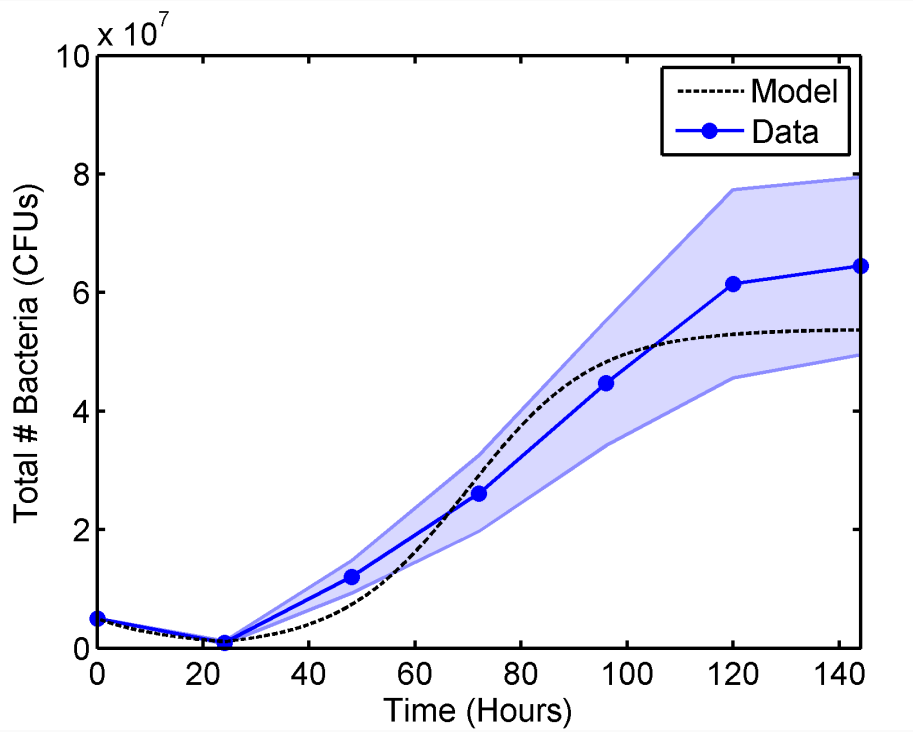
$$\frac{\mathrm{d}A_B}{\mathrm{d}t} = \alpha_A V A_F E - \beta_A A_B$$

Results — Case A : model fit

No Treatment

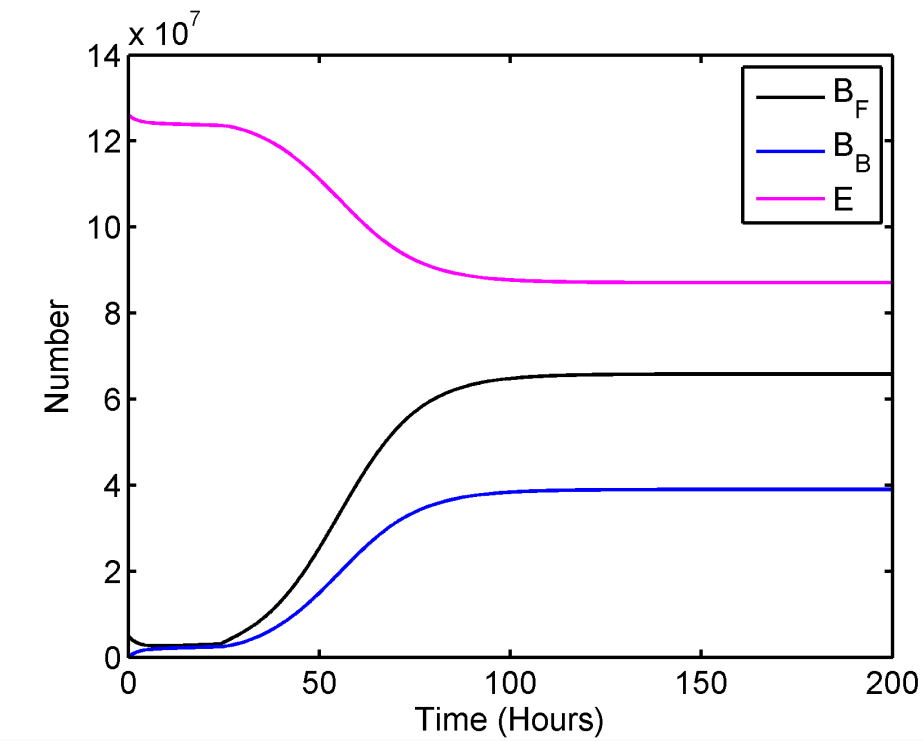


MAM7 Treatment



Results — Case A : model predictions beyond 7 days

No Treatment



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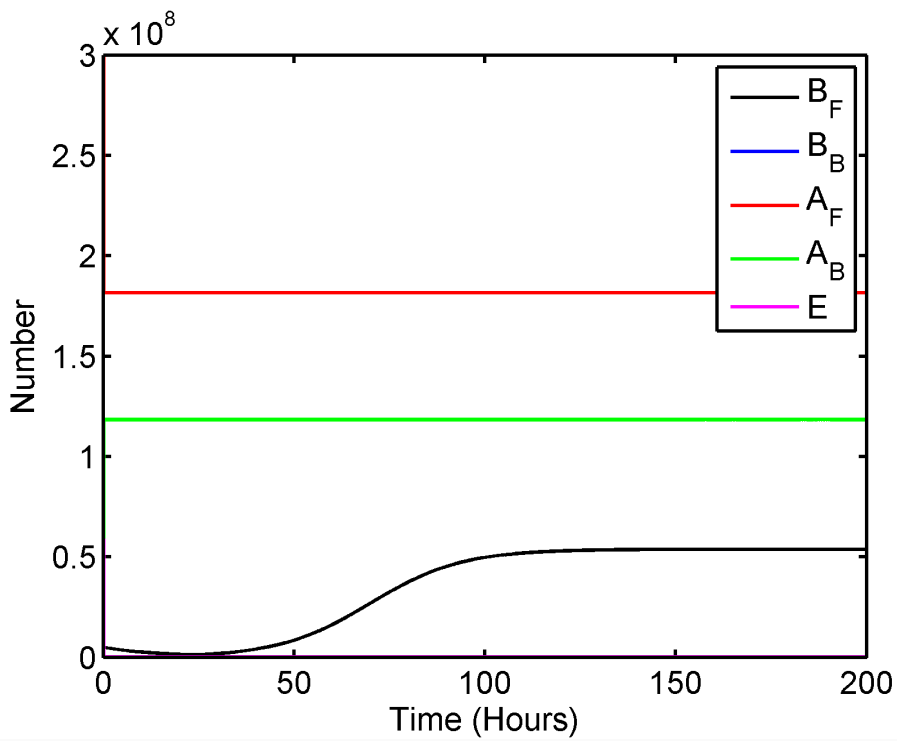
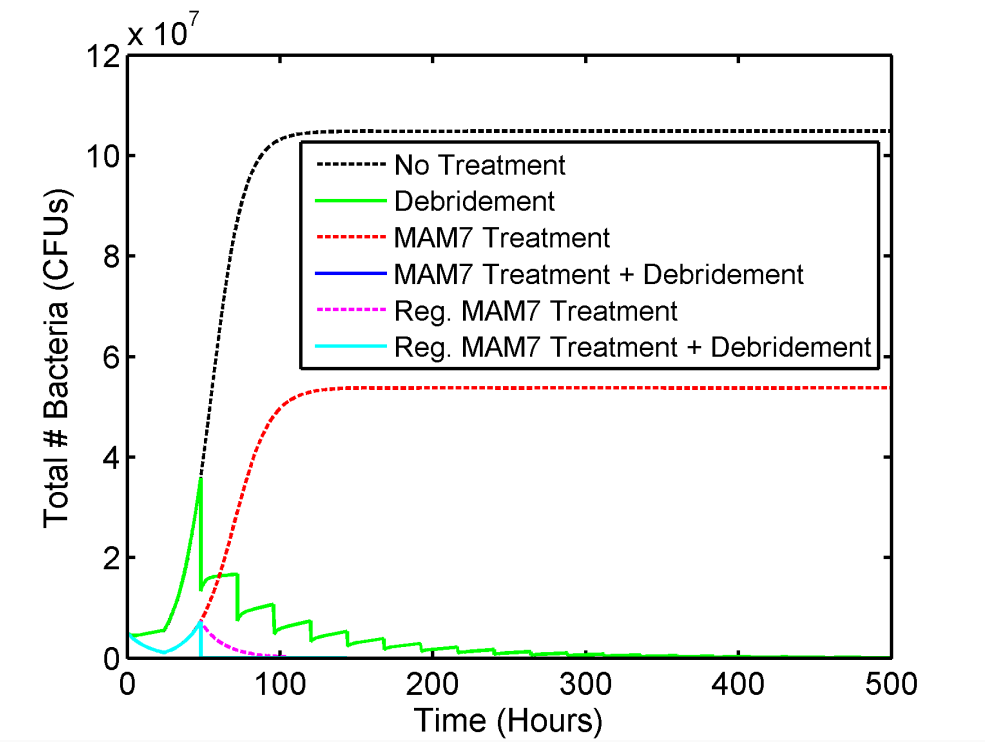


- No Treatment: Both free and bound bacteria are abundant
- MAM7 Treatment: Only free bacteria persist in significant numbers

Results — Case A : model predictions improving efficacy

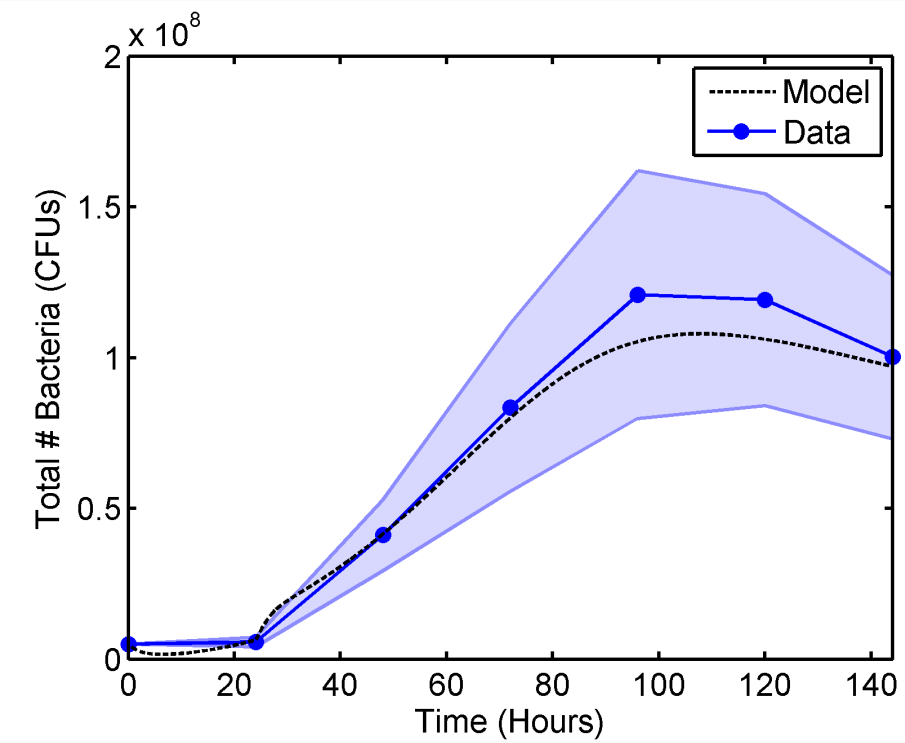
Debridement (washing)



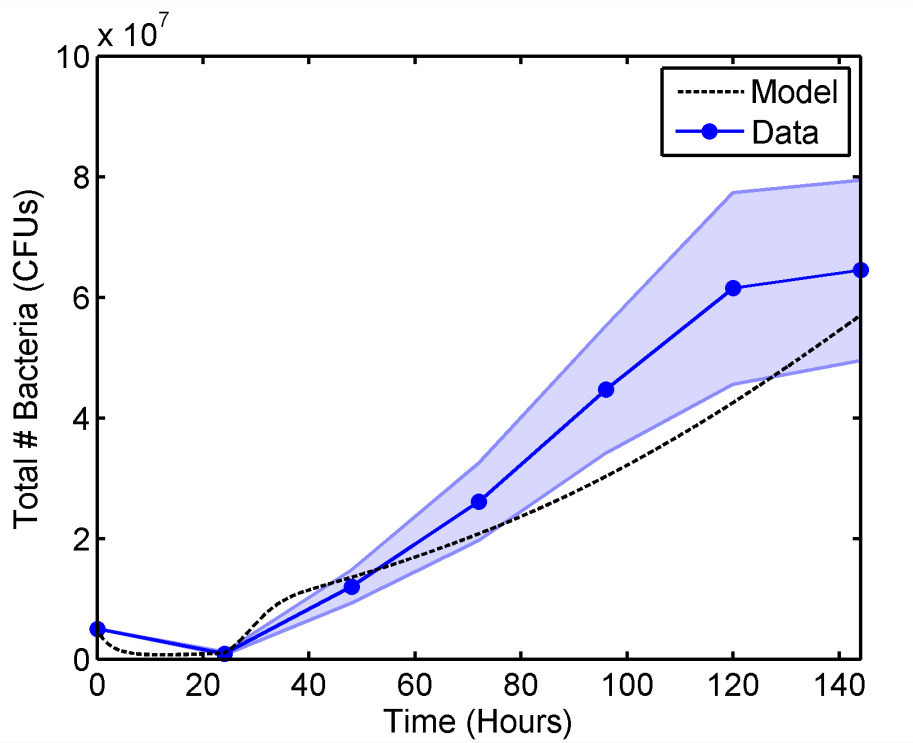
- Bacteria can be cleared by combining debridement with MAM7 beads

Results — Case B : model fit

No Treatment

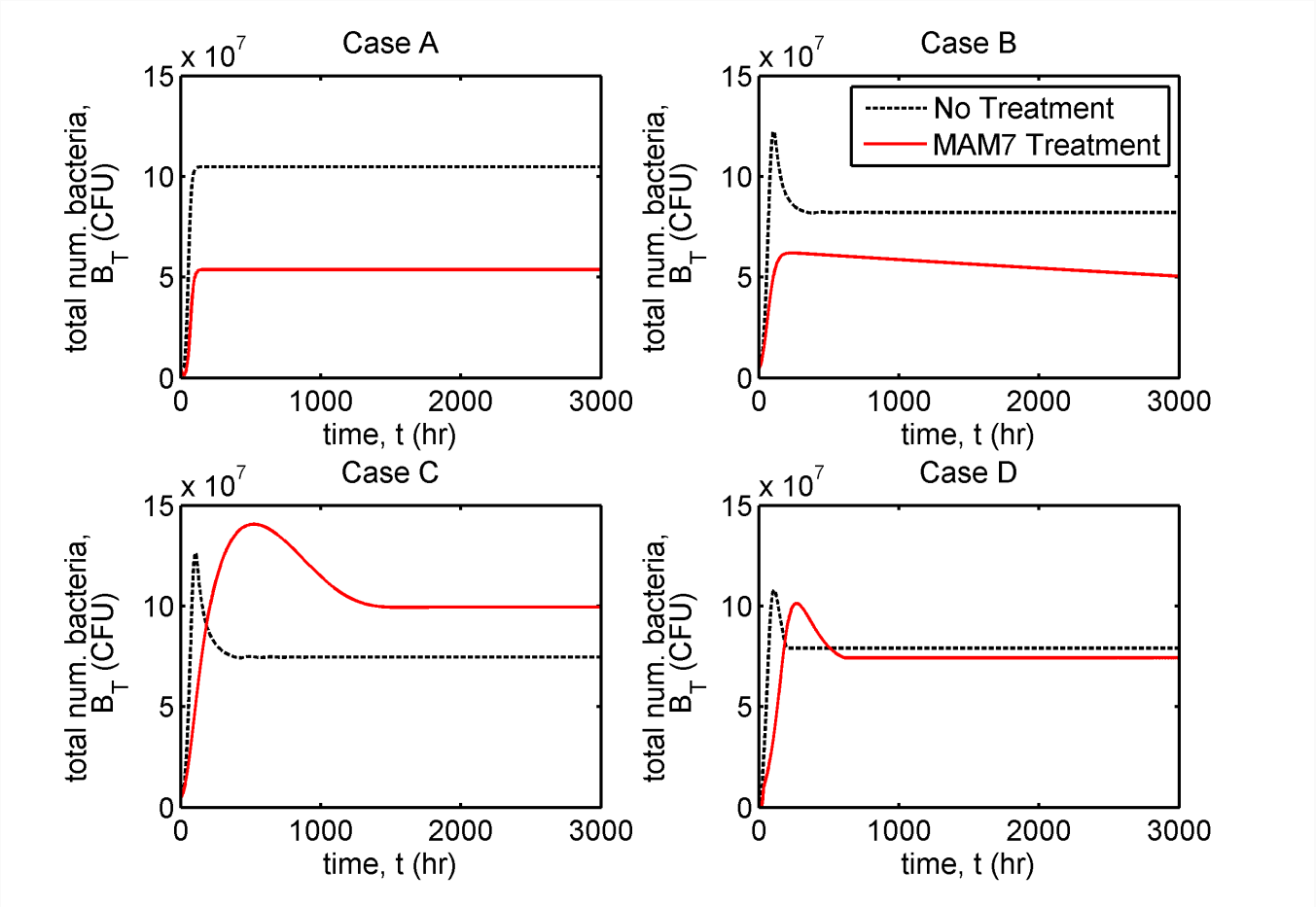


MAM7 Treatment



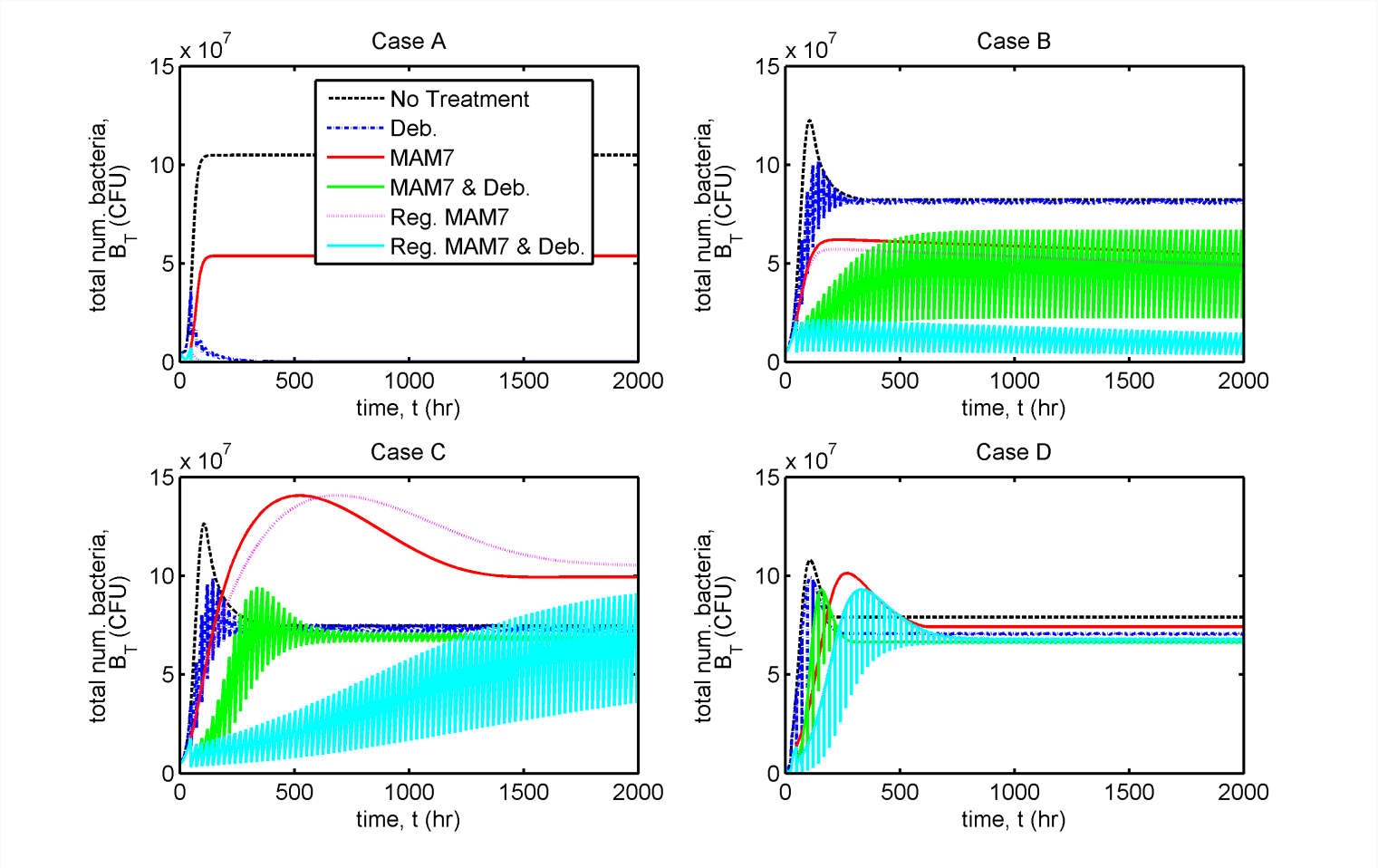
Entirely different set of estimated parameters!

4 Plausible parameter sets



- Cases A and B: treatment is effective
- Cases C and D: treatment is ineffective

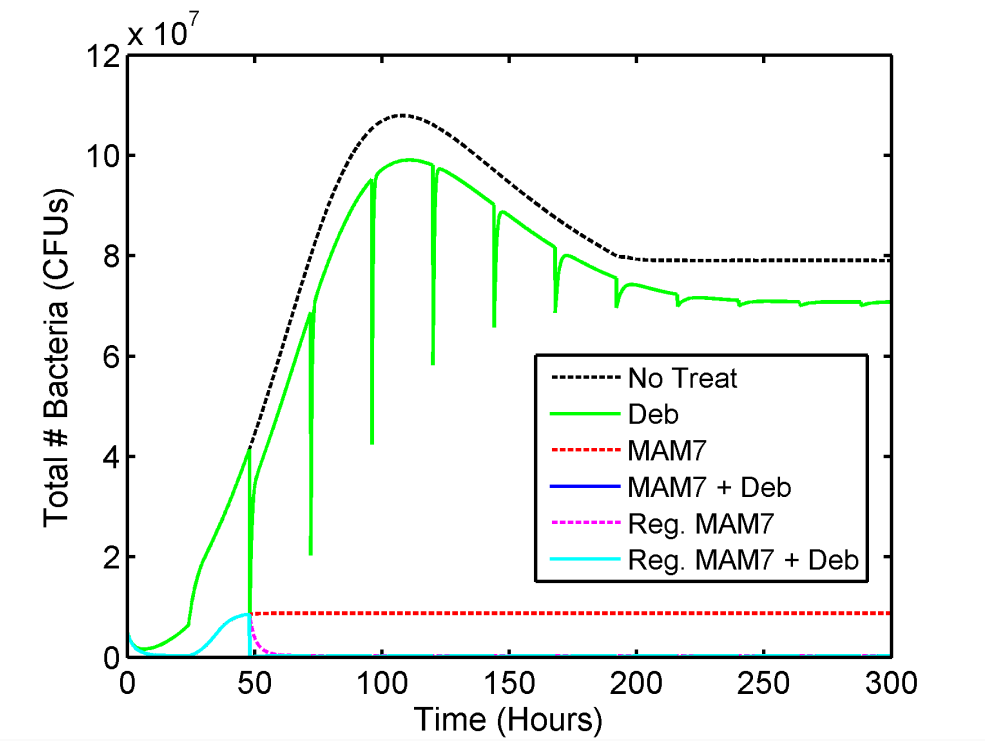
Treatment predictions



- Cases A and B: debridement is effective
- Cases C and D: debridement is ineffective

Making the treatment work — reduced β_A (Case D)

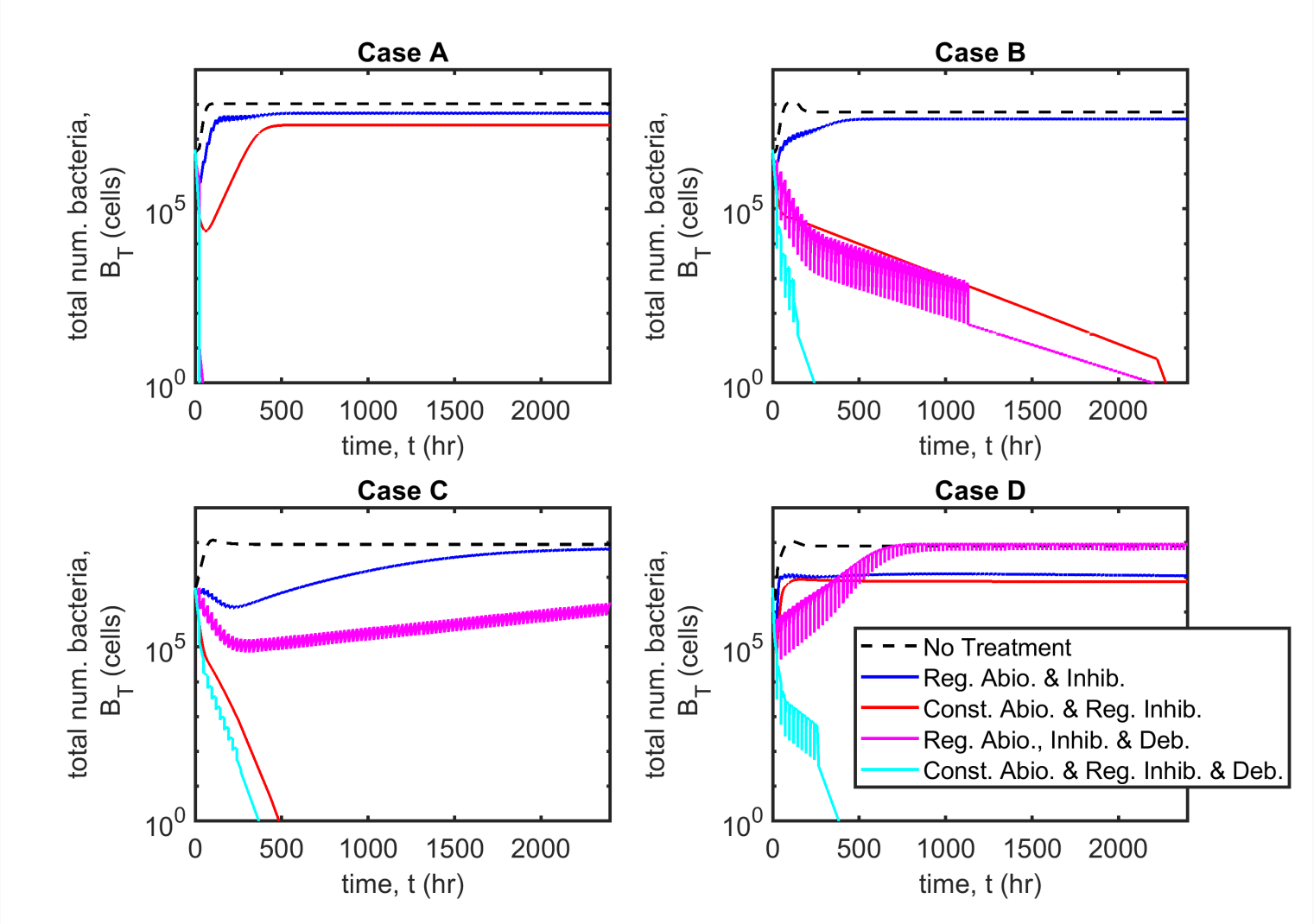
Debridement (washing)



- Almost all bacteria can now be eliminated when MAM7 treatment is used in combination with debridement

Combination treatment with antibiotics – treating an antibiotic resistant infection

Combination treatment with antibiotics – treating an antibiotic resistant infection



Optimising combined treatments

Continuous antibiotics, possible daily debridement, possible varying daily bead dose $\rightarrow$ 14,407 possibilities over a week

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- Antibiotics and anti-adhesion beads combine synergistically

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- To prevent infection: use all beads initially and debride daily

Optimising combined treatments

Continuous antibiotics, possible daily debridement, possible varying daily bead dose $\rightarrow$ 14,407 possibilities over a week

- Antibiotics and anti-adhesion beads combine synergistically
- To prevent infection: use all beads initially and debride daily
- To clear infection: use all beads initially and delay debridement

Optimising combined treatments

Continuous antibiotics, possible daily debridement, possible varying daily bead dose $\rightarrow$ 14,407 possibilities over a week

- Antibiotics and anti-adhesion beads combine synergistically
- To prevent infection: use all beads initially and debride daily
- To clear infection: use all beads initially and delay debridement
- Can significantly reduce the antibiotic usage

Anti-adhesion summary

- Treatment would work by preventing bacteria binding to host cells

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- Model predicts we can combine with debridement and/or change the bead design to improve efficacy

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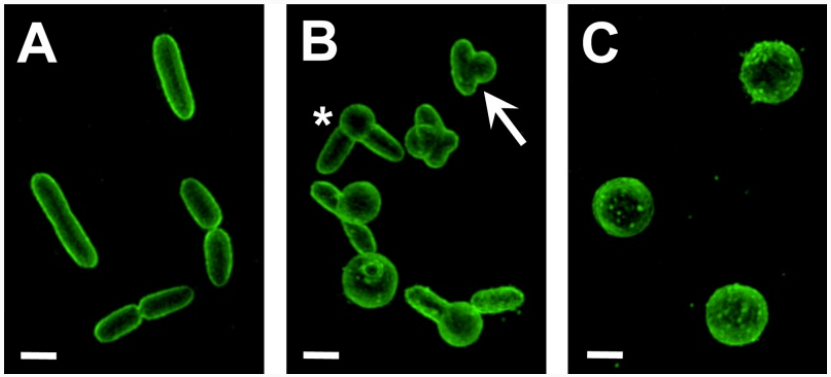
Anti-adhesion summary

- Treatment would work by preventing bacteria binding to host cells
- Model predicts we can combine with debridement and/or change the bead design to improve efficacy
- For full clearance, can combine with antibiotics (even on an antibiotic-resistant infection)
- Awaiting experimental testing...

Targeting changes in cell morphology – persister cells

Meropenem & *P. aeruginosa* cell morphology

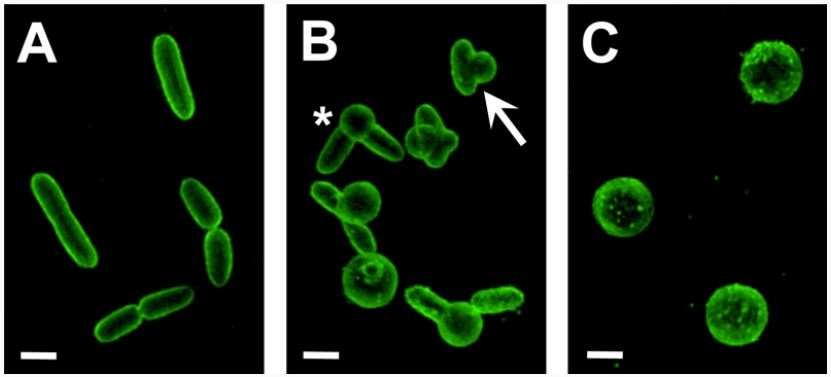


Monahan *et al.* Antimicrob. Ag. Chem. 58: 1956–62 (2014)

Chloe Spalding

P. aeruginosa cells transition to dormant spheres (persister cells?) upon exposure to certain antibiotics

Meropenem & *P. aeruginosa* cell morphology



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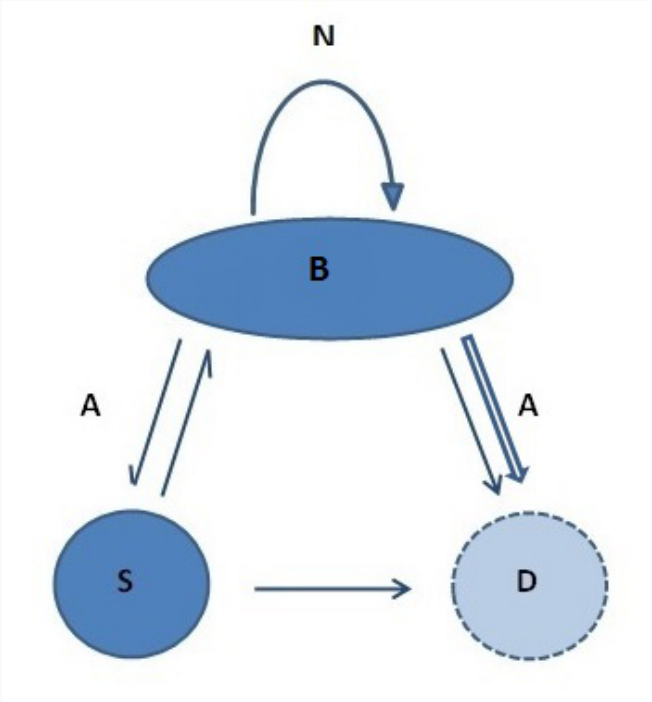

Chloe Spalding

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Model formulation

$$
\begin{aligned}
\frac{dB}{dt} &= rNB - \left(\frac{\gamma A}{T_{50} + A}\right) B + \delta S - \left(\frac{\rho A}{A_{50} + A}\right) B - \phi B, \\
\frac{dS}{dt} &= \left(\frac{\gamma A}{T_{50} + A}\right) B - \delta S - \psi S, \\
\frac{dD}{dt} &= \left(\frac{\rho A}{A_{50} + A}\right) B + \phi B + \psi S, \\
\frac{dA}{dt} &= -\alpha A - \left(\frac{\tilde{\rho} A}{A_{50} + A}\right) B, \\
\frac{dN}{dt} &= -\tilde{r} NB,
\end{aligned}
$$

$$
B(0) = B_0, \quad S(0) = 0, \quad D(0) = 0, \quad A(0) = A_0, \quad N(0) = 1.
$$

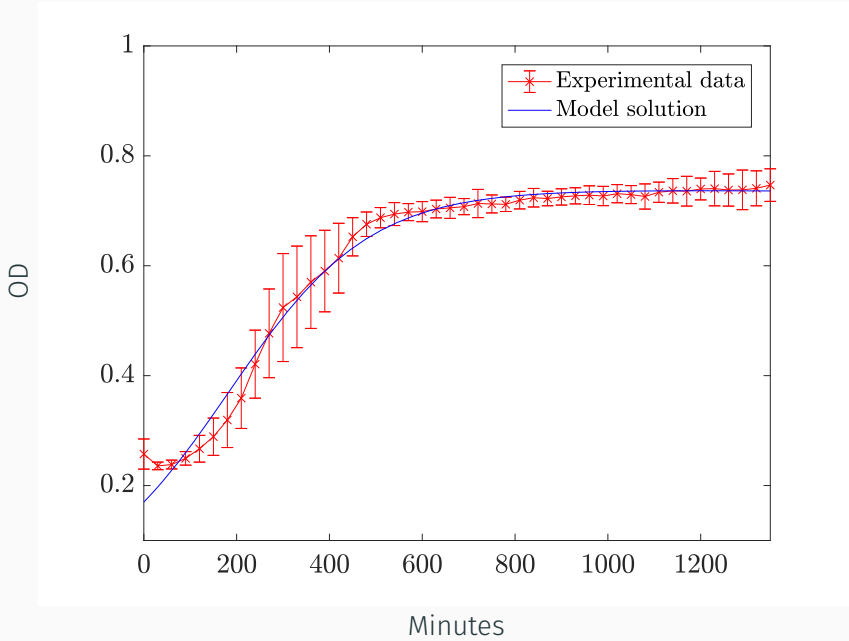
Parameterisation - growth curves

Parameterisation - growth curves

Emma Keen

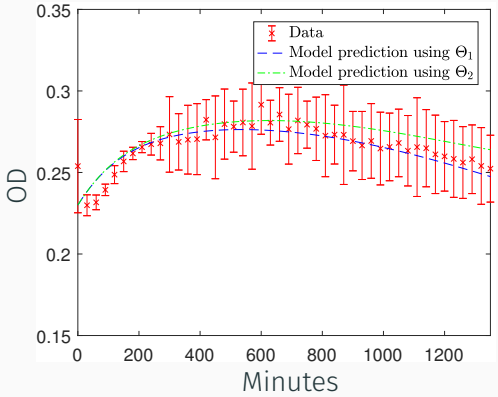
Parameterisation - growth curves

Emma Keen

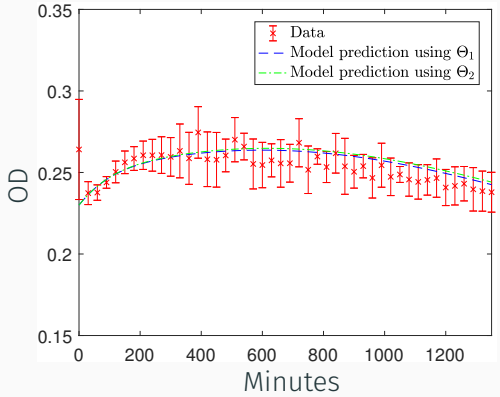


Parameterisation - kill curves

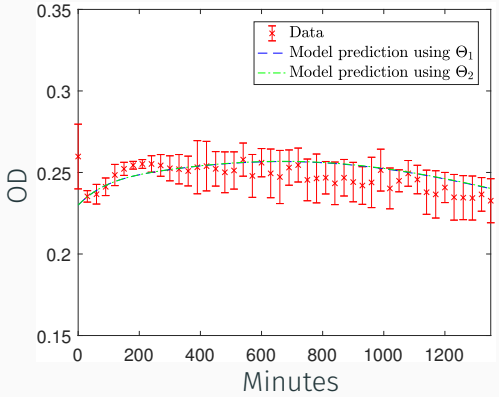
$2\mu g\,ml^{-1}$



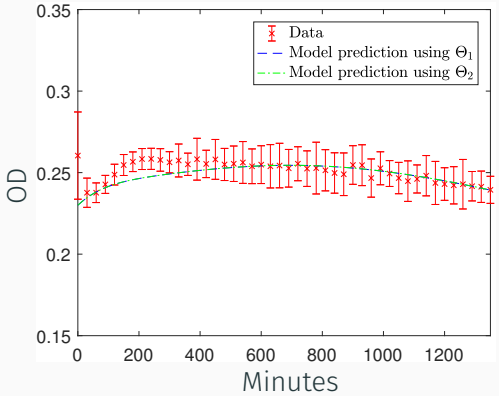
$4\mu g\,ml^{-1}$



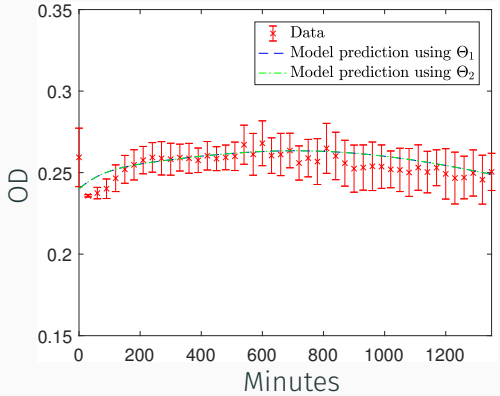
$10\mu g\,ml^{-1}$



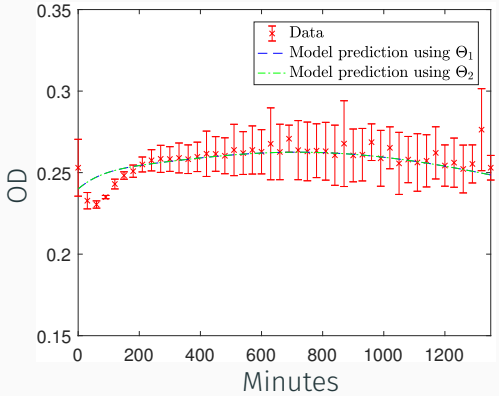
$20\mu g\,ml^{-1}$



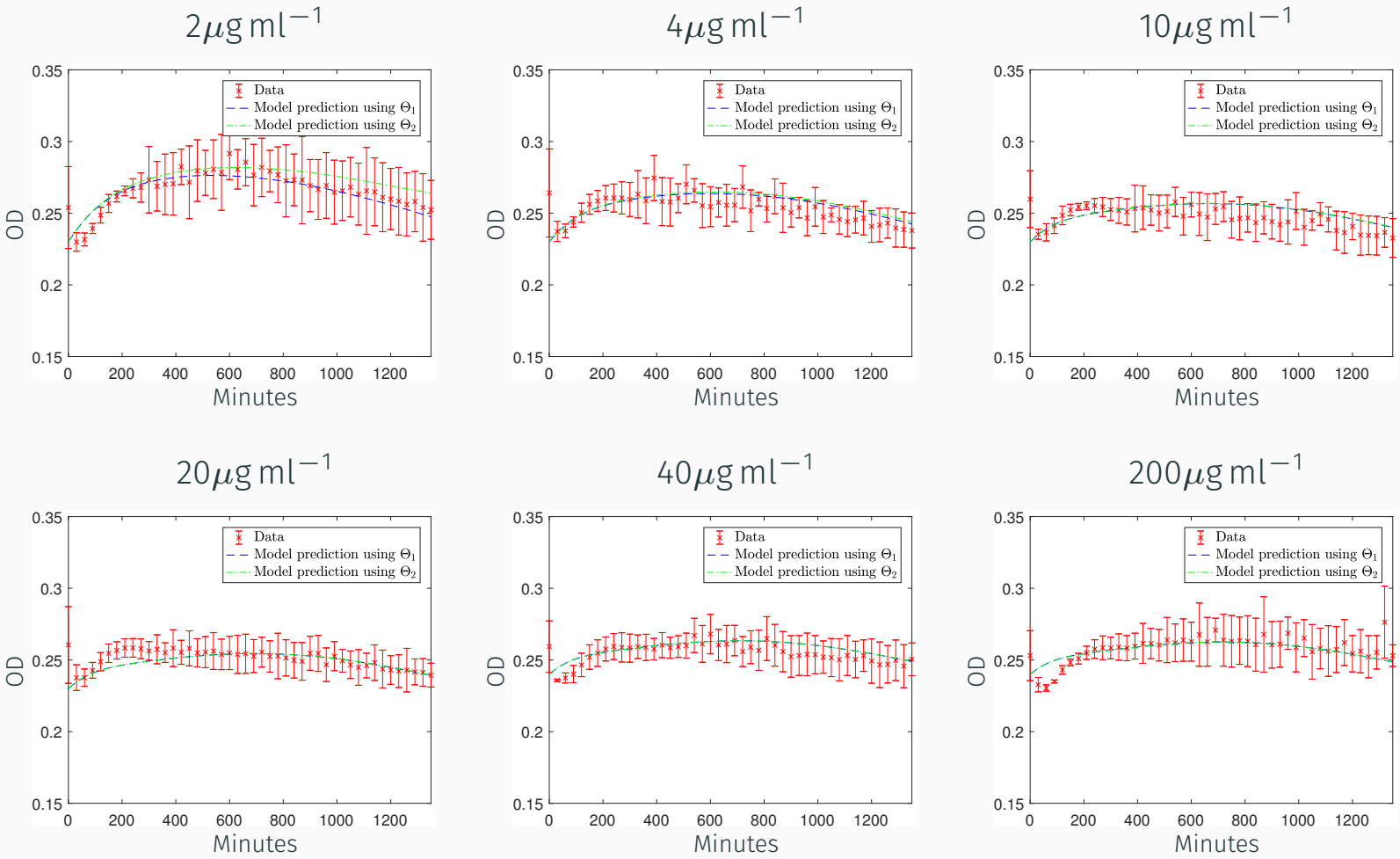
$40\mu g\,ml^{-1}$



$200\mu g\,ml^{-1}$



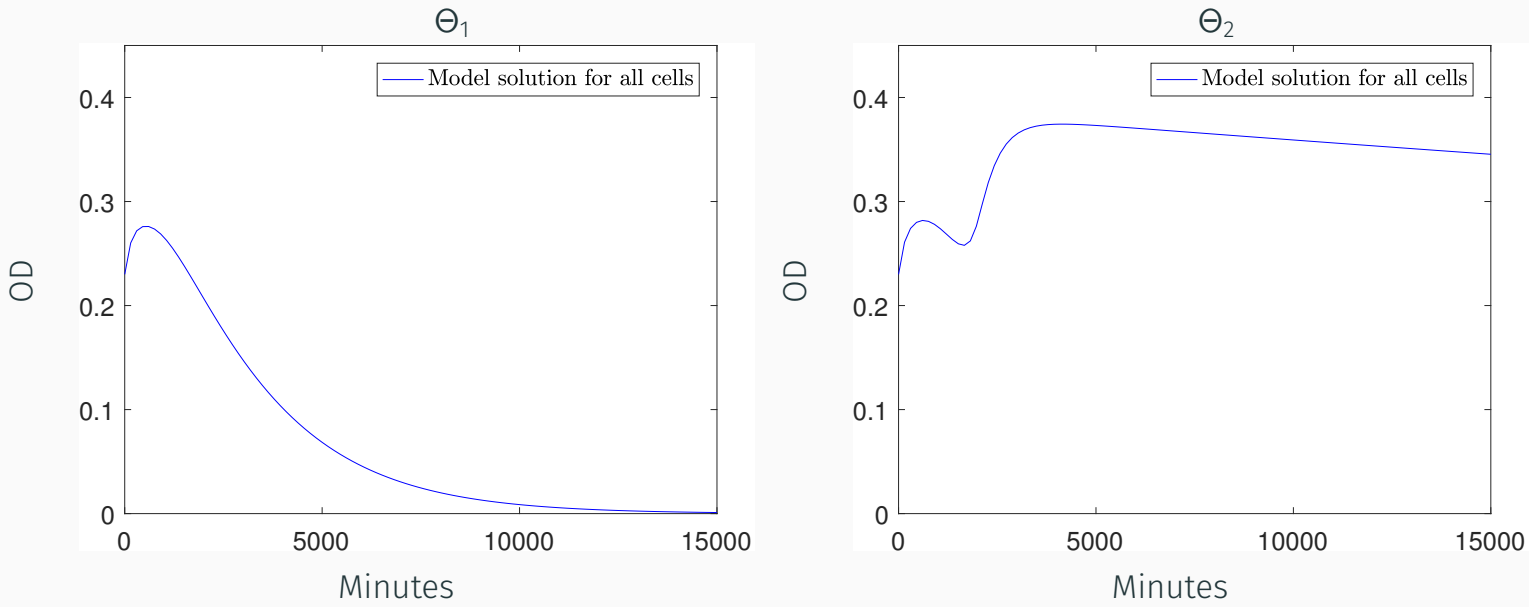
Parameterisation - kill curves



Two parameter sets: difference is in antibiotic sink terms

Long-term predictions

Qualitatively different long-term outcomes when $A_0 = 2\,\mu\text{g}\,\text{ml}^{-1}$.



Model validation - testing OD predictions

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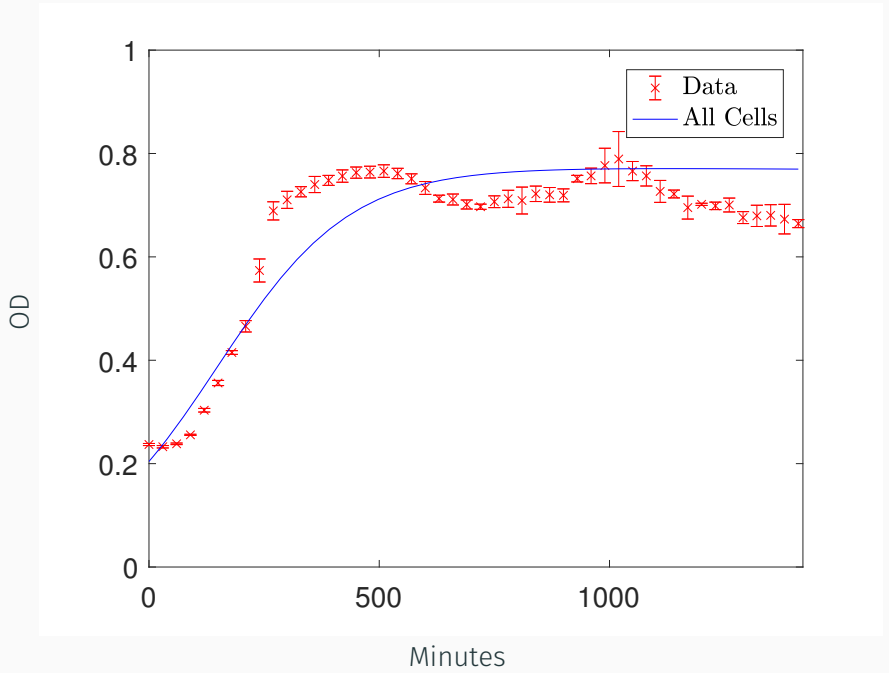
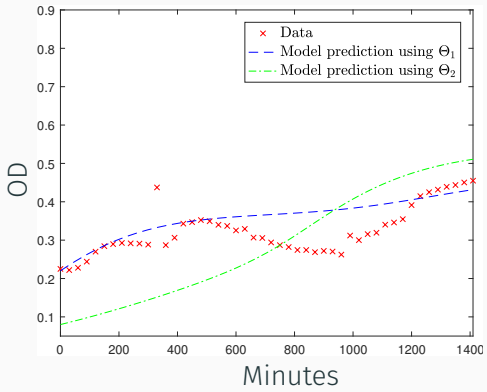
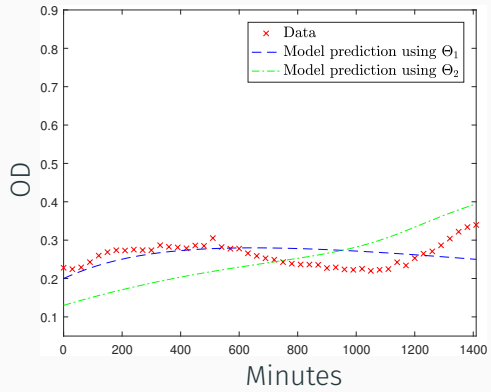


Model validation - testing OD predictions

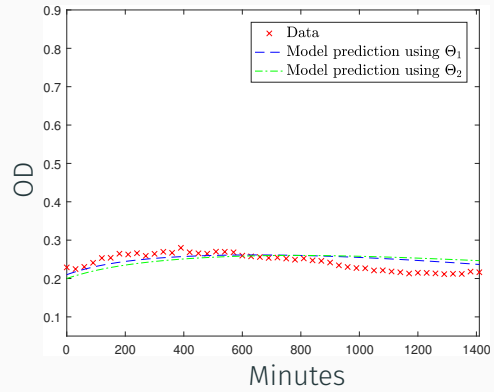
$0.5\mu g\,ml^{-1}$



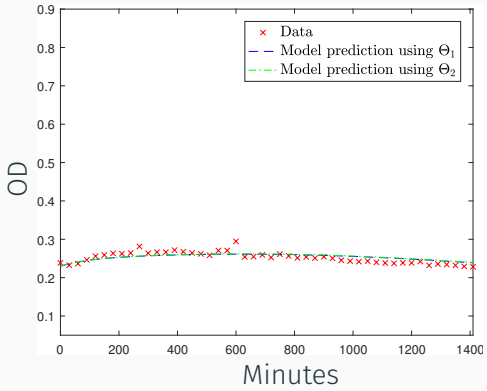
$1\mu g\,ml^{-1}$



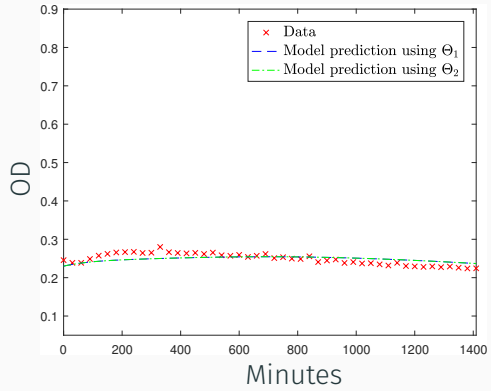
$2\mu g\,ml^{-1}$



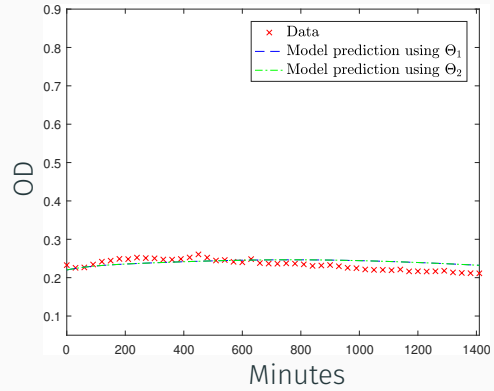
$5\mu g\,ml^{-1}$



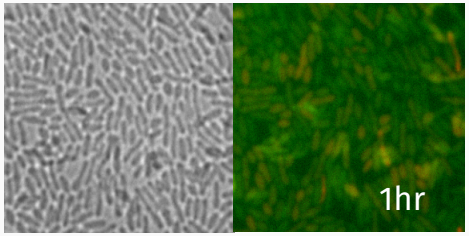
$50\mu g\,ml^{-1}$

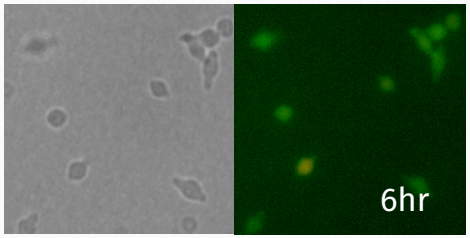


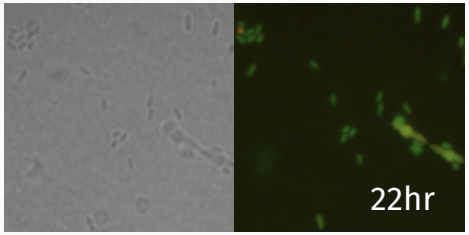
$100\mu g\,ml^{-1}$



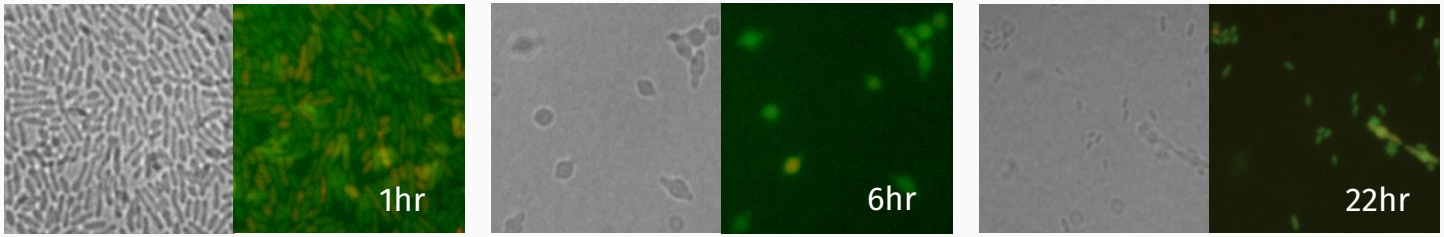
Microscopy data







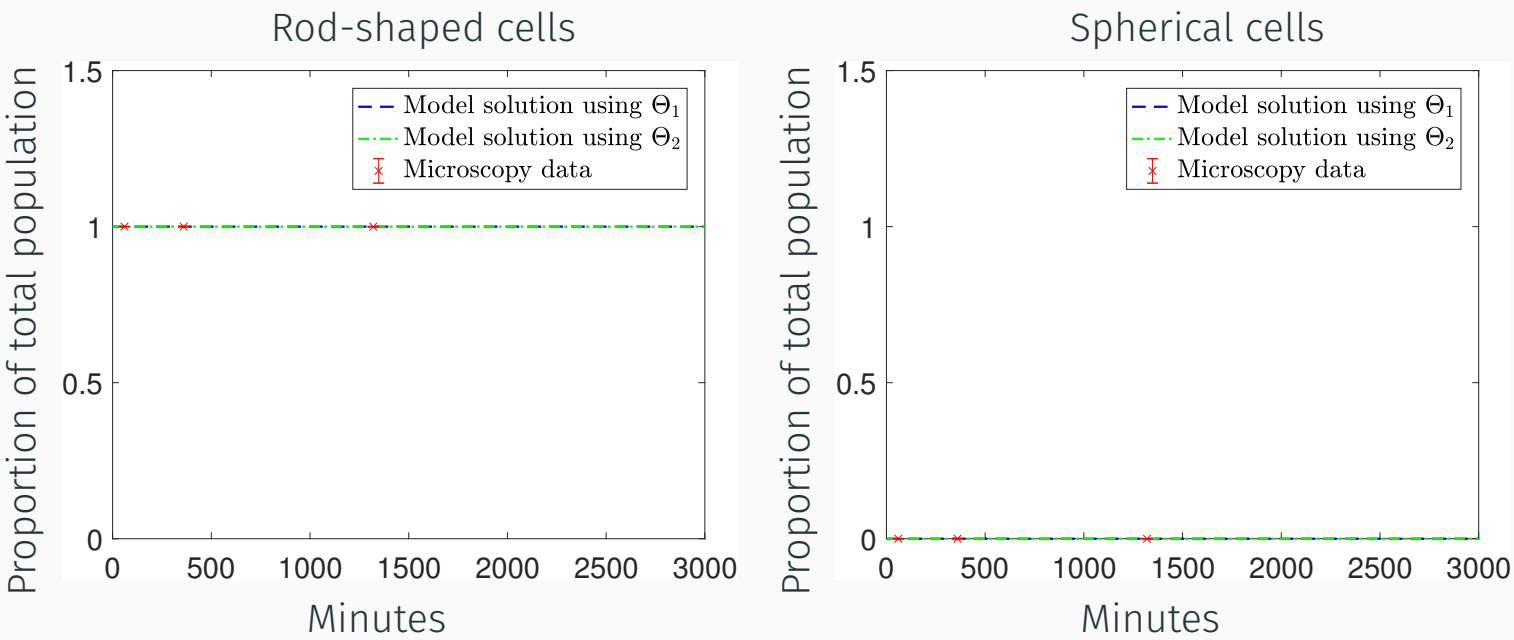
Microscopy data



$\rightarrow$ use the microscopy data to test our solutions for proportions of rods and spheres

Model validation – microscopy data

$0\,\mu g\,ml^{-1}$



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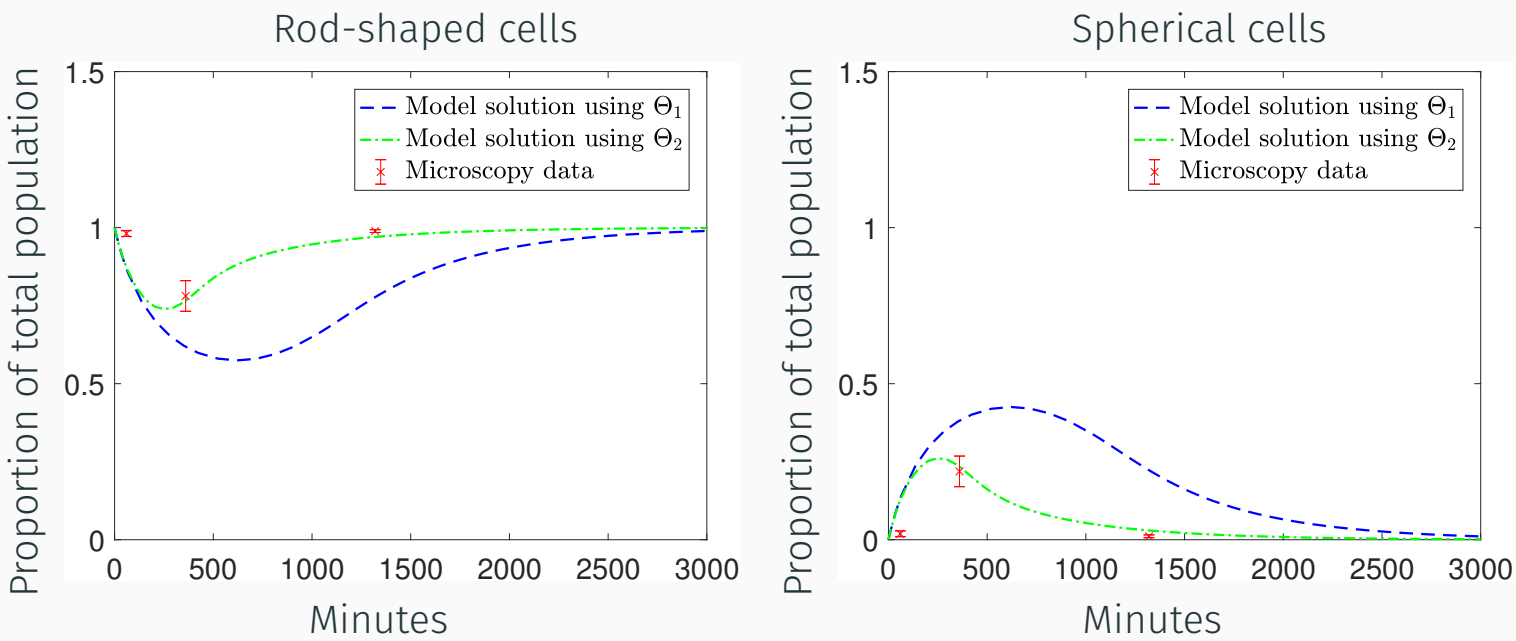
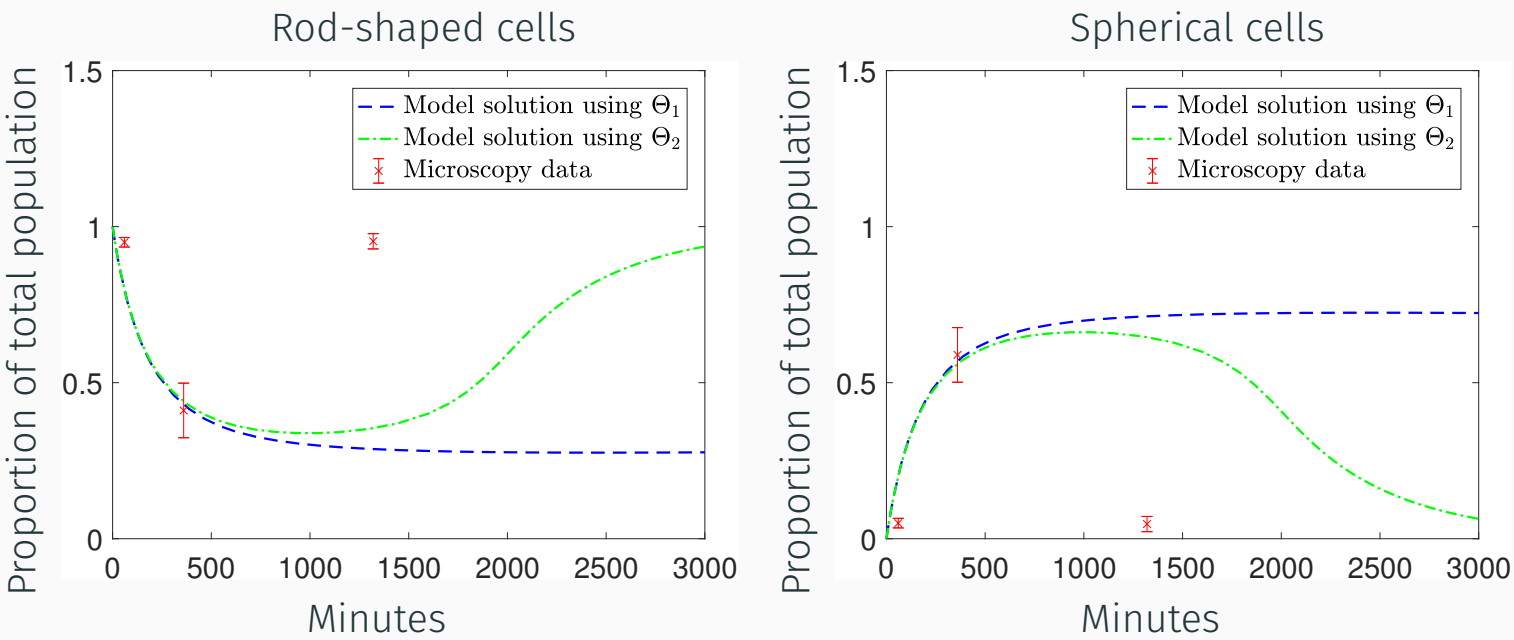


Model validation – microscopy data

$2\,\mu\mathrm{g\,ml^{-1}}$



Antimicrobial peptides as a potential adjuvant

We can simulate the use of antimicrobial peptides by increasing the death rate of the spherical cells, ψ.

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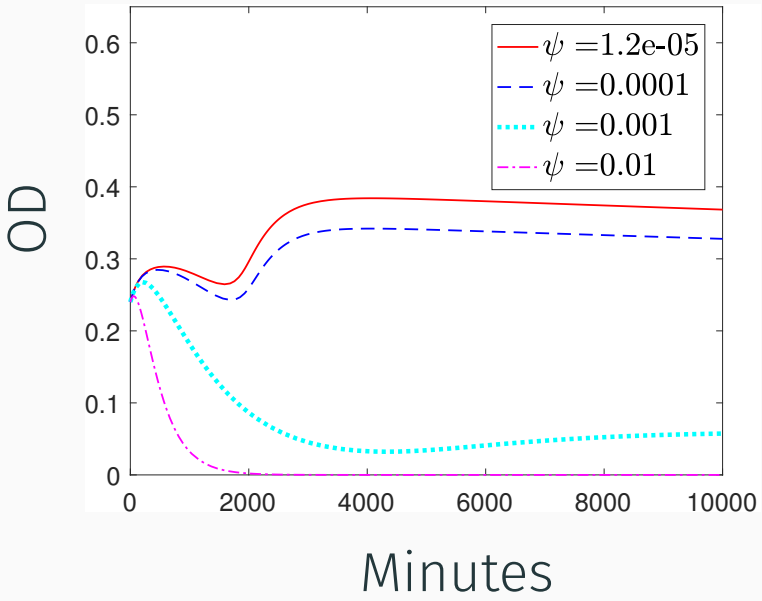

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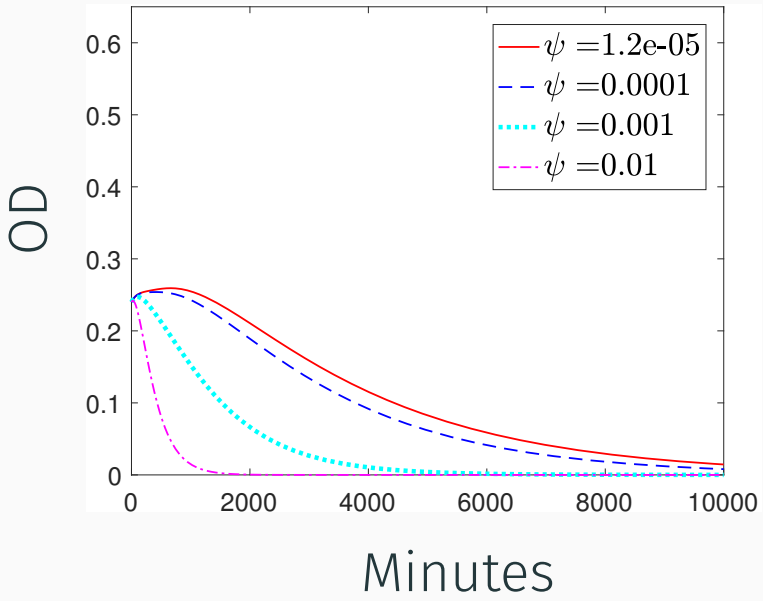
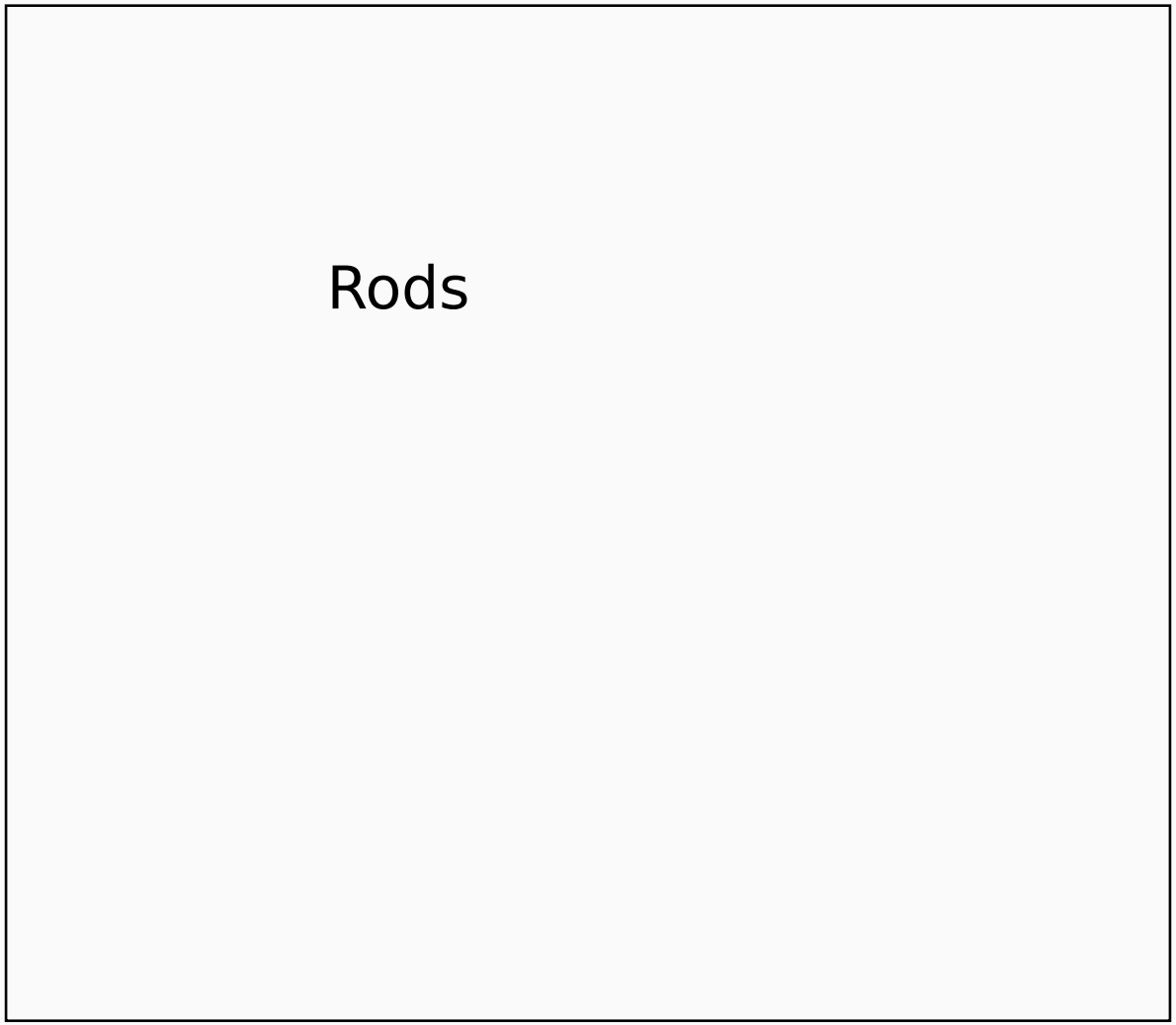


Predictions using Θ_2 and varying ψ

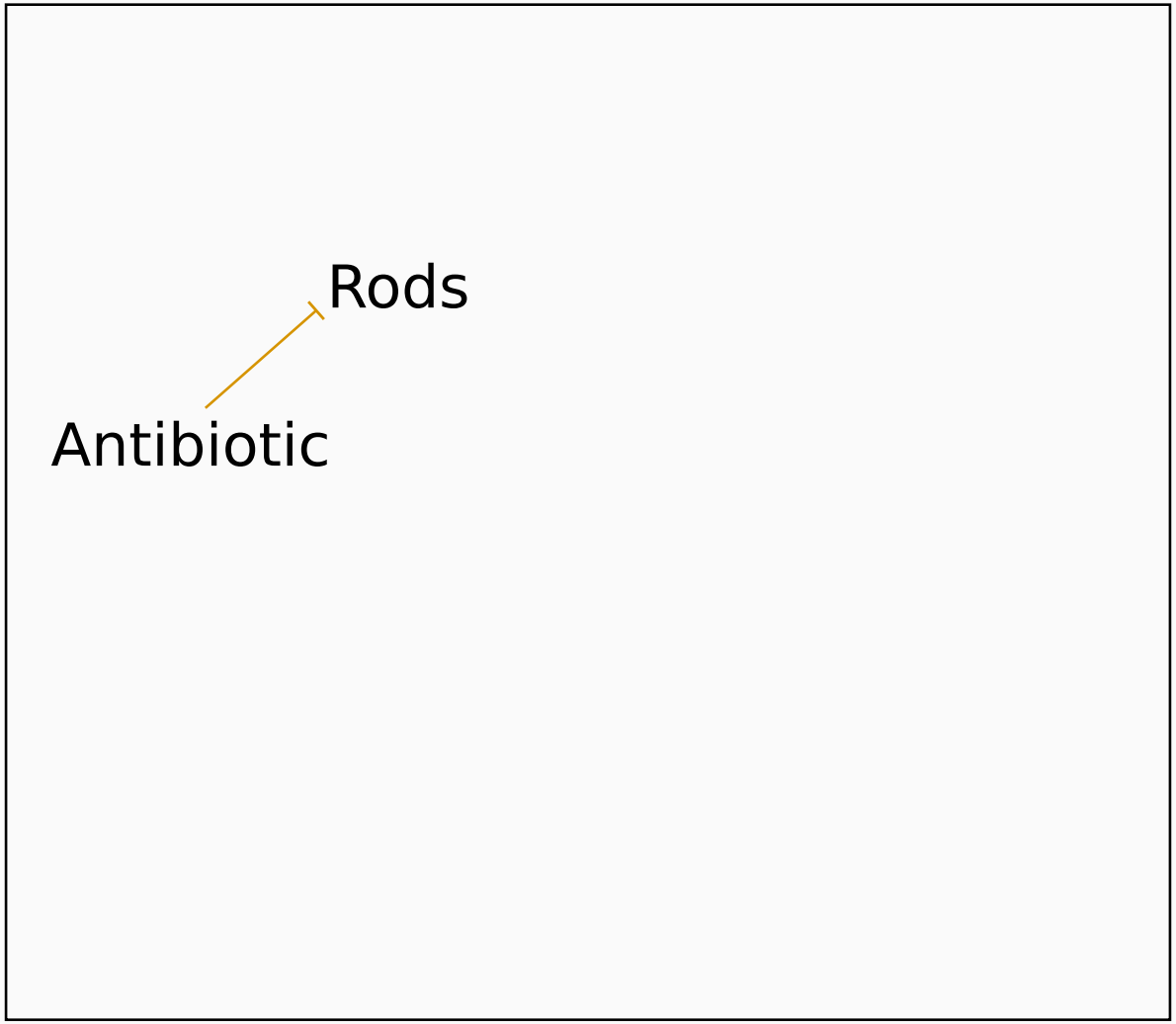
Infection level model

- Incorporate an immune response
  - immune cell recruitment rate the same for rods and spheres
  - phagocytosis rate lower for spheres
- Resistant bacteria may arise via mutation or by cross-contamination

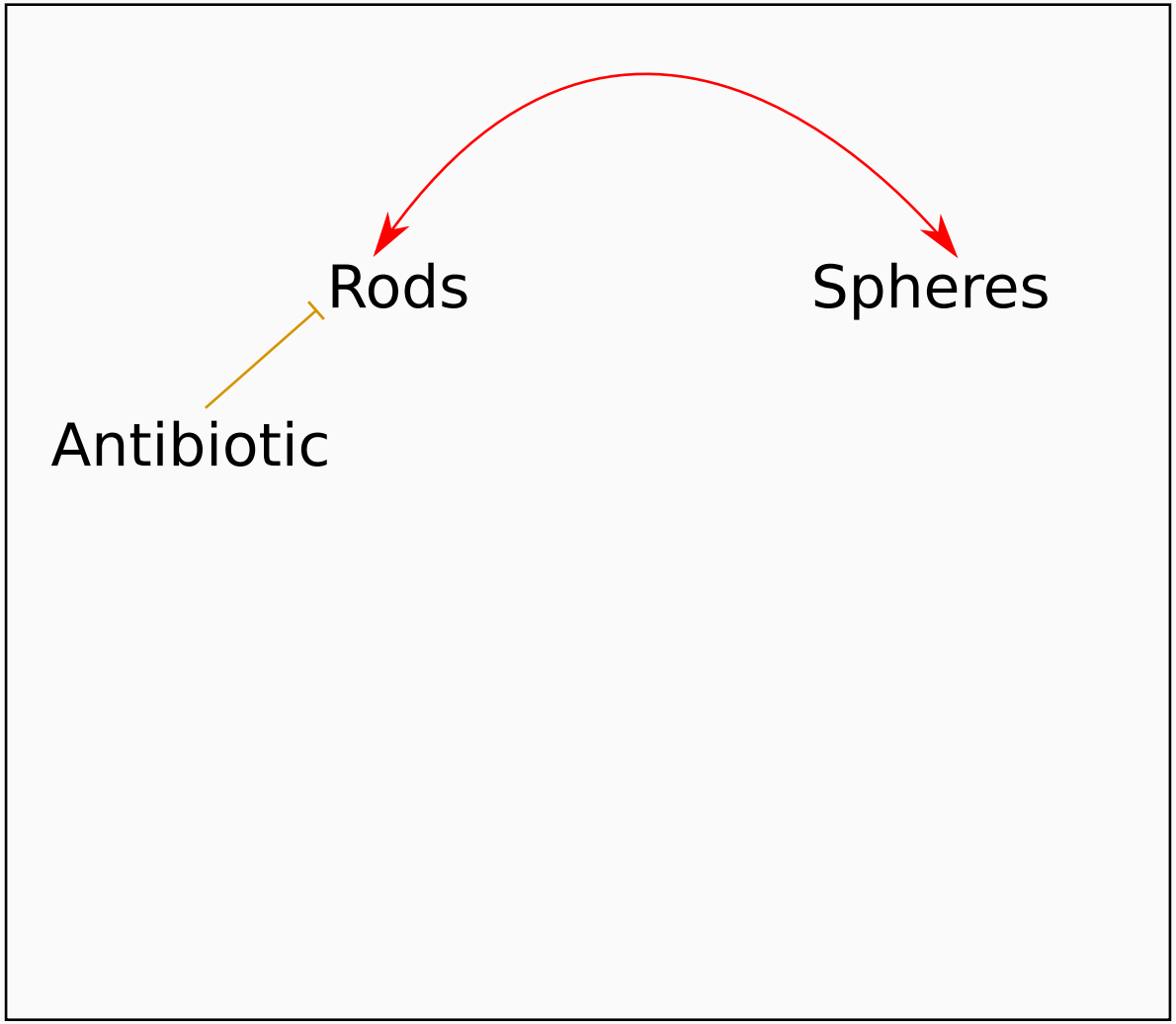
Infection level model



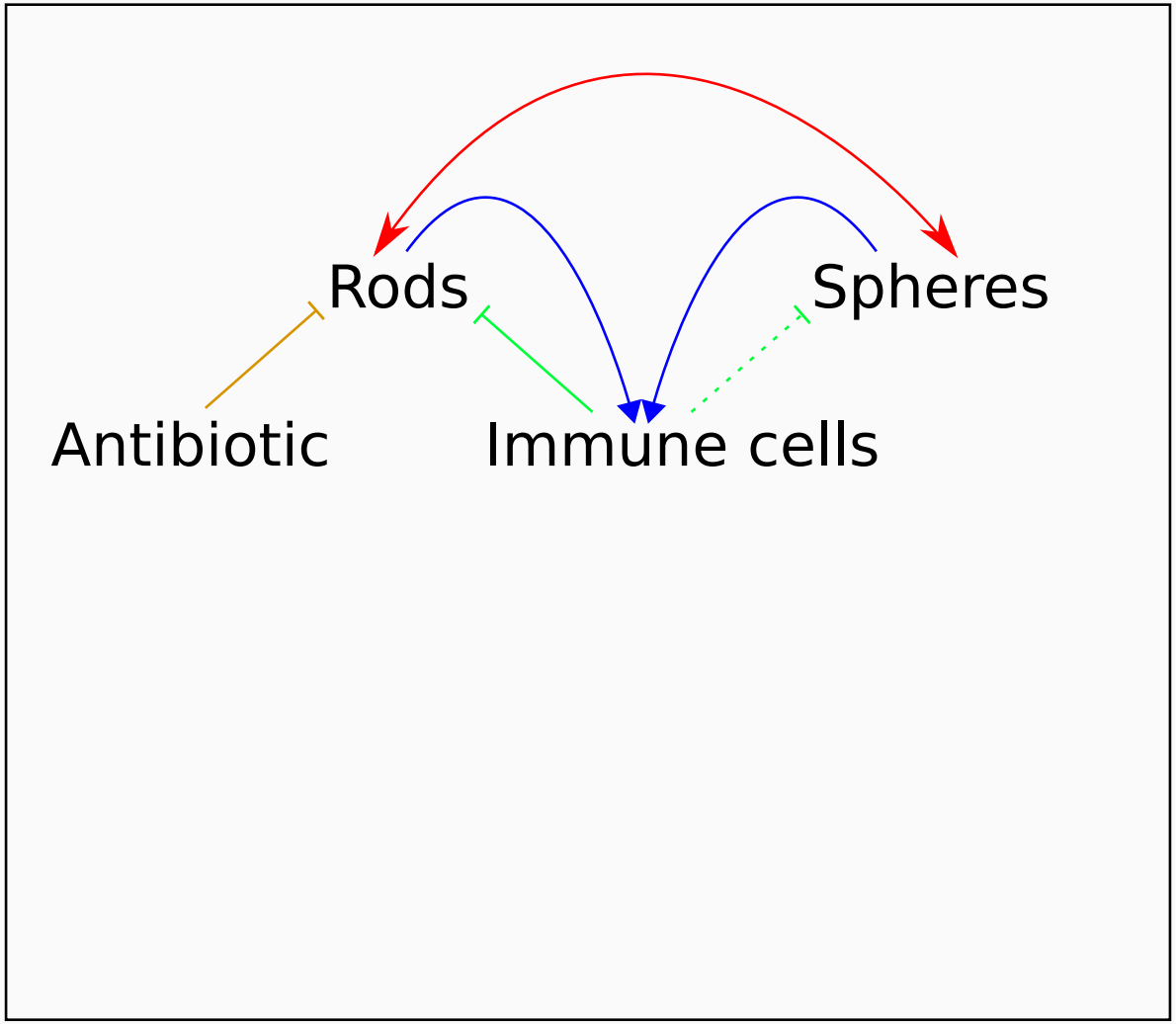
Infection level model



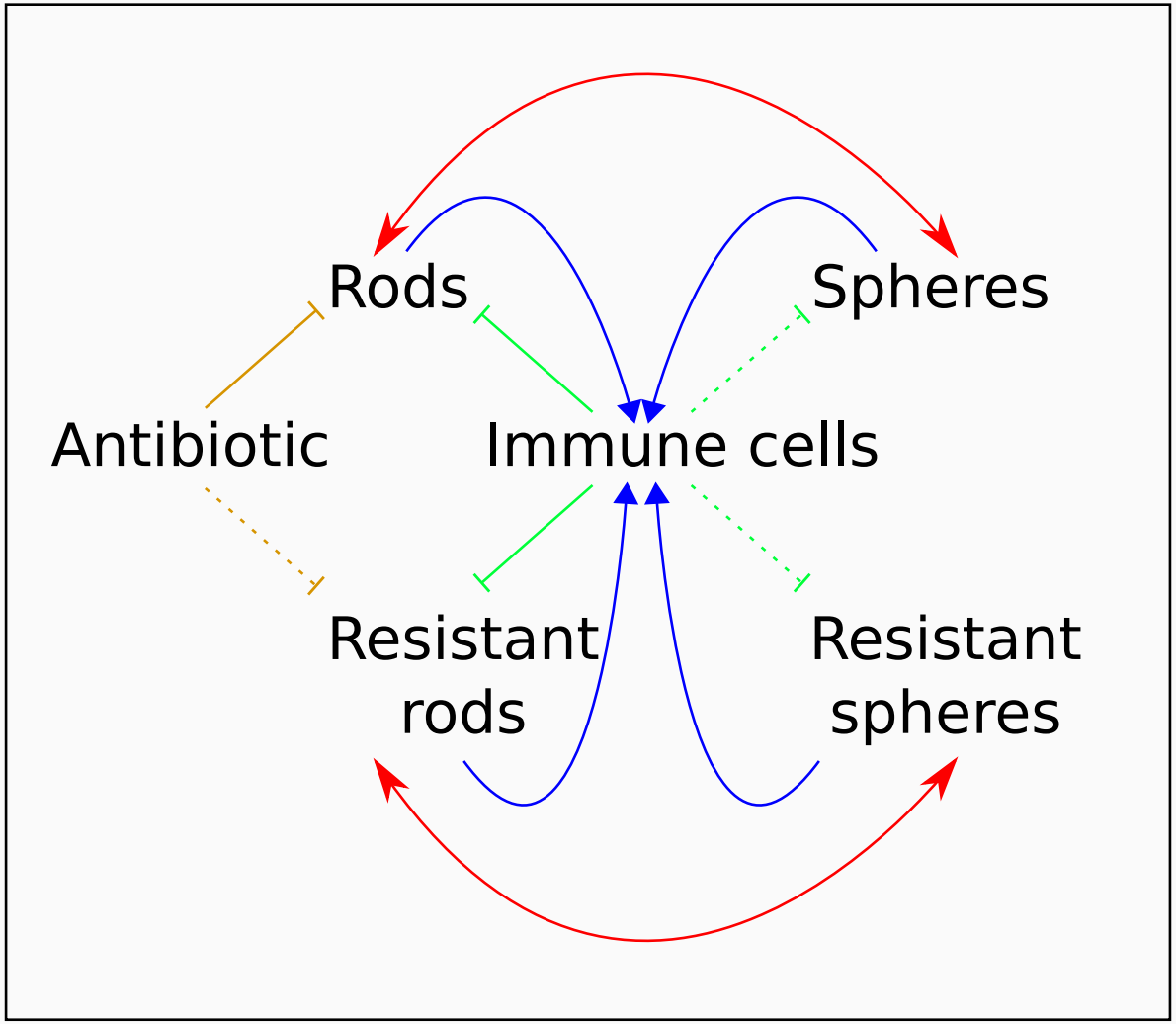
Infection level model



Infection level model



Infection level model



Adding antibiotics and antimicrobial peptides

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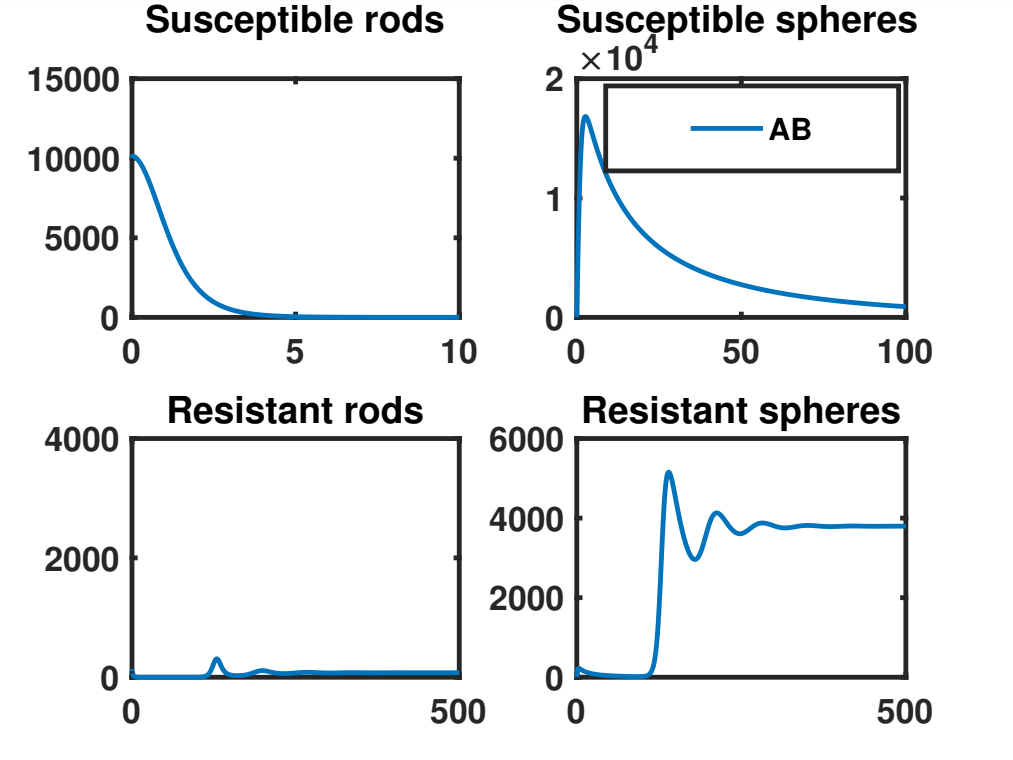
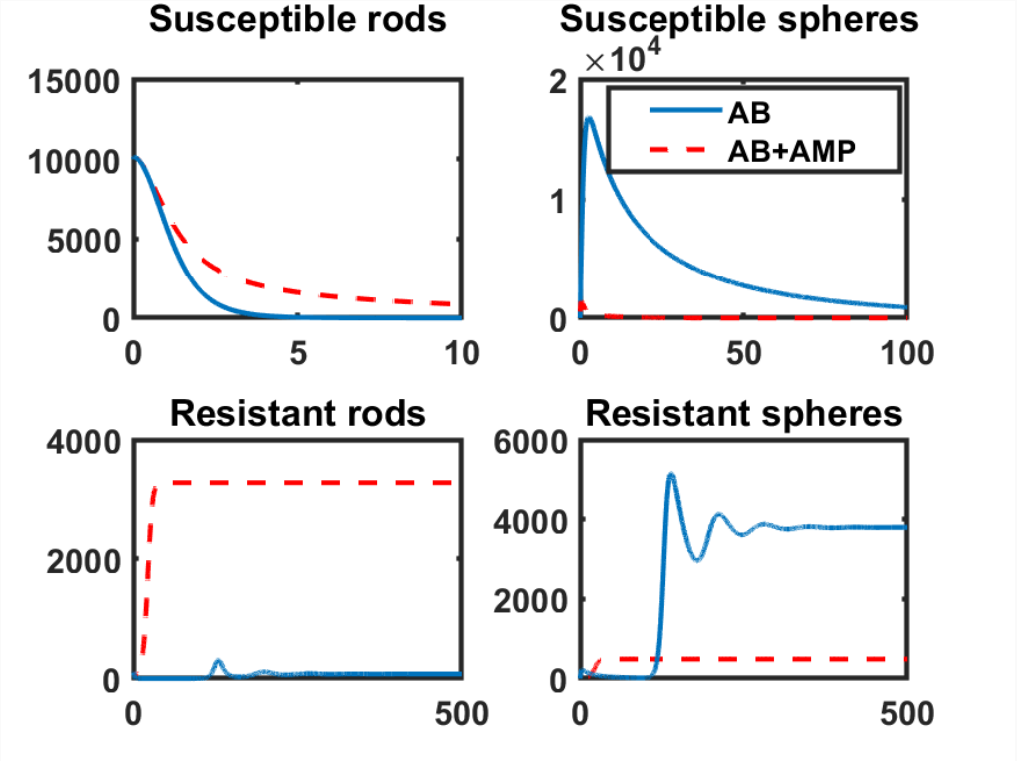


Adding antibiotics and antimicrobial peptides



Adding antimicrobial peptides may enhance the likelihood resistance can emerge

Adding antibiotics and a generic anti-virulence drug

We can simulate a generic anti-virulence drug by boosting the immune response against rods

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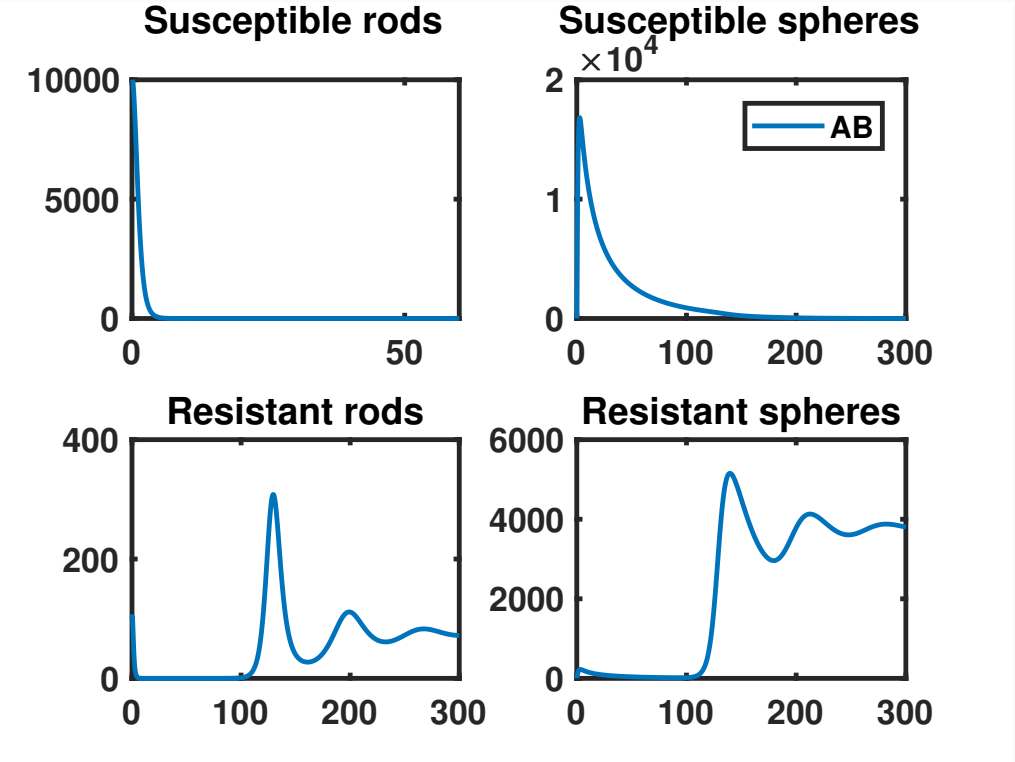

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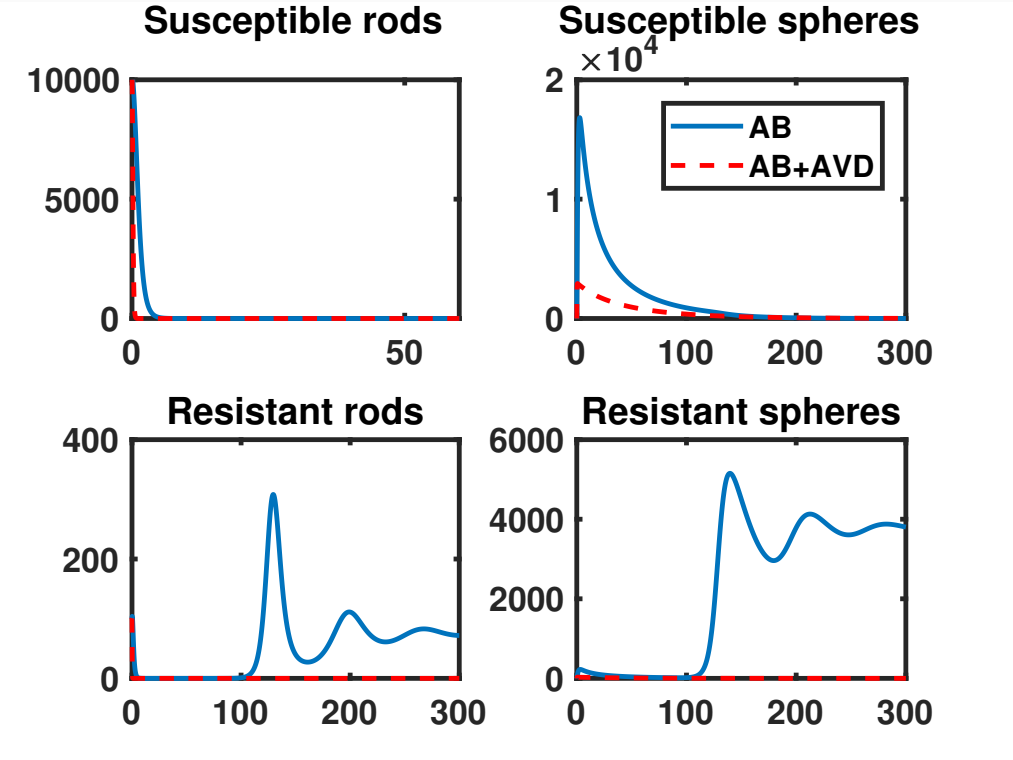

AVDs could suppress both resistant and susceptible subpopulations

Targeting persistence summary

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- *P. aeruginosa* changes its cell structure in response to certain antibiotics

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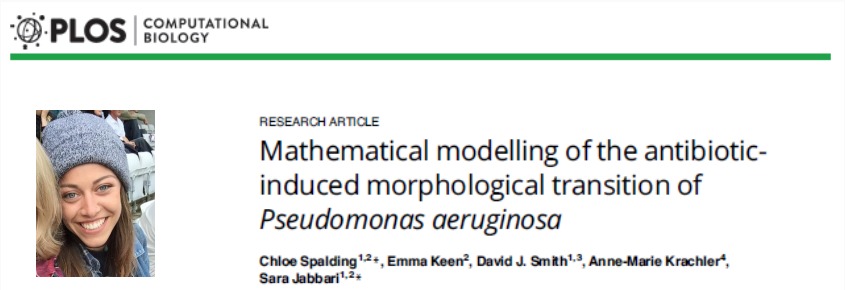
Targeting persistence summary

- *P. aeruginosa* changes its cell structure in response to certain antibiotics
- AMPs might be risky *in vivo*, anti-virulence drugs more promising
- Understanding the immune response is crucial



Summary

Summary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IMI
INSTITUTE OF
MICROBIOLOGY
AND INFECTION
EPSRC
BBSRC
bioscience for the future
wellcome

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Thank you for listening ☺