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In silico predictions of *in vitro* growth competition experiments between wild type and mutant *E.coli* MG1655 exposed to ciprofloxacin

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Background and Objective

- Valuable information on optimal dosing strategies can be obtained from *in silico* models based on *in vitro* time-kill curve experiments [1].
- An *in silico* model for *E. coli* MG1655 and three mutants thereof exposed to ciprofloxacin (Fig 1) has previously been presented [2]. This model has been expanded to fit three additional mutants and to include filamentation effects [3].
- The **aim** of this work was to predict the outcome of competition experiments between *E. coli* MG1655 wild type (WT) and each one of three mutants following ciprofloxacin exposure.
- The time-courses of bacterial kill for different dosing regimens using the previously developed model were also predicted.

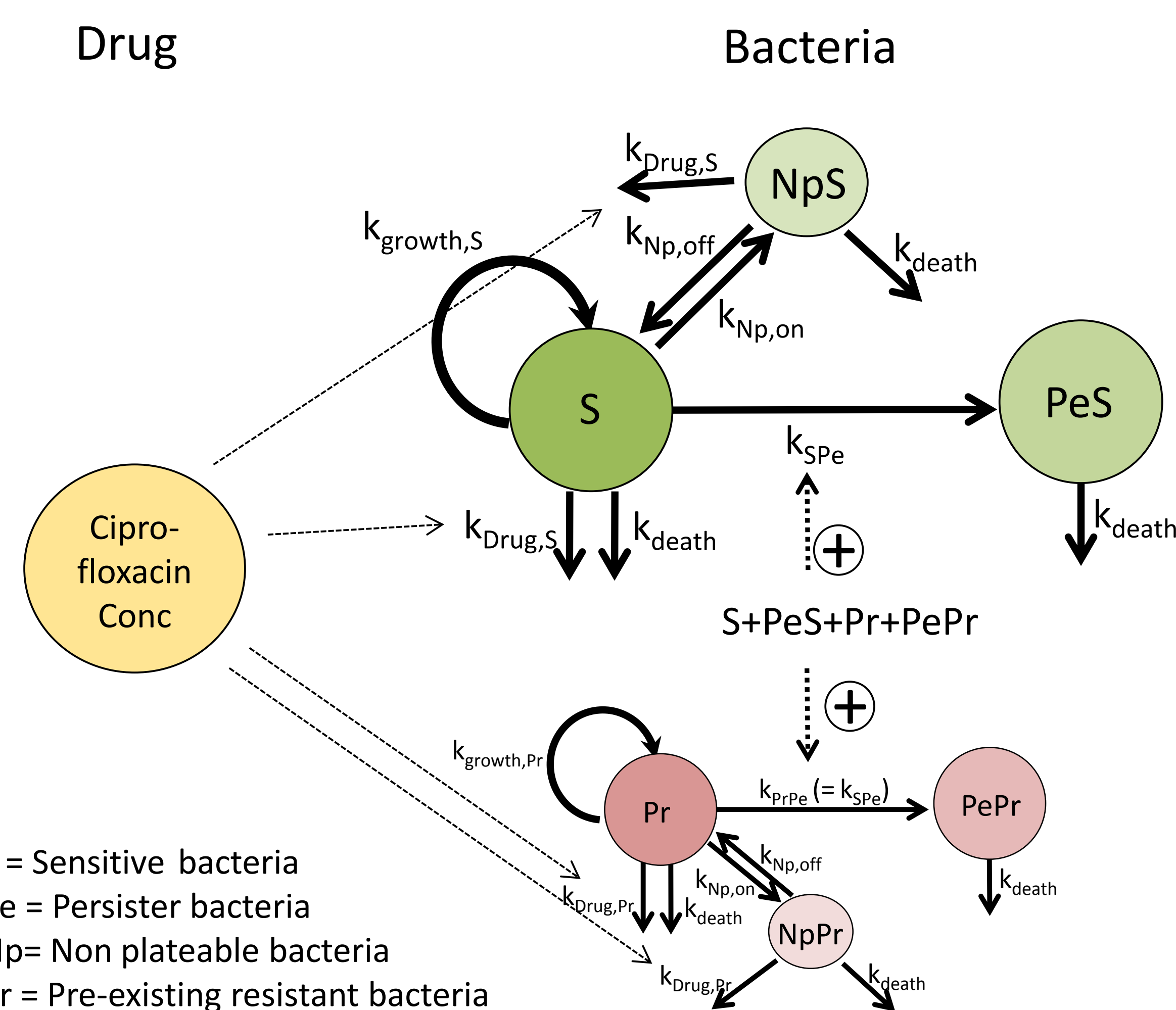


Fig 1. PKPD model developed for ciprofloxacin and *E.coli* [3]. Drug effect (k_{Drug}) was described by an Emax model with different EC_{50} for wild-type, resistant and pre-existing resistant bacteria. Bacteria can become non-plateable (Np). $k_{Np,on}$ was modeled with MTIME and turned off at 4 h.

Methodology

- Experimental data were obtained from 24h *in vitro* experiments with *E. coli* MG1655 WT in competition with one of three different well characterized MG1655 single mutants [4] (Table 1).
- Starting ratios of WT:mutant ranged from 10:1 to 10000:1.
- Ciprofloxacin concentrations were constant and chosen to be below, between and above the MICs of the WT and the mutant.
- The competition experiments were predicted from the model and compared to the observed data using NONMEM 7.
- The *in silico* model (Fig 1) included compartments for susceptible, persister, filamented (non-plateable) and pre-existing resistant bacteria, with one set of compartments for WT and one set for the competing mutant.
- Additionally, predictions of different once daily (QD) and twice daily (BID) dosing regimens of ciprofloxacin were performed, using a $t_{1/2}$ of 2, 4 and 7h.

Table 1. Bacteria characteristics.

^aMean fitness per generation, relative to WT.

Δ = knockout mutation.

<i>E.Coli</i> MG 1655 mutations, relative fitness and MIC					
Strain	gyrA1	gyrA2	marR	Relative fitness ^a	MIC mg/L
LM347 (WT)	-	-	-	1.0	0.023
LM202	-	-	Δ	0.83	0.047
LM378	S83L	-	-	1.01	0.38
LM534		D87N	-	0.99	0.25

References

- Nielsen EI et al., Antimicrob Agents Chemother 2011.
- Khan D et al., PAGE 2011.
- Khan D et al., ICAAC 2011.
- Marcusson LL et al., PLoS Pathogens 2009

Conclusions

- The model was shown to adequately predict *in vitro* competition experiments and can thus be a valuable tool in the search for dosing regimens that minimize the growth of resistant mutants existing in a bacterial population.
- Over a range of doses and half-lives, QD dosing was found to be superior over BID dosing by more efficiently killing of mutants, suppressing the overtake of the resistant mutants.

Results

- The model successfully predicted the time course of bacterial kill and growth in the competition experiments (Fig 2), except for one experiment where the difference in MIC for WT and mutant was only two-fold, and the drug concentration was between the two MICs (Fig 2a, 0.032 mg/L).

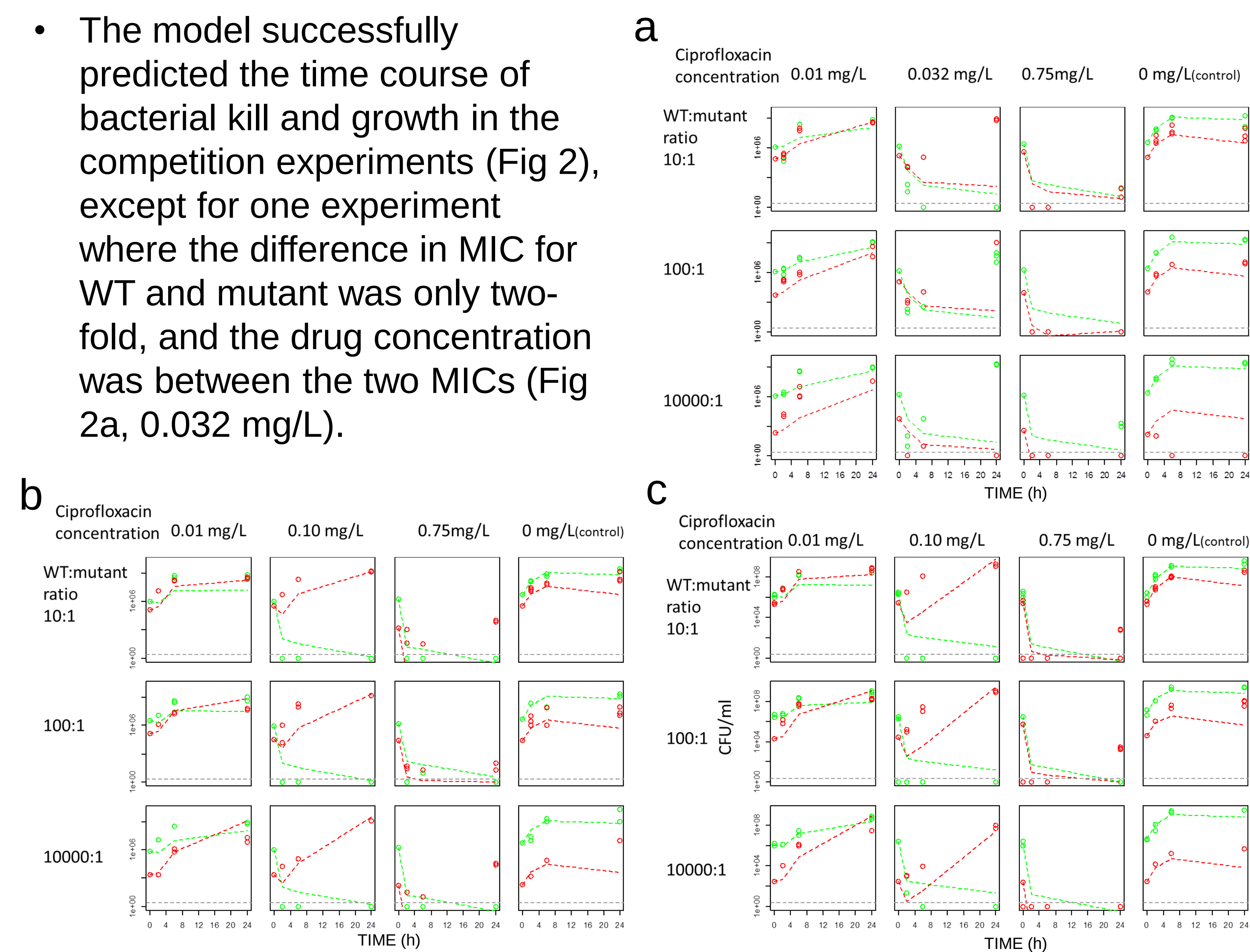


Fig 2. LM202 (a), LM378 (b) and LM534 (c) in competition with WT. Dashed lines are predictions and circles are observed data. Green is WT and Red is mutant.

- For all mutants, dose sizes, WT:mutant ratios (Fig 3) and half lives (not shown) the model predicted QD dosing regimens to be as efficient or superior compared to the BID regimens of the same total dose amount in overcoming resistance and resulting in an overall bacteria kill.

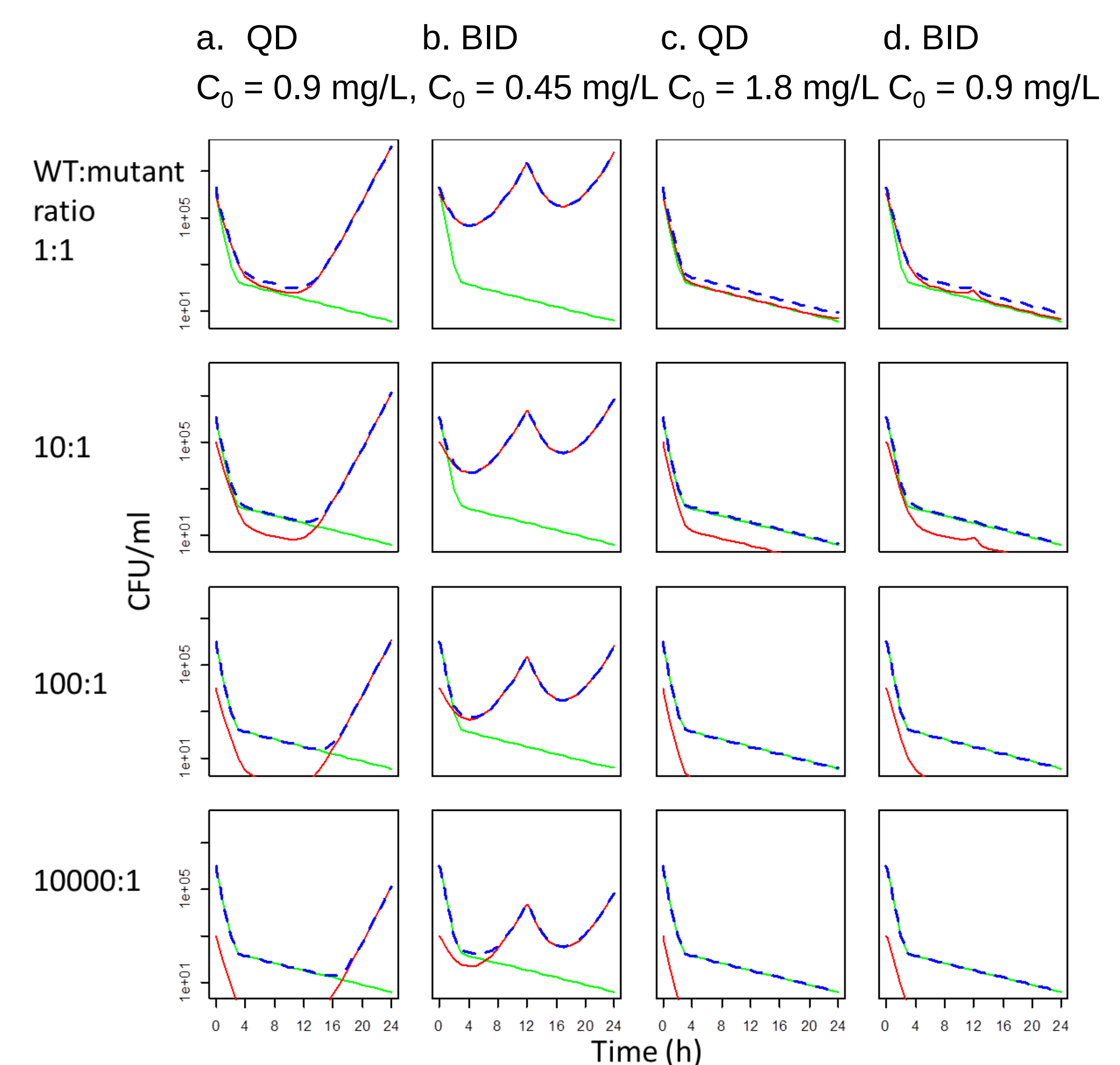


Fig 3. Predictions for QD and BID dosing of ciprofloxacin for WT in competition with mutant LM378 (16 x WT MIC). Green is WT, Red is mutant and Blue is total bacterial population.

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