PKPD-modeling of time-kill curves from *E. coli* mutants exposed to ciprofloxacin

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Background and Objective

- There is a need to better understand how antibiotics should be dosed to overcome and minimize development of resistance
- Information can be gained from modeling and simulations based on *in vitro* experiments of bacteria kill [1]
- A semi-mechanistic model describing *in vitro* antibiotic effects has previously been proposed based on a strain of *Streptococcus pyogenes* [2]. The semi-mechanistic model structure allows for application on other types of bacteria with parameter estimates dependent on the degree of bacterial resistance and fitness

- The aim of this work was to develop a PKPD-model describing the time-kill curves of wild-type and three well-characterized mutants of *E.Coli* MG1655.

Methodology

Time-kill curve experiments

- Data from 24h static *in vitro* experiments with *E. coli* MG1655 and three mutants thereof were used for model development
- Ciprofloxacin concentrations were constant during the experiment and ranged from 0.06 to 8 x MIC for each bacterial strain
- All data were modeled simultaneously in NONMEM 7

Well characterized strains

- Three well characterized mutants and wild type (LM347, MIC 0.02 µg/ml) bacteria were used in the time kill curve experiments
- Mutations for mutants LM378 and LM534 were in the *gyrA* gene (S83L and D87N respectively, MIC 0.3 µg/ml and 0.4 µg/ml). LM202 has a knockout mutation of the *marR gene* (MIC 0.03 µg/ml) [3]

Data analysis and model building

- Differences in parameters for wild type and mutants were searched for
- Regrowth in the end of the time-kill curve experiments were described as pre-existing resistant bacteria for a fraction of the starting inocula, see figure 1

Results

- Time-kill curves for all investigated strains and concentrations were well predicted by the model, see figure 2 and figure 3
- K_{growth} were 2-10% lower for the three mutants compared to wild type
- EC50 was 0.03 µg/ml for wild type (LM347), 0.06 µg/ml for LM202 and 0.5 µg/ml for both LM378 and LM534
- Emax was the same for LM347, LM202 and LM378. LM534 had a 20% lower Emax compared to LM347, LM202 and LM378
- Allowing for pre-existing resistant bacteria in the wild type starting inocula resulted in a decrease of 75 units in OFV
- Pre-existing resistant bacteria were estimated to 8 bacteria per 10^6 bacteria for wild type and 1, 2 and 9 bacteria per 10^6 bacteria for LM534, LM378 and LM202 respectively
- The model predicted regrowth of LM202 in a mixture population of LM202 and LM347 (wild type) with a dose corresponding to C0 at 0.04 µg/ml, see figure 4.

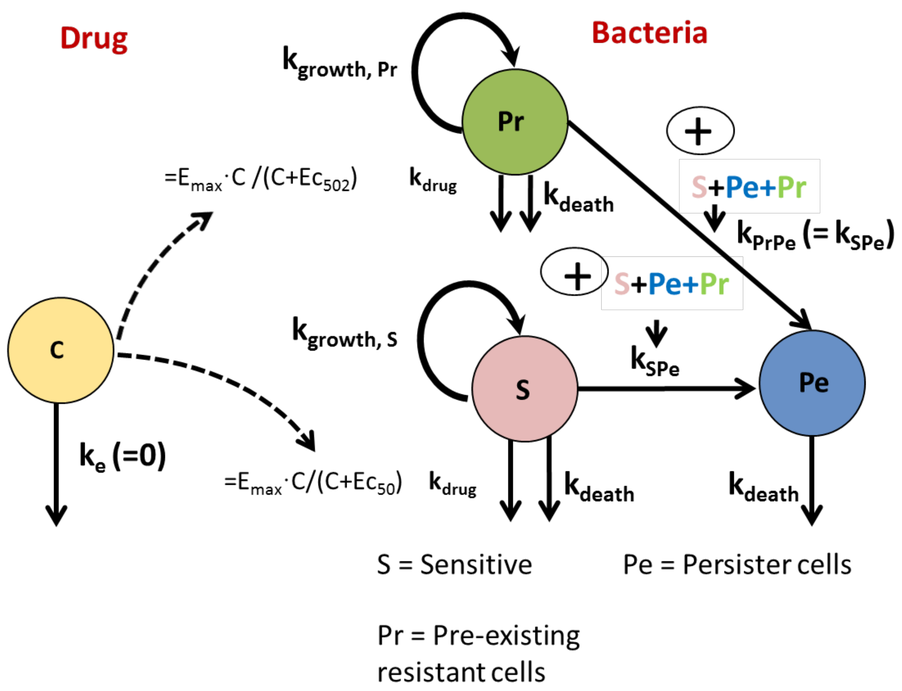

Fig 1: PKPD model developed for ciprofloxacin and *E.Coli* MG1655 wild type and three well characterized mutants

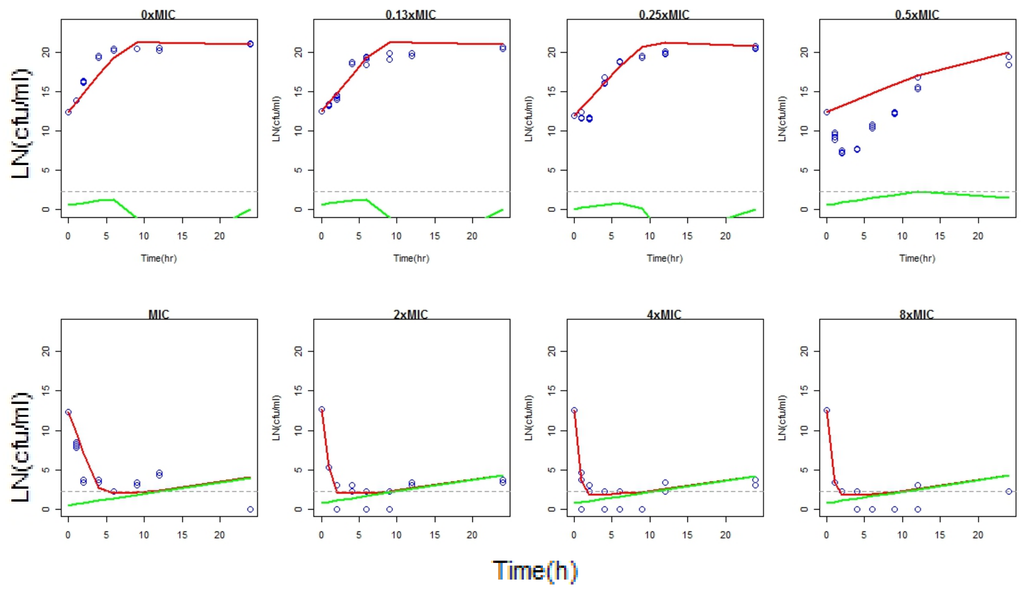

Fig 2: Example plots for wild type experiments with 8 static concentrations of ciprofloxacin.

Blue circles are observations, red lines are estimated total amount of bacteria and green lines are estimated total number of pre-existing resistant bacteria

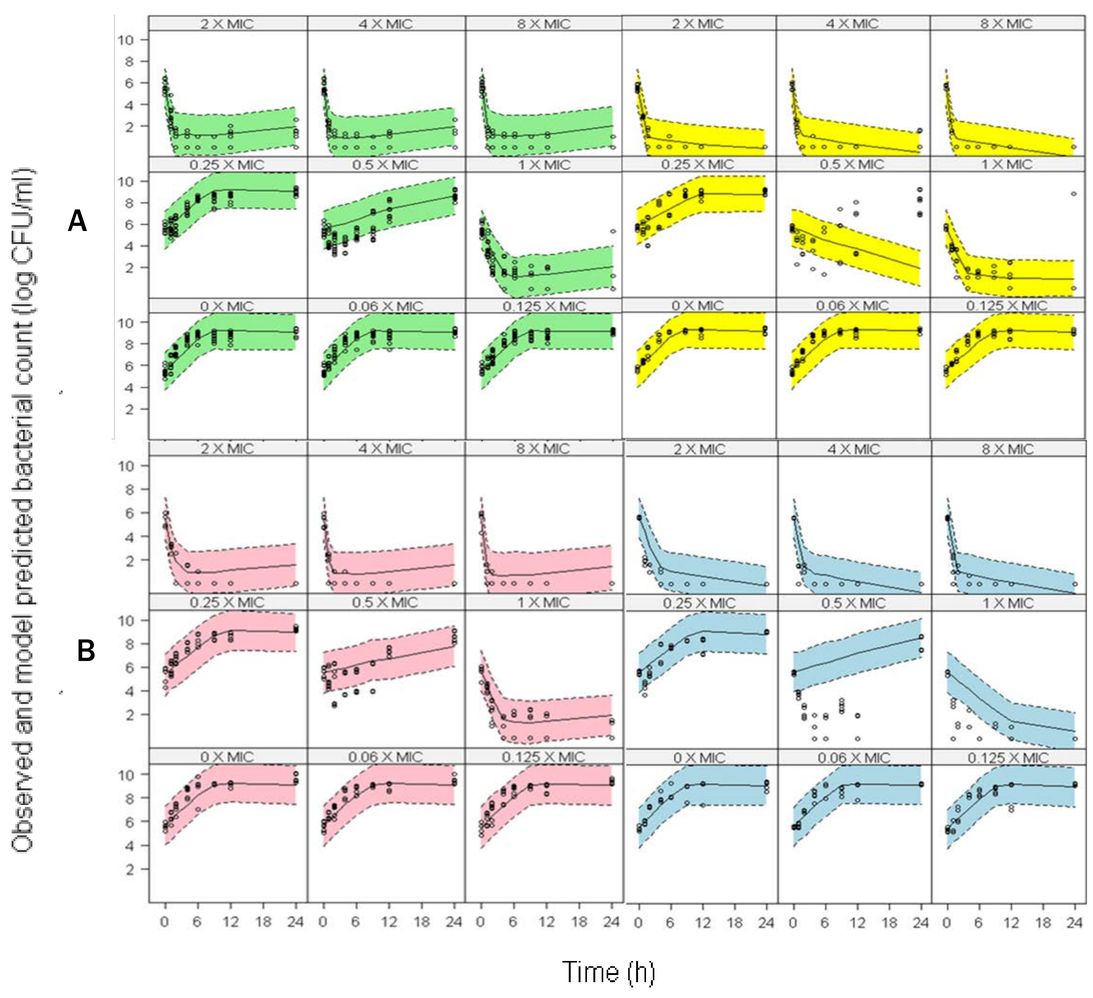

Fig 3: Visual predictive checks for:

A. LM347 wild type
B. LM202 mutant
C. LM378 mutant
D. LM534 mutant

○ **Observed**
— **Simulated median**
---- **90% prediction interval based on the simulated data**

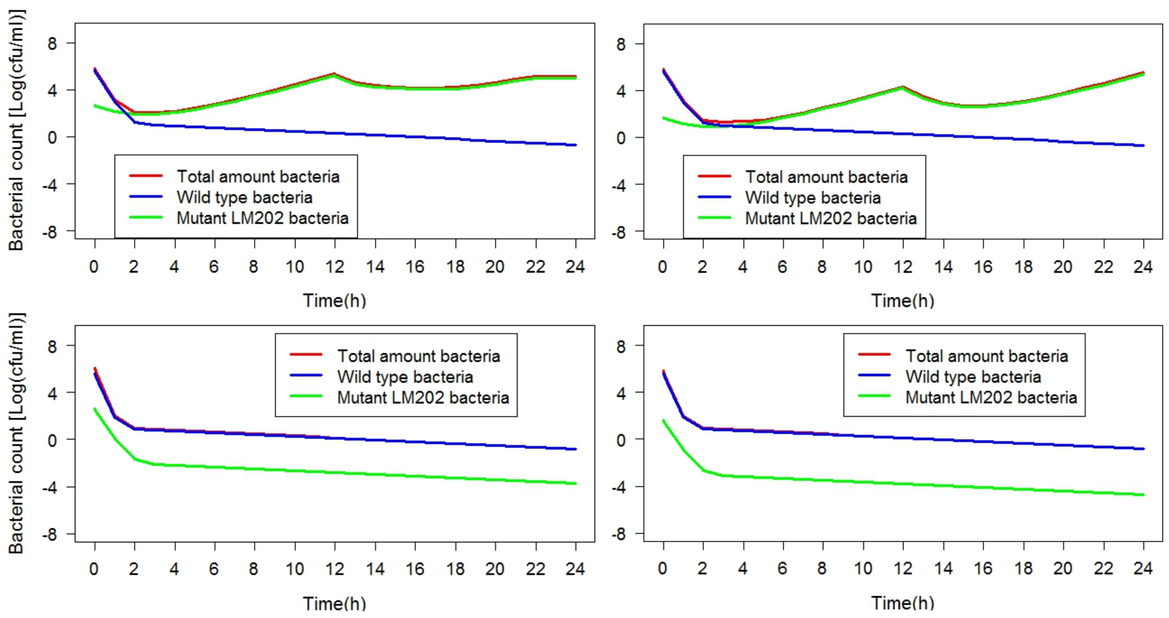

Fig 4: Predictions:

Top Left shows prediction for ciprofloxacin dose 1 on wild type and LM202 in a 999 to 1 ratio in the start inocula, bottom left is same ratio with dose 2

Dose 1 → C0 = 0.04 µg/ml

Dose 2 → C0 = 0.08 µg/ml

Top Right shows ciprofloxacin dose 1 on wild type and LM202 in a 9999 to 1 ratio in the start inocula, bottom right is same ratio with dose 2

$T_{1/2}$ = 4.5 h

Tau = 12 h

Conclusions

The model described the time-kill curves following ciprofloxacin exposure of all investigated mutants and wild type well for all concentrations except for 0.5xMIC. The model also explained the regrowth occurring in the experiments. The model could be useful in predicting dosing schedules in presence of different ratios of wild type and resistant bacteria

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References

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