Semi-mechanistic PK-PD modelling of combined polymyxin B and minocycline against a polymyxin-resistant strain of *A. baumannii*

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Introduction

- *Acinetobacter baumannii* is one of the most difficult to treat multi-drug resistant (MDR) pathogens responsible for opportunistic nosocomial infections all over the world and has the ability to become resistant to a wide variety of drugs [1]
- In face of these resistances, neglected and disused antibiotics like polymyxins may be used, especially in combination with other antibiotics, as the last line of defence against MDR *A. baumannii* [2]
- Polymyxin B (PMB) and minocycline (MIN) combination was shown to be synergistic on polymyxin-resistant *A. baumannii* strains in checkerboard screening experiments
- To further investigate this synergistic combination, a polymyxin-resistant clinical isolate (CR17) was selected to investigate the determinants of the polymyxin B + minocycline synergy observed in checkerboard experiments

Material & Methods

- Polymyxin-resistant *A. baumannii* clinical isolate CR17 was studied [3] MIC PMB = 8 mg/L – MIC MIN = 4 mg/L
- Heteroresistance to PMB and MIN was evaluated by plating a high inoculum (~10^9 CFU/mL) on plates containing 8 x MIC of drug (resistant subpopulation) and on drug free plates (total population) and counting after 24 h at 37°C.
- Fitness cost was evaluated by inoculating a 96 well plate with ~10^6 CFU/mL of total and resistant subpopulation of bacteria with OD reading at 600 nm over 24 h, and calculation of a growth rate constant [4].
- Single drug and combination time-kill experiments (TKE) were performed. The presence of resistant subpopulations was evaluated by population analysis profiles (PAPs), *i.e.* in this case count on plates containing 64 mg/L of PMB.
- A semi-mechanistic PK/PD model was built. Combination modelling was performed using the Global PharmacoDynamic Model approach [5]
- Effect parameters for each antibiotic and also for interaction were estimated. The effect of each model component on the area under the log10(CFU/mL) curve was computed.
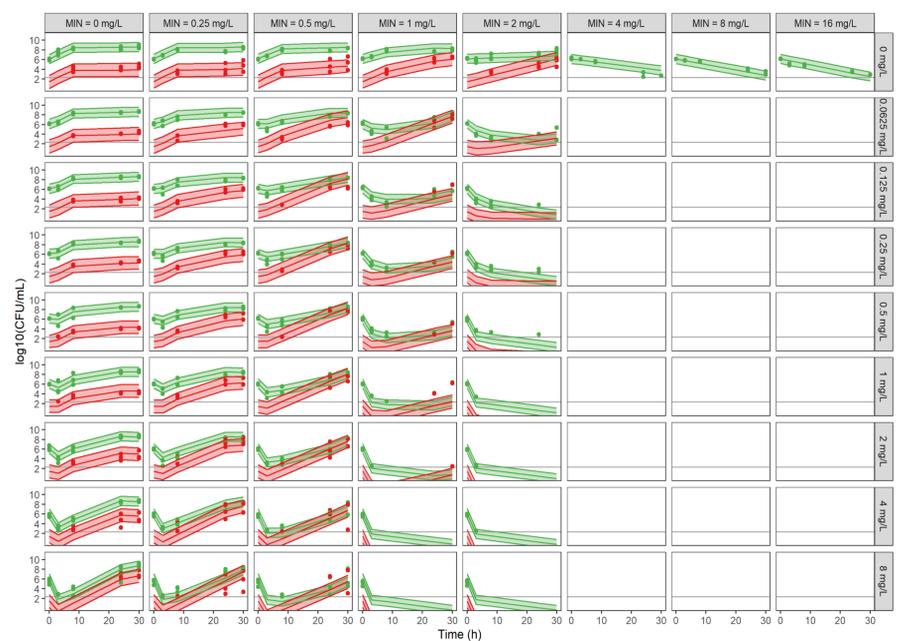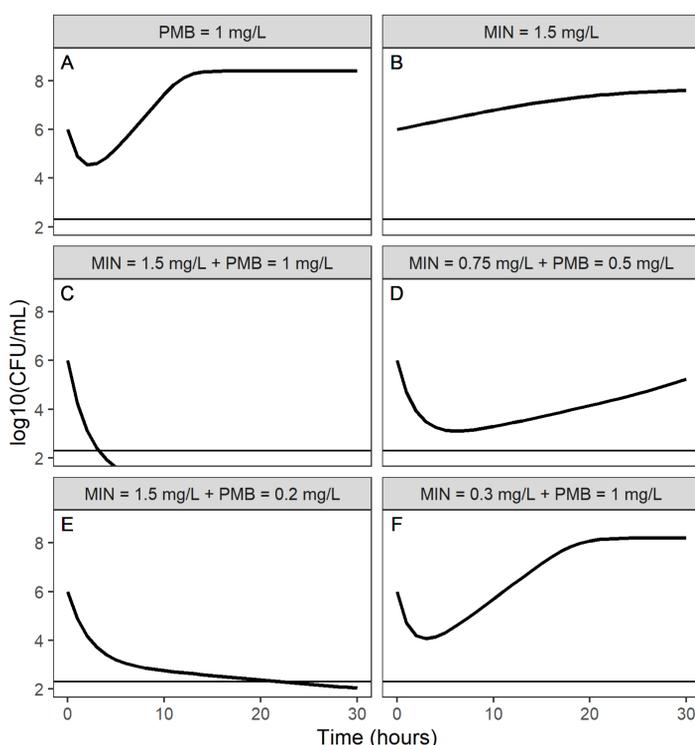
Results

1. Model description
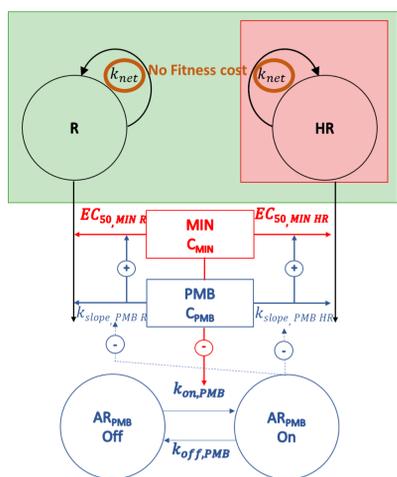
Figure 1 – Schematic representation of the final model. Green are bacteria counted on drug free plates – Red are bacteria counted on plates containing 64 mg/L of PMB. The model included : two subpopulations (R: Resistant – HR : Highly resistant). Sigmoidal effect of MIN, slope power effect of PMB and adaptive resistance to PMB of both subpopulations

- CR17 did not exhibit heteroresistance to MIN but to PMB (mean frequency: 5.07×10^{-6}, range [$1.22 \times 10^{-5} - 7.68 \times 10^{-7}$], n=6).
- No fitness cost was found.
- MIN was less active on the highly resistant subpopulation
- PMB alone exhibited a fast concentration-dependent effect followed by regrowth at all tested concentrations.
- A total of 253 TKE were performed. In single drug TKE, no effect was observed at concentrations <1 x MIC MIN while at concentrations ≥ 1 x MIC a concentration-independent effect was observed.

2. Visual Predictive Checks

Figure 2 – Visual Predictive Checks of the final model. Points represent observed data, lines and area represent 80% prediction interval of model simulations. Green are total bacteria, red are highly resistant bacterial subpopulation

- When combining MIN and PMB, total bacterial killing at 30 h was observed for concentrations as low as 1.5 mg/L MIN + 0.5 mg/L PMB.
- But MIN concentrations < 1 mg/L contributed to select the resistant subpopulation
- The effect of MIN reached 90% of its maximum at 2 x MIC MIN.
- Slope power effect model of PMB and adaptive resistance to PMB of both subpopulations.
- Adaptive resistance to PMB was fast with an almost complete resistance to polymyxin B after 3 hours.
- For concentrations of PMB >0.1 mg/L, MIN EC50 of both subpopulations was reduced by 50%.
- MIN reduced adaptation rate to PMB according to an Emax model, with a maximal decrease of the adaptation rate of 29 % reached for a MIN concentration of 1mg/L, and almost no synergy for lower concentrations.

3. Simulations of expected CFU

- As shown by **Figure 3**, while significantly improving the fit to data, the potentiation effect of MIN on PMB could be accounted for a smaller part of the total effect than the potentiation effect of PMB on MIN which was essential to the total effect

Conclusion & Perspectives

❖ A methodology enabling the qualitative and quantitative study of in vitro antibiotic combinations was developed.
❖ Heteroresistance to polymyxin B without fitness cost was observed.
❖ The combination was shown to be synergistic in in vitro time-kill curves but too low concentrations of minocycline contributed to resistant selection.
❖ By performing semi-mechanistic PK/PD modelling, target concentrations to maximize synergy were determined :. 0.2 mg/L of polymyxin B and 1.5 mg/L of minocycline.

References

1. Fournier et al. 2006. PLOS Genetics.
2. Nation et al. 2015. The lancet Inf. Dis.
3. López-Rojas et al. 2013. AAC
4. Bleibtreu et al. 2013. Infect Immun
5. Wicha et al. 2017. Nat Comm
6. Balaban et al. 2004. Nature

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