Use of a semi-mechanistic PK-PD model to quantify the combination effect of polymyxin B and minocycline against polymyxin-resistant *Acinetobacter 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

Materials/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 & Discussion

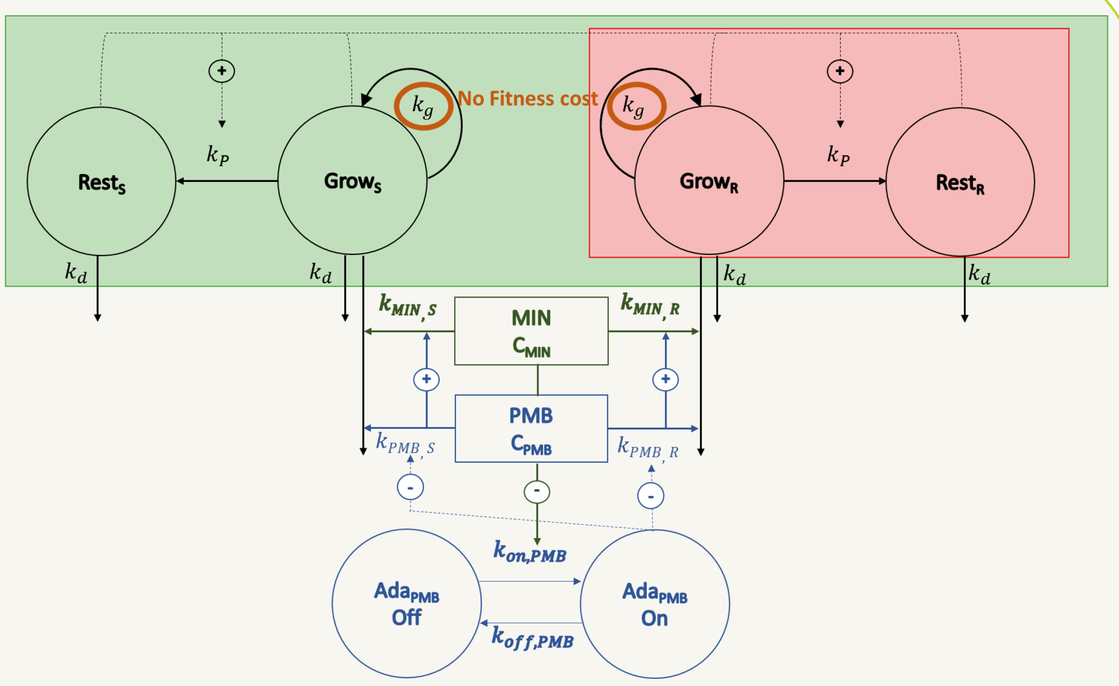

Figure 1 – Schematic representation of the final model. Green are bacteria counted on drug free plates – Red are bacteria counted on plates containing 64 mg/L of PMB. The model included : phenotypic switch to resting [6] form at high bacterial concentrations. Two subpopulations (PMB-susceptible and PMB-resistant). 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*10^{-5} - 7.68*10^{-7}], n=6).
- No fitness cost was found.
- MIN was less active on the PMB-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.

Results & Discussion

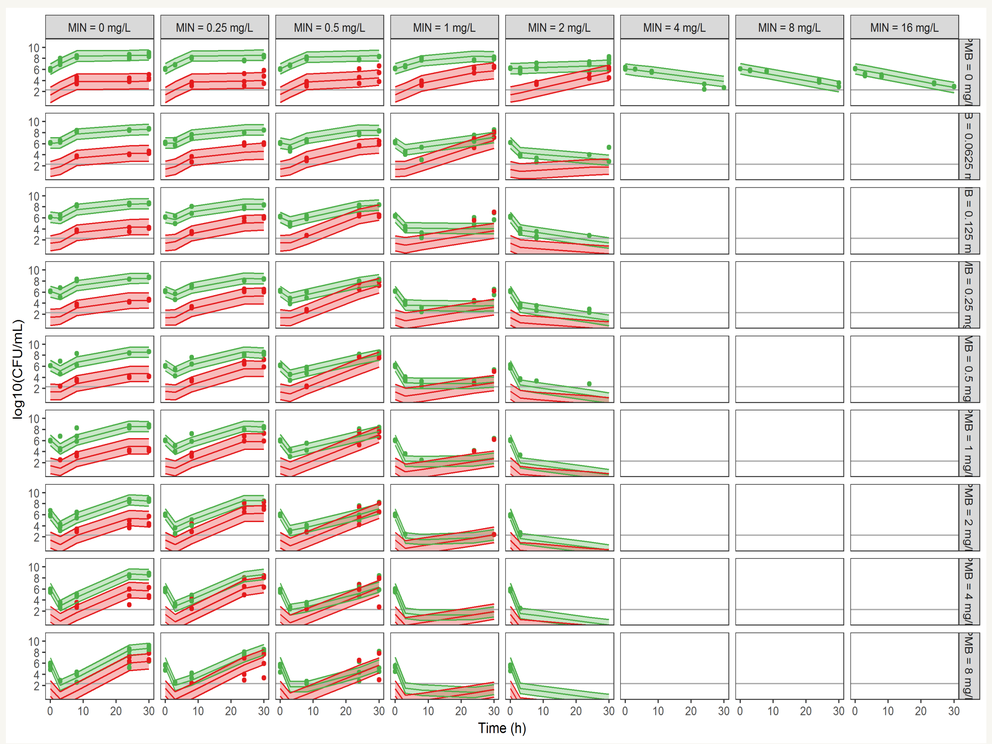

Figure 2 – Visual Predictive Checks of the final model. Points represent observed data, lines and area represent 90% prediction interval of model simulations. Green are total bacteria, red are resistant bacterial subpopulation

- When combining MIN and PMB, total bacterial killing at 30 h was observed for concentrations as low as 1/4 x MIC MIN + 1/16 x MIC 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 83 % and an interaction EC50 of 0.62 mg/L.

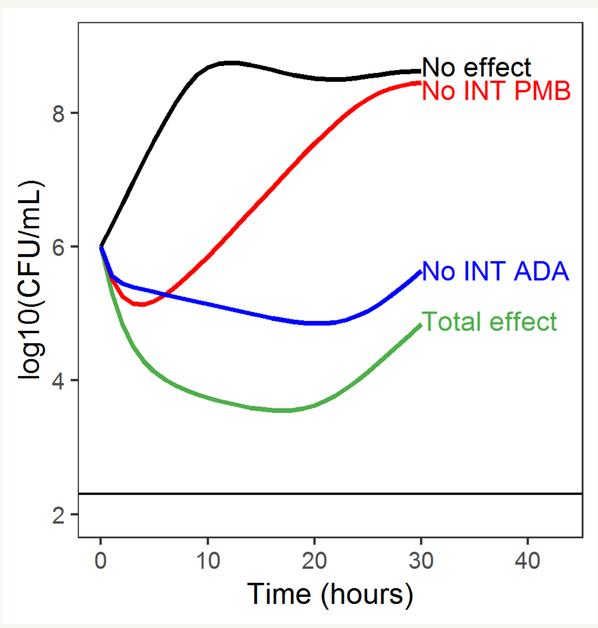

Figure 3 – Decomposition of model component contribution to the global effect - No INT PMB : simulations without potentiation of MIN effect by PMB – No INT ADA : simulation without MIN inhibition of adaptation to PMB – PMB = 0.125 mg/L – MIN = 1 mg/L

- 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

- 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, polymyxin B was shown to be the biggest contributor to synergy

References

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