Modelling rate and extent of resistance development against colistin in *Klebsiella pneumoniae*

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Introduction

- *Klebsiella pneumoniae* is a gram-negative pathogen associated with multidrug resistance.
- Colistin is one of the last resorts antibiotics for treatment of multidrug-resistant *K. pneumoniae* infections [1].
- Emergence of colistin resistance is a global issue [2] and can develop during colistin treatment [3].
- Quantitative modelling of time-kill experiments can guide the design of treatment schedules to control the emergence of resistance [4].

Aims

- Characterize the effect of colistin on kill and growth kinetics of *K. pneumoniae* using a static time-kill (STK) experiment and population analysis profiling (PAP).
- Develop a quantitative model to characterize the dynamics of colistin resistance during treatment based on STK and PAP data.

Methods

Experimental design

- STK experiments were carried out by exposing the carbepenemase-producing *K. pneumoniae* ATCC BAA-1705 to different colistin concentrations.
- Colony forming units (CFU) were determined at 0, 1, 2, 4, 8, and 24 h for antibiotic free and PAP-plates with increasing colistin concentrations.

Model development

- A previously developed pharmacodynamic model for colistin time-kill kinetics was used as base model [4], including:
  - drug-susceptible growing bacteria (S) and non-susceptible bacteria (R);
  - drug concentration (C_{col})-dependent rate influencing the transition from the state of no resistance (Re_{OFF}) to resistance (Re_{ON});
  - concentration-effect relationship for bacterial kill and resistance development according to an Emax model.
- The PAP data was modelled with an extension from the base model based on the ratio of colistin resistance and total CFU counts.

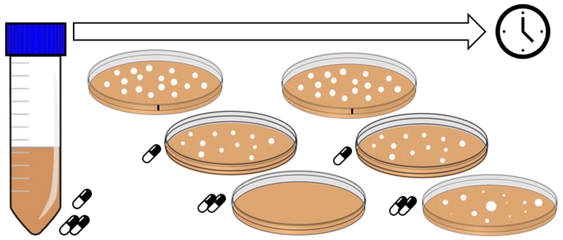

Figure 1. Overview of time kill experiment. Total CFU sampled at a given time point are quantified on multiple PAP plates which contain colistin concentration ranged from 0 to 20mg/L

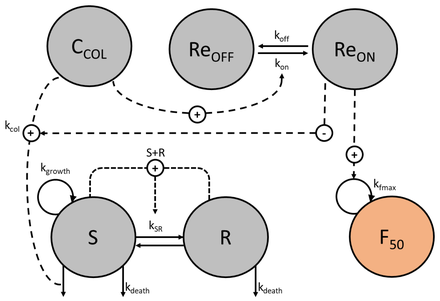

Figure 2. Schematic structure of the developed PD model. The gray compartment represents the existing model. We added a compartment for the F50 to incorporate PAP data.

Results

- Bacteria at the baseline were assumed to contain only sub-population S.
- The bacteria fraction (F) survived at specific concentrations of colistin PAP-plates (C_{colPAP}) was described in eq. 1-2.

$$\log_{10}(CFU_{PAP}) = F*\log_{10}(CFU_{total}) \quad (1)$$

$$F=1-(C_{colPAP}/(F_{50}(t)+C_{colPAP})). \quad (2)$$

- The emergence of resistance toward antibiotic on PAP plates described by an Emax equation with F_{50} at which half of the total population will survive at time t and PAP plate concentration (C_{colPAP}). F_{50} is a time-varying variable (Eq. 3)

$$d/dt(F_{50})=k_{fmax}*Re_{ON}\beta*F_{50} \text{ with } F_{50}(t=0) = EC_{50} \quad (3)$$

- k_{fmax} reflects the maximum emergence rate of F_{50}.
- The model adequately captured total CFU time-kill profiles and PAP CFU data.

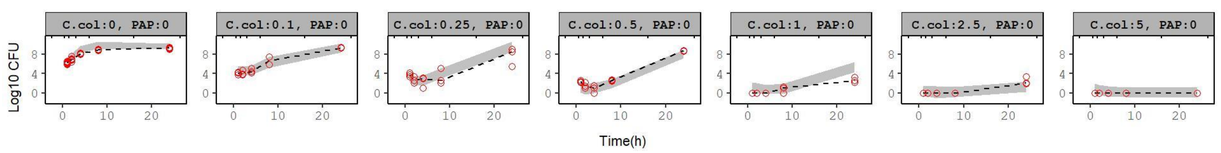

Figure 3. CFU observations and model predictions for the STK experiment. Grey areas indicate 95% model prediction interval of median (colistin concentration from left to right: 0, 0.1, 0.25, 0.5, 1, 2.5, 5mg/)

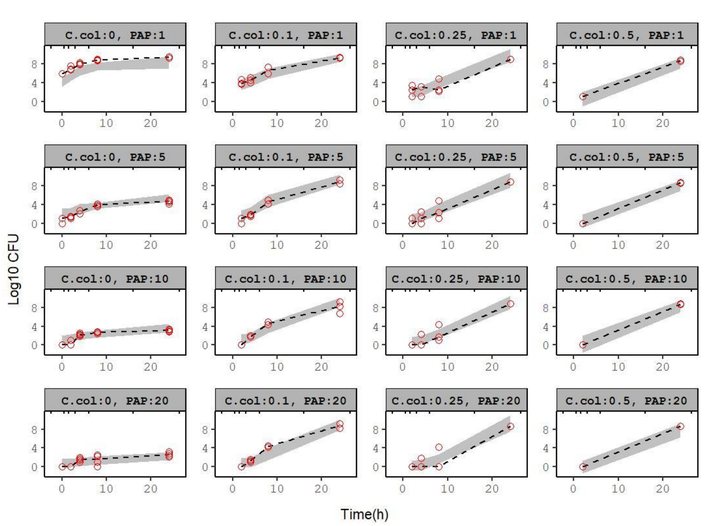

Figure 4. CFU observations and model predictions for PAP plates of the STK experiment. Grey areas indicate 95% model prediction interval of median model predictions (PAP colistin concentration top to bottom: 1, 5, 10, 20 mg/L)

Table 1. Population parameter estimates for the PD model.

Parameter (unit)	Description	Estimate	RSE%
k_{growth} (h^{-1})	Bacterial growth rate	1.9	16.1
k_{death} (h^{-1})	Natural bacterial death rate	0.179	fixed
B_{max} (log10 CFU/mL)	Maximum bacterial population	9.6	1.79
E_{max} (h^{-1})	Maximum kill rate	378	4.81
EC_{50} (mg/L)	Concentration that results in half of E_{max}	2.36	7.96
k_{onmax} (L/mgh)	Maximal resistance rate in the presence of colistin	3.71	7.08
C_{on50} (mg/L)	Antibiotic concentration that results in half of k_{onmax}	2.23	17.8
δ	Hill factor for drug concentration influencing rate constant for the development of adaptive resistance	0.748	8.88
β	Hill factor for drug inhibition due to resistance	0.158	20.3
k_{off} (h^{-1})	Rate constant for bacteria to return to the susceptible state after developing resistance	0.0791	32.8
$k_{f,max}$ (h^{-1})	Maximum rate of resistance development toward colistin on PAP plate	0.237	26.8
RES	Additive residual error (log10 scale)	0.964	

Conclusion

- Emergence of colistin resistance occurs at low concentration of 0.1mg/L in STK by PAP analysis.
- Our modelling approach based on time-kill studies and PAP assays, is relevant to characterize the rate and extent of resistance.
- The model can be used for simulations of the responses and the changes in (hetero-) resistance profile of *K. pneumoniae* population during colistin treatment.

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