Supplemental Document

Mathematical modeling complete methods:

Model development: To quantitatively characterize the inoculum effect with and without functional quorum sensing, previously developed pharmacodynamic models for colistin (1) were used as the basis to model the two different strains. Three pre-existing subpopulations with differencing susceptibilities to polymyxin B or colistin were considered as previously described (1) with the modification of the growth model. A life cycle growth model was used to describe the bacterial growth (2, 3). We assumed that bacteria existed in two states: the vegetative state (S1) (preparing for replication) and the replicating state (S2) (state immediately before replication). The transition from S2 to S1 (k_{21}) is assumed to be faster than transition from S1 to S2 (k_{12}) making k_{12} the rate-limiting factor. Hence, it is represented on the growth rate constant of bacteria. The growth model is described by equations 3 and 4. The replication factor (REP) (2) is a mathematical function to limit the total population (CFU_{ALL}) to the maximum population size or carrying capacity of *in vitro* system (CFU_{MAX}) (eq. 5).

$$\frac{d(S1)}{dt} = \text{Rep} \cdot k_{21} \cdot S2 - k_{12} \cdot S1, \quad \text{IC: CFUo} \qquad \text{eq. (S1)}$$

$$\frac{d(S2)}{dt} = \quad - k_{21} \cdot S2 + k_{12} \cdot S1 \quad \text{IC: 0} \qquad \text{eq. (S2)}$$

$$\text{REP} = 2 \cdot \left(1 - \frac{CFU_{ALL}}{CFU_{MAX} + CFU_{ALL}}\right) \qquad \text{eq. (S3)}$$

Previously developed target site binding model was used to describe the competitive interaction of polymyxin with Ca^{2+} and Mg^{2+} at the outer membrane of *P. aeruginosa* —initial site of polymyxin action (1). Free polymyxin concentration (f_p) is described by eq. 6 where C_{MGCA} is the concentration of cation in the media, KD_{MC} is the binding affinity of polymyxin to charged phosphate groups of lipid A, C_P is polymyxin concentration, KD_P is the polymyxin binding affinity. Binding parameters were fixed to literature values (1), and we assumed that the binding affinity of colistin and polymyxin B are the same. The effective polymyxin concentration (Cp_{eff}) was computed as a function of f_P (fraction of receptors unoccupied by cation), EC_{50} (concentration required to achieve 50% of effective colistin concentration relative to the concentration in broth), H (Hill coefficient), and C_P (polymyxin concentration) (eq. 7) (1). The polymyxin killing activity (KKS) was modeled as a second-order killing process as represented by eq. 8, where k_2 is a second-order rate constant, assumed to be different for each bacterial subpopulation.

$$f_P = 1 - \frac{C_{MGCA}}{KD_{MC} + C_{MGCA} + \frac{C_P}{MW_P} + \frac{KD_{MC}}{KD_P}} \qquad \text{eq. (S4)}$$

$$Cp_{eff} = \frac{f_p^{\,H}}{EC_{50}^{\,H} + f_p^{\,H}} \cdot C_P \qquad \text{eq. (S5)}$$

$$KKS = k_2 \cdot Cp_{Eff} \qquad \text{eq. (S6)}$$

Inoculum effect is incorporated by assuming a hypothetical signaling compartment that inhibits the growth rate (INH_{k12}) and killing activity (INH_{kill}) of polymyxin, as described in eq. 9 and eq. 10, respectively.

$$INH_{k12} = 1 - \frac{Imax_{k12}}{IC_{50} + C_{sig}} \cdot C_{sig} \qquad \text{eq. (S7)}$$

$$INH_{kill} = 1 - \frac{Imax_{kill}}{IC_{50} + C_{sig}} \cdot C_{sig} \qquad \text{eq. (S8)}$$

Estimation: Data were modeled using population approach in S-ADAPT software (version 1.57) with SADAPT-TRAN—pre- and post-processing tool to fit all data simultaneously. Importance sampling Monte Carlo parametric expectation maximization method (pmethod=4) algorithm (4, 5) in SADAPT was used. The additive residual error variance model on log_{10}-scale and the Poisson error model (eq. 9)) were used for the bacterial count greater or equal to 100 CFU/mL and below 100 CFU/mL, respectively (1, 3).

$$Var = \left(CFU_{Plate} \cdot SD_{CFU} \cdot Ln(10) + \sqrt{CFU_{Plate}} \cdot SD_{Pois} + SD_{Add} \right)^2 \qquad \text{eq. (11.)}$$

where, Var is residual variance, CFU_{plate} is number of colonies on the plate, SD_{CFU} is the standard deviation used to describe the additive error on log_{10} scale, and SD_{Pois} is standard deviation of Poisson error with a value of 1, and SD_{Add} is standard deviation of additive error with a value of 0.25.

Figure S1: The pharmacodynamic model for polymyxin antibiotic against *P. aeruginosa*. The model consisted of target binding site model of polymyxin (**A**), growth, kill and inoculum effect models (**B**), and a subpopulation model (**C**). Target site model consisted of a competitive interaction between polymyxin, Mg^{2+} and Ca^{2+} at the LPS binding site of outer membrane. The growth model assumed that bacteria existed in two states: vegetative state (state 1) and prior replicate state (state 2). The transition between state 2 to state 1 was assumed to be rapid while state 1 to state 2 is rate limiting step. The attenuation of bacteria killing was assumed due to the hypothetical inoculum effect compartment where the compartment inhibits the activity of polymyxin killing and bacterial growth. The bacterial population was assumed to consist of three pre-existing subpopulations: susceptible, intermediate, and least-susceptible ('resistant').

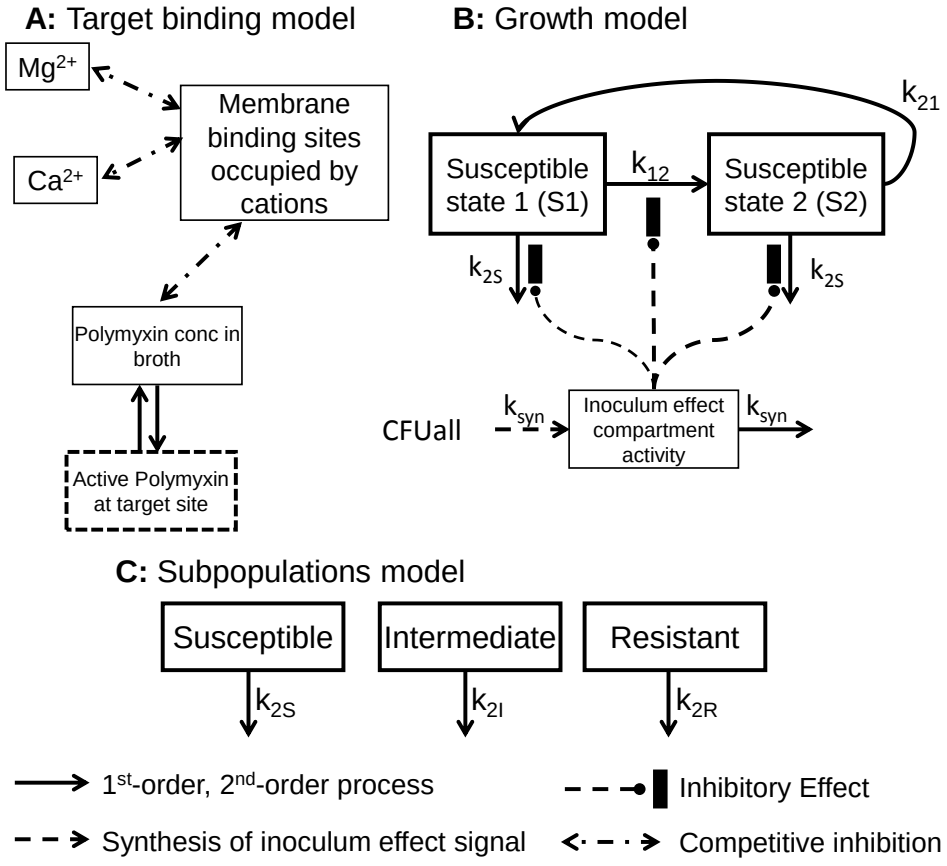

Table S1: Parameter estimates from the pharmacodynamic model

		Polymyxin B		Colistin	
Parameter	**Symbol**	**WT (BCV)**	**lasRrhlR KO (BCV)**	**WT (BCV)**	**lasRrhlR KO (BCV)**
Fraction of receptors occupied by cation, resulting in an effective colistin concn of 50% relative to the colistin concn in broth	EC_{506}	0.154 (0.503)	0.0290(1.12)	0.375 (0.407)	0.399 (0.0336)
	EC_{508}	0.320 (0.112)	0.510 (0.248)	0.473 (0.036)	0.334 (0.0437)
	EC_{509}	0.999 (0.777)	0.998 (0.583)	0.999 (0.735)	0.997 (0.348)
Second-order killing rate constant Susceptible population	k_{2S} (L/(mg·h))	4.23 (0.263)	5.22 (0.0587)	8.02 (0.0686)	5.00 (0.219)
Intermediate population	k_{2I} (L/(mg·h))	0.373 (0.0694)	0.336 (0.0962)	1.28 (0.0214)	0.911 (0.103)
Resistant population	k_{2R} (L/(mg·h))	0.00344 (0.0767)	0.0159 (1.21)	0.00145 (0.279)	0.0288 (0.0316)
Maximal inhibition of bacterial killing by signal molecule	$Imax_{kill}$	0.970 (0.134)	0.961 (0.359)	0.999 (0.510)	0.995 (0.606)
Maximal inhibition of bacterial growth by signal molecule	$Imax_{Rep}$	0.835 (1.19)	0.938 (0.078)	0.993 (0.993)	0.942 (0.0866)
Signal molecule concn resulting in 50% of Max. effect	$Log_{10}\,IC_{50}$	7.29 (0.158)	7.62 (0.439)	7.16 (0.02)	7.37 (0.122)
Degradation half-life of signal molecule	$T_{1/2}(k_{deg})$ (min)	5.59 (0.259)	10.62 (0.241)	81.6 (1.19)	31.6 (1.51)
Maximum population size	$Log_{10}\,POP_{max}$	9.19 (0.201)	9.5 (0.145)	11.2 (1.11)	9.6 (0.237)
Initial inoculum Size: 10^6 inoculum	$Log_{10}\,CFUo_6$	5.75(0.00649)	5.81 (0.0221)	5.87 (0.0401)	6.16 (0.131)
10^8 inoculum	$Log_{10}\,CFUo_8$	7.97 (0.0420)	8.04 (0.0338)	8.08 (0.0197)	8.19 (0.0124)
10^9 inoculum	$Log_{10}\,CFUo_9$	9.25(0.0300)	9.13 (0.0379)	9.14 (0.0126)	9.32 (0.0709)
Intermediate population as fraction of initial inoculum	$Log_{10}\,Fr_I$	-3.69 (0.316)	-4.94 (0.316)	-3.75 (0.316)	-4.49 (0.316)
Resistant population as fraction of initial inoculum	$Log_{10}\,Fr_R$	-6.90 (0.807)	-7.32 (0.316)	-6.09 (0.316)	-6.87 (0.316)
Bacterial growth: Mean generation time susceptible population	MTT_{12S} (min)	17.6 (0.288)	20.7 (0.15)	24.3 (0.0176)	24.4 (0.0205)
Mean generation time intermediate population	MTT_{12I} (min)	18.4 (0.331)	21.5 (0.12)	6.075 (0.475)	9.45 (0.0866)
Mean generation time resistant population	MTT_{12R} (min)	112 (0.170)	135 (0.0683)	101 (0.577)	60.0 (1.08)

Table S2: Parameter estimates for the hill-type functions corresponding to manuscript Figure 2 which shows the comparative pharmacodynamic responses between PAO1 wild-type and the isogenic *lasR*/*rhlR* QS double knockout at each inoculum to either polymyxin B or colistin. Emax is maximal effect, Eo represents baseline activity, IC_{50} is antibiotic concentration (mg/L) which achieves half of the maximum inhibition, and Hill is the Hill coefficient. The parameter estimates of the hill-type models are outlined (C) to mirror A1-B3.

	Inoculum		**10^6 CFU/mL**		**10^8 CFU/mL**		**10^9 CFU/mL**	
			Wild Type	**Δ*lasRrhlR***	**Wild Type**	**Δ*lasRrhlR***	**Wild Type**	**Δ*lasRrhlR***
Polymyxin B	E_{max}	-	4.198 (12.8 %)	5.28 (3.59 %)	2.67 (11.2 %)	3.36 (3.22 %)	2.69 (11.7 %)	1.74 (3.77 %)
	Eo	-	-0.135 (33.8 %)	-0.18 (12.0 %)	-0.169 (10.6 %)	-0.057 (7.01 %)	-0.014 (6.96 %)	-0.103 (4.67 %)
	IC_{50}	mg/L	4.24 (14.0 %)	5.58 (6.12 %)	12.5 (16.0 %)	5.61 (5.71 %)	50.3 (14.3 %)	19.6 (4.78 %)
	Hill	-	5 (FIXED)	5 (FIXED)	2.46 (33.7 %)	5 (FIXED)	1.91 (19.1%)	5 (FIXED)
Colistin	E_{max}	-	5.31 (1.51 %)	5.19 (2.00 %)	2.18 (14.3 %)	4.03 (1.48 %)	2.47 (2.75 %)	4.37 (6.97 %)
	Eo	-	0.230 (0.694 %)	-0.208 (0.936 %)	-0.166 (2.08 %)	0.467 (0.467 %)	-0.028 (0.287%)	0.080 (0.313 %)
	IC_{50}	mg/L	4.82 (2.02 %)	3.74 (2.23 %)	8.72 (24.6 %)	7.04 (2.64 %)	32.0 (3.50 %)	59.3 (12.3 %)
	Hill	-	3.93 (5.51 %)	5.99 (24.6 %)	2.63 (58.1 %)	4.77 (12.2 %)	2.89 (9.80 %)	1.20 (7.40 %)

References

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3. **Landersdorfer CB, Ly NS, Xu H, Tsuji BT, Bulitta JB.** 2013. Quantifying subpopulation synergy for antibiotic combinations via mechanism-based modeling and a sequential dosing design. Antimicrob Agents Chemother.
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