Supplemental material:

Table S1: Log_{10} mutation frequencies at 12 and 20 mg/L (3× and 5× the baseline MIC) piperacillin and 2.5 and 5 mg/L (3× and 5× the baseline MIC) tobramycin at various time points for each dosage regimen simulated (creatinine clearance = 250 mL/min) in the HFIM.[a]

	Time (h)	Control	PIP 4 g q4h	TOB 5 mg/kg q24h	TOB 7 mg/kg q24h	TOB 10 mg/kg q48h	PIP 4 g q4h + TOB 5 mg/kg q24h	PIP 4 g q4h + TOB 7 mg/kg q24h	PIP 4 g q4h + TOB 10 mg/kg q48h
12 mg/liter PIP (3× MIC)	0	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33
	23	-4.59	-2.53	-	-	-	< 0.7	< 0.7	< 0.7
	47	< -9.41	-4.13	-	-	-	< 0.7	< -0.3	< 0.7
	95	< -9.51	-2.49	-	-	-	< 0.7	< 0.7	< 0.7
	143	-4.48	-2.60	-	-	-	< -3.24	< -3.12	< -3.13
	191	-3.52	-2.66	-	-	-	< -3.02	< 0.7	< 0.7
20 mg/liter PIP (5× MIC)	0	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33
	23	-4.37	-3.27	-	-	-	< 0.7	< 0.7	< 0.7
	47	-4.64	-3.89	-	-	-	< 0.7	< -0.3	< 0.7
	95	-4.66	-3.55	-	-	-	< 0.7	< 0.7	< 0.7
	143	-4.64	-3.14	-	-	-	< -3.24	< -3.12	< -3.13
	191	-6.15	-4.15	-	-	-	< -3.02	< 0.7	< 0.7
2.5 mg/liter TOB (5× MIC)	0	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33
	23	-6.68	-	-5.93	< -2.58	< -1.83	< 0.7	< 0.7	< 0.7
	47	-6.73	-	-5.04	-7.25	-0.49	< 0.7	< -0.3	< 0.7
	95	-6.97	-	-2.61	-2.16	-1.63	< 0.7	< 0.7	< 0.7
	143	-6.05	-	-3.33	-1.55	-1.54	-1.59	-2.64	-2.36
	191	-7.28	-	-3.02	-3.19	-3.24	< -3.02	< 0.7	< 0.7

5 mg/liter TOB (10× MIC)	0	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33	< -6.33
	23	-7.62	-	< -6.24	< -2.58	< -1.83	< 0.7	< 0.7	< 0.7
	47	-7.94	-	-5.28	-7.55	-1.46	< 0.7	< -0.3	< 0.7
	95	-7.87	-	-3.06	-3.72	-2.10	< 0.7	< 0.7	< 0.7
	143	-6.56	-	-4.85	-3.73	-3.91	< -3.24	< -3.12	< -3.13
	191	-7.68	-	-4.62	-4.69	-4.72	< -3.02	< 0.7	< 0.7

[a] When no colonies were present on antibiotic-containing plates, mutation frequencies reported represent an upper limit based on the total viable count.

-: Viable counts on these antibiotic-containing plates were not assessed for the respective regimen.

Table S2. MIC values from colonies obtained from drug-containing agar plates (piperacillin at 12 and 20 mg/liter, equivalent to 3× and 5×MIC; tobramycin at 2.5 and 5 mg/liter, equivalent to 5× and 10×MIC) before treatment (0h) and at various time points for each dosage regimen simulated in the HFIM.

Treatment		Piperacillin			Tobramycin	
	Time (h)	12 mg/liter	20 mg/liter	Time (h)	2.5 mg/liter	5.0 mg/liter
Control	0	16	8	0	8	-
	143	8	8	143	8	-
	191	8	8	191	4	-
PIP 4 g q4h	143	32	64	.	.	.
	191	32	64	.	.	.
TOB 5 mg/kg q24h	.	.	.	143	8	8
	.	.	.	191	8	8
TOB 7 mg/kg q24h	.	.	.	143	8	16
	.	.	.	191	8	16
TOB 10 mg/kg q48h	.	.	.	143	8	8
	.	.	.	191	8	8
PIP 4 g q4h + TOB 5 mg/kg q24h	143	-	-	143	8	-
	191	-	-	191	-	-
PIP 4 g q4h + TOB 7 mg/kg q24h	143	-	-	143	8	-
	191	-	-	191	-	-
PIP 4 g q4h + TOB 10 mg/kg q48h	143	-	-	143	8	-
	191	-	-	191	-	-

- , no colonies on antibiotic-containing plates. PIP, piperacillin; TOB, tobramycin. The EUCAST MIC breakpoints were 16 mg/liter for piperacillin/tazobactam and 4 mg/liter for tobramycin.

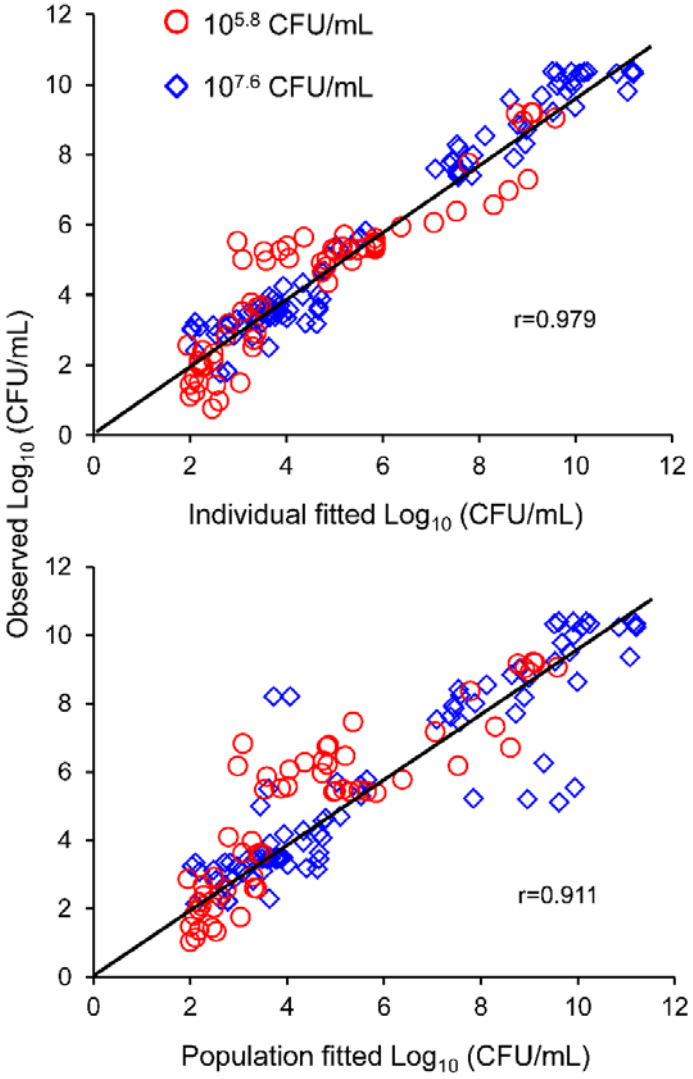

Fig. S1: Observed *versus* individual fitted (top) and population fitted (bottom) viable counts for piperacillin and tobramycin alone and in combinations in SCTK.

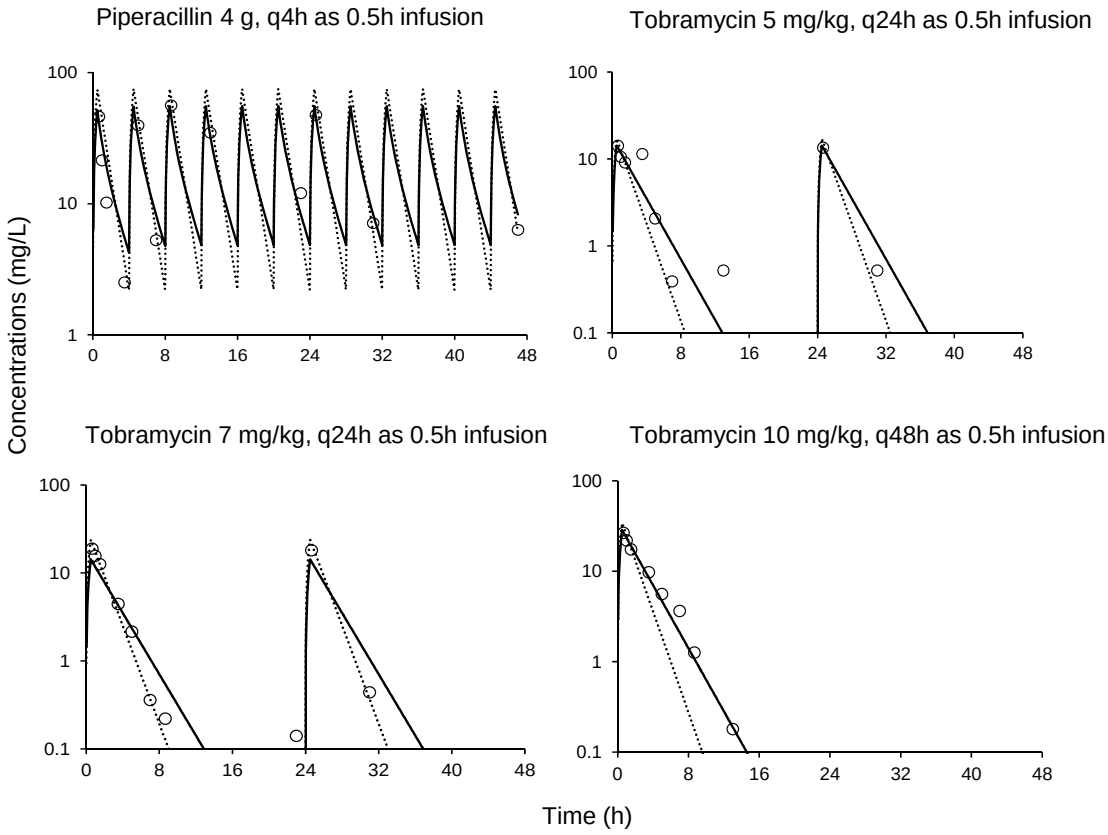

Figure S2: Typical simulated pharmacokinetic profiles showing the relationship between targeted (broken lines), measured (symbols) and model fitted (continuous lines) concentrations of piperacillin and tobramycin in the HFIM.

Mechanism-based population PK/PD modeling

Mechanism-based modeling (MBM) of SCTK data at both inocula was performed utilizing S-ADAPT (version 1.57, importance sampling, pmethod=4) (1, 2) and SADAPT-TRAN (1, 2). Competing models were evaluated based on the biological plausibility of the parameter estimates, the S-ADAPT objective function value (-1×log-likelihood), standard diagnostic plots, the coefficient of correlation and visual predictive checks (3-6).

A life-cycle growth model was utilized to describe the underlying biology of bacterial replication (7, 8). The first-order growth rate constant (k_{12}) determines the mean generation time (MGT) and reflects the transition from the vegetative state (*i.e.* state 1) to the replication state (*i.e.* state 2). The k_{21} was set to $50h^{-1}$, since replication was assumed to be fast (8). Models with two and three bacterial subpopulations that described the antibiotic effects *via* direct bacterial killing and inhibition of successful replication by piperacillin were considered. The final model for the combinations included three pre-existing subpopulations of different susceptibility to piperacillin and tobramycin (**Figure 1**). Each population was described in the two states (*i.e.* compartments) mentioned above. The total concentration of viable bacteria (CFU_{all}) was:

$$CFU_{all} = CFU_{SS1} + CFU_{SS2} + CFU_{RI1} + CFU_{RI2} + CFU_{IR1} + CFU_{IR2} \quad (1)$$

The $CFU_{NN\#}$ described the concentration of viable bacteria for population NN in state 1 or 2. The effect of piperacillin on inhibition of the probability of successful replication, denoted Inh_{REP}, was described as (9).

$$Inh_{REP} = \left(\frac{I_{max,REP} \cdot C^{Hill,_{REP}}}{C^{Hill,_{REP}} + IC_{50,REP}} \right) \quad (2)$$

where $I_{max,REP}$ is the maximum inhibition of the probability of successful replication, $IC_{50,REP}$ the concentration causing 50% of the maximum inhibition effect and $Hill,_{REP}$ the Hill coefficient, which describes the steepness of the concentration-effect relationship. Two different $IC_{50,REP}$ parameters are required ($IC_{50,SS,REP}$ and $IC_{50,IR,REP}$). An Inh_{REP} of 0.50 results in net stasis of the respective bacterial population and an Inh_{REP} of >0.50 results in bacterial killing, since bacteria that replicate unsuccessfully are eliminated.

The differential equation for the double susceptible population in state 1 (CFU_{ss1}) included killing by piperacillin (C_{PIP}; piperacillin concentration) and tobramycin (C_{TOB}; tobramycin concentration) (initial conditions described below):

$$\frac{d(CFU_{SS1})}{dt} = 2 \cdot PLAT \cdot (1 - Inh_{REP}) \cdot k_{21} \cdot CFU_{SS2} - k_{12,ss} \cdot CFU_{SS1} - \left(\frac{Kmax_{PIP} \cdot C_{PIP}^{Hill_{PIP}}}{C_{PIP}^{Hill_{PIP}} + KC_{50,SS,PIP}^{Hill_{PIP}}} + \frac{Kmax_{SS,TOB} \cdot C_{TOB}}{C_{TOB} + KC_{50,SS,TOB}} \right) \cdot CFU_{SS1} \quad (3)$$

The CFU_{SS2} is the bacterial concentration of the double susceptible population in state 2. The plateau factor (PLAT) represents the probability of successful replication and is defined as described previously (10, 11). The maximum killing rate constant (K_{max}), the associated antibiotic concentrations (KC_{50}) causing 50% of K_{max} and the Hill coefficient ($Hill_{PIP}$) for piperacillin are illustrated in **Figure 1** and **Table 1**. Killing by piperacillin and tobramycin was assumed to affect both states and thus the same killing terms were included for states 1 and 2. The double susceptible population in state 2 (CFU_{SS2}) was described as:

$$\frac{d(CFU_{SS2})}{dt} = -k_{21} \cdot CFU_{SS2} + k_{12,ss} \cdot CFU_{SS1} - \left(\frac{Kmax_{PIP} \cdot C_{PIP}^{Hill_{PIP}}}{C_{PIP}^{Hill_{PIP}} + KC_{50,SS,PIP}^{Hill_{PIP}}} + \frac{Kmax_{SS,TOB} \cdot C_{TOB}}{C_{TOB} + KC_{50,SS,TOB}} \right) \cdot CFU_{SS2} \quad (4)$$

The differential equations for the other two populations (RI and IR) were similar but included different estimates for K_{max}, KC_{50} and k_{12} compared to the double susceptible population.

Mechanism-based modeling of synergy: In this modeling analysis we evaluated two types of synergistic interactions: 1) subpopulation synergy (*i.e.* antibiotic A killing the bacteria resistant to antibiotic B and *vice versa*) and 2) mechanistic synergy (*i.e.* antibiotic A enhancing the killing by antibiotic B against one or multiple bacterial populations) (12-14). Mechanistic synergy was incorporated in the model assuming that disruption of the bacterial outer membrane by tobramycin increases the target site penetration of piperacillin (12-14). This permeabilizing effect of tobramycin was implemented in the model *via* the term OM_effect (13, 14). (parameters explained in **Table 1**):

$$OM\_effect = 1 - \left(\frac{I_{max,OM,TOB} \cdot C_{TOB}}{C_{TOB} + IC_{50,OM,TOB}} \right) \quad (5)$$

Between-curve variability was set to 10% coefficient of variation during the end of the estimation (15). The log_{10} viable counts were fitted using an additive residual error model. For observations below 100 CFU/mL (equivalent to fewer than 10 colonies per plate), a previously developed residual error model was utilized to fit the number of colonies per plate (15).

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