

Table S1. Log change in viable cell counts with polymyxin B (PMB) and enrofloxacin (ENRO) mono- and combination therapy against all three isolates of *Pseudomonas aeruginosa* in the static time-kill studies. The grey, green and orange backgrounds indicate activity (a ≥ 1 log₁₀ reduction compared to the initial inoculum), 1 to 2 log₁₀ more killing compared to the most active monotherapy, and synergy (>2 log₁₀ more killing compared to the most active monotherapy), respectively.

Time (h)	Log change [$\text{Log}_{10}(\text{CFU}_t) - \text{Log}_{10}(\text{CFU}_0)$]																	
	<i>P. aeruginosa</i> 12196						<i>P. aeruginosa</i> H131300444						<i>P. aeruginosa</i> LESB58					
	0.5	1	2	4	6	24	0.5	1	2	4	6	24	0.5	1	2	4	6	24
Growth control	0.11	0.61	1	1.49	1.87	2.52	0.08	0.24	0.59	0.97	1.75	2.59	0.1	0.44	0.69	1.24	1.68	2.42
PMB (4 mg/L)	-1.7	-1.74	-1.46	-1.36	-1.2	1.74	0.07	0.04	0.51	0.77	1.29	1.92	-0.62	-0.74	-0.58	-0.28	0.13	1.7
PMB (3 mg/L)	-1.63	-1.46	-1.18	-1.35	-1.01	1.53	0.04	-0.03	0.44	0.73	1.29	1.95	-0.43	-0.55	-0.51	-0.11	0.21	1.7
PMB (2 mg/L)	-1.52	-1.3	-1.15	-0.66	0.26	1.84	0.16	0.05	0.25	0.77	1.37	1.97	-0.24	-0.42	-0.31	0.01	0.65	1.83
PMB (1 mg/L)	-1.34	-1.17	-0.73	0.37	1.34	2.26	0.09	0.24	0.72	1.1	1.51	2.08	-0.26	-0.66	0.54	0.72	1.44	2.07

ENRO (4 mg/L)	-1.7	-1.9	-2.54	-2.94	-2.02	1.33	-0.52	-0.42	0.48	0.31	0.79	1.13	0.11	0.34	0.74	1.19	1.72	2.5
ENRO (3 mg/L)	-0.7	-0.73	-1.63	-1.72	-2.02	2.03	-0.49	-0.47	0.48	0.56	1.11	1.38	-0.1	0.37	0.74	1.2	1.83	2.44
ENRO (2 mg/L)	0.01	-0.11	-0.66	-0.46	-0.23	2.03	0.16	0.27	0.51	0.62	1.29	2.16	0.06	0.25	0.61	1.17	1.86	2.26
ENRO (1 mg/L)	0.09	0.12	0.27	0.97	1.06	1.94	0.16	0.38	0.47	1.01	1.51	2.52	-0.1	0.32	0.63	1.22	2	2.58
PMB (1 mg/L) + ENRO (1mg/L)	-3.49	-4.22	-4.4	-4.22	-3.22	-2.26	0.05	-0.19	-0.85	-2.4	-2.8	1.01	-0.78	-1.1	-1.81	-3.61	-2.79	-2.36
PMB (1 mg/L) + ENRO (4 mg/L)	-2.17	-3.11	-4.34	-5	-6.6	-3.5	-0.02	-0.85	-2.25	-3.41	-3.85	-2.76	-0.03	-0.28	-0.96	-2.12	-2.22	1.19
PMB (2 mg/L) + ENRO (2 mg/L)	-2.38	-3.7	-4.7	-5	-5.8	-5.4	0.01	-0.3	-1.85	-2.89	-3.25	-1.24	-0.33	-1.33	-2.81	-3.2	-2.79	-2.71
PMB (2 mg/L) + ENRO (3 mg/L)	-2.73	-4.19	-4.82	-4.82	-5.6	-4	-0.03	-0.79	-2.14	-3.55	-4.39	-2.28	-0.27	-1.06	-2.12	-1.84	-2.68	-1.89
PMB (3 mg/L) + ENRO (2 mg/L)	-3.69	-4.4	-4.7	-6.6	-6.6	-1.98	0.06	-0.3	-1.81	-3.62	-3.87	-1.66	-0.59	-1.41	-2.56	-3.24	-3	-2.12
PMB (3 mg/L) + ENRO (3 mg/L)	-3.94	-4.79	-5.18	-5.68	-6.18	-5.06	0.04	-0.84	-2.3	-4.87	-4.17	-1.99	-1.01	-2.14	-3.15	-4.17	-3.3	-2.12
PMB (4 mg/L) + ENRO (4 mg/L)	-4.7	-6.6	-6.6	-6.6	-6.6	-5.3	0.4	-1.03	-2.59	-4.32	-5.17	-4.57	-1.82	-2.81	-5.13	-3.16	-3.95	-3.37

$\text{Log}_{10}(\text{CFU}_0)$ = Initial inoculum

Table S2. Log change in viable cell counts with polymyxin B (PMB) and enrofloxacin (ENRO) mono- and combination therapy against *P. aeruginosa* 12196 in IVM. The grey, green and orange backgrounds indicate activity ($a \geq 1 \log_{10}$ reduction compared to the initial inoculum), 1 to 2 \log_{10} more killing compared to the most active monotherapy, and synergy ($>2 \log_{10}$ more killing compared to the most active monotherapy), respectively.

Time (h)	Log change [$\text{Log}_{10}(\text{CFU}_t) - \text{Log}_{10}(\text{CFU}_0)$]			
	Growth control	PMB $C_{ss} = 4 \text{ mg/L}$	ENRO $C_{max} = 3 \text{ mg/L}$	PMB $C_{ss} = 4$ mg/L + ENRO $C_{max} = 3 \text{ mg/L}$
1	-0.28	-1.52	-0.08	-4.48
2	-0.17	-1.56	-0.35	-4.01
4	0.02	-0.83	-0.69	-4.24
6	0.28	-0.28	-0.36	-3.97
23	-0.02	-0.58	-0.77	-3.62
25	-0.03	-0.65	-0.96	-4.79
28	-0.04	-0.37	-1.08	-4.69
48	0.19	-0.36	-0.62	-2.83

$\text{Log}_{10}(\text{CFU}_0)$ = Initial inoculum

Table S3. Log change in viable cell counts with polymyxin B (PMB) and enrofloxacin (ENRO) mono- and combination therapy against *P. aeruginosa* 12196 in the HFIM. The grey, green and orange backgrounds indicate activity (a ≥ 1 log₁₀ reduction compared to the initial inoculum), 1 to 2 log₁₀ more killing compared to the most active monotherapy, and synergy (>2 log₁₀ more killing compared to the most active monotherapy), respectively.

Time (h)	Log change [$\text{Log}_{10}(\text{CFU}_t) - \text{Log}_{10}(\text{CFU}_0)$]			
	Growth control	PMB $C_{ss} = 4 \text{ mg/L}$	ENRO $C_{max} = 3 \text{ mg/L}$	PMB $C_{ss} = 4$ mg/L + ENRO $C_{max} = 3 \text{ mg/L}$
1	0.86	-0.69	-1.50	-2.02
2	1.66	0.32	-1.34	-3.47
4	2.15	0.72	-2.07	-3.86
6	2.58	0.54	-2.37	-5.85
23	3.00	2.68	1.41	-5.25
25	3.00	2.56	1.77	-4.78
26	3.10	2.45	1.79	-7.16
28	3.14	2.62	1.66	-7.16
47	3.20	2.82	2.54	-7.16
49	3.30	2.90	2.73	-7.16

50	3.27	2.81	2.68	-7.16
52	3.11	2.87	2.70	-7.16
71	3.14	2.77	2.70	-5.25
73	3.24	2.86	2.86	-7.16
74	3.36	2.81	2.75	-7.16
76	3.29	2.71	2.68	-5.25
95	3.14	2.78	2.79	-4.51
97	3.14	2.50	2.79	-7.16
98	3.04	2.70	2.70	-7.16
100	3.17	2.56	2.65	-7.16
120	2.87	2.58	2.42	-7.16

$\text{Log}_{10}(\text{CFU}_0)$ = Initial inoculum

Table S4. Population mean parameter estimates for the synergistic combination of polymyxin B (PMB) and enrofloxacin (ENRO) models against three *P. aeruginosa* isolates. Values in parentheses are standard errors.

Parameter	Symbol	Unit	Population mean (SE [%]) for strain, treatment				
			<i>P. aeruginosa</i> 12196			<i>P. aeruginosa</i>	<i>P. aeruginosa</i>
			Static time-kill	IVM	HFIM	H131300444	LESB58
Log₁₀ Initial inoculum	Log ₁₀ CFU ₀	CFU/mL	6.67 (2.23%)	7.46 (2.46%)	6.74 (1.72%)	6.47 (1.63%)	6.31 (1.99%)
Log₁₀ Maximum population size	Log ₁₀ CFU _{max}	CFU/mL	8.73 (5.67%)	8 (2.87%)	10.1 (1.54%)	8.59 (2.22%)	8.76 (2.71%)
Mutation frequency							
Subpopulation 2	Log ₁₀ (MUT,S2)	CFU/mL	-2.65 (7.74%)	-3.11 (10.9%)	-5.46 (4.32%)	-4.4 (6.26%)	-3.33 (7.62%)
Subpopulation 3	Log ₁₀ (MUT,S3)	CFU/mL	-7 (4.14%)	-9.77 (1.87%)	-9.65 (3.98%)	-10.2 (1.53%)	-9.97 (2.89%)
Enrofloxacin concentration causing 50% of K_{max,ENRO}							
Subpopulation 1	KC _{50,ENRO,S1}	mg/L	10.4 (19.4%)	7.28 (16.9%)		7.38 (8.38%)	18.2 (20.8%)
Subpopulation 2	KC _{50,ENRO,S2}	mg/L	44.7 (6.16%)	19.3 (13.2%)		144 (5.85%)	181 (17.8%)

Subpopulation 3	KC _{50,ENRO,S3}	mg/L	182 (11%)	131 (16.5%)	132 (11%)	240 (13%)
Enrofloxacin maximum killing rate constant						
Subpopulation 1	K _{max,ENRO,S1}	1/h				
Subpopulation 2	K _{max,ENRO,S2}	1/h	26.5 (14.2%)	12.1 (22%)	3.17 (6.96%)	3.44 (14.3%)
Subpopulation 3	K _{max,ENRO,S3}	1/h				
Polymyxin B maximum killing rate constant						
Subpopulation 1	K _{max,PMB,S1}	1/h	28 (12.1%)	2.45 (21.8%)	8.04 (11.2%)	1.49 (108.4%)
Subpopulation 2	K _{max,PMB,S2}	1/h	3.44 (18.9%)	0.665 (37.1%)	0.742 (22.1%)	3.16 (45.6%)
Subpopulation 3	K _{max,PMB,S3}	1/h	0.05 (33.3%)	14.3 (40.7%)	2.05 (11.9%)	1.49 (108.4%)
Polymyxin B concentration causing 50% of K_{max,PMB}						
Subpopulation 1	KC _{50,PMB,S1}	mg/L	1.22 (35.4%)	4.75 (11%)	11.1 (18.8%)	0.117 (65.5%)
Subpopulation 2	KC _{50,PMB,S2}	mg/L	8.64 (26.5%)	6.26 (15%)	>100 ^a	>100 ^a
Subpopulation 3	KC _{50,PMB,S3}	mg/L	3.06 (26.1%)	5.7 (50.4%)	19.2 (16.6%)	77.5 (16.1%)

Enrofloxacin hill						
coefficient for bacterial killing	Log ₁₀ (Hill,ENRO)	-	0.263 (13.6%)	0.342 (25.9%)	0.365 (20.2%)	0.734 (42.9%)
Polymyxin B hill						
coefficient for bacterial killing	Log ₁₀ (Hill,PMB)	-	0.461 (39.8%)	0.728 (45.1%)	0.911 (19.4%)	0.712 (35.3%)
PMB concentration causing 50% of I_{max,Synergy,PMB}						
Subpopulation 1	IC _{50,Synergy, PMB, S1}	mg/L				^b
Subpopulation 2	IC _{50,Synergy, PMB, S2}	mg/L	0.428 (15.1%)	2.93 (21.9%)	0.163 (23.9%)	54.8 (19.5%)
Subpopulation 3	IC _{50,Synergy, PMB, S3}	mg/L				10.2 (29.6%)
Maximum fractional decrease of IC_{50,Synergy,PMB} by PMB						
decrease of IC_{50,Synergy,PMB} by PMB	I _{max,Synergy,PMB}	1/h	6.08 (4.12%)	9.17 (4.05%)	10.6 (1.13%)	8.33 (4.94%)

^aEstimated to be a very large value. ^bEstimated to be a very small value. ^cThe between-curve variability was set to a coefficient of variation of 10% for all parameters.

^dAdditional time kill data for polymyxin B (1 – 128 mg/L) and enrofloxacin (1- 32 mg/L) monotherapies were included during model development.

Table S8. Antibiogram of *P. aeruginosa* 12196.

Minimum Inhibitory Concentration (mg/L)	
Amikacin	16
Ampicillin/sulbactam	>16
Aztreonam	16
Cefepime	4
Ceftazidime	2
Ceftriaxone	16
Ciprofloxacin	>4
Doripenem	2
Gentamicin	>8
Imipenem	1
Levofloxacin	>4
Meropenem	8
Polymyxin	64
Piperacillin/tazobactam	16
Tigecycline	>4
Tobramycin	>16

Study Year: 2008; Site Code: 127; Bank Number: 503; Country Ireland; Source: JML Laboratories Iowa

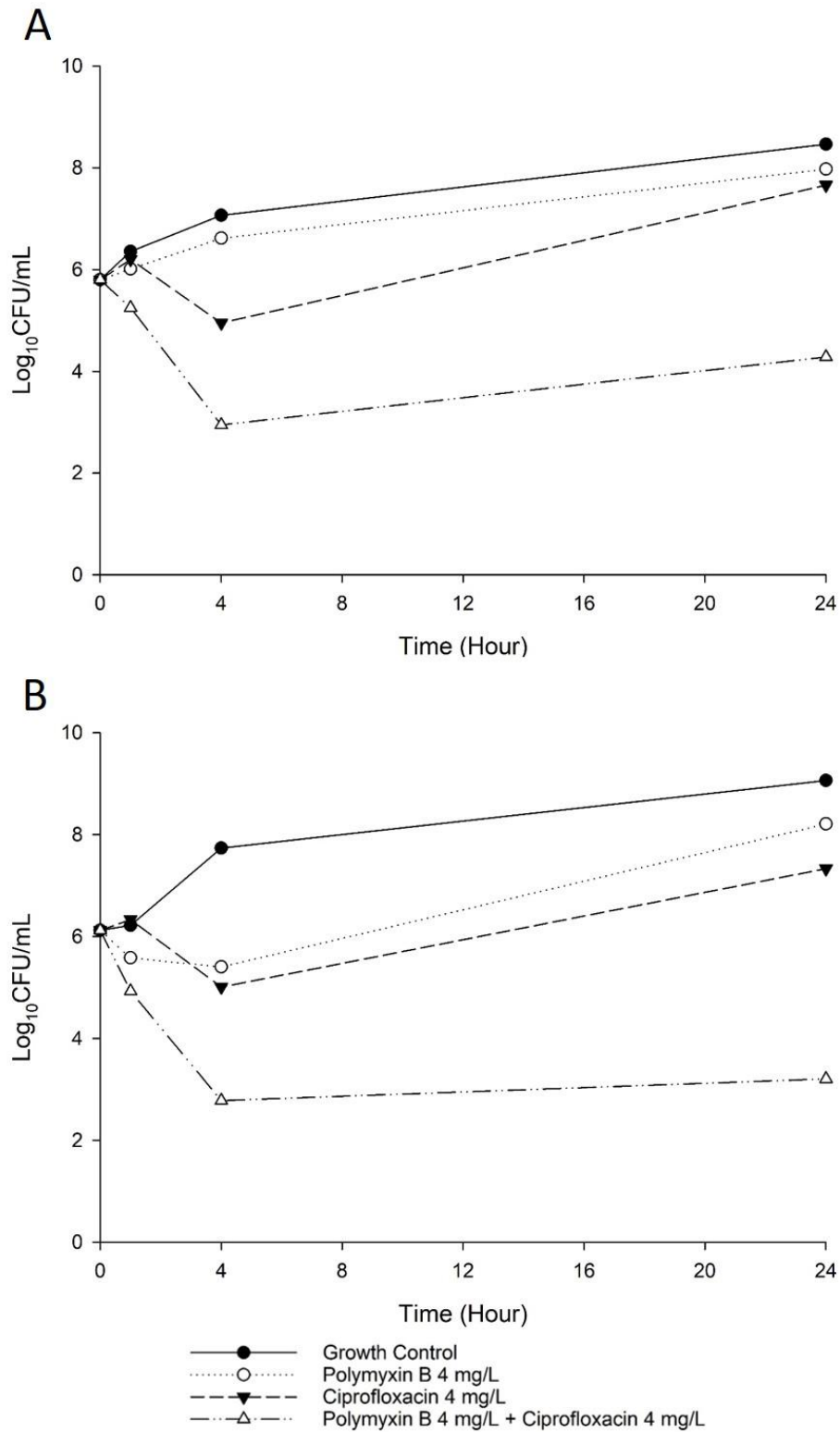


Figure S1. Static time-kill curves for polymyxin B and ciprofloxacin alone and in combination against **(A)** *P. aeruginosa* H131300444 and **(B)** *P. aeruginosa* LESB58 at an inoculum of $\sim 10^6$ CFU/mL.