Appendix 1; Table: Mapping of time-kill curve (TKC) data used for simultaneous PD analysis.

Bacteria	mcr-1	mcr-3	Inoculum (CFU/mL)	MIC, as measured at 1 x 10^5 CFU/mL (mg/L)	Multiple of MIC tested									Number of replicates
					0	0.125	0.25	0.5	0.75	1	1.5	2	4	
219	Negative	Negative	1.00E+05	0.125	x		x	x	x	x	x	x	x	4 TKCs
219	Negative	Negative	1.00E+05	0.125	x									1 growth curve
12241	Negative	Negative	1.00E+02	0.25	x									1 growth curve
12241	Negative	Negative	1.00E+04	0.25	x									1 growth curve
12241	Negative	Negative	1.00E+05	0.25	x		x	x	x	x	x	x	x	6 TKCs
12241	Negative	Negative	1.00E+05	0.25	x									1 growth curve
12241	Negative	Negative	1.00E+06	0.25	x									1 growth curve
N100	Negative	Negative	1.00E+02	0.125	x									1 growth curve
N100	Negative	Negative	1.00E+04	0.125	x									1 growth curve
N100	Negative	Negative	1.00E+05	0.125	x		x	x	x	x	x	x	x	5 TKCs
N100	Negative	Negative	1.00E+05	0.125	x									3 growth curves
N100	Negative	Negative	1.00E+05	0.125	x	x	x	x		x		x		3 TKCs
N100	Negative	Negative	1.00E+05	0.125	x			x		x				3 partial TKCs
N100	Negative	Negative	1.00E+06	0.125	x									1 growth curve
13846	Positive	Negative	1.00E+05	2	x		x	x	x	x	x	x	x	6 TKCs
13846	Positive	Negative	1.00E+05	2	x									1 growth curve
120h_B3_5	Positive	Negative	1.00E+05	2.4	x		x	x	x	x	x	x	x	4 TKCs
120h_B3_5	Positive	Negative	1.00E+05	2.4	x									4 growth curves
120h_B3_5	Positive	Negative	1.00E+05	2.4	x			x		x				3 partial TKCs
73h_B6_2	Positive	Negative	1.00E+05	3.2	x		x	x	x	x	x	x	x	4 TKCs
73h_B6_2	Positive	Negative	1.00E+05	3.2	x									1 growth curve
2013-SQ352	Negative	Positive	1.00E+02	2.4	x									1 growth curve
2013-SQ352	Negative	Positive	1.00E+04	2.4	x									1 growth curve
2013-SQ352	Negative	Positive	1.00E+05	2.4	x		x	x	x	x	x	x	x	5 TKCs
2013-SQ352	Negative	Positive	1.00E+06	2.4	x									1 growth curve

Appendix 2: PK/PD model description

Growth model

The model consists of two compartments, a susceptible growing population (S), and a non-susceptible, non-growing population. The initial susceptible population (starting inoculum) consists of two subpopulations representing a heterogenous bacterial population with a proportion (F_1) of bacteria being a highly susceptible dominant population (S1) and the remaining sub-dominant population (S2) having a lower susceptibility. F1 was estimated by the model. Apportioning of the starting inoculum (SLoad) to each of the initial sub-populations is defined by Equation 1 and Equation 2.

Equation 1: Proportion of initial inoculum that is apportioned to subpopulation S1.

$$S1 = \mathrm{SLoad} \times F_1$$

Equation 2: Proportion of initial inoculum that is apportioned to subpopulation S2.

$$S2 = \mathrm{SLoad} \times (1 - F_1)$$

A proportion of the susceptible population (S1 and S2) is irreversibly transferred to a non-susceptible, non-growing population at a rate constant (K_{SP}) as described by Equation 3.

Equation 3: Transfer rate equation describing change in susceptible population to non-susceptible, non-growing population.

$$K_{SP} = \frac{(k_{growth} - K_{death})}{B_{max}} \times (S1 + S2 + P)$$

The growth of susceptible bacteria (K_{growth}), the natural death (K_{death}) and the maximum total bacterial population achievable in the system (B_{max}) are factored. The non-susceptible, non-growing population although not growing is still subject to natural death at the same rate as the susceptible population. To adjust for potential growth delay at onset of time-kill experiment, attributed to physiological adaptation to the culture condition, a parameter (Alpha) describing a progressive increase in growth rate over time, such that at time 0, K_{growth} = 0 and K_{growth} increase until reaching a maximal growth rate ($K_{growthmax}$) is included according to Equation 4 (Pelligand et al., 2019). In the development of this model, the value for K_{death} , not identifiable, was fixed to 0.179 h^{-1} (a half-life of bacterial death of 3.87 h, as described by Nielsen et al. (2007)).

Equation 4: Bacterial growth rate as described by a progressive increase (alpha).

$$K_{GROWTH} = K_{GROWTHMAX} \times (1 - EXP(-Alpha \times Time))$$

Drug effect sub-model

Further to the natural death there is also a death rate associated with the colistin drug effect (K_{drug}). This is implemented through the E_{max} model described by **Equation 5**. The drug effect was described by a Hill model with three parameters namely a maximal killing rate of colistin (E_{max}), the concentration of colistin to achieve 50% of the maximal killing effect (EC_{50}) and a sigmoidicity (Hill) factor (Gamma; γ). A factor (F_2) increasing the value of E_{max} of K_{drug} for the dominant, highly susceptible, initial subpopulation was included according to Equation 6.

Equation 5: Kill rate associated with the antimicrobial effect of colistin on the less susceptible population, S2.

$$K_{DRUG(t)\_S2} = \frac{Emax \times C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}$$

Equation 6: Kill rate associated with the antimicrobial effect of colistin on the highly susceptible population, S1.

$$K_{DRUG(t)\_S1} = \frac{Emax \times F2 \times C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}$$

As multiple strains have been studied an individual EC_{50} is estimated for each strain with E_{max} considered the same for all strains, E_{max} of S1 and S2 being actually differentiated by the F2 factor. It was anticipated that the potency (EC_{50}) of colistin would be strain specific, based on the MIC differences measured between strains, to account for this variability individual estimates of the EC_{50} were calculated relative to reference strain (*E. coli* 219). Assuming no distribution and independent from MIC, using an equation of the form (**Equation 7**):

Equation 7: Estimation of individual EC_{50} for individual strains.

$$EC_{50(i)} = EC_{50(R)} + dEC_{50(i)}$$

Where the $EC_{50(R)}$ is the value associated with the reference strain. $dEC_{50(i)}$ is a fixed effect (covariate) for the i^{th} bacteria (with *i* ranging from 1 to 6) that describes the additive difference in EC_{50} between bacteria *i* and the reference strain.

***mcr*-status covariate model**

To allow for parameter variation related the categorical *mcr, a* covariate for strains harbouring *mcr* (*mcr*-1 or *mcr*-3) versus non-*mcr* was include in the PD parameters (E_{max} and slope) of the model.

These equations can be incorporated into differential equations describing the change in bacterial populations, S1 (Equation 8), S2 (Equation 9) and P (Equation 10).

Equation 8: The change in susceptible subpopulation 1 (S1) over time.

$$dS1/_{dt} = K_{growth} \times S1 - (K_{death} + K_{drug\_S1}) \times S1 - K_{SP} \times S1$$

Equation 9: The change in susceptible population (S2) over time.

$$dS2/_{dt} = K_{growth} \times S2 - (K_{death} + K_{drug\_S2}) \times S2 - K_{SP} \times S2$$

Equation 10: The change in the non-susceptible, non-growing population (P) over time.

$$dP/_{dt} = K_{SP} \times S1 + K_{SP} \times S2 - K_{death} \times P$$

Interindividual variability, residual error model and handling of data below the limit of quantification (BLQ)

Residual variability was modelled with an exponential error model (Pelligand et al., 2019). The Phoenix Naive Pooled (NP) engine was used to fit data. The NP engine treats all observations as if they came from a single individual in that it ignores inter-individual variations (no random components are computed) but it respects inter-individual differences in initial conditions (initial loads) and covariate values. As this engine was not able to return CV% of estimates (precision), the estimated median of fixed effect parameters (EC_{50}, E_{max}, alpha, gamma, $K_{growthmax}$, $B_{max,}$ F_1, F2, and all theta values of covariates) were also estimated using a bootstrap method.

Values below the limit of quantification (BLQ; ≤ 100 CFU/mL; 12.63% of the complete dataset) were retained in the analysis by using a likelihood-based approach according to the M3 method (Beal, 2001).

Adequacy of model fit was determined through the different diagnostic goodness-of-fit plots including Visual Precitive Check (VPC), the DV (dependent variable) versus PRED (population prediction), individual fitting and the overall fitting (-2LL and BIC). Through plotting of a visual predictive check (VPC) derived from the simulation of (200 datasets), graphical comparison of the observed data and prediction intervals (20, 50 and 80 quantiles) was performed. The estimated fixed effect parameters (EC_{50}, E_{max}, alpha, gamma, $K_{growthmax}$, $B_{max,}$ F_1, F_2, and all theta values of covariates) are reported as typical values with confidence intervals determined by bootstrap in **Error! Reference source not found.**.

The Phoenix shotgun tool was used to explore significance of covariate (E_{max}, gamma, $K_{growthmax}$, alpha, B_{max}, F_1, F2) by evaluating all covariate combinations (scenarios).

Calculation of secondary parameters

Additional secondary parameters were calculated directly from typical values of the model fitted with the NP engine, including MIC and MBC as describe by Mouton et al. (2005). The determination of these is reliant on a factor constant related to the experimental conditions including the initial inoculum, final count, and time of measurement (Equation 11).

Equation 11: Relationship constant for calculation of MIC and MBC.

$$\frac{1}{Time\ of\ measurement\ (24\ h)} \times \ln\left(\frac{N_{(t)}}{N_{(0)}}\right)$$

For MIC, time of measurement is fixed at 24 h, visible growth is assumed to indicate a bacterial density of 1 x 10^8 CFU/mL, and the initial inoculum was standardised at 5 x 10^5 CFU/mL resulting in a constant of 0.221. MIC is then calculated using Equation 12 (Mouton and Vinks, 2005).

Equation 12: Estimating MIC from PK PD model.

$$MIC = EC_{50} \times \left(\frac{K_{growth} - 0.221}{E_{max} - (K_{growth} - 0.221)}\right)^{\frac{1}{\gamma}}$$

With our model, it was possible to distinguish two initial subpopulations differing by their killing rate with a potentiation factor, F_2, increasing E_{max}. In addition, F_2 was different between strains harbouring, or not, an *mcr* gene, as was the Hill coefficient (Gamma; γ). Accordingly , **Equation 12** and 13 were used to compute individual MIC values for each of the two initial subpopulations; with **Equation 13** and **Equation 14** for non-*mcr* isolate and *mcr*-harbouring isolates, respectively.

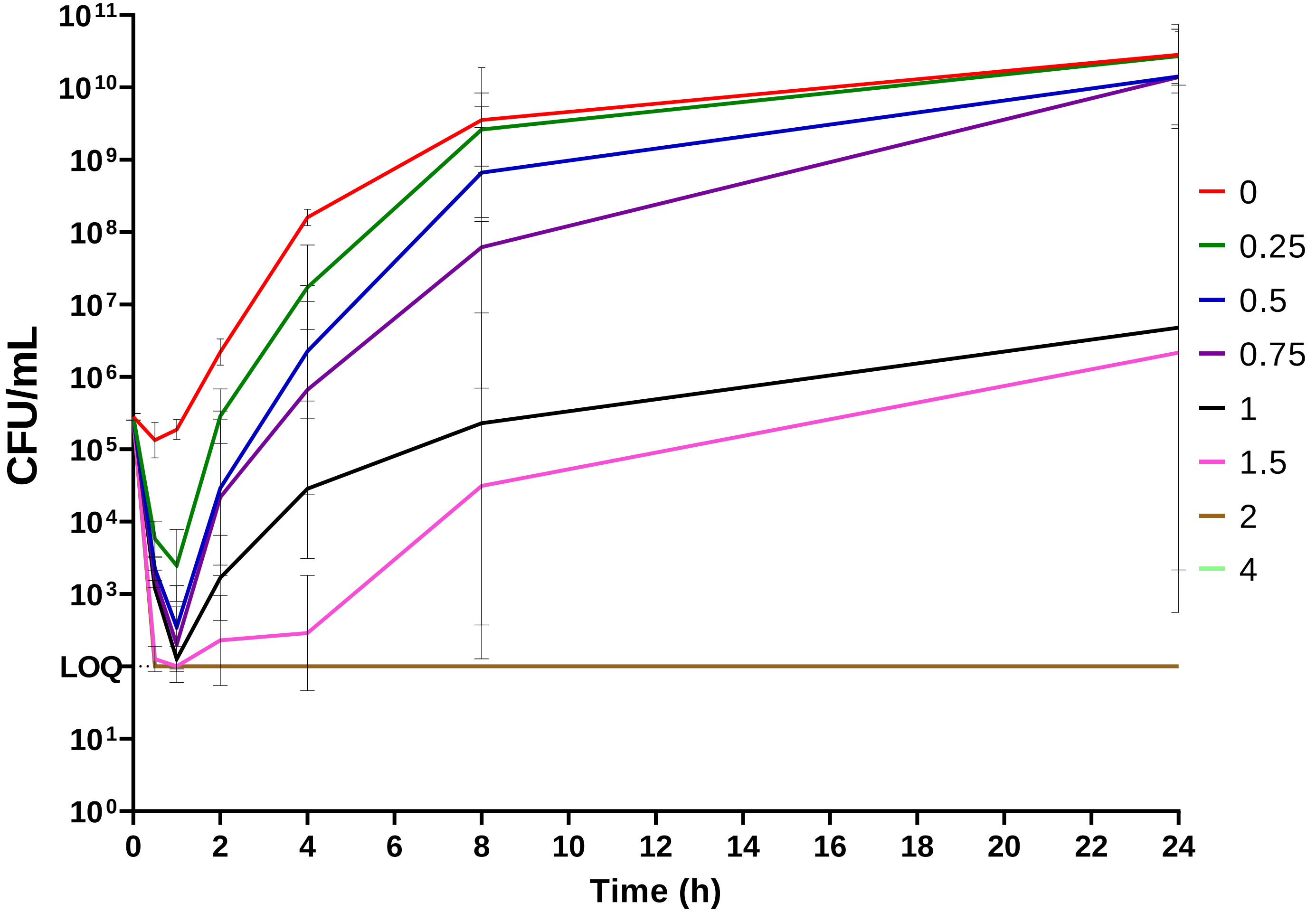
Equation 13: Estimating MIC for the highly susceptible subpopulation of non-mcr isolates.

$$MIC = EC_{50} \times \left(\frac{K_{growth} - 0.221}{E_{max} \times F_{2S} - (K_{growth} - 0.221)}\right)^{\frac{1}{\gamma S}}$$

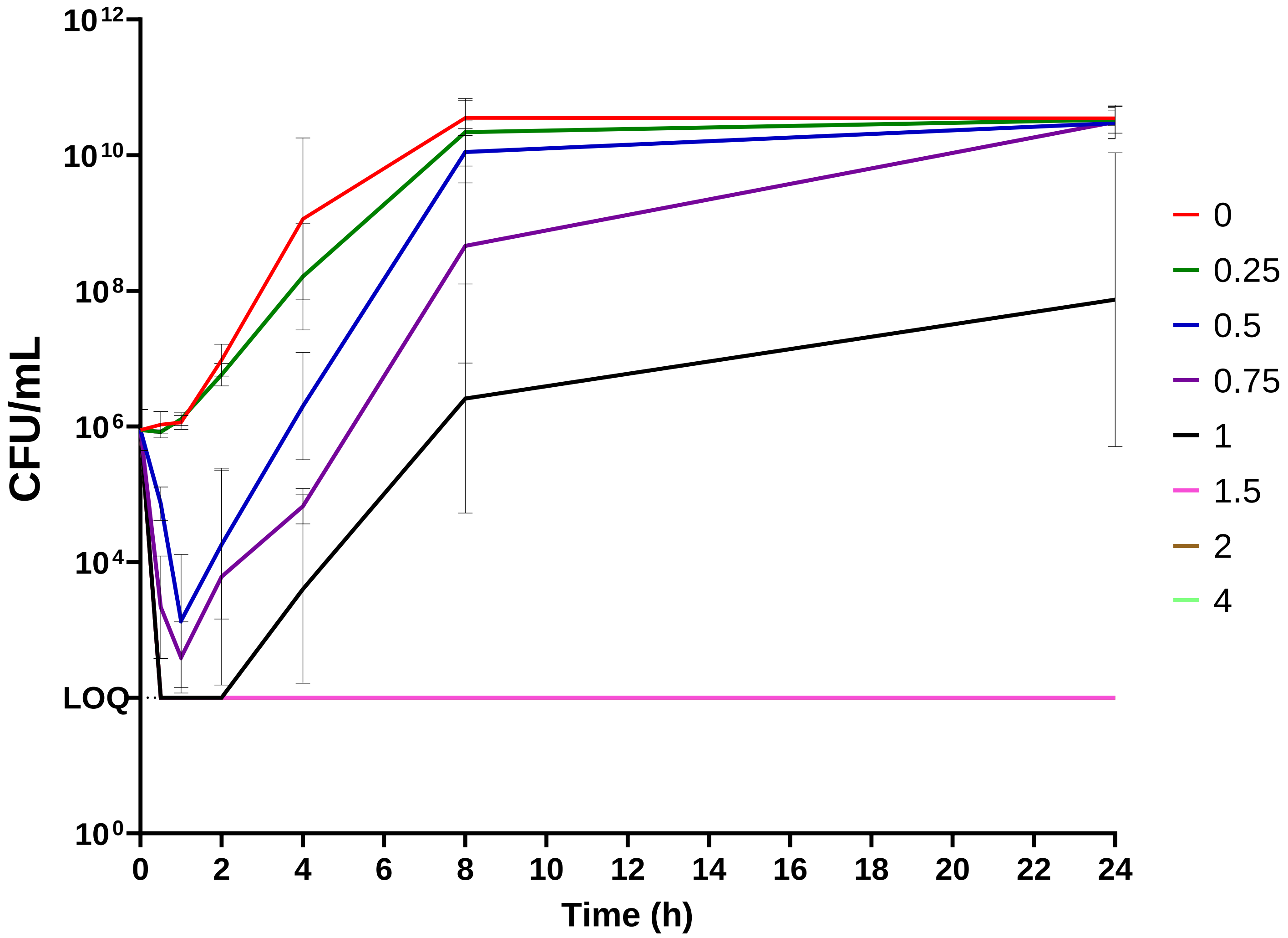
Equation 14: Estimating MIC for the highly susceptible subpopulation of isolates harbouring mcr genes (mcr-1; mcr-3).

$$MIC = EC_{50} \times \left(\frac{K_{growth} - 0.221}{E_{max} \times F_{2MCR} - (K_{growth} - 0.221)}\right)^{\frac{1}{\gamma MCR}}$$

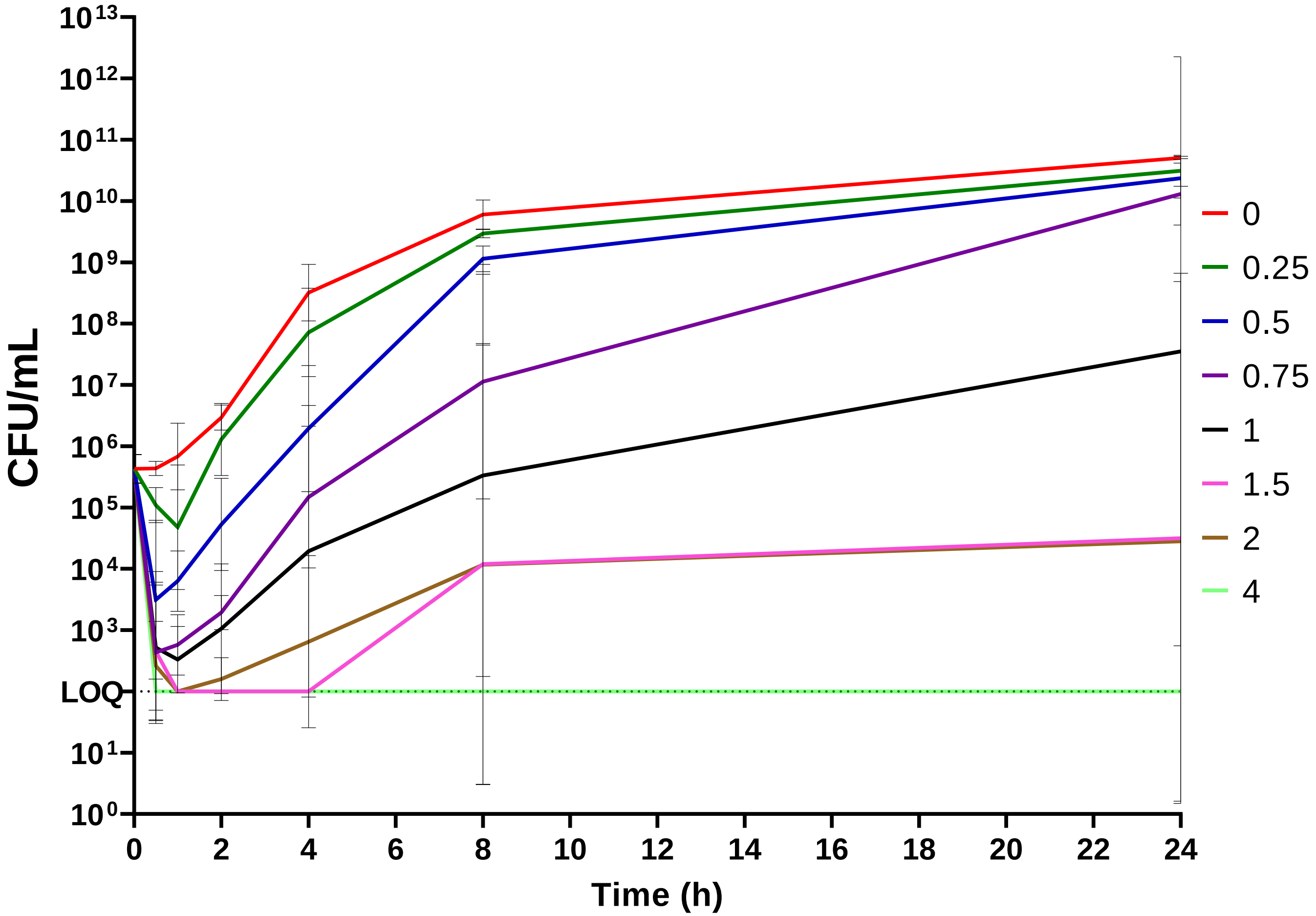
The potentiation factor for the highly susceptible population of the non-*mcr* isolates represented by F_{2S}, and the Hill coefficient γ_S; and the respective parameters for the highly susceptible population of *mcr*-harbouring isolates as F_{2MCR} and γMCR.



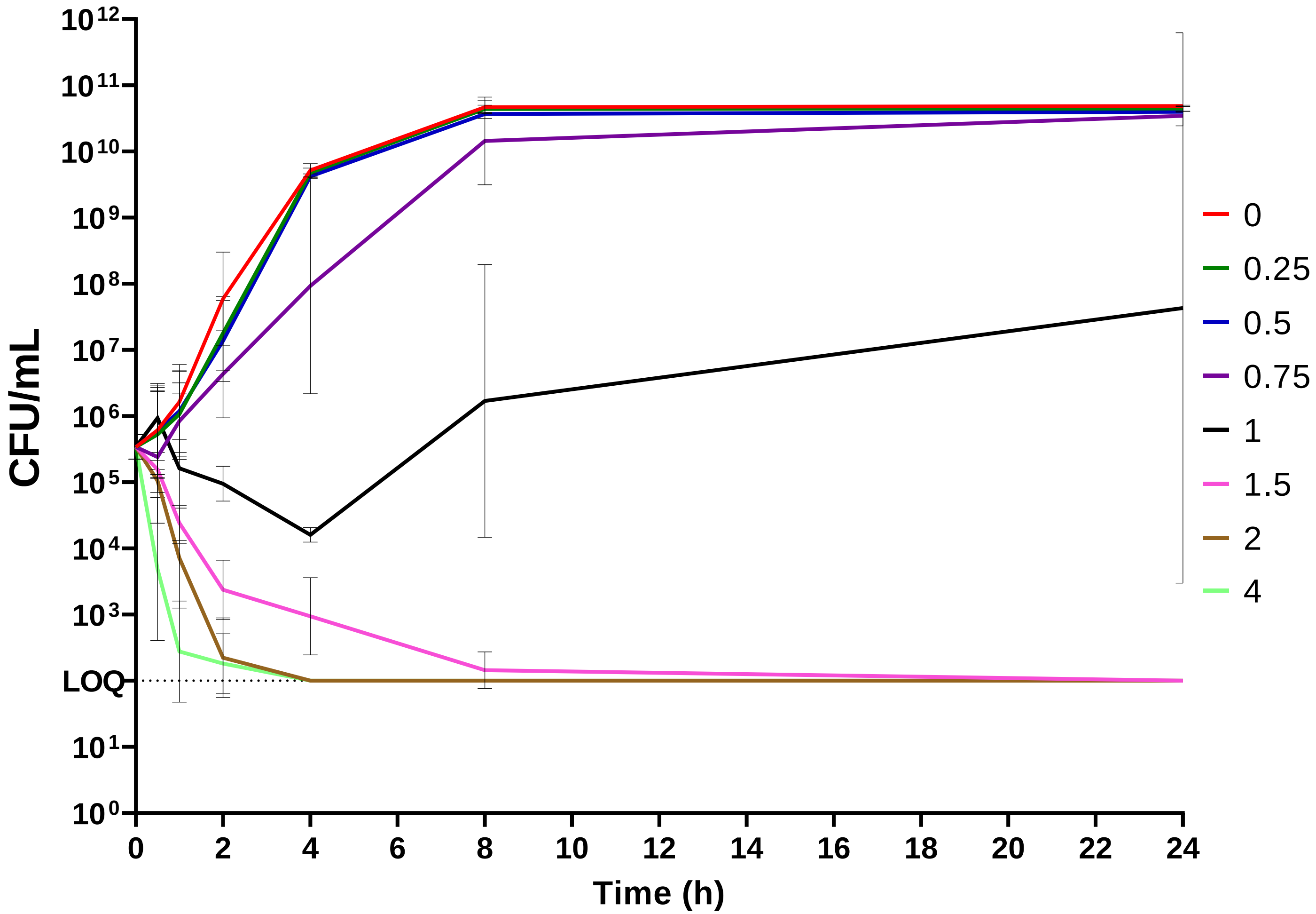
Appendix 3: Time-kill curve assay for E. coli 12241 (MIC = 0.25 mg/L); geometric mean of replicates (with SD) at an initial target inoculum of 5 x 105 CFU/mL at multiples (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4) of MIC. LOQ = 100 CFU/mL.



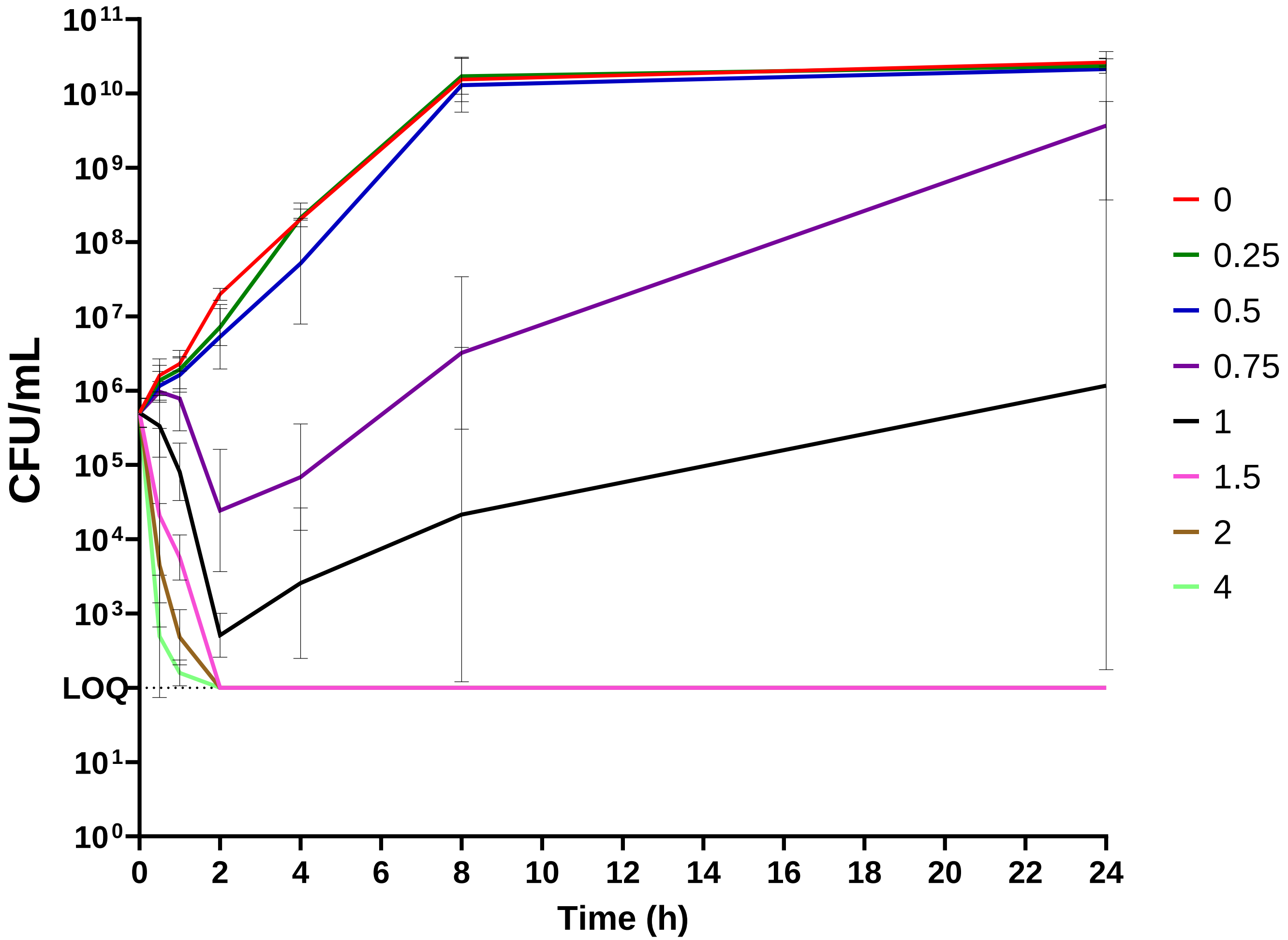
Appendix 4: Time-kill curve assay for E. coli N100 (MIC = 0.125 mg/L); geometric mean of replicates (with SD) at an initial target inoculum of 5 x 10^5 CFU/mL at multiples (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4) of MIC. LOQ = 100 CFU/mL.



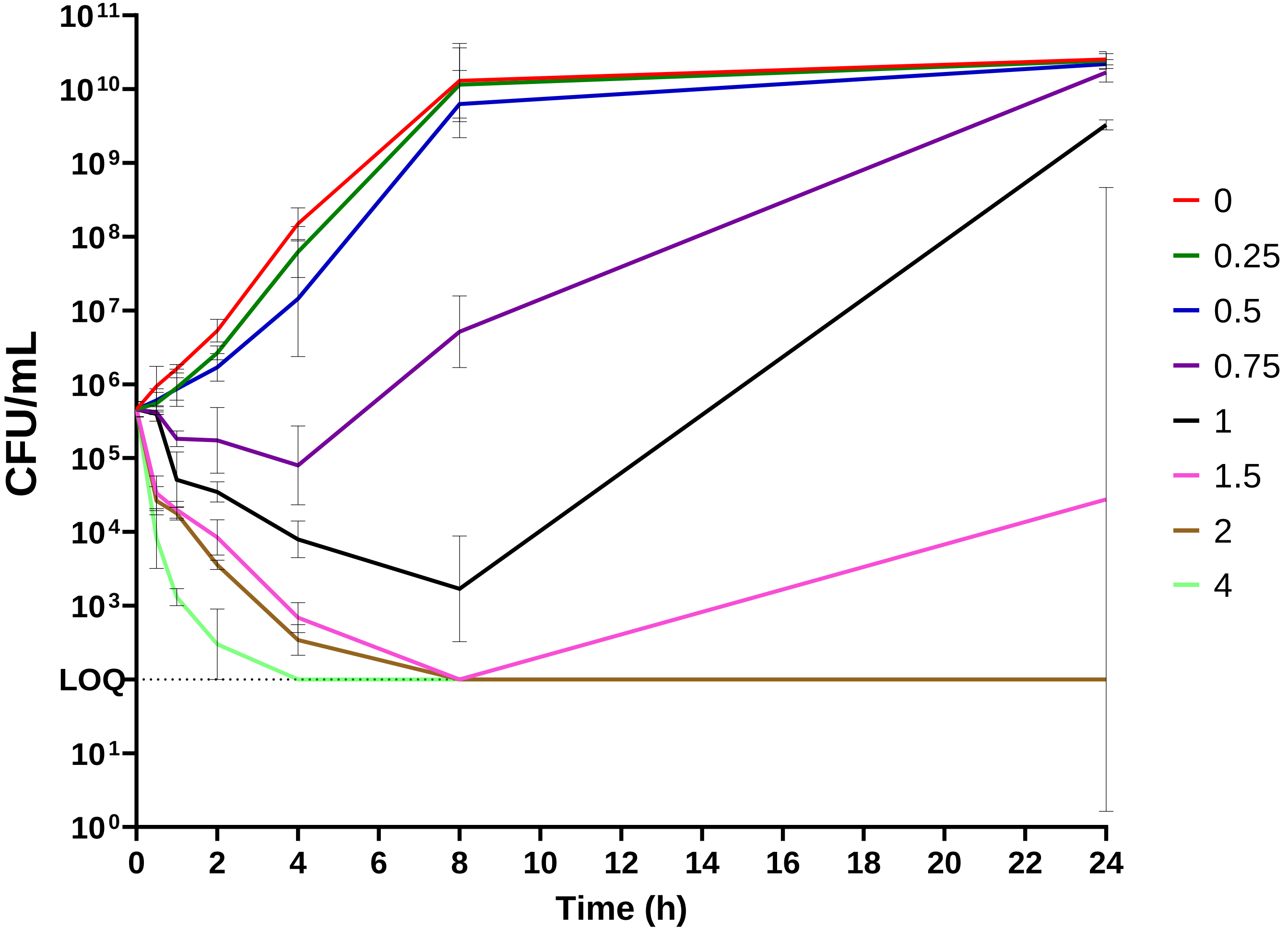
Appendix 5: Time-kill curve assay for E. coli 219 (MIC = 0.125 mg/L); geometric mean of replicates (with SD) at an initial target inoculum of 5 x 10^5 CFU/mL at multiples (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4) of MIC. LOQ = 100 CFU/mL.



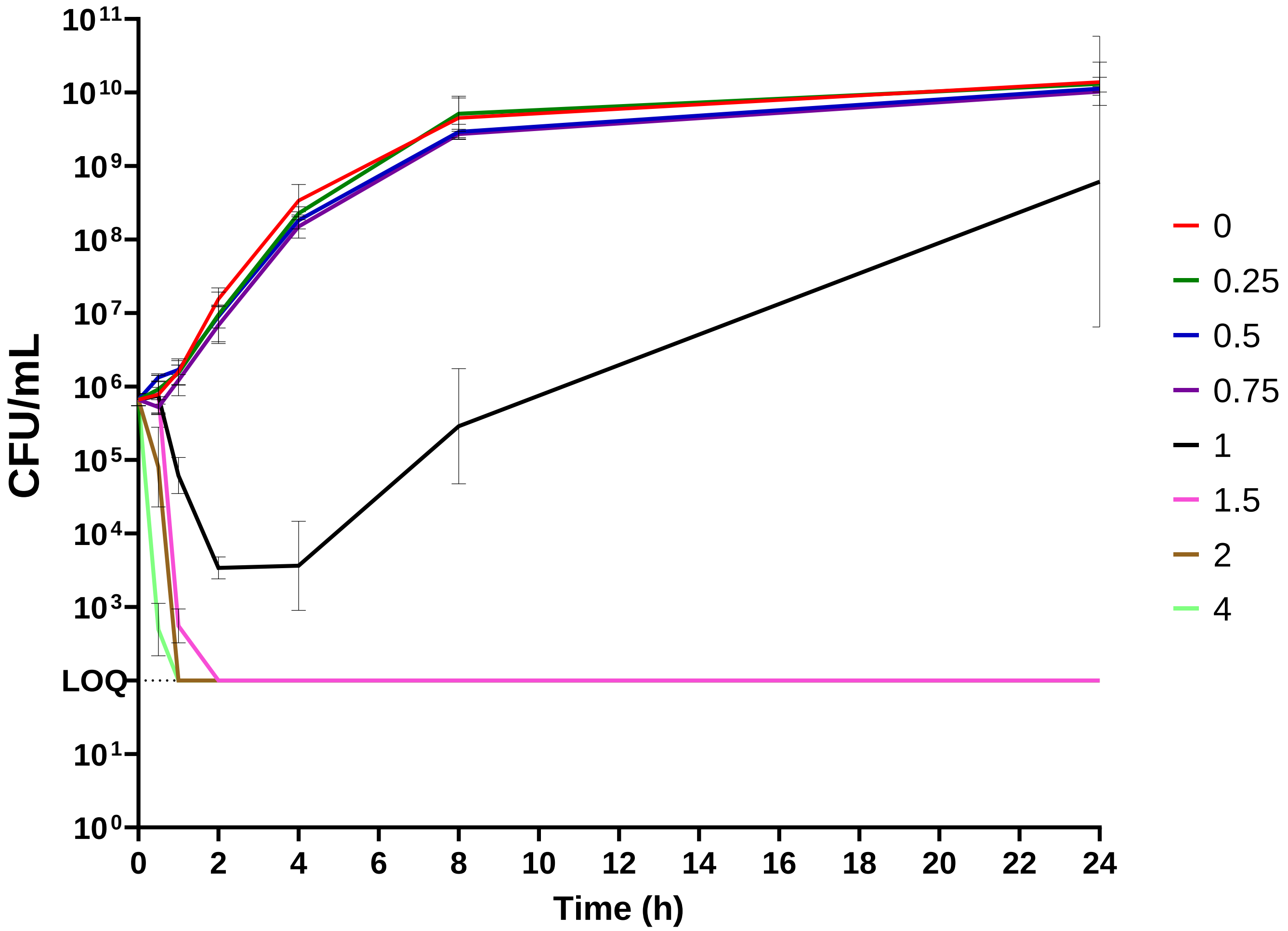
Appendix 6: Time-kill curve assay for E. coli 13846 (MIC = 2 mg/L); geometric mean of replicates (with SD) at an initial target inoculum of 5 x 10^5 CFU/mL at multiples (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4) of MIC. LOQ = 100 CFU/mL.



Appendix 7: Time-kill curve assay for E. coli 73h_B7_2 (MIC = 3.2 mg/L); geometric mean of replicates (with SD) at an initial target inoculum of 5 x 10^5 CFU/mL at multiples (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4) of MIC. LOQ = 100 CFU/mL.



Appendix 8: Time-kill curve assay for E. coli 120h_B3_5 (MIC = 2.4 mg/L); geometric mean of replicates (with SD) at an initial target inoculum of 5 x 10^5 CFU/mL at multiples (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4) of MIC. LOQ = 100 CFU/mL.



Appendix 9: Time-kill curve assay for E. coli 2013-SQ352 (MIC = 2.4 mg/L); geometric mean of replicates (with SD) at an initial target inoculum of 5 x 10^5 CFU/mL at multiples (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4) of MIC. LOQ = 100 CFU/mL.

Supplementary 1 – Time-kill curve replicates

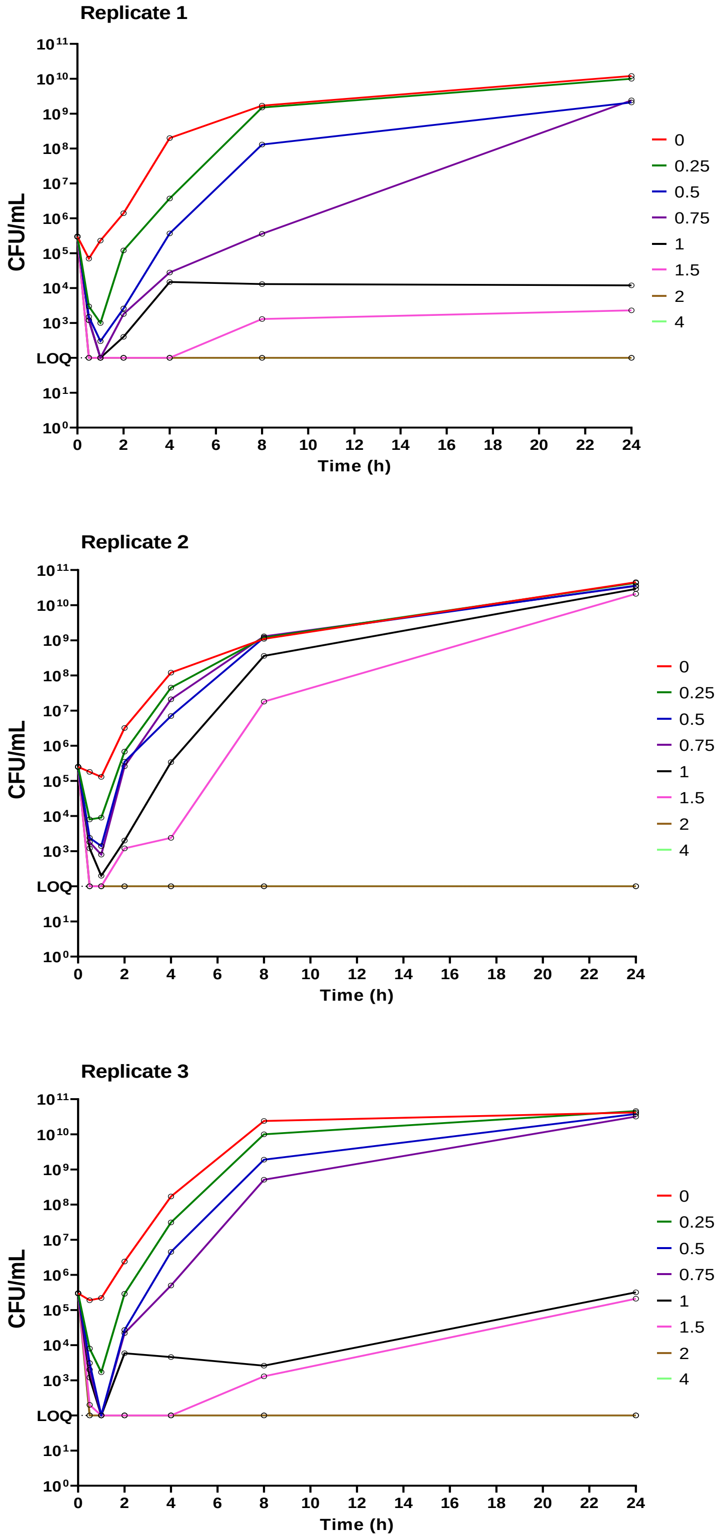


Figure S 1: Time-kill curve assay for E. coli 12241 individual replicates at an initial target inoculum of 5 x 10^5 CFU/mL.

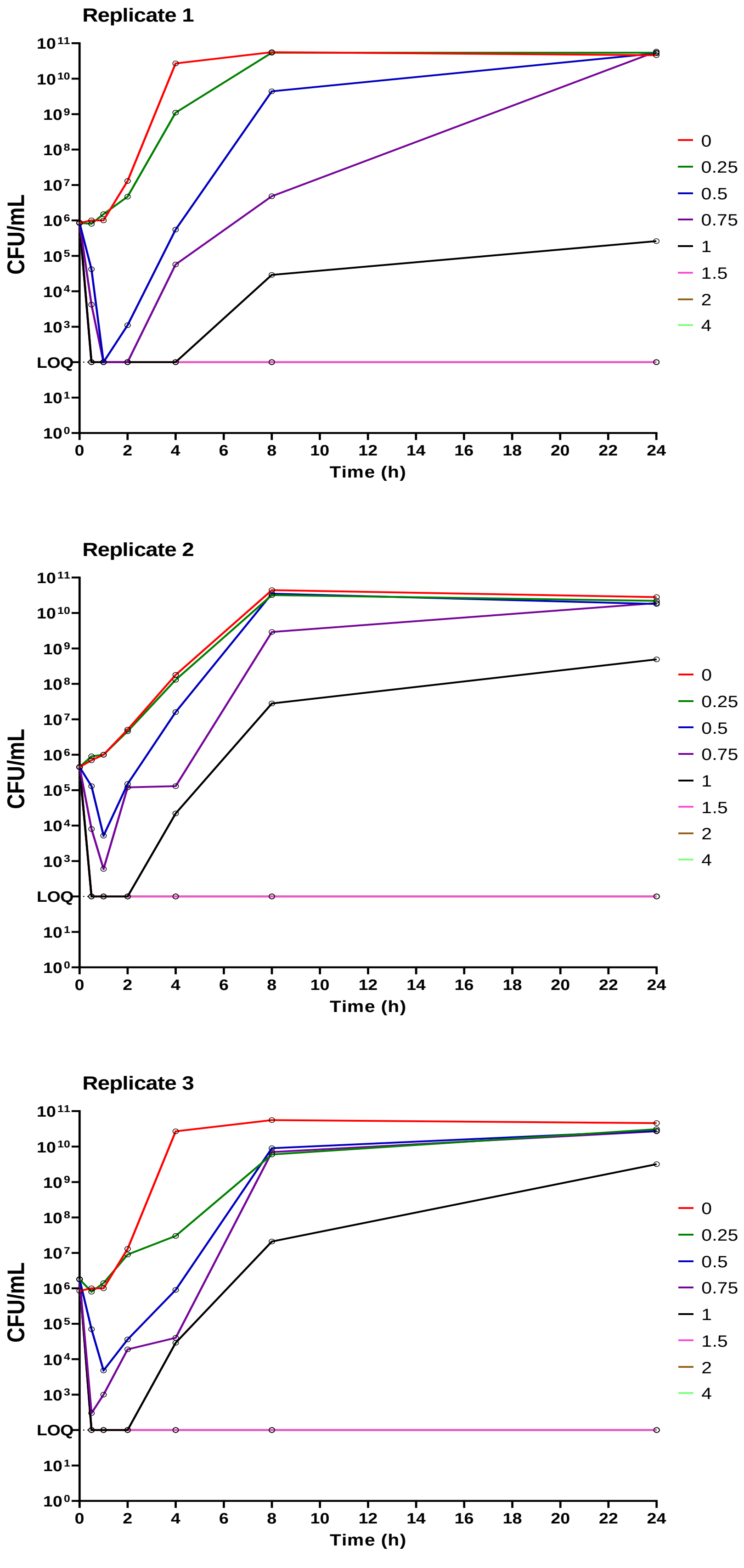


Figure S 2: Time-kill curve assay for E. coli N100 individual replicates at an initial target inoculum of 5 x 10^5 CFU/mL.

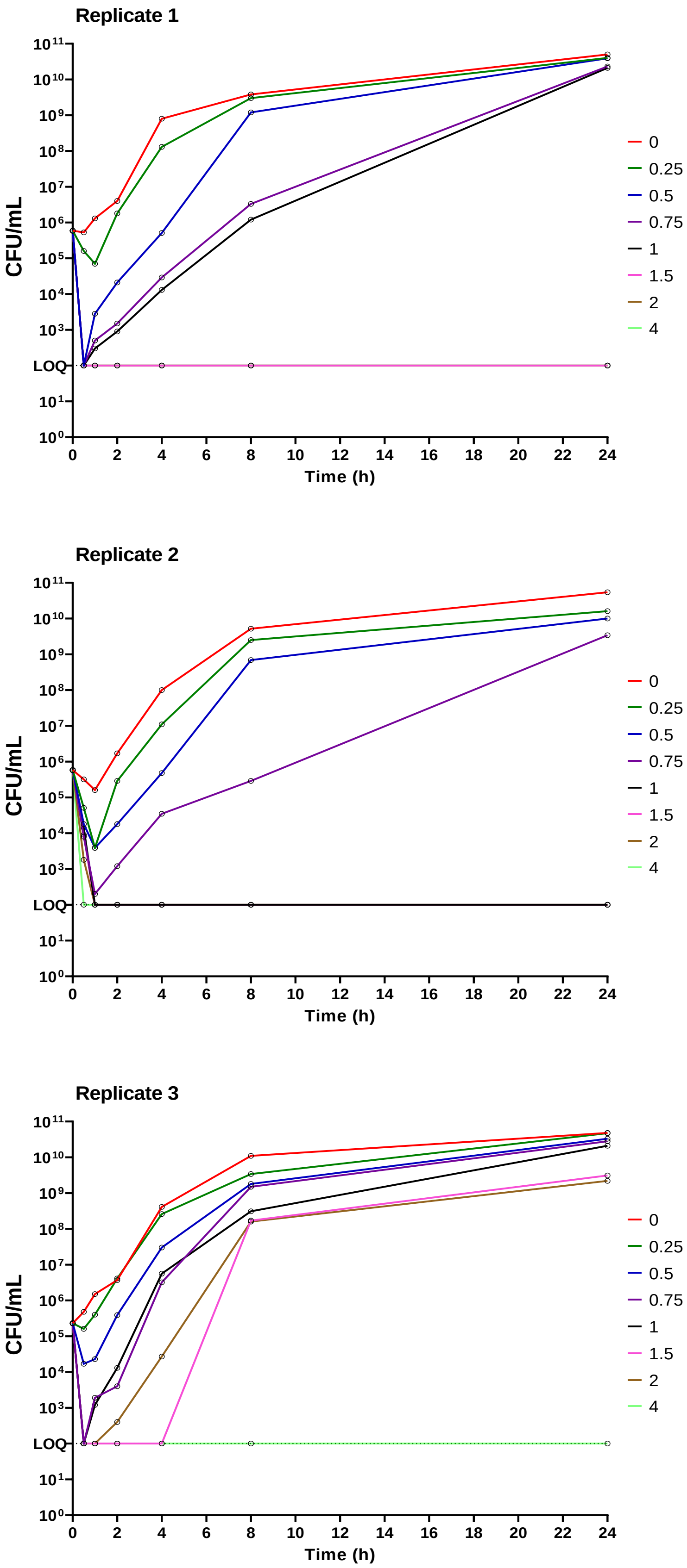


Figure S 3: Time-kill curve assay for E. coli 219 individual replicates at an initial target inoculum of 5 x 10^5 CFU/mL.

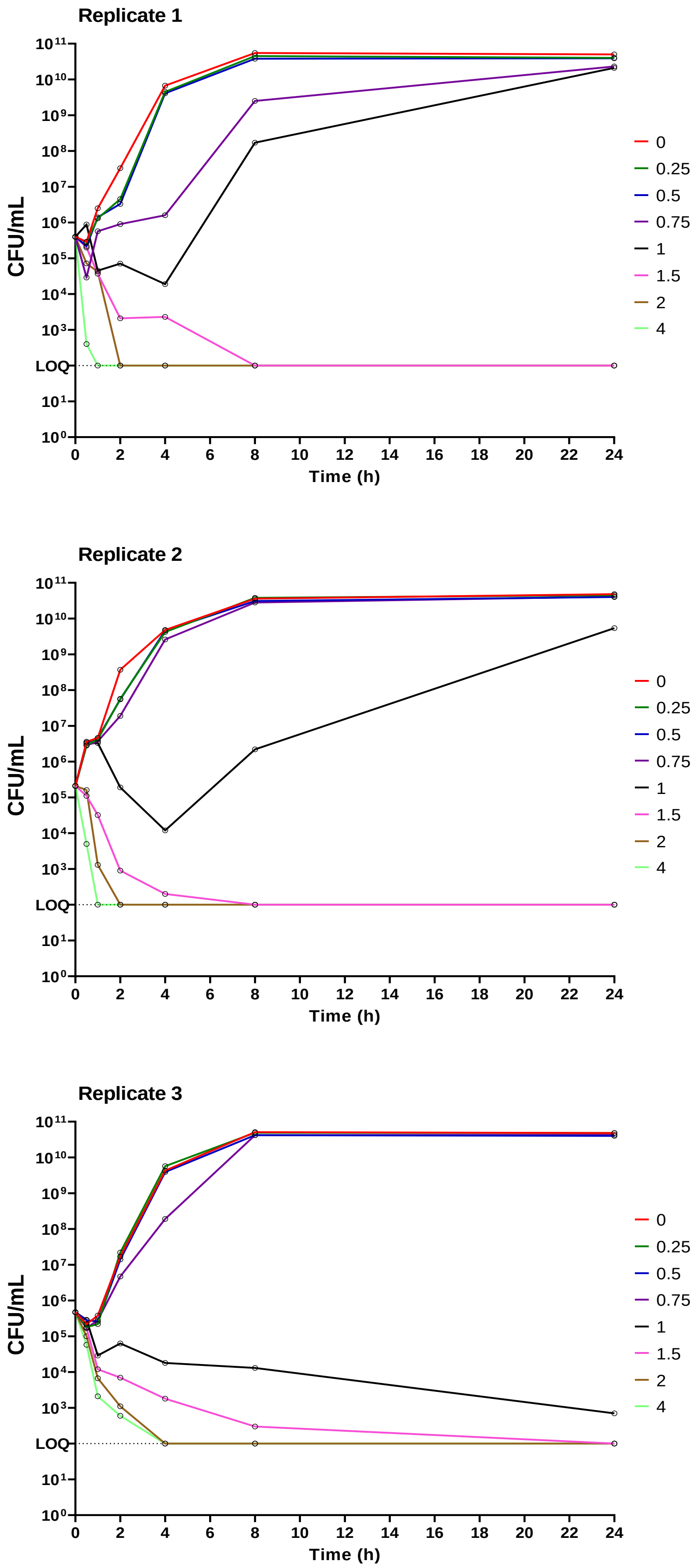


Figure S 4: Time-kill curve assay for E. coli 13846 individual replicates at an initial target inoculum of 5 x 10^{5} CFU/mL.

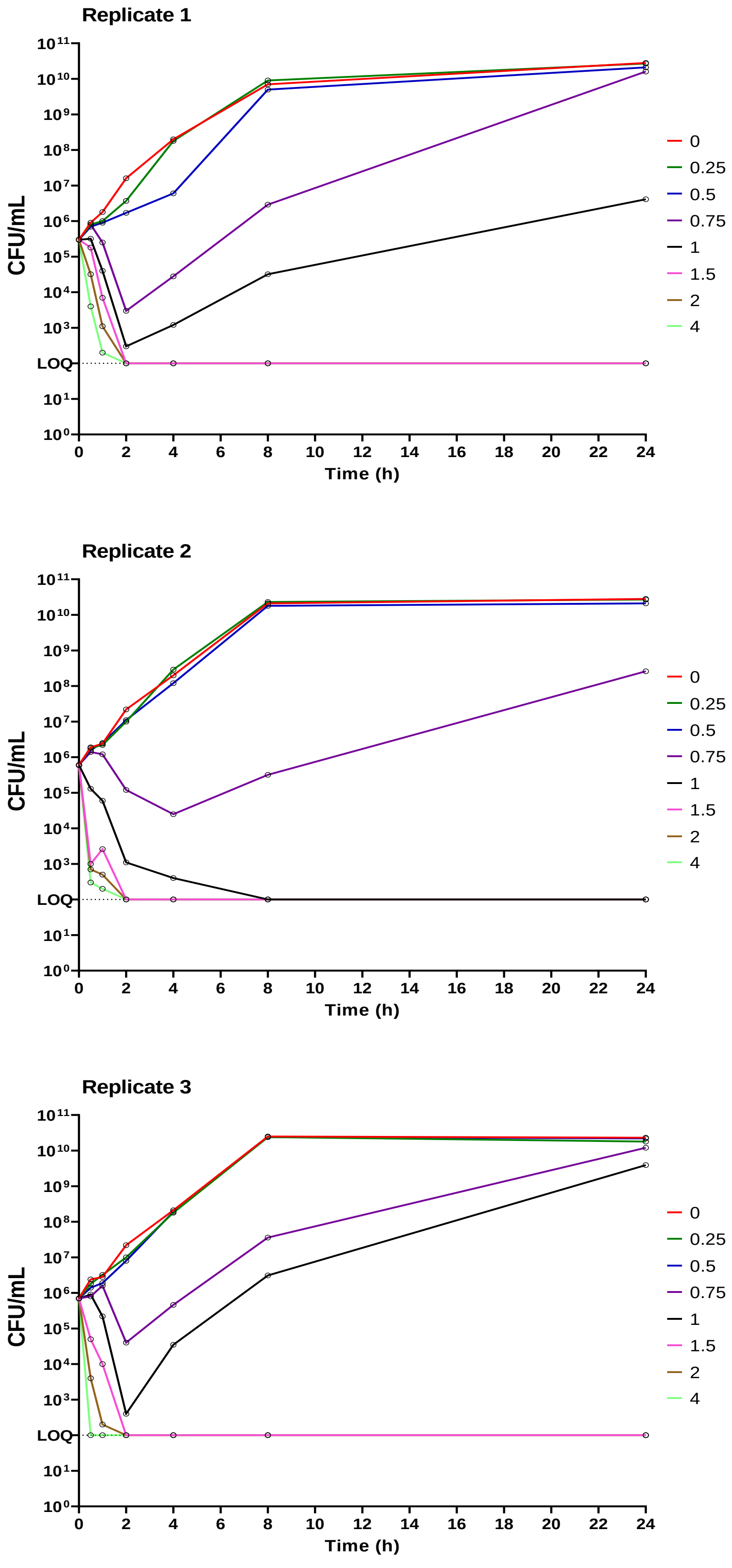


Figure S 5: Time-kill curve assay for E. coli 73h_B6_2 individual replicates at an initial target inoculum of 5 x 10^5 CFU/mL.

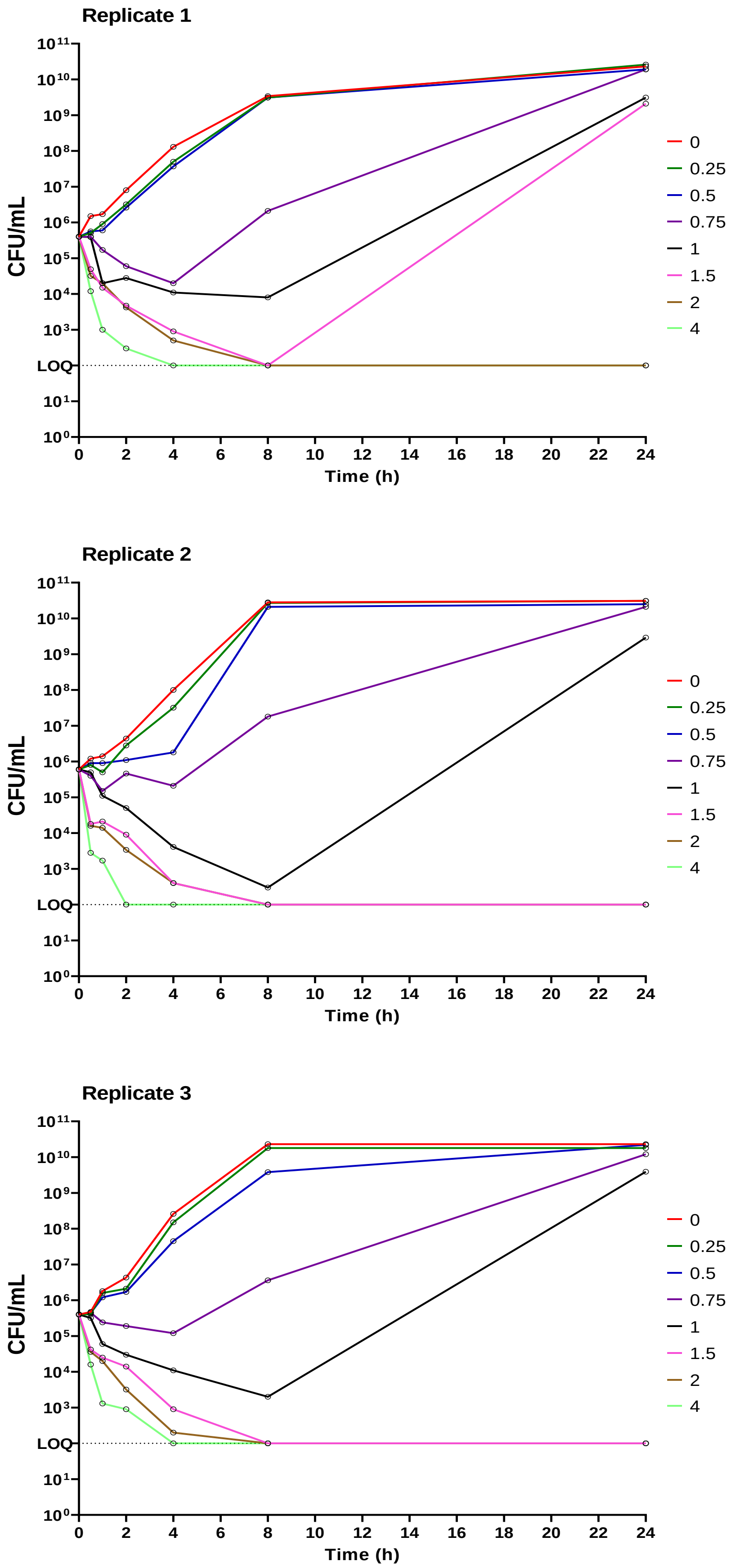


Figure S 6: Time-kill curve assay for E. coli 120h_B3_5 individual replicates at an initial target inoculum of 5 x 10^{5} CFU/mL.

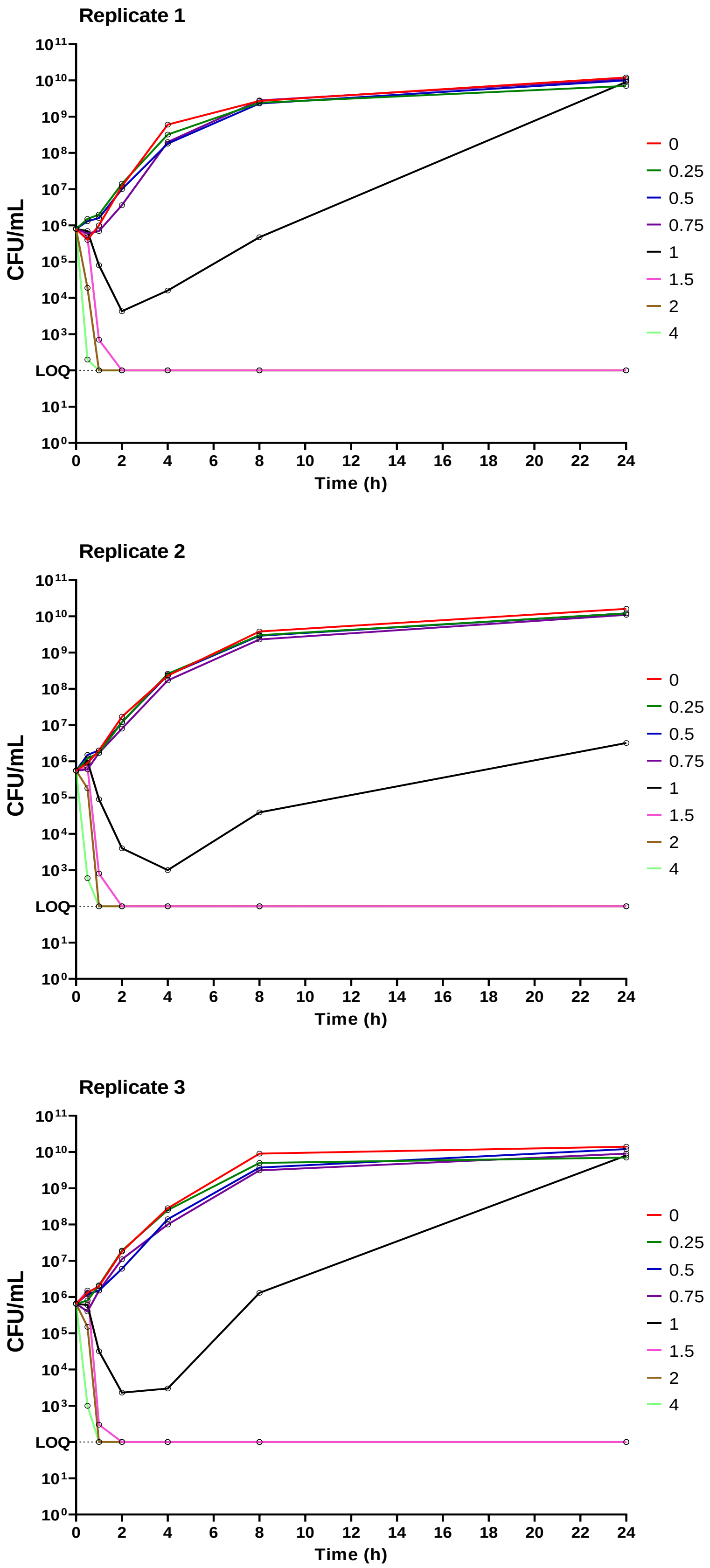


Figure S 7: Time-kill curve assay for E. coli 2013-SQ352 individual replicates at an initial target inoculum of 5 x 10^{5} CFU/mL.

Supplementary 2 - Model Code

Scenario: 0, 2, 3, 4, 5, 6, 7

```
test(){

# This is the basic model with constant AMD concentration (or with a possible degradation in the test tube over the duration of the assay; Kel). This model does not predict the development of resistance except for the adaptive resistance i.e. of non-susceptible, non-growing bacteria; P.

	# covariate "Dose_in_MIC" not in the model but required to stratify results (e.g. VPC)

	covariate(Dose_in_MIC())

	covariate(Replicate())

	# covariate "MCR" for the different parameters to distinguish resistant and non-resistant strains (i.e. mcr-negative and mcr-1/3-positive)

	covariate(MCR())

# This is the block giving the disposition model of the AMD (mono-exponential) describing the possible degradation of AMD in the test tube according to a first-order process (kel; per hour). Kel has been fixed to 0 meaning that we assumed no degradation of colistin.

	deriv(A1_A = - (A1_A *Kel))

# This is to declare that dosing (initial tested concentration) of the AMD is in compartment A1 as a bolus (initial condition).

	dosepoint(A1_A, idosevar = A1Dose)

		C1_A = A1_A #test tube concentrations of the AMD is C1 thus C1_A for dose A.

# This is the sub-model to describe the progressive increase of Kgrowth from time 0, up to a maximal value (Kgrowthmax), the rate of increase being controlled by alpha. Here the equation has a closed form and Time is declared as a covariate.

	# here alpha is different for S1 and S2 to assess a possible fitness cost for S2, i.e. alphaS2<alphaS1

	t

	KgrowthS1=Kgrowthmax*(1-exp(-alphaS1*t))

	KgrowthS2=Kgrowthmax*(1-exp(-alphaS2*t))
```

```
# This is the equation block to describe the test system with growing drug-sensitive bacteria;

      # "Drug" is the killing rate constant associated to the AMD concentration;

      #  kdeath is the natural death rate for S (sensitive pool) and P (non-susceptible, non-growing bacteria);

      # Kgrowth is the time dependent growth rate of S;

      # Bmax is the maximum possible size of the culture (S+P).

      # Ksr is the irreversible rate constant of transfer between S and P. Ksr is parametrized in term of Bmax, Kgrowth and Kdeath following Nielsen & Friberg (2013).


# The initial inoculum has two fractions: F (susceptible for concentration lower than the future MIC and (1-F), a smaller subpopulation that ultimately will control the MIC because of the its regrowth. With F1, the dominant subpopulation between 0 and 1 (to estimate) having an actual MIC lower than MIC as currently estimated by the 24h method i.e. a killing rate of F2*Kdrug i.e. a higher Killing rate than the one of the future dominant S population.

# Sequence statements declare initial apportioned values for pool i.e. S = S1 + S2

      covariate(SLOAD)

      # to apportion initial load between dominant and heteroresistant bacteria

      Sequence {S1= SLOAD * F1

            S2= SLOAD * (1-F1)}


# Sub model S1, dominant with lower MIC due to F2 potentiation; DrugA is the killing rate for S1 with F2 as potentiation factor

      deriv(S1 = KgrowthS1*S1 -(Kdeath+DRUG_A)* S1 - KsrS1*S1)


# Sub model S2, rival strain then becoming dominant, controlling the final MIC due to effect of colistin; drug B is the killing rate of S2

      deriv(S2 = KgrowthS2*S2 -(Kdeath+DRUG_B)* S2 -  KsrS2*S2)


      KsrS1=(((KgrowthS1-Kdeath)/Bmax)*(S1+P))

      KsrS2=(((KgrowthS2-Kdeath)/Bmax)*(S2+P))


      deriv(P = KsrS1*S1 + KsrS2*S2 - Kdeath*P)
```

The next block is to declare what is observed with the residual error model

The selected error model is a Log-additive model; this option corresponds to a form such as C*exp(epsilon). When the Log-additive error model is specified, and if there is only one error model as here, such as one observed statement, then the predictions and observations are log-transformed and are fit in that space by Phoenix. This is because the error model becomes additive in log-space, which allows for higher performance and accuracy. This affects all the plot results and residuals, because they are now in log-space.

Observe is what is observed during the experiment and should be mapped to the Main of the setup table

bql indicates that some data can be censored i.e. lower than the level of quantification (and the LL is computed with the M3 method by Phoenix); Laplacian method should be used as the engine for fitting when bql and a vector should document censored and non-censored data. Code is 0 for non-censored and 1 for censored data.

```
C = P+S1+S2

observe(Cobs = C *exp(CEps), bql=100)

error(CEps = 2.24096926524785)
```

This is the PD model: Emax is equivalent to Kdeath (units per h) and Drug is the concentration dependent killing rate associated with the AMD.

Emax determines the maximum increased killing rate of the bacteria in the susceptible stage (S); gamma is the Hill coefficient.

#DrugA is the killing rate for S1 with F2 as potentiation factor; F2 stimulates Emax

```
DRUG_A = Emax*F2*C1_A^gammaS1/((EC50S1)^gammaS1 +C1_A^gammaS1)
```

#DrugB is the killing rate for S2 with no potentiation factor

```
DRUG_B = Emax*C1_A^gammaS2/(EC50S2^gammaS2 +C1_A^gammaS2)
```

This is to declare the parameters of the model

#degradation rate of the antimicrobial drug

```
stparm(Kel = tvKel)
```

```
	# EC50 is the concentration for Emax/2 and measures AMD potency. There is one estimate per bacteria in this model and no distribution is assumed. Default is Bacteria 0, which is 219, MIC = 0.125. There are n-1 parameters (for each additional bacteria) estimated on top of EC50. This model is independent from MIC.

covariate(ID_covariate())


	# Here a single equation for EC50 with a covariate to distinguish the different strains (hence a single epsilon).

	#tvEC50 is for strain 219 that is ID==2

	stparm(EC50S1 = tvEC50S1

		+dEC50d12241S1 *(ID_covariate==3) /*for 12241, MIC 0.25*/

		+dEC50d13846S1 *(ID_covariate==4) /*for 13846, MIC 2*/

		+dEC50d120S1   *(ID_covariate==5) /*for 120h_B3_5, MIC 2.4*/

		+dEC50d2013S1  *(ID_covariate==6) /*for 2013-SQ352, MIC 2.4*/

		+dEC50d73S1    *(ID_covariate==7) /*for 73h_B6_2, MIC 3.2*/

		+dEC50d100S1   *(ID_covariate==8) /*for N100, MIC 0.125*/


	stparm(EC50S2 = tvEC50S2

		+dEC50d12241S2 *(ID_covariate==3) /* for 12241, MIC 0.25*/

		+dEC50d13846S2 *(ID_covariate==4) /* for 13846, MIC 2*/

		+dEC50d120S2   *(ID_covariate==5) /* for 120h_B3_5, MIC 2.4*/

		+dEC50d2013S2  *(ID_covariate==6) /* for 2013-SQ352, MIC 2.4*/

		+dEC50d73S2    *(ID_covariate==7) /* for 73h_B6_2, MIC 3.2*/

		+dEC50d100S2   *(ID_covariate==8) /* for N100, MIC 0.125*/



# covariate 0 is ID with 7 level (2 to 8 because ID coded from 2 to 8); EC50 for S1 subpopulation

	fixef(dEC50d12241S1(enable=c(0)) = c(, 0.0677999170204141, ))

	fixef(dEC50d13846S1(enable=c(0)) = c(, 2.85238465851354, ))

	fixef(dEC50d120S1  (enable=c(0)) = c(, 2.67731654267219, ))

	fixef(dEC50d2013S1 (enable=c(0)) = c(, 3.28762592161257, ))

	fixef(dEC50d73S1   (enable=c(0)) = c(, 3.5864899639135, ))

	fixef(dEC50d100S1  (enable=c(0)) = c(, 0.00116031789672895, ))
```

```
#covariate 0 is ID with 7 level (2 to 8 because ID coded from 2 to 8); EC50 for S2 subpopulation

      fixef(dEC50d12241S2(enable=c(0)) = c(, 0.173448258851468, ))

      fixef(dEC50d13846S2(enable=c(0)) = c(, 1.34815999329897, ))

      fixef(dEC50d120S2  (enable=c(0)) = c(, 2.05974007855565, ))

      fixef(dEC50d2013S2 (enable=c(0)) = c(, 1.9495106170011, ))

      fixef(dEC50d73S2   (enable=c(0)) = c(, 2.42532633594931, ))

      fixef(dEC50d100S2  (enable=c(0)) = c(, 0.000913300755223222, ))


#Emax is the maximal possible killing rate of the AMD and measure AMD efficacy

      stparm(Emax = tvEmax * exp(dEmaxdMCR1*(MCR==1)))

            fixef(dEmaxdMCR1(enable = c(1)) = c(, 0, ))


#Gamma, the Hill coefficient is the slope of the concentration-effect relationship

      stparm(gammaS1 = tvgammaS1 *exp(dgammadMCR1*(MCR==1)))

      stparm(gammaS2 = tvgammaS2 *exp(dgammadMCR1*(MCR==1)))

            fixef(dgammadMCR1(enable = c(2)) = c(, -0.234665772599484, ))


#Kgrowth is the growth rate (often about 1.2 per h)

      stparm(Kgrowthmax = tvKgrowthmax *exp(dKgrowthmaxdMCR1*(MCR==1)))

          fixef(dKgrowthmaxdMCR1(enable = c(3)) = c(,-0.0322820143504382, ))


#alpha is the rate of Kgrowth change i.e. lag phase

      stparm(alphaS1 = tvalphaS1 *exp(dalphadMCR1*(MCR==1)))

      stparm(alphaS2 = tvalphaS2 *exp(dalphadMCR1*(MCR==1)))

            fixef(dalphadMCR1(enable = c(4)) = c(, 0.594368703437492, ))


#Kdeath is the natural death rate for S and P (about 0.2 perh)

      stparm(Kdeath = tvKdeath)


#Bmax is the maximal possible size of the inoculum (S+P)

      stparm(Bmax = tvBmax *exp(nBmax)*exp(dBmaxdMCR1*(MCR==1)))

            fixef(dBmaxdMCR1(enable = c(5)) = c(, 0.208642819251874, ))
```

```
# fraction of dominant bacteria (most susceptible)

      stparm(F1 = ilogit(tvF1 + dF1dMCR1*(MCR==1)))

            fixef(dF1dMCR1(enable = c(6)) = c(, -0.301996146026096, ))


# higher killing effect on the dominant but more susceptible population

      stparm(F2 = tvF2 *exp(dF2dMCR1*(MCR==1)))

            fixef(dF2dMCR1(enable = c(7)) = c(, -1.21426692135279, ))


#The next block gives the initial values (without bounds) of the different fixed effect parameters:

      # Kel was fixed to 0 (freeze) because  it was  assumed that the AMD concentration is unchanged during the assay; but by editing the rate constant to another value, a degradation process can be introduced (e.g. replacing  0 by 0.05 as obtained in a satellite experiment); Kel  can also be introduced in the model as a parameter to be evaluated (for this you have simply to delete freeze); this can dramatically improve the fitting but be aware that Kel is not identifiable without actual measurement of AMD concentration in the “test tube”.

      fixef(tvKel(freeze)= c(, 0, ))


# These are the initial values for the EC50, gamma and Emax without lower or upper bounds

      fixef(tvEC50S1 = c(0, 0.0821283945492478, ))

      fixef(tvEC50S2 = c(0, 0.113846498972753, ))

      fixef(tvgammaS1 = c(0, 4.09361484849837, ))

      fixef(tvgammaS2 = c(0, 3.21725462676088, ))

      fixef(tvEmax = c(0, 2.6948164217004, ))


# potentiation coefficient of DRUG effect

      fixef(tvF2=c(, 12.6622263675955,))


#this is initial value for kgrowthmax and alpha

      fixef(tvKgrowthmax = c(0, 2.44126321088492, ))

      fixef(tvalphaS1 = c(, 0.882435537952875,))

      fixef(tvalphaS2 = c(, 3.73523125222903,))


# fraction of more susceptible strains, dominant approx. ≥90%
```

```
      fixef(tvF1=c(0, 7.69423025380142,))

#Kdeath is often fixed to some default value facilitating identifiability of
other parameters but here Kdeath is retained as a variable to evaluate

      fixef(tvKdeath (freeze)= c(0, 0.179, ))

      fixef(tvBmax = c(0, 14344240310.8269, ))


# Secondary parameters: model calculation of secondary parameters including
the calculated EC50 for each strain (with consideration of starting EC50
isoalte 219) and subpopulation (S1/S2), fraction F1 and the number of
bacteria in initial starting populations (based on average starting
population of 5.9 x 10^5),

      secondary(Ec50219S1     =tvEC50S1)

      secondary(EC50d12241S1  =tvEC50S1 + dEC50d12241S1)

      secondary(EC50d100S1    =tvEC50S1 + dEC50d100S1)

      secondary(EC50d13846S1  =tvEC50S1 + dEC50d13846S1)

      secondary(EC50d120S1    =tvEC50S1 + dEC50d120S1)

      secondary(EC50d73S1     =tvEC50S1 + dEC50d73S1)

      secondary(EC50d2013S1   =tvEC50S1 + dEC50d2013S1)


      secondary(Ec50219S2     =tvEC50S2)

      secondary(EC50d12241S2  =tvEC50S2 + dEC50d12241S2)

      secondary(EC50d100S2    =tvEC50S2 + dEC50d100S2)

      secondary(EC50d13846S2  =tvEC50S2 + dEC50d13846S2)

      secondary(EC50d120S2    =tvEC50S2 + dEC50d120S2)

      secondary(EC50d73S2     =tvEC50S2 + dEC50d73S2)

      secondary(EC50d2013S2   =tvEC50S2 + dEC50d2013S2)


      secondary(Emax_MCR0_S2  =tvEmax)

      secondary(Emax_MCR0_S1  =tvEmax*tvF2)

      secondary(Emax_MCR1_S1  =tvEmax*tvF2 *exp(dF2dMCR1))

      secondary(Emax_MCR1_S2  =tvEmax*exp(dEmaxdMCR1))


      secondary(F1_mcr0 = exp(tvF1) / (1+exp(tvF1)))

      secondary(Initial_fraction_HR1_mcr0 =1- F1_mcr0)

      secondary(Initial_count_HR_mcr0=590000*Initial_fraction_HR1_mcr0 )
```

```
secondary(ilogitF1_mcr1 = tvF1+dF1dMCR1)

secondary(F1_mcr1=exp(ilogitF1_mcr1)/(1+exp(ilogitF1_mcr1)))

secondary(Initial_fraction_HR1_mcr1 =1-F1_mcr1)

secondary(Initial_count_HR_mcr1=590000*Initial_fraction_HR1_mcr1 )



secondary(Killing_rateS1_mcr0=tvEmax*tvF2)

secondary(Time_min_LOQ_mcr0=-ln(100/590000)/(Killing_rateS1_mcr0/60))


secondary(F2_mcr1=tvF2*exp(dF2dMCR1))

secondary(Killing_rateS1_mcr1=tvEmax*F2_mcr1)

secondary(Time_min_LOQ_mcr1=-ln(100/590000)/(Killing_rateS1_mcr1/60))



#this is the equation to compute the MIC as a secondary parameter (initial load of 500,000, final 10^8), but for a reading at 24h, not 18h, the term 0.29 is replaced by 0.221

Kgrowthnet=tvKgrowthmax-tvKdeath


#MIC S1 mcr-negative (mcr0)

secondary(MIC_219_S1_mcr0=tvEC50S1*((Kgrowthnet-0.221)/(tvEmax*tvF2-(Kgrowthnet-0.021)))^(1/tvgammaS1))

secondary(MIC_12241_S1_mcr0=EC50d12241S1*((Kgrowthnet-0.221)/(tvEmax*tvF2-(Kgrowthnet-0.221)))^(1/tvgammaS1))

secondary(MIC_100_S1_mcr0=EC50d100S1*((Kgrowthnet-0.221)/(tvEmax*tvF2-(Kgrowthnet-0.221)))^(1/tvgammaS1))


#MIC S2 mcr-negative (mcr0)

secondary(MIC_219_S2_mcr0=tvEC50S2*((Kgrowthnet-0.221)/(tvEmax-(Kgrowthnet-0.021)))^(1/tvgammaS2))

secondary(MIC_12241_S2_mcr0=                EC50d12241S2*((Kgrowthnet-0.221)/(tvEmax-(Kgrowthnet-0.221)))^(1/tvgammaS2))

secondary(MIC_100_S2_mcr0=EC50d100S2*((Kgrowthnet-0.221)/(tvEmax-(Kgrowthnet-0.221)))^(1/tvgammaS2))
```

```
#MIC S1 resistant mcr-positive (mcr1 or mcr3)

      secondary(MIC_13846_S1_mcr1=EC50d13846S1*((Kgrowthnet-0.221)/(tvEmax*F2_mcr1-(Kgrowthnet-0.221)))^(1/tvgammaS1))

      secondary(MIC_120_S1_mcr1=EC50d120S1*((Kgrowthnet-0.221)/(tvEmax*F2_mcr1-(Kgrowthnet-0.221)))^(1/tvgammaS1))

      secondary(MIC_73_S1_mcr1=EC50d73S1*((Kgrowthnet-0.221)/(tvEmax*F2_mcr1-(Kgrowthnet-0.221)))^(1/tvgammaS1))

      secondary(MIC_2013_S1_mcr3=EC50d2013S1*((Kgrowthnet-0.221)/(tvEmax*F2_mcr1-(Kgrowthnet-0.221)))^(1/tvgammaS1))


#MIC S2 resistant mcr-positive (mcr1 or mcr3)

      secondary(MIC_13846_S2_mcr1=EC50d13846S2*((Kgrowthnet-0.221)/(tvEmax-(Kgrowthnet-0.221)))^(1/tvgammaS2))

      secondary(MIC_120_S2_mcr1=EC50d120S2*((Kgrowthnet-0.221)/(tvEmax-(Kgrowthnet-0.221)))^(1/tvgammaS2))

      secondary(MIC_73_S2_mcr1=EC50d73S2*((Kgrowthnet-0.221)/(tvEmax-(Kgrowthnet-0.221)))^(1/tvgammaS2))

      secondary(MIC_2013_S2_mcr3=EC50d2013S2*((Kgrowthnet-0.221)/(tvEmax-(Kgrowthnet-0.221)))^(1/tvgammaS2))


# MIC ratio S1/S2 for mcr-negative (mcr0)

      secondary(ratioS1S2_219=MIC_219_S1_mcr0/MIC_219_S2_mcr0)

      secondary(ratioS1S2_122419=MIC_12241_S1_mcr0/MIC_12241_S2_mcr0)

      secondary(ratioS1S2_100=MIC_100_S1_mcr0/MIC_100_S2_mcr0)


# MIC ratio S1/S2 for resistant mcr-positive (mcr1 or mcr3)

      secondary(ratioS1S2_13846=MIC_13846_S1_mcr1/MIC_13846_S2_mcr1)

      secondary(ratioS1S2_120=MIC_120_S1_mcr1/MIC_120_S2_mcr1)

      secondary(ratioS1S2_73=MIC_73_S1_mcr1/MIC_73_S2_mcr1)

      secondary(ratioS1S2_2013=MIC_2013_S1_mcr3/MIC_2013_S2_mcr3)


}
```