Modelling the synergistic effect of bacteriophage and antibiotics on bacteria: killers and drivers of resistance evolution

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ABSTRACT

Bacteriophage (phage) are bacterial predators which can also spread antimicrobial resistance (AMR) genes between bacteria by generalised transduction. Phage are often present alongside antibiotics in the environment, yet evidence of their joint killing effect on bacteria is conflicted, and the dynamics of transduction in such systems are unknown. Here, we combine *in vitro* data and mathematical modelling to identify conditions where phage and antibiotics act in synergy to remove bacteria or drive AMR evolution.

We adapt a published model of phage-bacteria dynamics, including transduction, to add the pharmacodynamics of erythromycin and tetracycline, parameterised from new *in vitro* data. We simulate a system where two strains of *Staphylococcus aureus* are present at stationary phase, each carrying either an erythromycin or tetracycline resistance gene, and where multidrug-resistant bacteria can be generated by transduction only. We determine rates of bacterial clearance and multidrug-resistant bacteria appearance, when either or both antibiotics and phage are present at varying timings and concentrations.

Although phage and antibiotics act in synergy to kill bacteria, by reducing bacterial growth antibiotics reduce phage production. A low concentration of phage introduced shortly after antibiotics fails to replicate and exert a strong killing pressure on bacteria, instead generating multidrug-resistant bacteria by transduction which are then selected for by the antibiotics. Multidrug-resistant bacteria numbers were highest when antibiotics and phage were introduced simultaneously.

The interaction between phage and antibiotics leads to a trade-off between a slower clearing rate of bacteria (if antibiotics are added before phage), and a higher risk of multidrug-resistance evolution (if phage are added before antibiotics), exacerbated by low concentrations of phage or antibiotics. Our results form hypotheses to guide future experimental and clinical work on the impact of phage on

37 AMR evolution, notably for studies of phage therapy which should investigate varying timings and

38 concentrations of phage and antibiotics.

INTRODUCTION

Bacteriophage (phage) are major bacterial predators and the most common organisms on the planet [1]. Phage are often present alongside antibiotics, naturally or in the context of antibacterial treatment [2–4], yet reports on their combined effect on bacteria are conflicting. Some previous studies showed that phage and antibiotics work synergistically to clear bacteria, while others have demonstrated that antibiotics reduce phage production [5–9]. This is further complicated by the fact that phage are also major drivers of bacterial evolution via horizontal gene transfer by transduction [10,11], which can notably contribute to antimicrobial resistance (AMR) spread [12,13]. If multidrug-resistant bacteria are generated by transduction, antibiotics present in the same environment may act as a synergistic selective pressure to increase their prevalence, yet to our knowledge the dynamics of transduction have not yet been investigated in such systems [14].

There are two types of transduction: specialised and generalised [15,16]. Specialised transduction occurs when a prophage accidentally picks up adjacent bacterial DNA upon excision from the bacterial chromosome, at the end of the lysogenic cycle. On the other hand, generalised transduction occurs during phage replication, when non-phage DNA is packaged by mistake in a new phage particle. The resulting transducing phage is released upon bacterial lysis, and injects this DNA in another bacterium. Generalised transduction is likely the most important type of transduction in the context of AMR, as it can lead to the horizontal transfer of any genetic material contained in a bacterium (including plasmids, major vectors of AMR genes) [10].

A major bacterial pathogen often exposed to phage is *Staphylococcus aureus*, which at any given time is colonising approximately 20% of humans [17]. Previous work suggests that all *S. aureus* carry integrated prophage [18], and at least 50% of individuals colonised by *S. aureus* also carry free phage capable of generalised transduction [19], the main mechanism of horizontal gene transfer for *S. aureus* [18]. This is particularly relevant for methicillin-resistant *S. aureus* (MRSA), a group of *S. aureus* present

in both the ESKAPE list and the World Health Organization priority list of antibiotic-resistant bacteria due to its large clinical burden [20–22]. *In vitro*, the generation rate of generalised transducing MRSA phage carrying an AMR gene has been estimated to be approximately one per 10^8 new phage produced, sufficient to consistently generate bacteria resistant to multiple antibiotics in less than 24h [23], and *in vivo* transduction rates are likely to be even higher [24].

Understanding the dynamics of bacteria, antibiotics, phage and transduction is especially important in the context of phage therapy, which aims to use phage as antibacterial agents, generally in combination with antibiotics [4]. Phage therapy is currently investigated as a solution to counter the threat of AMR, with ongoing clinical trials against MRSA infection [6,25–29]. Phage therapy guidelines recommend that only phage with a limited ability to perform transduction should be used [15,30,31], yet to our knowledge there is currently no technique to prevent phage from accomplishing generalised transduction, which is fundamentally a mispackaging and thus a biological error similar to a mutation. Hence, as previous reviews have highlighted, it is essential to explore the importance of this mechanism, and identify conditions under which it could affect the outcome of therapy and lead to multidrug-resistance evolution [14,30–32].

In this study, we aim to investigate the potential combined effect of antibiotics and phage capable of generalised transduction on bacteria. Making conclusions about this multidimensional chequerboard space of potential combinations and timings is difficult when using data from time-consuming, single-scenario, *in vitro* experiments. Instead, we generate hypotheses to guide future experimental work by simulating these conditions. We adapt a previously published mathematical model of phage-bacteria dynamics, including generalised transduction, to incorporate the effect of antibiotics on bacteria [23]. This model is parameterised using *in vitro* data from the same environment and set of conditions as when it was originally developed [23], making it a reliable tool to infer the dynamics governing this system.

87 We hypothesise that, depending on the timing and concentration at which they are added, phage and
88 antibiotics can either act synergistically to eradicate bacteria, or to create and select for multidrug-
89 resistant bacteria. We explore this in our model and generate guidelines to minimise the risk of
90 generating double-antibiotic-resistant *S. aureus* when two single-resistant strains are exposed to
91 antibiotics and phage, whilst maximising bacterial eradication.

MATERIALS AND METHODS

Laboratory methods

Bacterial strains and phage

Two *Staphylococcus aureus* strains were obtained from the Nebraska transposon library in the MRSA USA300 background [33]. These were NE327, with the *ermB* gene conferring resistance to erythromycin, and NE201KT7, a modified NE201 strain with a kanamycin resistance cassette replacing the *ermB* gene and a plasmid carrying the *tetK* gene conferring resistance to tetracycline. Horizontal gene transfer can only happen between these two strains via generalised transduction. When co-cultured with 80α phage, these give rise to double-resistant progeny (DRP) bacteria resistant to both erythromycin and tetracycline [23]. In the experiments conducted here, we used the double-resistant strain DRPET1, a DRP strain with a NE327 background generated during previous NE327, NE201KT7 and 80α co-cultures, containing both *ermB* and *tetK* genes [23]. Note that although 80α is a temperate phage, we have previously shown that lysogeny does not occur at a detectable level in our experiments, with a lysogenic frequency detection limit of 3.3×10^{-8} per non-lysogenic bacteria after 24h of co-culture, and that specialised transduction is unlikely to be responsible for horizontal gene transfer, due to the location of *tetK* on a plasmid and a ~1Mbp distance between *ermB* and the 80α integration site on the bacterial chromosome [23]. Hence the only interactions we consider between phage and bacteria in our environment are lysis and generalised transduction.

Time-kill curves

The growth conditions are identical to the ones previously used to generate data on lysis and transduction with these bacteria and phage [23]. Pre-cultures of each *S. aureus* bacterial strain (NE327, NE201KT7 and DRPET1) were separately generated overnight in 50mL conical tubes containing 10mL

114 of brain-heart infusion broth (BHIB, Sigma, UK). Unless otherwise stated, liquid cultures were
115 incubated in a warm shaking water bath (37°C, 90 rpm). Each pre-culture was then diluted in
116 phosphate-buffered saline (PBS) and mixed with 10mL of fresh BHIB in a 50mL conical tube to reach a
117 starting concentration of 10^4 colony-forming units (cfu)/mL. The new culture was incubated for 2h to
118 allow the bacteria to reach log-growth phase, following standard protocol for time-kill experiments
119 [34]. Erythromycin or tetracycline was then added to the culture, at a concentration of either 0
120 (control), 0.25, 0.5, 1, 2, 4, 8, 16, or 32 mg/L. At 0, 1, 2, 3, 4, 6 and 24h after antibiotic addition, 30µl
121 were sampled from the incubated culture, diluted in PBS, and plated on plain brain-heart infusion
122 agar. The plates were incubated overnight at 37°C. Colonies on the plates were then counted to derive
123 the concentration of bacteria in cfu/mL at the corresponding time point.

124 The experiment was repeated 3 times for each strain (NE327, NE201KT7 and DRPET1) and each
125 antibiotic (erythromycin and tetracycline).

126 Minimum inhibitory concentration

127 We measured the minimum inhibitory concentrations (MIC) of erythromycin and tetracycline for
128 NE327, NE201KT7 and DRPET1 by microbroth dilution [35]. Briefly, pre-cultures of each strain were
129 generated overnight in Mueller-Hinton broth (MHB-II, Sigma, UK). Antibiotic stocks were generated in
130 50% ethanol at concentrations doubling from 0.25 to 256 mg/L, and 10µL of each dilution were added
131 to separate wells on a 96-well plate. The overnight pre-cultures were diluted to a concentration of 10^5
132 cfu/mL, and 90µL were mixed with each antibiotic dilution in the 96-well plate. The plate was
133 incubated overnight at 37°C, and the MIC were then determined by eye, identifying the lowest
134 concentration of antibiotic which did not allow bacterial growth (i.e. the contents of the well were not
135 turbid).

Modelling methods

All analyses were conducted in the R statistical analysis software [36]. The underlying code and data are available in a GitHub repository: https://github.com/qleclerc/phage_antibiotic_dynamics.

Mathematical model

We adapted a previously published mathematical model of phage-bacteria dynamics, including generalised transduction [23]. This model was previously parameterised using the same bacterial and phage strains as in this study. Our three *S. aureus* strains are represented: B_E (erythromycin-resistant, corresponding to *in vitro* strain NE327), B_T (tetracycline-resistant, corresponding to NE201KT7), and B_{ET} (resistant to both erythromycin and tetracycline, corresponding to double-resistant bacteria) (Figure 1). The model also contains 80α lytic phage (P_L), which can give rise to transducing phage carrying either the erythromycin (P_E) or tetracycline (P_T) resistance gene. The transducing phage can give rise to B_{ET} bacteria via generalised transduction (Figure 1).

We added two compartments to the model to track the concentration of antibiotics: C_E for erythromycin, and C_T for tetracycline. The concentrations are expressed in mg/L. We can simulate the addition of a chosen concentration of antibiotic at any given time, and the antibiotics decay at a constant rate γ_E and γ_T for erythromycin and tetracycline, respectively (Figure 1, Equations 1-2). A similar constant rate γ_P is used for phage decay (Equations 7-9).

$$\frac{dC_E}{dt} = -\gamma_E * C_E \qquad (1)$$

$$\frac{dC_T}{dt} = -\gamma_T * C_T \qquad (2)$$

The antibiotic concentrations are then used to calculate $\varepsilon_{i,j}$, the effect of each antibiotic i (i ∈ {E, T}) on each bacterial strain j (j ∈ {E, T, ET}), according to a pharmacodynamic relationship parameterised through Hill equations [34,37] (Equation 3). This commonly used function calculates the effect of an

antibiotic using four parameters: the maximum effect ($\varepsilon max_{i,j}$), current concentration (C_i), half maximal effective concentration ($EC50_{i,j}$), and a Hill coefficient ($H_{i,j}$). Note that $\varepsilon max_{i,j}$ is relative to the maximum growth rate of the corresponding strain j (μmax_j, see below and Equation 10).

$$\varepsilon_{i,j} = \mu max_j * \varepsilon max_{i,j} * C_i^{H_{i,j}} / (EC50_{i,j}^{H_{i,j}} + C_{i,j}^{H_{i,j}}) \quad (3)$$

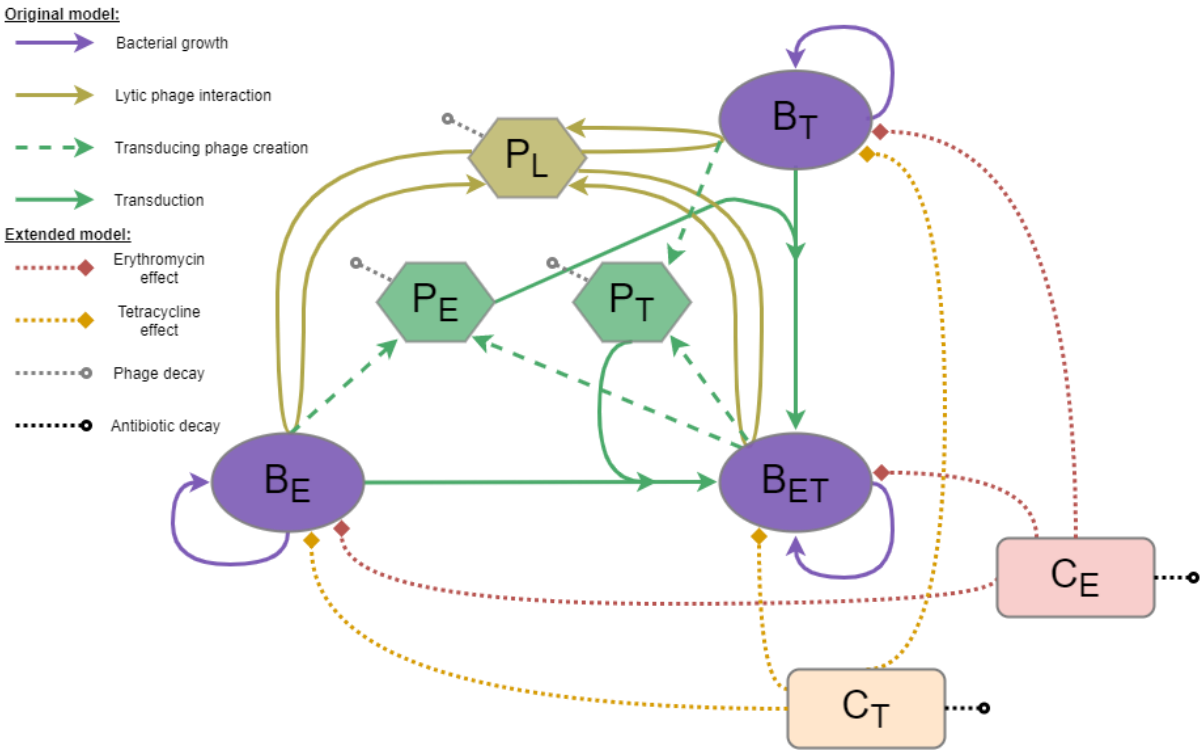

Figure 1: Mathematical model diagram. Each bacteria strain (B_E resistant to erythromycin, B_T resistant to tetracycline, or B_{ET} resistant to both) can replicate (purple). The lytic phage (P_L) multiply by infecting a bacterium and bursting it to release new phage (gold). This process can create transducing phage (P_E or P_T) carrying a resistance gene (*ermB* or *tetK* respectively) taken from the infected bacterium (dashed, green). These transducing phage can then generate new double-resistant progeny (B_{ET}) by infecting the bacteria strain carrying the other resistance gene (solid, green). The antibiotics, erythromycin (C_E) and tetracycline (C_T), decrease the growth rate of each bacteria strain to varying

extents, depending on their concentration and the resistance level of the strain (dotted, red and orange). Phage and antibiotics can decay at a fixed rate (dotted, grey and black).

The complete model equations can be found below.

$$\frac{dB_E}{dt} = \mu_E * (B_E - ((\varphi_L + \varphi_T) * B_E)) - (\varphi_L + \varphi_T) * B_E - (\varepsilon_{E,E} + \varepsilon_{T,E}) * B_E \quad (4)$$

{Change in B_E = growth of B_E – infections by P_L – infections by P_T - ery killing - tet killing}

$$\frac{dB_T}{dt} = \mu_T * (B_T - ((\varphi_L + \varphi_E) * B_T)) - (\varphi_L + \varphi_E) * B_T - (\varepsilon_{E,T} + \varepsilon_{T,T}) * B_T \quad (5)$$

{Change in B_T = growth of B_T – infections by P_L – infections by P_E - ery killing - tet killing}

$$\frac{dB_{ET}}{dt} = \mu_{ET} * (B_{ET} - (\varphi_L * B_{ET})) - \varphi_L * B_{ET} + \varphi_E * B_T + \varphi_T * B_E - (\varepsilon_{E,ET} + \varepsilon_{T,ET}) * B_{ET} \quad (6)$$

{Change in B_{ET} = growth of B_{ET} – infections by P_L + infections of B_T by P_E + infections of B_E by P_T - ery killing - tet killing}

$$\frac{dP_L}{dt} = \varphi_L(t-\tau) * \delta_E * (1-\alpha) * B_E +$$

$$\varphi_L(t-\tau) * \delta_T * (1-\alpha) * B_T +$$

$$\varphi_L(t-\tau) * \delta_{ET} * (1 - 2 * \alpha) * B_{ET} -$$

$$\lambda * P_L - \gamma_P * P_L \quad (7)$$

{Change in P_L = new P_L phage from B_E + new P_L phage from B_T + new P_L phage from B_{ET} – P_L phage infecting bacteria – P_L decay}

$$\frac{dP_E}{dt} = \varphi_L(t-\tau) * \delta_E * \alpha * B_E +$$

$$\varphi_L(t-\tau) * \delta_{ET} * \alpha * B_{ET} -$$

$$\lambda * P_E - \gamma_P * P_E \qquad (8)$$

{Change in P_E = new P_E phage from B_E + new P_E phage from B_{ET} –

P_E phage infecting bacteria – P_E decay}

$$\frac{dP_T}{dt} = \varphi_L(t-\tau) * \delta_T * \alpha * B_T +$$

$$\varphi_L(t-\tau) * \delta_{ET} * \alpha * B_{ET} -$$

$$\lambda * P_T - \gamma_P * P_T \qquad (9)$$

{Change in P_T = new P_T phage from B_T + new P_T phage from B_{ET} –

P_T phage infecting bacteria – P_T decay}

The bacterial growth rate μ_ϑ ($\theta \in \{E, T, ET\}$, $N = B_E+B_T+B_{ET}$) is modelled using logistic growth, with a maximum growth rate of μmax_ϑ and carrying capacity *Nmax*.

$$\mu_\theta = \mu max_\theta * (1 - \frac{N}{Nmax}) \qquad (10)$$

Phage predation is modelled as a frequency-dependent process. Previous work has suggested that this interaction is more biologically realistic than the more commonly used density-dependent interaction [23]. At any given time, the proportion λ of phage infecting bacteria is calculated according to Equation 11 (with β representing the phage adsorption rate), and the proportion φ_ϑ of bacteria infected by at least one phage P_ϑ ($\theta \in \{L, E, T\}$) according to Equation 12.

$$\lambda = (1 - exp\,(-\beta * N)\,) \qquad (11)$$

$$\varphi_\theta = \left(1 - exp\,\left(-\lambda * \frac{P_\theta}{N}\right)\right) \qquad (12)$$

This interaction ensures that, for any given time, the number of phage infecting bacteria and the number of bacteria infected by phage cannot be greater than the total number of phage and bacteria, respectively. The latent period τ corresponds to the delay between bacterial infection and burst, and the transduction probability α corresponds to the proportion of new phage released upon burst which are transducing phage carrying the erythromycin or tetracycline resistance gene.

In the model, phage burst size decreases as bacterial growth decreases. This happens as bacteria enter stationary phase when the population approaches carrying capacity *Nmax* (as demonstrated previously [23,38,39]), but here the additional effect of antibiotics must be included. To capture this, we use the same effective scaling as the bacterial growth rate (Equations 10 and 4-6) with inclusion of the relative effect of antibiotics $\varepsilon_{E,\theta}$ and $\varepsilon_{T,\theta}$ in the phage burst size estimation. Since antibiotics can kill bacteria, leading to a net negative growth rate, we limit the multiplier value to 0 as a minimum, to prevent a negative burst size. The phage burst size δ_θ for each bacteria strain θ (θ ∈{E, T, ET}) is then calculated as a fraction of the maximum phage burst size δmax (Equation 13).

$$\delta_\theta = \delta max * max\left(0, 1 - \frac{N}{Nmax} - \varepsilon_{E,\theta}/\mu max_\theta \ - \varepsilon_{T,\theta}/\mu max_\theta\right) \qquad (13)$$

Parameter estimation

Parameters for bacterial growth and phage predation were originally obtained by fitting our model to *in vitro* data for the same bacteria and phage strains as in this study [23].

Parameters for antibiotic effect were obtained in two steps using the least squares methods for model fitting, which aims to minimise the squared difference between data and model output. Firstly, the reduction in bacterial growth caused by a specific concentration of antibiotic (έ) was obtained by fitting a deterministic growth model (Equation 14) to the bacterial concentration B over time, estimated as the mean of three *in vitro* replicates.

$$\frac{d\mathrm{B}}{dt} = \mu * (1 - B/Nmax) \ * \mathrm{B} - \acute{\varepsilon} * B \qquad (14)$$

231 έ was then scaled to growth ($\varepsilon = \acute{\varepsilon}/\mu$), to represent the relative impact of the antibiotic on bacterial
232 growth rather than an absolute value. Since we obtained growth curve time series data for 8
233 concentrations for each strain, we generated 8 estimates of antibiotic effect for each strain.

234 Secondly, the three parameters of the Hill equation [34,37] (maximum effect ε_{max}, Hill coefficient *H*,
235 and half maximum effective concentration *EC50*), which calculates the antibiotic effect as a function
236 of concentration, were estimated by fitting Equation 3 to the 8 antibiotic effects for each strain.

237 Model scenarios considered

238 *Antibiotics alone*

239 We start with an environment containing both single-resistant strains at stationary phase (10^9 cfu/mL).
240 This mirrors the within-host diversity we could expect to see during bacterial infections [19], and the
241 fact that bacteria most often live at stationary phase in the environment [40]. We first investigate the
242 effect of the presence of either or both erythromycin and tetracycline at concentrations of 1 mg/L,
243 similar to antibiotic concentrations measured *in vivo* during treatment [41,42].

244 *Antibiotic and phage*

245 We then consider scenarios where phage are present. We first artificially inactivate transduction, by
246 setting the corresponding model parameter to 0. We add 10^9 pfu/mL of phage, similar to a
247 concentration that could be added during phage therapy, and equivalent to a multiplicity of infection
248 (ratio of phage to bacteria) of 1, similar to what could be found naturally in the environment [1]. We
249 consider scenarios where the phage are present alone, alongside only one antibiotic, or alongside both
250 antibiotics. Phage and antibiotics are assumed to be introduced concurrently at the start, and the
251 simulations run for 24h.

252 We then repeat these scenarios, but with transduction enabled to levels that were previously
253 observed *in vitro*, with approximately 1 transducing phage carrying an AMR gene generated for each
254 10^8 new lytic phage [23].

255 We repeat the analyses above with either single-resistant strain present at 10^9 cfu/mL, and the other
256 at 10^6 cfu/mL (0.1%). This could correspond to a scenario where bacterial diversity is underestimated,
257 with only one type of resistance detected [19].

258 *Antibiotic and phage level and timing variation*

259 To further investigate the scenario where transduction is enabled and both single-resistant bacterial
260 strains have a starting concentration of 10^9 cfu/mL, we vary the timing of introduction for antibiotic
261 and phage, with up to 24h delay between their respective additions, as well as varying the
262 concentration of antibiotics between 0.25, 0.5, 1 and 2 mg/L, and phage between 10^7, 10^8, 10^9, 10^{10}
263 pfu/mL, chosen to reflect realistic ranges [1,41,42]. We run these simulations for 48h after the
264 introduction of antibiotics or phage (whichever is added first).

265 *Phage and bacteria parameters variation*

266 Finally, we vary the probability for phage to perform generalised transduction to identify threshold
267 values which dictate whether multidrug-resistant bacteria appear above detectable levels within 24h.
268 To explore the sensitivity of our results to the parameter values estimated from this single
269 environment, we also conduct a partial rank correlation to assess the effect of varying phage predation
270 parameters (adsorption rate, latent period, burst size), bacteria growth rates, and antibiotic and phage
271 decay on total bacteria remaining after 48h, and maximum double-resistant bacteria generated. This
272 allows us to generalise our conclusions as these parameters likely vary for different combinations of
273 phage, antibiotics and bacteria. The ranges evaluated are shown in Table 1, and are either derived
274 from our initial parameterisation of the model [23], or from other studies [43,44].

275 # RESULTS

276 ## *In vitro* pharmacodynamics of erythromycin and tetracycline

277 Erythromycin at all concentrations caused a decrease in erythromycin-sensitive NE201KT7 numbers
278 over 6h, but only slowed down growth for erythromycin-resistant NE327 and double-resistant
279 DRPET1, even at 32 mg/L (Figure 2). Tetracycline caused a decrease in bacterial numbers over 6h at
280 concentrations greater than 0.5 mg/L for tetracycline-sensitive NE327, 8 mg/L for tetracycline-
281 resistant NE201KT7, and 4 mg/L for DRPET1 (Figure 2). As a comparison, the minimum inhibitory
282 concentrations (MICs) of erythromycin measured by microbroth dilution were 0.25, >256 and >256
283 mg/L for NE201KT7, NE327 and DRPET1 respectively. For tetracycline, the MICs were 32, 0.25 and 32
284 mg/L for NE201KT7, NE327 and DRPET1 respectively.

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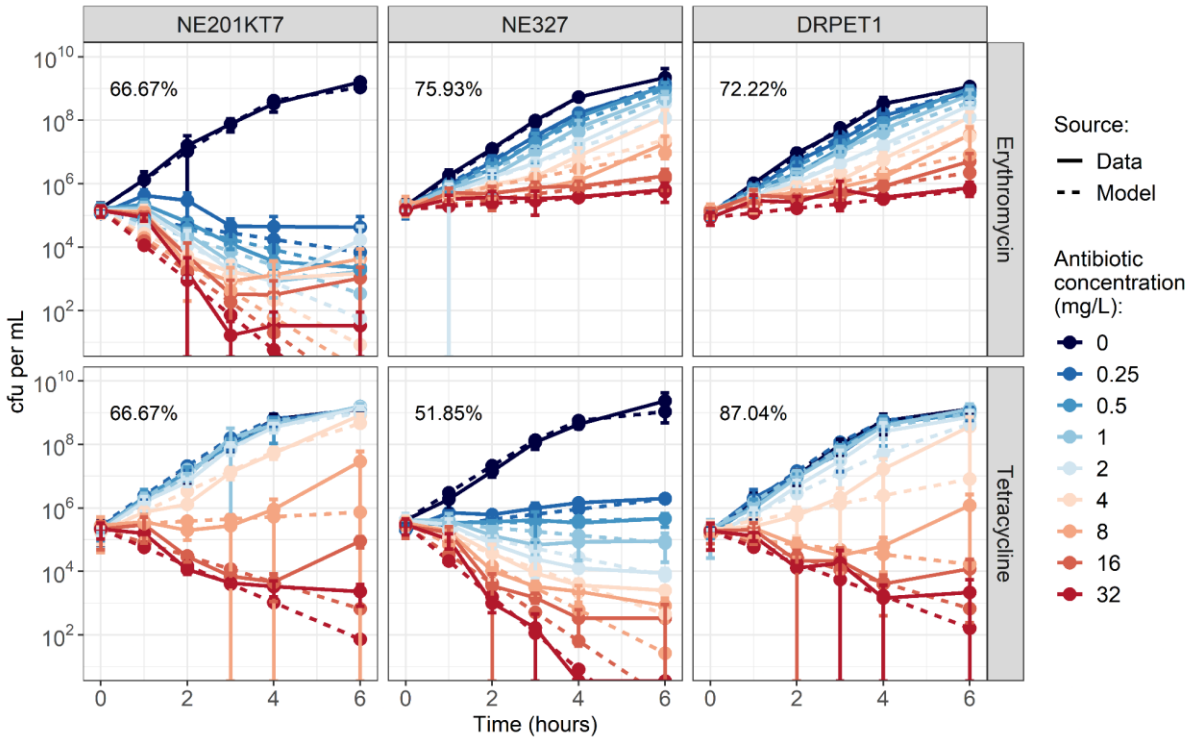


286

287 **Figure 2: Growth curves of NE201KT7 (tetracycline-resistant, left), NE327 (erythromycin-resistant,**
288 **middle) and DRPET1 (double-resistant, right), exposed to varying concentrations of erythromycin**
289 **(top) or tetracycline (bottom).** Solid lines show *in vitro* data, with error bars indicating mean +/-
290 standard deviation, from 3 replicates. Dashed lines show model output after fitting. Values indicate
291 the percentage of model points that fall within the range of the corresponding *in vitro* data point +/-
292 standard deviation. cfu: colony-forming units. Note that cfu per mL are shown on a log-scale.

293

294 The Hill equations fitted well to the effect of varying concentrations of antibiotics on the bacteria
295 (Figure 2, Supplementary Figure 1). The corresponding parameter values are presented in Table 1. The
296 antibiotic effect curves for the DRPET1 are similar to the one for NE201KT7 for tetracycline and NE327
297 for erythromycin (Supplementary Figure 1), which was expected since DRPET1 contains both
298 antibiotic-resistance genes from NE201KT7 (*tetK*) and NE327 (*ermB*).

299

300 **Table 1: Model parameter values.** Parameters with no units are dimensionless. All estimates were
301 obtained by fitting the model to *in vitro* data, except those marked with a * which are assumed.

Name (unit)		**Symbol**	**Estimate**	**Range for sensitivity analysis**	**Reference**
Bacterial growth parameters	Carrying capacity (bacteria)	Nmax	2.76×10^{9}	-	[22]
	B_E growth rate (h^{-1})	μmax_E	1.61	1.59 - 1.63	
	B_T growth rate (h^{-1})	μmax_T	1.51	1.49 - 1.53	
	B_{ET} growth rate (h^{-1})	μmax_{ET}	1.44	1.42 - 1.47	
Phage parameters	Phage adsorption rate ($phage^{-1}.h^{-1}$)	β	2.3×10^{-10}	2.1×10^{-10} - 2.4×10^{-10}	
	Phage burst size (phage)	δmax	76	70 - 83	

	Phage latent period (h)	τ	0.72	0.69 - 0.77	
	Transduction probability (phage^{-1})	α	1.0×10^{-8}	8.5×10^{-9} - 1.4×10^{-8}	
	Phage decay rate (h^{-1})	γ_P	0*	0 - 0.1	[43]
Antibiotic parameters	Erythromycin decay rate (h^{-1})	γ_E	0*	0 - 0.1	[44]
	Tetracycline decay rate (h^{-1})	γ_T	0*	0 - 0.1	
Effect of erythromycin on B_E	Max effect (h^{-1})	$\varepsilon max_{E,E}$	1.10	-	This study
	Half maximal effective concentration	$EC50_{E,E}$	3.42	-	
	Hill coefficient	$H_{E,E}$	0.67	-	
Effect of erythromycin on B_T	Max effect (h^{-1})	$\varepsilon max_{E,T}$	3.91	-	
	Half maximal effective concentration	$EC50_{E,T}$	9.26	-	
	Hill coefficient	$H_{E,T}$	0.22	-	
Effect of erythromycin on B_{ET}	Max effect (h^{-1})	$\varepsilon max_{E,ET}$	0.95	-	
	Half maximal effective concentration	$EC50_{E,ET}$	2.47	-	
	Hill coefficient	$H_{E,ET}$	0.79	-	
Effect of tetracycline on B_E	Max effect (h^{-1})	$\varepsilon max_{T,E}$	5.40	-	
	Half maximal effective concentration	$EC50_{T,E}$	81.43	-	
	Hill coefficient	$H_{T,E}$	0.30	-	
Effect of tetracycline on B_T	Max effect (h^{-1})	$\varepsilon max_{T,T}$	1.66	-	
	Half maximal effective concentration	$EC50_{T,T}$	7.27	-	
	Hill coefficient	$H_{T,T}$	2.41	-	
Effect of tetracycline on	Max effect (h^{-1})	$\varepsilon max_{T,ET}$	1.58	-	

B_{ET}	Half maximal effective concentration	$EC50_{T,ET}$	4.42	-
	Hill coefficient	$H_{T,ET}$	1.70	-

302

303 **Model-predicted antibacterial effect of concurrent erythromycin,**

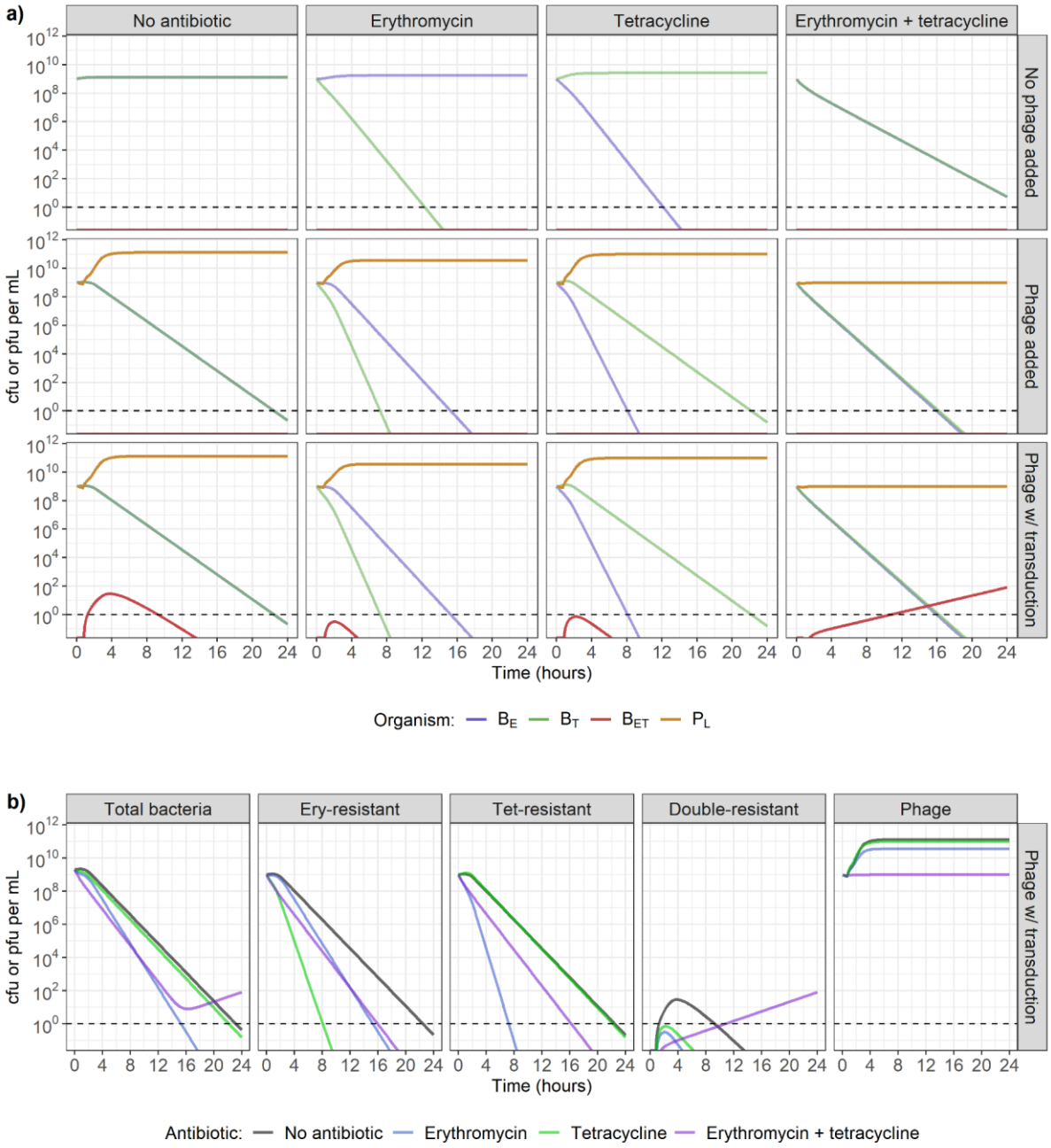
304 **tetracycline and bacteriophage presence**

305 When simulating the dynamics of two single-resistant *S. aureus* strains in our mathematical model, as
306 expected, the presence of only one antibiotic at 1 mg/L (2 x MIC for susceptible strains) leads to a
307 decrease in the susceptible strain, while the resistant strain does not decrease (Figure 3a, top row).
308 On the other hand, the presence of both antibiotics, or of only phage without transduction, causes a
309 decrease in both bacterial strains (Figure 3a, top and middle rows). The presence of phage and one
310 antibiotic leads to a decrease in both bacterial strains, with the antibiotic-susceptible strain decreasing
311 faster (Figure 3a, middle row). Out of all the conditions shown here, the presence of phage and both
312 antibiotics leads to the fastest decrease in both bacterial strains (Figure 3b). For comparison, to
313 replicate the combined effect of 10^9 pfu/mL of phage, 1 mg/L of erythromycin, and 1 mg/L of
314 tetracycline, we would need 4.58 mg/L of erythromycin and 1.14 mg/L of tetracycline in the absence
315 of phage (Supplementary Figure 2).

316 Transduction does not appear to affect the antibacterial activity of antibiotics and phage when phage
317 are either present alone, alongside erythromycin, or alongside tetracycline (Figure 3a, bottom row).
318 Double-resistant progeny bacteria (B_{ET}) appear, but only reach a maximum concentration of 30
319 cfu/mL, and do not remain higher than 1 cfu/mL for more than 8h (Figure 3a, bottom row). However,
320 when both erythromycin and tetracycline are present alongside phage capable of transduction, there
321 is a steady increase in the number of B_{ET} throughout 24h, reaching 80 cfu/mL after 24h (Figure 3a,
322 bottom row & Figure 3b). It is important to note here that when both antibiotics are present, phage

323 numbers do not increase throughout the 24h period, regardless of transduction ability (Figure 3a,

324 middle and bottom rows & Figure 3b, right).



325

326 **Figure 3: a) Model-predicted dynamics with two single-resistant strains starting at carrying capacity**

327 **(10^9 colony-forming units (cfu)/mL), in the presence of no antibiotics (1st column), erythromycin**

328 **only (2nd column), tetracycline only (3rd column), or both erythromycin and tetracycline (4th**

329 **column), combined with either no phage (top row), phage incapable of transduction (middle row),**

330 **or phage capable of generalised transduction (bottom row).** The starting strains are either
331 erythromycin-resistant (B_E) or tetracycline-resistant (B_T). Antibiotics and/or phage (P_L) are present at
332 the start of the simulation, at concentrations of 1 mg/L (2 x MIC for susceptible strains) and 10^9 plaque-
333 forming units (pfu)/mL respectively. Double-resistant bacteria (B_{ET}) can be initially generated by
334 generalised transduction only, and then by replication of existing B_{ET}. Dashed line indicates the
335 detection threshold of 1 cfu or pfu/mL. **b) Change in bacteria (single-resistant to erythromycin,**
336 **single-resistant to tetracycline, or double-resistant) and phage numbers depending on the antibiotic**
337 **exposure, in the presence of phage capable of generalised transduction.** Dashed line indicates the
338 detection threshold of 1 cfu or pfu/mL.

339

340 With a reduced starting concentration of either *S. aureus* strain to 10^6 cfu/mL, while the other remains
341 at 10^9 cfu/mL, the conclusions are similar as described above, regardless of which strain is in the
342 minority. Figure 4 shows the scenario where tetracycline-resistant bacteria are in minority, and
343 Supplementary Figure 3 shows the scenario where erythromycin-resistant bacteria are in minority.
344 When erythromycin, tetracycline, and phage capable of generalised transduction are all present, B_{ET}
345 are still generated, although they do not reach a concentration higher than 1 cfu/mL within 24h,
346 instead of 11h above (Figure 4, Figure 3).

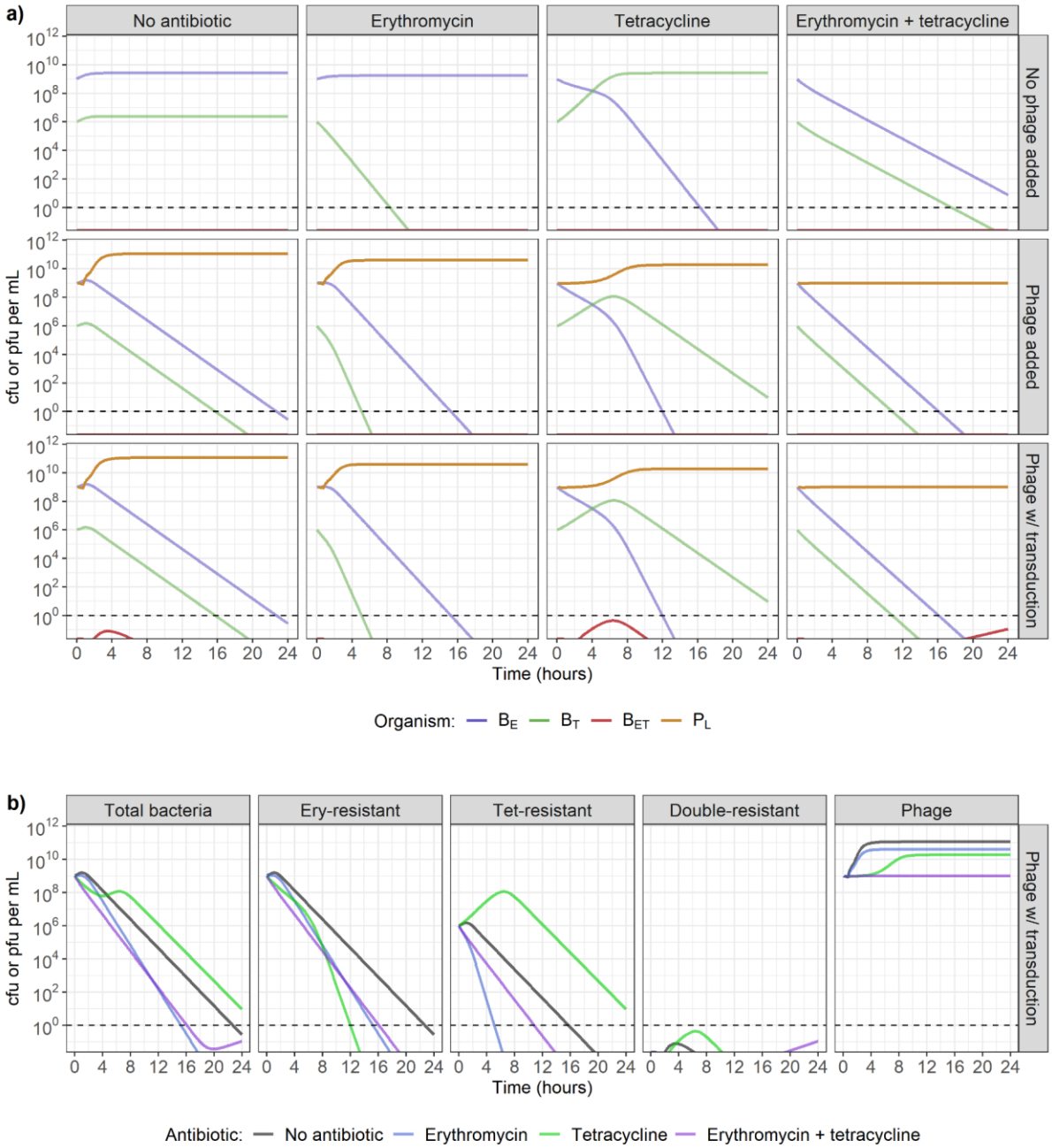


Figure 4: a) Model-predicted dynamics with one single-resistant strains starting at carrying capacity (10^9 colony-forming units (cfu)/mL) and the second in minority (10^6 cfu/mL), in the presence of no antibiotics (1st column), erythromycin only (2nd column), tetracycline only (3rd column), or both erythromycin and tetracycline (4th column), combined with either no phage (top row), phage incapable of transduction (middle row), or phage capable of generalised transduction (bottom row). Erythromycin-resistant bacteria (B_E) are initially present at a concentration of 10^9 cfu/mL, and tetracycline-resistant bacteria (B_T) at 10^6 cfu/mL. Antibiotics and/or phage (P_L) are present at the start

355 of the simulation, at concentrations of 1 mg/L (2 x MIC for susceptible strains) and 10^9 plaque-forming
356 units (pfu)/mL respectively. Double-resistant bacteria (B_{ET}) can initially be generated by generalised
357 transduction only, and then by replication of existing B_{ET}. Dashed line indicates the detection threshold
358 of 1 cfu or pfu/mL. **b) Change in bacteria (single-resistant to erythromycin, single-resistant to**
359 **tetracycline, or double-resistant) and phage numbers depending on the antibiotic exposure, in the**
360 **presence of phage capable of generalised transduction.** Dashed line indicates the detection threshold
361 of 1 cfu or pfu/mL.

362

363 Effect of variation in antibiotic and phage timing and concentration
364 on bacterial populations

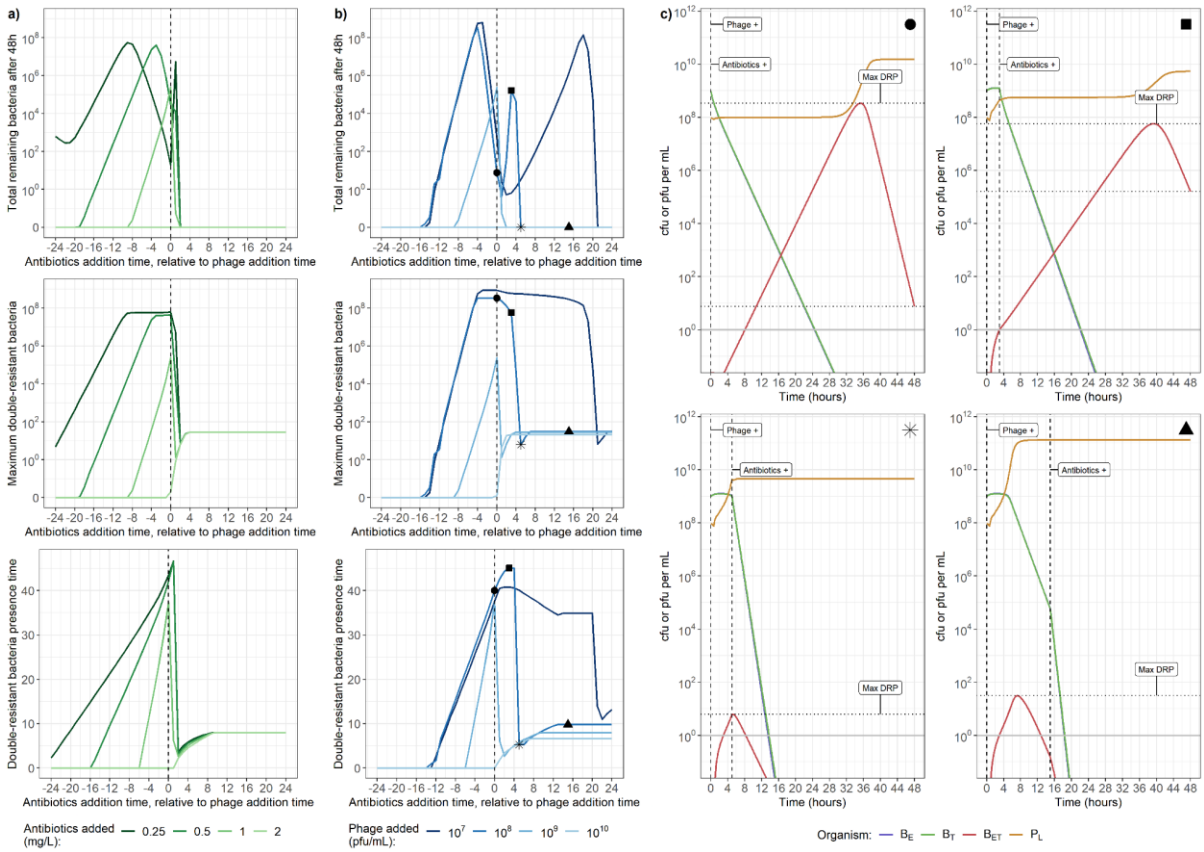
365 Under conditions where both erythromycin and tetracycline are present, the timing and concentration
366 of antibiotics and phage capable of generalised transduction drives substantial variation in dynamics,
367 with failure to clear all bacteria after 48h being a possibility (Figure 5a-b, top row).

368 With a phage concentration of 10^9 cfu/mL and for any antibiotics concentration between 0.25 and 2
369 mg/L (Figure 5a), the optimal conditions to clear bacteria within 48h are when phage are initially
370 present, and antibiotics are introduced at least 2h later (Figure 5a, top row). However, this
371 systematically leads to B_{ET} appearance, with a peak concentration of up to 30 cfu/mL (Figure 5a, middle
372 row), and a presence time (hours when cfu/mL > 1) of up to 8h (Figure 5a, bottom row). The presence
373 of antibiotics at the same time or shortly before phage leads to failure to clear bacteria within 48h (up
374 to 5.5 x 10^7 cfu/mL remaining after 48h - top row), and substantial B_{ET} appearance (maximum
375 concentration up to 6 x 10^7 cfu/mL - middle row; presence time up to 47h - bottom row). Regardless
376 of timings, the addition of at least 2 mg/L of antibiotics guarantees bacterial clearance within 48h (top

377 row, red line), and no detectable B_{ET} if the antibiotics are added at the same time as or before the
378 phage (bottom row, red line).

379 When keeping the antibiotic concentrations at 1 mg/L, but varying the phage concentration, the
380 impact of the delay between phage and antibiotic presence on the bacterial population is strongly
381 dependent on phage concentration (Figure 5b). We again see that bacteria are not cleared within 48h
382 if antibiotics are present at the same time as or shortly before a concentration of phage between 10^7
383 and 10^9 pfu/mL (Figure 5b, top row). However, we now note that this also occurs if antibiotics are
384 introduced after a concentration of phage between 10^7 or 10^8 pfu/mL (Figure 5b, top row). Regardless
385 of timing, the presence of a phage concentration of 10^{10} pfu/mL guarantees bacterial clearance within
386 48h (top row, red line), but leads to B_{ET} if the antibiotics are introduced after the phage (maximum
387 concentration up to 30 cfu/mL - middle row, red line; presence time up to 7h - bottom row, red line).

388



389

390 **Figure 5: a-b) Varying timing (x-axis) and dose of antibiotic and phage (line colour) affects total**
391 **bacterial count after 48h (top), maximum concentration of double-resistant bacteria (B_{ET}) (middle),**
392 **and time when the concentration of B_{ET} is greater than 1 colony-forming unit (cfu) per mL (bottom).**
393 **a)** Adding 10^9 plaque-forming units (pfu) per mL of phage, and between 0.25 and 2 mg/L of both
394 erythromycin and tetracycline. **b)** Adding 1 mg/L of both erythromycin and tetracycline, and between
395 10^7 and 10^{10} pfu/mL of phage. The x-axis indicates the time when antibiotics were added, relative to
396 when phage were added. For example, the value "4" indicates that phage were present at the start of
397 the simulation, and antibiotics were introduced 4h later. **c) Phage and bacteria dynamics over 48h for**
398 **4 conditions taken from panel b.** In all 4 conditions, indicated by the 4 shapes, phage are initially
399 present at a concentration of 10^8 pfu/mL, while erythromycin and tetracycline are both introduced at
400 concentrations of 1 mg/L after either 0h (circle), 3h (square), 5h (star) or 15h (triangle) with the timing
401 indicated by the vertical dashed lines. Horizontal dotted lines indicate bacteria remaining after 48h
402 (corresponding to the top row of a-b) and maximum double-resistant bacteria (B_{ET}) concentration
403 (middle row of a-b). Solid line indicates the detection threshold of 1 cfu or pfu/mL. The concentrations
404 of single-resistant bacteria (B_E, blue, and B_T, green) overlap and cannot be distinguished.

405

406 To investigate the dynamics behind these results, we selected four conditions from Figure 5b (points
407 indicated by the 4 shapes) with varying antibiotic addition times and plotted the underlying phage and
408 bacteria dynamics over 48h for each (Figure 5c). In all four conditions the starting concentrations are
409 10^8 pfu/mL for phage and 1 mg/L for antibiotics, but antibiotics are introduced either 0h (circle), 3h
410 (square), 5h (star) or 15h (triangle) after phage. These plots show that if phage are increasing, they
411 stop immediately following antibiotic addition (Figure 5c, star and square). If antibiotics are added too
412 soon after phage (Figure 5c - circle, square), phage do not reach a high enough number to exert a
413 sufficient killing pressure on bacteria. In that case B_{ET}, which are not substantially affected by either
414 antibiotic, replicate faster than they are killed by phage. After more than 35h, the B_{ET} population

415 reaches a sufficiently high number such that the phage population increases, and the resulting
416 pressure is enough to lead to a net negative bacterial growth rate. If antibiotics are added 15h or later
417 after phage (Figure 5b, Figure 5c - triangle), B_{ET} generation will not change, as during this period they
418 will have already arisen by transduction and been removed by phage predation. The presence of
419 antibiotics 5h after phage is optimal to ensure the lowest maximum number of B_{ET} (Figure 5c - star,
420 compare horizontal dotted lines). This timing allows phage to initially increase to a concentration of
421 almost 5 x 10^9, sufficiently high to exert a strong killing pressure on bacteria, while the added effect
422 of antibiotics prevents further B_{ET} generation by decreasing the single-resistant strains.

423 These results also apply to a scenario where one of the two bacterial strains is in the minority (starting
424 concentration of 10^6 instead of 10^9 cfu/mL), regardless of which strain is in the minority
425 (Supplementary Figures 4 & 5). However, B_{ET} peak, presence time, and total bacteria remaining after
426 48h decrease faster with a higher dose of antibiotic or phage, or with a higher delay between phage
427 and antibiotics, suggesting that phage and antibiotics are able to exert a greater killing pressure and
428 generate fewer B_{ET} when one strain is in the minority.

429

430 Effect of variation in phage and bacteria parameters on multidrug-
431 resistance evolution

432 Our results above rely on parameters estimated from phage and bacteria interactions *in vitro*, but
433 these may vary depending on the bacteria, phage, and environment. We can explore these different
434 conditions using our model to quantify the dynamics under varying values for parameters governing
435 phage-bacteria interactions (see Table 1 for the ranges used) to determine whether our results would
436 hold.

437 We first examine the impact of varying the transduction probability on our results (corresponding to
438 the probability that a transducing phage carrying an AMR gene is released instead of a lytic phage
439 during bacterial burst) as this is a vital and yet poorly quantified parameter. When 10^9 cfu/mL of each
440 single-resistant strain are simultaneously exposed to 10^9 pfu/mL of phage and 1 mg/L of erythromycin
441 and tetracycline, varying the transduction probability between 10^{-10} and 10^{-6} leads to a similar log-fold
442 increase in double-resistant bacteria numbers (B_{ET}, Figure 6a). Decreasing the probability only delays
443 the appearance of B_{ET} in the model, and does not prevent it. However, a probability lower than 10^{-9}
444 may prevent the appearance of B_{ET} in reality, since the single-resistant strains become undetectable
445 (< 1 cfu/mL) before the B_{ET} become detectable. If antibiotics are added more than 10h after phage, B_{ET}
446 will have already started declining due to phage predation, hence the antibiotics only contribute to
447 further increasing the decline in bacterial numbers (Figure 5, Figure 6b). Under these conditions, a
448 transduction probability lower than 10^{-9} is necessary to prevent B_{ET} from increasing past the detection
449 threshold (1 cfu/mL) (Figure 6b). If antibiotics are introduced 10h before phage, the resulting decline
450 in single-resistant bacteria prevents any B_{ET} from reaching a detectable level before single-resistant
451 bacteria are eradicated (< 1 cfu/mL), even with the highest transduction probability of 10^{-6} (Figure 6c).

452 Looking at how changes in other phage and bacteria parameters may affect our results, partial rank
453 correlation shows that an increase in phage predation either through an increase in phage adsorption
454 rate (β) or phage burst size (δmax) correlates with a decrease in maximum B_{ET} detected over 48h
455 (Figure 6d, blue). For example, the correlation coefficient of -0.65 between β and maximum B_{ET} implies
456 that a 100% increase in adsorption rate is correlated with a 65% decrease in maximum double-
457 resistant bacteria detected over 48h. An increase in phage burst size is also correlated with a decrease
458 in bacteria remaining after 48h. However, adsorption rate is not substantially correlated with bacteria
459 remaining after 48h (Figure 6d, red). Finally, an increase in latent period (τ), equivalent to a decrease
460 in predation since phage will take longer before lysing the bacteria, is correlated with an increase in
461 maximum B_{ET}, but is not substantially correlated with bacteria remaining after 48h.

462 Although we were not able to parameterise our model for antibiotic decay, our 24h time-kill curves
463 suggest that antibiotics no longer decrease bacterial numbers after 24h (Supplementary Figure 6).
464 Phage may also be affected by decay in the environment. Hence, we included these parameters in our
465 partial rank correlation analysis. An increase in phage or antibiotic decay (γ_P, γ_E, γ_T) is correlated with
466 an increase in maximum B_{ET} (Figure 6d, blue), but unexpectedly with a decrease in bacteria remaining
467 after 48h (Figure 6d, red). This is explained by the fact that such an increase leads to a weakened killing
468 pressure on B_{ET}, which are able to increase faster. This translates to a shorter time before the phage
469 population is able to increase again due to enough bacteria being available for predation, and thus a
470 shorter time before B_{ET} start decreasing due to phage killing (Supplementary Figure 7). This also
471 explains why an increase in the transduction probability (α) is correlated with a decrease in bacteria
472 remaining after 48h, as it leads to a faster increase in B_{ET}, and thus a shorter time before the phage
473 are able to increase and eradicate the B_{ET} population. Finally, single-resistant bacterial growth rates
474 (μmax_E, μmax_T) are not substantially correlated with either maximum B_{ET} or remaining bacteria, while
475 double-resistant growth rate (μmax_{ET}) is only weakly positively correlated with maximum B_{ET}, and
476 negatively with remaining bacteria.

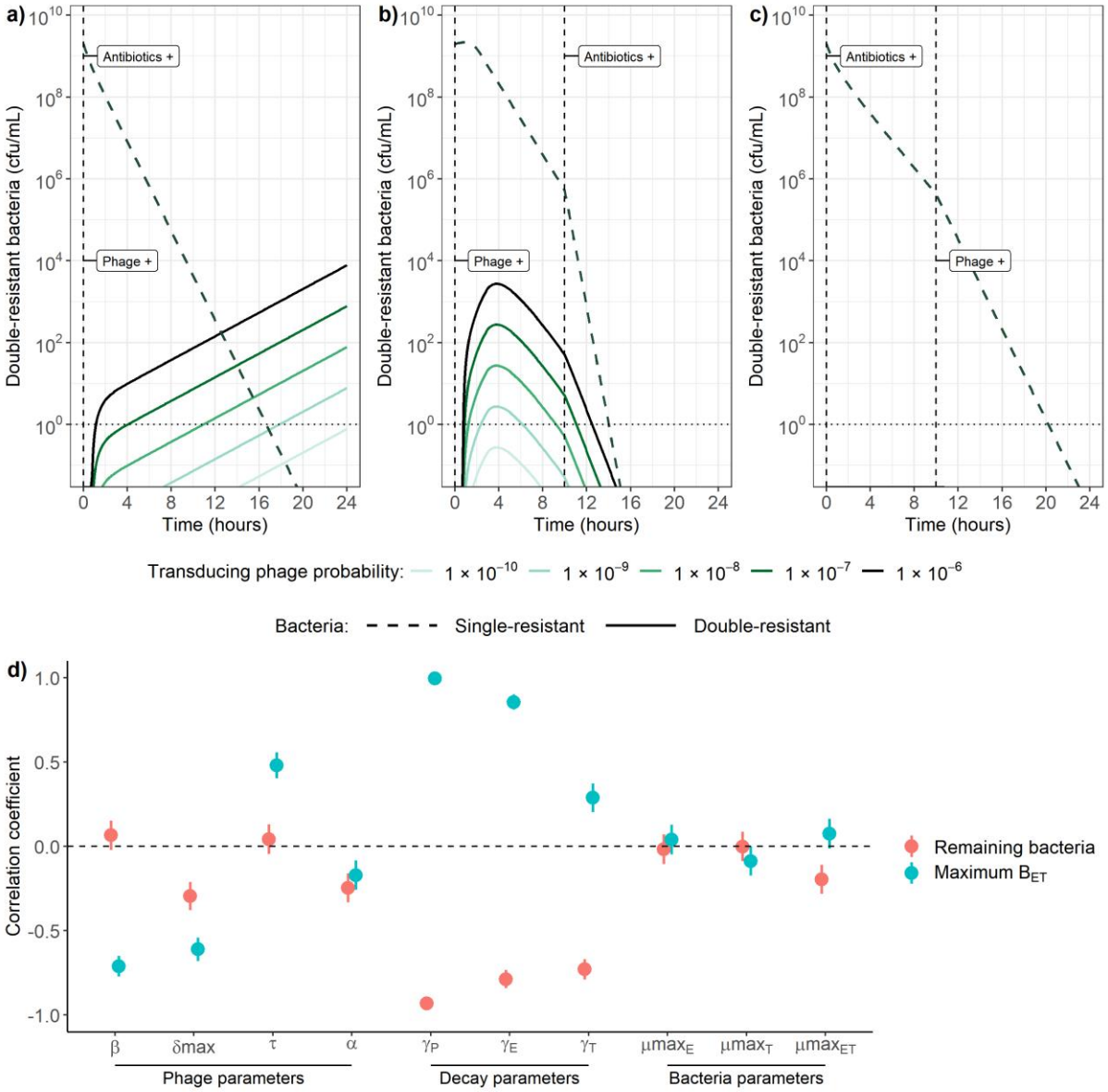


Figure 6: Sensitivity of phage-bacteria dynamics to changes in model parameters. Effect of varying the transduction probability between 10^{-10} and 10^{-6} when a) antibiotics and phage are present at the start of the simulation, b) phage are present at the start, antibiotics are introduced 10h later, and c) antibiotics are present at the start, phage are added 10h later. Transduction probability is defined as the probability that a transducing phage carrying an AMR gene is released instead of a lytic phage during bacterial burst. The dashed lines for single-resistant bacteria overlap and cannot be distinguished. Vertical dashed lines indicate timing of addition of antibiotics or phage. cfu/mL: colony-forming units per mL. **d) Partial rank correlation between model parameters, and remaining bacteria**

486 **after 48h (pink) or maximum double-resistant bacteria (B_{ET}) concentration (blue).** Information on the

487 parameter ranges investigated can be found in Table 1. β: adsorption rate, δmax: burst size, τ: latent

488 period, α: transduction probability, γ_P: phage decay, γ_E: erythromycin decay, γ_T: tetracycline decay,

489 μmax_E: B_E growth rate, μmax_T: B_T growth rate, μmax_{ET}: B_{ET} growth rate.

DISCUSSION

Summary of results

In this work, we reconcile the existing literature suggesting either that phage and antibiotics can synergistically kill bacteria, or that antibiotics reduce the efficacy of phage predation, whilst also considering the synergy of phage and antibiotics on antimicrobial resistance (AMR) evolution. We showed that although phage replication is limited in the presence of antibiotics, which negatively affect bacterial growth, phage are still able to exert a strong killing pressure on bacteria to complement the action of antibiotics. Under such conditions, the concentration and timing of antibiotics and phage are essential: phage introduced after antibiotics at a low concentration may not be able to replicate and hence not contribute to killing the bacterial population. Phage and antibiotics can act synergistically to drive AMR evolution when phage generate multidrug-resistant bacteria by transduction, and antibiotics act as a selection pressure. This is again exacerbated by the reduction in phage replication caused by antibiotics: a low concentration of phage capable of transduction introduced after antibiotics will not exert a strong killing pressure on bacteria, and instead generate multidrug-resistant bacteria at a background rate which are then selected for by the antibiotics.

Optimal conditions to clear bacteria and minimise AMR evolution

The best conditions to guarantee bacterial eradication within 48h whilst minimising the risk of multidrug-resistant bacteria evolution in our system are when antibiotics are present before phage, and either when erythromycin and tetracycline are present at a concentration of at least 2 mg/L (4 x MIC for susceptible strains), or phage at a concentration of at least 10^{10} pfu/mL. Multidrug-resistant bacteria generation is also restricted if phage are introduced at least 10h after antibiotics. This may be particularly relevant in the context of antibacterial treatment, further discussed below. The worst

512 outcome in which antibiotics and phage fail to rapidly clear all bacteria and instead act synergistically
513 to drive AMR evolution is a combination of a low antibiotic dose (< 1 mg/L) with a low phage dose (<
514 10^9 pfu/ml), introduced around the same time. Unfortunately, this may correspond to values seen in
515 natural environments where phage are commonly found and antibiotics are residually present due to
516 pollution [2,3].

517 Importance of transduction in the environment and during phage 518 therapy

519 Our results highlight the necessity to better understand the role of transduction in AMR spread and
520 evolution, and not assume by default that it is too rare to be relevant compared to other mechanisms
521 of horizontal gene transfer such as conjugation and transformation. We found that multidrug-
522 resistance evolution remained possible even at the lowest probability we considered here of a
523 transducing phage carrying an AMR gene being released instead of a lytic phage during burst (1
524 transducing phage per 10^9 lytic phage). Additionally, in this work we assumed that transduction rates
525 are constant, but previous research has shown that sub-MIC antibiotic exposure can lead to an
526 increase in transducing phage, but not lytic ones [45]. Hence, our results may still underestimate the
527 relative impact of transduction versus predation by phage when antibiotics are present. However, our
528 findings are encouraging for conditions under which AMR evolution is limited, since even with a high
529 risk of transduction (1 transducing phage per 10^6 lytic phage), if phage are only introduced to an
530 environment more than 10h after antibiotics, multidrug-resistant bacteria do not reach detectable
531 levels before single-resistant strains are eradicated (Figure 5c).

532 To the best of our knowledge, our model is the first to consider the potential consequences of
533 transduction in a system where concentrations of bacteria, phage, and antibiotic are similar to those
534 we may see during phage therapy. We echo the conclusions from previous studies which highlighted
535 that the timing of antibiotics and phage introduction during phage therapy can affect the rate at which

536 bacteria are cleared [6–9], but extend these to show that timing may also impact the risk for multidrug-
537 resistant bacteria to be generated. Here, we suggest that the timing leads to a trade-off between a
538 slower clearing rate of bacteria (if antibiotics are added before phage), and a higher risk of multidrug-
539 resistance evolution (if phage are added before antibiotics). Future studies and clinical trials of phage
540 therapy should investigate varying timings of phage and antibiotics, instead of only investigating their
541 simultaneous application, and consider the risk of transduction during treatment.

542 In any case, our ability to measure the impact of transduction as a driver of AMR evolution *in vivo* is
543 currently limited since individuals are not routinely screened for phage. A first step to measure this
544 despite the limitation may be to investigate evidence for within-patient changes in the resistance
545 profile of *S. aureus* isolates, as these would likely be caused by transduction [18]. In the case of
546 antibiotic treatment, the natural presence of phage capable of transduction may explain instances of
547 treatment failure, if these generate multidrug-resistant strains which are then selected for by the
548 antibiotics. Future studies monitoring therapeutic outcomes of antibacterial treatment in patients
549 where phage are also detected will be essential to better understand how our findings translate to *in*
550 *vivo* settings.

551 Limitations

552 The major limitation of our work is the deterministic nature of our model. While it does not account
553 for stochastic events which would play a large role when bacterial numbers are low, the deterministic
554 model is useful for analysis purposes, as it represents the average scenario we would observe. In
555 reality, we would likely see either bacterial clearance or unexpected increases at low numbers of
556 bacteria. We only generated model predictions for up to 48h, as our parameter values were obtained
557 using data from experiments over 24h. Beyond this time, bacteria and phage may be affected
558 negatively due to resource depletion, depending on the environment.

559 Our model does not include some dynamics which may be present *in vivo*, as we do not currently have
560 robust data available to parameterise these features, and would instead have had to rely on
561 assumptions or previously estimated parameter values from different settings. Notably, we have not
562 included the effect of the immune system, which may limit the number of multidrug-resistant bacteria
563 generated as it could suppress both bacteria and phage populations *in vivo* [46,47]. If the model was
564 extended to include the immune system, it would also have to consider potential detrimental effects
565 of large doses of phage and antibiotics, which would restrict these concentrations to prevent side
566 effects *in vivo* [48,49]. In addition, we assume that all the bacteria in our environment are equally
567 susceptible to phage infection, and have not considered the possibility for any further evolution (e.g.
568 adaptation mutation, resistance to phage). Such evolution may allow multidrug-resistant bacteria to
569 overcome any fitness cost and persist for longer durations in the environment, hence further
570 highlighting the necessity to minimise the risk of their initial appearance.

571 Although we varied the concentration of antibiotics in our results, we have consistently added
572 erythromycin and tetracycline in equal amounts. Our model would allow us to change this, yet we
573 have chosen not to for simplicity and because the antibacterial effect curves look similar for these two
574 antibiotics in our setting (Supplementary Figure 1). However, for other antibiotics it may be necessary
575 to revisit this assumption and investigate concentrations which may better reflect those to which
576 bacteria are exposed to in the environment or during antibacterial treatment.

577 Generalisability

578 Our model is extensively parameterised using data from a single phage and three *S. aureus* strains,
579 making it a robust tool to study the dynamics of these organisms, as it relies on a minimum number
580 of assumptions [23]. However, the parameters we have estimated (adsorption rate, burst size, latent
581 period and transduction probability) will likely vary depending on the phage, bacteria, and
582 environment studied. Our sensitivity analysis shows that the model outputs are reasonable with

583 alternative parameter values, predicting for example that phage with a higher predation capacity
584 (higher adsorption rate or burst size, or lower latent period) would clear more bacteria within 48h,
585 and reduce the maximum number of multidrug-resistant bacteria generated. This model has been
586 developed as part of an interdisciplinary project alongside *in vitro* experiments, hence it could be easily
587 re-parameterised using data for other strains of bacteria and phage showing similar dynamics of lysis
588 and generalised transduction. The structure of the model is generalisable to other systems of
589 generalised transducing phage and bacteria, as it captures the relevant biological characteristics of
590 phage predation and generalised transduction [23].

591 The unique dynamics of phage, bacteria, and antibiotics

592 We suggest that transduction and the effect of antibiotics should be considered in the context of the
593 previously described unique dynamics of phage and bacteria. These imply that phage must first reach
594 a certain concentration (previously referred to as "inundation threshold") before they can offset
595 bacterial growth and decrease the bacterial population, and bacteria must first reach a certain
596 concentration ("proliferation threshold") before the phage population can increase [9]. Generalised
597 transduction and antibiotics affect the size of the bacterial population, and therefore how phage
598 interact with bacteria to clear them and generate multidrug-resistant bacteria. Thus, multidrug-
599 resistant bacteria are able to increase in our model if phage are initially present at a concentration
600 lower than the inundation threshold (Figure 5d). This also explains our counterintuitive observation
601 that higher decay rates may lead to less bacteria remaining after 48h, as this would allow bacteria to
602 reach the proliferation threshold sooner, and therefore allow phage to increase up to the inundation
603 threshold sooner (Figure 6c, Supplementary Figure 7). Importantly, our results similarly suggest that
604 higher decay rates for antibiotics present alongside phage would reduce bacteria remaining after 48h,
605 at the cost of a higher peak concentration of multidrug-resistant bacteria, since this decay would allow
606 bacteria to reach the proliferation threshold sooner (Figure 6c, Supplementary Figure 7). This

607 knowledge may be further useful in the context of phage therapy, to select the antibiotics that will be
608 given alongside phage [44].

609 Conclusions

610 Our results demonstrate the complex synergy between phage and antibiotics to kill bacteria and drive
611 the evolution of AMR. We suggest this synergy leads to a trade-off between a slower clearing rate of
612 bacteria (if antibiotics are added before phage), and a higher risk of multidrug-resistance evolution (if
613 phage are added before antibiotics), further exacerbated by low concentrations of either phage or
614 antibiotics. Interdisciplinary frameworks such as ours combining *in vitro* data and mathematical
615 models are key to understanding both fundamental AMR evolution, and new interventions like phage
616 therapy or screening for phage in patients. Our conclusions form hypotheses to guide future
617 experimental work, notably for studies of phage therapy which should consider the risk for multidrug-
618 resistance evolution by transduction, and investigate varying timings and concentrations of phage and
619 antibiotics instead of only their simultaneous use.

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