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

of brain-heart infusion broth (BHIB, Sigma, UK). Unless otherwise stated, liquid cultures were incubated in a warm shaking water bath (37°C, 90 rpm). Each pre-culture was then diluted in phosphate-buffered saline (PBS) and mixed with 10mL of fresh BHIB in a 50mL conical tube to reach a starting concentration of 10^4 colony-forming units (cfu)/mL. The new culture was incubated for 2h to allow the bacteria to reach log-growth phase, following standard protocol for time-kill experiments [34]. Erythromycin or tetracycline was then added to the culture, at a concentration of either 0 (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 were sampled from the incubated culture, diluted in PBS, and plated on plain brain-heart infusion agar. The plates were incubated overnight at 37°C. Colonies on the plates were then counted to derive the concentration of bacteria in cfu/mL at the corresponding time point.

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

Minimum inhibitory concentration

We measured the minimum inhibitory concentrations (MIC) of erythromycin and tetracycline for NE327, NE201KT7 and DRPET1 by microbroth dilution [35]. Briefly, pre-cultures of each strain were generated overnight in Mueller-Hinton broth (MHB-II, Sigma, UK). Antibiotic stocks were generated in 50% ethanol at concentrations doubling from 0.25 to 256 mg/L, and 10µL of each dilution were added to separate wells on a 96-well plate. The overnight pre-cultures were diluted to a concentration of 10^5 cfu/mL, and 90µL were mixed with each antibiotic dilution in the 96-well plate. The plate was incubated overnight at 37°C, and the MIC were then determined by eye, identifying the lowest concentration of antibiotic which did not allow bacterial growth (i.e. the contents of the well were not 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 80 α 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).

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

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

The antibiotic concentrations are then used to calculate ε_{ij} , the effect of each antibiotic i ($i \in \{E, T\}$) on each bacterial strain j ($j \in \{E, T, ET\}$), 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 ($\epsilon_{max_{i,j}}$), current concentration (C_i), half maximal effective concentration ($EC50_{i,j}$), and a Hill coefficient ($H_{i,j}$). Note that $\epsilon_{max_{i,j}}$ is relative to the maximum growth rate of the corresponding strain j (μ_{max_j} , see below and Equation 10).

$$\epsilon_{i,j} = \mu_{max_j} * \epsilon_{max_{i,j}} * C_i^{H_{i,j}} / (EC50_{i,j}^{H_{i,j}} + C_i^{H_{i,j}}) \quad (3)$$

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}

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

$$\begin{aligned} 189 \quad & \varphi_L(t - \tau) * \delta_{ET} * \alpha * B_{ET} - \\ 190 \quad & \lambda * P_E - \gamma_P * P_E \end{aligned} \quad (8)$$

191 *{Change in P_E = new P_E phage from B_E + new P_E phage from B_{ET} -*

192 *P_E phage infecting bacteria - P_E decay}*

$$193 \quad \frac{dP_T}{dt} = \varphi_L(t - \tau) * \delta_T * \alpha * B_T +$$

$$194 \quad \varphi_L(t - \tau) * \delta_{ET} * \alpha * B_{ET} -$$

$$195 \quad \lambda * P_T - \gamma_P * P_T \quad (9)$$

196 *{Change in P_T = new P_T phage from B_T + new P_T phage from B_{ET} -*

197 *P_T phage infecting bacteria - P_T decay}*

198 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
199 maximum growth rate of μ_{max_θ} and carrying capacity N_{max} .

$$200 \quad \mu_\theta = \mu_{max_\theta} * \left(1 - \frac{N}{N_{max}}\right) \quad (10)$$

201 Phage predation is modelled as a frequency-dependent process. Previous work has suggested that this
202 interaction is more biologically realistic than the more commonly used density-dependent interaction
203 [23]. At any given time, the proportion λ of phage infecting bacteria is calculated according to Equation
204 11 (with β representing the phage adsorption rate), and the proportion φ_θ of bacteria infected by at
205 least one phage P_θ ($\theta \in \{L, E, T\}$) according to Equation 12.

$$206 \quad \lambda = (1 - \exp(-\beta * N)) \quad (11)$$

$$207 \quad \varphi_\theta = \left(1 - \exp\left(-\lambda * \frac{P_\theta}{N}\right)\right) \quad (12)$$

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 N_{max} (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 ($\theta \in \{E, T, ET\}$) is then calculated as a fraction of the maximum phage burst size δ_{max} (Equation 13).

$$\delta_\theta = \delta_{max} * \max\left(0, 1 - \frac{N}{N_{max}} - \varepsilon_{E,\theta}/\mu_{max_\theta} - \varepsilon_{T,\theta}/\mu_{max_\theta}\right) \quad (13)$$

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 (ε) 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.

$$\frac{dB}{dt} = \mu * (1 - B/N_{max}) * B - \varepsilon * B \quad (14)$$

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

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

Model scenarios considered

Antibiotics alone

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

Antibiotic and phage

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

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

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

Antibiotic and phage level and timing variation

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

Phage and bacteria parameters variation

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

RESULTS

In vitro pharmacodynamics of erythromycin and tetracycline

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

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

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

Table 1: Model parameter values. Parameters with no units are dimensionless. All estimates were 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)	N _{max}	2.76×10^9	-	[22]
	B _E growth rate (h ⁻¹)	$\mu_{\max_E}$	1.61	1.59 - 1.63	
	B _T growth rate (h ⁻¹)	$\mu_{\max_T}$	1.51	1.49 - 1.53	
	B _{ET} growth rate (h ⁻¹)	$\mu_{\max_{ET}}$	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)	$\delta_{\max}$	76	70 - 83	

	Phage latent period (h)	τ	0.72	0.69 - 0.77	
	Transduction probability (phage ⁻¹)	α	1.0×10^{-8}	$8.5 \times 10^{-9} - 1.4 \times 10^{-8}$	
	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	
<i>Effect of erythromycin on B_E</i>	Max effect (h ⁻¹)	$\epsilon_{\max_{E,E}}$	1.10	-	This study
	Half maximal effective concentration	EC50 _{E,E}	3.42	-	
	Hill coefficient	H _{E,E}	0.67	-	
<i>Effect of erythromycin on B_T</i>	Max effect (h ⁻¹)	$\epsilon_{\max_{E,T}}$	3.91	-	
	Half maximal effective concentration	EC50 _{E,T}	9.26	-	
	Hill coefficient	H _{E,T}	0.22	-	
<i>Effect of erythromycin on B_{ET}</i>	Max effect (h ⁻¹)	$\epsilon_{\max_{E,ET}}$	0.95	-	
	Half maximal effective concentration	EC50 _{E,ET}	2.47	-	
	Hill coefficient	H _{E,ET}	0.79	-	
<i>Effect of tetracycline on B_E</i>	Max effect (h ⁻¹)	$\epsilon_{\max_{T,E}}$	5.40	-	
	Half maximal effective concentration	EC50 _{T,E}	81.43	-	
	Hill coefficient	H _{T,E}	0.30	-	
<i>Effect of tetracycline on B_T</i>	Max effect (h ⁻¹)	$\epsilon_{\max_{T,T}}$	1.66	-	
	Half maximal effective concentration	EC50 _{T,T}	7.27	-	
	Hill coefficient	H _{T,T}	2.41	-	
<i>Effect of tetracycline on</i>	Max effect (h ⁻¹)	$\epsilon_{\max_{T,ET}}$	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 10⁹ 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

numbers do not increase throughout the 24h period, regardless of transduction ability (Figure 3a, middle and bottom rows & Figure 3b, right).

Figure 3: a) Model-predicted dynamics with two single-resistant strains starting at carrying capacity (10⁹ colony-forming units (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). The starting strains are either erythromycin-resistant (B_E) or tetracycline-resistant (B_T). Antibiotics and/or phage (P_L) are present at the start of the simulation, at concentrations of 1 mg/L (2 x MIC for susceptible strains) and 10^9 plaque-forming units (pfu)/mL respectively. Double-resistant bacteria (B_{ET}) can be initially generated by generalised transduction only, and then by replication of existing B_{ET} . Dashed line indicates the detection threshold of 1 cfu or pfu/mL. **b) Change in bacteria (single-resistant to erythromycin, single-resistant to tetracycline, or double-resistant) and phage numbers depending on the antibiotic exposure, in the presence of phage capable of generalised transduction.** Dashed line indicates the detection threshold of 1 cfu or pfu/mL.

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

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

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

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

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

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

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

Figure 5: a-b) Varying timing (x-axis) and dose of antibiotic and phage (line colour) affects total bacterial count after 48h (top), maximum concentration of double-resistant bacteria (B_{ET}) (middle), and time when the concentration of B_{ET} is greater than 1 colony-forming unit (cfu) per mL (bottom).

a) Adding 10^9 plaque-forming units (pfu) per mL of phage, and between 0.25 and 2 mg/L of both erythromycin and tetracycline. **b)** Adding 1 mg/L of both erythromycin and tetracycline, and between 10^7 and 10^{10} pfu/mL of phage. The x-axis indicates the time when antibiotics were added, relative to when phage were added. For example, the value “4” indicates that phage were present at the start of the simulation, and antibiotics were introduced 4h later. **c) Phage and bacteria dynamics over 48h for 4 conditions taken from panel b.** In all 4 conditions, indicated by the 4 shapes, phage are initially present at a concentration of 10^8 pfu/mL, while erythromycin and tetracycline are both introduced at concentrations of 1 mg/L after either 0h (circle), 3h (square), 5h (star) or 15h (triangle) with the timing indicated by the vertical dashed lines. Horizontal dotted lines indicate bacteria remaining after 48h (corresponding to the top row of a-b) and maximum double-resistant bacteria (B_{ET}) concentration (middle row of a-b). Solid line indicates the detection threshold of 1 cfu or pfu/mL. The concentrations of single-resistant bacteria (B_E , blue, and B_T , green) overlap and cannot be distinguished.

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

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

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

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

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

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

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

Although we were not able to parameterise our model for antibiotic decay, our 24h time-kill curves suggest that antibiotics no longer decrease bacterial numbers after 24h (Supplementary Figure 6). Phage may also be affected by decay in the environment. Hence, we included these parameters in our partial rank correlation analysis. An increase in phage or antibiotic decay (γ_P , γ_E , γ_T) is correlated with an increase in maximum B_{ET} (Figure 6d, blue), but unexpectedly with a decrease in bacteria remaining after 48h (Figure 6d, red). This is explained by the fact that such an increase leads to a weakened killing pressure on B_{ET} , which are able to increase faster. This translates to a shorter time before the phage population is able to increase again due to enough bacteria being available for predation, and thus a shorter time before B_{ET} start decreasing due to phage killing (Supplementary Figure 7). This also explains why an increase in the transduction probability (α) is correlated with a decrease in bacteria remaining after 48h, as it leads to a faster increase in B_{ET} , and thus a shorter time before the phage are able to increase and eradicate the B_{ET} population. Finally, single-resistant bacterial growth rates (μ_{max_E} , μ_{max_T}) are not substantially correlated with either maximum B_{ET} or remaining bacteria, while double-resistant growth rate ($\mu_{max_{ET}}$) is only weakly positively correlated with maximum B_{ET} , and 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, δ_{max} : burst size, τ : latent
 488 period, α : transduction probability, γ_P : phage decay, γ_E : erythromycin decay, γ_T : tetracycline decay,
 489 μ_{max_E} : B_E growth rate, μ_{max_T} : B_T growth rate, $\mu_{max_{ET}}$: 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

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

Importance of transduction in the environment and during phage therapy

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

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

bacteria are cleared [6–9], but extend these to show that timing may also impact the risk for multidrug-resistant bacteria to be generated. Here, we suggest that the timing 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). Future studies and clinical trials of phage therapy should investigate varying timings of phage and antibiotics, instead of only investigating their simultaneous application, and consider the risk of transduction during treatment.

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

Limitations

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

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

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

Generalisability

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

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

The unique dynamics of phage, bacteria, and antibiotics

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

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

Conclusions

Our results demonstrate the complex synergy between phage and antibiotics to kill bacteria and drive the evolution of AMR. We suggest this synergy 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), further exacerbated by low concentrations of either phage or antibiotics. Interdisciplinary frameworks such as ours combining *in vitro* data and mathematical models are key to understanding both fundamental AMR evolution, and new interventions like phage therapy or screening for phage in patients. Our conclusions form hypotheses to guide future experimental work, notably for studies of phage therapy which should consider the risk for multidrug-resistance evolution by transduction, and investigate varying timings and concentrations of phage and antibiotics instead of only their simultaneous use.

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