A generalised model for generalised transduction: the importance of co-evolution and stochasticity in phage mediated antimicrobial resistance transfer

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Abstract

Antimicrobial resistance is a major global challenge. Of particular concern are mobilizable elements that can transfer resistance genes between bacteria, leading to pathogens with new combinations of resistance. To date, mathematical models have largely focussed on transfer of resistance by plasmids, with fewer studies on transfer by bacteriophages. We aim to understand how best to model transfer of

resistance by transduction by lytic phages. We show that models of lytic bacteriophage infection with empirically derived realistic phage parameters lead to low numbers of bacteria, which, in low population or localized environments, lead to extinction of bacteria and phage. Models that include antagonistic co-evolution of phage and bacteria produce more realistic results. Furthermore, because of these low numbers, stochastic dynamics are shown to be important, especially to spread of resistance. When resistance is introduced, resistance can sometimes be fixed, and at other times die out, with the probability of each outcome sensitive to bacterial and phage parameters. Specifically, that outcome most strongly depends on the baseline death rate of bacteria, with phage-mediated spread favoured in benign environments with low mortality over more hostile environments. We conclude that larger-scale models should consider spatial compartmentalisation and heterogeneous microenviroments, while encompassing stochasticity and co-evolution.

Introduction

Antimicrobial resistance (AMR) is a major global health threat; at least 700,000 deaths per year are attributed to bacterial infections by drug-resistant strains globally (O'Neill 2016). Of particular concern are mobilisable elements, which are important in the spread of resistance genes between bacteria through horizontal gene transfer (HGT), as reviewed by Partridge *et al.* (2018). This is one of the salient factors responsible for rapid global spread of infections carrying resistance genes, e.g. NDM-1 (Dortet, Poirel and Nordmann 2014). Much research on spread of resistance, both empirical and modelling, has focussed on spread by conjugation, i.e. plasmids (Levin and Stewart 1980; Volkova *et al.* 2012; Volkova *et al.* 2013; Baker *et al.* 2016). However, very few models have considered gene mobilization by bacteriophage transduction, despite being the most abundant biological entities on the planet, with an estimated 10^{23} bacteriophage infections occurring every second (Pawluk 2017). Bacteriophage can

acquire gene segments from bacteria they infect and pass them on to other bacteria upon further infection (Snyder *et al.* 2013). Transduction can be primarily classified as specialised or generalised transduction. Specialised transduction results from the imprecise excision of a prophage in temperate phages, causing accidental packaging of the regions flanking the prophage insertion site in bacterial chromosome (Kwoh and Kemper 1978), while generalised transduction is the erroneous packaging of a random piece of bacterial DNA only. Thus, generalised transduction causes the formation of transducing particles, which may carry any genes including resistance genes. Phage communities are prevalent in environments important for spread of antimicrobial resistance, including human and animal intestines (Dhillon *et al.* 1976; Dhillon *et al.* 1980; Clokie *et al.* 2011; Caporaso, Knight and Kelley 2011) and consequently faecal waste and waste streams (Smith *et al.* 2018). Most of these phages are capable of generalised transduction *in vitro* (Schicklmaier and Schmieger 1995; Schicklmaier *et al.* 1998). Thus there is growing evidence provided by the metagenomic data to suggest that phages could play a vital role in the acquisition of resistance genes (Balcazar *et.* 2014; Moon *et al. 2015*; Haaber *et al.* 2016; Lekunberri *et al.* 2017; Keen *et al.* 2017; Lood, Ertürk and Mattiasson 2017), whether by generalised or specialised transduction or releasing transformable DNA on cell lysis (Keen *et al.* 2017). Prophages are capable of carrying resistance genes (Moon *et al. 2015*; Haaber *et al.* 2016), as are environmental bacteriophages (Balcazar *et.* 2014; Lekunberri *et al.* 2017), but resistance load in bacteriophages associated with bacterial communities is often neglected (Lood, Ertürk and Mattiasson 2017). However, the information that phages carry resistance genes was previously contested by Enault *et al.* (2017), where they use bioinformatic tools to evaluate relevant metagenome data for identifying known antibiotic resistance genes (ARGs) and determine that recent virome data suggesting greater role of phages in ARG carrying and transfer is due to high bacterial DNA or contains false positives due to relaxed thresholds of e-value in database searches. Thus, the study concludes that the growing concern might not be as vital as reported.

Mathematical modelling has been helpful in understanding factors associated with AMR emergence and spread (Gerrish and García-Lerma 2003; Murphy, Walshe and Devocelle 2008; Ayscue *et al*. 2009; Bell *et al*. 2014) and has brought to light the importance of HGT in the spread of AMR (Gehring *et al.* 2010; Baker *et al.* 2016). Most of these studies focused on conjugation as a means of HGT with only a few models on transduction as a means of HGT (Volkova *et al.* 2014; Tazzyman and Hall 2015; Moura De Sousa and Rocha 2019). Volkova *et al.* (2014) provide a good theoretical understanding of the roles of specialised and generalised transduction, and suggests that transduction contributes to HGT on the order of a thousand times less than conjugation. However, the study assumed a high density of enteric bacteria in a well-mixed system with continuous inflow and outflow of biomass, since the aim was to understand the dynamics in intestines of cattle. In contrast, in many environments, bacteria occur at lower densities and may live in spatially structured local communities for long periods of time. In these communities, phages co-exist with these bacteria, infecting and lysing the bacteria in their host range. Even in a gut, local biodiversity providing a wide host range for many phages and rapid viral turnover suggest that local dynamics might be important. Tazzyman and Hall (2015) focus on determining the long-term persistence of antibiotic resistance dependant on fitness cost and mutation rates, but ignores other parameters such as the adsorption, desorption and DNA injection rates. Moura de Sousa and Rocha (2015) discuss the affects of environmental structure on the resistance against antibiotics and phages, utilising an individual based model (eVIVALDI) to understand how environmental structure might have an effect on bacterial populations of different types. There is a clear difference in resistance fixation between well-mixed and spatially structured environments. Fixation refers to resistance persisting and spreading throughout the whole bacterial community instead of being lost after first few generations due to death of resistant bacteria before spread. Their results show that spatial structure plays a major role in phage-bacteria interactions, thus affecting long-term resistance

persistence, although the affects of other parameters are presented as part of a model for lysogenic phages but not for lytic phages.

We develop mathematical models for the spread of resistance by generalised transduction, which we have analysed specifically within a small volume compartment, in order to consider local effects. We focus specifically on lytic phages. Strictly lytic bacteriophages are not able to access the lysogenic life cycle and therefore can only transfer genes via generalised transduction, rather than specialised and/or lateral transduction of temperate phages, thus allowing for a model specifically focused on generalized transduction. However, while lytic phages are capable of generalised transduction, how important this is remains largely unknown and no models currently exist to predicts its role in the transfer of ARGs.

A bacterial community could be divided into sub-communities through physical compartmentalisation, or through characteristics of interest, such as strain, host range, phage immunity or antibiotic resistance. These bacteria of interest may be present in one location but not another. We compare output of deterministic versions (applicable to large well-mixed populations) with stochastic versions of the models (applicable to small populations where random events may be significant). While the aim of this work is to model the spread of resistance by lytic phages, purposefully start with a deterministic base model of phage infection without resistance. This model is defined by a set of Ordinary Differential Equations (ODE), similar to models used to understand and develop phage therapy (Cairns *et al.* 2009), but with separate adsorption and phage DNA injection terms as described by Smith and Trevino for multiple host binding sites in phage infection (Smith and Trevino 2009). We show that such a model predicts total extinction of bacterial and phage population for a wide and realistic range of parameter values, rather than the co-existence of phage and bacteria seen in the environment. Previous studies into well-mixed host phage systems corroborate this lack of stable co-existence (Levin, Stewart and Chao 1997). Antagonistic co-evolution – evolution of predator and prey species to adapt against

each other – of phages and bacteria (Luria and Delbrück 1943; Buckling and Rainey 2002; Gómez and Buckling 2011; Koskella and Brockhurst 2014) has been long-established as a mechanism to stabilize host-phage ecology, whether this co-evolution is mutational (Chaudhry *et al.* 2018; Pagliarini and Korobeinikov 2018), through CRISPR-Cas systems (Childs *et al.* 2012; Iranzo *et al.* 2013;, or through phase variation (Aidley *et al.* 2017). Therefore, we extend the simple model by introducing a stability mechanism in the form of a fluctuating mutational host immunity and susceptibility via "leaky (phage) resistance" (Chaudhry *et al.* 2018) – a general term to categorise the process of bacteria losing their phage immunity. We do not model the conditions behind this fluctuating immunity in bacteria so as to let the model be applicable to wide range of mechanisms. Thus, this model can be considered as a simplified representation of antagonistic co-evolution, where the host mutation for immunity corresponds to the evolution of bacteria whereas host mutation for susceptibility corresponds to phage evolution or it could even represent the phenotypic changes in immunity due to CRISPR-Cas systems (although again highly simplified). This formulation has the advantage of avoiding complicated systems of equations, for example for open-ended populations of phage and bacterial strains as a continous evolutionary process, and so is particularly useful for model analysis, e.g. through sensitivity analysis. We show that such mutational changes facilitate stable co-existence of host and phage populations, even in small compartments. We then use this phage dynamics model as a base to develop and analyze the model for phage-mediated spread of antimicrobial resistance, which shows the importance of stochastic affects for proliferation of resistance.

Methods

We used the R deSolve package's LSODA algorithm (Soetaert, Petzoldt and Setzer 2010) to solve the differential equations and the rootSolve, doParallel and foreach packages for sensitivity analysis. For the stochastic model we used COPASI (Hoops *et al.* 2006) to implement the Gibson-Bruck next reaction

algorithm (Gibson and Bruck 2000) and created shell scripts to run each model one thousand times. The output of each run was then imported in R and the graphs were created with the average of all the runs for comparison with the ODE model output. For creating the heatmap for sensitivity analysis, the ggplot2 (Wickham 2009) package of R was used.

Model description

We model a scenario with a predominant antimicrobial sensitive population in which a single resistant cell was introduced. The modelled volume is small (2.5 µL), such that the maximal carrying capacity is only 168 CFU ($6.71 \times 10^7 \left(\frac{CFU}{L}\right) \times 2.5 \times 10^{-6}(L)$). A schematic representation of the three models is provided in Fig. 1, with the base model represented in red, addition of phage immunity in blue and the transduction process in presence of antibiotic in green.

The latter two models build on the base model with further additions. Each circle represents the different populations of bacteria (S, S_{inf}, S_V, S_{imm}, S_{V_R}, R, R_{inf}, R_V and R_{imm}), phages (V) and transducing particles (V_R) whose concentrations are governed by kinetic processes denoted by arrows. The bacterial population sensitive to antimicrobial are denoted by 'S' and those resistant by 'R'. Bacteria with phage infected (inf), immune (imm) and with adsorped phage (V) or transducing particles (V_R) are denoted by the relevant subscripts. The base model defines the process of phage infection, with the kinetic processes of phage adsorption, desorption and infection separated. The second model builds on this by including equations for a phage immune bacterial population. Phage immunity can be gained (and lost) due to a range of possible mechanisms; as such the phage immune population is tied to the phage susceptible population by the mutational rates towards susceptibility and immunity. The third model – for transduction – builds on the second model by including equations for the corresponding antimicrobial resistant populations of bacteria along with the transducing particle adsorbed population as an intermediary population between antimicrobial resistant and susceptible bacteria.

The model equations can be found in Supplementary File 1; model parameters are given in Table 1. We use the logistic growth model for bacterial growth, i.e., bacteria will grow to a maximal carrying capacity (N_{max}), but have included a separate term for the baseline death rate of bacteria (δ_S and δ_R). The interaction between bacteria (S and R) and phage (V) or transducing particles (V_R), which determines the rate at which the phages and transducing particles adsorb (τ) to bacteria, follows the classic predator-prey interaction model defined by Lotka-Volterra equations as well as other phage infection dynamics models (Beretta and Kuang 1998). Bacteria with adsorbed phages (S_V and R_V) can either lose the phages via desorption (τ_{-1}) to revert to their native state (S and R) or become infected (S_{inf} and R_{inf}) dependent on phage DNA injection rate (ι). Bacteria with adsorbed transducing particles (S_{V_R}) become uninfected resistant bacteria (R) on DNA injection. The processes of phage DNA injection or desorption are much faster than phage adsorption on the bacterial cell surface, therefore we do not include the growth of bacteria with adsorbed phages in our equations.

Parameters used in the models

Table 1: All parameters used in the full model, some of which are also used in the simpler model variants, see equations (1)-(50). This table provides all the parameters used in the equations that are described here. All of the parameters define some process in the transduction model but only a few of them are used in the equations for the phage infection dynamics and antagonistic co-evolution models. Note that we explore the full range of fitness costs for the sake of complete model analysis, recognizing that fitness costs above 0.3 are unlikely to persist in nature, although could arise through spontaneous mutation. Note also that the mutation rate is given in units per hour rather than per cell division; for any given values of growth rate r and mutation rate M_B the per division mutation probability could be thought of as being $1-2^{-MB/r}$.

Parameter	Description	Value (Range)	Source
r	Specific growth rate	**0.5** (0.17-0.9) h^{-1}	Curds 1971; Godwin and Slater 1979; Levin, Stewart and Rice 1979.
N_{max}	Carrying capacity of liquid slurry	$\mathbf{6.71 \times 10^{7}}$ CFU/L	Ibrahim *et al.* 2016.
δ_R	Natural death rate of bacteria	**0.025** (0.0125-0.336) h^{-1}	Kudva, Blanch and Hovde 1998.
δ_S	Death rate of antibiotic sensitive bacteria	**0.025** (0.0125-0.336) h^{-1}	Kudva, Blanch and Hovde 1998.
τ	Phage adsorption rate constant	$\mathbf{4.32 \times 10^{-11}}$ (2.66×10^{-12}-1.26×10^{-10}) L h^{-1}	Moldovan, Chapman-McQuiston and Wu 2007.
τ_{-1}	Phage desorption rate constant	**3.06** (1.368-19.44) h^{-1}	Moldovan, Chapman-McQuiston and Wu 2007.
ι	Phage DNA injection rate constant	**2.88** (0.72-6.12) h^{-1}	Moldovan, Chapman-McQuiston and Wu 2007.
δ_I	Death rate due to phage infection	**1** (1-2.86) h^{-1}	Unpublished empirical data
B	Burst size of bacteriophage i.e., number of phage progeny from infected cell on its lysis.	**200** (16-200)	Unpublished empirical data
δ_V	Degradation rate of phages and	**0.003** (0.0015-0.0121) h^{-1}	De Paepe and

	transducing particles		taddei 2006.
α	Fitness cost for antibiotic resistance	**0.1** (0-0.99)	Godwin and Slater 1979; McDermott, Gowland and Gowland 1993; Subbiah *et al.* 2011.
ϕ	Fitness cost for phage immunity	**0.05** (0-0.99)	Volkova *et al.* 2014.
η	Fraction of transducing particles in total phages from burst cell – equal to probability of accidentally packaging antibiotic resistance genes by phage.	**0.02**	Volkova *et al.* 2014.
M_B	Mutation rate of phage susceptible bacteria to phage immune bacteria	**0.2** (0-0.99) h^{-1}	Assumed
M_V	Mutation rate of phages turning immune bacteria to susceptible	**0.1** (0-0.99) h^{-1}	Assumed
E_{max}	Maximum effect of antibiotics on bacterial growth	**2**	Volkova *et al.* 2012.
H	Hill coefficient in E_{max} model	**2**	Volkova *et al.* 2012.
MIC_S	MIC for sensitive bacteria	**8** $\mu g\ L^{-1}$	VMD DEFRA (2012)
MIC_R	MIC for resistant bacteria	**2000** $\mu g\ L^{-1}$	VMD DEFRA (2012)
A	Antibiotic concentration in microcosm	**5.6** $\mu g\ L^{-1}$	Baker *et al.* 2016.

The default parameter values are, where possible, taken from measurements for *E. coli* and coliphages isolated from slurry (Smith *et al.* 2015; Sazinas *et al.* 2018) and antibiotic related data for cefquinome, as an environmental example, with parameter values from other sources marked in Table 1; however, the sensitivity analyses test broad ranges of parameter values (Table 1) and so the results are applicable to a much wider range of environments and bacteria. The *E. coli* genome size is about 5,000 kbp (http://www.ncbi.nlm.nih.gov/). A transducing phage particle is assumed to carry 100 kbp of packaged DNA (based on available estimates of *E. coli* phages[8]), which amounts to approximately 2 percent of the total bacterial genome. We assume that if the transducing particle injects the DNA, the resistance carrying gene will always be incorporated in the recipient's genome (Fig 1).

The antibiotic concentration is necessarily kept constant in the model at a value lower than the MIC (sub-inhibitory) of sensitive bacteria to allow for the growth of bacteria, as well as match the observed values modelled by Baker et al (1996). Modelling antibiotic concentrations above the MIC would not provide meaningful results on spread of resistance as the bacterial populations would die out. Moreover, sub-inhibitory concentrations of bacteria are known to promote horizontal gene transfer, as well as provide selective pressure for resistant bacteria (Andersson and Hughes 2014).

Morris method and samples

A global sensitivity analysis was performed using the Morris sampling method (Morris 1991; Sin, Gernaey and Lantz 2009), as this method can be used for both deterministic and stochastic models; methods that rely on small perturbations (Baker *et al.* 2016) are not suitable for stochastic models. The method estimates the elementary effects of each input parameter on the desired model outcome. The elementary effect of each is calculated using Eq. (1) and then sigma-scaled (i.e., scaled by standard deviation of inputs and outputs) for standardized comparison of the elementary effects of different parameters.

$$EE_j = \frac{Y(x_1,x_2,x_j+\Delta,\ldots,x_m)-Y(x_1,x_2,x_j,\ldots,x_m)}{\Delta} \quad (1)$$

Where $Y(x_1,x_2,x_j,\ldots,x_m)$ is the model output at input parameters x_1, x_2, x_j, ..., x_m and $Y(x_1,x_2,x_j+\Delta,\ldots,x_m)$ is the output corresponding to a specific change (Δ) in input parameter x_j. The range for each parameter x_j is divided into p=20 levels and each perturbation of the input parameter chooses a value corresponding to this level. Δ is set as $\Delta = \frac{p}{2(p-1)} = 0.53$. Calculation of k elementary effects requires k+1 simulations, with a number of repetitions (r) for each giving a total of $r \times (k+1)$ simulations.

The elementary effect of a parameter can be negligible, a constant or a non-constant function of factor x_i or a non-constant function of more than one factor. The analysis is done using the mean and standard deviation of the scaled elementary effects. Parameters with linear effects will have a standard deviation of zero with non-zero mean. Parameters with a mean less than the standard error of the mean can be considered to have a negligible effect.

Results

Base model without phage immunity predicts host and phage extinction

The base model describes the rate of change of the populations of uninfected bacteria (S), bacteria with adsorbed phages (S_V), infected bacteria (S_{inf}) and free phages (V) (Fig. 1 in red). We represent these biological processes in two related models: a deterministic ODE model and a stochastic model. A detailed description of each is given in the supplementary file.

Steady-state analysis of the ODE model gives conditions for the stable existence of the three steady-states: extinction of both bacterial host population and phage (both populations are zero), phage extinction (phage population is zero, thus no infection, while the bacterial population is non-zero) and

co-existence (in which both populations are non-zero; see Supplementary Information for detailed mathematical analysis). Total extinction in the ODE model only occurs when the bacterial death rate is greater than the growth rate, as would be expected. However, model simulations (Fig 2) show that even in the co-existent state, bacterial numbers can be very low (< 3 CFU). These low numbers motivate the use of a stochastic model, because in stochastic models with random processes, bacterial or phage extinction might be a likely outcome. In the stochastic model, the biological processes shown in Fig. 1 are represented as a set of discrete events happening within the microcosm (see Supplementary Table S1 for details of the stochastic reaction scheme), and so it is possible to investigate whether the small numbers of bacteria lead to bacterial or phage extinction.

Simulating both the ODE and stochastic models for 100 days shows a clear difference between the two types of models (Fig 2) under the default parameter values. The stochastic model predicts extinction of the bacterial and phage populations, whereas the ODE model predicts coexistence between the bacterial and phage populations, albeit with low bacterial numbers (< 3 CFU in total). Therefore, it can be surmised that inclusion of stochastic effects can lead to extinction (zero individuals) in small populations even when the parameter values lie outside of the conditions for extinction of both bacterial host population and phage derived from the ODE model. This highlights the importance of including random events into these models.

Extinction is a dominant outcome in the base model for a wide range of realistic bacteriophage parameters

In order to demonstrate that the base model is inadequate, we simulate the base model not just for the default parameter values, but also for a wide range of realistic parameter values (Table 1). We focus on phage parameters because the size of the bacterial population in the co-existent steady state is sensitive

to all five phage parameters (burst size, adsorption, desorption, DNA injection and degradation rates), but not to bacterial parameters (see Supplementary Figure S1). To set up the simulations, we have taken realistic sets of 22 parameter values for phage adsorption, desorption and phage DNA injection rate (Supplementary Table S7) under varied growth conditions (including temperature and nutrient availability) (Moldovan, Chapman-McQuiston and Wu 2007), treating the three phage infection parameters as correlated, while allowing the phage degradation rate and burst size to vary independently (Table 1). This provides a sensitivity analysis of the outcome of stochastic simulation (extinction of bacteria and phage; phage extinction; co-existence) across all five parameters to which the outcome could be sensitive. For each parameter variation, 1000 simulations were performed. Fig 3 shows the outcomes for 9 of the 22 different cases, that between them cover the full range of parameter values investigated. The full set of 22 cases are provided in Supplementary Figure S3.

The simulations show a clear change in the output scenarios with increasing adsorption rate, with little change due to phage DNA injection rate (Figure 3). Out of the 22 cases in Supplementary Table S7, only one case (1°C in maltose media) does not result in extinction of both bacterial host population and phage. In all other cases, higher burst size and lower degradation rate cause extinction, whereas co-existence is seen only at low burst size and high degradation rate. This result is telling, because such coexistence may be evolutionarily unstable as a case of a "tragedy of the commons" (Hardin 1968; Kreft 2004; Kerr *et al.* 2006; MacLean and Gudelk 2006; Rankin *et al.* 2007), in which the shared resource are the bacteria (prey): the system would be driven to extinction because phages with higher burst size and lower degradation rate would outcompete phages with low burst size and high degradation rate. These results suggest that the base model cannot explain the environmental co-existence of bacteria and phage, because it provides unrealistic outcomes for all realistic values of phage parameters, and so is not a good starting point for modelling phage-mediated spread of resistance. This limitation can be overcome by introducing fluctuating host immunity into the model.

Co-existence requires antagonistic stable host immunity

It is long established that bacteria and phages are constantly co-evolving: the bacteria evolve to become immune to phage infection, then phages evolve to be able to infect the evolved bacteria (Luria and Delbrück 1943; Buckling and Rainey 2002; Gómez and Buckling 2011; Koskella and Brockhurst 2014). We introduce a minimal co-evolution model, in which phage susceptible bacteria evolve to become immune, while phage evolution is represented indirectly by phage immune bacteria evolving to become phage susceptible again, at a rate of mutation corresponding to the rate of mutation of phages (Fig. 1:Phage immunity model, blue outline). This gives a modified set of equations (Supplementary File 1) including a new population of phage immune bacteria (S_{imm}) and corresponding new terms which define the process of evolution from phage susceptible to phage immune bacteria and reversed evolution from phage immune back to phage susceptible.

The co-evolution model is deliberately as simple and general as possible: these processes could be seen as mutational (in which phage mutation is represented by a change in the bacterial population), or associated with phase variation (with only two variants) or a simple CRISPR-Cas system (noting that it is an extreme simplification). Phage immunity causes a fitness cost for the bacteria, reducing their growth rate. In view of the above differences between deterministic and stochastic simulations, we define an equivalent set of discrete events for the stochastic simulation algorithm (Table S6). Fig 4 shows the concentrations of different bacterial and phage populations. In contrast to the base model without co-evolution, these simulations consistently show co-existence of bacteria and phages, with full agreement between the deterministic and stochastic versions of the model. The phage immune bacteria buffer the bacterial population from which new phage sensitive variants continuously emerge. Therefore, bacterial phage immunity benefits both bacterial and phage populations.

Sensitivity analysis of co-evolution model highlight the importance of phage and host factors

To determine the sensitivity of the outcomes of the stochastic version of the co-evolution model to its 11 different parameters, we used the Morris method (Morris 1991) for sensitivity analysis, as described in the Methods. The parameter with the strongest effect on outcomes across all three scenarios is the adsorption rate, τ: increased adsorption increases the probability of extinction of both bacterial host population and phage while decreased adsorption increases the probability of co-existence or phage extinction (Fig 5). The death rates of sensitive cells (δ_S), and fitness cost of phage immunity (ϕ), show a similar pattern – increasing the values of these parameters increases chances of extinction of both bacterial host population and phage, and decreases the chances of co-existence. For the third scenario of phage extinction, both these parameters are not sensitive. The bacterial growth rate, r, shows the reverse pattern, with increased growth rate leading to increased co-existence. Phage extinction and extinction of both bacterial host population and phage are also sensitive to the phage decay rate (δ_V) and desorption rate (τ_{-1}), whose increase leads to increased probability of phage extinction, and whose decrease leads to increased probability of extinction of both bacterial host population and phage. It should be noted that none of the parameters have zero standard deviation with non-zero mean, indicating that all inputs either have a non-linear effect or are involved in interactions with other inputs.

Stochastic transduction model with antagonistic co-evolution facilitates quantification of risk of resistance gene spread

We now consider the impact of the transfer of antibiotic resistance genes in an environment containing an antibiotic by extending the antagonistic co-evolution model. In this model, spread of resistance occurs when lytic phages can pick up random gene segments from the host genome, whether

present on chromosome or plasmid, and transduce them into other cells rather than infect them. Details of this full model are given in Fig 1 (Transduction model, green outline).

Fig 6 shows that the deterministic model (blue lines), with default parameters as described in Table 1, predicts a phage-mediated spread of resistance through the bacterial population. In contrast, the stochastic simulations (red lines) predict two different outcomes, with transfer of resistance, also predicted by the ODE model, being somewhat more frequent (54.2%) (Fig 6(a)) than loss of resistance (Fig 6(b)) for these parameter values. This suggests that random events may have a considerable impact on whether phage mediated resistance will spread locally, even in the presence of antibiotic selection.

In order to assess the sensitivity of the spread of resistance to these parameters, we again use the Morris method on the stochastic model, to calculate elementary effects of 12 parameters, each parameter perturbed 50 times, giving a total of $50 \times (12 + 1) = 650$ input case scenarios. Again, each scenario is simulated 1,000 times and classified into two categories: no resistance spread and resistance spread. The mean of all outputs of a particular category gives the overall output for that input scenario. The most striking result is that the no-resistance steady state is most sensitive to the baseline bacterial death rate (Fig 7): increased death rate reduces spread of resistance. Similarly, increased fitness cost for carrying the resistance genes, and, to a lesser extent, increased fitness cost of phage immunity also promotes the no resistance steady state. Increasing bacterial growth rate has a much smaller, positive effect on resistance spread.

Discussion

In order to model the spread of ARGs through transduction by obligate lytic bacteriophages, we started with a base model of phage infection that describes the dynamics between phage and bacterial populations, and which includes phage adsorption, resorption and phage DNA injection terms. While we

used default parameter values for *E. coli*, where possible, and relevant phage populations, the model itself is quite flexible, and can easily be applied to other bacterial or phage populations by changing the parameter values. Using a realistic range of phage related parameter values, specifically, phage adsorption, resorption, DNA injection rates, burst size and phage degradation rate, we found that for most cases, the model predicts extinction of both bacteria and phage population, for continued co-existence, phages need to have a low burst size and high degradation rate to avoid over-exploiting their resource of host bacteria. Such restraint from over-exploitation is not an evolutionarily stable strategy (Smith and Price 1973) as mutant phage with higher burst size or lower degradation rate are likely to outcompete phage with lower burst size or higher degradation rate. This leads to a "tragedy of the commons" situation (Hardy 1968), in which selfish interest to increase exploitation of a resource (the bacterial host), would lead to selection for phages that exploit their host population more effectively, leading to over-exploitation of the resource; ultimately the resource can no longer support the population, to the detriment of all sharing the resource (Kreft 2004; Kerr *et al.* 2006; MacLean and Gudelj 2006; Rankin *et al.* 2007). In this case, due to a trade-off between latent period and burst size (it takes longer to make more phage), mutant phage that are less economical in their resource use outcompete the wild type at higher host densities (Abedon, Hyman and Thomas 2003). A similar trade-off between growth rate and growth yield of microorganisms means that a less resource consuming strategy with a higher growth yield but a lower growth rate is replaced in chemostat environments by the resource over-exploiting strategy of fast but inefficient growth. In biofilms, where spatial structure is important, the economical strategy is advantageous (Kreft 2004). We concluded that the simple base model is not sufficient to describe phage dynamics, and so not suitable for study of phage-mediated spread of resistance, which led us to includes host phage immunity, and phage evasion of immunity, into the model. The way we have modelled phage evasion of immunity is better explained

as loss of immunity. Another modeling study has shown that a CRISPR-Cas model of coevolution as well as loss of immunity is better suited to explain coexistence in certain cases (Weissman *et al.* 2018).

The second model includes antagonistic co-evolution between phage and bacterial host, and the results match the environmentally observed dynamics of continued co-existence. Because the second model explicitly includes mechanisms of phage-host interaction, we were able to use sensitivity analysis to identify those parameters to which continued coexistence are most sensitive. Adsorption rate to be the most sensitive parameter for co-existence, but it is not just the phage parameters which affect the output - bacterial growth rate also has an effect on chances of co-existence. Thus, bacteria in a resource rich environment with slow acting phages will survive longer than they would with fast acting phages. The other two scenarios for this model – phage extinction or extinction of both bacterial host population and phage are sensitive to more parameters than the co-existence scenario.

Having established a suitable modelling approach for phage-host interactions, we introduced antimicrobial resistant strains of bacteria to understand the dynamics of resistance spread via generalised transduction. We showed that stochasticity can play an important role in spread of resistance at low numbers of initial population, as resistance bacteria and/or transducing particles may die before transferring resistance carrying genes. Again, having a detailed phage model allowed us to use sensitivity analysis to identify whether spread of resistance is more sensitive to bacterial or phage parameters: spread is most sensitive to the environmental death rate of bacteria, the fitness cost for carrying the resistance genes, and the fitness costs for phage immunity. Thus, factors hampering the growth of resistant, phage immune bacteria and their rate of death have the most positive affect towards curbing resistance transfer, while other factors are less important.

Antagonistic co-evolution between bacteria and phage has long been known to occur (Luria and Delbrück 1943), and is easily studied in laboratory conditions (Luria and Delbrück 1943; Buckling and

Rainey 2002). However, there are many ways in which co-evolution could occur. We have chosen a minimal model, that treats viral evolution as equivalent (if faster) to bacterial evolution, and that could be interpreted as mutational, phase variational or CRISPR-Cas driven co-evolution. A more realistic model would consider multiple strains of bacteria and phage, with each new strain immune to the previous strains of phages but susceptible to the future strains of phages, essentially in an open ended way; such models have been used to model evolution of viral infections (Nowak *et al.* 1991), and can be readily analysed with some simplifying assumptions (May, Stekel and Nowak 1997). Another approach would be to consider strain type as a continuous variable, in a way that can demonstrate continuous evolution, at the expense of some realism (Pagliarini and Korobeinikov 2018). In principle, the model will assume continuous bacterial and phage evolution as suggested for an open-ended model, but drastically limit the number of equations by treating the strain types as continuous variables in an unbounded space instead of discrete variables. It is also possible to be more explicit about phase variation loci for bacterial escape from phage; this leads to bounded models (Aidley *et al.* 2017) - with only one particular strain of bacteria producing different proteins to confer immunity, hence no need for open-ended models or unbounded space considerations. Such a model would be easier to analyse and have a lower cost to simulate. Another possible biological mechanism for phage susceptibility would be a CRISPR-Cas system as modelled by Iranzo et al. (2013). With the growing knowledge of the CRISPR-Cas systems, their importance in different biological functions is also being discovered and when considering resistance spread due to phages it would be necessary to take into account not only the phage immunity provided but also the effect this will have on resistance transfer via transduction (Watson, Staals and Fineran 2018).

Our model considers a single environmental microcosm, in contrast to the Volkova model for phage-mediated resistance transfer, which considers a larger volume (Volkova *et al.* 2014) that, for microorganisms, is equivalent to a landscape scale (Battin *et al*. 2007) There is obviously a need for such

large-scale models for spread of resistance, whether in a mammalian gut, soil, slurry or other relevant environment. However, we would argue that none of these environments are well-mixed: they will all contain sub-compartments, either imposed by physical boundaries, or as a consequence of bacterial diversity interacting with phage host range. The extinction probabilities we have observed in the stochastic base model are likely to depend on the initial population of resistant cells in the environment. However, the key point we wish to make is that local extinction is often likely, especially with exchange of phages or resistant bacteria from nearby locales. For example, a completely sensitive bacterial population might be invaded by a single resistant cell or a number of resistant cells from neighbouring micro-populations. Our simulations demonstrate that these small-scale environmental considerations are likely to be important, and that larger models consisting of connected communities, e.g. a metacommunity model (Hanski 1998), are more likely to be realistic than homogeneous models described by ODEs. Moreover, the stochastic outcomes of our model, in particular that resistance might be fixed or eliminated from a microcosm, also suggest that the dynamics on larger spatial scales are likely to be spatially heterogeneous.

The probability of loss of resistance was found to be particularly sensitive to four parameters: bacterial death rate, fitness cost of carrying resistance genes, fitness cost of phage immunity, and (inversely) to the bacterial growth rate. Fitness costs are under evolutionary pressure, so we would expect to find that fitness costs of phage and antibiotic resistance would tend to decrease over time, and this would lead to increased phage-mediated spread of resistance. However, antibiotic or phage resistance mechanisms might have intrinsic costs that cannot be alleviated. The death and growth rate sensitivities suggest that phage-mediated spread of resistance will increase with decreased death rate and increased growth rate. This suggests that phage-mediated spread of resistance is more likely in favourable environments, for example the gut lumen, with high levels of nutrients, or sub-lethal concentrations of antimicrobials (Andersson and Hughes 2014). Phage mediated spread is less likely in

hostile environments, with low nutrient and high antimicrobial concentrations, for example in a slurry tank. Nonetheless, lytic phages bearing resistance genes have been identified in slurry (Smith *et al.* 2015), although these may have originated in mammalian guts.

In conclusion, we have shown that to model spread of resistance by transduction, it is necessary to consider antagonistic co-evolution, stochastic and local effects. Sensitivity analysis suggests that phage-mediated transfer of resistance is decreased in a more toxic environment, or when fitness costs of resistance or phage immunity are higher. Other factors have less effect on preventing spread of resistance by transduction.

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Acknowlegements

We are grateful for access to the University of Nottingham High Performance Computing Facility.

Competing Interests

The authors declare that they have no competing financial interests.

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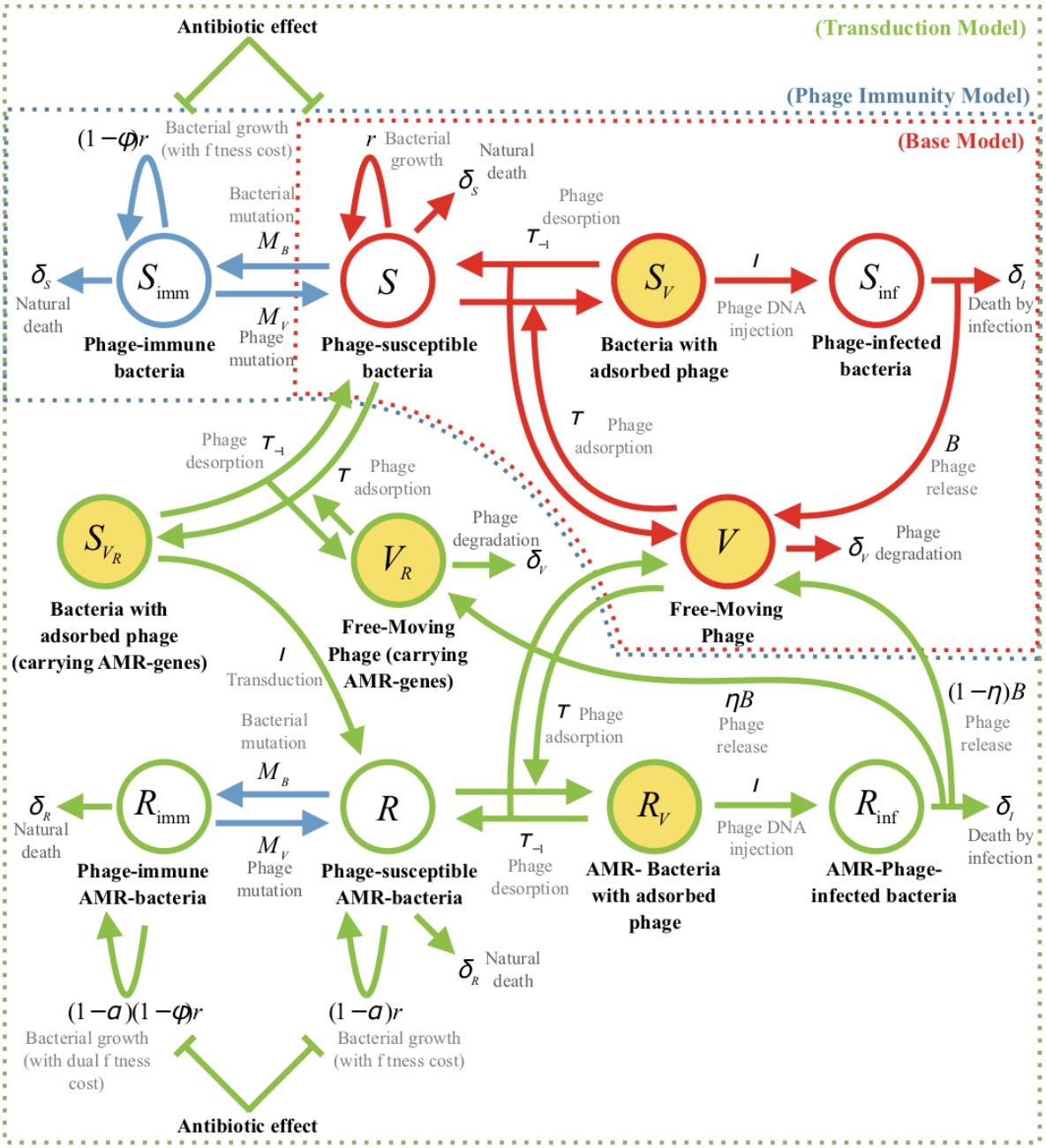

Fig. 1: **Schematic representation of the base model (red), phage immunity model (blue) and transduction model (green).** Each circle represents the different populations of bacteria (S, S_{inf}, S_V, S_{imm}, S_{V_R}, R, R_{inf}, R_V and R_{imm}), phages (V) and transducing particles (V_R) whose

concentrations are governed by kinetic processes denoted by arrows. The model equations can be found in Supplementary File 1; model parameters are given in Table 1. We use the logistic growth term for bacterial growth, i.e., bacteria will grow to a maximal carrying capacity (N_{max}), but have included a separate term for the baseline death rate of bacteria (δ_S and δ_R). The interaction between bacteria (S and R) and phage (V) or transducing particles (V_R), which determines the rate at which the phages and transducing particles adsorb (τ) to bacteria, follows the classic predator-prey interaction term used in Lotka-Volterra equations[64,65] as well as other phage models[66]. Bacteria with adsorbed phages (S_V and R_V) can either lose the phages via desorption (τ_{-1}) or become infected (S_{inf} and R_{inf}) on phage DNA injection (ι). Bacteria with adsorbed transducing particles (S_{V_R}) become uninfected resistant bacteria (R) on DNA injection. The processes of phage DNA injection or desorption are much faster than phage adsorption on the bacterial cell surface, therefore we do not include the growth of bacteria with adsorbed phages in our equations.

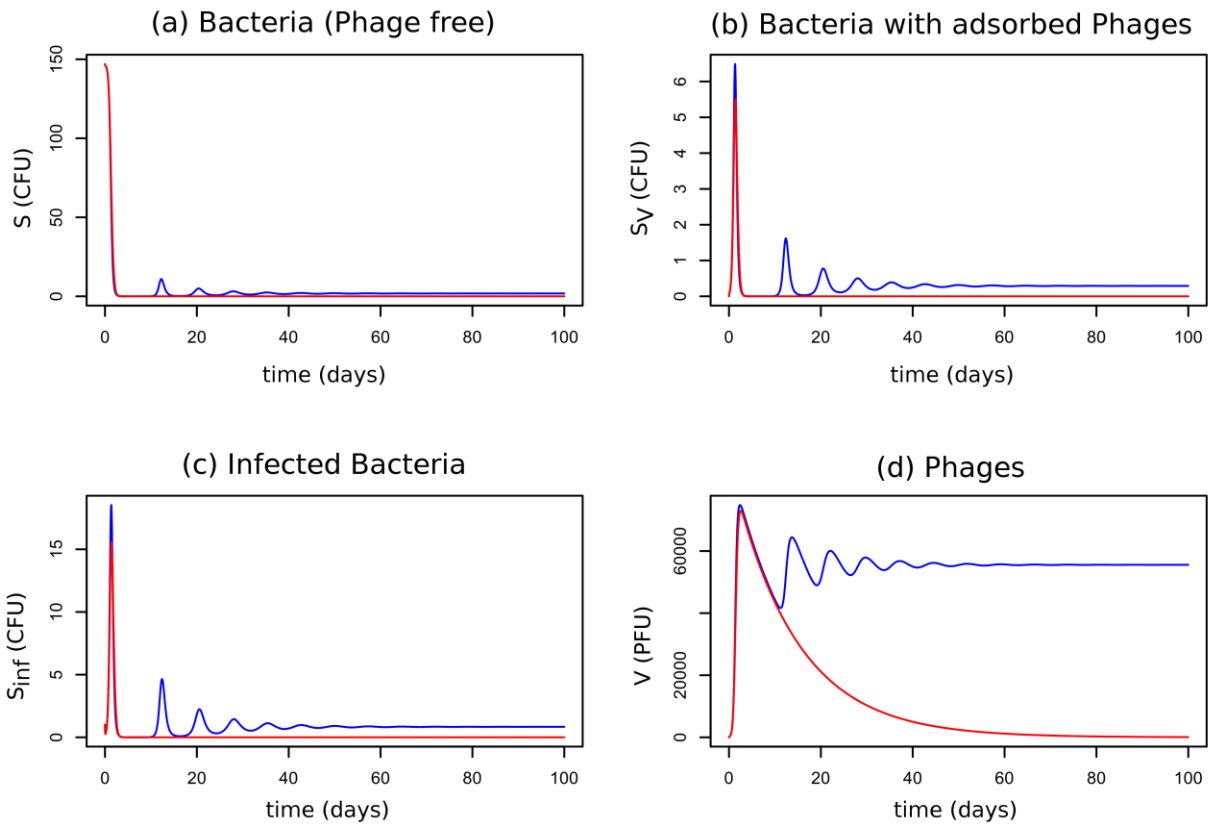

Fig. 2: **The ODE model (blue) predicts co-existence while the stochastic model (red) predicts extinction.** The blue lines represent the output from the solution of the differential equations whereas the red lines represent the arithmetic mean of the outputs from 1000 runs of the stochastic simulation using the Gibson-Bruck method. While the ODE model predicts co-existence (damped oscillations leading into a stable steady state), albeit at low bacterial numbers, the stochastic model shows that these low numbers are not sustainable, with the bacterial populations and then the phage populations becoming extinct. Thus, extinction is due to low abundance.

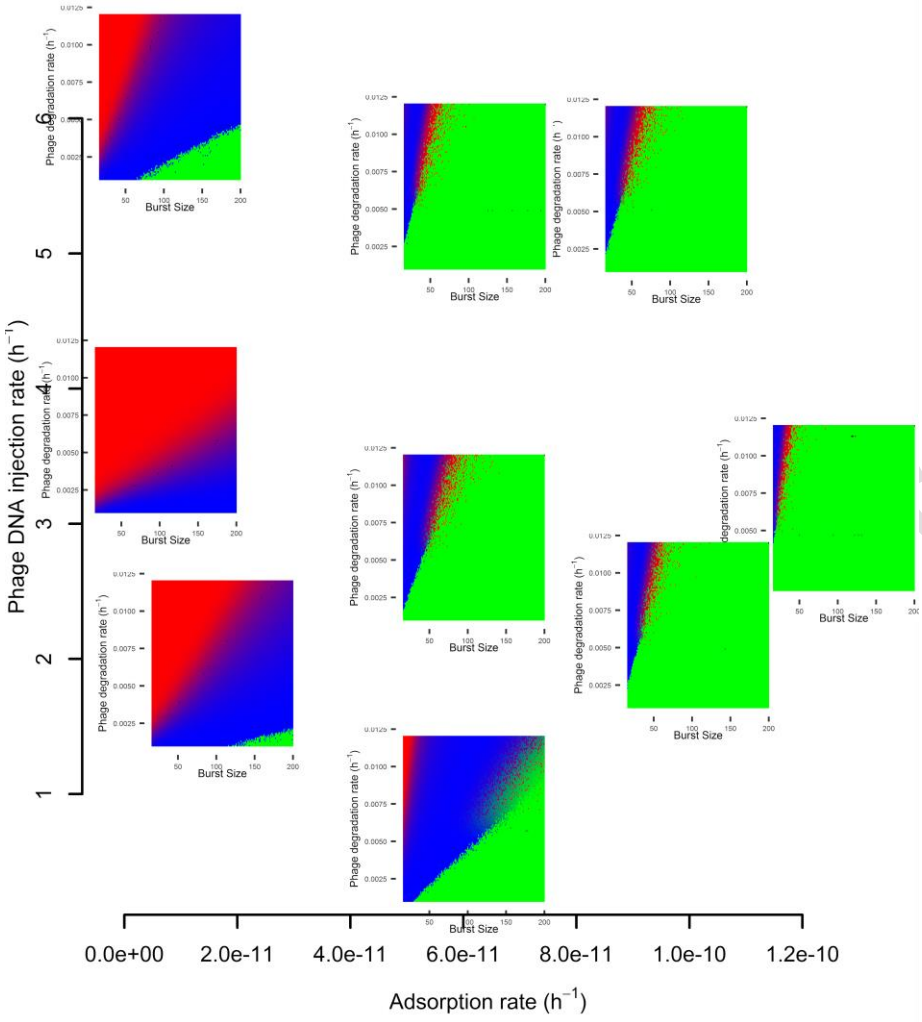

Fig. 3: **The outcome of simulating the stochastic version of the base model depends on phage degradation rate, burst size, phage DNA injection rate and phage adsorption rate.** The percentages of times the three different outcomes occurred at the same parameter setting are visualized as the intensity of the green (phage and bacterial host extinction), red (phage extinction) and blue (co-existence) channel of each pixel. Here we show a representative subgroup of the results arranged as a scatter plot for phage DNA injection rate vs adsorption rate. Each point of the scatter plot depicts the different cases with adsorption, desorption and degradation rates from Supplementary Table S7 with the degradation rate (mini y-axis) and

burst size (mini x-axis) varying over their respective ranges in each case. Note that there are two red regions of phage loss surrounding the coexistence region. The results suggest that a phage with low burst size and high degradation rate has an ecological advantage, quite contrary to what is observed in the environment. A complete result for all 22 cases is provided in Supplementary Figure S3.

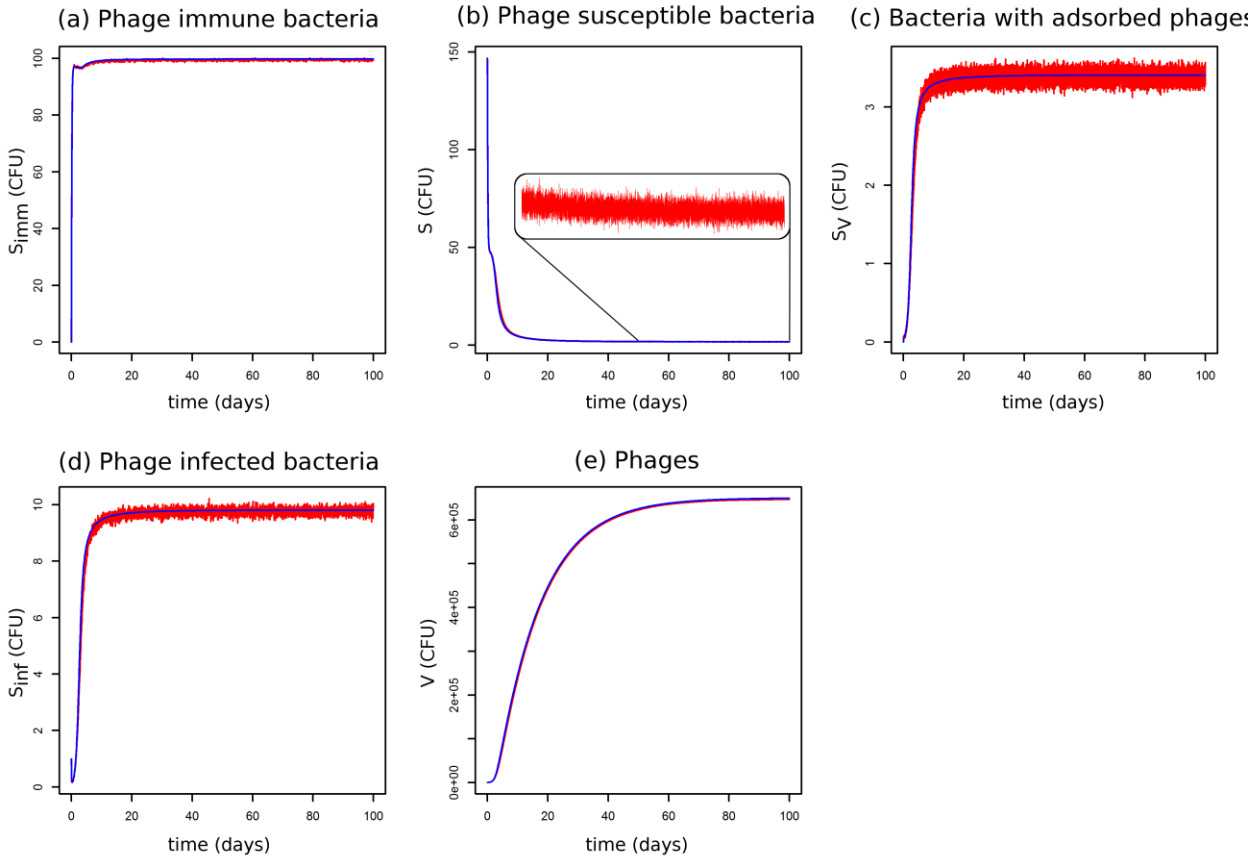

Fig. 4: **Co-existence of phage and bacteria in both the ODE version (blue line) and stochastic version (red line) versions of the co-evolution model.** The blue lines depict the output from the solution of the differential equations whereas the red lines show the arithmetic mean of the output of 1000 stochastic simulations run using the Gibson-Bruck method. Panel (b) also has a zoomed in view of the last 50 days of the stochastic simulation, showing a continuously fluctuating population. The parameter values are given in Table 1. These simulations show that the deterministic and stochastic versions of this model agree, indicating stable co-existence.

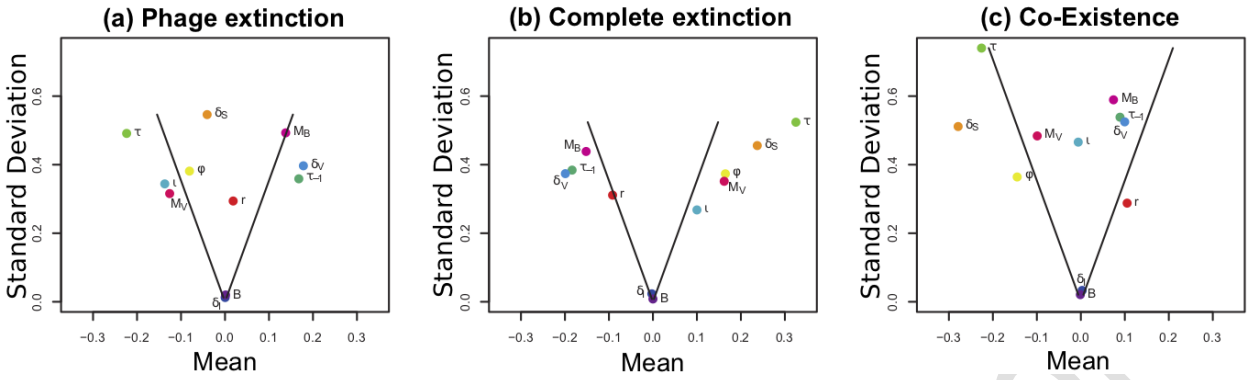

Fig. 5: **Parameter sensitivity of the stochastic version of the co-evolution model.** Standard deviation against mean of the sigma-scaled elementary effects of the input parameters on the different outcomes of the model. The lines forming the wedge correspond to $mean = \pm 2 \times sem = \pm \frac{SD}{\sqrt{50}}$. There are three panels for the three different outcomes predicted, (a) for phage extinction, (b) for complete extinction and (c) for co-existence. Outcomes are most sensitive to those parameters that lie outside the wedge, in order from top to bottom. None of the parameters have zero standard deviation with non-zero mean, indicating that all significant parameters are involved in non-linear interactions.

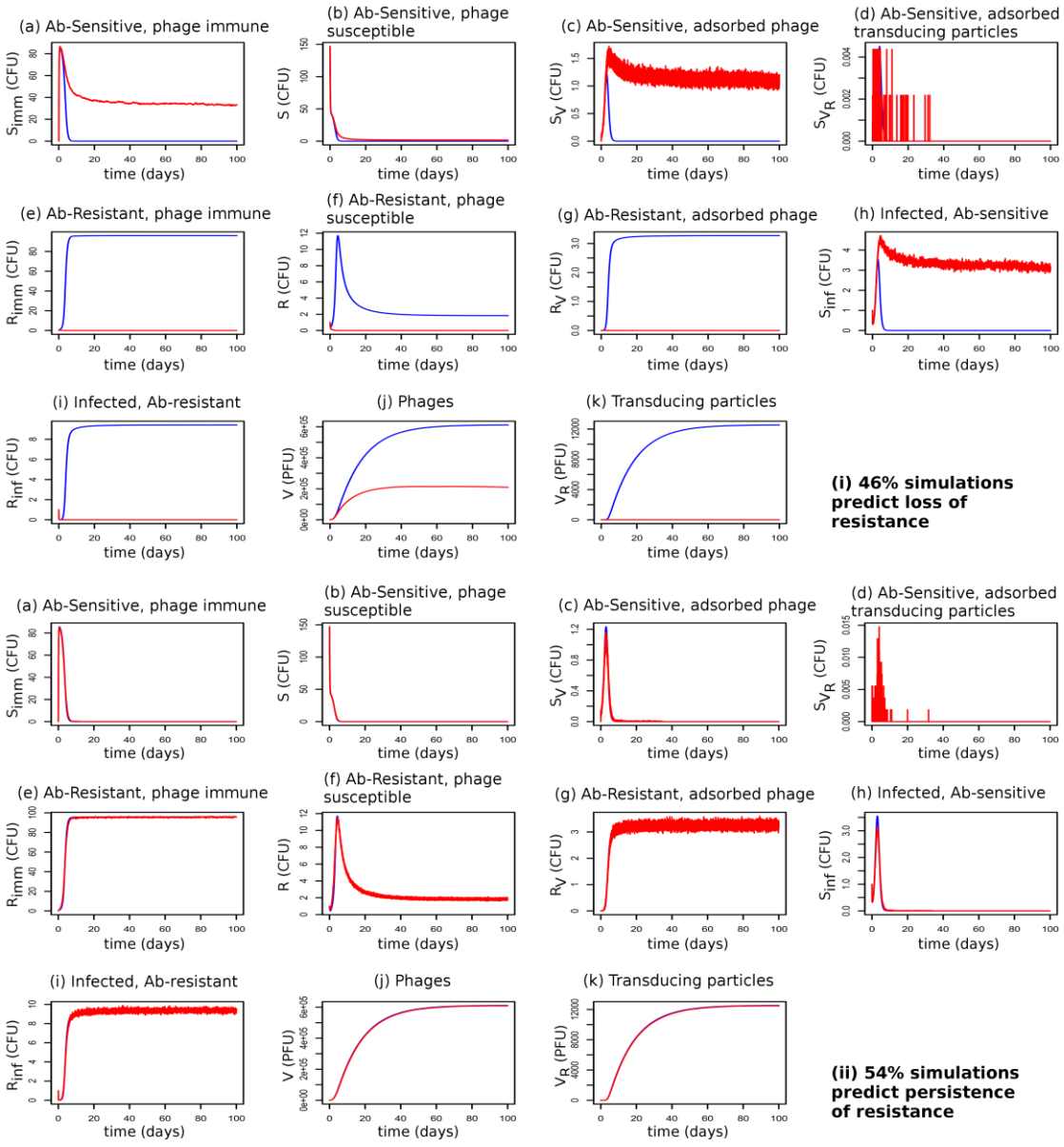

Fig. 6: **Stochastic simulations of the full model (red lines) show that spread of resistance is uncertain.** With the default parameter values, there is a (i) 46% chance of the resistant bacteria dying out before fixation of resistance but a (ii) 54% chance of fixation of resistance as also predicted by the deterministic model (blue lines). This motivates the application of sensitivity analysis to identify those parameters that most influence the spread of resistance.

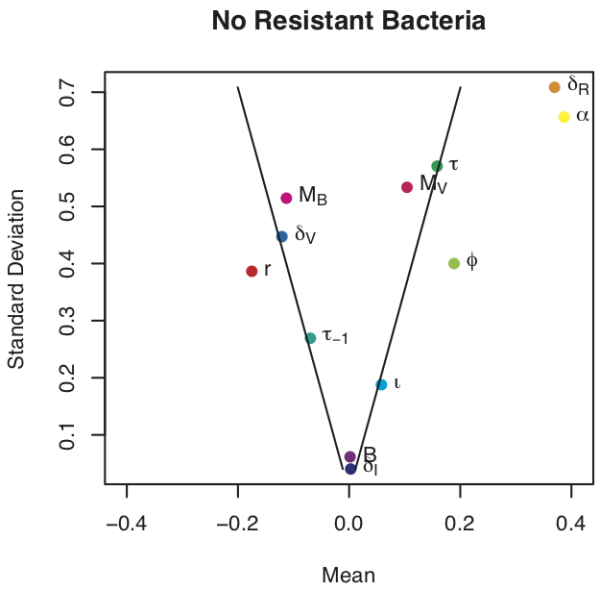

Fig. 7: **Parameter sensitivity for the loss of resistance outcome in the stochastic version of the full model.** Standard deviation against mean of the elementary effects of the input parameters on the percentage of No Resistance scenario predicted by the model simulated for 100 days. The line corresponds to $mean = \pm 2 \times sem = \pm \frac{SD}{\sqrt{50}}$.