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Coinfection and the evolution of drug resistance

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Abstract

Recent experimental work in the rodent malaria model has shown that when two or more strains share a host, there is competitive release of drug-resistant strains upon treatment. In other words, the propagule output of a particular strain is repressed when competing with other strains and increases upon the removal of this competition. This within-host effect is predicted to have an important impact on the evolution and growth of resistant strains. However, how this effect translates to epidemiological parameters at the between-host level, the level at which disease and resistance spread, has yet to be determined. Here we present a general, between-host epidemiological model that explicitly takes into account the effect of coinfection and competitive release. Although our model does show that when there is coinfection competitive release may contribute to the emergence of resistance, it also highlights an additional between-host effect. It is the combination of these two effects, the between-host effect and the within-host effect, that determines the overall influence of coinfection on the emergence of resistance. Therefore, even when competitive release of drug-resistant strains occurs, within an infected individual, it is not necessarily true that coinfection will result in the increased emergence of resistance. These results have important implications for the control of the emergence and spread of drug resistance.

Introduction

The term 'coinfection' refers to infections that consist of more than one pathogen genotype. Coinfection is extremely common, having been documented in at least 51 human and 21 non-human pathogens. These include bacteria, viruses, protozoa, helminths, and fungal pathogens and parasites (Balmer & Tanner, 2011). In addition, many of these multistrain infections have been shown to have important implications for the host (Balmer & Tanner, 2011).

A well-studied example of coinfection occurs in the rodent malaria model *Plasmodium chabaudii*. Experiments have shown that when drug-sensitive and drug-resistant strains share a host, the drug-sensitive strains can competitively exclude drug-resistant strains in untreated hosts (Wargo *et al.*, 2007; Huijben *et al.*, 2010, 2011) and that drug treatment leads to competitive release or competitive facilitation of these resistant strains (Wargo *et al.*, 2007; Huijben *et al.*, 2010, 2011). For example, Wargo *et al.* (2007) showed that the resistant strain in a mixed infection (i.e. an infection with resistant and sensitive strains present) did even better upon treatment than if a sensitive strain was never present. In other words, having to initially share a host gave the resistant strain an additional boost in numbers upon treatment. These experiments suggest that having an increased understanding of the effect of coinfection and competitive release might be important for our ability to manage malaria and other diseases where competitive suppression/release occurs.

Although some mathematical models have used a coinfection framework (Spicknall *et al.*, 2013), only a few have specifically explored the effect of coinfection and competitive release on the emergence of drug resistance. Most of these models have focused specifically

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on malaria and have been population-genetics-based. For example, using a population genetics model Hastings (1997) has shown that when there is competitive suppression between clones, or what Hasting refers to as the generalized immunity model, the rate of evolution of drug resistance increases as the number of clones increases and also as the magnitude of drug treatment increases.

Later, using a population genetic model, Hastings (2006) contended that intense competition between coinfecting clones may cause the frequency of drug resistance to increase and then stabilize at low frequencies. Mackinnon's model (Mackinnon, 1997) used a combination of branching processes and population genetics to incorporate multiclonal infections and the effect of unlinked loci that code for resistance. Later, Mackinnon & Hastings (1998) studied another population genetic model, this time incorporating particular life stages of the malaria parasite. As previously, they found that resistance increases with the number of mutants present. They also found that a cost of resistance can reduce the overall rate of growth of resistance.

Despite the advantages of these models, including the fact that it is relatively easy to incorporate multiple clones within a host as well as fitness costs, they do not incorporate population dynamical feedbacks (Mackinnon, 2005). In contrast, epidemiological models allow for such feedbacks by explicitly modelling the population dynamics of susceptible and infected individuals. Our purpose here is to use such a mathematical model to explore the emergence of drug resistance. We show that these epidemiological feedbacks give rise to an unanticipated 'between-host' effect of coinfection that acts independently of within-host competitive release. Furthermore, we show that this 'between-host' effect can work in concert or in opposition with competitive release, meaning that coinfection can enhance or hinder the spread of drug resistance depending on the pathogen and situation in question.

Model derivation and analysis

We use the theoretical framework developed by van Baalen & Sabelis (1995), but adapted to model the emergence of drug-resistant pathogens under drug treatment pressure. Our goal is to derive the conditions under which a drug-resistant pathogen can spread when rare, and to determine how these conditions are affected by the presence of coinfection.

Model

Following van Baalen & Sabelis (1995), we begin by extending the standard susceptible and infected (SI) model (Hethcote, 1989; Anderson & May, 1991) to allow for multiple infections. We consider the simplest case of coinfection by allowing a host to be infected by up to two strains. Let S denote the number of susceptibles. These can become infected with the drug-sensitive strain, W (for wildtype), or with the drug-resistant strain R. The number of individuals infected with strain R or W is denoted by I_R and I_W, respectively, and these individuals can then become coinfected with strain R or W. This results in four additional infectious classes, whose numbers are denoted by I_{RR}, I_{WW}, I_{RW} and I_{WR}. The inclusion of I_{RR} and I_{WW} classes is important, although the reason is subtle. We will be analysing the invasion condition of the resistant strain over a wide range of epidemiological parameters; however, if for example the only difference between strain R and W is their susceptibility to drugs, then if the drug resistance is set to zero the invading resistant strain should be neutral. If we did not allow wildtype strains to coinfect other wildtype strains, then the invading resistant strain would always have an inherent advantage, even when its resistance properties were identical to those of the wildtype strain. This is because, even though the two strains are then biologically identical, the rare invader can nevertheless take over wildtype infections, whereas a wildtype would not be able to do this.

As we are interested in the invasion of drug-resistant pathogens when coinfection is present, our model also includes treatment. This results in six additional treated classes whose numbers are denoted by T_R, T_W, T_{RW}, T_{WR}, T_{RR} and T_{WW} where the subscripts denote the type of infection that was present prior to treatment. Figure 1 depicts the flow through infectious classes to treated classes.

For simplicity, we assumed that the population is maintained by a constant influx Θ of susceptible individuals and they die at a constant per capita mortality rate μ. Infection is assumed to occur according to the

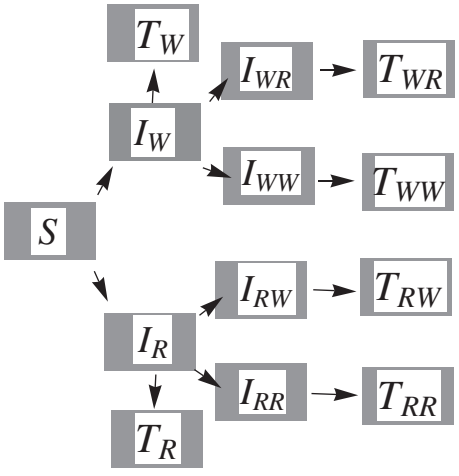

Fig. 1 Flow Diagram of the Model.

law of mass action, and the forces of infection of strains R and W are denoted by h_R and h_W, respectively, with

$$h_R = \beta_{R,R}I_R + \beta_{RR,R}I_{RR} + I_{RW}\beta_{RW,R} + I_{WR}\beta_{WR,R} + T_R\beta_{TR,R} + T_{RR}\beta_{TRR,R} + T_{RW}\beta_{TRW,R} + T_{WR}\beta_{TWR,R} \quad (1)$$

$$h_W = \beta_{W,W}I_W + \beta_{WW,W}I_{WW} + I_{RW}\beta_{RW,W} + I_{WR}\beta_{WR,W} + T_W\beta_{TW,W} + T_{WW}\beta_{TWW,W} + T_{RW}\beta_{TRW,W} + T_{WR}\beta_{TWR,W} \quad (2)$$

where $\beta_{X,Y}$ is the transmission rate of strain Y from infectious class X and has units of (time^{-1}/infected). Likewise, $\beta_{TX,Y}$ is the transmission rate of strain Y from treated class X. We note that competitive effects between strain R and W can affect these transmission rates.

Individuals infected by a single type of pathogen (either R or W) can die or clear the infection at a per capita rate μ_R or μ_W, respectively, where μ_R or μ_W is a combination of the background mortality rate μ, the infection induced mortality rate δ_R or δ_W, and the clearance rate c_R or c_W. Thus, for a general infectious class X, we have:

$$\mu_X = \mu + \delta_X + c_X. \quad (3)$$

We assume that once an infection is cleared, the host develops full immunity and therefore does not re-enter the susceptible population.

Instead of dying, or clearing the infection, an infectious individual can become coinfected with strain R at a rate of $\sigma I_R h_R$ or $\sigma I_W h_R$, or become coinfected with strain W at a rate of $\sigma I_R h_W$ or $\sigma I_W h_W$. Here σ is a parameter that represents the relative efficiency of coinfection. For example, if given a contact has occurred a susceptible is as likely to be infected as a singly infected individual, then $\sigma = 1$. Once a susceptible individual has been infected twice, the individual can only exit the class through death or clearance at a per capita rate of μ_X.

Treatment occurs at a per capita rate v, and any infected individual can enter a treated class. Treated individuals can, potentially, infect nontreated individuals, but we assume that treatment results in reinfection immunity from both pathogens, meaning that treated individuals cannot become reinfected (see appendix for the invasion condition analysis where this assumption has been relaxed). Once treated, the host can either clear the infection or die at a rate of μ_{TX}, where X denotes the infectious class. Similar to μ_X, μ_{TX} is a combination of the background mortality rate, the per capita mortality of a treated class X and the clearance rate:

$$\mu_{TX} = \mu + \delta_{TX} + c_X. \quad (4)$$

Here we have assumed that the clearance rate is independent of the treatment state. Although treatment may affect clearance rate as well, for simplicity we assume that treatment only affects the severity of the infection (i.e. δ_X and $\beta_{X,Y}$). Future work will explore the effects of relaxing this assumption.

The above assumptions lead to the following system of differential equations:

$$\begin{aligned}
\dot{S} &= \Theta - \mu S - h_R S - h_W S \\
\dot{I}_R &= h_R S - \sigma h_R I_R - \sigma h_W I_R - \mu_R I_R - v I_R \\
\dot{I}_W &= h_W S - \sigma h_W I_W - \sigma h_R I_W - \mu_W I_W - v I_W \\
\dot{I}_{WW} &= \sigma h_W I_W - \mu_{WW} I_{WW} - v I_{WW} \\
\dot{I}_{RR} &= \sigma h_R I_R - \mu_{RR} I_{RR} - v I_{RR} \\
\dot{I}_{RW} &= \sigma I_R h_W - \mu_{RW} I_{RW} - v I_{RW} \\
\dot{I}_{WR} &= \sigma I_W h_R - \mu_{WR} I_{WR} - v I_{WR} \\
\dot{T}_R &= v I_R - \mu_{TR} T_R \\
\dot{T}_W &= v I_W - \mu_{TW} T_W \\
\dot{T}_{WW} &= v I_{WW} - \mu_{TWW} T_{WW} \\
\dot{T}_{RR} &= v I_{RR} - \mu_{TRR} T_{RR} \\
\dot{T}_{RW} &= v I_{RW} - \mu_{TRW} T_{RW} \\
\dot{T}_{WR} &= v I_{WR} - \mu_{TWR} T_{WR},
\end{aligned} \quad (5)$$

Derivation of invasion condition

Now that we have an epidemiological model that incorporates coinfection and treatment, we wish to investigate how the presence of coinfection affects the emergence of resistant pathogens. We begin by assuming that there is no resistance in the population and therefore all variables involving strain R in system (5) are set to zero. We assume this system reaches a stable endemic W equilibrium, and introduce a small amount of strain R infectious material into our system and ask whether or not it will invade. This is mathematically equivalent to asking whether the endemic W equilibrium of our system (5) is stable. We do this using the next-generation theorem (NGT) (Diekmann & Heesterbeek, 1999; van den Driessche & Watmough, 2002). The NGT provides a quantity $\mathcal{R}_R$ that is a threshold quantity for the stability of the endemic equilibrium (Appendix A).

Although the precise mathematical derivation of $\mathcal{R}_R$ is presented in Appendix A, an intuition for this quantity can be gained by deriving it in a more heuristic manner. To do this, we consider a propagule newly released in the population and ask how many new propagules it is expected to produce (van Baalen & Sabelis, 1995). When an R propagule is released into the population, it can either infect a susceptible, S, or infect a host that is singly infected with W. Therefore, we can write $\mathcal{R}_R$ as

$$\mathcal{R}_R = F_S\hat{S} + F_W\hat{I}_W \quad (6)$$

where we will call F_S and F_W the per-host transmission factors and $\hat{S}$ and $\hat{I}_W$ denote the number of susceptibles and singly infected W strains, respectively, at

the endemic W equilibrium. They represent the expected number of new R propagules produced if the R propagule infects an S host or an I_W host, respectively. F_W is therefore the probability that a propagule infects an I_W host multiplied by the expected number of propagules produced by such an infection, where this incorporates both the number of propagules produced by an I_{WR} infection and the number of propagules produced by those I_{WR} infections that enter treated classes.

To make our derivation more transparent, we will break up the transmission rate, $\beta_{X,Y}$, in the following manner:

$$\beta_{X,Y} = \frac{b\kappa_{X,Y}}{u} \tag{7}$$

where we define b such that $\hat{S}b$ is the rate at which a propagule encounters and infects susceptibles (S), and therefore $\hat{I}_W\sigma b$ is the rate at which a propagule encounters and infects singly infected classes (Bonhoeffer *et al.*, 1996). The quantity $\kappa_{X,Y}$ is the rate at which an infected host I_X produces new Y propagules, and $\frac{1}{u}$ is the expected lifetime of a propagule.

Now the expected number of R propagules produced in an I_{WR} class is the rate of W propagule production $\kappa_{WR,R}$ multiplied by the expected lifespan of a I_{WR} infection $1/(\mu_{WR}+\nu)$. The probability of transitioning to a treated class is $\nu/(\mu_{WR}+\nu)$, and the number of propagules produced while in that class is $\kappa_{TWR,R}$, multiplied by the expected lifespan of a I_{TWR} class $1/\mu_{TWR}$. Therefore:

$$\begin{aligned} F_W &= \sigma\frac{b}{u}\left(\frac{\kappa_{WR,R}}{\mu_{WR}+\nu} + \frac{\nu}{(\mu_{WR}+\nu)}\frac{\kappa_{TWR,R}}{\mu_{TWR}}\right) \\ &= \frac{\sigma\beta_{WR,R}}{\nu+\mu_{WR}} + \frac{\nu\sigma\beta_{TWR,R}}{(\nu+\mu_{WR})\mu_{TWR}} \end{aligned}$$

We can derive F_S in precisely the same manner, keeping in mind that in addition to becoming treated, an I_R class can also become infected by W. Recall that the rate of becoming doubly infected, per singly infected class, is σh_W. Therefore, the expected amount of time spent in an I_R class is $1/(\nu+\sigma\hat{h}_W+\mu_R)$, and the probability of transitioning to an RW class from an R class is $\sigma\hat{h}_W/(\nu+\sigma\hat{h}_W+\mu_R)$. Hence, the probability of transitioning from an I_R class to an I_{RW} class to a T_{RW} class is $\left(\sigma\hat{h}_W/(\nu+\sigma\hat{h}_W+\mu_R)\right)(\nu/(\nu+\mu_{RW}))$. We can therefore write our expression for F_S as

$$\begin{aligned} F_S = &\frac{\beta_R}{\nu+\sigma\hat{h}_W+\mu_R} + \frac{\sigma\hat{h}_W\beta_{RW,R}}{(\nu+\mu_{RW})\left(\nu+\sigma\hat{h}_W+\mu_R\right)} \\ &+ \frac{\nu\beta_{TR,R}}{\mu_{TR}\left(\nu+\sigma\hat{h}_W+\mu_R\right)} \\ &+ \frac{\nu\sigma\hat{h}_W\beta_{TRW,R}}{(\nu+\mu_{RW})\mu_{TRW}\left(\nu+\sigma\hat{h}_W+\mu_R\right)}. \end{aligned} \tag{8}$$

Combining expressions, we obtain our expression for the invasion condition for the resistant strain $\mathcal{R}_R$,

$$\begin{aligned} \mathcal{R}_R = &\frac{\sigma\hat{I}_W\beta_{WR,R}}{\nu+\mu_{WR}} + \frac{\hat{S}\beta_R}{\nu+\sigma h_W+\mu_R} \\ &+ \frac{\hat{S}\sigma\hat{h}_W\beta_{RW,R}}{(\nu+\mu_{RW})\left(\nu+\sigma\hat{h}_W+\mu_R\right)} + \frac{\hat{S}\nu\beta_{TR,R}}{\mu_{TR}\left(\nu+\sigma\hat{h}_W+\mu_R\right)} \\ &+ \frac{\hat{S}\nu\sigma\hat{h}_W\beta_{TRW,R}}{(\nu+\mu_{RW})\mu_{TRW}\left(\nu+\sigma\hat{h}_W+\mu_R\right)} + \frac{\nu\sigma\hat{I}_W\beta_{TWR,R}}{(\nu+\mu_{WR})\mu_{TWR}}. \end{aligned} \tag{9}$$

Now our problem of stability has been reduced to analysing $\mathcal{R}_R$, where $\hat{S}$, $\hat{I}_W$ and $\hat{h}_W$ denote the values of S, I_W and h_W at the endemic equilibrium when there is no R strain present. To determine the endemic equilibrium, we assume that a class that is singly infected has the same transmission rate and mortality rate as a class that is coinfected with the same strain (i.e. $\beta_{ii,i} = \beta_{i,i}$, $\mu_{ii} = \mu_i$). To simplify, our $\mathcal{R}_R$ expression even further, we also assume that the order of infection does not affect the transmission rate or mortality rate, $\beta_{ij,i} = \beta_{ji,i}$ and $\mu_{ij} = \mu_{ji}$.

Applying these assumptions and substituting in our expression for the endemic equilibrium (Appendix S1), we obtain the following expression for $\mathcal{R}_R$:

$$\mathcal{R}_R = \Gamma_R\left(\frac{\nu+\mu_R}{\nu+\sigma\hat{h}_W+\mu_R} + \frac{\sigma\hat{h}_W}{\nu+\sigma\hat{h}_W+\mu_R}\frac{\Gamma_{RW}}{\Gamma_R}\left(1+\frac{\nu+\sigma\hat{h}_W+\mu_R}{\nu+\sigma\hat{h}_W+\mu_W}\right)\right) \tag{10}$$

where we have made use of the following notational convention:

$$\Gamma_X = \frac{\left(\beta_{X,R}\mu_{TX} + \nu\beta_{TX,R}\right)}{(\nu+\mu_X)\mu_{TX}} \Big/ \frac{\left(\nu\beta_{TW,W} + \beta_{W,W}\mu_{TW}\right)}{(\nu+\mu_W)\mu_{TW}} \tag{11}$$

Notice that if the efficiency of double infections is zero (i.e. $\sigma = 0$), then $\mathcal{R}_R$ reduces to Γ_R, which is precisely the invasion condition of the resistant strain when only single infections are permitted (Appendix S1).

Results

Two effects of coinfection

Recall that we have assumed that strain W is a drug-sensitive strain and that strain R is a completely drug-resistant strain; that is $\beta_R = \beta_{TR}$ and $\mu_{TR} = \mu_R$. We can see from equation (10) that $\mathcal{R}_R$ has the following structure:

$$\mathcal{R}_R = \mathcal{R}_S(c + (1-c)\mathcal{W}\mathcal{B}), \tag{12}$$

where $\mathcal{R}_S$ denotes the invasion condition in the single infection case (Appendix S1) and

$$c = \frac{v + \mu_R}{v + \mu_R + \sigma\hat{h}_W}, \tag{13}$$

which is the probability an infected R class dies, clears or is treated instead of becoming coinfected by a W strain.

Where $\mathcal{W}$ is defined as

$$\mathcal{W} = \Gamma_{RW}/\Gamma_R \tag{14}$$

and is the ratio between the number of resistant, R, propagules produced by a composite class to that produced by a class with a single R infection (we used the word 'composite' to denote a coinfected class that contains two different strains, an R and a W strain, whereas 'coinfected' is a more general term that refers to any infection made of two strains, including identical ones). In other words, $\mathcal{W}$ is a measure of how productive the R strain is in a composite infection compared with when it does not share a host. We refer to this as the 'within-host' effect of coinfection.

The quantity $\mathcal{B}$ is defined as:

$$\mathcal{B} = 1 + \frac{v + \sigma\hat{h}_W + \mu_R}{v + \sigma\hat{h}_W + \mu_W} \tag{15}$$

where

$$\frac{v + \sigma\hat{h}_W + \mu_R}{v + \sigma\hat{h}_W + \mu_W} \tag{16}$$

in expression (15) can be interpreted as a meaningful ratio as well. It is how often strain R 'invades' strain W infections relative to how often strain W 'invades' strain R infections (see appendix). We refer to this as the 'between-host' effect of coinfection. In other words, $\mathcal{B}$ is a measure of how effective strain R is at coinfecting W infections compared with how effective strain W is at coinfecting R infections.

From (12), we can see that to determine whether coinfection increases or decreases the value of $\mathcal{R}_R$, it suffices to look at the magnitude of $\mathcal{WB}$ compared to 1. In other words, it is the product of the within- and the between-host effects that determines the overall outcome. If $\mathcal{WB} > 1$, then coinfection increases $\mathcal{R}_R$. If $\mathcal{WB} < 1$, then coinfection decreases $\mathcal{R}_R$. And if $\mathcal{WB} = 1$, then coinfection has no effect. We note that although our analysis deals primarily with the invasion condition, $\mathcal{R}_R > 1$, we can also show that the qualitative behaviour of the growth rate of the resistant strain is similar to that of $\mathcal{R}_R$, at least when the amount of coinfection is relatively small (Hansen, 2011).

These results show that we can understand the effect of coinfection on $\mathcal{R}_R$, compared with the single infection case, as being the result of two factors: (i) how much production a resistant strain gets out of a composite class relative to the amount it gets from a single infection $\mathcal{W}$; and (ii) how often R 'invades' W infections relative to how often W 'invades' R infections, $\mathcal{B}$. For example, if a composite class produces half as many R propagules compared to a single infection, then $\mathcal{W} = 1/2$. And if R and W equally coinfect one another, then $\mathcal{B} = 2$. As a result, $\mathcal{WB} = 1$ and coinfection have no effect on the overall $\mathcal{R}_R$ value of the resistant strain. In this case, coinfection results in no advantage or disadvantage for the R strain. In a composite class, its overall propagule output is halved $\mathcal{W} = 1/2$, but for every instance in which an R only infection gets infected with a W strain and its overall output is reduced by half, an R propagule is able to infect a single W infection and gain back this half that it lost.

On the other hand, if an R strain produces the same amount of propagules in a coinfected class as it would in the single infection case, then $\mathcal{W} = 1$. And again if R and W equally coinfect one another, then $\mathcal{B} = 2$ and therefore $\mathcal{WB} = 2$. In this case, coinfection increases $\mathcal{R}_R$. We now take a closer look at these two distinct mechanisms of coinfection.

Within-host competition

If we examine expressions $\mathcal{W}$ and $\mathcal{B}$, we notice that the only place that the transmission rate of a composite infection plays a role is in the expression $\mathcal{W}$. What determines the transmission rate of a resistant strain within a composite infection? Certainly depending on the particular situation and infection, the answer can be varied and complex. For example, it may depend on the timing and ordering of subsequent infections of a host (Read & Taylor, 2001; de Roode *et al.*, 2005), the general or strain-specific immune response of a host (Read & Taylor, 2001; de Roode *et al.*, 2005), competition between strains for host resources such as blood cells or vital elements (Read & Taylor, 2001; de Roode *et al.*, 2005), or even interference-based competition such as strain-specific bacteriocins (Read & Taylor, 2001).

Although we will not be exploring how timing or order of infection affects resistance, we can encompass the other three in a general expression. Let us introduce the non-negative parameters ϕ and ϕ_τ, such that:

$$\begin{aligned} \beta_{RW,R} &= \phi\beta_{R,R} \\ \beta_{TRW,R} &= \phi_\tau\beta_{R,R} \end{aligned} \tag{17}$$

The parameter ϕ can be understood as a 'competition parameter' as it indicates what fraction of propagules strain R outputs in a composite infection relative to the amount it would output if it was exclusively occupying a host. If strain R experiences no net competition when sharing a host with strain W, then $\phi = 1$. In other words, the same propagule output is expected whether

or not the pathogen shares the host with another strain. Alternatively, if $\phi < 1$, then we would say that strain R experiences within-host competition, that is its propagule production is reduced when it shares its host with another strain. If $\phi > 1$, this would be the scenario where the presence of another strain has a facilitative effect on propagule production.

Similarly, ϕ_τ can be understood as a 'competitive release' parameter as it indicates what fraction of propagules strain R outputs in a treated composite infection relative to when it is exclusively occupying a host. Competitive release can then be defined as occurring whenever there is positive competition between strains R and W when there is no treatment, $\phi < 1$, and upon treatment the propagule production of strain R then increases, $\phi < \phi_\tau$. For example, in the competitive release experiments of Wargo *et al.* (2007), in a host coinfected with both the resistant and sensitive strain, competitive suppression of the resistant strain occurred (i.e. $\phi < 1$). Upon treatment, competitive release of the resistant strain occurred because $\phi < \phi_\tau$. If we examine expression (10), we see that the only place that within-host competition and competitive release play a role is in expression $\mathcal{W}$. Substituting in our expressions for Γ_R and Γ_{RW}, and including our competition parameters, we obtain:

$$\mathcal{W} = \frac{(\phi\mu_{TRW} + v\phi_\tau)\ (v + \mu_R)(\mu_{TR})}{(\mu_{TR} + v)\ \ (v + \mu_{RW})(\mu_{TRW})}. \tag{18}$$

Let's assume for the moment that the mortality rate is equal among the following classes, $\mu_R = \mu_{TR} = \mu_{RW} = \mu_{TRW}$. This simplifies $\mathcal{W}$ further into the following expression:

$$\mathcal{W} = \frac{\phi\mu_R + \phi_\tau v}{\mu_R + v} \tag{19}$$

Now, examining $\mathcal{W}$, we note that it is independent of the transmission rate and that it only depends on the amount of competition within an untreated composite host, ϕ, the amount of competitive release in a treated host, ϕ_τ, the mortality and clearance rate of a R host, μ_R, and the treatment rate, v.

Clearly, as the amount of competitive release experienced by a resistant strain, ϕ_τ, increases, $\mathcal{W}$ will also increase, and therefore $\mathcal{R}_R$ will also increase. The converse is also true. If the amount of competitive release is reduced, the overall $\mathcal{R}_R$ of the resistant strain will also decrease. Thus, this identifies competitive release as an important factor in the emergence of drug resistance at the population level, and it also suggests that it could be a potential resistance management target.

As an example, Huijben *et al.* (2010) showed that lower dose chemotherapy can reduce the overall amount of competitive release experienced in a composite infection, in the rodent malarial model while still not compromising host health. Thus, reducing the amount of treatment an individual host receives could reduce ϕ_τ and therefore potentially prevent drug-resistant strains from emerging at the population level.

Also notice that ϕ_τ is weighted by the population treatment rate v. Therefore, the overall effect of competitive release on the emergence of resistance on a population-wide scale will depend on the total rate of treatment. This is expected as, if we have a higher treatment rate, more hosts will be treated at any one time and therefore more hosts will potentially experience competitive release. This is consistent with the observation, in models that do not consider coinfection, that when the fraction of treated individuals is above a threshold value, then resistance will spread within a population (Koella & Antia, 2003).

Within-host competition in untreated individuals also plays an important role in determining the overall value of $\mathcal{R}_R$. This of course is an additional sophistication that is not present in standard SI models that do not consider coinfection. The greater the cost of resistance in terms of within-host competition, the lower the value of ϕ and thus the lower the value of $\mathcal{R}_R$. Of course, the higher the treatment rate, the more hosts that will experience competitive release and the less important this effect will be.

Up until now, we have assumed that the mortality rates were equal among the different classes, $\mu_R = \mu_{TR} = \mu_{RW} = \mu_{TRW}$. Allowing mortality rates between a composite class and a single infection class to differ can also potentially increase or decrease the value of $\mathcal{W}$. For example, if μ_{RW} is greater than μ_R, we expect less propagule production from our composite class compared to a single strain R infection and therefore a lower $\mathcal{W}$ value, and similarly for a treated composite class when μ_{TRW} is higher then μ_R.

Figure 2, left panel, shows $\mathcal{R}_R$ as a function of the competitive release parameter ϕ_τ, when $\mathcal{B} = 2$, for various levels of σ. Here we assume that the resistant strain pays a cost of resistance through a reduced rate of propagule production $\beta_R < \beta_W$. When there is no coinfection, $\mathcal{R}_R < 1$ and we do not get invasion of the resistant strain. If there is some coinfection however (i.e. $\sigma \neq 0$), then as the strength of competitive release ϕ_T increases, $\mathcal{R}_R$ increases as well. This shows how the presence of competitive release can make the difference between drug resistance emerging or not. Figure 2, right panel, shows the prevalence of resistance in the population as a function of time. Here too we can see that increasing the strength of competitive release increases the rate at which the resistant strain spreads through the population. Thus, all else equal, controlling the amount of competitive release has the potential to prevent or delay the spread of resistant strains.

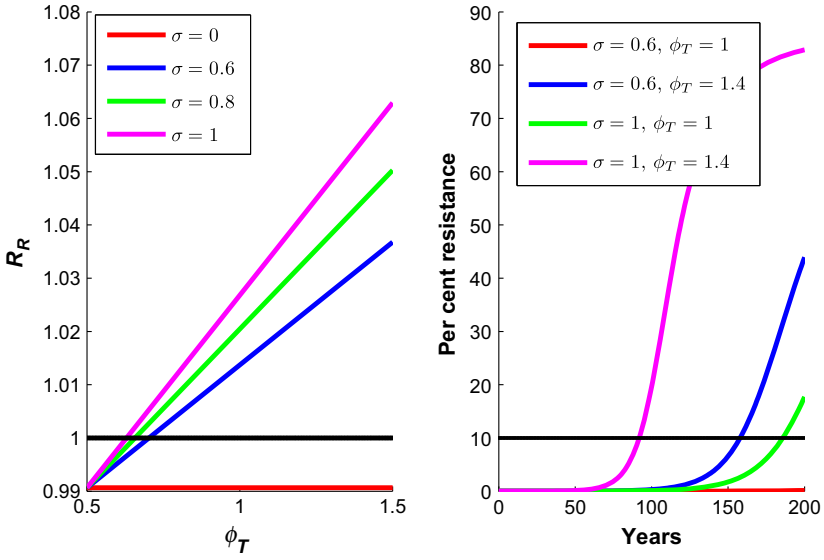

Fig. 2 Left panel: $\mathcal{R}_R$ as a function of the competitive release parameter ϕ_T, for various amounts of coinfection σ, where $\mathcal{B} = 2$. For $\sigma = 0$, no coinfection $R_R < 1$ and we will not get invasion of the resistant strain. For $\sigma > 0$ $\mathcal{R}_R$ increases for increasing ϕ_T. As σ increases, we get increasing $\mathcal{R}_R$ values as well. Right panel: per cent resistance in the population as a function of time. Each curve corresponds to a point on the $\mathcal{R}_R$ vs. ϕ_T graph (left). For a fixed sigma, increasing ϕ_T increases the growth rate, and we reach 10% resistance faster. Similarly, fixing ϕ_T and increasing σ also increase the growth rate of the resistant strain. See supplementary information for simulation details and a detailed list of parameters.

Between-host competition

The effect of coinfection described above (i.e. $\mathcal{W}$, the within-host effect) is likely the most obvious, and it is also the most discussed effect in the literature (Read & Taylor, 2001; Read *et al.*, 2011). This is likely because it is a feature of coinfection that is present at the scale of a single infected host and therefore it is readily observable in laboratory experiments (de Roode *et al.*, 2005; Huijben *et al.*, 2010, 2011). On the other hand, $\mathcal{W}$ is entirely independent of within-host competition between strains. Instead, its value, relative to 2, is determined entirely by the value of μ_R vs. that of μ_W. Recall that if a composite class produces half as many propagules as a singly infected class, then $\mathcal{W} = 1/2$ and so if $\mathcal{B} = 2$ then coinfection has no effect on the invasion of the resistant strain. If $\mu_R > \mu_W$, then strain R is more able to coinfect strain W than the reverse because, on average, strain W infections will last longer than strain R infections and therefore they have a greater opportunity to become coinfected (resulting in a $\mathcal{B} > 2$). Conversely, if $\mu_W > \mu_R$, then strain W is more able to coinfect strain R. This is the 'between-host' effect of coinfection.

Figure 3, left panel, shows $\mathcal{R}_R$ as a function of μ_R when there is no competitive release (i.e. $\phi_T = \phi$, and therefore $\mathcal{W} = 1/2$). When $\mu_R > \mu_W$, we see that coinfection increases $\mathcal{R}_R$. Here increasing the amount of coinfection can result in the emergence of resistance that would otherwise not spread in the absence of coinfection. Figure 3 right panel shows the prevalence of resistance in the population as a function of time, for various levels of σ and a fixed μ_R. We can see that increasing the amount of coinfection, σ, increases the rate of spread of resistance as well.

Alternatively, when $\mu_R < \mu_W$, Fig. 4 left panel shows that coinfection decreases the value of $\mathcal{R}_R$. In this case, increasing the amount of coinfection can prevent the emergence of a resistant strain that would otherwise spread in the absence of coinfection. Likewise, Fig. 4, right panel, shows that populations with lower levels of coinfection also display a greater rate of spread of resistance through the population.

Together, Figs 3 and 4 show that coinfection can make the spread of resistance easier or more difficult as a result of the between-host effect depending on parameter values. Of course, the overall effect of coinfection will be determined by the product of the within- and between-host effects, but these results demonstrate the effect of coinfection can go either way depending on the relative magnitude of these factors.

Discussion

We have identified two distinct mechanisms through which coinfection can increase or decrease the value of $\mathcal{R}_R$ compared to a single infection case. The first is a 'within-host effect' that takes into account the effect of competition/competitive release and determines how many propagules a resistant strain that shares its host with a sensitive strain is able to produce. The second mechanism is a 'between-host effect' and takes into account the difference between an already established infection being invaded by an additional strain, vs. invading an already established infection. It is a measure of how effective the resistant strain is at coinfecting sensitive infections compared with how effective the sensitive strain is at coinfecting resistant infections. In other words, it is better to exploit another strain's

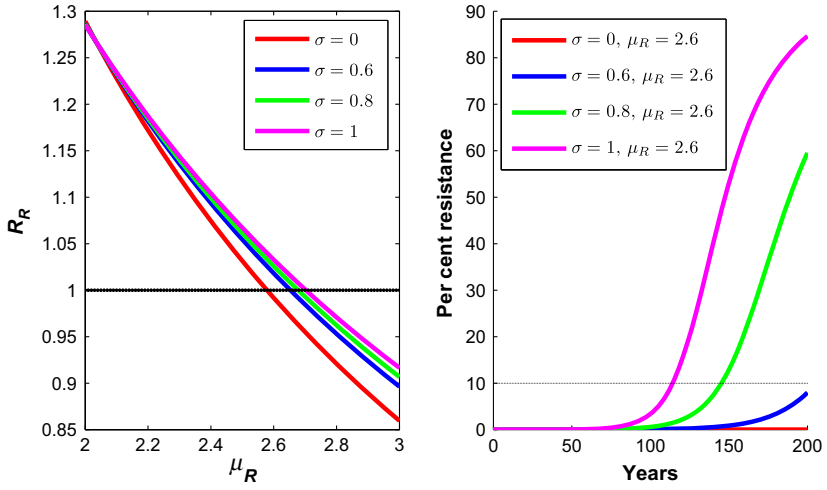

Fig. 3 Left panel: $\mathcal{R}_R$ as a function of μ_R for various amounts of coinfection σ, where there is no competitive release $\phi = \phi_T = 1/2$, and $\mu_W = 2.0360$. When $\mu_R > \mu_W$, $\mathcal{R}_R$ increases for increasing levels of coinfection (σ). Right panel: per cent resistance vs. time for $\mu_R = 2.6$ years^{-1} and various levels of σ. As σ increases, the growth rate increases. See supplementary information for simulation details and a detailed list of parameters.

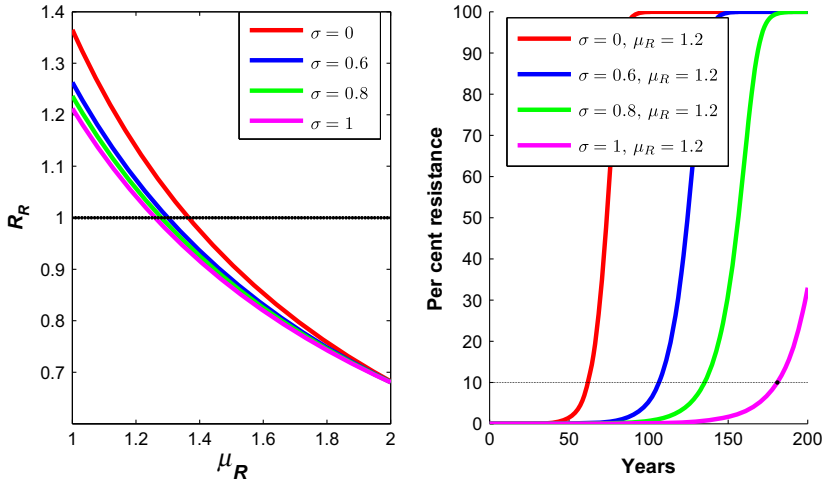

Fig. 4 Left panel: $\mathcal{R}_R$ as a function of μ_R for various amounts of coinfection σ, where there is no competitive release $\phi = \phi_T = 1/2$, and $\mu_W = 2.0360$. When $\mu_R < \mu_W$, $\mathcal{R}_R$ increases for decreasing levels of coinfection, σ. Right panel: per cent resistance vs. time for $\mu_R = 1.2$ years^{-1} and various levels of σ. As σ increases, the growth rate decreases. See supplementary information for simulation details and a detailed list of parameters.

host rather than have your own host be exploited by another strain.

We realize that in deriving these results, we made particular modelling assumptions that may not be reasonable for all types of infections. For example, we assumed that once a susceptible cleared an infection, it was immune for life and did not re-enter the susceptible population. If, however, we chose to have some loss of immunity and immune individuals re-entered the susceptible class, the exact expression of $\mathcal{R}_R$ would change but the overall form, that is that the overall effect of coinfection, depends on the result of a 'within-host effect' and a 'between-host' effect would remain the same. Another assumption was that the coinfection efficiency σ was strain-independent. If this assumption was relaxed, we would expect this to affect the value of $\mathcal{B}$, and the between-host effect would in addition be determined by these differences in σ, but again its overarching role would remain the same. Again, we note that having only a maximum of two strains infecting a host at a time may not necessarily be realistic for every type of infection, but we would expect still that the amount of output that a strain gets when it shares a host and its ability to exploit already infected hosts will play an important role.

Thinking about these two mechanisms and the impact they have on resistance invasion offers us a new lens through which to look at intervention strategies. The first and most discussed of these is the effect of competitive release (de Roode *et al.*, 2005; Huijben *et al.*, 2010, 2011; Read *et al.*, 2011). Competitive release can have a significant effect on the invasion of new resistant strains as well as the growth rate of already-present strains. Managing this effectively through reducing the treatment length or intensity of treatment (Huijben *et al.*, 2010; Read *et al.*, 2011) may have a very large and meaningful impact on resistance management strategies.

The second mechanism occurs at the between-host level. This effect has to do with the difference between individual clearance rates and/or mortality rates between strains. Its main implication is that, although increasing competitive release will always increase $\mathcal{R}_R$, the overall effect of competitive release is not the same as the overall effect of coinfection. We have shown that sometimes coinfection can reduce the $\mathcal{R}_R$ value of a resistant strain, and sometimes it can increase the $\mathcal{R}_R$ value of the resistant strain.

These results may have practical implications. One clear conclusion that follows from our results is that attempting to reduce coinfection need not be a good idea in terms of stemming the spread of resistance. This is because the overall effect depends on both the within- and between-host effects of coinfection. However, targeting competitive release is always a good idea, if this can be done without affecting the between-host effect of coinfection.

That said, reducing coinfection itself can sometimes be beneficial if the between-host effect works in concert with competitive release (or is small in magnitude). For example, treating with prophylaxis may increase the likelihood that a patient is singly infected with a resistant strain (as opposed to coinfected). Therefore, enough patients taking prophylaxis could effectively reduce the amount of coinfection in a population. Thereby, this might not only be protective from the standpoint of disease spread, but it might also prevent the invasion of resistant strains. Vaccinations are another intervention strategy known to reduce the overall genetic diversity of infections (Read & Taylor, 2001), and this too might not only reduce the spread of disease, but also prevent the emergence of resistance.

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Appendix A

Biological meaning of $\mathscr{B}$

The rate at which *WR* infections are produced by an *R* propagule is:

$$b\sigma\hat{I}_W \tag{A1}$$

and the rate at which *RW* infections are produced by an *R* propagule is:

$$b\hat{S}\left(\frac{\sigma\hat{h}_W}{v+\sigma\hat{h}_W+\mu_R}\right). \quad \text{(A2)}$$

Taking the ratio of the two rates and substituting in the endemic *W* equilibrium we get:

$$\frac{v+\sigma\hat{h}_W+\mu_R}{v+\sigma\hat{h}_W+\mu_W.} \quad \text{(A3)}$$

Supporting information

Additional Supporting Information may be found in the online version of this article:
Appendix S1 Supporting Derivations and Figure Details and Parameters.

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