

# Drug Resistance in Acute Viral Infections: Rhinovirus as a Case Study

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**ABSTRACT.** The emergence and spread of drug resistant virus variants reflects both within-host and between-host processes. We develop an epidemiological model that can be used to address the spread of resistance at the population level, and a virus dynamics model that can be used to study the dynamics of virus over the time course of an individual's infection. The dynamics depend in an important way on the competition between drug sensitive and drug resistant virus strains. A key observation is that the strength of competition between strains is strongly modulated by the degree of cross-immunity that infection with one strain confers against infection with the other. At the within-host level, we see that an efficient immune response can reduce the likelihood of the emergence of resistant virus. Consequently, resistance poses more of a problem for chronic infections in which there is significant immune impairment than for acute infections. These findings are discussed in the setting of rhinovirus infections, which are an important cause of infection in humans and for which novel antiviral drugs are being developed.

## 1. Introduction

Viruses are responsible for a large number of infectious disease cases each year. Many viruses are associated with severe disease and high levels of mortality: examples include the human immunodeficiency virus (HIV) and pandemic strains of influenza such as the 1918 Spanish flu. Even when the case fatality ratio is not so high, the sheer number of cases can lead to a large number of disease-related deaths. Many other viruses, on the other hand, are associated with much less severe disease, but their high levels of morbidity lead to their having a major economic impact (Fendrick et al., 2003). As an example, rhinovirus infections—responsible for roughly a half of ‘common cold’ cases—are one of the leading reasons for people to visit their physician in the US (Hayden et al., 1999; Couch, 2001).

Until recently, there were only a few drugs that could be used to combat viral infections. This situation has changed with the development of a large number of antiviral drugs, for instance in the settings of HIV, hepatitis B and influenza. The potential benefit of these drugs is enormous, both in terms of improving the prognosis for infected individuals and for the potential reduction of transmission of the infection at the population level (Blower et al., 2000; Velasco-Hernandez et al., 2002).

The use of an effective drug treatment imposes a strong selection pressure on a virus (or any other infectious disease agent). Variants of the virus that are less sensitive to the action of the drug have a considerable replicative advantage over more sensitive variants. As a consequence, their relative frequency will increase. The emergence of viral variants that are resistant to one or more drugs threatens to curtail the benefits that might be gained from the use of antivirals: concern over the emergence of resistant forms of HIV and influenza virus has long been expressed and indeed such forms have often been found in the wake of the introduction of drugs against these infections (Larder and Kemp, 1989; Hayden and Hay, 1992; Richman, 1994; Coffin, 1995; Pillay et al., 2000).

Many viruses have the potential to undergo rapid genetic changes. For instance, in the case of HIV, the reverse transcription of viral RNA to DNA during the virus's replication cycle lacks a proof reading mechanism and so is error-prone, leading to the generation of many mutants. Since, in many instances, drug resistance can be conferred by small genetic changes, such as even a single point mutation, there is ample opportunity for the acquisition of resistance. (In the case of bacteria, genetic material can be acquired from other bacteria or the wider environment, offering them yet more opportunities to acquire resistance.) The acquisition of resistance, however, often comes at a cost: the resistant type is typically less fit (i.e. has a lower replicative ability) in the absence of the drug than the sensitive virus from which it evolved (see, for example, Back et al., 1996; Goudsmit et al., 1996).

This combination of potentially rapid genetic change and the strong selection pressure imposed by drug treatment provides an ideal environment within which drug resistant variants can emerge and spread. Any attempt to deploy drug treatment on a wide scale should, therefore, take resistance, and its management, into account (Levin et al., 1998). In particular, issues surrounding resistance are an important consideration when new drugs are brought into clinical use.

In order to fully understand drug resistance, two distinct issues must be considered. The emergence of a novel drug resistant viral variant typically occurs within an infected individual over the time course of their infection. The population level impact of this new resistant variant can only be assessed by understanding its spread from individual to individual in the population. Mathematical modeling provides an ideal framework within which such questions can be addressed, as witnessed by the growing literature in this area (see, for example, Frost and McLean, 1994; Levin et al., 1998; Bonhoeffer and Nowak, 1997; Bonhoeffer et al., 1997; Blower and Gerberding, 1998; Blower et al., 1998; Stilianakis et al., 1998; Gershengorn and Blower, 2000; Lipsitch et al., 2000b,a; Ribeiro and Bonhoeffer, 2000; Blower et al., 2001; Ferguson et al., 2003b).

In this chapter we shall address both of these issues, using modeling approaches to examine the likelihood of emergence and spread of resistance. We shall focus on the setting of human rhinovirus infections, for which an effective drug, pleconaril, has only recently been developed (Hayden et al., 1999; Rotbart, 2002; Hayden et al., 2003). We shall see that the epidemiology and natural history of rhinovirus infections plays a crucial role in terms of resistance. Consequently, we shall compare and contrast behavior seen here with what occurs in other settings such as HIV and influenza.

This chapter is organized as follows. After discussing pertinent details of rhinovirus epidemiology, we shall introduce the basic epidemiological framework that we employ. After discussing the spread of resistant virus at the epidemiological level, we turn to the dynamics of resistant and sensitive virus within an infected individual.

## 2. Rhinovirus Epidemiology

Roughly 100 serotypes of human rhinoviruses have been described (Couch, 2001; Gwaltney, 1997). Epidemiological surveys typically indicate that a large number of these co-circulate within a given community at any given time, with little indication of clear geographic or temporal patterns of their distribution (Hamre et al., 1966; Gwaltney et al., 1968; Monto and Johnson, 1968; Fox et al., 1975; Monto et al., 1987). Not all serotypes are equally transmissible in all settings, but the impact of any differences in transmissibility on population-level patterns of serotype prevalence is not clear.

An interesting observation is that new serotypes do not appear to be emerging over time (Fox et al., 1985; Monto et al., 1987). This is in marked contrast to influenza, where the appearance of new strains, which over a period of time replace the previously existing strains, is well documented (Bush et al., 1999; Ferguson et al., 2003a).

Infection with a given rhinovirus typically leads to the development of cold symptoms following a short (1-2 day) incubation period (Hamre et al., 1966; Couch, 2001). Symptomatic individuals have been shown to shed large amounts of virus, suggesting that the duration of infectiousness typically echoes the exhibition of symptoms. About 25% of infections appear to be subclinical, with few symptoms being exhibited: such individuals are presumably much less infectious.

Recovery from infection leads to long-lived, if not lifelong, immunity against the infecting serotype (Taylor-Robinson, 1963; Cate et al., 1964; Gwaltney et al., 1967; Hendley et al., 1969). Cross-immunity between serotypes appears to be low since individuals repeatedly acquire rhinovirus infections, but the infection rate declines with age (adults typically experience roughly 2-4 colds per year, and children between 4 and 6, Gwaltney, 1997). The higher infection rate amongst children is consistent with this picture of immunity: repeated infection leads to immunity against an ever larger repertoire of serotypes over time.

The antiviral agent pleconaril interrupts the viral replication cycle by blocking the attachment of virus to its cellular target. Clinical trials of the drug have shown that its use can reduce both the duration and severity of cold symptoms (Hayden et al., 1999; Rotbart, 2002; Hayden et al., 2003). Given the observed relationship between disease symptoms and infectiousness, this will presumably also reduce the transmissibility of infection. An interesting *in vitro* observation is that pleconaril is not effective against all rhinovirus serotypes (Pevear, 1999): there are what might be termed ‘naturally resistant’ serotypes.

The limited clinical trials that have studied the use of pleconaril have yet to yield much information on the emergence of resistance. In a small fraction of treated individuals, virus recovered after the end of infection exhibited somewhat decreased drug susceptibility (Hayden et al., 2003). The clinical significance of these virus variants is not, however, clear from these studies: neither the level to which these variants grew nor the timing of their appearance were ascertained.

Additional information has been gleaned from *in vitro* cell culture studies, from which drug resistant mutants have also been recovered. Such mutants often show severely reduced growth (Heinze et al., 1989; Pevear et al., 1999).

An important observation in the rhinovirus setting, therefore, is that there are two distinct types of resistant variants. Naturally resistant variants existed and circulated long before the introduction of treatment, whereas novel resistant variants would only appear in the wake of the introduction of treatment. Notice that these novel resistant variants are likely to be serotypically similar or identical to existing forms of the virus. The form of our model in the ‘novel variant’ setting echoes existing models for the population-level spread of resistance in which it is typically assumed that individuals are either infected with resistant or sensitive variants, but not both. The natural resistance setting, however, has received much less attention in the literature.

Clinical trials involving pleconaril have focused on therapeutic use of the drug, where already infected patients are treated. Beyond the setting of clinical trials, its availability would likely be restricted to prescription usage, requiring a visit to a physician. In the early stages of its deployment, therefore, it would be reasonable to assume that its use would not be extremely widespread. The possibility of its use as a prophylactic has been considered as a means of reducing the potential for secondary infections. In this study we restrict attention to therapeutic use of pleconaril.

### 3. The Epidemiological Model

Our population-level model is based on the well-known multi-strain model of Castillo-Chavez et al. (1989). Their model is built upon the standard SIR (susceptible/infective/recovered) framework (see, for example, Anderson and May, 1991), but incorporates two distinct strains of infection. (A further extension to additional strains is given by Andreasen et al., 1997). We extend this model to include treatment so that it can be used to describe the transmission dynamics of drug sensitive and resistant virus (Lloyd and Wodarz, 2005).

The infections are assumed to be non-fatal, with recovery from a particular strain leading to life-long immunity against reinfection with that strain. An important feature of the model is that recovery from one infection confers partial protection against infection with another strain. One way to model this cross-immunity is by reducing the susceptibility of an individual to infection by other strains (Castillo-Chavez et al., 1989; Andreasen et al., 1997). The model must keep track of the infection histories of individuals: for combinatorial reasons, this rapidly becomes difficult as the number of strains in the model increases (Andreasen et al., 1997). For this reason, we restrict attention to a two strain setting.

The fractions of the population that are susceptible, infective and recovered are denoted by  $S$ ,  $I$  and  $R$ , respectively. We subdivide the  $S$  and  $I$  classes according to infection history (superscripts) and the current strain with which an individual is infected (subscripts). So, for example,  $S$  denotes the fraction of individuals that have never experienced infection.  $S^1$  denotes individuals that have recovered from strain one but are still susceptible to strain two. In a similar way,  $I_2$  denotes individuals who are infected with strain two and who have not experienced strain one, while  $I_2^1$  denotes individuals that are infected by strain two but who have

experienced strain one in their past. Notice that the total fraction of individuals that are infected with strain two equals  $I_2 + I_2^1$ .

All of the infection classes, and the possible transitions between them, are illustrated in figure 1. Notice that for simplicity we neglect any latent period between infection and the start of infectiousness, assume that individuals can only be infected with one strain at a time and that individuals who recover from one strain are immediately susceptible to the other strain (if they have not previously encountered that strain).

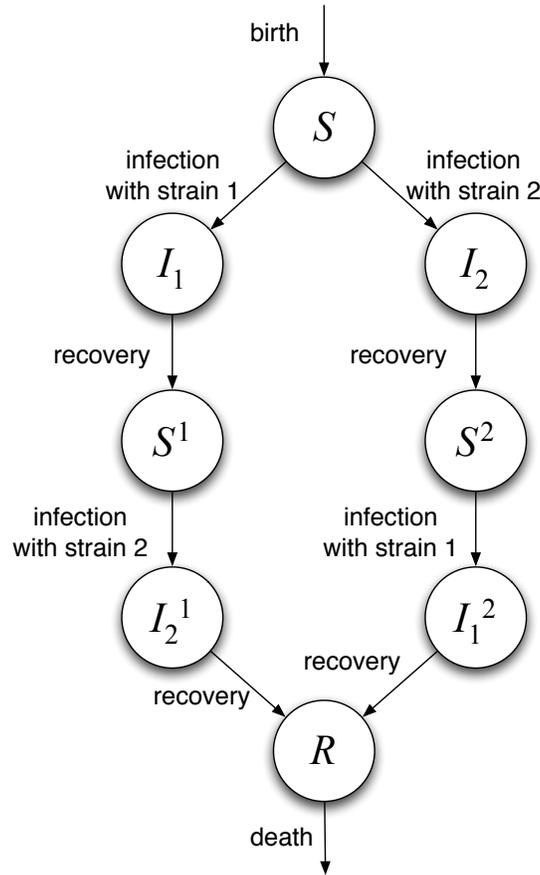


FIGURE 1. Flowchart showing the population subdivided according to infection status and the possible transitions between classes. The model assumes a constant disease-independent background mortality rate of  $\mu$ . For reasons of clarity, this figure does not depict deaths from classes other than the recovered class.

Making standard assumptions (for instance that the population is well-mixed and that recovery probabilities are independent of the time since infection) Castillo-Chavez et al. obtain the following set of equations for the epidemiological model

$$\begin{aligned}
 (1) \quad \dot{S} &= \mu - \beta_1 S(I_1 + I_1^2) - \beta_2 S(I_2 + I_2^1) - \mu S \\
 (2) \quad \dot{I}_1 &= \beta_1 S(I_1 + I_1^2) - (\mu + \gamma)I_1 \\
 (3) \quad \dot{I}_2 &= \beta_2 S(I_2 + I_2^1) - (\mu + \gamma)I_2 \\
 (4) \quad \dot{S}^1 &= \gamma I_1 - \sigma \beta_2 S^1(I_2 + I_2^1) - \mu S^1 \\
 (5) \quad \dot{S}^2 &= \gamma I_2 - \sigma \beta_1 S^2(I_1 + I_1^2) - \mu S^2 \\
 (6) \quad \dot{I}_2^1 &= \sigma \beta_2 S^1(I_2 + I_2^1) - (\mu + \gamma)I_2^1 \\
 (7) \quad \dot{I}_1^2 &= \sigma \beta_1 S^2(I_1 + I_1^2) - (\mu + \gamma)I_1^2 \\
 (8) \quad \dot{R} &= \gamma I_2^1 + \gamma I_1^2 - \mu R.
 \end{aligned}$$

Here, the parameters  $\beta_1$  and  $\beta_2$  are the transmission parameters of the strains,  $\gamma$  is the rate of recovery (assumed to be the same for both strains) and  $\mu$  is the birth and death rate of the population. We assume the birth and death rates to be equal, corresponding to the assumption of a constant population size.

The remaining parameter,  $\sigma$ , is an inverse measure of cross immunity: under the mass-action (perfect mixing) assumption, the rate at which naive individuals become infected by strain one is given by the product  $\beta_1 S(I_1 + I_1^2)$ . In contrast, the rate at which individuals that have already experienced infection with strain two become infected with strain one is  $\sigma \beta_1 S^1(I_1 + I_1^2)$ . If  $\sigma$  equals zero, recovery from strain two leads to complete protection against strain one. If  $\sigma$  equals one, recovery from strain two offers no protection against strain one. (In this case, the model of Castillo-Chavez et al. (1989) reduces to an earlier model that was employed by Dietz (1979).)

Mutation between strains can be considered by allowing a small fraction of infections,  $\nu$ , with one strain to lead to infection with the other strain. The parameter  $\nu$  attempts to capture the complex process by which a strain might mutate over the course of an individual's infection. A more realistic description of this process requires the development of a within-host model, as we outline towards the end of this chapter. This mutation term is most likely to be important when one of the strains is either absent or present at a low frequency.

## 4. Behavior of the Epidemiological Model

**4.1. Behavior in the Absence of Treatment.** As is often the case with epidemiological models of this sort, the behavior of the system depends on the basic reproductive numbers of the two strains (Dietz, 1979; Castillo-Chavez et al., 1989).  $R_0^1$  quantifies the transmissibility of strain one: in a completely susceptible population, the introduction of one individual infected with strain one would lead to an average number of  $R_0^1 = \beta_1/(\gamma + \mu)$  secondary infections of type 1. Similarly,  $R_0^2 = \beta_2/(\gamma + \mu)$ . A necessary (but not sufficient) condition for the establishment of a given strain is that its  $R_0$  value is greater than one.

The two strains compete for susceptible hosts, with the level of competition dependent on the level of cross-immunity (Castillo-Chavez et al., 1989; Andreasen et al., 1997). If there were complete cross-immunity, individuals that became infected with one strain could never become infected with the other strain. In this case, the strain with the larger  $R_0$  value would out-compete the other strain, driving it to extinction (Castillo-Chavez et al., 1989): this is an example of competitive

exclusion. At the other extreme, if there were no cross-immunity, competition between strains would be very weak: the only effect of strain one on strain two arises because coinfection is assumed not to occur (Dietz, 1979). If the duration of infection is short then this effect would be very small. The dependence of the equilibrium (long-term) behavior of this model, in the absence of drug treatment, on the two values  $R_0^1$  and  $R_0^2$  is illustrated in figure 2. Analytic expressions for the curves that separate the different regions in figure 2 can be found in Castillo-Chavez et al. (1989) in the general case and in Dietz (1979) in the  $\sigma = 1$  case.

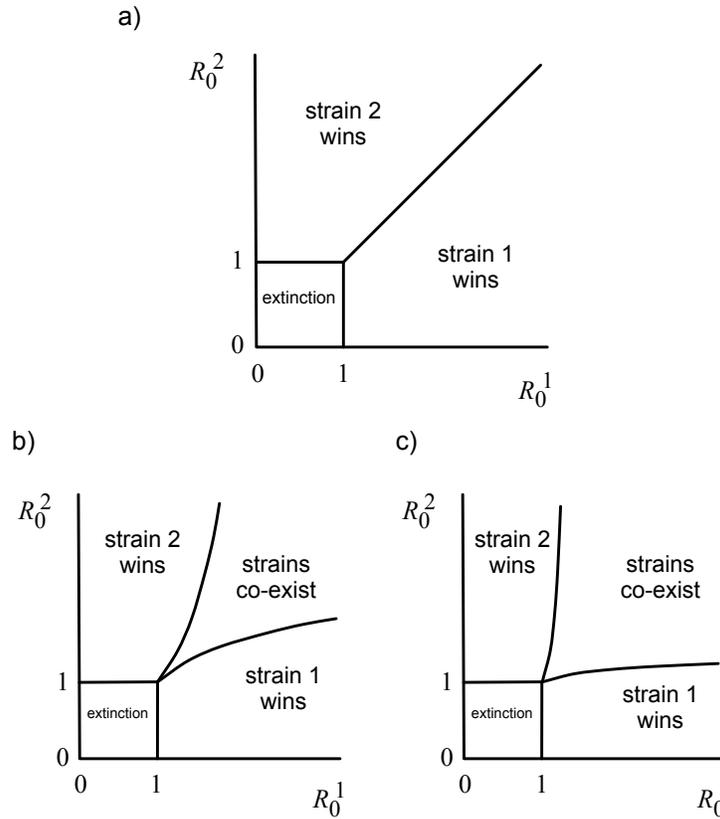


FIGURE 2. Schematic figure illustrating the outcome of the competition between strains in the two strain epidemiological model, equations (1-8). The three panels depict three different strengths of cross-immunity: (a) complete cross-immunity ( $\sigma = 0$ ), (b) partial cross-immunity, (c) no cross-immunity ( $\sigma = 1$ ). The curves separate different regions of the  $(R_0^1, R_0^2)$  plane according to the long-term (stable equilibrium) behavior seen. (The exact boundary curves that appear in this figure can be calculated exactly using the analytic results of Dietz (1979) and Castillo-Chavez et al. (1989), as outlined in the text.)

The inclusion of mutation within this model prevents the less fit strain from going extinct when it would otherwise do so. It will be continually produced by mutation from the other strain and so will be present at the low equilibrium level that results from the balance between mutation (producing the strain) and selection (which reduces the strain’s relative frequency).

**4.2. Inclusion of Treatment.** Treatment is included within the model by allowing for treated classes of individuals. We imagine that a certain fraction of infections,  $\epsilon$ , are treated and that the effect of treatment is to increase the rate at which treated individuals recover from  $\gamma$  to  $\gamma_T$ . This corresponds to a reduction in the average duration of infection. We assume that strain one is drug sensitive and that strain two is completely drug resistant.

In this extended model, the class  $T_1$  denotes infectious individuals whose first infection is with strain 1 and are being treated. The class  $T_1^2$  denotes infectious individuals whose second infection is with strain 1 and are being treated. The classes  $I_1$  and  $I_1^2$  now refer to untreated infectious individuals. (Since treatment has no effect on infections with strain 2, there is no need to make a similar distinction for those individuals.) The total fraction of individuals that are infected with strain 1 is given by  $I_1 + T_1 + I_1^2 + T_1^2$ . Equation (2) (the previous equation for  $\dot{I}_1$ ) is replaced by the following pair of equations

$$(9) \quad \dot{I}_1 = (1 - \epsilon)\beta_1 S(I_1 + I_1^2 + T_1 + T_1^2) - (\mu + \gamma)I_1$$

$$(10) \quad \dot{T}_1 = \epsilon\beta_1 S(I_1 + I_1^2 + T_1 + T_1^2) - (\mu + \gamma_T)T_1.$$

A similar pair of equations replaces the previous  $\dot{I}_1^2$  equation.

As mentioned earlier, an important feature of many resistant variants is that the acquisition of resistance comes at some fitness cost. In terms of the model parameters, this means that  $\beta_2 < \beta_1$ . An important realization in the rhinovirus setting is that resistance is not always associated with a fitness cost: some serotypes are naturally resistant to pleconaril. Given that these strains have been circulating in the human population for some time, and there is no evidence that these strains are present at lower prevalence than drug sensitive strains, we argue that their transmissibility must be comparable to other, drug sensitive, serotypes.

**4.3. Behavior of the Model in the Presence of Treatment.** The imposition of treatment reduces the basic reproductive number of strain 1 by reducing the average duration of infections with that strain. Treatment, therefore, shifts the competitive balance in favor of strain 2. Since the strength of competition is modulated by the degree of cross-immunity between strains, the impact of treatment depends on whether we are considering the setting of natural resistance (two serotypically distinct strains with low or no cost of resistance) or the setting of novel resistant strains (two serotypically identical strains with a significant cost of resistance).

When cross-immunity between strains is not too high, as in the ‘natural resistance’ setting, the imposition of treatment can do little to alter the prevalence of drug sensitive and drug resistant strains. The model predicts that the use of antiviral drugs is unlikely to lead to a major change in the relative frequencies of sensitive and resistant strains, provided that the fraction of individuals treated is not too high. This situation is illustrated in figure 3a, in which we assume that

there is no transmission cost to having resistance. If a large fraction of individuals is treated (a situation which is unlikely to hold if people have to visit a physician in order to obtain a drug for what is in most cases a mild infection), then the use of the drug can have a more significant impact.

If there is strong cross-immunity between strains (i.e. when the competition between strains is strong), the imposition of treatment can have a major impact (figure 3b). This scenario, in which the resistant strain is serotypically similar to the sensitive strain, is most likely to arise when the resistant strain is a novel mutant derived from an existing drug sensitive strain. The acquisition of resistance in this setting, however, is typically associated with a fitness cost. This reduces the competition benefit that the resistant strain experiences due to the imposition of treatment. Consequently figure 3b provides an overly pessimistic estimate of the increase of the resistant strain since the parameter values chosen did not include any cost of resistance. The drug-resistant strain will only increase its relative frequency if the reduction in  $R_0$  that it experiences due to the cost of resistance is lower than the reduction in  $R_0$  that the sensitive strain experiences due to the use of treatment. In this case, we would expect the drug resistant strain to largely replace the sensitive strain because of the strong competition. (The sensitive strain will not completely disappear, assuming that back mutation of the resistant strain can generate the sensitive strain.) A previously sensitive serotype would be largely replaced by a resistant serotype.

In order for this second scenario to occur, a novel resistant mutant must have appeared over the course of an individual's infection. For us to consider the likelihood of this event, we must turn to a within-host model that can describe the time course of infection in an individual. Before doing so, we remark that this discussion has only examined long-term (equilibrium) dynamics. More details of the short-term dynamics, in particular a discussion of the time-scale on which resistance spreads, can be found in Lloyd and Wodarz (2005).

## 5. The Within-Host Model

The time course of infection within an individual is modeled by describing the interaction between the virus, the cells that it infects (the so-called 'target cells') and an immune response that develops against the infection. Our model is based on the basic model of virus dynamics that has received much attention in the setting of HIV infection (early examples include Frost and McLean, 1994; Nowak and Bangham, 1996). As a consequence, we can draw upon the literature that models the emergence of drug resistant HIV strains (see, for example, Frost and McLean, 1994; Bonhoeffer and Nowak, 1997; Bonhoeffer et al., 1997; Ribeiro and Bonhoeffer, 2000). There are, however, important differences that arise because rhinovirus infections are acute infections that are resolved by the immune system, as opposed to chronic HIV infections in which the immune system experiences significant impairment.

Two processes must occur in order for a novel drug resistant viral strain to be generated within a patient and then transmitted to another individual. First, the resistant virus strain must be created by mutation: this event occurs over the course of the virus's transmission and replication cycle. Second, the mutant virus must replicate to a sufficiently high level within the infected individual in order to allow the possibility of transmission to occur. We shall consider these two

processes separately, and since the second process is in some ways analogous to the population-level process already studied, our discussion will first focus on this issue.

The basic model of virus dynamics for a single strain can be written as

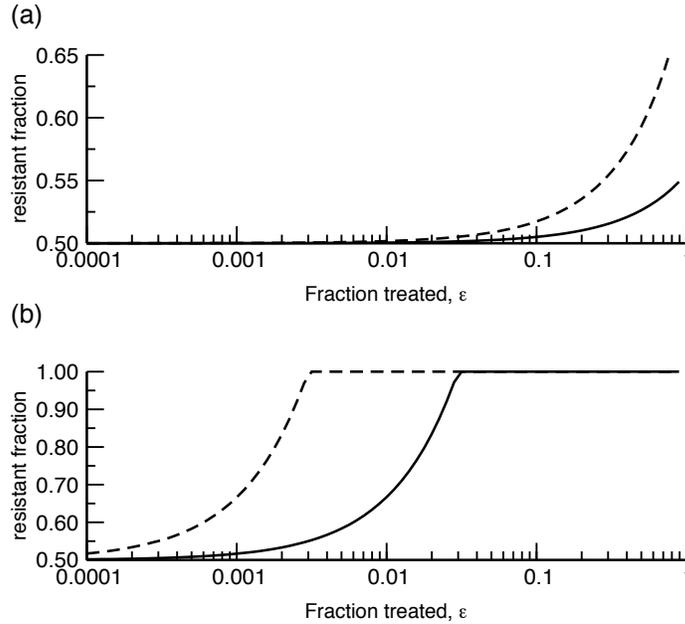


FIGURE 3. Dependence of the equilibrium fraction of infections that are due to the resistant strain on the fraction of infections that are treated ( $\epsilon$ ). Panel (a) depicts the cases of no cross-immunity ( $\sigma = 1$ , solid curve) and partial cross-immunity ( $\sigma = 0.1$ , broken curve). The impact of treatment is small or modest in these cases. Panel (b) illustrates strong cross-immunity ( $\sigma = 0.001$ , solid curve and  $\sigma = 0.0001$ , broken curve), and shows that treatment can have a major impact on the relative prevalence of resistant infections if cross-immunity is strong. In the limiting case of complete cross-immunity ( $\sigma = 0$ ), all infections, at equilibrium, are with the resistant strain whenever  $\epsilon$  is positive. In this figure, we assume that both strains have an  $R_0$  value of six: since the basic reproductive number of rhinovirus infections has yet to be quantified, we take this value for illustrative purposes. We assume that untreated infections last, on average, six days while treated infections last five days. The average lifespan of the population is taken to be 70 years. All curves were generated by numerical integration of the equations governing the system.

$$(11) \quad \dot{x} = \lambda - dx - \beta xy$$

$$(12) \quad \dot{y} = \beta xy - ay - pyz$$

$$(13) \quad \dot{z} = cyz - bz.$$

Here,  $x$  denotes the number of uninfected (target) cells,  $y$  the number of infected cells and  $z$  measures the strength of the immune response. We make standard assumptions regarding uninfected and infected cell dynamics. Free virus particles are assumed to undergo a rapid turnover, so their numbers closely echo those of the infected cell populations (in the model, we make a quasi-steady state assumption between free virus and infected cells). The rate at which target cells become infected is assumed to be described by the mass action term  $\beta xy$ , i.e. is proportional to both the numbers of target cells and infected cells, with constant of proportionality  $\beta$ . Uninfected cells are produced at a constant rate  $\lambda$  and die at a constant per-capita rate  $d$ . Infected cells die at rate  $ay$  (so that their lifespan is  $1/a$ ) in the absence of an immune response. The immune response that develops against the infection further removes infected cells at rate  $pyz$ . The presence of infected cells stimulates the immune response: for simplicity and definiteness, we take this stimulation to be described by the bilinear term  $cyz$ . (For more detailed discussions of alternative forms of this immune response, see De Boer and Perelson (1998) or Wodarz (2001).) Finally, the immune response (in the absence of stimulation) is reduced at rate  $bz$ .

The behavior of this model is determined by the basic reproductive number of the within-host system. During the initial stages of infection, when the target cell population is close to its infection free equilibrium level and the immune response is weak, the average number of secondary infected cells that result from the introduction of a single infected cell is given by  $R_0 = \lambda\beta/da$ . When  $R_0$  is less than one, the level of virus within an individual can never increase. If  $R_0$  is greater than one, the level of virus can increase and an infection can be established: the patient's viral load will reach an equilibrium level over time. It is important to keep in mind that this within-host measure of the virus's replicative ability is different to the population-level (between-host)  $R_0$  discussed above. (The two quantities are linked, however: if the virus is unable to replicate effectively within an individual, then an infected individual would be very unlikely to be able to transmit infection.)

An important observation is that the immune response cannot completely clear the infection within this model. This reflects the predator-prey dynamics between the immune response and the infection: if the level of infection falls, the level of the immune response falls which in turn allows the level of infection to rise. Eventually, an equilibrium is established between the two. The level of this equilibrium, however, is higher or lower depending on how quickly the immune response can kill infected cells.

Since we are interested in the dynamics of an acute infection, our primary interest is in the short term behavior of the within-host model in response to the introduction of infection. It is difficult to perform much mathematical analysis on these dynamics, but it turns out that some insight can be gained by understanding the equilibrium behavior of the model. An equilibrium approach is also informative when comparing the behavior of the rhinovirus system with that previously described in the setting of HIV infection. Our discussion will initially focus on

equilibrium dynamics before later returning to the short term dynamics of acute infection.

**5.1. The Two Strain Within-Host Model and Drug Treatment.** The within-host model can be extended to consider the interaction between two virus strains and the impact of treatment on the strains (Wodarz and Lloyd, 2004). We account for wild-type (drug sensitive) strain and a drug resistant strain, and the numbers of cells infected with these strains are written as  $y_w$  and  $y_r$ . Furthermore, we assume that these strains will be equally well recognized by the immune response: this assumption corresponds to the belief that a novel resistant strain will most likely be serotypically identical to the strain from which it evolved. We also assume that the lifespan of infected cells (in the absence of an immune response) is the same for cells that are infected with either strain.

The infection parameter  $\beta$  is allowed to differ between the two strains, as expressed by the two parameters  $\beta_w$  and  $\beta_r$ . As mentioned before, the acquisition of resistance usually comes at a fitness cost, so we have that  $\beta_r < \beta_w$ . The imposition of treatment reduces the replication of virus: we have that  $\beta'_w < \beta_w$ , where  $\beta'_w$  denotes the treatment value of the infection parameter. In order for the mutant strain to be considered resistant, we require that  $\beta'_r$  is greater than  $\beta'_w$ : the ‘resistant’ strain is better able to replicate during treatment than the wild-type. Notice that the replication rate of the resistant strain may be affected by treatment, but the operational definition of resistance is that treatment overturns the competitive advantage that the wild-type enjoys (due to the cost of resistance) in the absence of treatment (Bonhoeffer and Nowak, 1997; Bonhoeffer et al., 1997).

From these infection parameters, we can write down the basic reproductive numbers of the strains in the presence and absence of treatment. We denote these quantities by  $R_0^{(w)}$ ,  $R_0^{(w)'}$ ,  $R_0^{(r)}$  and  $R_0^{(r)'}$ , where the superscripts denote wild-type and resistant and the primes denote values during treatment. Since we have assumed that the infected cell lifespan (and indeed the rate at which the immune response clears infected cells) is the same for both strains, we see that the basic reproductive numbers are obtained by multiplying the corresponding infection parameters by  $\lambda/da$ .

Mutation can lead to the generation of resistant strains in patients that were only initially infected with drug sensitive virus. To model this possibility, we assume that infection of cells by the sensitive strain can lead to the appearance of a resistant strain. The per infection mutation probability (usually assumed to be small) is written as  $\mu$ . Since we are interested in the emergence and spread of resistant virus, we ignore back mutation from the resistant to the sensitive strain.

The two strain model, in the presence of mutation from wild-type to resistant virus, can be written as

$$(14) \quad \dot{x} = \lambda - dx - \beta_w xy_w - \beta_r xy_r$$

$$(15) \quad \dot{y}_w = (1 - \mu)\beta_w xy_w - ay_w - py_w z$$

$$(16) \quad \dot{y}_r = \beta_r xy_r + \mu\beta_w xy_w - ay_r - py_r z$$

$$(17) \quad \dot{z} = c(y_w + y_r)z - bz$$

in the absence of treatment. During treatment, the same model holds except that the  $\beta$  parameters are replaced by the  $\beta'$ .

**5.2. Equilibrium Behavior of the Within-Host Model.** As for the between-host model, the outcome of the within-host model depends on the competition between strains. Our first step towards understanding the within-host dynamics involves investigating the competition between sensitive and resistant strains. In this section we shall assume that resistant strains are already present at an early stage of infection: discussion of the appearance of resistant strains will be deferred until later.

An important difference between the within-host and between-host models is that target cells only experience infection with one strain or the other: the within-host model is in some ways similar to the between-host model with complete cross immunity ( $\sigma = 0$ ). Competition between strains, therefore, is strong. In the absence of mutation and treatment, the wild-type strain out-competes the resistant strain, driving it to extinction. Assuming that  $R_0^{(w)}$  is greater than one, an equilibrium level of infection is approached in the long term. As in the between-host model, mutation allows for the maintenance of the resistant strain at a low level, reflecting the within-host balance between mutation and selection.

When treatment is applied, the sensitive strain loses its competitive advantage and so the frequency of the resistant strain may increase. The presence of an effective immune response, however, can temper this increase. Assuming that the target cell level quickly rebounds to its pre-treatment level following the start of therapy and that the level of the immune response does not fall appreciably over this time, the following condition for the growth of resistant virus can be derived (Wodarz and Lloyd, 2004)

$$(18) \quad R_0^{(r)'} - 1 - (p/a)z^* > 0.$$

Here  $z^*$  is the equilibrium level of the immune response that developed in response to the growth of the sensitive virus. As would be expected, since the immune response effectively increases the death rate of infected cells, the established immune response makes it more difficult for the resistant strain to grow (its  $R_0$  must lie well above one).

In the limit of a strong enough immune response, it can be shown that this condition reduces to

$$(19) \quad R_0^{(r)'} > R_0^{(w)}.$$

In order for resistant virus to grow during treatment, its basic reproductive number must be greater than that of the sensitive virus in the absence of treatment. This is very unlikely to be the case in reality: the cost of resistance means that the resistant virus should have a lower replicative ability than the wild-type.

If the immune response is weaker, this condition is relaxed somewhat. The degree to which this condition is relaxed depends on the relative importance of the immune response, compared to the natural death of infected cells, in the turnover of infected cells. It has been argued (Wodarz and Lloyd, 2004) that if the immune response is efficient, i.e. is largely responsible for the removal of infected cells, then condition (19) provides a good approximation.

The level of resistant virus can be kept in check by two distinct mechanisms. In the absence of therapy, resistant virus is out-competed by sensitive virus. In the presence of therapy, resistant virus can be kept at low values by the immune response that was developed in response to the sensitive virus. This second effect assumes that the immune response does not quickly fade upon treatment (and the



We assume that there is a fixed probability that any viral replication cycle will lead to a cell becoming infected with the novel resistant mutant. The chance of generating a resistant mutant, therefore, is proportional to the number of replication events that occur. We can estimate the relative probabilities of a resistant mutant

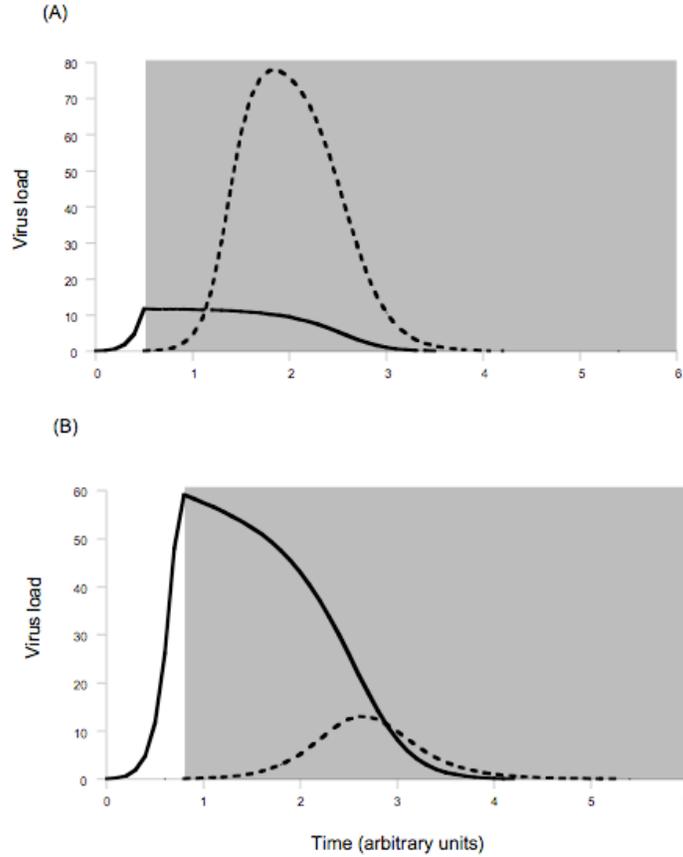


FIGURE 4. The time at which therapy is started affects the level to which resistant virus (dashed lines) can grow over the course of a treated infection. (Solid lines denote the level of sensitive, wild-type, virus.) In panel (a), therapy is started early, before the immune response has fully developed. Consequently, drug resistant virus can grow to a high level. In panel (b), therapy is started later and so a well developed immune response hinders the growth of resistant virus. In both cases it is assumed that resistant virus exists before the start of treatment. An illustrative set of parameter values was chosen as follows:  $\lambda = 10$ ,  $d = 0.1$ ,  $a = 0.2$ ,  $p = 1$ ,  $c = 0.05$ ,  $b = 0.01$ ,  $\beta_w = 0.1$ ,  $\beta_r = 0.09$ ,  $\beta'_w = 0.0021$ ,  $\beta'_r = 0.09$ . The shaded area denotes the time over which treatment is deployed.

being generated before the start of therapy and during therapy by simply counting the number of times that wild-type virus replicates before and after the start of therapy. This question of whether resistant virus is more likely to evolve before or during treatment has been studied in detail for HIV infection (Bonhoeffer and Nowak, 1997).

Figure 5 illustrates the scaled number of replication events that occur before and after the start of therapy for a wild-type infection, assuming a range of treatment efficacies (defined as  $1 - R_0^{(w)'}/R_0^{(w)}$ ). The number of replication events before therapy is, as should be expected, a monotonic function of the time at which treatment is started.

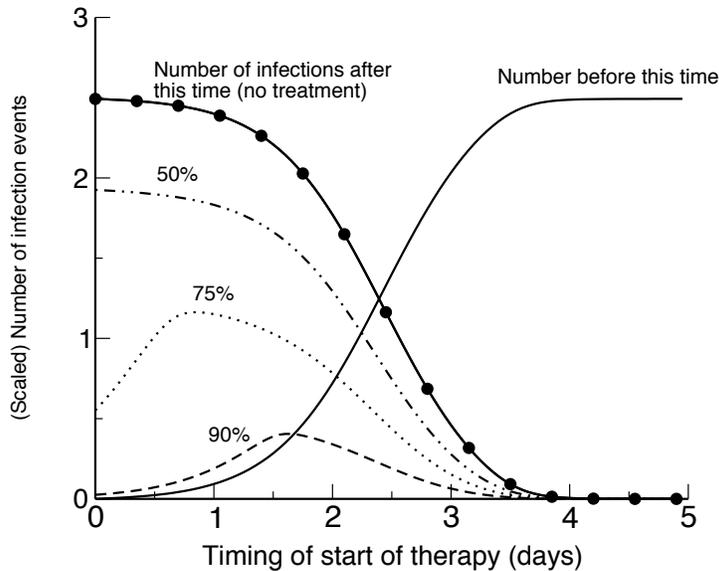


FIGURE 5. Number of infection events occurring before and during therapy for a wild-type infection, as a function of the time at which therapy is started. The solid curve depicts the number of infection events that occur before a given time point in the absence of therapy. The solid curve with symbols depicts the complementary quantity, namely the number of infection events that occur after the given time point. The remaining curves show the number of infection events that occur after the start of therapy, as a function of the time at which therapy is started, assuming that treatment is either 50, 75 or 90 percent effective. All curves were obtained by numerical simulation of the within-host model using the parameter values  $\lambda = 0.25$ ,  $d = 0.1$ ,  $\beta_w = 1.0$ ,  $a = 0.25$ ,  $c = 4.0$ ,  $p = 4.0$  and  $b = 0.1$ .

Notice that we can easily produce a second curve that shows the number of infection events that occur *after* a given time point. This second curve is shown on figure 5 as the curve with symbols superimposed. In the limit of zero treatment

efficacy, this second curve gives the number of infection events that occur after the onset of therapy. The corresponding curves for moderately effective therapies follow a similar shape, although with lower levels since the drug now diminishes wild-type replication.

If the drug efficacy is high, wild-type replication is efficiently curtailed following the start of therapy. In this case, the number of replication events during therapy echoes the viral load achieved before the start of therapy. Consequently, as can be seen in figure 5, the curves for high drug efficacy essentially mimic the plot of viral load against time seen in a wild-type infection. The generation of resistant mutants in this case appears to be most likely if treatment is started near the peak level of viraemia.

## 6. Discussion

The key point that emerges from these models is the crucial role played by competition between strains, either for susceptible hosts on the epidemiological level or for target cells on the immunological level. In turn, we see that this competition depends on the details of the epidemiology and virology/immunology of the infection. At both the within and between host levels, competition is modulated by some factor. At the epidemiological level, competition is modulated by the degree of cross-immunity conferred by infection. In the rhinovirus context, this gives rise to two quite different scenarios when naturally resistant serotypes and novel resistant variants are considered. At the immunological level, competition is modulated by the immune response that is generated against the wild-type infection. Consequently, the effectiveness of the immune response and whether the infectious agent leads to acute or chronic infection has a major impact on the emergence of resistance.

Despite gaining these insights, much work remains to be done here. We have yet to provide a complete description of the emergence and rise of resistance at the within-host level: the work described here considers these problems as two separate issues. These viewpoints need to be integrated more closely. This will likely require the deployment of a stochastic modeling framework. This approach has been described in the setting of HIV infection (Ribeiro and Bonhoeffer, 2000), but it leads to considerable additional complexity. Furthermore, as discussed earlier, the simple immune response that we employed in the model is in many ways unsatisfactory for describing an acute infection.

The most appropriate parameter values for this system are unclear at both the epidemiological and within-host levels. In the absence of these parameter values, we view the model predictions presented here as giving qualitative, rather than quantitative, insights into the dynamics of the system. Epidemiological parameters could be elucidated from more detailed transmission experiments. These could easily be accommodated within the framework of future clinical trials that assess the efficacy of drug treatment with pleconaril (or indeed similar drugs that are developed). Within-host parameters could, in analogy with the development of HIV models, be measured if trials investigated the viral load seen in patients over the time course of their infection. Furthermore, the use of well-designed clinical trials (such as family-based transmission studies) could yield important information regarding both the impact of treatment on reducing secondary transmissions and the likelihood of the generation and transmission of resistance.

Both the epidemiological and within-host models ignore heterogeneity within the population. A key heterogeneity is likely to be the strength of individuals' immune responses: these differ across a population, with certain groups (such as the immunocompromised or elderly) having significantly weakened immune responses. Resistance is much more likely to emerge in immunocompromised individuals, so from the viewpoint of managing resistance we might be less inclined to treat infection in this subgroup. But these are precisely the people who are likely to benefit most from treatment. This raises an interesting conflict between an individual's benefit of treatment and the population-level costs of treatment (i.e. the emergence of resistance). Mathematical frameworks such as the one presented here provide an ideal way to study such conflicts in more detail. (See Bauch et al. (2003) for a game-theoretic approach to a somewhat related individual/population conflict in the context of a mass-vaccination policy.)

Although this study has presented both between- and within-host models, the two were considered in isolation. An ambitious goal is to combine the two within a single, multi-scale, framework. Clearly, this will provide a much more realistic depiction of the dynamics of resistance since there is a close connection between the emergence of resistance within a patient and its spread at a population level. Such multi-scale models, however, pose significant challenges, particularly in terms of how we might gain general understanding of general patterns of behavior.

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