



Antiviral resistance and the control of pandemic influenza: The roles of stochasticity, evolution and model details

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ABSTRACT

Antiviral drugs, most notably the neuraminidase inhibitors, are an important component of control strategies aimed to prevent or limit any future influenza pandemic. The potential large-scale use of antiviral drugs brings with it the danger of drug resistance evolution. A number of recent studies have shown that the emergence of drug-resistant influenza could undermine the usefulness of antiviral drugs for the control of an epidemic or pandemic outbreak. While these studies have provided important insights, the inherently stochastic nature of resistance generation and spread, as well as the potential for ongoing evolution of the resistant strain have not been fully addressed. Here, we study a stochastic model of drug resistance emergence and consecutive evolution of the resistant strain in response to antiviral control during an influenza pandemic. We find that taking into consideration the ongoing evolution of the resistant strain does not increase the probability of resistance emergence; however, it increases the total number of infecteds if a resistant outbreak occurs. Our study further shows that taking stochasticity into account leads to results that can differ from deterministic models. Specifically, we find that rapid and strong control cannot only contain a drug sensitive outbreak, it can also prevent a resistant outbreak from occurring. We find that the best control strategy is early intervention heavily based on prophylaxis at a level that leads to outbreak containment. If containment is not possible, mitigation works best at intermediate levels of antiviral control. Finally, we show that the results are not very sensitive to the way resistance generation is modeled.

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1. Introduction

It is almost certain that sooner or later, a new influenza A virus will emerge against which humans have little or no immunity and that is able to spread through human populations and potentially cause a pandemic (Webster, 2002; Cox and Subbarao, 2000). In the face of this threat, researchers have been studying control strategies that might prevent or mitigate such a pandemic (Longini et al., 2005; Ferguson et al., 2005, 2006; Longini et al., 2004; Germann et al., 2006; Duerr et al., 2007). Most proposed intervention strategies rely to some extent on the use of antivirals, most notably the neuraminidase inhibitors (Gani et al., 2005; Moscona, 2005). Unfortunately, the strong selection pressure exerted by the extensive use of drugs often leads to the evolution of drug resistance (Livermore, 2005; Levy and Marshall, 2004;

Dancer, 2004). Most situations encountered so far in the realm of antibiotic resistance involve time-scales on the order of years before a large fraction of hosts harbors a resistant strain (Livermore, 2005; Lipsitch, 2001). However, the high mutation rate of viruses can lead to a much more rapid evolution of resistance. One premier example is the evolution of resistance that occurs in HIV during treatment with a single drug (Clavel and Hance, 2004). Since influenza is also a relatively fast evolving virus with a high mutation rate (Parvin et al., 1986; Nobusawa and Sato, 2006), it is possible that drug resistance can become a problem during the course of a single pandemic outbreak.

A number of modeling studies investigated the possible impact of resistance emergence and spread during an influenza outbreak (Stilianakis et al., 1998; Ferguson et al., 2003; Regoes and Bonhoeffer, 2006; Lipsitch et al., 2007; Alexander et al., 2007; Debarre et al., 2007; Xu et al., 2007; Moghadas et al., 2008). While these studies have provided important insights, a few aspects remain to be fully addressed. Most importantly, the majority of studies are based on deterministic models. This ignores the stochastic nature of the rare events that lead to initial resistance generation and spread, while a few recent studies were based on

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stochastic models (Debarre et al., 2007; Xu et al., 2007), these studies only considered outbreaks in small populations (less than $10^3 - 10^4$ individuals). Further, these studies did not consider continued evolution of the resistant strain. While resistance usually carries a fitness cost, the resistant mutants can undergo further evolution, acquiring so called compensatory mutations that restore their fitness while retaining the resistant phenotype (Andersson and Levin, 1999; Maisnier-Patin and Andersson, 2004). The result can be a strain that is at the same time drug resistant and has a fitness close to—and in the worst case even higher than—the original drug-sensitive strain. Limited *in vitro* evidence suggests that compensatory mutations might occur for neuraminidase inhibitor resistant influenza (Yen et al., 2006). Only one study considered compensatory mutations for influenza drug resistance (Moghadas et al., 2008). However, this study is based on a deterministic framework, and due to the rarity of these compensatory mutation events, a stochastic framework is more appropriate (Handel et al., 2006).

Here, we study a stochastic model of neuraminidase inhibitor resistance emergence and consecutive evolution of the resistant strain in response to antiviral control during an influenza pandemic in a large population. Our study shows that taking stochasticity into account leads to results that can differ from deterministic models. Specifically, we find that rapid and strong control can contain not only a drug sensitive outbreak but also prevent a resistant outbreak from occurring. We find that the best control strategy to prevent resistance emergence and reduce the total number of infecteds is early intervention heavily based on prophylaxis at a level that leads to outbreak containment. If containment is not possible, mitigation works best at intermediate levels of control. Taking into consideration the ongoing evolution of the resistant strain does not increase the probability of resistance emergence, however it increases the total number of infecteds if a large resistant outbreak occurs. We also show that the results are largely insensitive with respect to the detailed implementation of the resistance generation process.

2. The model

We model the outbreak using a stochastic, compartmental, SIR-type model. A schematic flow diagram of the model is shown in Fig. 1, Table 1 gives the transitions and their propensities which fully specify the model, while Table 2 summarizes the variables and parameters of the system.

We consider a pandemic outbreak in the United States. We assume that for a novel, pandemic strain, no immunity exists, the whole population is susceptible (*S*). Susceptible hosts receive prophylaxis with a uniform probability, or phrased differently, a fraction f_p of susceptible hosts receive prophylaxis, which has an efficacy of e_p . If prophylaxis fails, hosts become infected. We assume that all infected persons will become ill and show symptoms, we ignore the possibility of asymptomatic infections. Infecteds are divided into five different compartments. A fraction f_t of hosts infected with the drug sensitive strain receive antiviral treatment (I_t), while the remainder of the hosts infected with the sensitive strain do not (I_u). Following Lipsitch et al. (2007), we assume that failed prophylaxis leads to a course of infection

Table 1
Possible transitions and their propensities (the propensity multiplied with the time step gives the probability that a given event occurs)

Transitions	Propensity
$S \rightarrow S - 1, I_u \rightarrow I_u + 1$	$(1 - f_p)(1 - f_t)(\beta_u(1 - c_u)I_u + \beta_t(1 - c_t)I_t)S$
$S \rightarrow S - 1, I_t \rightarrow I_t + 1$	$(f_p(1 - e_p) + (1 - f_p)f_t)(\beta_u(1 - c_u)I_u + \beta_t(1 - c_t)I_t)S$
$S \rightarrow S - 1, I_1 \rightarrow I_1 + 1$	$(\beta_u c_u I_u + \beta_t c_t I_t)S + \beta_1(1 - c_1)I_1 S$
$S \rightarrow S - 1, I_2 \rightarrow I_2 + 1$	$\beta_1 c_1 I_1 S + \beta_2(1 - c_2)I_2 S$
$S \rightarrow S - 1, I_3 \rightarrow I_3 + 1$	$\beta_2 c_2 I_2 S + \beta_3 I_3 S$
$I_u \rightarrow I_u - 1$	$v_u I_u$
$I_t \rightarrow I_t - 1$	$v_t I_t$
$I_1 \rightarrow I_1 - 1$	$v_1 I_1$
$I_2 \rightarrow I_2 - 1$	$v_2 I_2$
$I_3 \rightarrow I_3 - 1$	$v_3 I_3$

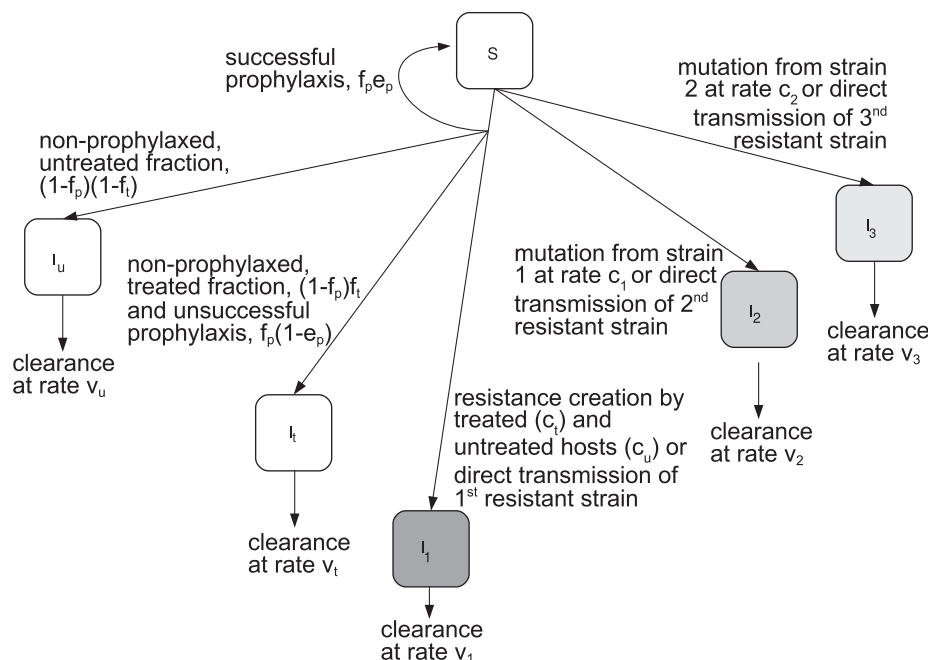


Fig. 1. Schematic of the compartmental model describing the infection dynamics. The compartments are susceptibles, *S*, persons infected with the drug sensitive strain that are untreated, I_u , persons infected with the drug sensitive strain that receive treatment, I_t , and persons infected with the first, second and third resistant strain, I_1 , I_2 and I_3 . The first resistant strain is the one initially generated, ongoing evolution leads to further mutations that increase fitness of the resistant strain, resulting in I_2 and subsequently in I_3 . Table 1 show the possible transitions and their propensities, Table 2 summarizes the model parameters. Further details are given in the text.

Table 2
Model parameters

Symbol	Meaning	Values	Comment
f_p	Fraction of uninfecteds receiving prophylaxis	0–1	Varied
f_t	Fraction of infecteds receiving treatment	0–1	Varied
e_p	Efficacy of prophylaxis	0.8	Based on AVE ₅ in Yang et al. (2006), AVE _{5d} in Halloran et al. (2007)
ν_u	Clearance rate (1/mean duration of infection) of untreated infected hosts	$1/4.8d^{-1}$	Based on Carrat et al. (2008)
ν_t	Clearance rate of treated infected hosts	$1/3.4d^{-1}$	Reduction of infectious period by $\approx 30\%$, based on Treanor et al. (2000) and Whitley et al. (2001)
ν_1, ν_2, ν_3	Clearance rate of resistant infected hosts	$1/4.8d^{-1}$	Assumption that resistant strain leads to same duration of infection as sensitive strain
c_t	Probability of resistance generation for treated hosts	10^{-3}	Based on Fig. 4A in Handel et al. (2007b), assuming treatment at day one for the more realistic (IR) model
c_u	Probability of resistance generation for untreated hosts	10^{-5}	Similar to value for ineffective (late) treatment in Handel et al. (2007b, Fig. 4A), IR model
c_1, c_2	Probability of resistance generation of compensatory mutants	10^{-3}	See text
R_u	Reproductive number of susceptible strain (in the absence of treatment)	2.0	Viboud et al. (2006) and Wallinga and Lipsitch (2007)
R_t	Reproductive number of susceptible strain (in the presence of treatment)	0.68	Based on Yang et al. (2006)
R_1, R_2, R_3	Reproductive numbers of resistant strains	1.5, 1.75, 2.0	Assumed
$\beta_u, \beta_t, \beta_1, \beta_2, \beta_3$	Transmission parameters		Calculated as $R_i \nu_i / N_0$
N_0	Population size	3×10^8	US population

Values are specific for neuraminidase inhibitor treatment and resistance emergence in influenza A.

comparable to a treated host. Additionally, three compartments for resistant mutants are considered, (I_1, I_2 and I_3). We assume that treatment has no effect on the resistant strains. All infected hosts leave the infected stage after some time, either through recovery or death. The rates of “clearing” the infection by either means are listed in Table 2.

There are several important differences that distinguish our model from previous ones. Most previous models include a conversion rate from wild-type infected to resistant infected hosts. This assumes that once a host converts to a resistant one, the infection “starts over”. In contrast, we assume here that resistance can emerge during treatment, and with probability c_t cause new infections that are dominated by the resistant strain. We believe that this way of implementing resistance generation is more realistic. Additionally, we allow resistance to arise and spread with a small probability, c_u , in untreated patients. Values for c_t and c_u are chosen based on estimates we obtained in a previous study (Handel et al., 2007b). Specifically, we chose the value for c_t as obtained from the immune response model with treatment occurring one day after infection, while the value for c_u was chosen slightly lower than that obtained for the no treatment case (see Handel et al., 2007b, Figure 4). Also note that in our model, prophylaxis has no direct effect on the generation of resistant infecteds. Instead, prophylaxis influences resistance generation through the fact that failed prophylaxis places infecteds into the treated class (which is more likely to give rise to resistance than the untreated class).

Our model includes the evolution of the resistant strain. While back-mutations to the fitter, susceptible strain are possible, it is often more likely that instead of reversion to the original, drug sensitive genotype, the resistant mutant undergoes further, so called compensatory mutations (Levin et al., 2000; Maisnier-Patin and Andersson, 2004; Handel et al., 2006). These mutations reduce the fitness cost that comes with resistance, while at the same time retaining the resistant mutation. The result can be a strain that is at the same time resistant and has a fitness similar to the initial, sensitive strain. Evolution of compensatory mutants could occur along a single linear pathway or there could be

multiple routes with multiple possibilities for compensatory mutations to increase fitness. For illustrative purposes, we choose a simple, linear pathway with three levels of fitness for the resistant strain. While the positive selection pressure experienced by the fitter, compensated mutants could be less strong compared to the selection pressure induced by drug treatment, there is no data for estimates of the rate at which compensated mutants arise and spread. As a conservative estimate, we assume that resistant mutants with increased fitness are generated at the same rate as the initial generation of resistance during treatment, i.e. we choose $c_1 = c_2 = c_t$.

Since the creation of resistance is a rare event, stochasticity is important. Therefore, we use a stochastic model. The model is a variation of a discrete time, Monte-Carlo simulation, often referred to as the Gillespie (1977) algorithm. The Gillespie algorithm produces exact trajectories of the stochastic process. Since a straightforward implementation of the Gillespie algorithm would be computationally too expensive for the population size we consider, we instead use a recently introduced hybrid stochastic solver known as partitioned leaping algorithm (Harris and Clancy, 2006). The algorithm uses the exact Gillespie method for low numbers and reaction rates, i.e. when stochasticity is important, but switches automatically to computationally more efficient methods using Poisson, Langevin and deterministic approximations when appropriate (Harris and Clancy, 2006). This leads to a significant reduction in execution time, while still essentially retaining the “exactness” of the Gillespie algorithm. The simulations are implemented in Fortran 90, the code is available from the authors upon request.

3. Results

Antiviral control affects the drug sensitive strain by reducing its fitness, defined in our setting as the average number of secondary infections caused by an infected host, the reproductive number (Diekmann et al., 1990; Anderson and May, 1991; Hethcote, 2000). The reproductive number of the sensitive strain

is given by the largest eigenvalue of the matrix $M = FV^{-1}$ (van den Driessche and Watmough, 2002), where

$$F = \begin{pmatrix} (1-f_p)(1-f_t)\beta_u & (1-f_p)(1-f_t)\beta_t \\ (f_p(1-e_p) + (1-f_p)f_t)\beta_u & (f_p(1-e_p) + (1-f_p)f_t)\beta_t \end{pmatrix}$$

and

$$V = \begin{pmatrix} v_u & 0 \\ 0 & v_t \end{pmatrix}.$$

From this one finds that the reproductive number in the presence of control is

$$R_f = (1-f_t)(1-f_p)R_u + (f_t + f_p(1-e_p-f_t))R_t. \quad (1)$$

Note that we ignored the negligible contributions of resistance generation, i.e. we set $c_i \approx 0$. The reproductive numbers for untreated and treated hosts R_u and R_t , as well as the other parameters are given in Table 2. If $R_f < 1$, on average less than one new host gets infected with the sensitive strain and therefore the outbreak will die down. For $R_f > 1$, containment of the outbreak is likely to fail. However, treatment or prophylaxis will still reduce the number of hosts infected with the sensitive strain. Note that the antiviral has no effect on the fitness of the resistant strains.

For any infectious disease outbreak, there will be a time lag between the occurrence of the first infection, the recognition of the outbreak as such, and implementation of control measures. Since the probability that resistance is generated depends on the number of infected hosts, rapid containment of the outbreak will reduce the probability that a resistant mutant is generated and spreads. In the following sections, we study how resistance emergence depends on the number of infecteds before control starts. We consider this for different scenarios by varying the evolutionary pathway of the resistant strain and the type (prophylaxis versus treatment) and strength of the antiviral control.

3.1. Compensatory mutations do not change the probability of resistance emergence, but increase the number of infecteds in large outbreaks

We start by considering how ongoing evolution of the resistant strain can influence the probability of resistance generation and the size of a pandemic outbreak. For the first scenario, we assume that antiviral control is not strong enough to control the drug sensitive strain, that is we have $R_f > 1$. The control effort leads to only a mitigation in outbreak size. Since in this situation, a large number of infections occur, resistance is always generated. In the presence of compensatory mutations, the resistant strain can evolve to higher fitness and therefore contribute to a larger outbreak, increasing the attack rate by $\approx 20\%$ (Fig. 2). Including ongoing evolution also increases the variance in the outbreak size, which is expected since stochastic effects are most important when a new resistant strain is created, which happens three times for the scenario with ongoing evolution, versus only one time in the absence of further evolution. Since the dynamics is nonlinear, it is not expected that the mean of the stochastic simulations agrees with the result found from the equivalent deterministic model. However, as Fig. 2 shows, there is relatively good agreement. If control starts early, almost all infections are caused by the resistant strain(s). If control starts later, the sensitive strain causes a significant outbreak before the resistant strains emerge and cause their own outbreaks. These multiple smaller outbreaks cause less infections compared to one large outbreak, leading to the observed decline in overall attack rate for late control. This phenomenon has been noted previously (Handel et al., 2006; Lipsitch et al., 2007) and we will return to it in a later section.

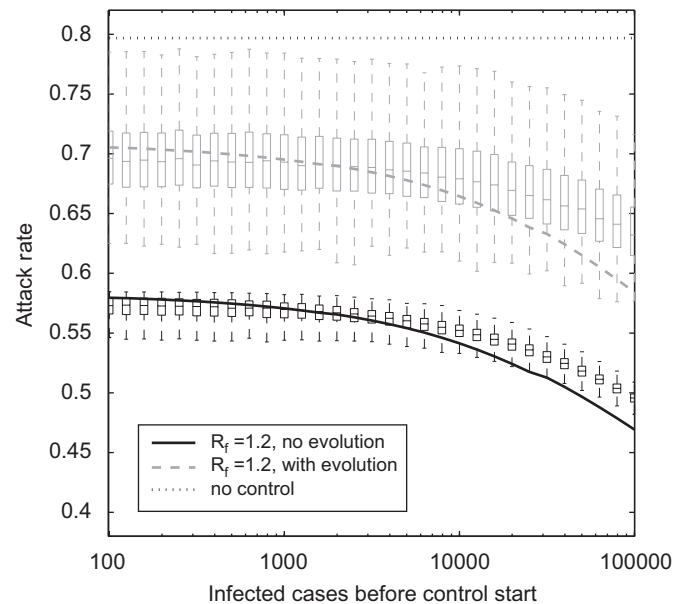


Fig. 2. Attack rate in the absence and presence of compensatory mutations. Control starts after the indicated number of infections have occurred, with treatment and prophylaxis chosen at equal levels, ($f_t = f_p$). Control can only mitigate the outbreak ($R_f = 1.2$). Attack rate is defined as the total number of infecteds divided by the population size. Boxplots are results from 2000 stochastic simulations, lines show results from the equivalent deterministic model. The black boxes and solid line are results in the absence of ongoing evolution through compensatory mutations, the gray boxes and dashed line show results in the presence of ongoing evolution. The dotted line shows the attack rate in the absence of control. The resistant strains have $R_1 = 1.5$, $R_2 = 1.75$ and $R_3 = 2.0$, for the case with compensatory mutations, $c_1 = c_2 = c_t$.

In the next scenario, we assume that the control effort is strong enough to lead to $R_f < 1$, i.e. the outbreak caused by the sensitive strain can be contained. If containment occurs before the resistant strain has emerged, no major epidemic occurs. If, on the other hand, the resistant strain is generated and starts to spread, antiviral control efforts become ineffective and a large epidemic caused by the resistant strain occurs. We find that including evolution of the resistant strain barely changes the probability of resistance emergence Fig. 3. However, as was the case in the mitigation scenario above, the increase in fitness of the resistant strain due to compensatory mutations leads to an increase in attack rate by as much as $\approx 20\%$.

3.2. Evolution through compensatory mutations can be mapped onto a one-step process

While continued evolution of a resistant strain is without doubt going to occur, the details of the evolutionary process cannot be predicted. Above, we assumed that fitness increases in equal steps, from $R_1 = 1.5$ over $R_2 = 1.75$ to $R_3 = 2.0$, the original fitness of the sensitive strain. We further assumed that the probability of these events happening was the same as the probability of emergence during treatment, i.e. $c_t = c_1 = c_2$. However, other scenarios are equally likely. The increase in fitness could for instance occur in unequal steps or the probabilities for these events could differ. While it is impossible to explore all these scenarios (see Handel et al., 2006 for some more details), it is worth investigating if and how the evolutionary trajectory can be mapped onto a simple process where a resistant strain emerges and does not undergo further compensatory mutations. Two mappings might be expected to be possible. First, a 3-step trajectory with fitness levels $R_1 = 1.5$, $R_2 = 1.75$ and $R_3 = 2.0$ could be equivalent to a single step to a resistant strain with some

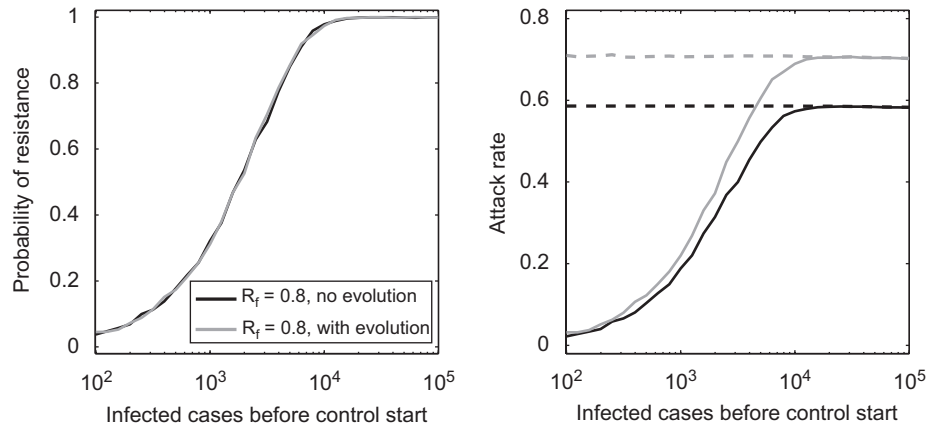


Fig. 3. Probability of resistance emergence and attack rate in the absence and presence of compensatory mutations. Control is strong enough to contain the sensitive outbreak ($R_f = 0.8$). Left: Probability of resistance emergence in the absence (black) and presence (grey) of compensatory mutations. Resistance is considered to have emerged if at least 5% of the total number of infections that have occurred during the outbreak are resistant infecteds (a higher/lower percentage leads to a right/left shift of the curves). Right: Solid lines show attack rate averaged over all 2000 stochastic simulations, dashed lines show attack rate averaged only over those simulations where a (resistant) outbreak occurred. The variance in attack rate for the stochastic simulations when an outbreak occurs is very small, we therefore only plot the mean instead of showing boxplots. These mean values agree closely with the deterministic results (not plotted). Rest as explained previously.

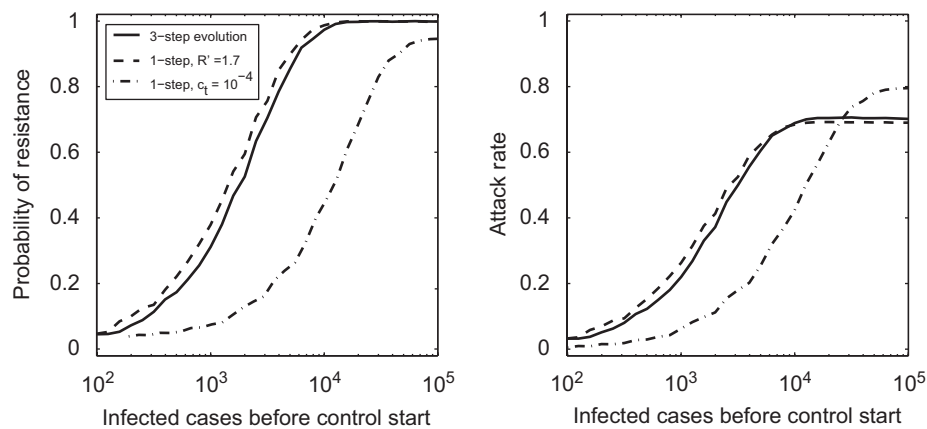


Fig. 4. Different evolutionary trajectories of the resistant strain. Control is strong enough to contain the sensitive outbreak ($R_f = 0.8$), as previously shown in Fig. 3. Left: Probability of resistance emergence for the 3-step evolutionary trajectory previously shown in Fig. 3 (solid line), a 1-step process with $c_t = 10^{-3}$ as before and $R' = 1.7$ (dashed line), and a 1-step process with $c' = 10^{-4}$ leading to a strain with fitness $R' = 2$ (dash-dotted). Right: Average attack rate. Attack rate here and in the following figures is the average over all stochastic simulations, independent of the occurrence of a resistant outbreak.

different fitness R' . Alternatively, the three probabilities c_t , c_1 and c_2 , might be mapped into a single probability c' , directly leading to the final strain. As Fig. 4 shows, while it is indeed possible to find a 1-step process with resistant fitness R' that produces a result similar to that of the 3-step process, it is not possible to map the jump probabilities into a single one. The reason for this latter finding is that the main “bottleneck” in the process is caused by the initial generation of resistance. Once the resistant strain has been generated, it starts to spread and quickly reaches levels at which the generation of fitter strains is almost certain. Therefore, the initial rate of resistance generation is crucial in determining the probability of resistance emergence, and therefore the average attack rate. Changing this rate to a different value, c' , does not lead to dynamics that resembles a process with three transition rates.

3.3. Early control based on strong prophylaxis is the best control strategy to prevent resistance emergence

While treatment with neuraminidase inhibitors will be important to reduce morbidity and mortality of individuals,

epidemiological control can be achieved with treatment or prophylaxis. While resistance is more likely to emerge during treatment, the fact that prophylaxed individuals are only susceptible to the resistant strain leads to strong selective pressure for resistance (Lipsitch et al., 2007). Nevertheless, we find that if control is strong enough to contain the outbreak caused by the sensitive strain, prophylaxis fares better in preventing resistance emergence and therefore reducing the attack rate Fig. 5. Stronger control measures (i.e. a further reduction in R_f) contain the sensitive outbreak faster, thereby further reducing the probability that resistance emerges. This leads to a shift of the curves in Fig. 5 towards the right (not shown). Equivalently, resistance emergence becomes more likely and the curves shift to the left if control is less strong and containment takes longer. Note that again, the distribution underlying the average for the attack rate is bimodal, consisting of a fraction of simulations for which no resistant outbreak occurred, and another fraction (given by the probability of resistance emergence) for which resistant outbreaks occur. Changing the level of control does not change the size of a resistant outbreak once it occurs,

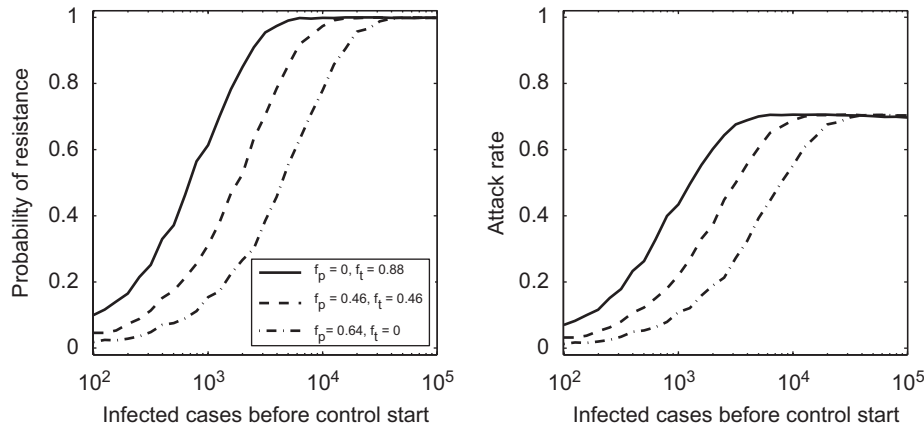


Fig. 5. Probability of resistance emergence and attack rate for different control strategies. Control starts after the indicated number of infections have occurred. It leads to $R_f = 0.8$, which is strong enough to contain the outbreak caused by the sensitive strain. Control strategies are: only treatment (solid line), equal levels of prophylaxis and treatment (dashed line) and only prophylaxis (dash-dotted line). Evolution through compensatory mutations is included.

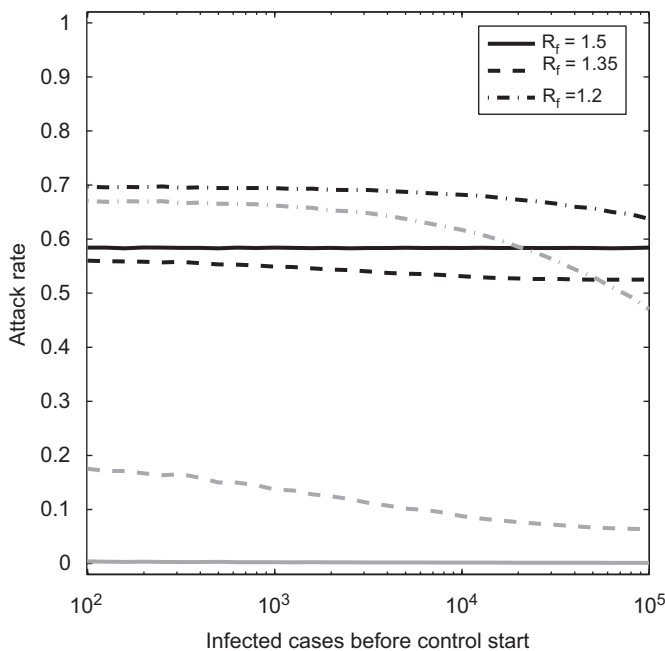


Fig. 6. Average attack rate for different levels of control. Treatment and prophylaxis are chosen equal ($f = f_p + f_t$). Control starts after the indicated number of infections have occurred. Control can only reduce, not contain the outbreak caused by the sensitive strain. The solid, dashed and dash-dotted lines show results for $R_f = 1.5, 1.35$ and 1.2 . Black lines are total attack rate, gray lines indicate fraction of cases caused by the resistant strains. Evolution through compensatory mutations is included as previously described.

it only changes the probability of such a resistant outbreak to occur.

3.4. Optimal mitigation occurs at intermediate control strength

If control cannot contain the outbreak, but instead can only mitigate its strength, resistance is very likely to be generated. However, if control does not bring the fitness of the sensitive strain below that of the initially generated resistant mutant, the sensitive strain will dominate. The resistant strain will cause few infections, not enough to have a significant chance of generating further mutants with fitness levels above that of the sensitive

strain. The pandemic is almost certain to end before resistance can emerge (i.e. account for more than 5% percent of infecteds) and virtually all infections are caused by the sensitive strain (Fig. 6, $R_f = 1.5$). Increasing control measures to a level where $R_f < R_1$ leads to a decrease of sensitive infecteds, but now resistance will emerge and contribute to the attack rate (Fig. 6, $R_f = 1.35$). Once control measures are strong enough to sufficiently suppress the sensitive strain, the resistant strain will dominate and lead to a large resistant outbreak, which in turn leads to an overall increase in infecteds (Fig. 6, $R_f = 1.2$). Therefore, if it is not possible to contain the outbreak, an intermediate level of control is optimal. We will discuss this point in more detail in the next section. In contrast to the containment scenario ($R_f < 1$), for the mitigation scenario ($R_f > 1$) the type of control (prophylaxis or treatment) has almost no impact on the overall attack rate (not shown).

3.5. Optimal treatment strategies differ between stochastic and deterministic models

Above results suggest, and previous studies have shown, that if there are two outbreaks, one caused by the drug sensitive strain and one by a drug resistant strain, an intermediate level of antiviral control can lead to a minimum in the total number of infecteds (Lipsitch et al., 2007; Moghadas et al., 2008). This can be explained by one of our previous studies, where we showed that the minimization of an “overshoot”—defined as the excess infections that occur during the waning phase of an outbreak—will lead to an optimal control strategy for multiple outbreaks, such as a drug sensitive outbreak followed by a drug resistant one (Handel et al., 2007a). Essentially, two small outbreaks, sensitive and resistant, lead to less overshoot and therefore a smaller overall number in infecteds compared to one large outbreak (Handel et al., 2007a; Lipsitch et al., 2007). These studies are based on a deterministic modeling framework, for which resistance is always generated. We decided to see if these proposed strategies are still optimal when stochasticity is taken into account.

Fig. 7 shows that for $R_f > 1$ (area left of the dotted vertical line in Fig. 7), there is indeed an intermediate level of control which minimizes the attack rate, in agreement with the results obtained for the mitigation scenario above. However, if control can be implemented such that $R_f < 1$, Fig. 7 suggests that more control is better, since it can reduce the probability of resistance generation (area right of the dotted vertical line in Fig. 7). This is in contrast

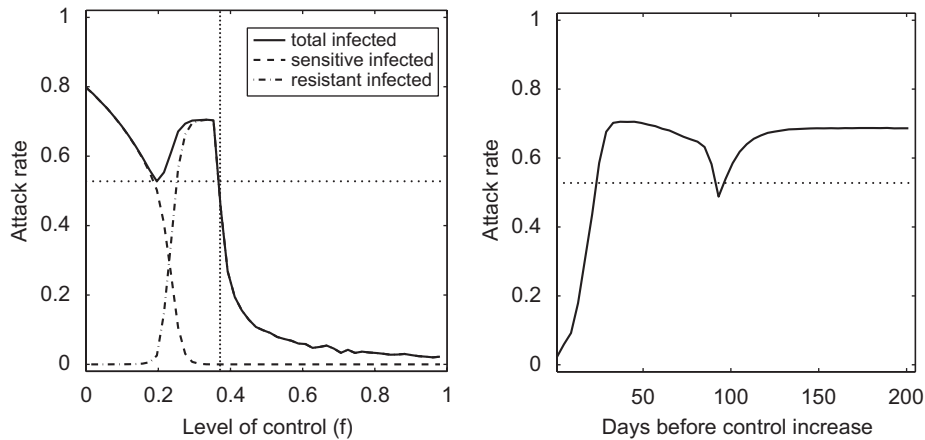


Fig. 7. Attack rate for varying levels of control. Control starts after 500 infected cases have occurred, treatment and prophylaxis are used equally ($f = f_t = f_p$). Left: Control strength is varied from 0 to 1 and kept constant throughout the outbreak. The vertical dotted line indicates the level of control for which $R_f = 1$. Right: Low level of control ($f = 0.1$) for the indicated number of days, followed by a switch to strong control ($f = 0.9$). The horizontal dotted line indicates the minimum attack rate obtained for the constant treatment schedule. Evolution through compensatory mutations is included.

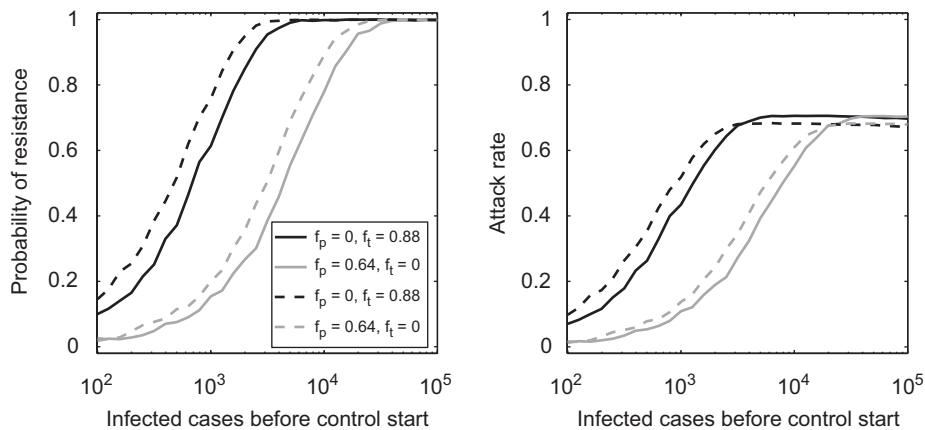


Fig. 8. Probability of resistance emergence and attack rate for different implementation of resistance generation. Control based on treatment only (black) or prophylaxis only (gray) for the model with resistance generation as used here (solid) and as used in previous studies (dashed). See text for details on the two model implementations. Evolution through compensatory mutations is included.

to results obtained using a deterministic framework (Moghadas et al., 2008), for which resistance is always generated and causes a second outbreak. In such a deterministic scenario, high levels of antiviral use lead to rapid generation of resistance and an increased overall attack rate, with an intermediate control level producing the lowest attack rate. The stochastic framework suggests that rapid and strong control that might lead to quick containment of the outbreak is best.

It was also shown that for a deterministic model, a strategy of initial low control, followed by a sudden increase in control strength once enough sensitive infecteds are depleted, could perform better compared to a strategy that is based on a constant level of treatment (Moghadas et al., 2008). However, little initial control is more likely to lead to generation of a secondary resistant outbreak, while rapid and strong control might contain not only the sensitive outbreak, but also prevent resistance generation. Fig. 7 confirms this. We plot attack rate for a situation where treatment starts at $f = 0.1$ and increases to $f = 0.9$ at the indicated time. The figure shows that a scenario at which the switch to stronger control occurs at around 90 days leads to a local minimum in the attack rate, again due to minimization of the overshoot (Handel et al., 2007a). However, rapid switch to strong control leads to the largest reduction in attack rate. This suggests

that using a “start low, then increase” control strategy as suggested in Moghadas et al. (2008) might be suitable if a secondary resistant outbreak is unavoidable. However, if control can reduce the number of infecteds enough to prevent generation and spread of resistance, then one should implement strong control measures at high levels as soon as possible.

3.6. Details of modeling resistance emergence lead to small differences in results

Our study implements the process of resistance generation in a way that differs from previous studies. Most previous models assume that a fraction of treated hosts exit the class of sensitive infecteds and enter the class of resistant infecteds, thereby essentially “starting over”. In contrast, we assume that resistance can emerge during treatment (and at much lower levels in the absence of treatment), and with a certain probability cause new infections that are dominated by the resistant strain. We believe that our implementation of resistance generation is more realistic. To see if the different implementations of the resistance generation process are important, we compared our model to previous ones. Specifically, we assumed—as done in previous models—that as

infected hosts leave their compartment, a small fraction, c'_i , enter a new resistant infected compartment (i.e. treated and untreated hosts enter the resistant compartment I_1 , the first resistant strain enters compartment I_2 , etc.). It is not clear what the rates for c'_i should be; especially since we would argue that modeling resistance generation in this way does not correspond directly to a biologically realistic mechanism. To allow some comparison, we assume here that the fractions c'_i are equal to the c_i . Fig. 8 shows that using the two different ways of implementing resistance generation leads to small differences but overall close agreement. We find the same for other types and levels of control (not shown).

4. Discussion

Several conclusions can be drawn from our study. First, we find that the ongoing evolution of the resistant strain can contribute significantly to an increase in outbreak size. The fitness of the resistant mutants is not known. If the initially generated resistant mutant spreads poorly (i.e. $R_1 < R_f$), it could take very long before compensatory mutations are created that improve the fitness to a level where the strain can spread widely (Antia et al., 2003; Handel et al., 2006). While there was initial hope that strains resistant to the neuraminidase inhibitors have strongly reduced fitness, recent data suggest that at least some resistant mutants spread almost as well as the wild-type (Herlocher et al., 2004; Yen et al., 2005; Sheu et al., 2008). Therefore, assuming that resistant strains with fitness value similar to the ones we choose here will emerge is (unfortunately) reasonable. For the (assumed but plausible) scenario where the rates of compensatory mutation are as high as those of resistance generation during treatment and the fully compensated resistant strain has a fitness the same as the drug sensitive strain, we find that the number of infecteds can increase by as much as 20% owing to the evolution of the resistant strain.

Second, our results show that if it is possible to quickly contain an outbreak caused by a drug sensitive strain, it might also be possible to prevent resistance generation and an outbreak by the resistant strain. For that to occur, it is crucial to start control early and at high levels. Additionally, our results suggest that prophylaxis is the better control strategy to prevent resistance emergence. However, prophylaxis of a fraction of the total population will likely require many more doses of antivirals and is more problematic logistically, compared with treatment of infecteds. This could be prevented by using targeted prophylaxis (Longini et al., 2004). In any case, additional factors will likely influence the question of treatment versus prophylaxis. If a pandemic strain with a high level of virulence were to spread, treatment might be crucial to reduce mortality and could take precedence over prophylaxis.

Third, we find—in agreement with earlier studies (Lipsitch et al., 2007; Moghadas et al., 2008)—that if containment is not possible and outbreak mitigation is the best possible outcome, intermediate levels of control minimize the number of infecteds, owing to a reduction in overshoot caused by two smaller outbreaks (a sensitive and a resistant one) compared to one large outbreak (either a sensitive or a resistant one) (Handel et al., 2007a).

Fourth, we find that details in which resistance generation is implemented in the model do not significantly affect the results. This is reassuring, as it suggests some robustness of the results obtained by different models.

The inclusion of stochasticity and the consideration of evolution of the resistant virus gives a somewhat more realistic model compared to most previous ones that were used to study generation and spread of neuraminidase inhibitor resistance in influenza. Nevertheless, we still made a number of simplifying assumptions. Our model assumes a homogeneous population.

Based on results by others, we expect that heterogeneity will likely change the detailed dynamics of the outbreak, but the overall qualitative results will probably not change (Lipsitch et al., 2007). We also assume that every infected case is symptomatic. If asymptomatic cases do not spread the virus, then including those into our model simply reduces the reproductive numbers and therefore makes a given level of control more effective. If, however, asymptomatic cases spread, and at the same time are not detected (i.e. do not receive treatment), it could undermine treatment-based control strategies. This would argue further for the importance of prophylaxis as the better control strategy from an epidemiological standpoint. Implicit in our model formulation is the assumption that infectious periods are exponentially distributed. It has been shown that the assumption that infectious periods are exponentially distributed can lead to different results in for instance parameter estimation and dynamical details, compared to models that assume gamma-distributed infection periods (Wearing et al., 2005; Lloyd, 2001). One way our results could be affected is that an exponential distribution leads to a few hosts with unrealistically long infection times. These hosts could potentially impact the probability that resistance is generated, especially in the multi-step process including compensatory mutations. Based on our experience using models with both exponential and gamma-distributed infection times, we believe that a gamma-distributed model would not affect the qualitative results. However, we have not formally tested this for the scenarios studied here, and it might merit further investigation.

If an outbreak were to occur, treatment or prophylaxis will not be random and uniform as we implemented it in our simple model, but instead public health authorities will likely use a combination of targeted antiviral prophylaxis, contact tracing, preferential treatment of certain groups, etc. Therefore, to carefully assess intervention methods that take into account drug availability, as well as details in drug delivery, e.g. at what day post-infection people start taking the drug, for how long they continue to do so, and how that affects transmission, requires more detailed, agent-based models (Longini et al., 2005; Ferguson et al., 2005; Ferguson et al., 2006; Germann et al., 2006; Halloran et al., 2008). Such models that include resistance into agent-based models are in development (Neil Ferguson, personal communication).

To summarize, our results suggest that if we are able to detect an outbreak early and intervene quickly, it might be possible to not only control a sensitive outbreak, but also to prevent the emergence and spread of resistance. If on the other hand intervention is not quick enough, or control measures are not able to stop the outbreak, then the emergence of resistance is very likely. Therefore, while antivirals will certainly be an important component of pandemic control, we should not rely on them too much. Instead, a comprehensive approach based on good surveillance and rapid response with first-line control mechanisms such as antivirals and behavior changes such as social distancing measures, as well as a concerted effort to rapidly produce a potent vaccine, will be the best answer to an influenza pandemic (Halloran et al., 2008). In fact, such a multi-pronged approach seems the most promising approach against most future, novel emerging pathogens.

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