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PERSPECTIVE

Emergence of Drug-Resistant Influenza Virus: Population Dynamical Considerations

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Given the considerable challenges to the rapid development of an effective vaccine against influenza, antiviral agents will play an important role as a first-line defense if a new pandemic occurs. The large-scale use of drugs for chemoprophylaxis and treatment will impose strong selection for the evolution of drug-resistant strains. The ensuing transmission of those strains could substantially limit the effectiveness of the drugs as a first-line defense. Summarizing recent data on the rate at which the treatment of influenza infection generates resistance de novo and on the transmission fitness of resistant virus, we discuss possible implications for the epidemiological spread of drug resistance in the context of an established population dynamic model.

Two classes of drugs are currently available for chemoprophylaxis and treatment of influenza. The neuraminidase (NA) inhibitors oseltamivir (brand name Tamiflu) and zanamivir (brand name Relenza) impair the efficient release of virus from infected cells. The M2 protein inhibitors amantadine (known under several brand names) and rimantadine (brand name Flumadine) target the viral M2 protein, which is required for efficient uncoating of the virus inside the cell. The NA inhibitors are effective against all NA subtypes of influenza, whereas the M2 inhibitors are effective only against influenza A virus (1).

In the absence of transmitted drug resistance, the efficacy of chemoprophylaxis is comparable to that of vaccines. The efficacy of vaccines in preventing laboratory-confirmed illness is around 80% in children and adults but is substantially lower in elderly people and depends on how well the vaccine matches the antigenic characteristics of the circulating virus. The efficacy of prophylaxis with amantadine against the development of illness is around 80 to 90% during inter-pandemic influenza (2, 3) but may be only 60 to 70% for pandemic influenza (4). Among NA inhibitors, only oseltamivir is approved for chemoprophylaxis, but both types of NA inhibitors have been shown to be similarly effective (zanamivir, 84%; oseltamivir, 82%) (5). Thus, if available in sufficient quantities, anti-influenza drugs could play an important role as a first-line defense in the case of a new pandemic.

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The typical course of influenza is self-limiting and lasts for about a week. Antiviral treatment has only a moderate effect on shortening the infection. Aggregate analysis of a large number of trials shows that the time to alleviation of symptoms is shortened by 1 day by M2 inhibitors (6) and by 1.3 days by NA inhibitors (7). However, treatment of patients within the first 30 hours of the onset of symptoms can lead to a more substantial reduction of the time to alleviation of symptoms (8).

High-level drug resistance to both types of inhibitors is typically conferred by single amino acid substitutions in the M2 protein and the NA (9, 10). Mutations conferring resistance to M2 inhibitors generally provide full cross-resistance to both amantadine and rimantadine (11), whereas several mutations conferring resistance to NA inhibitors are drug-specific (12).

Two key factors affect the epidemiology of drug resistance in influenza. The first factor is the rate at which treatment generates resistance de novo. M2 inhibitor-resistant mutants have been reported to occur in about 30% of treated patients (5), but the estimates may be even higher if more rigorous detection techniques are used (13). Until recently, it seemed that de novo resistance against NA inhibitors occurs only rarely. Resistance against zanamivir has been found only in one immunocompromised child (14), whereas resistance against oseltamivir was found in 0.7 to 4% (15, 16) of adults and in 4 to 8% of children (15, 17). However, a more recent study found resistant isolates in 18% of oseltamivir-treated children (18).

The second factor is the fitness costs associated with drug resistance mutations.

Studies of the transmission of amantadine-resistant virus in birds in the absence of the drug suggest that M2 inhibitor resistance mutations incur no or little cost in terms of transmissibility and pathogenicity (19). Moreover, M2 inhibitor-resistant mutants have been shown to be transmissible in humans (2). Several studies suggest that resistance to NA inhibitors typically involves high fitness costs (20–22), nourishing the hope that in comparison to M2 inhibitors, resistance against NA inhibitors will be less of an epidemiological concern (23, 24). However, recent studies in ferrets show that transmission and growth characteristics vary substantially between different oseltamivir-resistant mutants (25). Although the Arg²⁹²→Lys²⁹² (R292K) and His²⁷⁴→Tyr²⁷⁴ (H274Y) mutations in NA were associated with substantially impaired growth and transmission (21, 26), the Glu¹¹⁹→Val¹¹⁹ (E119V) mutant displayed growth and transmissibility similar to that of wild-type virus (25), demonstrating that fitness costs for resistance to NA inhibitors need not be high. Moreover, it is conceivable that with increased use of NA inhibitors, compensatory mutations could arise that restore fitness without affecting drug resistance.

Despite the high level of de novo resistance and the apparently low transmissibility cost of resistance mutations for M2 inhibitors, resistance among circulating isolates was generally rare until 5 years ago (approximately 1 to 2% of isolates globally) (3). However, over the recent years, resistance to M2 inhibitors has grown rapidly. In 2004, 12% of globally collected isolates were resistant [with 70 to 74% of isolates being resistant in Hong Kong or China (3)]. During the initial months of the 2005/2006 influenza season in the United States, 92% of the H3N2 isolates tested were resistant (27). This rapid rise of M2 inhibitor resistance is presumably due to high rates of de novo resistance and low transmission fitness costs. Therefore, the recent findings regarding NA inhibitors that de novo resistance may be higher and fitness costs may be lower than previously thought raise justifiable concerns that the extensive use of NA inhibitors may induce a greater resistance problem than anticipated so far.

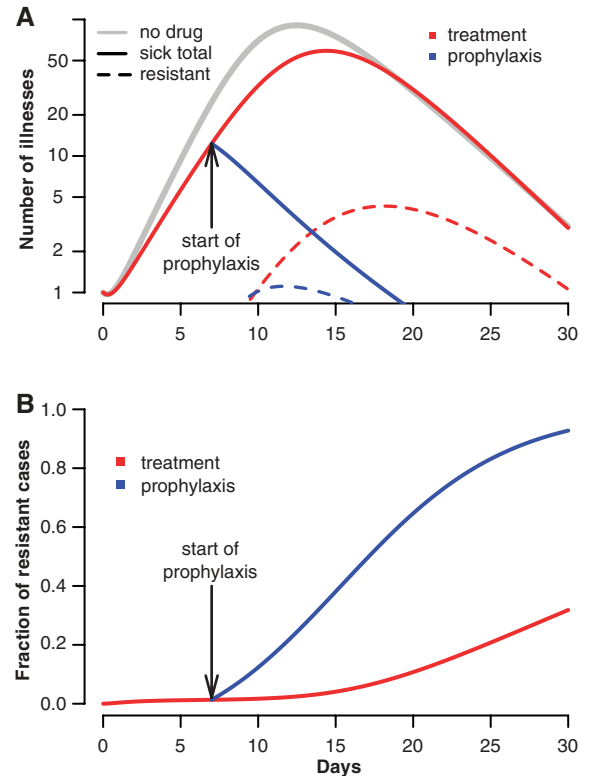
Epidemiologists frequently use the concept of the basic reproductive number, R_0 , as a measure of the transmission fitness of an infectious pathogen (28). R_0 is defined as the expected number of secondary cases generated by the primary case in a wholly susceptible population. Consequently, a pathogen with $R_0 < 1$ cannot sustain transmission, and thus an epidemic caused by such a pathogen would inevitably die out. Estimates of

R_0 for pandemic influenza strains range from 1.8 to 3 for the 1918 Spanish flu (29, 30) and are around 1.9 for the H3N2 pandemic (31). Clearly, the R_0 of a novel pandemic strain cannot be predicted, but it is conceivable that a novel strain would at least initially be poorly adapted for human-to-human transmission and hence that its R_0 would be only marginally larger than 1. On the other hand, such a strain could rapidly acquire higher values of R_0 after only a few rounds of transmission (32). Considering that treatment leads to only a modest shortening of the course of disease, it is highly unlikely that a new pandemic could be contained solely by treatment of symptomatic individuals. Indeed, detailed computer simulations modeling the outbreak of pandemic influenza in Southeast Asia suggest that even with targeted prophylaxis, a pandemic can only be contained if $R_0 < 1.6$ to 1.8 (29, 33).

Given that a large number of individuals will need treatment or prophylaxis in the case of a new pandemic, it is relevant to study the rise of resistance under conditions of high drug selection pressure. Mathematical models have been used to study the epidemiology of drug resistance for both amantadine (34), and oseltamivir (23) treatment, but these models have not accounted for the recently described higher *de novo* resistance rates and low transmission fitness costs of oseltamivir-resistant mutants. To illustrate some general principles of the effects of treatment and prophylaxis, we show simulation results based on a model developed by Stilianakis *et al.* (34), adapted here to model interventions with oseltamivir (Fig. 1). This model describes the dynamics of resistance in a closed population, such as a school or a nursing home, and thus allows analysis of the effects of drug intervention for the control of small epidemic foci. A detailed description of the simulations is provided in the supporting online material (SOM) (35).

In the following discussion, we use the term “prophylaxis” to describe the use of prophylaxis in addition to treatment. Figure 1A shows a simulated local outbreak in a closed population of 500. For the chosen parameters, the simulation shows that using prophylaxis in addition to treatment reduces both the total number of infected cases and the absolute number of resistant cases. However, prophylaxis increases the fraction of resistant cases (Fig. 1B). Figure 2, A and B, shows the fraction of resistant cases accumulated over the entire outbreak as a function of the rate at which treatment generates *de novo* resistance and the relative transmission fitness of resistant and sensitive virus. The simulations shown in Fig. 2A suggest that

Fig. 1. Epidemic curves as predicted by the model of Stilianakis *et al.* (34) for parameters characterizing interventions with oseltamivir. [For model equations and parameter values, see the SOM (35).] (A) Number of infected individuals using treatment only (red) or using prophylaxis in addition to treatment (blue) over the first 30 days of the epidemic. The gray line shows the course of the epidemic without any intervention. The solid lines depict the total number of infected individuals, whereas the dashed lines depict the number of individuals infected with resistant virus. The total number of illnesses during the 30-day period is 247, 227, or 26 in the epidemic with no intervention, with treatment, or with prophylaxis, respectively. (We assume that there is no pre-existing immunity in the population.) All symptomatic individuals receive treatment on average 1.4 days after the onset of symptoms. Prophylaxis starts at day 7. (B) Fraction of individuals infected with resistant virus under treatment (red) and prophylaxis (blue).



when only symptomatic individuals are treated, the fraction of resistant cases increases only slowly with both increasing rate of *de novo* resistance and increasing levels of relative transmission fitness. The reason is that treatment has only a moderate effect in terms of shortening the period of viral shedding, and therefore its effect on the transmission fitness of the sensitive strain is limited. Sensitive virus spreads rapidly in the unprotected population and interferes with the transmission of resistant virus, because individuals who are or have been infected with sensitive virus are protected against infection by resistant virus. However, if prophylaxis is used in addition to treatment, then changes in the rate of *de novo* resistance and the relative transmission fitness have markedly different effects (Fig. 2B). Although the fraction of resistant cases increases only slowly with increasing rate of *de novo* resistance, small changes in relative transmission fitness can lead to a substantial increase of the fraction of resistant cases. The reason for this abrupt increase is that once the resistant strain has sufficiently high transmission fitness (that is, once it has an $R_0 > 1$), it can generate a self-sustaining epidemic in the population receiving prophylaxis (fig. S2). In this case, using prophylaxis in addition to treatment leads to a marked increase

in resistance in terms of both the fraction (Fig. 2B) and the absolute number (fig. S1) of resistant cases.

Prophylaxis has two important effects on the epidemiology of resistance. First, prophylaxis has a more pronounced effect than treatment on the relative transmission fitness, because it greatly reduces the number of individuals susceptible to the sensitive strain. Second, prophylaxis reduces the competitive interference between sensitive and resistant strains, because only resistant virus can infect individuals receiving prophylaxis. The simulations illustrate the critical role of transmission fitness in determining the epidemiology of resistance. If the transmission fitness of the resistant virus is sufficiently low, then prophylaxis will reduce the incidence of resistance. Conversely, if the transmission fitness of the resistant strain is high, then prophylaxis will enhance the spread of resistance, in contrast to the common notion that treatment but not prophylaxis generates resistance.

The mathematical model discussed here was developed to describe epidemiological data from a small outbreak in a boarding school in 1978 (34). To extrapolate the above points to a pandemic, we need to consider the limitations of the model. First, the model assumes a single well-mixed homogeneous

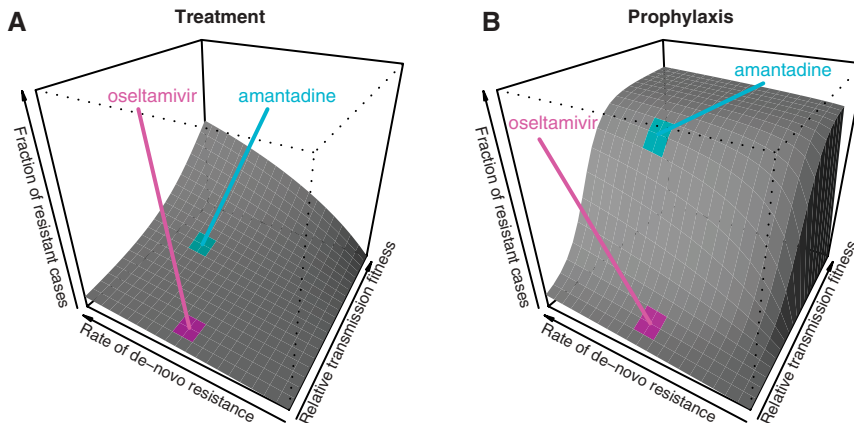


Fig. 2. Fraction of resistant cases as a function of the relative transmission fitness of resistant virus and the rate at which treatment generates de novo resistance with (A) treatment of symptomatic individuals and (B) prophylaxis in addition to treatment. The top of the z axis corresponds to 100%, which is the theoretical maximum. The relative transmission fitness ranges from 0 to 1, and the rate of de novo resistance ranges from 0 to 0.072 per day. For other parameters, see the SOM (35). The most likely parameter ranges for oseltamivir (violet) and amantadine (cyan) are indicated on the resistance surfaces. (For oseltamivir, these are also the parameters used in Fig. 1.) Because of our rudimentary knowledge with regard to these parameters, these ranges need to be regarded with caution.

population rather than a network of interconnected heterogeneous subpopulations, as would be more appropriate to model a large-scale outbreak. However, the threshold behavior when resistant virus is capable of causing a self-sustaining epidemic (Fig. 2B and fig. S2) has also been observed in models with a more complex population structure (23). Second, the model makes the simplifying assumption that all individuals in the population receive prophylaxis. In a pandemic, prophylaxis will be targeted only at exposed individuals. However, it is only these individuals that generate a selection pressure for resistance, because individuals who receive prophylaxis but are never exposed will obviously not contribute to the development and spread of resistance. Hence, the simplifying assumption is expected to be of little consequence for the extrapolation to the pandemic situation. However, to go beyond the conceptual points we make here and to quantitatively predict the emergence of resistance will require more detailed models.

Assessing the transmissibility of resistant virus in human infection remains a challenge and currently provides only rough estimates. To date, it is therefore difficult to predict exactly how intensive drug use for treatment and prophylaxis will affect the epidemiology of resistance. Should the R_0 of a new pandemic strain be lower than 2, then a 50% reduction in transmission fitness of the resistant strain would be sufficient to render the resistant virus incapable of causing a self-sustaining epidemic. The observation that

resistance to M2 inhibitors has increased substantially in recent years (3, 27) suggests that these strains do indeed have sufficiently high transmission fitness to cause a self-sustaining epidemic. For NA inhibitors, however, the situation is less clear. The more common R292K or H274Y mutations may be associated with sufficiently impaired transmission fitness (21, 26). However, the comparatively high transmissibility of the E119V mutant (25) and the possibility of compensatory mutations raise the concern that intense use of NA inhibitors for treatment and prophylaxis could also generate a large number of resistant cases. Unfortunately, taking a broader view of the use of novel anti-infective drugs against microbial pathogens reinforces this concern, because resistance has typically emerged faster than anticipated when the drugs were first introduced (36).

To get a better understanding of the consequences associated with the use of antiviral drugs as a first-line defense against a novel pandemic strain, we see a need for further research in two specific areas. (i) Experimental and epidemiological studies need to be intensified to allow a more accurate quantification of the transmission fitness of resistant virus. (ii) Computer models, based on a realistic human population structure, are required to simulate the emergence of resistance at the outset of a pandemic for various scenarios of drug use. Such models would also allow the testing of alternative intervention strategies such as combination therapy/prophylaxis or the use of different drugs for treatment and prophylaxis.

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