Monitoring the fitness of antiviral-resistant influenza strains during an epidemic: a mathematical modelling study

Kathy Leung, Marc Lipsitch, Kwok Yung Yuen, Joseph T Wu

Summary

Background Antivirals (eg, oseltamivir) are important for mitigating influenza epidemics. In 2007, an oseltamivir-resistant influenza seasonal A H1N1 strain emerged and spread to global fixation within 1 year. This event showed that antiviral-resistant (AVR) strains can be intrinsically more transmissible than their contemporaneous antiviral-sensitive (AVS) counterpart. Surveillance of AVR fitness is therefore essential. Our objective was to develop a simple method for estimating AVR fitness from surveillance data.

Methods We defined the fitness of AVR strains as their reproductive number relative to their co-circulating AVS counterparts. We developed a simple method for real-time estimation of AVR fitness from surveillance data. This method requires only information on generation time without other specific details regarding transmission dynamics. We first used simulations to validate this method by showing that it yields unbiased and robust fitness estimates in most epidemic scenarios. We then applied this method to two retrospective case studies and one hypothetical case study.

Findings We estimated that the oseltamivir-resistant A H1N1 strain that emerged in 2007 was 4% (95% credible interval [CrI] 3–5) more transmissible than its oseltamivir-sensitive predecessor and the oseltamivir-resistant pandemic A H1N1 strain that emerged and circulated in Japan during 2013–14 was 24% (95% CrI 17–30) less transmissible than its oseltamivir-sensitive counterpart. We show that in the event of large-scale antiviral interventions during a pandemic with co-circulation of AVS and AVR strains, our method can be used to inform optimal use of antivirals by monitoring intrinsic AVR fitness and drug pressure on the AVS strain.

Interpretation We developed a simple method that can be easily integrated into contemporary influenza surveillance systems to provide reliable estimates of AVR fitness in real time.

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Introduction

Antiviral drugs for influenza are important for mitigating influenza epidemics. The neuraminidase inhibitor oseltamivir is the most commonly used influenza antiviral[1] and has been extensively stockpiled by many countries for pandemic preparedness.[2] The effectiveness of antivirals is threatened by emergence and spread of antiviral-resistant (AVR) viruses. For oseltamivir, the most commonly detected resistance mutation in influenza A H1N1 viruses is the neuraminidase H275Y substitution. Before 2007, emergence of oseltamivir-resistant influenza viruses were sporadically reported, and the fitness of detected resistant viruses had always been substantially compromised.[3] As such, there was a consensus that AVR influenza viruses would always be outcompeted by their antiviral-sensitive (AVS) counterparts, and hence posed only minimal threat to public health.

Such conventional wisdom was refuted by events in 2007–08 when a new oseltamivir-resistant A H1N1 virus emerged and displaced its contemporaneous oseltamivir-sensitive counterpart to become the dominant A H1N1 strain globally within only 12 months.[4] The emergence and rapid fixation of this oseltamivir-resistant virus was not driven by widespread use of oseltamivir.[4,5] This event thus proved that AVR viruses are not necessarily less transmissible than their AVS counterparts. Furthermore, in the context of large-scale antiviral intervention during a pandemic, AVR fitness could be enhanced by the drug pressure on the AVS strain such that an intrinsically less transmissible AVR strain might become more fit than the AVS strain. Timely and accurate assessment of AVR fitness is therefore essential for informing situational awareness and optimal use of antivirals during both interpandemic and pandemic periods.[6]

The spread of AVR influenza viruses can increase morbidity and mortality. For example, case-fatality risk might increase because antivirals would be ineffective for treating AVR cases. Furthermore, if AVR viruses spread during the early stage of a pandemic, populations at the downstream of global spread will be subject to substantial importation and hence higher incidence of AVR cases.[7] In view of such risks, national and supranational agencies, especially the WHO's Global

Research in context

Evidence before this study

Antiviral drugs for influenza (eg, oseltamivir) are one of most important pharmaceutical interventions for controlling influenza epidemics and pandemics. The effectiveness of antivirals is threatened by the emergence and spread of antiviral resistance. For example, in 2007, an oseltamivir-resistant seasonal A H1N1 virus emerged and quickly displaced its co-circulating antiviral-sensitive (AVS) counterpart in 12 months. In view of such risks, national and supranational agencies, especially the WHO's Global Influenza Surveillance and Response System, have emphasised the need for robust antiviral-resistant (AVR) surveillance to provide timely and accurate assessment of AVR fitness, which is defined as the transmissibility of AVR strains by comparison with their AVS counterpart. However, few advances have been made in the development of data analytics, assessment of resource requirement, and performance evaluation for AVR surveillance systems. We searched PubMed for the terms "influenza", "antiviral", "resistant", "fitness OR transmissibility OR reproduct* number", and "surveillance" for literature in English published before May 18, 2016. We did not find any systematic method for estimating AVR fitness from influenza surveillance data.

Added value of this study

We developed a novel and simple method for real-time estimation of AVR fitness from surveillance data. We illustrate the value of this method in three case studies. In the first case study, we estimate that the oseltamivir-resistant seasonal A H1N1 strain that emerged in 2007 was 4% more transmissible than its oseltamivir-sensitive predecessor, indicating that such seemingly small fitness advantage is sufficient for the AVR strain to spread to fixation within months. If large-scale antiviral intervention is implemented during a pandemic, the resulting drug pressure on the AVS strain might confer such magnitude of fitness advantage to an intrinsically less transmissible AVR strain. In the second case study, we estimate that the oseltamivir-resistant pandemic influenza A H1N1 virus detected in a large community cluster in Japan during the 2013–14 season was 24% less transmissible than its contemporaneous oseltamivir-sensitive counterpart. Such a fitness cost was not detected in previous laboratory experiments, which concluded that the virological characteristics of the two strains were similar. In the third case study, we show that in the event of large-scale antiviral interventions during an influenza pandemic with co-circulation of AVS and AVR strains, our method can be used to inform optimal use of antivirals in real-time by monitoring the intrinsic fitness of the AVR strain and the drug pressure on the AVS strain.

Implications of all the available evidence

This study is the first to present a systematic method for estimating fitness of AVR influenza strains from surveillance data. This method can be easily integrated into the current influenza surveillance systems. Timely and accurate estimates of AVR fitness is particularly important in the context of large-scale antiviral intervention during pandemics because the spread of AVR could substantially reduce the effectiveness of antivirals in prophylaxis and treatment of severe infections.

Influenza Surveillance and Response System (GISRS), have emphasised the need for timely and accurate assessment of AVR fitness.[8] However, few advances have been made in data analytics and performance evaluation for AVR surveillance systems. Our objective is to help fill this knowledge gap by developing a simple method for estimating AVR fitness from surveillance data.

Methods

The model

We assume that there is only one transmissible AVR strain over the course of a single epidemic wave constituted by the A subtype or B lineage to which the AVR strain and its AVS counterpart (the AVS strain) belong. We define the intrinsic AVR fitness as the ratio of the basic reproductive number of the AVR strain to that of the AVS strain ($\sigma_0 = R_0^R / R_0^S$). Similarly, we define AVR fitness as the ratio of their reproductive numbers ($\sigma = R^R / R^S$), which encapsulates the combined effect of intrinsic fitness and any reduction in AVS transmissibility due to antiviral interventions.

See Online for appendix

We formulate our model under the following base case assumptions: (1) the AVS and AVR strains co-circulate during the epidemic; (2) without antiviral treatment, AVS and AVR infections have the same severity such that all infections are equally likely to be selected for AVR testing; (3) recovery from infection with either strain provides complete cross-protection against both strains during the epidemic; (4) the effect of viral interference (if any) caused by all other circulating influenza viruses (ie, those from other subtypes and lineages) and pathogens are the same for both strains; (5) AVR fitness does not depend on age; and (6) age-specific susceptibility to the AVR virus is the same as that to the AVS virus.

Assumptions 5 and 6 are relatively less likely to hold. For example, high-risk groups might be more likely to receive antiviral prophylaxis, and susceptibility to the AVR virus might be different from that to the AVS virus.[9] Our method can be extended to allow relaxation of these two assumptions (appendix p 5).

Under the base case assumptions, the next generation matrix of AVR infections is simply σ times that of AVS infections. This relationship remains true in the presence of seasonal forcing and interventions such as vaccination and school closure because transmission of the AVS and AVR strain are identically affected by these factors (appendix p 2). As the epidemic unfolds, the proportion of infections that are

AVR at time t, denoted by $\rho(t)$, will increase towards 1 if $\sigma>1$, remain at the same level if $\sigma=1$, and decline towards 0 if $\sigma<1$. The key step of our method is to approximate $\rho(t)$ using the equation:

$$\rho(t)=\frac{\int_0^t \sigma g^R(t-a)\rho(a)i(a)da}{\int_0^t \sigma g^R(t-a)\rho(a)i(a)da+\int_0^t g^S(t-a)(1-\rho(a))i(a)da} \quad (1)$$

where $i(t)$ is the total incidence rate of AVR and AVS infections, and g^R and g^S are the generation time distributions for AVR and AVS infections, respectively. To verify the accuracy of this approximation, we randomly generate 100 epidemic scenarios driven by the UK contact matrix[10] with four age groups (0–5 years, 6–18 years, 18–65 years, and >65 years) using Latin-hypercube sampling from the following parameter space, which covers a wide range of plausible epidemics: initial susceptible proportion of each age group between 0·3 and 1; initial reproductive number of the AVS strain ($R^S(0)$) between 1·2 and 3; mean generation time (T_g) between 2 and 4 days; intrinsic AVR fitness (σ_0) between 0·8 and 1·2; and the proportion of seeding infections that are AVR between 0·1 and 0·9.

The approximation in equation (1) is very accurate (appendix p 8). As such, given $i(t)$ or a proxy of it (see below) and the generation time distribution for both strains, equation (1) allows us to accurately describe $\rho(t)$, without knowing other epidemiological details such as basic reproductive number, contact matrix, symptomatic proportion, and seasonality.

Inference of AVR fitness

Our method requires two streams of data (for the subtype or lineage under investigation). The first data stream is the incidence rate $i(t)$ or its proxy—eg, based on the daily number of laboratory confirmed infections in the Hong Kong E-Flu system,[11] Flu Near You,[12] or other proxies used for calculating influenza excess mortality.[13] We denote this data stream by $\tilde{i}(t)$. These data are typically confounded with temporal fluctuation in reporting rate and laboratory testing capacity. Our method, however, is robust against such fluctuation (see Results). The second data stream is from AVR surveillance where Z_d^R and Z_d^S are the number of influenza-positive isolates tested on day d that are found to be positive and negative for AVR, respectively. The patients selected for AVR testing should have not been treated with antivirals for their infection and have no recent travel history to avoid misclassifying imported cases as local cases.

We substitute $i(t)$ with its proxy $\tilde{i}(t)$ in equation (1) and denote the resulting approximation by $\tilde{\rho}(t)$. The approximate likelihood is:

$$\prod_d \binom{Z_d^S+Z_d^R}{Z_d^R} p_d^{Z_d^R}(1-p_d)^{Z_d^S}$$

where:

$$p_d=p_{sens}\int_d^{d+1}\tilde{\rho}(t)dt+(1-p_{spec})\left(1-\int_d^{d+1}\tilde{\rho}(t)dt\right)$$

and p_{sens} and p_{spec} are the sensitivity and specificity of AVR testing. With this likelihood and uniform priors, we estimate AVR fitness σ using Markov chain Monte Carlo methods (appendix p 3).

Validation of the AVR fitness inference method

To validate our method, we simulated 100 stochastic realisations of the data streams for each of the 100 epidemic scenarios generated earlier assuming that daily reporting proportions were uniform random variables ranging between 0·5% and 2%; and daily AVR testing capacity was two, five, ten, 20, or 80 isolates. AVR fitness was then inferred at the end of each epidemic.

Case studies

After validating our method, we applied it to three case studies. Case study 1 is a retrospective study of the oseltamivir-resistant influenza A H1N1 virus in 2007–08. To estimate the (intrinsic) fitness of this oseltamivir-resistant strain in comparison with its oseltamivir-sensitive predecessor, we retrieved the data on influenza virus activity and AVR surveillance for ten countries or regions from published literature and public online data (appendix pp 13–17). We assumed that AVS and AVR

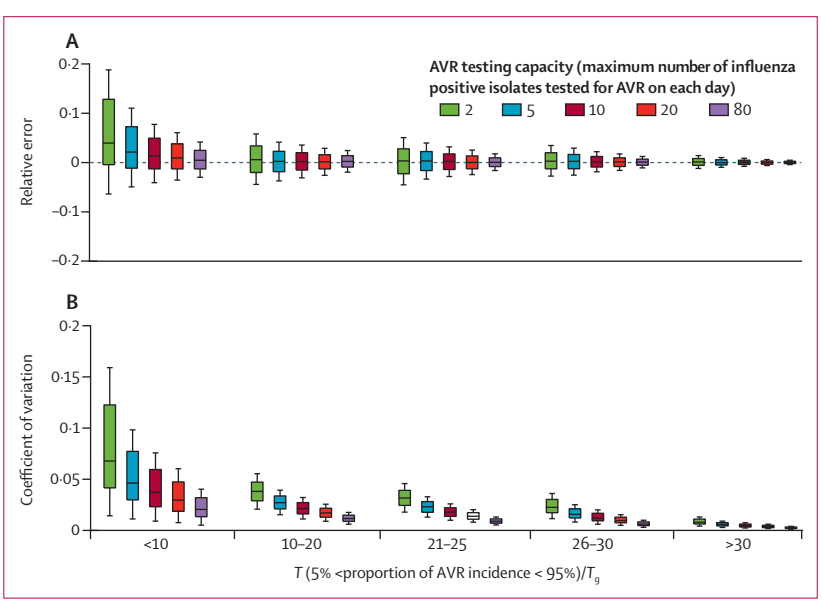

Figure 1: **Validating the accuracy and precision of antiviral-resistant (AVR) fitness estimates when the sensitivity and specificity of AVR testing are both 100%**

100 epidemic scenarios were randomly generated and 100 stochastic realisations of the data streams were simulated for each scenario (see Methods). AVR fitness was inferred at the end of each simulated epidemic. (A) Frequency distribution of the relative error in the fitness estimates $\hat{\sigma}$ (ie, $1-E[\hat{\sigma}]/\sigma$) across all scenarios and realisations when the daily AVR testing capacity was two, five, ten, 20, and 80 samples. The smaller the relative error, the more accurate the estimates. (B) Frequency distribution of the coefficient of variation of $\hat{\sigma}$. The smaller the coefficient of variation, the more precise the estimates.

infections had the same generation time distribution because there is no published evidence that indicates the contrary. Based on published serial interval estimates, we assumed that the generation time distribution was log-normal with mean 2·8 days and coefficient of variation 0·54.[14] We first obtained a pooled estimate of AVR fitness by assuming that AVR fitness was the same in all populations. We then estimated AVR fitness in each population separately and compared them.

Case study 2 is a retrospective study of the oseltamivir-resistant influenza A H1N1 pdm09 virus in Japan during 2013–14. Although 98% of the tested A H1N1 pdm09 virus isolates were sensitive to oseltamivir by 2014,[8] large clusters of oseltamivir-resistant variants were detected in Newcastle, Australia in 2011,[15] and Hokkaido, Japan in 2013–14.[16] In the Japan cluster, the oseltamivir-resistant virus was causing community outbreaks until it was displaced by its oseltamivir-sensitive counterpart. We applied our method to estimate the fitness of this oseltamivir-resistant strain using published data[16] and the generation time distribution in case study 1.

Case study 3 is a hypothetical study of AVR fitness and drug pressure under large-scale antiviral interventions during a pandemic. Oseltamivir resistance is not uncommon among influenza viruses with pandemic potential (eg, avian influenza A H5N1[17] and A H7N9 viruses[18]). We consider a hypothetical but realistic situation in which large-scale antiviral interventions, both prophylaxis and treatment, are implemented during a pandemic that comprises co-circulation of AVS and AVR viruses.[7,19–21] The epidemic parameters are $R^s(0)=1·4$ and $T_g=2·8$ days, with all individuals susceptible at time 0. We consider situations in which the AVR strain is intrinsically less transmissible than the AVS strain with $\sigma_0=0·95$; and large-scale antiviral interventions reduce the AVS reproductive number by a proportion μ such that drug pressure renders the AVS strain less transmissible than the AVR strain (ie, $\sigma=\sigma_0/(1-\mu)>1$). We consider 10%, 15%, and 20% coverage of antiviral prophylaxis that reduces susceptibility to the AVS virus by 81%;[22] this corresponds to μ=0·08, 0·12, and 0·16, respectively. We assume that σ_0, σ, and μ are unknown a priori and show how our method can be used to estimate them in real time to inform optimal use of antivirals. Specifically, if AVR fitness is consistently estimated to exceed 1 with high probability (eg, above 0·9 for 1 week), then there is compelling evidence that an increasing proportion of severe cases would be AVR and hence not treatable with the antiviral. We assume that in response to this alert, antiviral use would be suspended except for treating high-risk and severe cases as policy makers deliberate how to strategically adjust antiviral use to strike a balance between reducing transmission of AVS infections and increasing the number of severe AVR infections, and whether alternative treatment options such as convalescent plasma and antivirals with different resistance mechanisms should be considered.[7,20,21,23] The objective of this case study is to show how estimates of σ_0 and μ can be used to build an evidence base for this decision making process.

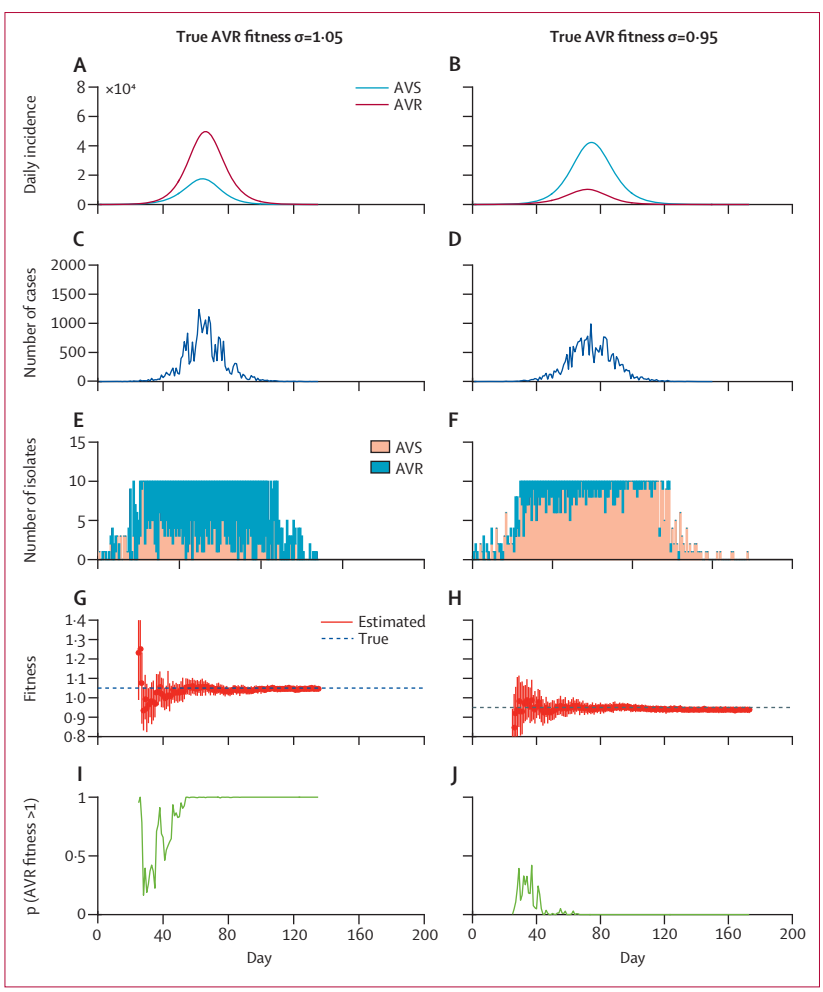

Figure 2: **Simulated example to show the timeliness of reliable antiviral-resistant (AVR) fitness estimates**
The epidemic parameters are $R^s(0)=1·4$ and $T_g=2·8$ days. At time 0, 50% of each age group are susceptible and the epidemic is seeded with ten antiviral-sensitive (AVS) and ten AVR infections. (A, B) Incidence of AVS and AVR infections in two fitness scenarios: σ=1·05 or 0·95. (C, D) The daily number of reported cases. (E, F) The daily number of influenza-positive isolates that are AVS and AVR with a testing capacity of ten samples per day. (G, H) Posterior distribution of the fitness estimate $\hat{\sigma}$ on each day. Circles and error bars indicate the posterior medians and the 95% credible intervals, respectively. (I, J) The posterior probability that AVR fitness is above 1.

Role of the funding sources
The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
Figure 1 summarises the accuracy and precision of AVR fitness estimates across a wide range of plausible epidemic scenarios when AVR testing sensitivity and specificity are both 100% (a reasonable assumption for

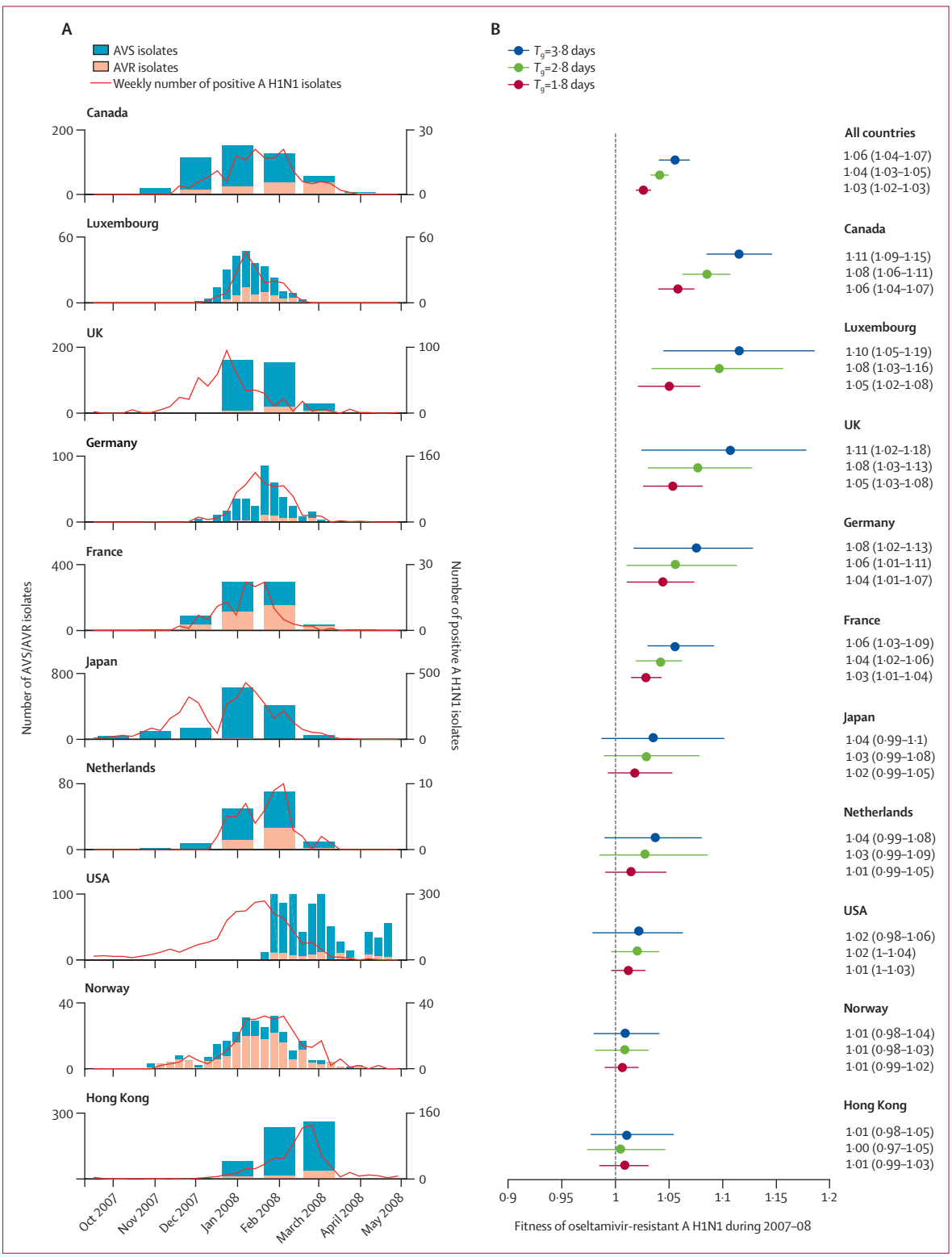

Figure 3: **Surveillance data for seasonal influenza A H1N1 and fitness estimates for the oseltamivir-resistant strain during 2007–08 in Canada, Luxembourg, UK, Germany, France, Japan, Netherlands, USA, Norway, and Hong Kong**

(A) The number of positive A H1N1 virus isolates and the number of oseltamivir-sensitive and resistant A H1N1 isolates over time in each population. (B) Fitness estimates (95% credible interval) for the oseltamivir-resistant A H1N1 virus under three assumed generation time distributions. The pooled antiviral-resistant (AVR) fitness estimate (at the top) is obtained by assuming that AVR fitness was the same in all populations.

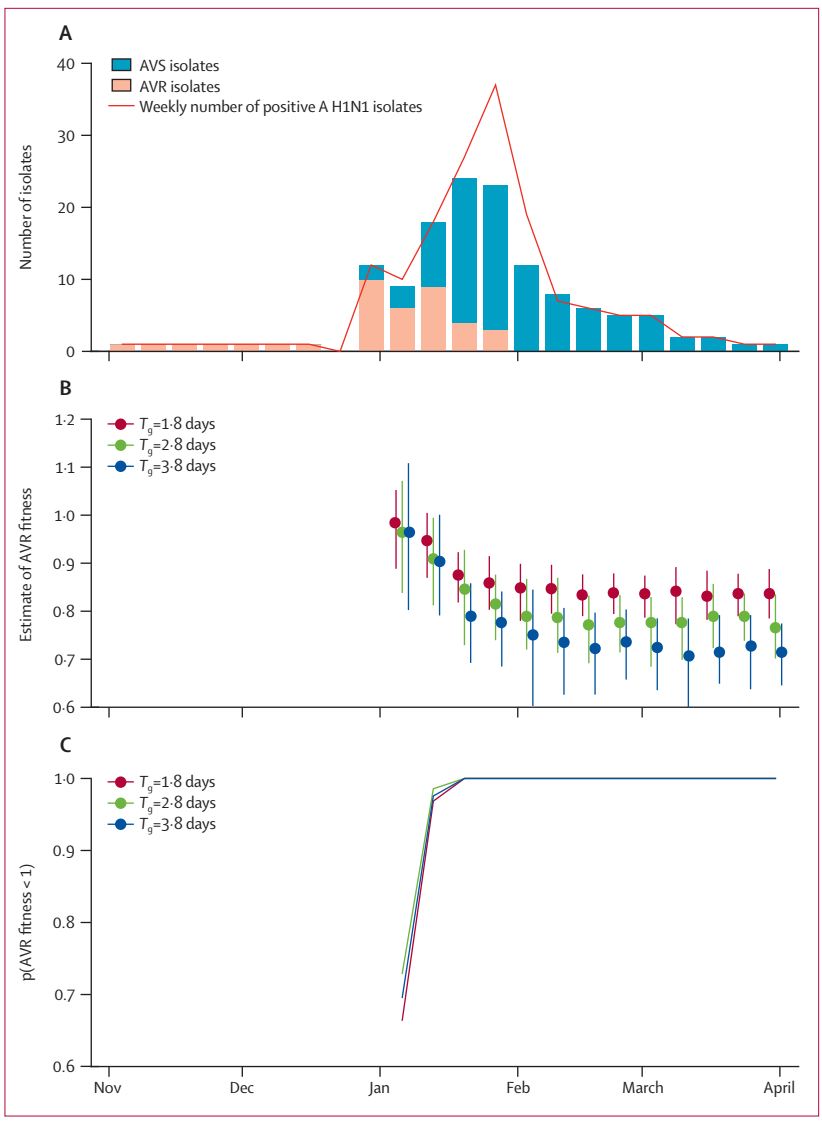

Figure 4: **Retrospective real-time fitness estimate for the oseltamivir-resistant A H1N1 pdm09 virus that circulated in Hokkaido, Japan, during the 2013–14 influenza season**
(A) Data on influenza A H1N1 activity and AVR surveillance. (B) Weekly fitness estimate using the same generation time distributions considered in figure 3. Circles and error bars indicate the posterior medians and the 95% credible intervals, respectively. (C) The posterior probability that AVR fitness was above 1.

genotypic testing). The reliability of fitness estimates depends on epidemic characteristics mainly via the time span, expressed in terms of number of generation intervals, during which the AVS and AVR strains are both circulating in substantial proportions. Fitness estimates are largely unbiased unless this time span is below ten generation intervals (around 30 days) and AVR testing capacity is low (less than five samples per day). Increasing the daily testing capacity beyond 20 samples provides little improvement in the fitness estimates. The accuracy and precision of fitness estimates deteriorate substantially when testing sensitivity and specificity are both reduced to 90%, which has a similar effect as halving the testing capacity (appendix p 9).

Figure 2 shows the timeliness of reliable AVR fitness estimates for one stochastic realisation of an exemplary epidemic scenario. The AVS and AVR reproductive numbers differ by 5%, which is sufficiently high to result in fixation within a single epidemic wave. The daily AVR testing capacity is ten samples, a modest level for well resourced populations like Hong Kong. Our method correctly predicts which virus would become dominant with posterior probability consistently above 0·9 as early as 3 weeks before the epidemic peak. However, stochasticity has a strong effect on the timeliness of reliable fitness estimates. Two alternative realisations of the same epidemic scenarios in which reliable fitness estimates are available a couple of weeks sooner or later than in figure 2 are shown in the appendix (p 10).

Results from case study 1 are shown in figure 3. The pooled (intrinsic) AVR fitness estimate is 1·04 (95% credible interval 1·03–1·05)—ie, the oseltamivir-resistant strain was 4% (3–5) more transmissible than its contemporaneous oseltamivir-sensitive counterpart. The fitness estimate increases (decreases) by 0·01 when we increase (decrease) T_g by 1 day. If the data were available in real time, reliable fitness estimates would have been available by late February, 2008, which was 15 weeks after the oseltamivir-resistant virus was first identified in Norway and months before it became dominant in populations outside Europe.[24] If we estimate AVR fitness in each population separately, the results suggest that the oseltamivir-resistant strain was more transmissible than the oseltamivir-sensitive strain only in Canada, Luxembourg, the UK, Germany, and France, but not in the other five populations (figure 3). In particular, there is no strong evidence that the oseltamivir-resistant strain was more transmissible than its oseltamivir-sensitive counterpart in Japan.[25] The intrinsic AVR fitness estimates remain unchanged when the effect of drug pressure in Japan is explicitly modelled (appendix p 4).

Results from case study 2 are shown in figure 4. We estimate that this oseltamivir-resistant A H1N1 pdm09 virus was 24% (95% credible interval 17–30) less transmissible than the oseltamivir-sensitive strain that displaced it. Such differential transmissibility was not detected by in-vitro competitive growth and in-vivo ferret transmission experiments.[16] In retrospect, our method could have correctly predicted that the AVR virus was less transmissible that its AVS counterpart (with posterior probability >0·95) after both viruses had co-circulated for 2 weeks, which corresponds to 4 weeks before the AVR virus was displaced.

Results from case study 3 (estimating AVR fitness and drug pressure on the AVS strain under large-scale antiviral interventions during a pandemic) are shown in figure 5. Reliable estimates of σ_0 and μ are typically available within 1–2 weeks after antiviral interventions are suspended. These estimates can be used to inform the optimal use of antivirals. For example, if policy

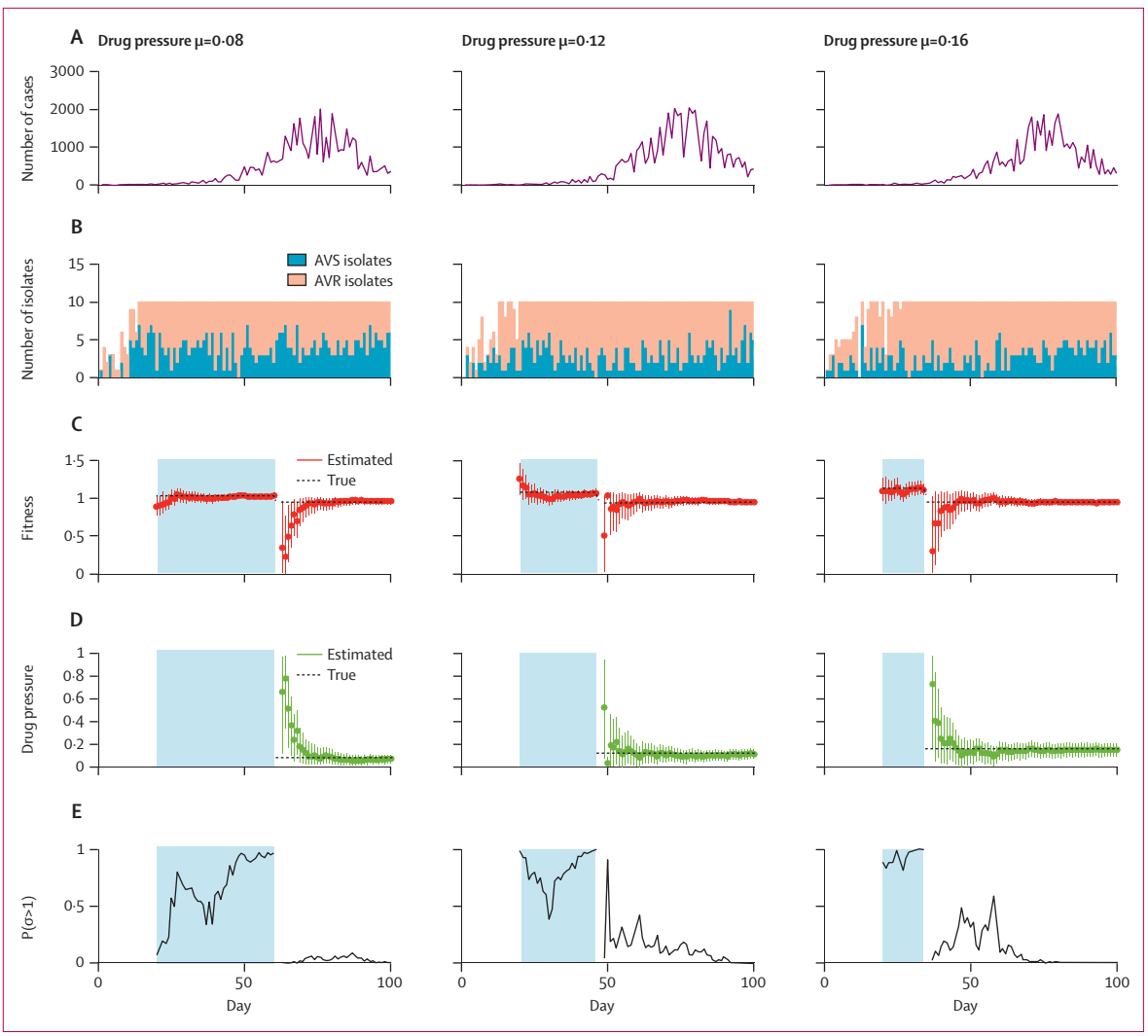

Figure 5: **Estimating antiviral–resistant (AVR) fitness and drug pressure on the antiviral-sensitive (AVS) strain posed by large-scale antiviral prophylaxis**
The epidemic parameters are the same as that in figure 2 with intrinsic AVR fitness σ_0=0·95. We assume that antiviral prophylaxis reduces susceptibility by 81% and the prophylaxis coverage is 10%, 15%, and 20% so that the drug pressure μ is 0·08, 0·12, and 0·16, respectively. Large-scale antiviral intervention is suspended after the posterior probability of σ>1 is greater than 0·9 for 7 consecutive days. Light blue shade indicates the time period during which large-scale antiviral intervention is implemented. (A) Daily number of reported cases. (B) Daily number of influenza-positive isolates that are AVS and AVR with a testing capacity of ten samples per day. (C) Posterior distribution of the AVR fitness estimate on each day. Circles and error bars show the posterior medians and the 95% credible intervals, respectively. (D) Posterior distribution of the estimates for drug pressure on the AVS strain at the baseline level (ie, before large-scale antiviral intervention is suspended). Circles and error bars show the posterior medians and the 95% credible intervals, respectively. (E)The posterior probability that AVR fitness is above 1.

makers resume large-scale antiviral prophylaxis with coverage equal to γ times the baseline level, then the resulting AVR fitness would be $\sigma_0/(1–\gamma\mu)$, which can be used to assess the downstream effect of increased AVR incidence (eg, increase in case-fatality risk due to more cases not treatable with antivirals).

Discussion

We have developed a simple method for estimating AVR fitness from influenza AVR surveillance data. Characterisation of the non-linear epidemic dynamics underlying surveillance data typically requires inference of multiple parameters in transmission models (eg, basic reproductive number, reporting rate).[26] Our method bypasses such complexity and is therefore easy to implement.

Conventionally, AVR fitness is assessed based on in-vitro experiments examining kinetics of neuraminidases and virus replications in cell cultures, or in-vivo experiments examining viral load and virus transmission in animal models.[27] As shown in our second case study, fitness estimates from such laboratory settings do not necessarily conform with that observed in actual community transmission settings.[16] Moreover, as the 2007 experience showed, experiments done using different genetic background might give different results.[28] Nonetheless,

these experiments are indispensable for early detection of transmissible AVR viruses. Our method complements these experiments by providing population-level fitness estimates when both AVS and AVR viruses co-circulate.

Timeliness of AVR surveillance depends on the capacity and turnaround time of AVR testing. Current influenza AVR surveillance mainly relies on the WHO collaborating centres in GISRS with antiviral susceptibility testing capacity available mainly in five WHO collaborating centres—namely, Atlanta, Beijing, London, Melbourne, and Tokyo.[8] National influenza centres collect clinical specimens and send representative virus isolates to one of the WHO collaborating centres for more advanced analyses. However, patient-specific clinical and epidemiological data for these isolates, such as sex, age, geographical location, health-care setting, antiviral treatment history, and vaccination status, are often incomplete or missing, especially when these samples are not collected by the sentinel surveillance systems. Routine collection of these data (eg, antiviral treatment history) can enhance the performance of AVR surveillance.

The turnaround time of AVR testing depends on our knowledge regarding the genetic mechanisms that confer AVR. If the genetic markers associated with AVR are known a priori (eg, the neuraminidase H275Y mutation[27]), the turnaround time for genotypic tests are usually 1–2 days. By contrast, phenotypic tests for antiviral susceptibility (eg, neuraminidase inhibition assay[8]) are necessary for monitoring emergence of AVR strains with previously unknown AVR mechanisms.[27] Phenotypic tests are much more labour intensive than genotypic tests with a turnaround time of 1–2 weeks. Following the discovery of a new strain with an unknown AVR mechanism, further investigations would be needed to characterise the associated genetic markers. As such, real-time surveillance for novel AVR strains will probably incur a lead time of at least several weeks.

In our first case study, we estimate that the oseltamivir-resistant influenza A H1N1 virus that emerged and became globally dominant in 2007–08 was 4% more transmissible than its oseltamivir-sensitive predecessor. This result is consistent with the findings of Chao and colleagues[29] in which the fitness advantage of the oseltamivir-resistant strain was estimated to be 1·7–2·4% based on the rate at which it spread around the globe. Both studies indicate that an AVR strain with a fitness advantage of as little as 2–4% would spread to fixation both locally and globally within months. If large-scale antiviral intervention is implemented during a pandemic, the resulting drug pressure on the AVS strain might confer such magnitude of fitness advantage to an intrinsically less transmissible AVR strain. In such context, timely and robust surveillance of AVR fitness is essential for informing optimal use of antivirals. For example, given that antiviral therapy will likely be the first-line treatment for severe cases during a pandemic, an increase in the AVR/AVS incidence ratio and growing ineffectiveness of antivirals in treating AVR cases might increase the overall pandemic mortality. Estimates of intrinsic AVR fitness and drug pressure on the AVS strain provided by our method would thus be useful for assessing the risk of such outcome, though a comprehensive evaluation of optimal antiviral use would require knowledge of additional parameters (eg, reproductive number and antiviral efficacy in reducing mortality).[30]

In our method, AVR fitness corresponds to the combined effect of intrinsic AVR fitness and the drug pressure posed on the AVS strain by population-wide antiviral interventions. AVR fitness will vary across populations if the drug pressure in each locality is different. Therefore, comparison of AVR fitness estimates from different populations should account for heterogeneities in drug pressure. We have shown how to do this in our case study 1 in which we jointly estimate intrinsic AVR fitness and drug pressure in Japan using data from ten populations (appendix p 4).

Our study has several important limitations. First, our method is applicable only when AVS and AVR strains co-circulate and hence cannot be used to estimate the fitness of a newly emerged AVR strain that has not yet spread in the community. Second, our method requires accurate specification of the generation time distribution. If data on exposure or onset times of infector–infectee pairs are available, our method can be extended to jointly infer the generation time distribution (appendix p 4). The resulting fitness estimate remains largely unbiased, but its precision would be lower due to uncertainty in the generation time distribution. Third, our method has not accounted for importation of AVS and AVR viruses. In the presence of such importation, our method would still be valid if cases with recent travel history are excluded from AVR surveillance and the number of imported cases is small compared with incidence from local transmission (which is generally the case after the local epidemic has undergone exponential growth for 1–2 weeks).

Timely and accurate estimates of AVR fitness are important during both interpandemic and pandemic periods because the spread of AVR viruses can substantially attenuate the effectiveness of antivirals. Robust real-time interpretation of AVR surveillance data for estimating AVR fitness is thus an essential but currently missing function of AVR surveillance. Our method has the potential to fill this knowledge gap and can be easily integrated into contemporary surveillance systems.

Contributors
JTW, ML, and KL designed the experiments. KL and JTW did the data collection and analysis. KL, ML, KYY, and JTW interpreted the results and wrote the manuscript.

Declaration of interests
We declare no competing interests.

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