approach that combines clinical research with the use of animal models will allow scientists to understand how we catch the flu.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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■ BENCH TO BEDSIDE

# Drug-resistant influenza viruses: why fitness matters

**Anne Kelso & Aeron C Hurt**



Influenza viruses can spread explosively and cause devastating disease. Of the few drugs we have to reduce the impact of influenza, oseltamivir is the most widely used and stockpiled. However, just over four years ago, A(H1N1) influenza viruses circulating in humans rapidly acquired a mutation that conferred oseltamivir resistance without a loss of viral fitness—their ability to infect, replicate in and be transmitted by the host—in the absence of the drug[1,2]. This lineage of seasonal H1N1 viruses vanished from human circulation as the pandemic H1N1 virus spread around the world in 2009–2010. In the two years before their disappearance, however, the former seasonal H1N1 viruses gave us an important lesson in influenza virology. That lesson is relevant again today.

Since 1999, the influenza neuraminidase (NA) inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza) have been widely available for the treatment of influenza, and many countries have acquired stockpiles for use in a pandemic. Before 2008, few countries other than Japan and the US had used oseltamivir on a large scale, and resistance to the drug was detected only rarely. Resistance occurred most commonly in oseltamivir-treated patients, particularly the immunosuppressed who received extended drug treatment because they could not clear the infection quickly. Many oseltamivir-resistant H1N1 viruses had an amino acid substitution near the enzyme active site of their NA, from histidine to tyrosine at position 275 (H275Y) (274 in N2 numbering), which impaired the ability of NA to undergo the rearrangement that is necessary for effective oseltamivir binding (summarized by Moscona[3]). Initial studies showed that H1N1 viruses with the H275Y mutation were less fit than wild-type viruses *in vitro* and in animals in the absence of the drug[4,5], suggesting that sustained transmission between untreated people was unlikely (**Fig. 1**).

This comfortable notion was overturned in early 2008 when Norway reported that a high proportion of H1N1 viruses analyzed in that country were oseltamivir resistant and carried the H275Y mutation, despite negligible clinical use of oseltamivir. Over the next year, H275Y variants progressively became predominant among H1N1 viruses circulating in both hemispheres[1,2], apparently arising independently in several locations[1]. Although it is not known whether they originated in oseltamivir-treated patients, there was no escaping the conclusion that these viruses could compete effectively with their oseltamivir-sensitive counterparts and that one of the few drugs available for influenza prophylaxis and treatment was no longer useful for H1N1 viruses.

The unexpected ability of the new drug-resistant variants to spread between untreated people was partly explained by studies investigating the function of the resistant NA[6,7]. Bloom *et al.*[6] showed that the reduced expression of H275Y NA at the infected cell surface and concomitant loss of viral fitness in the absence of drug were overcome by substitutions at positions 222 and 234 of the NA. These 'permissive' mutations had preceded the widespread emergence of H275Y in circulating H1N1 viruses, apparently enabling acquisition of oseltamivir resistance without loss of viral fitness in the absence of the drug (**Fig. 1**). The fact that most oseltamivir-resistant (and many sensitive) H1N1 viruses circulating in 2007–2008 belonged to a new antigenically distinct group (represented by the vaccine virus A/Brisbane/59/2007) may be relevant, particularly if the rapid spread was assisted by their antigenic novelty or other functional changes in the hemagglutinin[1,7].

Why does this story matter now that the former seasonal H1N1 lineage is no longer circulating? For the first year after their emergence in early 2009, the pandemic H1N1 (H1N1pdm09) viruses behaved like the former seasonal H1N1 viruses before 2008 with only sporadic oseltamivir resistance, usually in treated patients. In 2010 and 2011, however, oseltamivir-resistant H275Y H1N1pdm09 viruses were detected in several countries at elevated frequencies among untreated community cases with no known contact with treated patients[8–10]. The largest cluster identified to date occurred in and around the city of Newcastle in eastern Australia between May and August 2011 (31 cases), with a single case in September about 4,000 km away[8,11]. High sequence similarity suggested the spread of a single variant. No further cases have been detected. It is not known whether the cluster died out because the influenza season came to an end or because these viruses were less fit than oseltamivir-susceptible viruses concurrently circulating in the same region.

These observations raise the possibility that recently circulating H1N1pdm09 viruses that acquire tyrosine at position 275 may not suffer much loss of fitness in untreated patients. It is notable that several candidate permissive NA mutations, predicted by computational modeling to offset the negative effect of the H275Y substitution on NA stability, are now present in these currently circulating viruses[11,12].

Against the background of the experience in 2008, the Newcastle cluster and other recent

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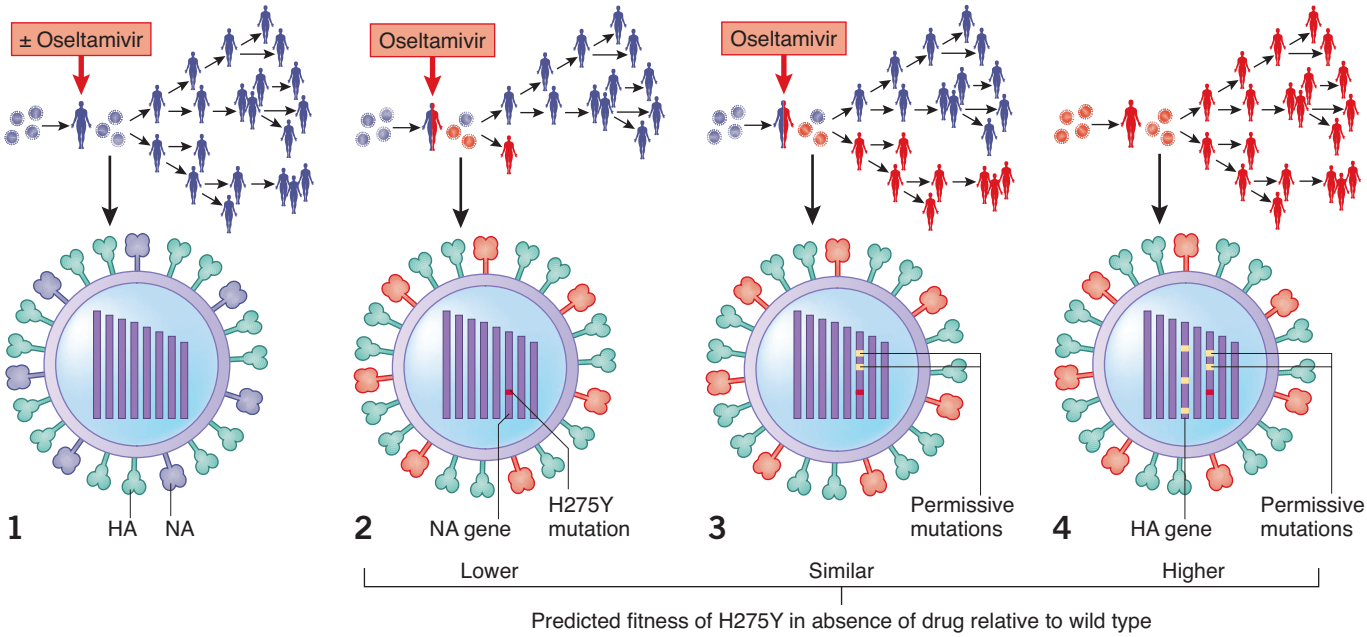


**Figure 1** The spread of oseltamivir-resistant influenza viruses depends on their fitness relative to susceptible wild-type viruses. The genome of influenza A viruses comprises eight single-stranded RNA segments, which encode two surface glycoproteins (hemagglutinin (HA) and NA) and several internal proteins. Amino acid substitutions in any of these proteins may alter the ability of the virus to infect and be transmitted (fitness) relative to the wild-type virus. A wild-type H1N1 or H1N1pdm09 virus (blue) can infect a susceptible individual who may or may not be treated with the neuraminidase inhibitor oseltamivir. Drug-resistant viruses do not emerge, and, before the first infected individual clears the infection, wild-type viruses are transmitted to other susceptible hosts (1). Oseltamivir treatment of a person infected with wild-type virus can lead to the emergence of a subpopulation of drug-resistant viruses carrying the H275Y substitution (red) in the NA gene. In this case, the fitness of the H275Y variant is too low to support its transmission beyond a first recipient in the absence of the drug (2). However, treatment of a person infected with a virus carrying permissive mutations in its NA gene leads to the emergence of a subpopulation of H275Y mutant viruses without compromised fitness that are capable of extended onward transmission to untreated individuals (3). The H275Y mutation has previously occurred on a background of permissive mutations in the NA and other genes (including mutations in the HA gene causing antigenic drift) (4), resulting in H275Y variants that can be readily transmitted between untreated individuals, enabling it to become the dominant virus lineage.

reports of oseltamivir-resistant viruses in the community sound an alert that widespread resistance in H1N1pdm09 viruses may be possible and is perhaps imminent. There are several implications for public health and clinical practice.

First, active and timely sentinel surveillance for antiviral drug resistance should be encouraged, and evidence of community spread of resistant viruses should be reported rapidly. It may be particularly important to monitor resistance when transmission of H1N1pdm09 is favored by antigenic drift. Although it may not be feasible to arrest the spread of resistant viruses in the community, it is important for patient care that clinicians are aware of emerging resistance so that alternative drugs are considered in the event of a poor response to oseltamivir.

Second, care should be taken to minimize the risk of virus transmission from hospitalized patients undergoing oseltamivir treatment. H1N1pdm09 viruses infecting immunocompromised patients who are undergoing oseltamivir treatment are more likely to acquire drug resistance because of the prolonged duration of viral replication in the presence of the drug.

Third, a wider choice of anti-influenza drugs and clinical studies of the efficacy of drug combinations are needed to reduce reliance on oseltamivir and to lower the risk of emergence and/or spread of resistant viruses. Oseltamivir has been the drug of choice, mainly because of its ease of administration in tablet form. The alternative, zanamivir, is inhaled and has not been used nearly as extensively as oseltamivir, even though resistance has been detected rarely. Two other recently developed NA inhibitors, peramivir and laninamivir, are currently approved for use in Japan, with additional clinical trials planned elsewhere[13]. Other anti-influenza drugs that target different stages of viral replication such as favipiravir (T-705) and nitazoxanide (Alinia) are also in late-stage clinical trials[13].

The spread of antiviral drug resistance among H1N1pdm09 viruses in the absence of selective drug pressure would also present a new challenge for pandemic planning. Since its emergence from swine in 2009, a remarkable property of this human-adapted viral lineage has been its capacity for transmission back to swine and other domestic and wild animals and birds[14], substantially increasing the risk of gene shuffling (reassortment) with other avian or swine influenza viruses to create a new pandemic virus. If such a virus were resistant to oseltamivir from the outset, we would lose one of our most important weapons for containing its spread and clinical impact.

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