



The Evolution of Influenza Resistance and Treatment

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IN FEBRUARY 2006, THE US CENTERS FOR DISEASE CONTROL and Prevention (CDC) reported that 92.3% of the circulating influenza A(H3N2) at that time was resistant to the adamantanes (amantadine and rimantadine), 1 of 2 pharmacological classes available for the treatment of influenza.¹ The resistant viruses harbored an S31N amino acid substitution in the influenza M2 protein that confers resistance but does not affect virulence. Although resistance to adamantanes increased to 14.5% in the prior year,² the dramatic increase in 2005-2006 came as a shock to both the medical and scientific communities and the public.³

At the time, it was confidently held that influenza was unlikely to develop similar resistance to neuraminidase inhibitors (oseltamivir and zanamivir), the second class of influenza-directed agents.^{4,5} The neuraminidase inhibitors were designed with an understanding of the structural interaction between neuraminidase and its natural substrate sialic acid. Because these antivirals closely resemble sialic acid, any mutation within neuraminidase that reduced affinity for the pharmacological inhibitors was believed to invariably compromise viral fitness.

The available evidence supported this notion. In vitro selection of neuraminidase inhibitor-resistant mutants is generally difficult, which argues that resistance-associated mutations compromise viability. In a 2002 study,⁵ the H274Y (histidine-to-tyrosine at codon 274 in N2 nomenclature) mutation in neuraminidase significantly reduced the replication of H1N1 strains and their virulence in mice and ferrets, regardless of whether the mutation was derived by site-directed mutagenesis, through serial passage in culture under drug pressure or during treatment in humans. In clinical trials of oseltamivir, resistance was relatively rare (0.32% in adults and 4.1% in children)⁴ and the few oseltamivir-resistant isolates recovered during treatment seemed clinically unimportant. As recently as the

2006-2007 influenza season, transmission of oseltamivir-resistant influenza was exceedingly uncommon.^{6,7}

Nevertheless, studies by Dharan et al⁸ and Gooskens et al⁹ in this issue of JAMA demonstrate that change was in the air. On December 19, 2008, the CDC reported that nearly all cases of influenza A(H1N1), the predominant circulating strain for the season thus far, are resistant to oseltamivir.¹⁰ Dharan et al⁸ expand that analysis to 268 H1N1 isolates, of which 264 (98.5%) are oseltamivir resistant. Of all influenza isolates typed by the CDC this season, approximately 55% are oseltamivir-resistant H1N1.¹¹ These isolates carry the neuraminidase H274Y mutation that confers resistance to oseltamivir but does not affect susceptibility to zanamivir. The oseltamivir-resistant H1N1, which is now circulating on all major continents,¹² is similar to the A/Brisbane/59/2007 strain that circulated during the 2007-2008 season¹³ and is included in the 2008-2009 influenza vaccines.

The studies by Dharan et al⁸ and Gooskens et al⁹ dispel the notion that oseltamivir resistance compromises virulence. Four of the 142 patients (2.8%) with oseltamivir-resistant H1N1 isolates submitted to the CDC during the 2007-2008 influenza season died of influenza. In a comparison between 99 oseltamivir-resistant cases and 182 matched oseltamivir-susceptible cases, there were no discernible differences in the predisposing factors, clinical symptoms, or complications related to influenza infection.

Gooskens et al⁹ describe a typical nosocomial outbreak of influenza at their center during the 2007-2008 season with an alarming new wrinkle—the outbreak strain was oseltamivir-resistant H1N1. At least 3 patients who were immunocompromised acquired influenza from the index case, each developed pneumonia and 2 died. Five health care workers also developed an influenza-like illness but were not tested for H1N1.

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The widespread belief that oseltamivir would retain activity against epidemic influenza strains has crumbled, but this should come as no surprise. Global and regional surveillance networks have tracked the incidence of neuraminidase-inhibitor resistance among circulating influenza for several years. The available epidemiology indicated that oseltamivir resistance was increasing on a trajectory that precisely paralleled the surge of adamantane resistance 3 years earlier (FIGURE). During the 2007-2008 season, 10.9% of H1N1 viruses tested in the United States were oseltamivir resistant.¹³ Even higher rates were reported elsewhere, including Canada (26%), Europe (25%), and Hong Kong (12%).¹⁴ The highest rate of oseltamivir resistance (67.3%) was in Norway, a country where oseltamivir can only be acquired with a prescription and is rarely used.¹⁴ In a study of the cases in Norway, oseltamivir resistance did not affect the phenotype of influenza infection,¹⁵ similar to the study by Dharan et al.⁸ During the summer of 2008, H1N1 strains related to A/Brisbane/59/2007 predominated in the Southern hemisphere and 100% of the H1N1 isolates in South Africa were resistant to oseltamivir.¹⁴

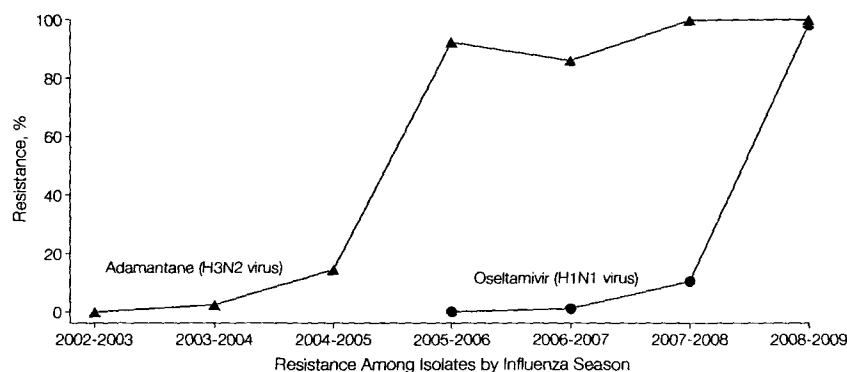
If the epidemiology was not sufficiently compelling, newer studies of H1N1 virulence have confirmed that H274Y does not necessarily compromise substrate binding, enzyme activity, viral replication, or virulence in animal models.¹⁶⁻¹⁸ Binding of neuraminidase to oseltamivir involves a conformational change in the side chain of the glutamic acid residue at codon 276 (E276). Importantly, the same conformational change is not required for zanamivir binding. Substitution of a tyrosine, which is bulkier than histidine, at codon 274 pushes

the carboxyl group of E276 further into the binding site.¹⁸ Group 1 neuraminidases (N1, N4, N5, N8) have another tyrosine below the active site at codon 252. The combination of the 2 tyrosines (Y252 and Y274) disrupts the pocket that normally accommodates oseltamivir. In contrast, group 2 neuraminidases (N2, N3, N6, N7, N9) have a smaller threonine at codon 252 and can still accommodate oseltamivir in the binding pocket in the presence of Y274.¹⁸

The structural study offers several insights. First, resistance to oseltamivir among strains with group 1 neuraminidases was predictable. Second, this resistance was less likely to affect strains with group 2 neuraminidases, consistent with the complete susceptibility of H3N2 strains to oseltamivir this season.¹¹ Third, zanamivir will maintain activity against some oseltamivir-resistant strains. Fourth, new inhibitors can be intelligently crafted to overcome specific mutations within neuraminidase.¹⁹ Fifth, oseltamivir resistance is likely to develop during the treatment of other N1-containing strains, including avian influenza A(H5N1).²⁰ Thus, stockpiles to mitigate an influenza pandemic should not be limited to oseltamivir.

For an influenza strain to achieve global predominance, it must outcompete other strains. A study by Rameix-Welti et al¹⁶ supports the intriguing notion that the H274Y mutation provides a competitive advantage to the circulating H1N1 strain that is completely independent of its effect on oseltamivir. The neuraminidase from this strain is distinguished by a unique combination of at least 8 amino acid substitutions, exclusive of H274Y. Both the enzymatic activity and substrate affinity of this neuraminidase (with or without H274Y) are significantly higher compared with neuraminidase proteins from strains that circulated during

Figure. Frequency of Resistance to Adamantane Among Influenza A(H1N1) Isolates and Oseltamivir Among Influenza A(H3N2) Isolates Submitted to the Centers for Disease Control and Prevention in the United States



Adamantane, No. Resistant isolates	3	9	92	193	NA	524	49
Isolates tested	174	466	636	209	395	525	49
Oseltamivir, No. Resistant isolates				0	4	111	264
Isolates tested				NA	588	1020	268

Frequency of resistance among isolates submitted to the US Centers for Disease Control and Prevention.^{1,2,7,8,11,13} NA indicates not available. The number of H1N1 isolates tested for oseltamivir resistance in 2005-2006 and the number of adamantane resistant H3N2 isolates in 2006-2007 were not available. The oseltamivir resistance before the 2007-2008 influenza season was less than 1%.

previous years, although the affinity is reduced somewhat by the H274Y mutation.¹⁶ The latter point is especially noteworthy. The “fitness” of an influenza strain is affected by the functional balance between its hemagglutinin and neuraminidase proteins. The additional mutations within the circulating H1N1 strain greatly enhance neuraminidase function, which may throw off the balance. The H274Y mutation, by attenuating neuraminidase substrate affinity, could bring the relative activities of hemagglutinin and neuraminidase back into balance, thereby enhancing the strain’s overall fitness.¹⁶

In an accompanying editorial to the 2006 report of adamantane resistance, we stated that “an axiom of good infectious disease practice [is that] inappropriate use of anti-infectives invariably results in resistance.”³ But if drug-resistant strains can outcompete susceptible ones in the absence of anti-infective pressure, it follows that for this organism and this agent, the most basic “truth” about anti-infective resistance may be wrong.

Between 2002 and 2007, the antigenic and genetic evolution of influenza A(H3N2) at distant locations around the world was remarkably homogenous.²¹ This homogeneity supports a model in which epidemic viruses circulate globally rather than persisting within a region and evolving locally. In fact, there is a continuous network of temporally overlapping H3N2 epidemics in East and Southeast Asia. The epidemics within temperate regions are seeded from this network, generally first in Oceania, North America and Europe, and then later in South America.²¹ Reseeding back into East and Southeast Asia is uncommon, such that once H3N2 strains leave East and Southeast Asia they rarely contribute to viral evolution. If the global dissemination of H1N1 follows similar patterns to those for H3N2, the widespread use of oseltamivir is not the cause of widespread resistance. Instead, oseltamivir use outside of East and Southeast Asia, appropriate or not, is unlikely to affect the epidemics within East and Southeast Asia, and thus the next wave of global dissemination.

This new understanding of influenza dynamics helps to explain the discordance between regional frequencies of oseltamivir resistance and oseltamivir use during the 2007-2008 influenza season. For example, oseltamivir resistance among H1N1 strains was 67.3% in Norway, where oseltamivir is rarely used, but was only 3% in Japan, the country with the highest per capita oseltamivir use.¹⁴

While rates of oseltamivir-resistant H1N1 remain high, patients who are candidates for influenza treatment or chemoprophylaxis should receive either zanamivir or the combination of oseltamivir and rimantidine (or amantidine, if rimantidine is not available).¹⁰ Essentially all influenza A(H3N2) strains circulating this year are resistant to adamantanes but susceptible to both neuraminidase inhibitors. Thus, single-agent oseltamivir is only appropriate for the rare patient with infection that is known to be caused by influenza A(H3N2) or influenza B.

Considering the need for dual-therapy or inhaled zanamivir, which can be difficult to administer and tolerate, the risk/benefit calculus for empirically treating a healthy adult with an influenza-like illness needs to be assessed on a case-by-case basis. Rapid assays to diagnose influenza are insensitive, so the decision should primarily rest on the patient’s risk for influenza-related complications and the local influenza epidemiology.

Vaccination remains the cornerstone of global efforts to control the dissemination of influenza. A study by Wang et al²² provides some additional insight into the absolute and relative benefits of the trivalent inactivated vaccine (TIV) and live attenuated influenza vaccine (LAIV). The authors reviewed the health care encounters for influenza and pneumonia among more than a million active-duty members of the US military between 2004 and 2007. In this young healthy population with annual vaccination rates between 50% and 80%, TIV was associated with reduced influenza and pneumonia encounters by 30% to 55% compared with placebo. Those outcomes were similar to previous studies and were better than LAIV, which only outperformed placebo for vaccinees who were relatively vaccine-naive.

The authors speculate that because the attenuated vaccine strains in LAIV must replicate within the recipient to effectively stimulate influenza-specific immunity, the presence of neutralizing antibodies from either previous vaccination or natural infection may prevent this replication and thereby compromise the efficacy of LAIV. Both a randomized study^{23,24} and a meta-analysis²⁵ have compared TIV with LAIV in adults and both reported an advantage for TIV. Not surprisingly, LAIV is equally or possibly more efficacious in children, who presumably lack preexisting influenza-specific immunity.^{26,27}

How these new findings by Wang et al²² will affect recommendations for influenza vaccination remain to be seen. During typical influenza seasons, it may be prudent to use TIV in patients who were vaccinated at least once in the previous 2 years. On the other hand, live attenuated vaccines against pandemic strains may be more effective than inactivated vaccines, as the population will presumably lack pre-existing immunity.

The understanding of influenza biology and epidemiology has advanced markedly; however, the global dissemination of oseltamivir-resistant influenza came as a great surprise. Undoubtedly, new surprises await in the perpetual struggle with influenza as one thing is certain—the organism will continue to evolve. Anticipating the rapid and endless changes in influenza biology and dynamics will require faster diagnostics to molecularly characterize specimens, extensive surveillance among humans and animals, and more rapid and malleable systems for translating basic and epidemiological discoveries into clinically applicable interventions. For now, the best tools to mitigate influenza infection are tried-and-true—vaccination, social distancing, hand washing, and common sense.

Published Online: March 2, 2009 (doi:10.1001/jama.2009.324).
 Financial Disclosures: None reported.

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