

# Antiviral drug resistance in seasonal and pandemic influenza



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**Two classes of anti-influenza drugs are currently available for the treatment or prophylaxis of influenza. These are the adamantanes (amantadine and rimantadine), which block the activity of the M2 ion channel of influenza A viruses (but not influenza B viruses)<sup>1</sup>, and the neuraminidase inhibitors (NAIs), which act by binding to the enzymatic site of the influenza neuraminidase (NA) thereby preventing progeny virions from being released from the host cell during viral replication<sup>2</sup>. Antiviral resistance can occur in influenza viruses and render the drug ineffective for the treatment of patients. Virtually all influenza A viruses currently circulating in the human population are resistant to the adamantanes, while in comparison these viruses remain susceptible to the NAIs. In particular, very low NAI resistance has been observed in pandemic A(H1N1) 2009 viruses, even though unprecedented amounts of these drugs were used.**

## NAI susceptibility of circulating influenza viruses

### Seasonal influenza viruses

Since 1999, two NAIs have been available for use throughout many parts of the world, oseltamivir (Tamiflu, Hoffmann-La Roche) and zanamivir (Relenza, GlaxoSmithKline), and two newer NA inhibitors, peramivir (BioCryst Pharmaceuticals) and laninamivir (Daiichi Sankyo/Biota Holdings), were recently approved for use in Japan. Unlike the resistance observed to the adamantanes, early clinical trials of oseltamivir detected resistance in only 1–4% of viruses from adults under oseltamivir treatment, and 5–6% of viruses from children<sup>3,4</sup>, while resistance was even lower following zanamivir use, with only one zanamivir-resistant virus reported to date from a clinical setting<sup>5</sup>. No resistance was detected in community isolates collected globally prior to the release of the NAIs<sup>6</sup>, and only 0.3% resistance (8 out of 2287) in viruses collected during the first three years of clinical use of the NAIs (1999–2002)<sup>7</sup>. The frequency of resistance in community isolates from Japan was 3% in 2005–2006, but 0% in 2004–2005 and 2006–2007, even though it had the highest global per capita

use of oseltamivir<sup>8</sup>. However, in late 2007, a higher than normal frequency of oseltamivir resistance was detected in seasonal A(H1N1) viruses from Norway and other countries throughout Europe<sup>9</sup>. All of these strains contained a H275Y mutation in the NA, which confers reduced susceptibility to both oseltamivir and peramivir, but has no impact on zanamivir susceptibility<sup>10</sup>. Importantly, the resistant strains were isolated from untreated patients, and were spreading in the absence of drug-selection pressure<sup>11</sup>. Oseltamivir-resistant A(H1N1) strains spread rapidly to other regions of the world including the USA in early 2008 and the southern hemisphere by the middle of 2008<sup>12,13</sup>. By the end of 2008, only 12 months after the initial detection of the emergent strain, virtually all seasonal A(H1N1) strains around the world were oseltamivir-resistant. The rapid global spread of the seasonal A(H1N1) oseltamivir-resistant strain occurred largely in the absence of drug-selective pressure, demonstrating the ability of an NAI-resistant strain to retain full viral fitness<sup>14</sup>. The oseltamivir-resistant strain continued to circulate globally until the A(H1N1)pdm strain emerged, after which time detection of the seasonal A(H1N1) decreased substantially, and is now considered to be no longer circulating. During the first pandemic wave in New Zealand, the oseltamivir-resistant seasonal A(H1N1) and A(H1N1)pdm viruses co-circulated, posing a theoretical, but unrealised, risk of reassortment between the strains leading to oseltamivir resistance in A(H1N1)pdm viruses<sup>15</sup>.

### Pandemic A(H1N1) 2009 viruses

Unprecedented amounts of oseltamivir, and to a lesser extent zanamivir, were used during the 2009 A(H1N1) pandemic. Due to this, and the experience of oseltamivir resistance in seasonal A(H1N1), there was significant concern that NAI-resistant A(H1N1)pdm viruses would emerge. Global testing data collated by the WHO during the first year of the pandemic found that only ~1% of the 20,000+ A(H1N1)pdm viruses tested were oseltamivir-resistant and none was zanamivir-resistant<sup>16</sup>. Virtually all of the oseltamivir-resistant strains contained the H275Y NA mutation, the same mutation responsible for conferring resistance in the 2007–2008 seasonal A(H1N1) virus. This mutation also confers cross-resistance to peramivir, one of the newer NAIs, but has no impact on zanamivir susceptibility<sup>10</sup>. A small number of A(H1N1)pdm viruses contained NA mutations at residue I223 (I223R, I223V and I223K), which conferred a ~10-fold reduction in susceptibility to both zanamivir and oseltamivir compared to the wild type<sup>17–19</sup>. The clinical significance of the I223 mutations remain unknown, although it is important to note their impact on susceptibility is considerably less than that caused by the H275Y mutation, which results in a 500 to 1500-fold reduced oseltamivir susceptibility compared to the wild type<sup>17,18,20</sup>. However, it is noteworthy that the relationship between the *in vitro* susceptibility result of a virus and the efficacy of treatment of a patient shedding that virus is not well understood.

Osetamivir-resistant A(H1N1)pdm viruses have been detected in at least 22 different countries worldwide<sup>16</sup>, including 13 osetamivir-resistant strains out of 1776 tested (0.7 %) from Australia. Almost half of the osetamivir-resistant strains detected globally have been isolated in either Japan or the USA, most probably due to the relatively large amounts of osetamivir used in these countries. During the period of April to December 2009, Japan used ~9 million doses of osetamivir and the USA ~8 million doses, compared to ~3 million doses used by the rest of the world (personal communication, Dr. James Smith, Hoffmann-La Roche). Patient and treatment information is known for 231 of the 302 osetamivir-resistant viruses detected globally, and shows that the majority (203/231) of these strains were isolated from patients that were undergoing treatment (Figure 1), nearly half of whom were immunocompromised (Figure 1). Clinical case reports from Australia have also identified osetamivir-resistant strains in immunocompromised individuals under treatment<sup>21-23</sup>. At the start of the pandemic, many of the resistant strains detected globally were selected during post-exposure prophylaxis of patients, a treatment strategy that, under certain conditions, can promote the spread of resistant viruses<sup>24</sup>. Twenty-eight patients shedding osetamivir-resistant A(H1N1)pdm virus had no known association with osetamivir use (Figure 1), and included community and hospital clusters where transmission of osetamivir-resistant A(H1N1)pdm strains occurred between individuals in close contact for prolonged periods<sup>25,26</sup>. To date there are no reports of sustained community transmission of osetamivir-resistant A(H1N1)pdm viruses. On occasions during the pandemic, antiviral combination therapy was used to avoid or reduce the selection of resistant variants, these included osetamivir/zanamivir combinations<sup>27</sup> and oral combinations of osetamivir/tibavirin/amantadine as part of an ongoing clinical trial<sup>28</sup>.

### Adamantane susceptibility of circulating influenza viruses

Adamantane resistance can occur as rapidly as two days post-treatment<sup>29</sup> and in up to 80% of treated patients<sup>30</sup>. In addition, adamantane-resistant viruses, which show complete

cross-resistance to both amantadine and rimantadine<sup>31</sup>, are not compromised in their ability to infect, replicate or transmit<sup>32</sup>. Adamantane resistance was first detected in circulating A(H3N2) viruses from China and the USA between 2003 and 2005<sup>33,34</sup>. The resistant A(H3N2) strains continued to spread worldwide, and since 2008 have virtually all been resistant to the adamantanes (Table 1). Although the initial emergence of adamantane-resistant strains may have been associated with increased use of the drugs in China and the USA, the global spread was independent of adamantane use and was not driven by drug selective pressure<sup>33</sup>. The pandemic A(H1N1) virus (A(H1N1)pdm) that emerged in 2009 is another example of adamantane resistance occurring in the absence of drug pressure. The matrix segment (and therefore the M2 protein) of the A(H1N1)pdm virus, is derived from a Eurasian-swine lineage virus, and contained an adamantane resistance mutation that had existed for many years in the swine virus prior to the transfer into humans<sup>35</sup>. To date this mutation has been maintained in the A(H1N1)pdm viruses (Table 1).

### Conclusions

The emergence of adamantane-resistant A(H3N2) viruses, and osetamivir-resistant seasonal A(H1N1) viruses demonstrated the ability for such strains to spread rapidly around the world in the absence of drug selection pressure. Although the A(H1N1)pdm developed adamantane resistance prior to emergence in the human population, the vast majority of strains remain susceptible to the NAIs, even though large amounts of osetamivir and zanamivir were used during the pandemic. Due to the high frequency of adamantane resistance in currently circulating influenza A viruses, the NAIs remain the most appropriate antiviral for the treatment or prophylaxis of influenza. Although ongoing surveillance to monitor the susceptibility of currently circulating strains remains important to ensure that the most appropriate antiviral is used.

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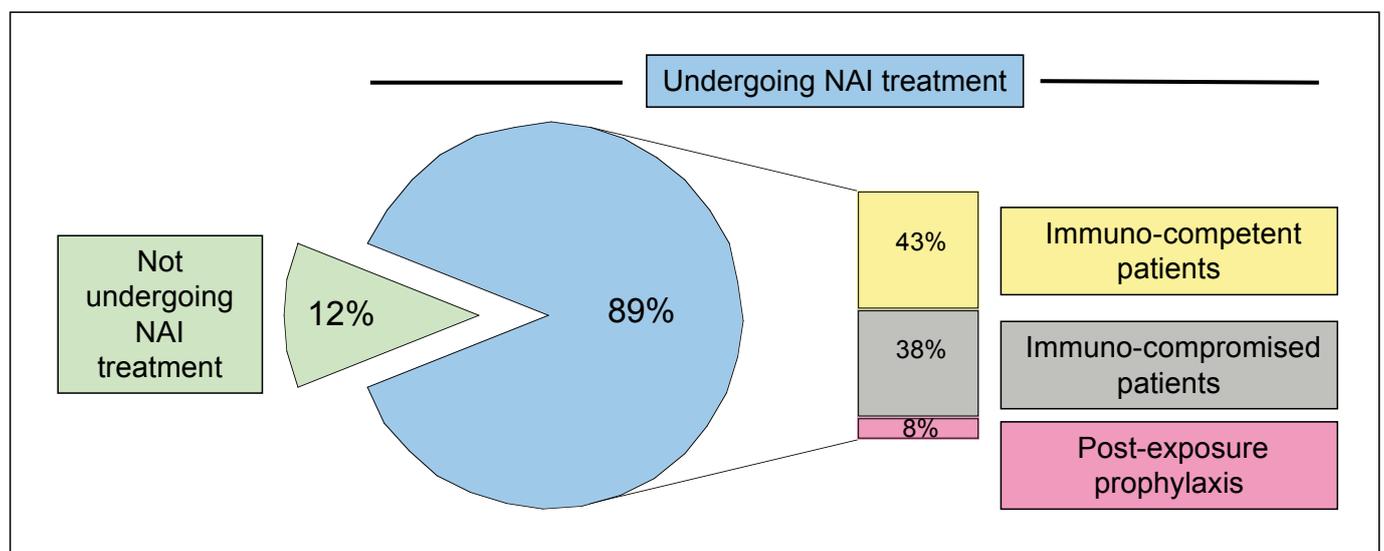


Figure 1. Treatment and clinical history of patients shedding osetamivir-resistant A(H1N1)pdm viruses. The percentages are based on global data reported by WHO<sup>16</sup>. The majority of osetamivir-resistant strains were isolated from patients under treatment, with over half of these either being immunocompromised or receiving osetamivir post-exposure.

Table 1.

Antiviral drug	Mode of action	Proportion of each type/subtype that were resistant to a particular antiviral drug			
		A(H3N2)	Seasonal A(H1N1)	Pandemic A(H1N1) 2009	Influenza B
Oseltamivir (Tamiflu)	NA inhibitor	0%	96%	1%	0%
Zanamivir (Relenza)	NA inhibitor	0%	0%	0%	0%
Adamantanes (amantadine and rimantadine)	M2 ion channel inhibitor	100% <sup>a</sup>	4%	100% <sup>a</sup>	Not effective for influenza B viruses
<sup>a</sup> Resistance due to the S31N M2 mutation			<b>Strain is no longer circulating</b>		

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