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Short communication

Emergence and spread of oseltamivir-resistant A(H1N1) influenza viruses in Oceania, South East Asia and South Africa

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ABSTRACT

The neuraminidase inhibitors (NAIs) are an effective class of antiviral drugs for the treatment of influenza A and B infections. Until recently, only a low prevalence of NAI resistance (<1%) had been detected in circulating viruses. However, surveillance in Europe in late 2007 revealed significant numbers of A(H1N1) influenza strains with a H274Y neuraminidase mutation that were highly resistant to the NAI oseltamivir. We examined 264 A(H1N1) viruses collected in 2008 from South Africa, Oceania and SE Asia for their susceptibility to NAIs oseltamivir, zanamivir and peramivir in a fluorescence-based neuraminidase inhibition assay. Viruses with reduced oseltamivir susceptibility were further analysed by pyrosequencing assay. The frequency of the oseltamivir-resistant H274Y mutant increased significantly after May 2008, resulting in an overall proportion of 64% (168/264) resistance among A(H1N1) strains, although this subtype represented only 11.6% of all isolates received during 2008. H274Y mutant viruses demonstrated on average a 1466-fold reduction in oseltamivir susceptibility and 527-fold reduction in peramivir sensitivity compared to wild-type A(H1N1) viruses. The mutation had no impact on zanamivir susceptibility. Ongoing surveillance is essential to monitor how these strains may spread or persist in the future and to evaluate the effectiveness of treatments against them.

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Table 1
Frequency of detection of H274Y mutants among A(H1N1) viruses in samples collected between 1st January and 1st November 2008.

	Number of H1N1 viruses tested	Number of H274Y mutants (%)	Proportion of H1N1 viruses circulating[a]
Australia	111	95 (86%)	11%
Macau	34	2 (6%)	47%
Malaysia	34	15 (44%)	17%
New Caledonia	7	7 (100%)	100%
New Zealand	4	3 (75%)	1%
Philippines	11	10 (91%)	14%
Singapore	10	3 (30%)	14%
South Africa	26	26 (100%)	72%
Taiwan	5	0 (0%)	33%
Thailand	22	7 (32%)	20%
Total	264	168 (64%)	12%

[a] Proportion based on the number of A(H1N1) viruses compared to A(H3N2) and influenza B viruses submitted to the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, from each country over the collection period.

The neuraminidase inhibitors (NAIs) zanamivir (Relenza™) and oseltamivir (Tamiflu™) are representatives of the most effective class of antiviral drugs for the treatment and prevention of influenza A and B infections (Gubareva et al., 2000). Until recently, a low prevalence of NAI resistance (<1%) has been detected in isolates from untreated patients (Hurt and Barr, 2008; Monto et al., 2006), although resistant viruses have been detected in up to 18% of oseltamivir-treated children (Kiso et al., 2004). Analysis of A(H1N1) viruses isolated during the Northern Hemisphere 2007–2008 influenza season (October 2007–March 2008) in Europe revealed a striking increase in the frequency of circulating oseltamivir-resistant viruses. The overall frequency of oseltamivir resistance in A(H1N1) strains from Europe was 25%, although it varied between countries, with Norway detecting the highest proportion (67%), and others, including Spain, as low as 2% (Lackenby et al., 2008; WHO, 2008). All resistant strains tested had a histidine (H) to tyrosine (Y) mutation at position 274 (275 in N1 numbering) of the NA gene, a mutation known to result in high-level oseltamivir resistance (Ives et al., 2002). Given the emergence of this resistant strain in Europe and reports of its increased frequency in the USA (Sheu et al., 2008), Canada (WHO, 2008) and South Africa (Besselaar et al., 2008) it was important to monitor the global spread of the H274Y mutant in A(H1N1) viruses from Oceania and South East Asia during the Southern Hemisphere 2008 season (Monto, 2008).

A total of 264 A(H1N1) viruses collected in 2008 were received at the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne from 10 countries and territories in Oceania and South East Asia (Table 1), as part of the WHO Global Influenza Surveillance Network. A(H1N1) viruses represented 11.6% of isolates received between January and November 2008, the remainder being influenza B (50.9%) and A(H3N2) (35.9%) viruses, although the prevalence of A(H1N1) viruses differed significantly between countries ranging from 1% to 100%. New Caledonia reported only A(H1N1) viruses during their influenza season, whereas only 1% of influenza viruses detected in New Zealand were of the A(H1N1) subtype (Table 1). Virus isolates or clinical samples were passaged in Madin–Darby canine kidney (MDCK) cells and tested for susceptibility to the NA inhibitors oseltamivir, zanamivir and peramivir (a NAI currently undergoing clinical development in parenteral formulations) in a fluorescence-based NA enzyme inhibition assay (Hurt et al., 2004). All viruses with reduced oseltamivir sensitivity in the NA inhibition assay were then analysed using a pyrosequencing assay (Deyde et al., 2009) to confirm the presence of the H274Y mutation. The mean concentration of oseltamivir required to inhibit 50% of NA activity (IC_{50}) for viruses containing the H274Y mutation was 1363 nM (388 ng/ml) (range 401–3070 nM), which exceeds the expected maximum concentration in plasma following normal oseltamivir dosing (348 ng/ml) (Roche Pharmaceuticals, 2006), and represents a 1466-fold increase compared to wild-type A(H1N1) strains (Table 2). The H274Y mutation caused a 527-fold increase in peramivir IC_{50} (mean IC_{50} = 94.9 nM, equivalent to 21.6 ng/ml) (Table 2). However this value is significantly lower than the plasma concentrations of peramivir which exceed 10,000 ng/ml following a 300 mg parenteral dose (Alexander et al., 2007). Importantly, the H274Y mutation had no impact on zanamivir susceptibility.

Table 2
IC_{50} values in NA inhibition assays with oseltamivir, zanamivir and peramivir for H274 and Y274 A(H1N1) viruses collected between 1st January and 1st November 2008.

	n	Oseltamivir IC_{50} (nM)	Zanamivir IC_{50} (nM)	Peramivir[a] IC_{50} (nM)
H274 viruses	96	0.93 ± 0.54	0.77 ± 0.52	0.20 ± 0.13
Y274 viruses	168	1363.4 ± 485.8	0.96 ± 0.63	111.6 ± 50.0

[a] Mean and standard deviation of peramivir IC_{50} values based on 50 H274 viruses and 85 Y274 viruses.

Of the A(H1N1) viruses tested, the frequency of H274Y mutant viruses was 64% (168/264), although the incidence of the mutant viruses differed considerably between countries (Table 1). The frequency was high in Australia, New Zealand, New Caledonia, the Philippines and South Africa, moderate in Singapore, Malaysia and Thailand, but low or not detected in Macau and Taiwan (Table 1). It should be noted that only low numbers of A(H1N1) viruses were available from some countries and these may have been isolated during a narrow time period. Analysis of the prevalence of H274Y mutant viruses for all countries over time revealed a significant increase in the proportion of resistant strains from May 2008 (Fig. 1). Overall, the percentage of H274Y mutants among viruses collected between October 2007 and April 2008 was 5% (4/84) compared to 78% (167/213) of strains collected between May and October 2008. This was particularly pertinent for countries such as Thailand and Australia where samples were available for the majority of the 12-month period (Fig. 1).

Of particular note were three H274Y mutant strains detected in Australia during late 2007. The first virus, A/Victoria/0715920/2007, was collected on 3rd October 2007 from a patient with no known travel history. This virus showed close genetic similarity in both the haemagglutinin (HA) and NA genes to an oseltamivir-resistant Hawaii strain isolated later in 2007 (Sheu et al., 2008) (Fig. 2) and pre-dates detection of the H274Y mutants reported from the Northern Hemisphere (Lackenby et al., 2008). Two more H274Y mutants isolated in Australia during 2007, from siblings who had recently

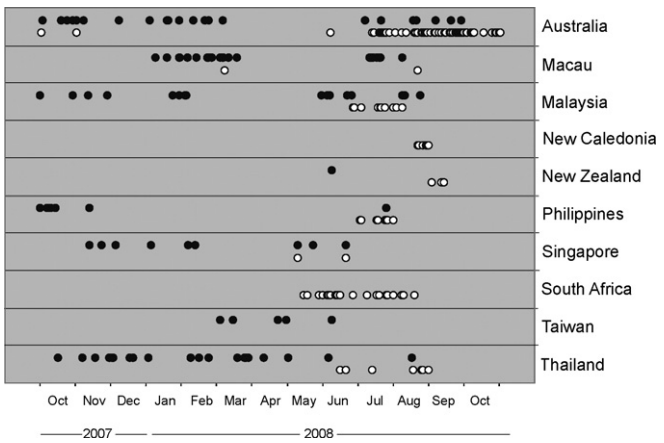

Fig. 1. Sample collection dates of oseltamivir-sensitive A(H1N1) viruses (black circles) and H274Y mutant oseltamivir-resistant A(H1N1) viruses (white circles) detected in each country between 1st October 2007 and 1st November 2008.

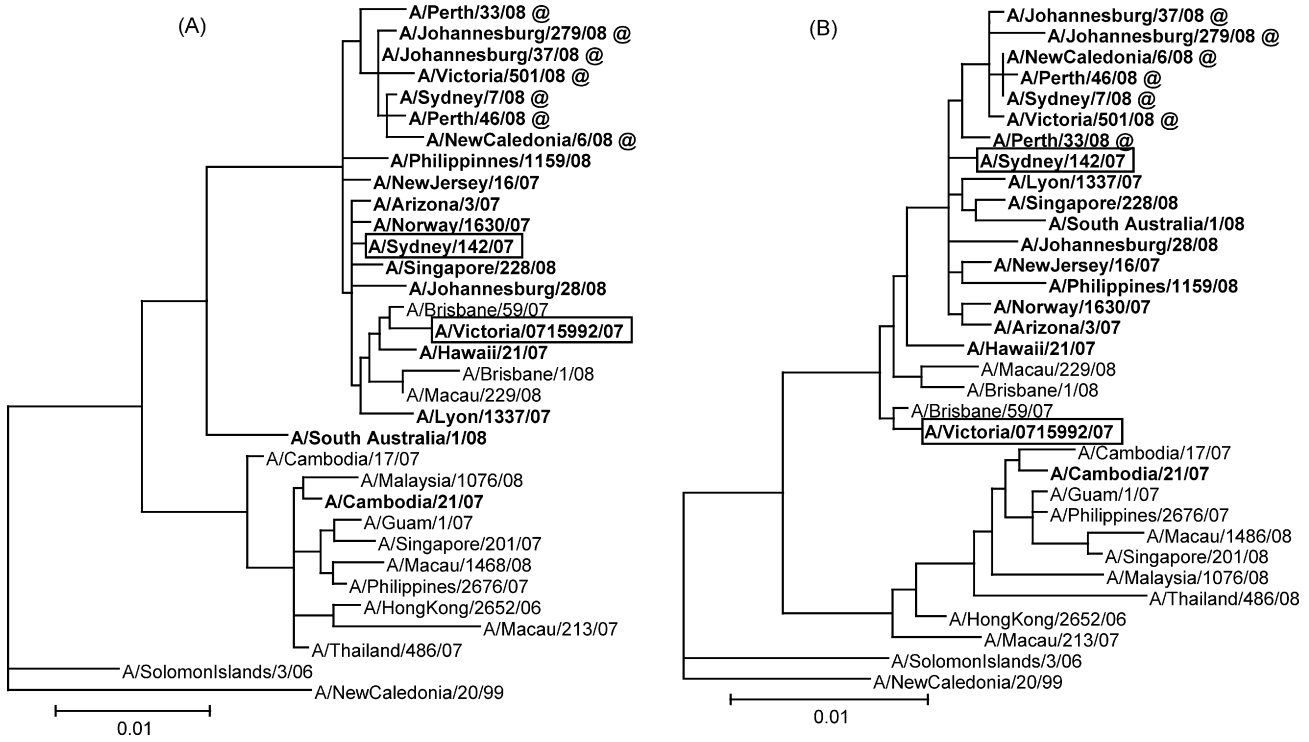

Fig. 2. Phylogenetic trees for (A) HA sequences and (B) NA sequences, showing oseltamivir-resistant A(H1N1) viruses with the H274Y NA mutation in bold. The symbol @ indicates viruses with N187S and G189N HA mutations. Australian H274Y A(H1N1) viruses collected in 2007 are boxed. Sequence data were aligned using Clustal W and maximum-likelihood trees generated using Phylip (Felenstein, 1989) and Tree Explorer (Tamura, 1997). Genbank accession numbers for HA and NA sequences, respectively, from the following viruses are: A/Johannesburg/279/2008 – FJ743452 and FJ743451, A/Johannesburg/37/2008 – FJ743454 and FJ743453, A/Victoria/501/2008 – FJ743456 and FJ743455, A/Sydney/7/2008 – FJ743457 and FJ743460, A/Perth/46/2008 – FJ743459 and FJ743458, A/New Caledonia/6/2008 – FJ743462 and FJ743461, A/Johannesburg/28/2008 – FJ743464 and FJ743463, A/South Australia/1/2008 – FJ743466 and FJ743465, A/Sydney/142/2007 – CY031378 and FJ743467, A/Philippines/1159/2008 – FJ743469 and FJ743468, A/Cambodia/21/2007 – FJ743471 and FJ743470, A/Perth/33/2008 – FJ743473 and FJ743472, A/Victoria/07159220/2007 – FJ743475 and FJ743474.

returned from Sudan at the end of October 2007, also clustered with the Northern Hemisphere H274Y mutants (Fig. 2). Although these isolated cases involving resistant viruses were detected in Australia during late 2007, H274Y strains did not become predominant until July 2008, coinciding with the rise in influenza activity in Australia, marking the start of the 2008 influenza season. As well as the H274Y mutation in the NA conferring oseltamivir resistance, the majority of mutant viruses (87%) collected in Australia after July 2008 also contained two amino acid differences in the HA gene (N187S and G189N) compared to H274Y viruses circulating in the Northern Hemisphere or Australia prior to that time (Fig. 2A). Viruses with these amino acid substitutions in the HA were also detected from South Africa and New Caledonia. Nevertheless, all of these H274Y mutant viruses remained in the phylogenetic clade represented by the vaccine reference strain A/Brisbane/59/2007. Phylogenetic analysis of the NA gene sequences also showed that all H274Y viruses grouped in the A/Brisbane/59/2007-like clade, with no consistent NA sequence differences between the pre- and post-July 2008 H274Y strains (Fig. 2B).

Atypically, one H274Y mutant virus collected in Cambodia in early October 2007 was genetically different to the other H274Y strains and instead belonged to the clade represented by the reference strain A/Hong Kong/2652/2006 for both the HA and NA genes (Fig. 2). Given these genetic differences it appears that this virus is not a variant of the H274Y strain detected elsewhere, but instead has arisen independently, as has been occasionally reported previously (Hurt and Barr, 2008; Monto et al., 2006). This Cambodian strain was also resistant to the adamantane class of inhibitors as a result of a S31N mutation in its M2 gene. This mutation has been present in the majority of other circulating A/Hong Kong/2652/2006-like A(H1N1) viruses (Barr et al., 2008) but is rare in A/Brisbane/59/2007-like viruses including the H274Y viruses. No influenza B (0/243) or A(H3N2) viruses (0/234) with reduced susceptibility to either oseltamivir or zanamivir were detected at the Melbourne Centre in 2008. The oseltamivir and zanamivir mean IC_{50} values ±1 standard deviation for the 2008 influenza B viruses tested were 23.5 ± 12.9 nM and 3.2 ± 2.3 nM, respectively, and for the A(H3N2) viruses were 0.4 ± 0.3 nM and 0.6 ± 0.5 nM, respectively.

The H274Y A(H1N1) viruses which first emerged in Europe in late 2007 have now caused high levels of influenza activity in South Africa (Besselaar et al., 2008) and outbreaks in Australia, New Zealand and some SE Asian countries, all of which use little or no oseltamivir for the treatment of influenza. The circulation of these strains suggests that these viruses are fully fit, are easily transmissible in the absence of drug pressure and are not generated as a result of oseltamivir treatment in the patient in whom they are detected. To better understand the origin and subsequent spread of the H274Y mutant A(H1N1) strains on a global scale would be of significant interest, particularly given the data on the global circulation of A(H3N2) viruses that suggest new variants originate in East or SE Asia (Russell et al., 2008). Importantly the H274Y mutants do not appear to cause more severe clinical outcomes than contemporary oseltamivir-sensitive A(H1N1) viruses (Hauge et al., 2009), although this requires formal study (Besselaar et al., 2008; Lackenby et al., 2008). Fortunately these viruses remain sensitive to zanamivir and also to amantadine and rimantadine. Clearly all influenza strains, but especially A(H1N1) and A(H5N1) viruses, will need to be monitored for resistance to NAIs to evaluate the effectiveness of treatments for both seasonal and potential pandemic influenza viruses. Methods for rapid influenza A typing or detection of oseltamivir-resistant influenza A strains will be necessary to assist clinicians in selecting appropriate influenza antiviral therapy. Based on the outcomes of these future studies, authorities may

need to modify antiviral recommendations for the treatment of seasonal influenza and the composition of pandemic stockpiles (Nicoll et al., 2008).

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