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Adamantane resistance in influenza A(H1) viruses increased in 2007 in South East Asia but decreased in Australia and some other countries

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ABSTRACT

The adamantanes (amantadine and rimantadine) were the initial antivirals licensed for use against influenza A viruses and have been used in some countries to control seasonal influenza and have also been stockpiled for potential pandemic use. While high rates of resistance have been observed in recent years with A(H3) viruses, the rates of resistance with A(H1) viruses has varied widely. In this study we analysed 281 human influenza A viruses isolated in 2007 that were referred to the WHO Collaborating Centre for Reference and Research in Melbourne, mainly from Australia and the surrounding regions, for evidence of resistance to adamantanes and a subset of these was examined for resistance to the neuraminidase inhibitors (NIs). We found that the rates of adamantane resistance in A(H3) viruses continued to increase in most countries in 2007 but a distinct variation was seen with A(H1) resistance levels. A(H1) viruses from Australia, New Zealand and Europe had low rates of resistance (2–9%) whereas viruses from a number of South East (SE) Asian countries had high rates of resistance (33–100%). This difference can be attributed to the spread of A/Brisbane/59/2007-like viruses to many parts of the world with the exception of SE Asia where A/Hong Kong/2652/2006-like viruses continue to predominate. When these two A(H1) subgroups were compared for their *in vitro* sensitivity to the other class of influenza antiviral drugs, the neuraminidase inhibitors, no difference was seen between the groups with both showing normal levels of sensitivity to these drugs, The finding of reducing A(H1) resistance rates in Australia and rising levels in SE Asia in 2007, reverses the trend seen in 2006 when A(H1) resistance levels were rising in Australia and elsewhere but remained low in most of SE Asia.

1. Introduction

Since the mid-2000s the level of resistance to the adamantanes, a class of antivirals active against influenza A viruses, has been increasing for both the A(H3N2) and A(H1N1) influenza viruses in most countries. This has been especially evident for the A(H3) viruses which have reached almost 100% resistance in several countries (Bright et al., 2005; Deyde et al., 2007; Barr et al., 2007) and resistance in A(H1) viruses has also been rising in recent years (Bright et al., 2006; Deyde et al., 2007; Barr et al., 2007). This class of drugs, which includes the licensed products amantadine (Symmetrel™) and rimantadine (Flumadine™), has been widely used in many countries to combat seasonal influenza and in some countries has been stockpiled by for use in future pandemics (Aoki, 1998; Tsiodras et al., 2007). Due to the persistence of high rates of adamantane-resistant viruses, the use of the newer group of influenza antivirals known as neuraminidase inhibitors (NIs) (Reece, 2007), oseltamivir (Tamiflu™) and zanamivir (Relenza™), was recommended for the treatment or prevention of influenza for the last two influenza seasons by the US CDC (Centers for Disease Control and Prevention) and ACIP (Advisory Committee on Immunization Practices) (CDC, 2006; Fiore et al., 2007). Recently however there has been an increase in the proportion of A(H1N1) viruses with resistance to oseltamivir found in Europe, North America, Australia, Hong Kong, Japan, and especially in Norway where resistance was reported to be as high as 66% (155/234 viruses tested) of the 2007–2008 A(H1) viruses (last quarter 2007–April 4 2008, WHO, 2008a). There have also been reports of increased levels of resistance to zanamivir in circulating viruses in Australia and South East Asia (Hurt and Barr, 2007).

With the increasing levels of resistance to both licensed NI it is important to monitor the rates of resistance to the adamantanes in both circulating seasonal and potentially pandemic strains of influenza. The adamantanes work by blocking the ion channel formed by the M2 protein of influenza A viruses and inhibiting the early stages of virus replication (Hay, 1992; Pinto et al., 1992;

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Aoki, 1998). The mechanism of resistance is well understood and revolves around mutations in the M2 protein that lead to the loss of binding or action of these drugs (Hay et al., 1985; Aoki, 1998,). Several amino acid substitutions (at positions 26, 27, 30, 31 and 34) in the M2 protein and prevent binding of the adamantanes or change the structure of the ion channel to allow it to operate even in the presence of bound drug, with both types of change resulting in the generation of resistant viruses (Hay et al., 1986; Astrahan et al., 2004). This occurs when patients with influenza are treated with these drugs where there is a rapid generation of adamantane-resistant viruses and these resistant viruses are fully capable of transmission to other humans (Shiraishi et al., 2003). Abed et al. (2005) used reverse genetics to generate recombinant influenza A A(H1N1) viruses with the commonly observed adamantane resistance mutations in the M2 gene (L26F, V27A, A30T, S31N, G34E, and V27A/S31N). They showed not only did all of these mutations cause amantadine resistance but also the M2 mutants had no impairment in their replicative capacities *in vitro* and were at least as virulent as the wild-type virus in experimentally infected mice.

The recent proliferation of adamantane-resistant A(H3) viruses appears to have occurred by a chance reassortant event between influenza A(H3) viruses, which has made these viruses fully fit, possibly even more fit than other circulating A(H3) viruses, resulting in their current predominance (Simonsen et al., 2007). It is unclear at this stage what events have occurred to increase the A(H1) resistance levels although recombination with adamantane-resistant A(H3) viruses or increased use of the drug appear unlikely causes. Resistance among A(H1) viruses has generally not increased as quickly as it has among A(H3) viruses, with only a few regions having high levels of resistance in 2006 (e.g. Taiwan, Province of China with 82% (9/11) resistant strains) and most having low levels of resistance. For example in Malaysia and the Philippines in 2006, no A(H1) adamantane-resistant viruses were detected among 26 viruses tested. In Australia levels of resistance in A(H1) viruses grew from 0% in 2005 to 40% in 2006 (Barr et al., 2006a, 2007) compared to the global levels of 4.1% in 2004–2005 rising to 15.5% in 2005–2006 (Deyde et al., 2007). In the present study we examined influenza A viruses isolated in 2007 from patients with influenza in Africa, Australia, France, New Zealand, South East Asia, Macau (SAR of China), Pacific Islands and Taiwan (Province of China) and compared these results to adamantane resistance levels seen in these regions in previous years.

2. Materials and methods

2.1. Viruses

Influenza A(H3) and A(H1) viruses were received from WHO National Influenza Centres, WHO Influenza Collaborating Centres and other regional laboratories and hospitals from Australia, New Zealand, and the Asia/Pacific region. Viruses were received as isolates passaged in cell culture (MDCK cells) or as original clinical samples in which influenza A had been detected by immunofluorescence or by RT-PCR. Once received at the Centre, the isolates were cultured in MDCK cells and monitored for growth by CPE and the presence of haemagglutination activity using turkey red blood cells (RBCs) as previously described (Barr et al., 2003). Positive samples were typed using the haemagglutination inhibition (HAI) assay against a panel of known standard reference viruses and their homologous ferret antisera. Viruses were generally tested after two or three passages in MDCK cells however some were tested after a single passage and a small number tested after more than three passages.

2.2. Sequencing

RNA extraction, RT-PCR and sequencing of the HA1 domain of the HA gene and the full matrix (M) gene were performed as previously described (Barr et al., 2003). Details of the primers used to amplify these genes are available on request. Sequences were assembled using the Lasergene Seqman package IV (DNAStar 7) and the phylogenetic relationships determined using PHYLIP V3.5.7 (Felsenstein, 1989) using the neighbour-joining method on ANGIS (Australian National Genomic Information Service) and dendrograms were drawn using TreeExplorer V2.12 (Kumar et al., 2004). Bootstrap confidence values were calculated using 1000 replicates before determining phylogenetic distances with PHYLIP. The M2 amino acid sequences were analysed to determine the presence of mutations known to confer resistance (Hay et al., 1985). All sequences detailed in this report have been submitted to GenBank.

2.3. Neuraminidase inhibitor drug sensitivity assays

A selection of viruses was also tested for sensitivity to the NIs oseltamivir carboxylate (the active component of oseltamivir; kindly provided by Hoffman-La Roche, Ltd., Switzerland), zanamivir and peramivir (an unlicensed NI currently in clinical trials; kindly provided by Biocryst, Birmingham, USA) as previously described (Hurt et al., 2004). Briefly, NA inhibition was measured using the fluorescent product 4-methylumbelliferone from the substrate 2-(4-methylumbelliferyl)-a-D-*N*-acetylneuraminic acid (MUNANA) (Sigma) as a measure of NA activity. IC_{50} values (the concentrations required to inhibit 50% of NA activity) were calculated using a logistic curve fit program "Robosage" kindly provided by GlaxoSmithKline, UK. Sensitive and resistant control strains were included in each assay.

3. Results

An increased proportion (34.2%) of influenza A(H1) viruses (from the total number of influenza A(H1), A(H3) and B viruses) was received at the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne in 2007 compared to recent years (2006 16.8%; 2005 12.8%; 2004 8.4%; 2003 0.9%). This increase was particularly high for some countries such as New Zealand, Australia and Sri Lanka compared to 2006. A total of 127 A(H1) viruses and 154 A(H3) viruses sent to the Centre during 2007, were examined for the specific mutations known to correlate with resistance to the antiviral drugs amantadine and rimantadine (Table 1). These were selected from 847 A(H1) and 1276 A(H3) viruses received at the Centre during 2007 based on their country of origin and date of isolation. The sequenced A(H3) and A(H1) viruses therefore represented 15% and 12%, respectively, of the total number received of each subtype in 2007. The median age of the patients from which the A(H1) viruses were sequenced was 16 years (mean: 18.5 years) with a range of 1 month to 88 years (ages were only available for 104/127 patients (82%)) and the median age from patients from whom A(H3) viruses were isolated was 12 years (mean: 22.3 years) with a range of 1 month to 94 years (ages were only available for 126/154 patients (82%)). While it cannot be excluded, it is unlikely that many of these patients were taking amantadine or rimantadine at the time of sampling, either because these drugs are not widely used in certain countries (e.g. Australia and New Zealand) or due to their relatively high cost (e.g. developing countries). For viruses isolated in 2007, 48/127 (37.8%) of influenza A(H1) viruses and 122/154 (79.2%) of A(H3) viruses had substitutions which would confer resistance to adamantanes (Table 1).

Table 1
Geographical origin and proportion of A(H1) and A(H3) adamantane-resistant viruses isolated in 2007

Country	A(H1) viruses		A(H3) viruses	
	# resistant/# tested	% resistant	# resistant/# tested	% resistant
Macau (SAR)	5/5	100	8/9	89
Taiwan	3/7	43	4/5	80
Thailand	3/9	33	3/12	25
Malaysia	10/10	100	8/11	73
Singapore	10/12	83	5/7	71
Philippines	10/10	100	5/11	45
Australia	1/43	2	57/65	88
New Zealand	1/11	9	11/11	100
New Caledonia	–	–	10/10	100
Sri Lanka	0/1	0	2/2	100
South Africa	0/2	0	5/5	100
Korea	2/2	100	2/2	100
French Polynesia	0/1	0	–	–
Guam	2/2	100	–	–
France	1/12	8	2/4	50
Total	48/127	37.8	122/154	79.2

The highest frequency of A(H1)-resistant strains was present in viruses obtained from Macau (SAR) [5/5 (100%)], Malaysia [10/10 (100%)], Philippines [10/10 (100%)] and Singapore [10/12 (83%)], while a number of countries had low levels (e.g. New Zealand 1/11 (9%), Australia 1/43 (2.3%), France 1/12 (8.3%)) (Table 1). For the A(H3) viruses a high level (>70%) of resistance was present in all countries with the exception of Thailand, Philippines and France. All of the 2007 A(H1) and A(H3)-resistant viruses had the same single nucleotide change (AGT to AAT) resulting in an S31N substitution in the M2 protein. Four H274Y (N2 numbering, by N1 numbering H275Y), A(H1) oseltamivir-resistant viruses were also tested for resistance to adamantanes by sequence analysis. All four had an adamantane-sensitive genotype (Table 2). A number of adamantane-resistant and -sensitive viruses isolated in 2007 were also tested for resistance to the NI using the fluorescence-based neuraminidase enzyme inhibition assay. All adamantane-sensitive and -resistant H1 viruses were susceptible to both zanamivir and oseltamivir (Table 2) as were the H3 viruses (data not shown).

Sequences from the HA1 domain of the HA gene of the A(H1) and A(H3) viruses that were assessed for adamantane resistance were compared phylogenetically (Fig. 1). For the A(H1) viruses virtually all of the 2007 sensitive viruses grouped in a clade represented by A/Brisbane/59/2007 (with signature amino acid changes at D45N, K149R—H3 numbering) with a small number grouping around earlier viruses such as A/Brisbane/193/2004 or A/Solomon Islands/3/2006. In contrast nearly all of the resistant viruses grouped around the reference virus A/Hong Kong/2652/2007 (with signature amino acid changes at S46N, R192M, T197K, K144E). For the A(H3) viruses small groups of sensitive viruses were scattered through the HA1 tree amongst mainly A/Brisbane/10/2007-like viruses with no clearly distinct subgroup apparent (Fig. 1). The phylogenetic analysis of the matrix genes of A(H1) and A(H3) viruses gave clearly distinguishable groups for each subtype which were further subdivided into clades representing either resistant or sensitive viruses (Fig. 1).

In 2007, influenza A(H1) became more prevalent in a number of regions including Europe, United States, Australia and New Zealand. These viruses were antigenically and genetically similar to either the reference A(H1N1) strain A/Brisbane/59/2007 (which is the WHO recommended A(H1) vaccine strain for the Northern Hemisphere for 2008–2009) or to A/Hong Kong/2652/2006. Interestingly there was a geographic separation with these two groups with viruses circulating in Australia and Europe falling into the A/Brisbane group while A(H1) viruses from number of countries in South East Asia/Pacific including Malaysia, Singapore, Philippines, Cambodia, Macau (SAR China), Korea and Guam, fell into the A/Hong Kong group. In Japan and the USA, viruses from both groups circulated in 2007, however A(H1) A/Brisbane/59/2007-like adamantane-sensitive viruses predominated with the USA reporting only 80/660 (12.1%) resistant viruses (CDC, 2008). The only 2007 A(H1)-resistant viruses from Australia and New Zealand (A/Victoria/501/2007 isolated 12 February 2007 from an Indonesian visitor and A/Auckland/8/2007 isolated on 18 June 2007) fell into the A/Hong Kong/2652/2006-like group genetically (Fig. 1). Four oseltamivir-resistant 2007 strains bearing the H274Y NA mutation were also genetically assessed for resistance to adamantanes. None of these strains had a resistant genotype nor did the zanamivir-resistant strains with a Q136K mutation in the NA gene (data not shown). Adamantane-sensitive and -resistant 2007 A(H1) and A(H3) viruses were equally sensitive to NI oseltamivir carboxylate and zanamivir.

4. Discussion

Adamantane resistance in A(H3) strains has continued to increase in many countries from 2005 to 2007 (e.g. Australia, Japan, Malaysia, New Caledonia, New Zealand, Philippines, and Singapore) while remaining very high in other places (e.g. Macau, Taiwan, and South Africa) (Barr et al., 2007; Saito et al., 2008) including the USA (A(H3) were 99.4% resistant in 2007–2008, CDC, 2008). Resistance in A(H1) strains however has been quite variable during this period. For example in Australia there was no detected resistance in 2005, then in 2006 this rose to 40% but in 2007 this dropped to only 2.3%. In other countries levels of resistance rose markedly from 2006 to 2007 in Malaysia (0 to 100%), Philippines (0 to 100%) and Singapore (0 to 83%) while some remained high (e.g. Macau, 82 to 100%) and others increased gradually (e.g. Thailand 10 to 33%, Barr et al.,

Table 2
Oseltamivir-resistant strains of A(H1N1) are sensitive to adamantanes and adamantane-resistant viruses are sensitive to all NI

Designation	M2 Res/ Sens	M2 gene mutations	Date of isolation	Oseltamivir IC_{50} (nM) Res/Sens		Zanamivir IC_{50} (nM) Res/Sens		Peramivir IC_{50} (nM) Res/Sens		NA gene mutation
A/Sydney/142/2007[a]	Sens	None	2/11/2007	797.5	Res[b]	0.4	Sens[b]	56.8	Sens	H274Y[c]
A/Sydney/144/2007[a]	Sens	None	2/11/2007	807.2	Res	0.3	Sens	49.7	Sens	H274Y
A/Victoria/07159220/2007	Sens	None	3/10/2007	1043.1	Res	0.5	Sens	ND	ND	H274Y
A/Lyon/1337/2007	Sens	None	11/12/2007	850.2	Res	0.3	Sens	51.2	Sens	H274Y
A/Brisbane/59/2007-like ($n=28$)	Sens	None	2007	0.57 ± 0.45	Sens	0.29 ± 0.23	Sens	ND	ND	None
A/Hong Kong/2652-like ($n=21$)	Res	31N	2007	0.48 ± 0.30	Sens	0.30 ± 0.15	Sens	ND	ND	None

ND: not determined.

[a] Viruses collected from siblings.

[b] Res, resistant. Defined as a strain(s) with an IC_{50} value greater than 100 nM (not clinically confirmed); sens, sensitive. Values are means of at least two determinations.

[c] Numbering according to equivalent residue in the N2 gene, by N1 numbering the equivalent amino acid occurs at amino acid 275.

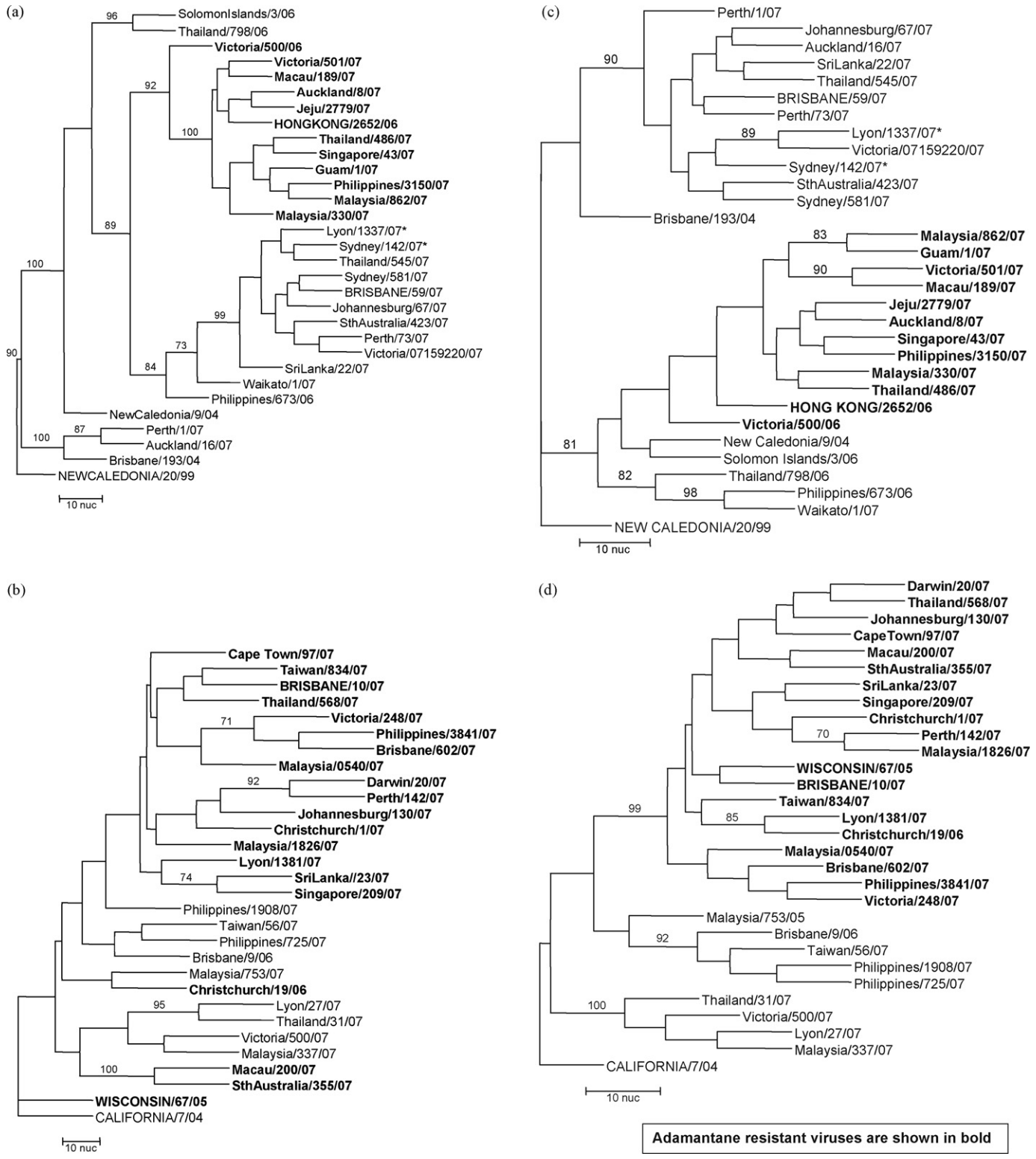

Fig. 1. Phylogenetic analysis of HA1 domain of HA gene from 2007 A(H1) (a) and A(H3) (b) viruses and the full matrix gene of A(H1) (c) and A(H3) viruses (d), based on nucleotide differences. Bootstrap values are shown for nodes having values >70%. Reference/vaccine viruses are capitalized. *A(H1N1) viruses with the H274Y (according to N2 numbering, actually H275Y for N1) mutation in their NA gene. Viruses shown in bold are resistant to adamantanes based on their M2 amino acid sequence. Bar indicates a 10-nucleotide difference.

2007). Japan also saw increased rates of A(H1) resistance from the 2005–2006 to the 2006–2007 season with rates rising from 0 to 64.2%, respectively (Saito et al., 2008), while in the US, Canada and South America rates remained low (Deyde et al., 2008).

The mutation in the M2 gene responsible for adamantane resistance in both the A(H1) viruses and the A(H3) viruses was identical resulting in an S31N change in all 2007 viruses examined. This is despite the fact that a substitution in any one of five amino acids

in the M2 protein in influenza A viruses is capable of conferring resistance (Hay et al., 1985; Hay, 1992; Aoki, 1998). Phylogenetically the matrix genes for the A(H1) and A(H3) were distinct indicating that there has been no reassortment of the matrix gene from A(H3) viruses to the A(H1) viruses as shown in this study and previous studies (Barr et al., 2007; Deyde et al., 2007). When the resistant and sensitive strains were examined phylogenetically, based on the HA gene, it was clear that there was a geographical split between the strains with the resistant strains derived mainly from South East Asia and the sensitive strains derived mainly from Australia, Europe and the USA. The resistant viruses were represented by A/Hong Kong/2652/2006-like viruses (also described by Deyde et al., 2007, as clade 2a viruses) and the sensitive strains by A/Brisbane/59/2007-like viruses. Both lineages continue to circulate, although there has been an increasing predominance of the A/Brisbane/59/2007 lineage globally and in the 2007–2008 Northern Hemisphere influenza season, this resulted in this virus being selected for the 2008–2009 Northern Hemisphere influenza vaccine by the WHO in February 2008 (WHO, 2008b). Deaths in children have also been associated with A(H1) viral infections during 2007–2008 in Australia, Hong Kong and USA. The reason for the differences seen with the A(H1) and A(H3) resistance patterns remains to be determined. For A(H1) viruses we have previously seen the co-circulation of two lineages in the late 1990 to the early 2000s where both the A/Bayern/7/95-lineage and the A/Beijing/262/95-lineage co-circulated in many countries before being replaced by the A/New Caledonia/20/99 viruses. This has been suggested by Rambaut et al. (2008) as a consequence of A(H1N1) viruses being less prone to seasonal genetic bottlenecks compared to A(H3N2) viruses resulting in more genetically diverse lineages co-existing. Two distinct lineages of B viruses have also co-circulated since the late 1980s (Barr et al., 2006b). It is clear that both current A(H1N1) lineages are fully fit viruses and it remains to be seen if one will predominate over the other in time.

In terms of resistance to other antiviral drugs, the increased proportions of oseltamivir-resistant A(H1) strains in many countries, especially some in Europe (WHO, 2008a), may limit the use of this drug in future years, so it is very fortunate that these viruses appear to be sensitive to both the adamantanes and to zanamivir. Other recent zanamivir-resistant A(H1) strains appear also to be susceptible to both adamantanes and to oseltamivir (Hurt and Barr, 2007). It is notable that these oseltamivir-resistant viruses are A/Brisbane/59/2007-like viruses and so should also be covered with the 2008–2009 influenza vaccine in the Northern Hemisphere, as this strain will be included in the vaccine (WHO, 2008b). The adamantanes also remain a treatment option for some of the A(H5N1) viruses that have infected humans in some countries which have adamantane-sensitive viruses present. The resistance patterns appear to be relatively static with recent viruses from Indonesia (Clade 2.1) remaining approximately 80% resistant while others from Europe, China and Vietnam (Clades 2.2 and 2.3) appearing to be sensitive to the adamantanes (Cheung et al., 2006; Hurt et al., 2007; WHO, 2008c). Dual antiviral treatments have also been suggested where the strains are sensitive to the adamantanes in both seasonal and H5N1 infections (Galabov et al., 2006; Ilyushina et al., 2006, 2007; Masihi et al., 2007; Morrison et al., 2007).

Adamantanes form an important resource for use against seasonal influenza and potentially against pandemic influenza. Due to the extremely high levels of resistance to these drugs seen with A(H3) since 2005–2006, they have become less favoured for the treatment and prevention of seasonal influenza. However, with the emergence of oseltamivir-resistant, adamantane-sensitive A(H1) viruses in Europe and many other parts of the world during 2007–2008, adamantanes and zanamivir may become the drugs of choice if a resistant virus is observed in an immunocompromised or severely ill patient or if oseltamivir-resistant A(H1) viruses predominate. There is also the recent emergence of zanamivir-resistant (adamantane-sensitive) A(H1) viruses (Hurt et al., 2008) to consider. Clearly in order to select the most appropriate antiviral drug for use both in seasonal and non-seasonal influenza, in individual cases and for the community in general, it will be important to continue to closely assess the rates of resistance for both the M2 and neuraminidase inhibitor drugs in A(H1), A(H3) and A(H5) viruses that are circulating in both human and avian species.

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References

Abed, Y., Goyette, N., Boivin, G., 2005. Generation and characterization of recombinant influenza A (H1N1) viruses harboring amantadine resistance mutations. Antimicrob. Agents Chemother. 49, 556–559.
Aoki, F.Y., 1998. Amantadine and rimantadine. In: Nicholson, K.G., Webster, R.G., Hay, A.J. (Eds.), Textbook of Influenza. Blackwell Science, Oxford, pp. 457–476.
Astrahan, P., Kass, I., Cooper, M.A., Arkin, I.T., 2004. A novel method of resistance for influenza against a channel-blocking antiviral drug. Proteins 55, 251–257.
Barr, I.G., Komadina, N., Hurt, A.C., Shaw, R., Durrant, C., Iannello, P., Tomasov, C., Sjogren, H., Hampson, A., 2003. Reassortments in recent human influenza A and B isolates from South East Asia and Oceania. Virus Res. 98, 35–44.
Barr, I.G., Hurt, A.C., Iannello, P., Tomasov, C., Deed, N., Komadina, N., 2006a. Increased adamantane resistance in influenza A(H3) viruses in Australia and neighbouring countries in 2005. Antiviral Res. 98, 35–44.
Barr, I.G., Komadina, N., Durrant, C., Sjogren, H., Hurt, A.C., Shaw, R.P., 2006b. Circulation and antigenic drift in human influenza B viruses in SE Asia and Oceania since 2000. Commun. Dis. Intell. 30, 350–357.
Barr, I.G., Hurt, A.C., Deed, N., Iannello, P., Tomasov, C., Komadina, N., 2007. The emergence of adamantane resistance in influenza A(H1) viruses in Australia and regionally in 2006. Antiviral Res. 75, 173–176.
Bright, R.A., Medina, M.J., Xu, X., Perez-Oronoz, G., Wallis, T.T., Davis, X.M., Poyinelli, L., Cox, N.J., Klimov, A.I., 2005. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. Lancet 366, 1175–1181.
Bright, R.A., Shay, D.K., Shu, B., Cox, N.J., Klimov, A.I., 2006. Adamantane resistance among influenza A viruses isolated during the 2005-2006 influenza season in the United States. JAMA 295, 891–894.
Centre for Disease Control and Prevention (CDC), 2006. High levels of adamantane resistance among influenza A(H3N2) viruses and interim guidelines for use of antiviral agents—United States, 2005–06 influenza season. Morb. Mortal. Wkly. Rep. 55, 44–46.
Centre for Disease Control and Prevention (CDC). 2008. Fluview. 2007–2008 Influenza Season Week 12, ending March 22, 2008. http://www.cdc.gov/flu/weekly/fluactivity.html (accessed 31 March 2008).
Cheung, C.L., Rayner, J.M., Gavin, J.D., Wang, P., Naipospos, T.S.P., Zhang, J., Yuen, K.Y., Webster, R.G., Peiris, M.K., Guan, Y., Chen, H., 2006. Distribution of amantadine-resistant H5N1 avian influenza variants in Asia. J. Infect. Dis. 193, 1626–1629.
Deyde, V.M., Xu, X., Bright, R.A., Shaw, M., Smith, C.B., Zhang, Y., Shu, S., Gubareva, L., Cox, N.J., Klimov, A.I., 2007. Surveillance of resistance to adamantanes among influenza A(H3) and A(H1N1) viruses isolated worldwide. J. Infect. Dis. 196, 249–256.
Deyde, V.M., Garten, R., Sheu, T.G., Gubareva, L.V., Klimov, A.I., 2008. Reply to Saito et al. J. Infect. Dis. 197, 632–633.
Felsenstein, J., 1989. PHYLIP—phylogeny inference package (Version 3.2). Cladistics 5, 164–166.
Fiore, A.E., Shay, D.K., Haber, P., Iskander, J.K., Uyeki, T.M., Mootrey, G., Bresee, J.S., Cox, N.J., 2007. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 56 (RR-6), 1–54.
Galabov, A.S., Simeonova, L., Gegova, G., 2006. Rimantadine and oseltamivir demonstrate synergistic combination effect in an experimental infection with type A (H3N2) influenza virus in mice. Antivir. Chem. Chemother. 17, 251–258.
Hay, A.J., Wolestenholm, A.J., Skehel, J.J., Smith, M.H., 1985. The molecular basis of the specific anti-influenza action of amantadine. EMBO 4, 3021–3024.
Hay, A.J., Zambon, M.C., Woltstenholme, A.J., 1986. Molecular basis of resistance of influenza A viruses to amantadine. J. Antimicrobiol. Chemother. 18, 19–29.

Hay, A.J., 1992. The action of adamantanamines against influenza A viruses: inhibition of the M2 ion channel protein. Virology 3, 21–30.
Hurt, A.C., Barr, I.G., Hartel, G., Hampson, A.W., 2004. Susceptibility of human influenza viruses from Australasia and South East Asia to neuraminidase inhibitors zanamivir and oseltamivir. Antiviral Res. 62, 37–45.
Hurt, A.C., Selleck, P., Komadina, K., Shaw, R., Barr, I.G., 2007. Susceptibility of highly pathogenic H5N1 avian influenza viruses to the neuraminidase inhibitors and adamantanes. Antiviral Res. 73, 228–231.
Hurt, A.C., Barr, I.G. 2007. Novel mutations in the '150-cavity' of N1 neuraminidase confer reduced sensitivity to the neuraminidase inhibitors. Options for the Control of Influenza. Toronto, Ontario, Canada. Abstract 067.
Ilyushina, N.A., Bovin, N.V., Webster, R.G., Govorkova, E.A., 2006. Combination chemotherapy, a potential strategy for reducing the emergence of drug-resistant influenza A variants. Antiviral Res. 70, 121–131.
Ilyushina, N.A., Hoffmann, E., Salomon, R., Webster, R.G., Govorkova, E.A., 2007. Amantadine-oseltamivir combination therapy for H5N1 influenza virus infection in mice. Antiviral Ther. 12, 363–370.
Kumar, S., Tamura, T., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5, 150–163.
Masihi, K.N., Schweiger, B., Finsterbusch, T., Hengel, H., 2007. Low dose oral combination chemoprophylaxis with oseltamivir and amantadine for influenza A virus infections in mice. J. Chemother. 19, 295–303.
Morrison, D., Roy, S., Rayner, C., Amer, A., Howard, D., Smith, J.R., Evans, T.G., 2007. A randomized, crossover study to evaluate the pharmacokinetics of amantadine and oseltamivir administered alone and in combination. PLoS ONE 12 (2), e1305, PMID: 18074029.
Pinto, L.H., Holsinger, L.J., Lamb, L.A., 1992. Influenza virus M2 protein has ion channel activity. Cell 69, 517–528.
Rambaut, A., Pybus, O.G., Nelson, M.I., Viboud, C., Taubenberger, J.K., Holmes, E.C., 2008. The genomic and epidemiological dynamics of human influenza A virus. Nature, doi:10.1038/nature06945.
Reece, P.A., 2007. Neuraminidase inhibitor resistance in influenza viruses. J. Med. Virol. 79, 1577–1586.
Saito, R., Suzuki, Y., Li, D., Zaraket, H., Sato, I., Masaki, H., Kawashima, T., Hibi, S., Sano, Y., Shobugawa, Y., Oguma, T., Suzuki, H., 2008. Increased incidence of adamantane-resistant influenza A(H1N1) and A(H3N2) viruses during the 2006–2007 influenza season in Japan. J. Infect. Dis. 197, 630–632.
Shiraishi, K., Mitamura, K., Sakai-Tagawa, Y., Goto, H., Sugaya, N., Kawaoka, Y., 2003. High frequency of resistant viruses harbouring different mutations in amantadine-treated children with influenza. J. Infect. Dis. 188, 57–61.
Simonsen, L., Viboud, C., Grenfell, B.T., Dushoff, J., Jennings, L., Smit, M., Macken, C., Hata, M., Gog, J., Miller, M.A., Holmes, E.C., 2007. The genesis and spread of reassortment human influenza A(H3N2) viruses conferring adamantane resistance. Mol. Biol. Evol. 24, 1811–1820.
Tsiodras, S., Mooney, J.D., Hatzakis, A., 2007. Role of combination antiviral therapy in pandemic influenza and stockpiling implications. BMJ 334, 293–294.
WHO, 2008a. http://www.who.int/csr/disease/influenza/h1n1_table/en/index.html (accessed 10 April 2008).
WHO, 2008b. http://www.who.int/csr/disease/influenza/recommendations2008_9north/en/index.html (accessed 28 March 2008).
WHO, 2008c. WHO writing committee of the Second World Health Organization Consultation on clinical aspects of human infection with avian influenza A (H5N1) virus, 2008. Update on Avian Influenza A(H5N1) Virus infection in humans. NEJM 358, 261–273.