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Short communication

The emergence of adamantane resistance in influenza A(H1) viruses in Australia and regionally in 2006

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Abstract

The adamantanes (amantadine and rimantadine) were the first antivirals licensed for use against influenza A viruses and have been used in some countries to control seasonal influenza. While increasing resistance of A(H3) viruses to this class of drug has been reported in recent years, only low levels of resistance were seen with A(H1) viruses until the 2005–2006 influenza season in the USA. In this study we analysed 101 human influenza A viruses isolated in 2006 that were referred to the WHO Collaborating Centre for Reference and Research in Melbourne, from Australia and the surrounding regions, for evidence of resistance to adamantanes. We found that whereas previously A(H1) resistant viruses were rare, 21.8% of the 2006 viruses had a resistant genotype. By comparison, 58.6% of influenza A(H3) viruses isolated in 2006 that were tested at the Centre, had a resistant genotype.

Keywords: Amantadine; Rimantadine; Matrix gene; Resistance; Australia; South East Asia

Recently we and others have reported that while an increasing proportion of influenza A(H3) viruses show resistance against the adamantane class of antiviral drugs little resistance has been seen amongst the A(H1) viruses (Bright et al., 2005; Barr et al., 2007). This class of drugs, represented by the licensed products amantadine (Symmetrel™) and rimantadine (Flumadine™) (Aoki, 1998) has been widely used in many countries to combat seasonal influenza. With this high level of adamantane-resistant A(H3) viruses persisting, the use of the newer group of influenza antivirals known as neuraminidase inhibitors (NI) (Gubareva et al., 2000), oseltamivir (Tamiflu™) and zanamivir (Relenza™), is currently recommended for the treatment or prevention of influenza (CDC, 2006).

The adamantanes work by blocking the ion channel formed by the M2 protein of influenza A viruses which inhibits the early stages of virus replication (Hay, 1992; Pinto et al., 1992; Aoki, 1998). The mechanism of resistance is well understood and revolves around mutations in the M2 protein and leads to 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, 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. 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) however, for unknown reasons these viruses have failed to spread widely until very recently (Belshe et al., 1989).

Bright et al. (2005) reported a high percentage of influenza A(H3) viruses resistant to adamantanes had emerged recently, especially in China where 73.8% of strains tested were resistant in 2004 (Bright et al., 2005) and in the USA, where 92.3% of strains tested during the 2005–2006 influenza season were found to be resistant (Bright et al., 2006). For the A(H1) viruses tested between 1998 and 2004, almost all were sensitive to the adamantanes but there was a hint of growing resistance in recent times with 2/8 of the 2005–2006 A(H1) viruses isolated in the USA bearing a resistant genotype (Bright et al., 2006).

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In this present study we examined influenza A viruses isolated from patients with influenza in Africa, Australia, New Zealand, South East Asia, Macau (SAR of China), Pacific Islands and Taiwan (Province of China), in 2006 and compared these results to adamantane resistance levels seen in these regions in recent years.

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 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 (RBC's) as previously described (Barr et al., 2003). Positive samples were typed using the haemagglutination inhibition assay (HAI) against a panel of known standard reference viruses and their homologous ferret antiserum (CDC, 1982). 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 three or more passages. RNA extraction, RT-PCR and sequencing were performed as previously described (Barr et al., 2003). Sequences were assembled using the Lasergene Seqman package IV (DNAStar 6). A selection of viruses predicted by their genotype to be resistant or sensitive to adamantanes were tested in a bioassay for sensitivity to Rimantadine (Sigma–Aldrich, Australia) as previously described (Barr et al., 2007).

The proportion of influenza A(H1) viruses (from the total number of influenza A(H1), A(H3) and B viruses) received at the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne in 2006 was 16.8% an increased proportion compared to the previous 3 years (12.8% in 2005, 8.4% in 2004, 0.9% in 2003). This increase was more marked in some countries such as the Philippines, Singapore and Macau (SAR) where the predominant viruses isolated in 2006 were A(H1). A total of 87 A(H3) viruses and 101 A(H1) viruses sent to the Centre during 2006, were examined for the specific mutations known to correlate with resistance to the antiviral drugs amantadine and rimantadine (Table 1). These were selected from 804 A(H3) and 243 A(H1) viruses received at the Centre up to 3 November 2006 based on their country of origin and date of isolation. The sequenced A(H3) and A(H1) viruses therefore represented 10.8% and 42.8% respectively of the total number received of each subtype to date in 2006. The average age of the patients from which the A(H3) viruses were sequenced was 21.6 years with a range of 0.2–87 years (ages were only available for 65/87 patients) and for A(H1) viruses was 14.8 years with a range of 0.1–82 years (ages were only available for 66/101 patients). 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 2006, 51/87 (58.6%) of influenza A(H3) viruses and 22/101 (21.8%) of A(H1) viruses had substitutions which would confer resistance to adamantanes.

Table 1
Geographic origin and proportion of A(H1) and A(H3) adamantane resistant viruses isolated in 2006

Country	2006 A(H1) viruses		2006 A(H3) viruses	
	# Resistant/# tested	Resistant (%)	# Resistant/# tested	Resistant (%)
Macau (SAR)	9/11	82	1/1	100
Taiwan	1/5	20	2/2	100
Thailand	1/10	10	6/10	60
Malaysia	0/10	0	2/9	22
Cambodia	0/3	0	–	–
Singapore	1/10	10	0/1	0
Philippines	0/14	0	1/1	100
Australia	8/20	40	20/40	50
New Zealand	1/7	14	9/12	75
New Caledonia	0/5	0	0/1	0
South Africa	1/3	33	10/10	100
Solomon Islands	0/3	0	–	
Total	22/101	21.8	51/87	58.6

The highest frequency of resistant strains were noted in A(H1) viruses obtained from Macau (SAR) 9/11 (82%), Australia 40% (8/20) and South Africa 1/3 (33%), while a number of countries had low (e.g. Thailand 1/10 (10%), Singapore 1/10 (10%)) or no apparent resistance (e.g. Malaysia 0/10 (0%), Philippines 0/14 (0%), New Caledonia 0/5 (0%)). For A(H3) viruses isolated in 2006, resistance levels were high in New Zealand 9/12 (75%), Australia 20/40 (50%), South Africa 10/10 (100%) and Taiwan 2/2 (100%). Analysis of the isolation dates for the A(H1) resistant viruses showed that they were first detected in January 2006 in both Australia and Macau and they then persisted throughout 2006 in Australia and until May 2006 in Macau (Fig. 1). Interestingly in those countries where resistant viruses circulated, sensitive viruses also co-circulated. All of the 2006 A(H3) viruses had the same single nucleotide change (AGT to AAT) resulting in an S31N substitution in the M2 protein as did all of the A(H1) viruses except for a single isolate from Macau (SAR) (A/Macau/7334/2006 isolated on 21 February

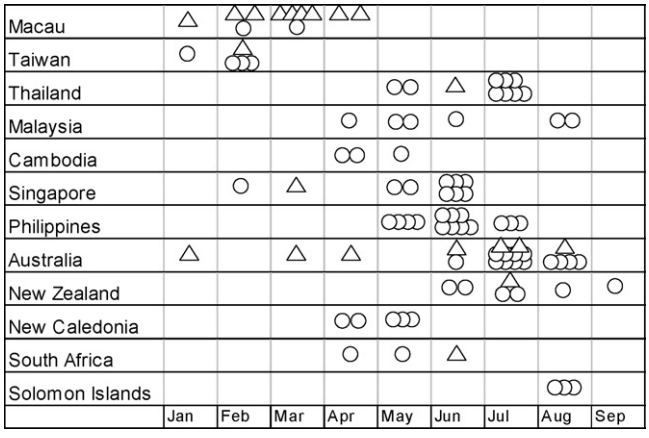

Fig. 1. The identification of adamantane resistant (△) and adamantane sensitive: (◯), influenza A(H1) viruses isolated between January and September 2006.

2006) which had a V27A mutation. Six A(H1) adamantane-sensitive and 6 A(H1) adamantane-resistant viruses (as predicted by genotyping) that were isolated in 2006 were tested in a bioassay for sensitivity to rimantadine. The viruses predicted to be adamantane-sensitive all had their growth inhibited in MDCK cultures with 0.2 μg/ml rimantadine while the viruses predicted to be adamantane-resistant were all able to grow well in 2 or 20 μg/ml of rimantadine.

In 2006, influenza A(H1) viruses have become more prevalent in many countries in the region such as the Philippines, Singapore and Macau (SAR) with moderate increases in Thailand but no increase in other countries like Australia. The majority of these viruses were similar to the reference and vaccine A(H1N1) strain A/New Caledonia/20/99. Adamantane resistant strains were common among A(H1) isolates in Australia and some Asian countries, e.g. Macau (SAR) but not in the Philippines, even though the latter had many A(H1) cases in 2006. This appears to be a relatively new phenomenon as no adamantane resistant influenza A(H1) viruses were identified in our laboratory in 2005 (0/37 isolates tested) or previously, while in 2006 the level of resistance had grown to 21.8% of the strains tested. Overall in 2006, A(H1) resistant viruses were found in 6/12 of the countries from which samples were obtained while for A(H3) viruses, adamantane resistance was more widespread with 8/10 countries from which samples were obtained. The A(H1) and A(H3) viruses were obtained from geographically distinct sites and generally represent all or a representative proportion of the 2006 viruses from these sites available at the Centre for testing. Although the majority (58.6%) of influenza A(H3) strains isolated in 2006 were resistant to adamantanes and this was higher than the levels seen in 2005 (42% resistant), this proportion was still lower than that seen in the USA in 2005–2006 influenza season where 92.3% of strains were found to be resistant (Bright et al., 2006).

A substitution in any one of five amino acids in the M2 protein in influenza A viruses can confer resistance to amantadine or rimantadine (Hay et al., 1985; Hay, 1992; Aoki, 1998), but in this present study of influenza A viruses isolated in 2006, only one substitution (S31N) was evident in all of the influenza A(H3) viruses and for all of the A(H1) viruses except for one virus from Macau (SAR). This is consistent with our previous findings on A(H3) viruses (Barr et al., 2007) and with other reports (Bright et al., 2005, 2006) and may indicate a common origin of these resistant subtypes. Prior to this report the most common mutation in A(H1) viruses conferring resistance to adamantanes reported was the V27A change that was present in 6/9 resistant viruses isolated from Japanese children in the winters of 1999–2000 and 2000–2001 (Saito et al., 2003). In contrast recent studies on A(H5N1) viruses from SE Asia, found the S31N change was almost invariably associated with an additional L26I substitution (Cheung et al., 2006; Hurt et al., 2007). Analysis of the full matrix genes from A(H1) and A(H3) 2006 resistant viruses show that there are still distinct differences between the subtypes (there are conserved nucleotide differences at more than 58 sites in the matrix gene), indicating that the two resistant genotypes do not share the same origin.

None of the adamantane-resistant A(H3) or A(H1) viruses identified in this study that were tested, showed increased levels of resistance to the two licensed NI antivirals, oseltamivir or zanamivir as determined using a neuraminidase-inhibition assay (data not shown). Clearly influenza A(H1) and A(H3) viruses with the S31N substitution are capable of spreading within the community with no viral fitness compromises and may now even have a selective advantage. As the adamantanes have proven useful in prevention of seasonal influenza A and may play a role if a pandemic eventuated, it would be ideal to reduce the frequency of resistance of human and avian influenza viruses to these drugs as soon as possible. This may be achievable if use of these drugs is limited for the next few years to allow resistant strains to be possibly be replaced by non-resistant influenza A viruses which are still widely circulating. This would reduce the chance of an A(H5) virus reassorting with an A(H1) or A(H3) virus or the further proliferation of A(H5) adamantane resistant viruses, which have already been isolated in a number of countries (Cheung et al., 2006; Hurt et al., 2007). To monitor these changes, it will be important to assess the level of resistance for the M2 inhibitors in influenza A(H1), A(H3) and A(H5) viruses circulating in both human and avian species in the coming years.

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