Indian J Med Res 140, August 2014, pp 244-251

Antiviral drug profile of human influenza A & B viruses circulating in India: 2004-2011

V.A. Potdar[1], M.R. Dakhave[1], P.B. Kulkarni[1], S.A. Tikhe[1], S. Broor[2], P. Gunashekaran[3], M. Chawla-Sarkar[4], A. Abraham[5], D. Bishwas[6], K.N. Patil[1], A.A. Kadam[1], S.S. Kode[1], A.C. Mishra[1] & M.S. Chadha[1]

[1]National Institute of Virology (ICMR), Pune, [2]All India Institute of Medical Sciences, New Delhi, [3]King Institute of Preventive Medicine & Research, Chennai, [4]National Institute of Cholera & Enteric Diseases (ICMR), Kolkata, [5]Christian Medical College, Vellore & [6]Regional Medical Research Centre (ICMR), Dibrugarh, India

Received December 12, 2012

***Background & objectives*: Recent influenza antiviral resistance studies in South East Asia, Europe and the United States reveal adamantane and neuraminidase inhibitor (NAIs) resistance. This study was undertaken to evaluate antiviral resistance in influenza viruses isolated from various parts of India, during 2004 to 2011.**

***Methods*: Influenza viruses were analyzed genetically for known resistance markers by M2 and *NA* gene sequencing. Influenza A/H1N1 (n=206), A/H3N2 (n=371) viruses for amantadine resistance and A/H1N1 (n=206), A/H3N2 (n=272) and type B (n=326) for oseltamivir resistance were sequenced. Pandemic (H1N1) (n= 493) isolates were tested for H274Y mutation by real time reverse transcription (rRT)-PCR. Randomly selected resistant and sensitive influenza A/H1N1 and A/H3N2 viruses were confirmed by phenotypic assay.**

***Results*: Serine to asparagine (S3IN) mutation was detected in six isolates of 2007-2008.One dual-resistant A/H1N1 was detected for the first time in India with leucine to phenylalanine (L26F) mutation in *M2* gene and H274Y mutation in *NA* gene. A/H3N2 viruses showed an increase in resistance to amantadine from 22.5 per cent in 2005 to 100 per cent in 2008 onwards with S3IN mutation. Fifty of the 61 (82%) A/H1N1 viruses tested in 2008-2009 were oseltamivir resistant with H274Y mutation, while all A/H3N2, pandemic A/H1N1 and type B isolates remained sensitive. Genetic results were also confirmed by phenotypic analysis of randomly selected 50 resistant A/H1N1 and 40 sensitive A/H3N2 isolates.**

***Interpretation & conclusions*: Emergence of influenza viruses resistant to amantadine and oseltamivir in spite of negligible usage of antivirals emphasizes the need for continuous monitoring of antiviral resistance.**

Key words Amantadine - drug resistance - India - influenza - oseltamivir - real time reverse transcription

Antiviral drugs play an essential role in the prophylaxis and treatment of influenza infections. Currently two classes of antiviral drugs M2 blockers and neuraminidase inhibitors (NAIs) are licensed for prevention and treatment of seasonal, zoonotic and pandemic influenza. Adamantanes (amantadine and rimantadine) are extensively used for influenza A in some countries[1,2]. Mutations in M2 protein at positions 26, 27, 30, 31 or 34 prevent binding of adamantanes and become resistant[3]. Rapid increase in the frequency of influenza A/H3N2 virus resistant to M2 inhibitors was identified globally during 2002/2003 season, reaching almost 100 per cent since 2006/2007 season[4,5]. Amantadine resistance in South East Asia remained elevated (33-100%) since 2007[6].

Resistance to M2 inhibitors has also been identified for seasonal A/H1N1 influenza virus between seasons and countries. This variability has been associated with the co-circulation of 2 haemagglutinin (HA) A/H1N1 lineages; Subclade 2C (A/Hong Kong/2652/2006) was typically oseltamivir sensitive and carried S31N marker of amantadine resistance in M2 gene, whereas subclade 2B (A/Brisbane/59/2007) was amantadine sensitive and had oseltamivir resistant mutation H274Y (N2 numbering) in *NA* gene[7]. The Hong Kong data showed the emergence of dual resistant A/H1N1 viruses[7].

With the persistence of amantadine-resistant viruses, use of the newer group of antiviral 'neuraminidase inhibitors' oseltamivir and zanamavir has been recommended for treatment and/or prevention of influenza A and B since 2007. Neuraminidase inhibitors block the release of progeny virions from a host cell by selectively binding to the active site of the neuraminidase enzyme. This inhibits cleavage of the sialyl-acid bond to the host receptor, thus the virus is unable to be released from infected host cells and spread to new cells. Mutations giving rise to NAI resistance are both influenza subtype and drug-specific. Various mutations at amino acid positions 118,119,151, 152, 222, 224, 227, 274, 276, 292, 294, and a deletion at positions (Δ)244-247 of *NA* gene of influenza A have been implicated towards resistance to oseltamivir and/or zanamavir[8].

Resistance to NAIs among seasonal influenza virus was low (<0.1%) in the field isolates until 2006-2007 season. In late 2007 unexpected emergence and spread of oseltamivir resistance in seasonal A/H1N1, characterized by mutation H274Y of the NA gene was observed globally[9,10]. The rise in resistance appeared to be due to the spontaneous emergence and transmission of H274Y mutant viruses rather than selection pressure due to increased oseltamivir use[10]. A similar mutation has also been shown to be associated with clinical failure of drug treatment in A/H5N1 zoonotic infections[11]. The A/H3N2 viruses remained sensitive to oseltamivir.

Two lineages of influenza B viruses Victoria and Yamagata (HA/NA based) have been co-circulating since 1980s. Emergence of resistance to NAIs in influenza B and reduced susceptibility to NAIs have been detected through virus surveillance[12-14], and in clinical settings following drug treatment[15,16].

Most of the pandemic (H1N1) 2009 viruses are susceptible to NAIs but resistant to adamantanes[17]; however, oseltamivir-resistant pandemic (H1N1) 2009 viruses, have been detected in persons receiving oseltamivir treatment. They have been detected in less than 1 per cent of untreated patients in the community, and transmission has been documented only in closed settings or settings involving close contact with infected persons[18,19].

The widespread use of NAIs for pandemic control may create increasing selective pressure for the emergence and spread of drug-resistant influenza. The present study was carried out to evaluate antiviral drugs susceptibility for seasonal influenza A and B viruses circulating in India from 2004 to 2011.

Material & Methods

In India, influenza surveillance has been carried out in multisite regional centres located at different parts of India (Pune and Nagpur in West, Delhi and Lucknow in North, Kolkata and Dibrugarh in East, Chennai, Vellore and Kerala in South)[20]. The National Institute of Virology (NIV) Pune, monitors genetic variations and drug susceptibility in circulating influenza viruses received from regional centres. Influenza viruses, isolated from 2004 to 2011 were analyzed genetically for known resistance markers by *M2* and *NA* gene sequencing.

Viruses: Virus isolation and antigenic characterization was carried out at respective regional laboratories by inoculating nasal/throat swabs into Madin Darby Canine Kidney (MDCK) cells[21]. Isolates were then shared with referral centre and passaged further for molecular characterization. A total of A/H1N1 (n=206), A/H3N2 (n=371) viruses were studied for amantadine resistance and A/H1N1 (n=206), A/H3N2 (n=272), pandemic (H1N1) (n= 493) and type B (n=326) were studied for oseltamivir resistance.

Fluorescent neuraminidase inhibition assay (NI): The half maximal inhibitory concentration **(**IC_{50}) to oseltamivir carboxylate was determined by a fluorescence-based neuraminidase inhibition assay[22]. Eleven sensitive and 50 resistant A/H1N1 virus isolates of 2007–2009 seasons, and 40 A/H3N2 sensitive viruses of 2009-2010 were tested along with reference viruses. Oseltamivir carboxylate (GS4071) was provided by F. Hoffmann-La Roche Ltd. (Basel, Switzerland). The reference viruses were provided by WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia. Type B viruses were not tested for NAI due to unavailability of reference strains.

Molecular detection of M2 and NA gene

RNA extraction and RT-PCR - Viral RNA was extracted from culture supernatant using the QIAamp Viral RNA Mini Kit (Qiagen, USA). One step PCR [reverse transcription (RT)-PCR)] (Invitrogen Superscript III Platinum kit country) was performed to amplify *M2* and *NA* gene for detection of established molecular markers using published and gene-specific primers[23-26] and *HA1* gene using CDC (unpublished) for phylogenetic analysis.

Sequencing - The amplicons for *M2* (270 bp covering amino acid positions 26 to 31), *N1* (253 bp covering 274 amino acid positions), *N2* (1103 bp covering all known mutation sites from 118 to 294 amino acid position) and type B *NA* (1146bp covering all known mutation sites from 119 to 371 amino acid position) genes were purified using PCR purification kits (Qiagen). DNA sequencing was carried out using Big Dye terminator V 3.1 cycle sequencing ready reaction kit (ABI, Foster City, CA, USA) and unincorporated labelled ddNTPs (dideoxy nucleotides) were removed using Dye-X removal column purification kit (Qiagen). The sequencing was done on ABI 3730 DNA analyzer (ABI, Foster City, CA, USA) and pair wise sequence alignment and protein translation was performed with MEGA 4 program[27].

Drug susceptibility markers: *M2* gene sequencing of A/H1N1 (n=206) and A/H3N2 (n=371) and *NA* gene sequencing of A/H1N1 (n=206), A/H3N2 (n=272), and type B (n=326) were performed to analyze the established molecular markers. Amantadine susceptibility of A/H1N1 and A/H3N2 viruses was examined for mutations at positions 26, 27, 30, 31 or 34 in *M2* gene; for N1 subtype oseltamivir susceptibility was examined for amino acid position 274 (N2 numbering) and for N2 subtype amino acid positions 118, 119, 151, 152, 222, 224, 227, 274, 276, 292 and 294 and Δ244-247 were examined. Type B influenza positions 119, 152, 198, 222, 274, 371 (N2 numbering) were checked.

Allelic real-time RT-PCR assay for detection of H274Y in pandemic H1N1 - Susceptibility to oseltamivir for pandemic (H1N1) viruses was evaluated by allelic discrimination real-time RT-PCR. The primers and probes used in this assay were designed by South-East Asia Regional influenza reference laboratory, Thailand[28].

Phylogenetic analysis of HA1 gene: Phylogenetic analysis of A/H1N1 and A/H3N2 viruses used in this study along with WHO reference strains was done. MEGA version 4 was used for constructing neiughbour-joining (NJ) trees using the Kimura's two-parameter distance model, with 1000 bootstrap replicates[27].

Results

Amantadine susceptibility of influenza A viruses

Seasonal A (H3N2) viruses - As summarized in Table I, A/H3N2 viruses circulating in 2004 were sensitive to amantadine. In 2005, resistant viruses (23%), were introduced and were co-circulating along with sensitive viruses in Pune, Delhi and Kolkata. At the same time, the viruses from Chennai remained sensitive to amantadine. In 2006, proportion of resistant viruses increased to 48 per cent in Pune, Delhi, Chennai, Kolkata and Dibrugarh. In 2007, it reached to 90 per cent and 2008 onwards it became 100 per cent in India. The most common mutation S31N responsible for amantadine resistance was observed in all A/H3N2 isolates.

Seasonal A (H1N1) viruses - In contrast to A/H3N2 viruses most of A/H1N1 viruses remained sensitive to amantadine till mid 2007 (Table I). A single resistant H1N1 virus was first detected from Delhi in August 2007 followed by Vellore in November. In 2008, three isolates from Kolkata were found to be resistant to amantadine and two of these had common mutation S31N in the *M2* gene and single virus A/K/0951720/2009 had less common L26F mutation in *M2* gene along with H274Y mutation in *NA* gene leading to dual-resistant.

Phylogenetic analysis of HA1 gene of resistant A/H1N1 and A/H3N2 - Phylogenetic analysis of HA1 coding region of A/H1N1 viruses showed that the amantadine resistance viruses grouped with clade 2B, A/Brisbane/59/2007 with signature amino acid changes

Table I. Amantadine susceptibility testing of influenza A virus isolates (2004-2011)

Year	Type	Centre and virus type wise No. of resistant samples detected/Total no. of samples tested									Total resistant samples/ Total samples (% positivity)
		Pune	Delhi	Chennai	Kolkata	Vellore	Dibrugarh	Nagpur	Lucknow	Kerala	
2004	H3N2	0/4	0/2	0/4	-	-	-	-	-	-	0/10
	H1N1	-	-	-	-	-	-	-	-	-	-
2005	H3N2	0/5	4/18	0/5	2/3	-	-	-	-	-	6/31 (22.5)
	H1N1	0/14	0/9	0/19	0/2	-	0/1	-	-	-	0/45
2006	H3N2	4/7	1/7	2/2	1/2	1/1	3/6	-	-	-	12/25 (48)
	H1N1	0/6	0/2	0/18	0/12	-	-	-	-	-	0/38
2007	H3N2	10/10	-	2/2	1/2	4/5	-	1/1	-	-	18/20 (90)
	H1N1	0/23	1/15	-	0/18	**1**/1	-	0/4	-	-	2/61 (3)
2008	H3N2	8/8	4/4	37/37	18/18	5/5	-	-	-	-	72/72 (100)
	H1N1		0/1	0/15	3/7	-	-	-	-	-	3/23 (13)
2009	H3N2	28/28	65/65	-	20/20	15/15	2/2	2/2	-	-	132/132 (100)
	H1N1	0/12	0/14	0/5	1/7	-	-	-	-	-	1/38 (2)
2010	H3N2	9/9	-	20/20	-	-	-	-	-	1/1	30/30 (100)
	H1N1	0/1	-	-	-	-	-	-	-	-	0/1
2011	H3N2	30/30	6/6	1/1	5/5	-	3/3	1/1	3/3	2/2	51/51 (100)
	H1N1	-	-	-	-	-	-	-	-	-	-

Centre and year-wise A/H3N2 and A/H1N1 virus isolates sequenced for *M2* gene and amino acid positions 26,27,30,31 or 34 conferring the resistance to amantadine were checked

D45N, K149R (H3 numbering). On the contrary sensitive H3N2 viruses were scattered throughout the HA1 phylogenetic tree. Amantadine resistant A/H3N2 viruses with double mutations at position S193F and D225N were grouped in clade N.

Neuraminidase drug susceptibility markers of influenza A and B viruses

A/H1N1 viruses - *NA* gene sequencing of 206 A/H1N1 isolates was done to assess oseltamivir resistance mutation H274Y (Table II). Up to November 2008 all A/H1N1 isolates were sensitive to oseltamivir while in December 2008 resistant seasonal A/H1N1 viruses were observed from Chennai. In 2009, resistant viruses were detected from Delhi (13/14), Kolkata (7/7), Chennai (5/5), Pune (10/12) and similar findings were reported from Kolkata. No other substitutions conferring resistance to oseltamivir were found in these isolates.

A/H3N2 viruses - A total of 272 A/H3N2 isolates were studied for the presence of substitutions at positions R118K, E119Q, D151E, R152K, I222V, R224K, E227D, H274Y, E276D, R292K, N294S and a Δ 244-247, and none of the isolates had any of the reported oseltamivir-resistance conferring mutations. (Accession Nos: KF 314400-KF314591; KF 314609-KF314682; KF014004,6,8,10) (Table II).

The analysis of the A/H1N1 NA sequences revealed the presence of H274Y mutation in 15/23 of 2008 isolates and 35/38 of 2009 isolates. This mutation is known to be associated with an oseltamivir resistance in NA1 subtype virus. Its identification in the NA sequence of A/H1N1 virus isolates which exhibited high fluorescence IC_{50} values between 299.8 to 2513 nM when compared with wild type reference strain (0.6-3.0 nM) allowed to confirm 15/23 (65.22%) and 35/38(92.10%) A/H1N1 virus isolates from 2008 and

Table II. Neuraminidase drug susceptibility testing of influenza A and influenza B

Year	Type	Centre and virus type-wise: No. of resistant samples detected/total no. of samples tested									Total resistant samples/Total samples (% positivity)
		Pune	Delhi	Chennai	Kolkata	Vellore	Dibrugarh	Nagpur	Lucknow	Kerala	
2004	H3N2	0/4	0/2	-	-	-	-	-	-	-	0/6
	Type B	-	0/1	-	-	-	-	-	-	-	0/1
	H1N1	-	-	-	-	-	-	-	-	-	-
2005	H3N2	0/5	0/8	0/3	0/1	-	-	-	-	-	0/17
	Type B	0/13	0/2	0/2	-	-	-	-	-	-	0/17
	H1N1	0/14	0/9	0/19	0/2	-	0/1	-	-	-	0/45
2006	H3N2	0/5	0/2	-	-	-	0/3	-	-	-	0/10
	Type B	0/3	0/4	-	-	-	-	-	-	-	0/7
	H1N1	0/6	0/2	0/18	0/12	-	-	-	-	-	0/38
2007	H3N2	0/8	-	0/1	0/2	-	-	-	-	-	0/11
	Type B	0/1	-	-	-	-	-	-	-	-	0/1
	H1N1	0/23	0/15	-	0/18	0/1	-	0/4	-	-	0/61
2008	H3N2	0/5	0/4	0/37	0/15	0/5	-	-	-	-	0/66
	Type B	0/3	0/15	0/15	0/3	-	-	-	-	-	0/36
	H1N1	-	0/1	15/15	0/7	-	-	-	-	-	15/23 (65.22)
2009	H3N2	0/22	0/65	-	0/20	0/11	0/2	0/2	-	-	0/122
	Type B	0/1	0/7	0/2	0/4	0/5	0/1	-	-	-	0/20
	H1N1	10/12	13/14	5/5	7/7	-	-	-	-	-	35/38 (92.10)
	P (H1N1)	0/134	0/28	0/25	0/13	-	0/17	0/03	-		0/220
2010	H3N2	0/6	-	0/13	-	-	-	-	-	0/1	0/20
	Type B	0/61		0/45	0/34	0/42	0/6	0/8			0/196
	H1N1	1/1	-	-	-	-	-	-	-	-	1/1 (100)
	P (H1N1)	0/235	0/6	0/6	0/1	-	0/7	0/2		0/6	0/263
2011	H3N2	0/7	0/5	-	0/2	-	0/3	-	0/3	-	0/20
	Type B	0/29	0/3	0/2	0/4	0/2	0/3	0/5	-	-	0/48
	H1N1	-	-	-	-	-	-	-	-	-	-
	P (H1N1)	0/3	-	-	0/2	0/2	0/2	0/1	-	-	0/10

Amino acid sites were checked as follows: H3N2:R118K, E119Q, D151E, R152K, I222V, R224K, E227D, H274Y, E276D, R292K, N294S and a Δ 244-247 , Type B: E119A, R152K, D198N/E, I222T, H274Y and R371K, H1N1 and P(H1N1): H274Y

Table III. IC_{50} (half maximal inhibitory concentration) values for influenza viruses from India

Influenza type/subtype	A(H1N1) seasonal		A(H3N2)
	2008	2009	2009
Number of isolates tested	23	38	36
Mean IC_{50} ± SD (nM)	272.36 ± 482.9	278.3 ± 514.29	3.47 ± 2.47
Range	0.4 - 963.2	0.4 - 2513	0.215 - 9.67
Oseltamivir resistance	Yes	Yes	No

2009, respectively resistant to oseltamivir. The NA sequence of oseltamivir-resistant viruses had T81N, D354G when compared with A/Brisbane/59/2007. Majority of A/H3N2 subtype viruses had IC_{50} values between 0.215 - 9.67 nM and remained susceptible to oseltamivir when compaired with IC_{50} values of resistant reference strain (48 to 180 nM). Table III presents year-wise number of isolates tested along with mean IC_{50} (nM), standard deviation (nM), minimum IC_{50} (nM) and maximum IC_{50} (nM). Four isolates showed IC_{50} values 24.52, 55.24, 50.36 and 27.64 nM resulting as minor outliers in fluorescent assay. These outliers were further assessed for *NA* gene mutations and all had D151. Though Asn(N) at position 151 has been associated with a reduction in the susceptibility of influenza A and B viruses to oseltamivir and/or Zanamavir; our study showed that the sensitive H3N2 viruses had either Asp (D),/Asn (N) or Gly (G) at residue 151. So we could not correlate the presence of D151 in four minor outliers with their IC_{50} values.

Type B viruses - A total of 326 type B isolates were sequenced for *NA* gene and lack of changes at positions E119A, R152K, D198N/E, I222T, H274Y and R371K indicated that all type B isolates were sensitive to oseltamivir (Accession Nos: KF314197- KF314399; KF364360-KF364482) (Table II).

Pandemic (H1N1) viruses - Pandemic (H1N1) isolates (n=493) were screened for H274Y mutation in *NA* gene by allelic discrimination and all were sensitive to oseltamivir

Discussion

The acquisition of a mutation in influenza A viruses conferring resistance to an antiviral agent may occur as a result of drug selection, spontaneous mutation or through genetic reassortment with another drug resistant influenza A virus. High prevalence of amantadine resistance in influenza A viruses was observed in countries, irrespective of its use[5,6].

Globally, a marked increase in amantadine drug resistant virus isolates was reported from Asian countries especially for A/H3N2 subtype[4]. However, majority of A/H1N1 viruses remained sensitive till 2006 season and 2 per cent of A/H1N1 viruses worldwide showed resistance to amantadine[29]. In the present study, resistance to amantadine was mainly observed for A/H3N2 subtype, with identification of common mutation S31N of *M2* gene. In the present study, A/H1N1 viruses remained sensitive up to 2006. Resistant A/H1N1 viruses detected in India were 3 per cent in 2007, 13 per cent in 2008 with S31N substitution and agreeing with global data[4]. In 2009, a single isolate from Kolkata had less common mutation L26F in M2 gene and H274Y mutation in *NA* gene which conferred dual resistance. Indian isolates with S31N mutation indicated that strains already harbouring drug resistant mutation were most likely introduced in the community. Since in India the use of amantadine for influenza treatment was extremely rare during the study period; the possibility of emergence of amantadine resistance due to drug pressure is remote.

Before 2008, oseltamivir-resistance for any influenza type/subtype was not observed in India, due to negligible use of oseltamivir till May 2009. Widespread use of oseltamivir for suspected H1N1 patients in India started after June 2009 when cases of pandemic A (H1N1) 09 were detected in India. Prior to this, use of antivirals for influenza was negligible indicating that there was no potential for drug resistance due to selective pressure. However, no evidence was found to suggest that increased access to oseltamivir had promoted resistance or lack of it. A similar study from Japan, where oseltamivir is more widely used, reported no significant effect on the occurrence of resistance[30].

In India, a probable importation or detection of global 2008 oseltamivir-resistant seasonal A/H1N1 virus was documented nine months after it was first reported in Europe in January 2008[9,10]. Oseltamivir

resistance was observed for seasonal A/H1N1 influenza viruses with an increased frequency of 65.227 per cent in 2008 to 100 per cent in 2010. These resistant viruses exhibited extremely high IC_{50} values, between 299.8 to 2513 nM and carried H274Y mutation. It was well studied that oseltamivir susceptibility to influenza viruses differed significantly depending on the NA subtypes. This was expected to occur due to minor structural differences between the NA active sites of each NA subtype viruses which could result in different oseltamivir binding affinities[22]. The reduction in susceptibility to oseltamivir observed worldwide from 2005-2006 to 2007-2008 in A/H1N1 subtype was most probably a result of NA antigenic drift from A/Caledonia/20/1999 to A/Brisbane/59/2007. We detected oseltamivir resistance in N1 subtype from 2008 and it could be a result of same antigenic drift.

Oseltamivir was important for controlling the transmission and dissemination of pandemic viruses before a vaccine became widely available. No vaccine was available in India until one year after the first case of influenza A(H1N1) pdm09 was detected. This study showed that all 493 influenza A(H1N1) pdm09 viruses from 2009 and 2011 were sensitive to oseltamivir.

Even though Indian isolates had permissive secondary mutations R194G, E214G, R222Q and V234M in *NA* gene from 2005-2007; which were thought to have occurred before the emergence and spread of H274Y NAI resistant viruses[31]; A/H1N1 isolates remained sensitive to oseltamivir till 2007. The role of observed additional mutations T81N and D354G when compared with A/Brisbane/59/2007 is still unclear. Our findings were in agreement with the widespread and sustained transmission of oseltamivir resistant A/H1N1 viruses observed worldwide since 2007/2008[9,10].

Like influenza A viruses, influenza B viruses also undergo continuous evolution with accumulation of point mutations and reassortment events between co-circulating lineages. It was observed worldwide that the community acquired isolates of influenza B virus remained sensitive to NAI drugs. However, sporadic cases of reduced NAI susceptibility in the NA inhibition assays have been reported from drug treated patients[13]. Our results showed that the influenza B viruses were sensitive to oseltamivir with agreement to global data.

Continued surveillance for antiviral drug resistance in influenza viruses is required to ensure that stockpiled neuraminidase inhibitors are effective and clinicians can be kept informed of the efficacy of neuraminidase inhibitors when treating patients for influenza.

References

1. Dolin, R. Antiviral chemotherapy and chemoprophylaxis. *Science* 1985; *227* : 1296-303.
2. Hayden FG, Treanor JJ, Fritz RS, Lobo M, Betts RF, Miller M, *et al*. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. *JAMA* 1999; *282* : 1240-6.
3. Hay AJ, Zambon MC, Wolstenholme AJ, Skehel JJ, Smith MH. Molecular basis of the resistance of influenza A viruses to amantadine. *J Antimicrob Chemother*1986; *18* (Suppl B) : 19-29.
4. Deyde VM, Xu X, Bright RA, Shaw M, Smith CB, Zhang Y, *et al*. Surveillance of resistance to adamantanes among influenza A (H3N2) and A (H1N1) viruses isolated worldwide. *J Infect Dis* 2007; *196* : 249-57.
5. Bright RA, Medina MJ, Xu X, Wallis TR, Davis XM, Povinelli L, *et al*. Incidence of adamantine resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet* 2005; 366 : 1175-81.
6. Barr IG, Deng YM, Iannello P, Hurt AC, Komadina N. Adamantane resistance in influenza A(H1) viruses increased in 2007 in South East Asia but decreased in Australia and some other countries. *Antiviral Res* 2008; *80* : 200-5.
7. Cheng PKC, Leung TWC, Ho ECM, Leung PCK, Ng AYY, Lai MYY, *et al*. Oseltamivir- and amantadine-resistant influenza viruses A (H1N1). *Emerging Infect Dis* 2009; *15* : 966-8.
8. Hay AJ, Collins PJ, Russell J. Antivirals and resistance. *Monogr Virol* 2008; *27* : 251-70.
9. Hauge S, Dudman S, Borgen K, Lackenby A, Hungnes A. Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. *Emerging Infect Dis* 2009; *15* : 155-62.
10. Lackenby A, Hungnes O, Dudman SG, Meijer A, Paget WJ, Hay AJ, *et al*. Emergence of resistance to oseltamivir among influenza A(H1N1) viruses in Europe. *Euro Surveill* 2008; *13* (S) : 1-2 pii.
11. Jong De M, Thanh TT, Khanh TH, Hien VM, Smith GJD, Chau NV, *et al*. Oseltamivir resistance during treatment of influenza A (H5N1) infection. *N Engl J Med* 2005; *353* : 2667-72.
12. Hurt AC, Iannello P, Jachno K, Komadina N, Hampson AW, Barr IG, *et al*. Neuraminidase inhibitor-resistant and sensitive influenza B viruses isolated from an untreated human patient. *Antimicrob Agents Chemother* 2006; *50* : 1872-4.
13. Sheu TG, Deyde VM, Adhiambo MO, Garten RJ, Xu X, Bright RA, *et al*. Surveillance for neuraminidase inhibitor resistance among human influenza A and B viruses circulating worldwide from 2004 to 2008. *Antimicrob Agents Chemother* 2008; *52* : 3284-92.
14. Sleeman K, Sheu TG, Moore Z, Kilpatrick S, Garg S, Fry AM, *et al*. Influenza B viruses with mutation in the neuraminidase active site, North Carolina, USA, 2010-11. *Emerging Infect Dis* 2011; *17* : 2043-6.

15. Hatakeyama S, Sugaya N, Ito M, Yamazaki M, Ichikawa M, Kimura K, *et al.* Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. *JAMA* 2007; *297* : 1435-42.

16. Ison, MG, Gubareva LV, Atmar RL, Treanor J, Hayden FG. Recovery of drug-resistant influenza virus from immunocompromised patients: a case series. *J Infect Dis* 2006; *193* : 760-4.

17. Drug Susceptibility of Swine-Origin Influenza A (H1N1) Viruses, April 2009. Centers for Disease Control and Prevention (CDC). *MMWR Morb Mortal Wkly Rep* 2009; *58* : 433-5.

18. Hurt AC, Hardie K, Wilson NJ, Deng YM, Osbourn M, Gehrig N, *et al.* Community transmission of oseltamivir-resistant A(H1N1)pdm09 influenza. *N Engl J Med* 2011; *365* : 2541-2.

19. Storms AD, Gubareva LV, Su S, Wheeling JT, Adhiambo M.O., Pan C.Y., *et al*. Oseltamivir-resistant pandemic (H1N1) 2009 virus infections, United States, 2010-11. *Emerging Infect Dis* 2012; *18* : 308-11.

20. Chadha MS, Broor S, Gunasekaran P, Potdar VA, Krishnan A, Chawla-Sarkar M, *et al.* Multi site virological influenza surveillance in India: 2004-2008. *Influenza Other Respir Viruses* 2011; *6* : 196-203.

21. World Health Organization, WHO Manual in Animal Influenza Diagnosis and Surveillance 2001. Available from: *http://www.wpro.who.int/NR/rdonlyres/EFD2B9A7-22654AD0-BC98-97937B4FA83C/0/manualon animal aidiagnosisandsurveillance.pdf*., accessed on July 12, 2012.

22. Hurt AC, Barr IG, Hartel G, Hampson AW. Susceptibility of human influenza viruses from Australasia and South East Asia to the neuraminidase inhibitors zanamivir and oseltamivir. *Antiviral Res* 2004; *62* : 37-45.

23. Alvarez AC, Brunck ME, Boyd V, Lai R., Virtue E., Chen W, *et al*. A broad spectrum, one-step reverse-transcription PCR amplification of the neuraminidase gene from multiple subtypes of influenza A virus. *Virol J* 2008; *5* : 77

24. Pachucki CT, Khurshid MA, Nawrock J. Utility of reverse transcriptase PCR for rapid diagnosis of Influenza A virus infection and detection of amantadine resistant influenza A virus Isolates. *J Clin Microbiol* 2004; *42* : 2796-8.

25. Sheu TG, Deyde VM, Garten R.J.,Klimov A.I.,Gubareva L.V. Detection of antiviral resistance and genetic lineage markers influenza B virus neuraminidase using pyrosequencing. *Antiviral Res* 2010; *85* : 354-60.

26. Lin YP, Gregory V, Collins P, Kloess J, Wharton S, Cattle N, *et al*. Neuraminidase receptor binding variants of human influenza A(H3N2) viruses due to substitution of aspartic acid 151 in the catalytic site - Role in virus attachment? *J Virol* 2010; *84* : 6769-81.

27. Tamura K, Dudley J, Nei M, Kumar S. Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007; *24* : 1596-9.

28. Monitoring drug resistance in influenza viruses Report of the Regional Workshop Nonthaburi, Thailand 23-28 August 2010. Available from: *http://www.searo.who.int/LinkFiles/BCT_Reports_HLM-409.pdf*, accessed on August 31, 2010.

29. Nelson M I, Simonsen L, Viboud C, Miller MA, Holmes EC. The origin and global emergence of adamantane resistant A/H3N2 influenza viruses. *Virology* 2009; *388* : 270-8.

30. Tashiro M, McKimm-Breschkin JL, Saito T, Klimov A, Macken C, Zambon M, *et al*. Neuraminidase Inhibitor Susceptibility Network; Surveillance for neuraminidase-inhibitor-resistant influenza viruses in Japan, 1996-2007. *Antiviral Ther* 2009; *14* : 751-61.

31. Bloom J, Gong L, Baltimore D. Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. *Science* 2010; *328* : 1272-5.

Reprint requests: Dr V.A. Potdar, Scientist C, National Institute of Virology (ICMR), Pune 411 001, India
e-mail: varshapotdar9@yahoo.co.in