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Update: Drug Susceptibility of Swine-Origin Influenza A (H1N1) Viruses, April 2009

Since April 21, 2009, CDC has reported cases of respiratory infection with a swine-origin influenza A (H1N1) virus (S-OIV) that is being spread via human-to-human transmission (*1*). As of April 28, the total number of confirmed S-OIV cases in the United States was 64; these cases occurred in California (10 cases), Kansas (two), New York (45), Ohio (one), and Texas (six). The viruses contain a unique combination of gene segments that had not been reported previously among swine or human influenza viruses in the United States or elsewhere (*1*). Viruses from 13 (20%) of 64 patients have been tested for resistance to antiviral medications. To date, all tested viruses are resistant to amantadine and rimantadine but are susceptible to oseltamivir and zanamivir. The purpose of this report is to provide detailed information on the drug susceptibility of the newly detected S-OIVs, which will aid in making recommendations for treatment and prophylaxis for swine influenza A (H1N1) infection. These data also will contribute to antiviral-resistance monitoring and diagnostic test development.

Adamantane susceptibility was assessed by conventional sequencing or pyrosequencing assay (*2*) with modifications (*3*), using viral RNA extracted from original clinical specimens and/or virus isolates. Susceptibility of virus isolates to the neuraminidase inhibitors (NAIs), including oseltamivir and zanamivir and two investigative NAIs (peramivir and A-315675), was assessed by chemiluminescent neuraminidase inhibition assay using the NAStar Kit (Applied Biosystems, Foster City, California) (*4*). The generated IC_{50} values (i.e., drug concentration needed to inhibit 50% of neuraminidase enzyme activity) of test viruses were compared with those of sensitive seasonal control viruses. In addition, because H274Y is the most commonly detected mutation in oseltamivir-resistant viruses (*4,5*), a set of new primers for pyrosequencing of the N1 gene was designed to monitor a residue of the neuraminidase protein at 274 (275 in N1 numbering) in viruses of swine origin (*6,7*) (Table 1).

All 13 specimens tested contained the S31N mutation in the M2 protein, which confers cross-resistance to the adamantane class of anti-influenza drugs (Table 2). In addition, a partial sequence deduced from the M2 pyrograms revealed changes characteristic for the M gene of S-OIVs. Existing primers used for the detection of adamantane resistance in seasonal viruses do not work with all tested S-OIVs. Optimized primers have been designed and are currently being validated. All 13 tested virus isolates exhibited IC_{50} values characteristic of oseltamivir- and zanamivir-sensitive influenza viruses. A/Georgia/17/2006 (H1N1), which is a seasonal virus, was used as a control (Table 2). The IC_{50} for oseltamivir ranged from 0.28 nM to 1.41 nM, whereas those for zanamivir ranged from 0.30 nM to 1.34 nM. All tested viruses also were susceptible to peramivir and A-315675. A subset of viruses (n = 2) tested in the fluorescent neuraminidase inhibition assay showed IC_{50} for oseltamivir and zanamivir ranging from 1.50 nM to 2.40 nM, similar to the sensitive control. Among the 36 specimens tested to date with pyrosequencing for the H274Y mutation in N1, none had mutations at residue 274.

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Editorial Note: In the United States, two classes of antiviral drugs are approved by the Food and Drug Administration (FDA) for use in treating or preventing influenza virus infections: M2 ion channel blockers and NAIs. The M2 blockers (adamantanes) are effective against influenza A viruses, but not influenza B viruses, which lack the M2 protein (*8*). However, use of the M2 blockers has been associated with the rapid emergence of drug-resistance mutations of the M2 protein among human influenza A viruses of H3N2 subtype, and in H1N1 subtype viruses circulating in certain geographic areas (*2,3,9*). Adamantane resistance also has been detected in A (H5N1) viruses in Southeast Asia (*10,11*). In addition, adamantane resistance has been reported for swine viruses in

TABLE 1. Sequences of swine-origin influenza A (H1N1) primers for pyrosequencing targeted NA codon 274

Primer	Primer sequence (5' to 3')
Forward primer (Uni-sw-N1-B-F780)	GGG GAA GAT TGT YAA ATC AGT YGA
Reverse primer (Uni-sw-N1-B-R1273-biot)	CWA CCC AGA ARC AAG GYC TTA TG
Sequencing primer (Uni-sw-N1-B-F804seq)	GYT GAA TGC MCC TAA TT

TABLE 2. Drug susceptibility of human influenza A (H1N1) viruses of swine origin

CDC identification no.	Strain designation	Date specimen collected	Adamantane susceptibility	M2 mutation	NAI* susceptibility (IC_{50}, nM)†			
					Oseltamivir	Zanamivir	Peramivir	A-315675
2009712047	A/California/04/2009	04/01/09	Resistant	S31N	1.37	1.34	0.13	0.66
2009712097	A/California/05/2009	03/30/09	Resistant	S31N	1.41	1.30	0.15	1.78
2009712110	A/California/06/2009	04/16/09	Resistant	S31N	0.28	0.49	0.08	0.11
2009712111	A/California/07/2009	04/09/09	Resistant	S31N	0.56	0.31	0.10	0.18
2009712113	A/California/08/2009	04/09/09	Resistant	S31N	0.73	0.93	0.09	0.19
2009712175	A/Texas/04/2009	04/14/09	Resistant	S31N	0.64	0.62	—	—
2009712177	A/Texas/05/2009	04/15/09	Resistant	S31N	0.54	0.44	0.10	0.35
2009712190	A/Mexico/4482/2009	04/14/09	Resistant	S31N	0.39	0.51	0.06	0.63
2009712191	A/Mexico/4486/2009	04/14/09	Resistant	S31N	0.42	0.50	0.12	0.39
2009712192	A/Mexico/4108/2009	04/03/09	Resistant	S31N	0.39	0.56	0.12	0.50
2009712389	A/Mexico/4516/2009	04/03/09	Resistant	S31N	1.01	0.86	0.26	1.94
2009712390	A/Mexico/4603/2009	04/14/09	Resistant	S31N	0.34	0.35	0.07	1.03
2009712391	A/Mexico/4604/2009	04/14/09	Resistant	S31N	0.44	0.30	0.07	0.68
Control (seasonal)	A/Georgia/17/2006	—	Sensitive	S31	0.61	0.56	0.16	0.67
Control (seasonal)	A/Georgia/20/2006§	—	Sensitive	S31	200.73	0.80	13.87	1.59

* Neuraminidase inhibitor.
† Drug concentration needed to inhibit 50% of neuraminidase enzyme activity (determined by chemiluminescent NAI assay).
§ Oseltamivir resistant, zanamivir sensitive.

Eurasia (*12–14*) but not in North America. This rapid increase in resistance has reduced the usefulness of this class of drugs for the management of influenza A infections, and since 2005, CDC has not recommended their use (*15*), although the emergence of resistance to oseltamivir in seasonal influenza viruses circulating during the 2008–09 season led to changes in CDC recommendations.*

Two NAIs, oseltamivir (Tamiflu [Hoffman-La Roche, Ltd, Basel, Switzerland]) and zanamivir (Relenza [GlaxoSmithKline, Stevenage, United Kingdom]) are FDA-approved drugs for use against type A and type B influenza infections (*16*). The two drugs differ structurally, resulting in oseltamivir being orally bioavailable, whereas zanamivir is not and must be inhaled (*17,18*). A third NAI, peramivir (BioCryst, Inc., Birmingham, Alabama), is formulated for intravenous administration and is undergoing clinical trials, and a fourth, called A-315675 (Abbott Laboratories, Abbott Park, Illinois) has only been investigated in preclinical studies.

Compared with M2 blockers, NAIs previously exhibited lower frequency of antiviral resistance during therapeutic use (*16,19*). However, during the 2007–08 influenza season, emergence and transmission of oseltamivir-resistant A (H1N1) viruses, with a H274Y mutation in the neuraminidase protein, was simultaneously detected in several countries in the Northern Hemisphere (*4,20–22*) and spread globally (*7,9,23*). As of April 2009, similar trends have been observed in the 2008–09 influenza season, with many countries reporting up to 100% oseltamivir resistance in A (H1N1) viruses. As a result, the World Health Organization Global Influenza Surveillance Network (GISN) and CDC have emphasized the urgent need for close monitoring of resistance to NAIs. Current interim antiviral recommendations for treatment and chemoprophylaxis of swine influenza A (H1N1) viruses include the use of either zanamivir or oseltamivir and are available at http://www.cdc.gov/swineflu/recommendations.htm.

* Available at http://www.cdc.gov/features/dsfluview2009.

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