

SURVEILLANCE OF ANTIVIRAL RESISTANT INFLUENZA FROM 2006-2008 BY A NETWORK OF U.S. STATE PUBLIC HEALTH LABORATORIES

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Introduction

- Early in the 2005-2006 influenza season a dramatic increase in the prevalence of adamantane (amantadine and rimantadine) resistance was detected in circulating influenza A viruses in the U.S. (1).
- The Centers for Disease Control and Prevention (CDC) issued recommendations against the use of adamantanes and recommended oseltamivir (Tamiflu) or zanamivir (Relenza) for treatment and prophylaxis of influenza infection (2).
- During the 2006-2007 influenza season New York State (NYS) and Wisconsin State (WS) began sharing influenza susceptibility data from their respective states.
- In the 2007-2008 influenza season there was a dramatic increase of the oseltamivir resistance mutation H274Y among circulating influenza A/H1N1 viruses (3).

Methods

- NYS and WS screened influenza primary specimens and tissue culture isolates collected in respective states for mutations conferring resistance to adamantanes and oseltamivir.

Adamantane Susceptibility Surveillance

- Using a unidirectional pyrosequencing method (4), NYS and WS screened influenza A viruses for mutations affecting amino acids 26, 27, 30, 31, and 34 in the M2 gene which are known to confer adamantane resistance. Pyrograms obtained with this assay are shown in Figure 1.
- In NYS this method was expanded to bidirectional pyrosequencing with a newly designed reverse pyrosequencing primer (Figure 2) designed from an aligned dataset of 168 influenza A matrix gene sequences spanning 72 years.

Neuraminidase Inhibitor Susceptibility Surveillance

- WS performed a CDC-designed mutation-specific (H274Y) pyrosequencing assay to screen influenza A/H1N1 viruses for oseltamivir resistance. Pyrograms obtained with this assay are shown in Figure 3.
- NYS aligned the neuraminidase gene sequences of over 460 strains of influenza viruses A/H1N1, A/H2N2, A/H3N2, A/H5N1, and B spanning more than 85 years. This alignment was further analyzed in four distinct, virus type-specific groupings (1-A/H1N1, 2-A/H2N2 and A/H3N2, 3-H5N1, and 4-B). For each alignment group, one pair of external primers was designed for RT-PCR and bidirectional Sanger dideoxy sequencing of approximately 95% of the NA gene. Additionally, 3 internal primer pairs were designed to ensure sequencing accuracy (as well as to prepare for sequence variation among the strains) (Figure 4). PCR products of 1378bp, 1333bp, and 1326bp of seasonal influenza viruses A/H1N1, A/H3N2, and B respectively, were dideoxy sequenced and analyzed for characterized influenza antiviral resistance mutations and amino acid altering mutations in conserved regions and functional domains (Figure 5) (5).

Results

Adamantane Susceptibility Surveillance

- Collectively, for the 2006-2007 influenza season, network participants detected mutations conferring adamantane resistance at rates of 82.7% and 0% for influenza A(H3N2) and A(H1N1) respectively (Table 1)
- Collectively, for the 2007-2008 influenza season, network participants detected mutations conferring adamantane resistance at rates of 100% and 1.2% for influenza A(H3N2) and A(H1N1). The CDC reported nationwide rates of 99.6% and 11.1%.
- All influenza A(H3N2) viruses contained the S31N resistance mutation in the matrix gene.

Neuraminidase Inhibitor Susceptibility Surveillance

- During the 2006-2007 influenza season NYS did not detect any neuraminidase-inhibitor resistance mutations among influenza viruses A(H1N1), A(H3N2), or B (Table 2). The CDC also did not detect neuraminidase-inhibitor resistance mutations among influenza A(H3N2) and B, but detected an oseltamivir resistance mutation rate of 0.7% in influenza A(H1N1).
- Collectively, for the 2007-2008 influenza season, network participants detected a neuraminidase inhibitor (oseltamivir) resistance mutation among influenza A(H1N1) viruses at a rate of 14.3%, compared to the CDC rate of 10.2%. All oseltamivir resistant influenza A(H1N1) viruses contained the H274Y mutation, which does not confer zanamivir resistance, and retained susceptibility to adamantanes.
- For the 2007-2008 influenza season NYS has not detected any neuraminidase-inhibitor resistance mutations among influenza A(H3N2) and B, consistent with CDC data.

Figure 1. Pyrosequencing Influenza A M2 Gene

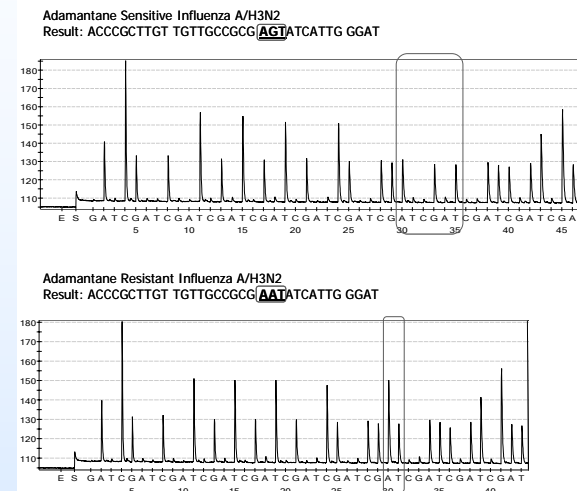


Figure 3. Pyrosequencing Influenza A(H1N1) NA Gene

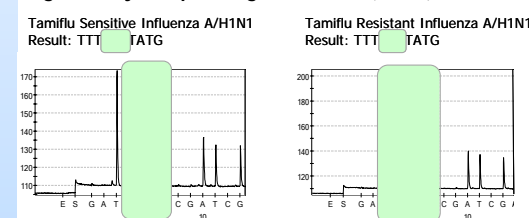


Figure 5. Dideoxy Sequencing Influenza A(H1N1) NA Gene

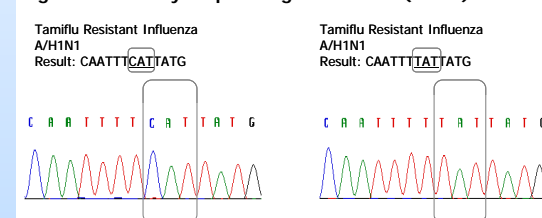


Figure 4. Influenza NA Gene Primer Location

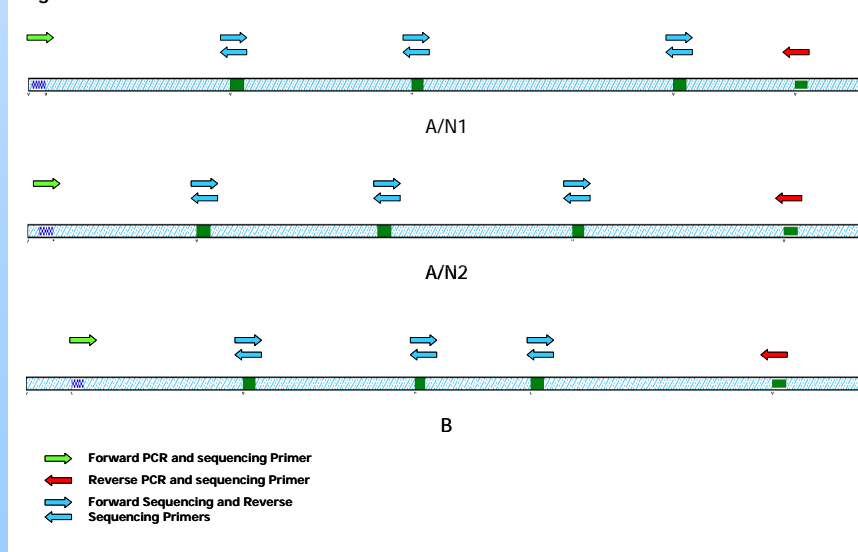


Figure 2. Pyrosequencing Methods

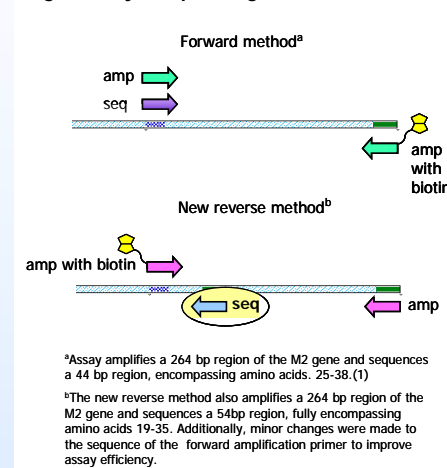


Table 1. Adamantane Resistance Mutations (Influenza A) 2006-2007 and 2007-2008 Influenza Seasons

	Influenza Season 2006-2007			Influenza Season 2007-2008		
	H1	H3 ^a	UD	H1	H3 ^a	UD
New York	0/37	38/42	6/11	0/44 ^b	101/101	na
+/total (%)	(0.0)	(90.5)	(54.5)	(0.0)	(100.0)	
Wisconsin	0/107	29/39	na	1/42 ^c	44/44	na
(0.0)	(0.0)	(74.4)		(2.4)	(100.0)	
Total	0/144	67/81	6/11	1/86	145/145	na
(0.0)	(0.0)	(82.7)	(54.5)	(1.2)	(100.0)	

^a All mutations detected were S31N

^b One uncharacterized-mutation in conserved region, in one sample, currently under investigation

^c One adamantane resistant A/H1N1 was susceptible to oseltamivir

UD, undetermined (Influenza A positive, unable to subtype)

na, not applicable

Table 2. Neuraminidase Inhibitor Resistance Mutations^a Detected in 2006-2007 and 2007-2008 Influenza Seasons

	Influenza Season 2006-2007			Influenza Season 2007-2008		
	N1	N2	B	N1	N2	B
New York^b	0/18	0/17	0/18	5/45	0/92	0/45
+/total (%)	(0.0)	(0.0)	(0.0)	(11.1)	(0.0)	(0.0)
Wisconsin^c	na	na	na	8/46	na	na
(0.0)				(17.4) ^c		
Total^d	0/18	0/17	0/18	13/91	0/92	0/45
(0.0)	(0.0)	(0.0)	(0.0)	(14.3)	(0.0)	(0.0)

^a All mutations detected were H274Y

^b Genotyped by dideoxy sequencing of NA gene

^c Genotyped by H274Y specific pyrosequencing. Recent testing late in the season increased the positivity rate from 9.1 to 17.4%.

^d All samples resistant to oseltamivir were susceptible to adamantanes

na, not applicable

Conclusions

- These results indicate that adamantane and oseltamivir antiviral-resistance mutations, among circulating influenza viruses in participant states, are increasing at a rate comparable to CDC's national averages.
- Implications of these results include the possibility of the development of sub-type specific rapid influenza tests to guide therapeutic decisions.
- The state public health laboratory surveillance network for antiviral susceptibility resistance should be expanded to include other states in order to monitor influenza resistance activity.
- Such a network could provide antiviral test results in support of CDC testing efforts.

References

- Bright RA, Shay DK, Shu B, Cox NJ, Klimov AI. Adamantane resistance among influenza A viruses isolated early during the 2005-2006 influenza season in the United States. *JAMA* 2006;295(8):891-94.
- Centers for Disease Control and Prevention. High levels of adamantane resistance among influenza A (H3N2) viruses and interim guidelines for use of antiviral agents - United States, 2005--06 influenza season. *MMWR Morbidity Mortality Weekly Report* 2006;55(2):44-46
- Centers for Disease control and Prevention. Update: influenza activity - United States, September 30, 2007 - February 9, 2008. *MMWR Morbidity Mortality Weekly Report* February 15, 2008;57:1-5
- Bright RA, Medina M, Xu X, Perez-Orozco G, Wallis TR, Davis XM, Povinelli L, Cox NJ, Klimov AI. Incidence of adamantane resistance among influenza A(H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet* 2005;366:1175-81
- Colman PM, Hoyne PA, Lawrence MC. Sequence and structure alignment of paramyxovirus hemagglutinin-neuraminidase with influenza virus neuraminidase. *J.Virology* 1993;67(6):2972-80.

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