Influenza antivirals: surveillance and resistance

Alexander Klimov
Influenza Division
National Center for Immunization and Respiratory Diseases
Centers for Disease Control and Prevention

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Influenza Surveillance - Global

- **Global Influenza Surveillance Network - GISN**
- **Established in 1952**
- **Comprises**
  - 4 WHO Collaborating Centers (WHO CCs)
  - 121 WHO NICs in 93 countries
  - NICs collect specimens, perform primary virus isolation and preliminary antigenic characterization
  - Ship newly isolated strains to WHO CCs for comprehensive antigenic and genetic analysis
  - Basis for WHO recommendations on the composition of influenza vaccine for the Northern and Southern Hemispheres each year
- **Alerts** emergence of new virus variants (antigenic, drug-resistant) and/or viruses with pandemic potential
WHO Collaborating Centers for Influenza

WHO Collaborating Centers - Atlanta, London, Melbourne, and Tokyo

Countries containing at least 1 WHO influenza laboratory
**Human Infections with Avian Influenza Detected by GISN**

<table>
<thead>
<tr>
<th>Year</th>
<th>Virus</th>
<th>Location</th>
<th>Cases</th>
<th>Fatalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>H5N1</td>
<td>Hong Kong, SAR China</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>1998</td>
<td>H9N2</td>
<td>China</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>H9N2</td>
<td>Hong Kong, SAR China</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>H7N2</td>
<td>Virginia</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>H5N1</td>
<td>Hong Kong, SAR China</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2003</td>
<td>H5N1</td>
<td>China, Vietnam</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2003</td>
<td>H7N7</td>
<td>Netherlands</td>
<td>89</td>
<td>1</td>
</tr>
<tr>
<td>2003</td>
<td>H9N2</td>
<td>Hong Kong, SAR</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>H7N2</td>
<td>New York</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>H7N3</td>
<td>Canada</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>H5N1</td>
<td>Thailand, Vietnam</td>
<td>46</td>
<td>32</td>
</tr>
<tr>
<td>2005</td>
<td>H5N1</td>
<td>Cambodia, China, Indonesia, Thailand, Vietnam</td>
<td>98</td>
<td>43</td>
</tr>
<tr>
<td>2006</td>
<td>H5N1</td>
<td>Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Thailand, Turkey</td>
<td>115</td>
<td>79</td>
</tr>
<tr>
<td>2007</td>
<td>H5N1</td>
<td>Cambodia, China, Egypt, Indonesia, Laos, Myanmar, Nigeria, Pakistan, Thailand, Vietnam</td>
<td>85</td>
<td>58</td>
</tr>
<tr>
<td>2007</td>
<td>H7N2</td>
<td>United Kingdom</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Most cases - direct contacts with infected poultry  
Limited H-to-H transmission  

(January 3, 2008)
WHO CC for Surveillance, Epidemiology and Control of Influenza, Atlanta, USA

- Identification and characterization of circulating influenza viruses using serologic and molecular techniques
  - Monitoring appearance of new antigenic or potentially pandemic variants
  - WHO and FDA vaccine strain selection
  - Monitoring resistance to FDA-approved drugs
    - Adamantanes (M2 blockers) - amantadine, rimantadine
    - NA inhibitors - zanamivir, oseltamivir
- Pandemic preparedness
- Develop methodology for influenza diagnosis
  - Preparation and distribution of reagents
    - Serological (antigenic drift; antigenic shift)
    - Molecular (seasonal; animal; pandemic)
- Laboratory training
- Research
Specimens Received by Influenza Division, CDC

2006-07:
46 US states
73 foreign countries
WHO CC for Surveillance, Epidemiology and Control of Influenza, Atlanta, USA

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- Monitoring resistance to FDA-approved drugs
  - Adamantanes (M2 blockers) - amantadine, rimantadine
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- Pandemic preparedness
- Develop methodology for influenza diagnosis
  - Preparation and distribution of reagents
    - Serological (antigenic drift; antigenic shift)
    - Molecular (seasonal; animal; pandemic)

- Laboratory training
- Research
Two Classes of FDA Approved Drugs

- **Adamantanes or M2 blockers**
  - amantadine and rimantadine

- **Neuraminidase inhibitors**
  - oseltamivir (Tamiflu) and zanamivir (Relenza)

- While both classes are effective at treating and preventing influenza illness, they differ by mechanisms of action, resistance development and transmission.
Adamantanes (M2 blockers)

- **Amantadine, rimantadine** *(influenza A only)*
  - Decrease Symptoms and shedding by 1-2 days
  - Prophylaxis - 70-90% effective
  - Cross-resistance (Amantadine & Rimantadine)
  - Rapid development of resistance
    - 10-40% in 2-5 days
  - **Mechanisms of action:**
    - blocking virus “uncoating” at stage of infection
      *(H^+ flow across the M2 channel)*
  - Molecular markers of resistance:
    - AA changes within transmembrane domain of M2
      *(26F/I, V27A, A30V/T, S31N, G34E)*
Monitoring Resistance to M2 Blockers: Seasonal and Avian Influenza Viruses

- Detection of established molecular markers of resistance in the virus genome (M2 gene)

- **Pyrosequencing** is current assay of choice:
  - High throughput
  - CDC: >11,000 viruses tested
  - Could be done with clinical specimen
  - Rapid
  - Accurate
Rapid Spread of Adamantane Resistance in A(H3N2) Viruses

Fiscal year 2000-2008

- China
- Hong Kong
- Japan
- Korea
- Canada
- US
- S. America
Rapid Spread of Adamantane Resistance in A(H3N2) Viruses

- Present system of surveillance allowed us to timely detect a rise of M2 resistance

- Rapid change in policy recommendations
  - CDC advised against prescribing amantadine and rimantadine in 2005-06, 2006-07, and 2007-08 seasons
Spread of Adamantane Resistance in A(H1N1) Viruses

![Graph showing the spread of Adamantane resistance in A(H1N1) viruses across different regions from 2004 to 2008. The graph includes data from China, Hong Kong, Japan, Korea, the US, and South America. Each region is represented by a different line and color, with the percentage of resistance increasing over the fiscal years.](image-url)
Adamantane resistance in 2006-07: geographic diversity

<table>
<thead>
<tr>
<th></th>
<th>A(H3N2)</th>
<th></th>
<th>A(H1N1)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>353</td>
<td>63</td>
<td>180</td>
<td>83</td>
</tr>
<tr>
<td>China</td>
<td>69</td>
<td>100</td>
<td>62</td>
<td>100</td>
</tr>
<tr>
<td>S. Korea</td>
<td>101</td>
<td>57</td>
<td>42</td>
<td>100</td>
</tr>
<tr>
<td>Europe</td>
<td>100</td>
<td>39</td>
<td>58</td>
<td>52</td>
</tr>
<tr>
<td>Canada</td>
<td>143</td>
<td>30</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>US</td>
<td>395</td>
<td>86</td>
<td>551</td>
<td>6</td>
</tr>
<tr>
<td>Global</td>
<td>1348</td>
<td>72</td>
<td>892</td>
<td>24</td>
</tr>
</tbody>
</table>

Resistance emergence in H1N1 is an independent event
No evidence for reassortment between H3N2 and H1N1 viruses
Resistance to M2 Blockers: Seasonal Influenza

- Rapid development of influenza A drug resistance under treatment
- Rapid increase of resistance among influenza A viruses in recent years
- Transmissibility of resistant mutants in humans
- Several independent introductions of resistant viruses into human population
  - Different from bacterial resistance
- Unaltered fitness of drug resistant viruses
Global spread of M2 resistant seasonal influenza A viruses - factors?

- Increased use of drugs
  - due to SARS outbreak and bird flu scare?
- Accessibility of drugs
  - no prescription needed in some countries
  - cheap
- Changes in other genes may facilitate spread of resistant viruses
  - HA antigenic change
  - favorable gene constellation?
    - Holmes et al., 2005; Simonsen et al., 2007;
    - CDC unpublished data
NA Inhibitors

• **Zanamivir, Oseltamivir** (Influenza A and B)
  • Treatment*: decrease symptoms by 1-2 d, hospitalizations by ~50%; seasonal mortality among elderly
  • Prophylaxis (seasonal)*: 50-70% effective
  • **H5N1** cases - small numbers
    • early treatment may reduce mortality
  • Mechanism of action:
    • block virus release from infected cells
    • different from M2 blockers
  • Molecular markers of resistance:
    • not well defined (especially for H5N1)
    • changes in NA structure
      • drug specific; virus type/ subtype specific

* Cooper at al., 2003
NA Inhibitors

- **Zanamavir, oseltamivir** (influenza A and B)
  - Emergence of resistance during treatment
    - 5.5% - US children *(Whitley et al, 2001)*
    - 18% - Japanese hospitalized children *(Kiso et al, 2004)*
    - 0.2% - adults *(Treanor et al, 2000)*

- Resistant viruses appear to be less transmissible and possibly less viable, but human data are limited
  - Reduced transmissibility in ferrets (seasonal)
    - only a few mutants tested (R292K; E119V; H274Y)

- Cross-resistance is variable
  - mutations in NA catalytic site confer cross-resistance
  - mutations in framework (e.g. H274Y) do not
Monitoring NAI-susceptibility: seasonal and H5N1 influenza

• Changes in the interactions between NA enzyme and inhibitor
  • IC$_{50}$, a concentration of NAI which reduces the enzyme activity by 50% (nM)

• High throughput screening method:
  NA inhibition assay with chemiluminescent substrate (NAStark kit, Applied Biosystems)
  • >5,000 influenza A and B viruses tested at CDC
  • Limitations:
    - Complicated data interpretation
    - Artificial, small substrate
    - Requires a grown virus

• Confirmation: Sequence analysis of NA to detect mutations at NA active site
Detected NAI resistant viruses - seasonal

- **2004-05**
  - 1 oseltamivir-resistant virus:
    - H274Y: from the US (no epi data)
  - 1 oseltamivir- and zanamivir-resistant virus:
    - R371K: from China (no epi data)
- **2005-06**
  - 1 oseltamivir-resistant virus:
    - H274Y (H1N1) from China
- **2006-07**
  - US: 5 oseltamivir-resistant viruses
    - 4 H274Y (H1N1)
      - 1 from oseltamivir-treated, 3 unknown
    - 1 E119V (H3N2): oseltamivir-treated
  - China: 1 oseltamivir-resistant virus
    - H274Y (H1N1) (no epi data)
## Frequency of oseltamivir resistance among seasonal influenza A and B viruses

<table>
<thead>
<tr>
<th>Location</th>
<th>FY 2007</th>
<th>FY 2008*</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>5/ 1350 (0.4%)</td>
<td>3/ 80 (3.8%)</td>
</tr>
<tr>
<td>Foreign</td>
<td>1/ 797 (0.1%)</td>
<td>-</td>
</tr>
</tbody>
</table>

* October 1 - December 31, 2007
Resistance to NI Inhibitors: Seasonal Influenza

- Frequency of resistance to NA inhibitors is low

In 2006-07
- 2121 isolates were tested using NAStar Kit
- 859 H3N2; 776 H1N1; 486 B
- 0.5% oseltamivir-resistance
- all but one sensitive to zanamivir

- H3N2 viruses (0.04-5.2 nM) were the most sensitive to oseltamivir, followed by H1N1 (0.16-3.9 nM) and B (0.19-10.5 nM) viruses

- H1N1 (0.15-11.6 nM) viruses were the most sensitive to zanamivir, followed by B viruses (0.03-12.0 nM) and H3N2 (0.15-24.5 nM)
Influenza H5N1 Viruses

Clade 2.2
birds: Asia, Africa, Europe
humans: Egypt, Nigeria

Clade 2.3
birds, humans: China

Clade 1
Birds, humans: Vietnam, Thailand, Cambodia

Clade 2.1
birds, humans: Indonesia

Evolution of the H5 HA Gene

884 full length (1-1659) ORF of H5 HA sequences
Resistance of H5N1 Viruses to M2 blockers

Published M gene sequencing data

* 1% resistant (avian)
** 5% resistant (avian)

Clade 1
- Human 100%
- Avian 93%

Clade 2.1
- Resistant
- Human 83%
- Avian 22%

Clade 2.2
- Sensitive*

Clade 2.3
- Sensitive**

Other clades: 17%

* 1% resistant (avian)
** 5% resistant (avian)
Sensitivity of H5N1 Viruses to NIs *

Clade 1
Sensitive
Resistant mutants from treated p’ts

Vietnam/1194/04
Vietnam/1203/04

Hong Kong/213/03

Indonesia/5/05

Clade 2-1
Sensitive
Resistant mutant from treated (?) p’ts

w s/Mongolia/244/05

bh gs/Qinghai/1A/05

Turkey/Turkey/1/05

Clade 2-2
Sensitive
Resistant mutants from treated (?) p’ts

Anhui/1/05

Hong Kong/156/97

Clade 2-3
Sensitive
Resistant mutants from birds

gs/Guangdong/1/96

CDC
Influenza Division
**H5N1 Viruses Resistant to Oseltamivir** (chemiluminescent assay)*

<table>
<thead>
<tr>
<th>Clade</th>
<th>#R/ #total</th>
<th>IC$_{50}$</th>
<th>NA mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/ 33</td>
<td>23 vs 0.4 (~57 fold)</td>
<td>274</td>
</tr>
<tr>
<td>2.1</td>
<td>1/ 33</td>
<td>29 vs 1.4 (~21 fold)</td>
<td>136**</td>
</tr>
<tr>
<td>2.2</td>
<td>2/ 19</td>
<td>13 vs 0.8 (~15 fold)</td>
<td>294</td>
</tr>
<tr>
<td>2.3</td>
<td>2/ 58</td>
<td>10-40 vs 1.5 (7-27 fold)</td>
<td>150+222***</td>
</tr>
</tbody>
</table>

**Clinical significance is not clear**

* CDC preliminary data  
** Resistant to zanamavir (~270 fold)  
*** Avian isolates
### H5N1: I C<sub>50</sub> - Fluorescent NAI assay

<table>
<thead>
<tr>
<th>Clade</th>
<th>N</th>
<th>Oseltamivir</th>
<th>Zanamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>0.4 ± 0.2</td>
<td>1.9±2.2</td>
</tr>
<tr>
<td>2.1</td>
<td>35</td>
<td>7.1±4.7</td>
<td>3.2±2.2</td>
</tr>
<tr>
<td>2.2</td>
<td>2</td>
<td>1.3; 7.2</td>
<td>0.8; 1.8</td>
</tr>
<tr>
<td>2.3</td>
<td>46</td>
<td>10.3±7.1</td>
<td>1.1±0.2</td>
</tr>
</tbody>
</table>

Clinical significance of elevated I C<sub>50</sub> values is not clear.
H5N1: IC$_{50}$ - Chemiluminescent NAI assay

<table>
<thead>
<tr>
<th>Clade</th>
<th>N</th>
<th>Oseltamivir</th>
<th>Zanamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0.7 ± 1.5</td>
<td>1.3 ± 2.7</td>
</tr>
<tr>
<td>2.1</td>
<td>31</td>
<td>1.4 ± 0.9</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>2.2</td>
<td>11</td>
<td>0.7 ± 0.3</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>2.3</td>
<td>50</td>
<td>1.5 ± 0.7</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

Clinical significance of elevated IC$_{50}$ values is not clear
H5N1 viruses - Conclusions

- H5N1 viruses remain a pandemic threat but have not yet developed the ability to be transmitted efficiently from person-to-person
- Distinct geographical distribution of H5N1 genetic and antigenic variants have been identified
- It is not known which, if any, H5N1 variant might acquire the ability to be transmitted efficiently
- Difficulties in WHO pre-pandemic vaccine recommendations
H5N1 viruses - Conclusions

- High level of resistance to amantadine and rimantadine detected in some genetic groups clade 1; clade 2.1

- Low level of resistance to NIs
  - several resistant mutants identified
  - clinical significance of some R mutants is not clear

- New techniques for rapid detection of known mutations is needed
  - pyrosequencing (274, 294)

- Continued global surveillance for antiviral resistance is critical
  - even without H5N1 pandemic threat (seasonal influenza, other potentially pandemic subtypes)
Acknowledgements

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Thank you

H5N1

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