# Drugs Resistance of Influenza Viruses

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***Antiviral and Nonprescription Drugs Advisory Committee - Influenza Medkit***
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# Resistance to Adamantanes

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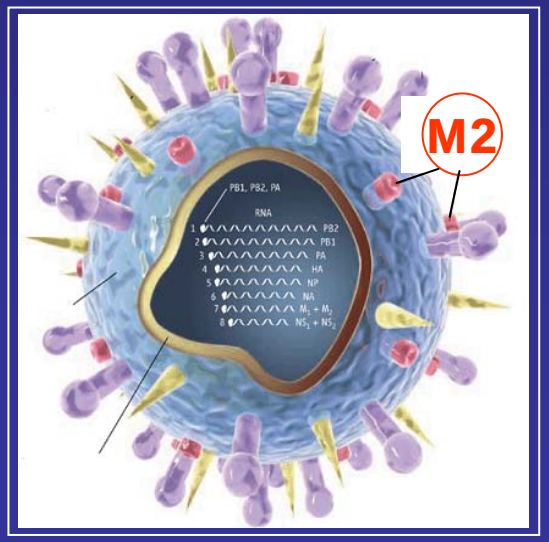
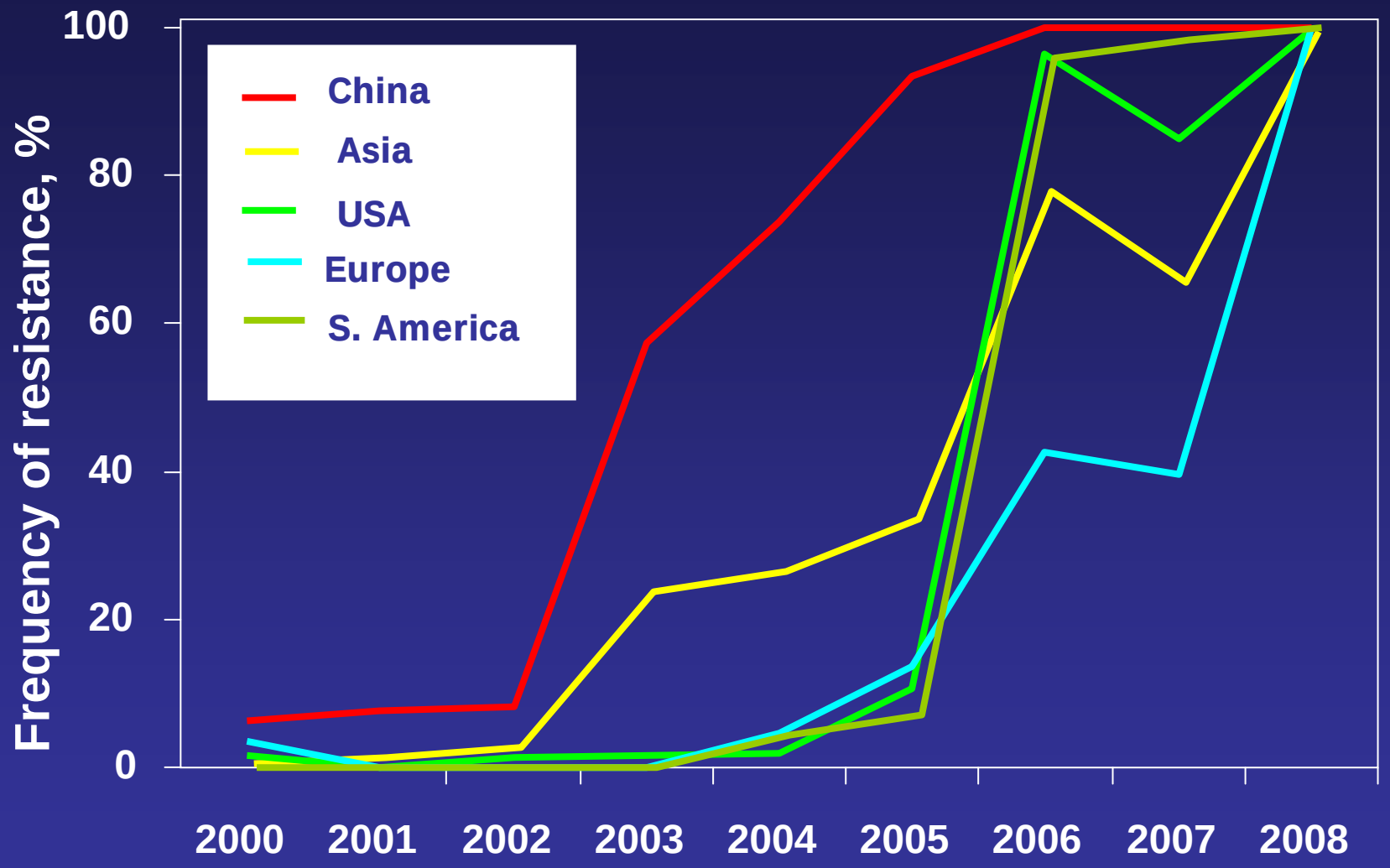
# Resistance to Adamantanes: Background

- Reduce replication of influenza A viruses by inhibiting function of M2 ion channel (M2 blockers)
- Amantadine and rimantadine, chemically related, orally administered drugs referred to as adamantanes
- No activity against influenza B
- Resistance develops rapidly after treatment
- High prevalence of resistance among circulating human influenza A(H1N1) and A(H3N2) viruses
- Adverse effects include gastrointestinal and neurological symptoms
- **Not currently recommended for treatment of influenza A**



# Adamantane Resistance in A(H3N2) Viruses




**Since 2005, CDC has advised against prescribing adamantanes, recommended use of NAIs**

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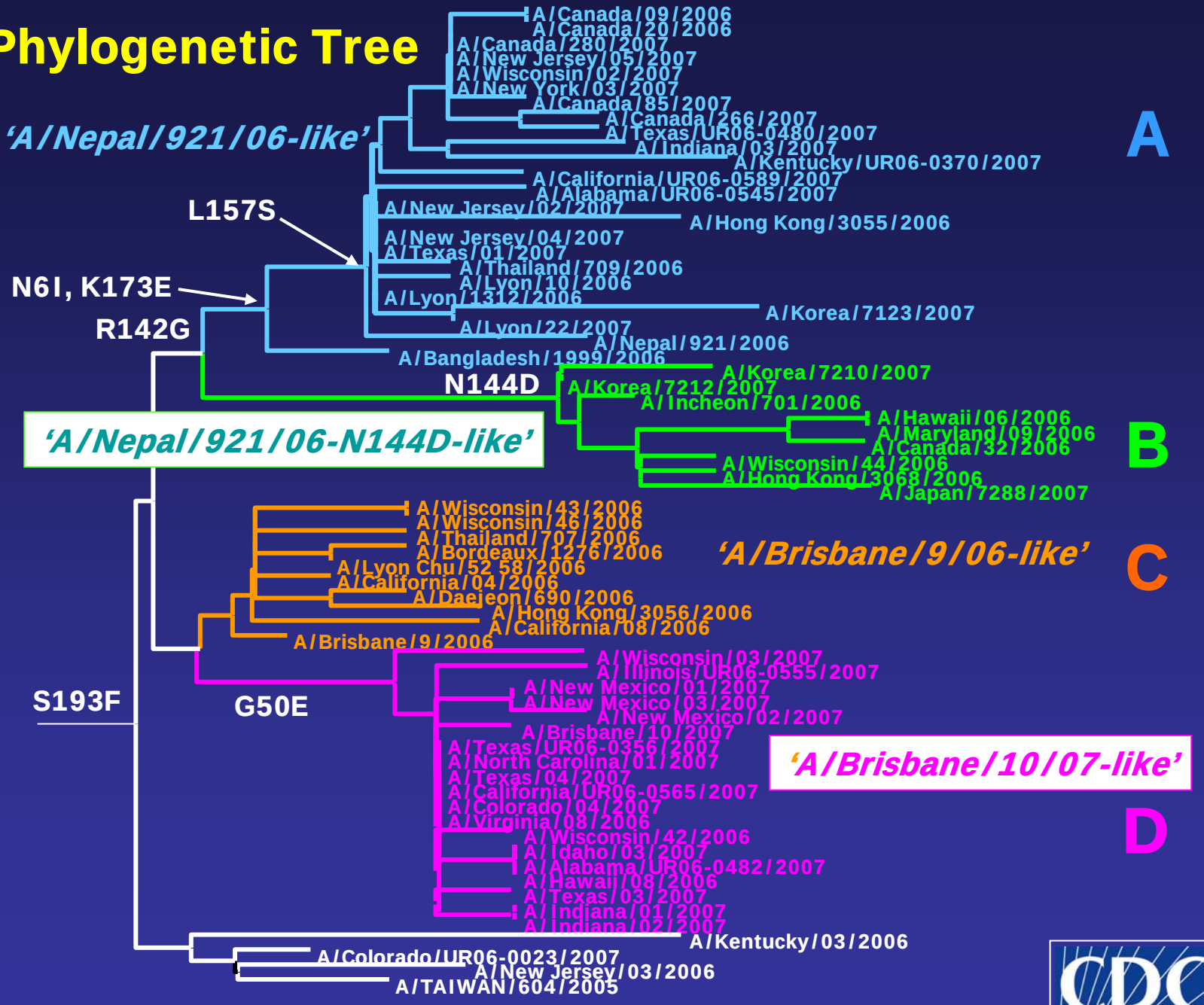
# H3 HA Phylogenetic Tree

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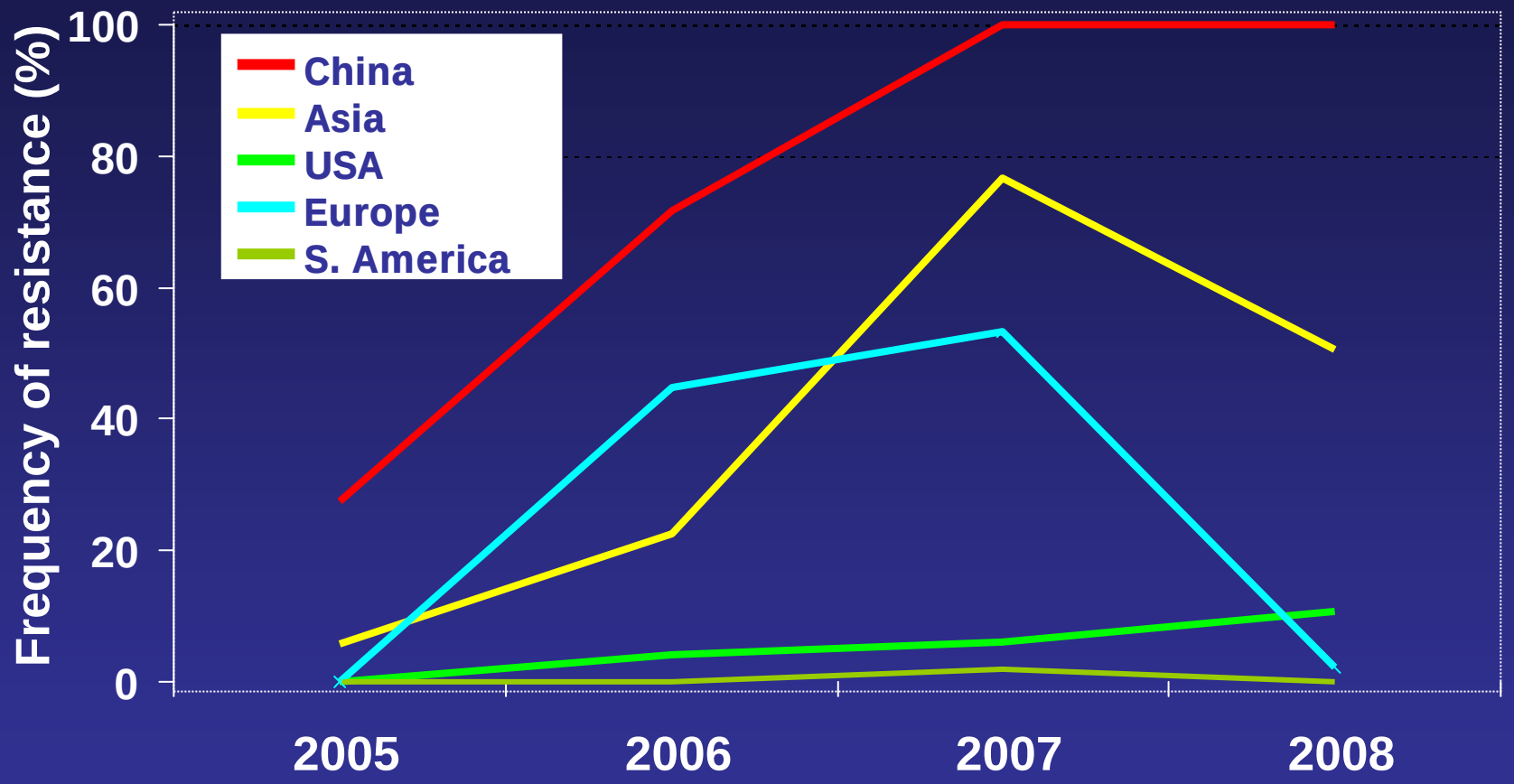
# Adamantane Resistance in A(H1N1) Viruses




**Geographic factor in emergence and spread of adamantane resistance**

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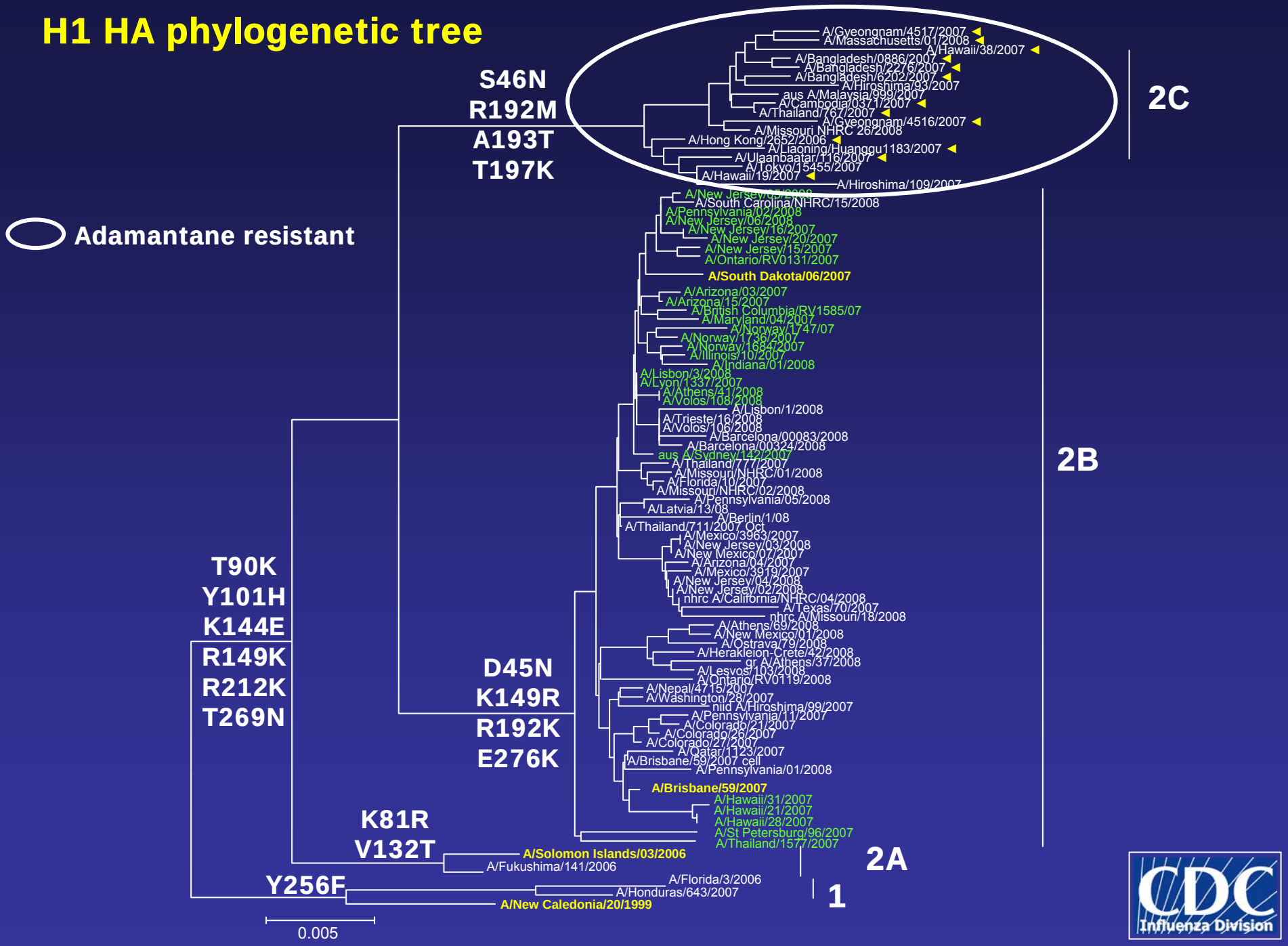
# H1 HA phylogenetic tree



- Although the use of adamantanes likely contributed to the initial emergence of resistant variants, other factors (antigenic drift, gene reassortment) appear to contribute to the global spread of adamantane resistant influenza A(H3N2) and A(H1N1) viruses

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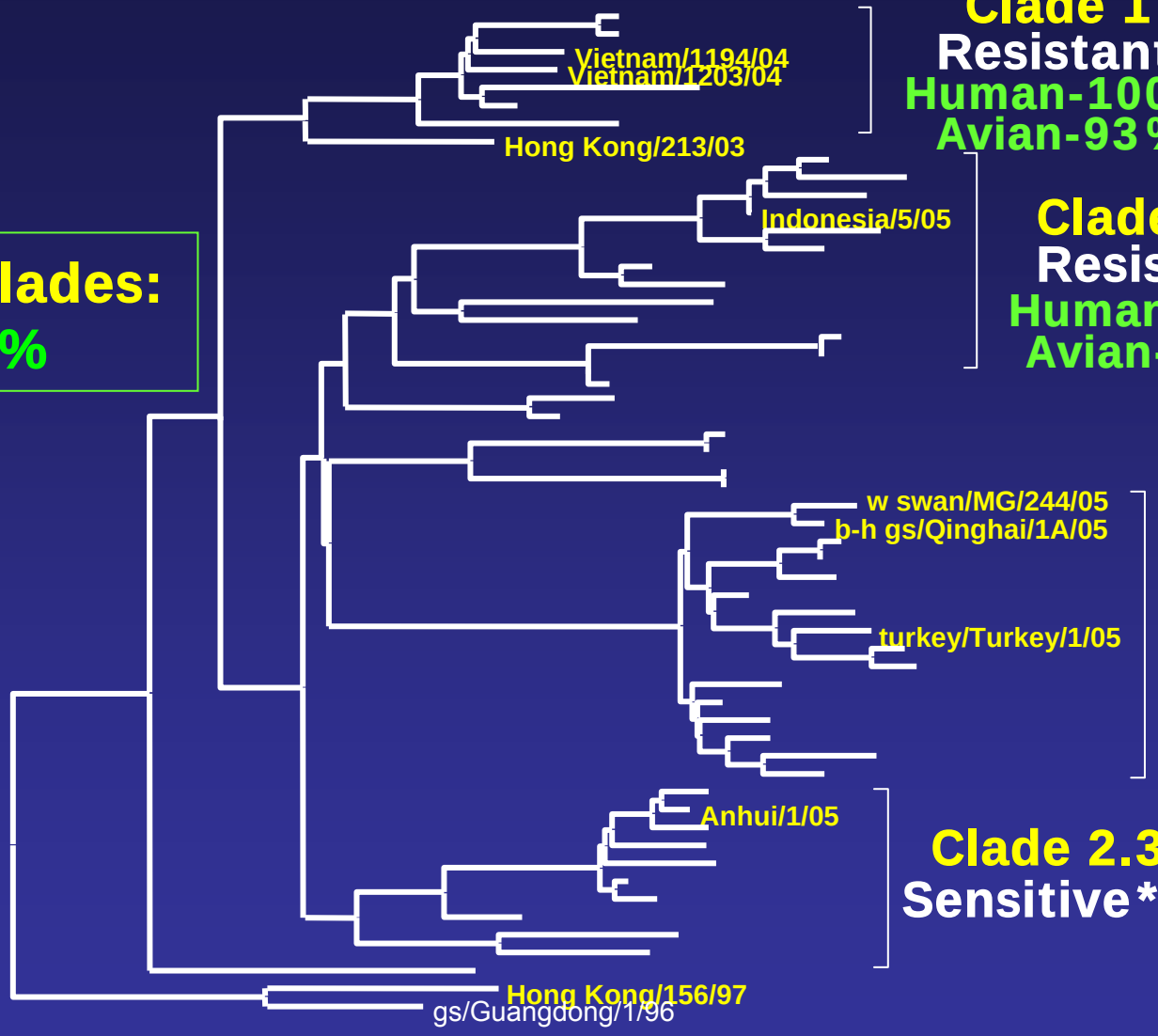
# Resistance to Adamantanes in H5N1 Viruses




**Clade 1**
**Resistant**
**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)

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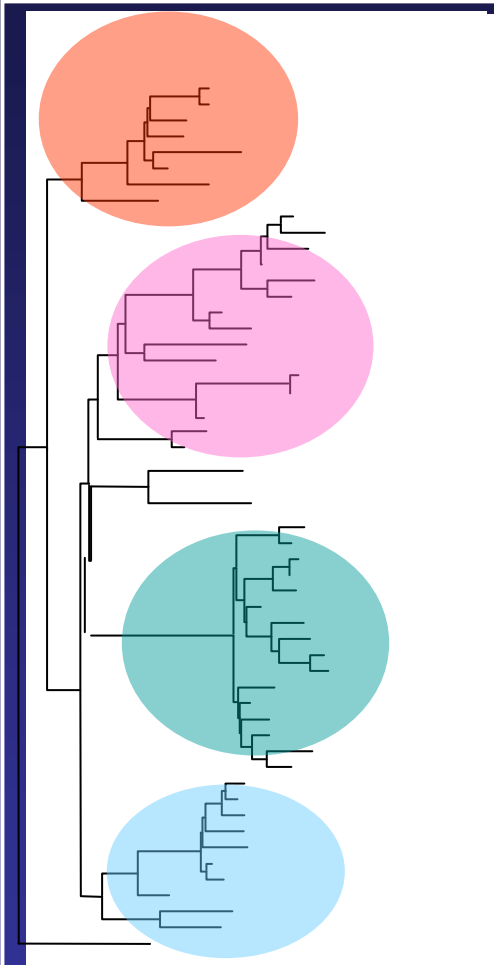
# Markers of Adamantane Resistance in H5N1



**Clade 1:**
- S31N
- L26I + S31N
- L26I + V27A + S31N

**Clade 2.1:**
- S31N
- V27A + S31N
- V27A

**Clade 2.2:**
- S31N
- V27A

**Clade 2.3:**
- S31N
- L26I + V27A + S31N

**Evidence for amantadine use in poultry farms in China (*He et al. 2008*)**

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# Resistance to Neuraminidase Inhibitors

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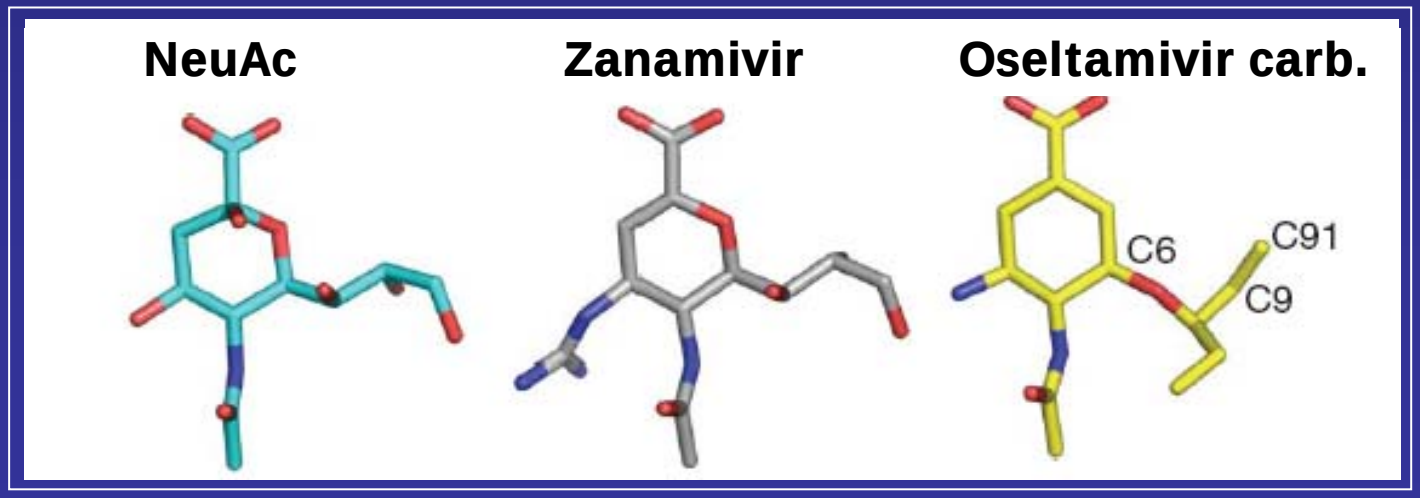
# Resistance to NAIs: Background

- Zanamivir (inhaled) and Oseltamivir (prodrug, pill)
- Since 1999, FDA-approved for prophylaxis and/or treatment of influenza A and B infections
- Structurally, NAIs mimic the natural enzyme substrate, neuraminic acid
- Zanamivir was designed according to a minimalist approach, *(Varghese 1998),* whereas oseltamivir is further departed from the substrate
- Zanamavir and oseltamivir drug design was based on the NA molecule structures of N2 and N9 subtypes

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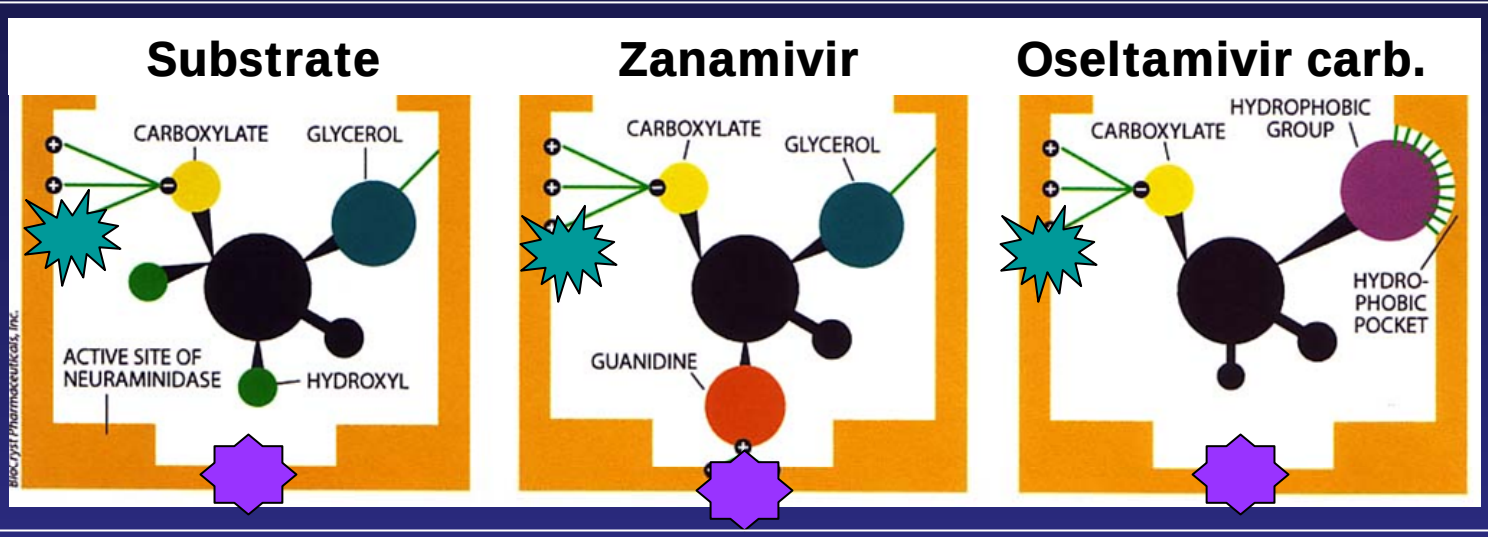
# Drug-Specific Nature of NAI-Resistance




Mutation at catalytic residue (R292K) typically confers cross-resistance to all NAIs and reduces the NA activity

Mutation at framework residue (E119G) typically confers resistance to one NAI

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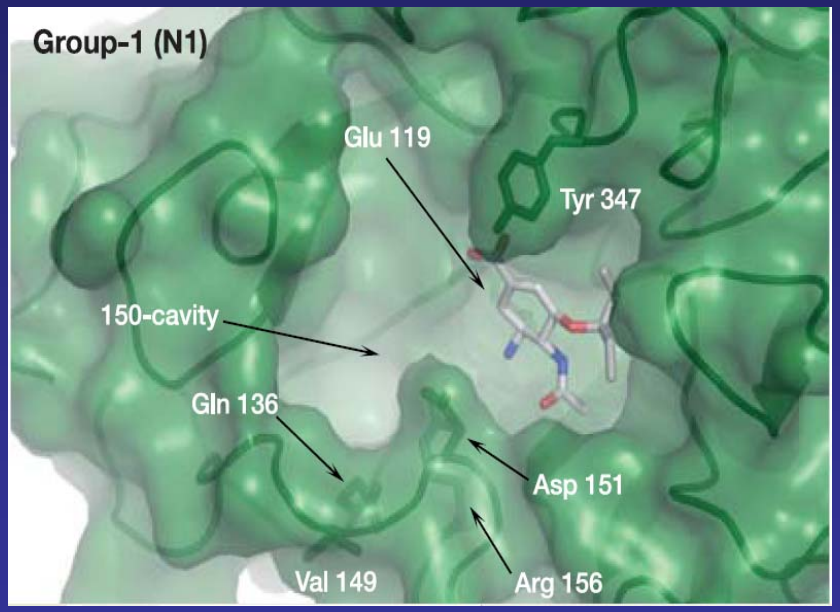
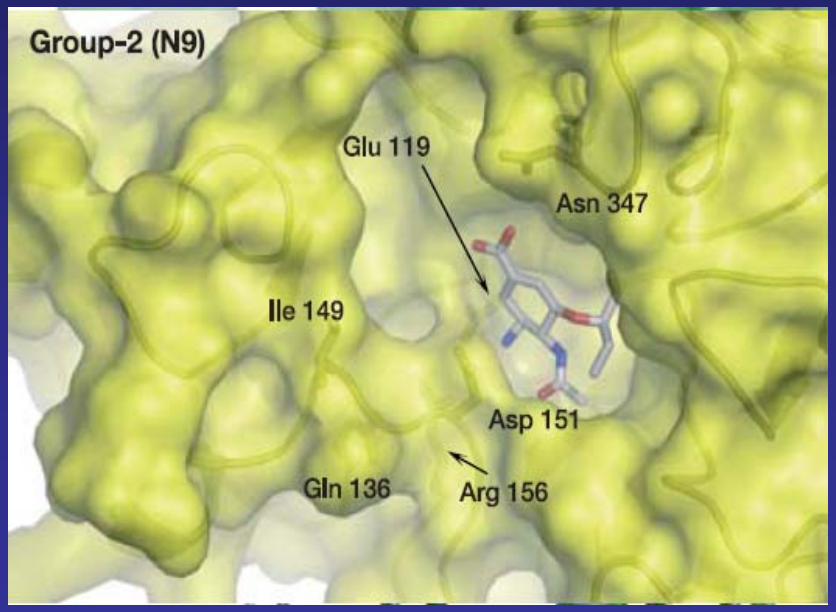
# NA Crystal Structure and Implications for Drug Resistance

- N1-N9 subtypes in avian viruses (N1 and N2 in human viruses)
- Group 1: N1, N4, N5, and N8
- Group 2: N2, N3, N6, N7, and N9 (N2 and N9 used for drug design)
- N1 crystal structure resolved (*Russell, Nature, 2006*)
- Difference in conformation of N1 and N2 active sites




150-loop conformation, residues E119, Q136, D151...
The N1-N2 difference caused by amino acids that lie **outside the active site**

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- Little or no resistance emergence to zanamivir in treated patients (limited use)
- Emergence of oseltamivir-resistance in treated patients (1-4% in adults, 4-8% in children, 18% in young children)
- Reduced transmissibility and infectivity of NAI-resistant viruses but degree is mutation-specific

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# Resistance to Oseltamivir

- Since 2000, CDC has monitored influenza A and B virus susceptibility to zanamivir and oseltamivir (over 7,000 viruses tested)
- Before 2007-2008, <1% US influenza viruses NI-resistant
- In Japan: 2004-07 – 1% (4/336)
- Beginning 2007-08 influenza season, marked rise in oseltamivir-resistance in H1N1 viruses in US and other countries

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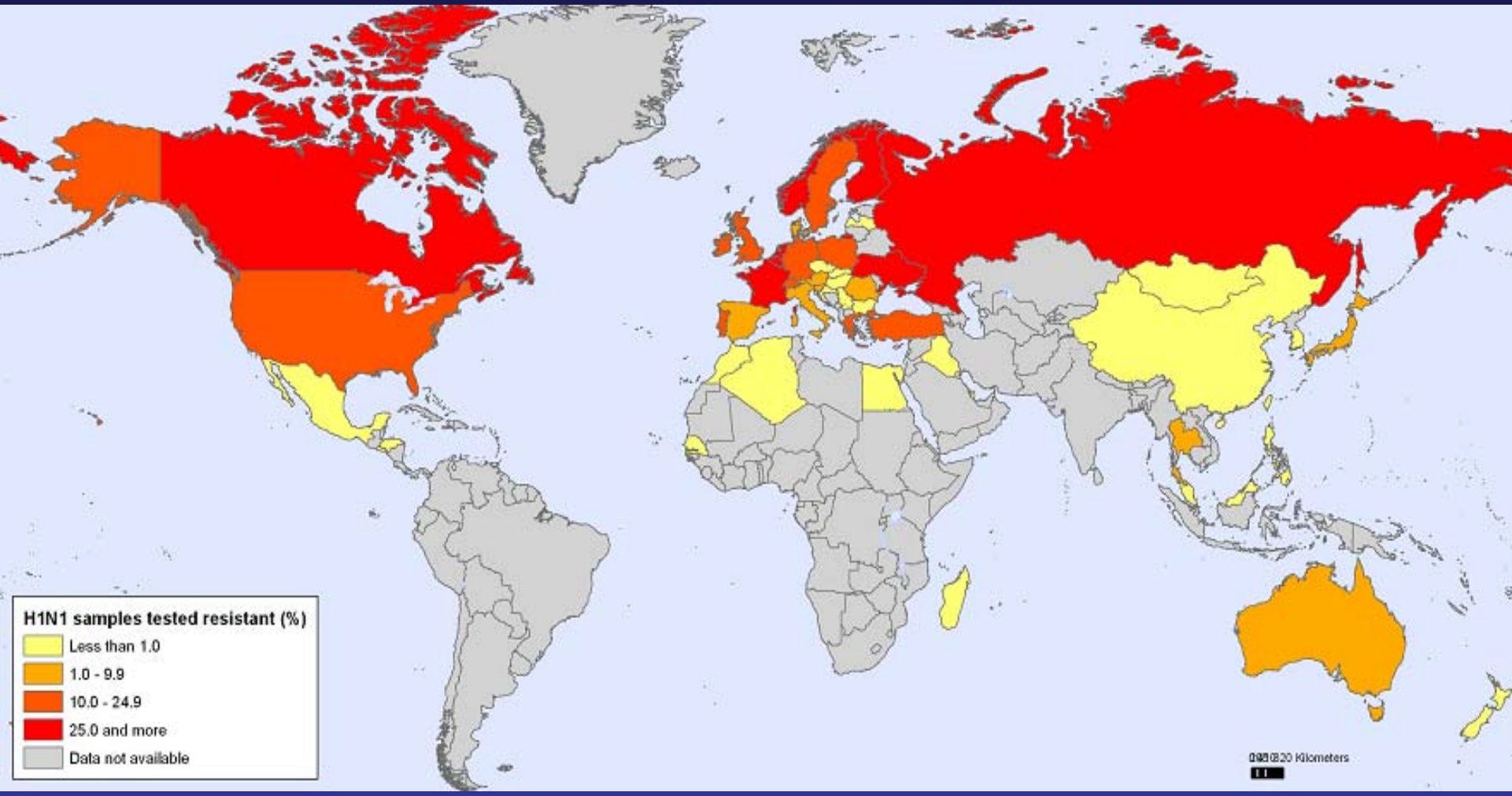
# Prevalence of Oseltamivir-Resistant H1N1 Viruses
## (as of July 1, 2008)

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# Emergence of Oseltamivir Resistance in H1N1 Viruses: 2007-08 *

## Resistance in U.S. (CDC)

| Virus | A(H1N1) | A(H3N2) | B |
|---|---|---|---|
| USA (45 states) | 10.9% 109/1003 | 0% 0/397 | 0% 0/305 |

## Resistance in Canada:

| Virus | A(H1N1) | A(H3N2) | B |
|---|---|---|---|
| Canada | 26% 125/481 | 0% 0/221 | 0% 0/599 |

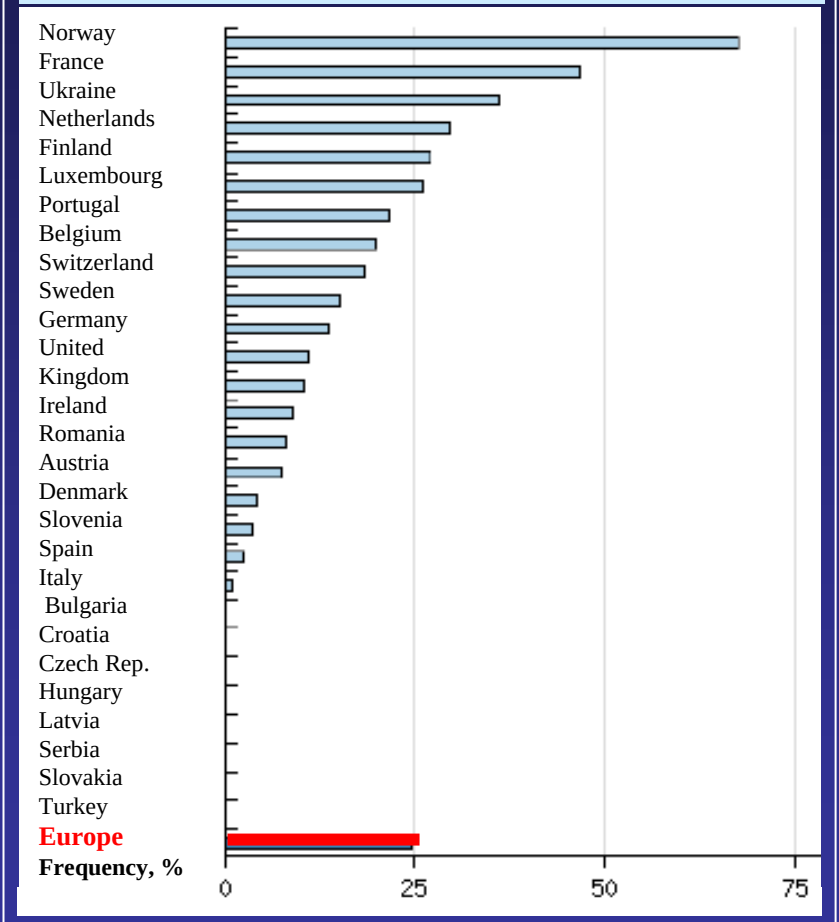
## H1N1 Oseltamivir Resistance:

China: 11%

Australia: 4%

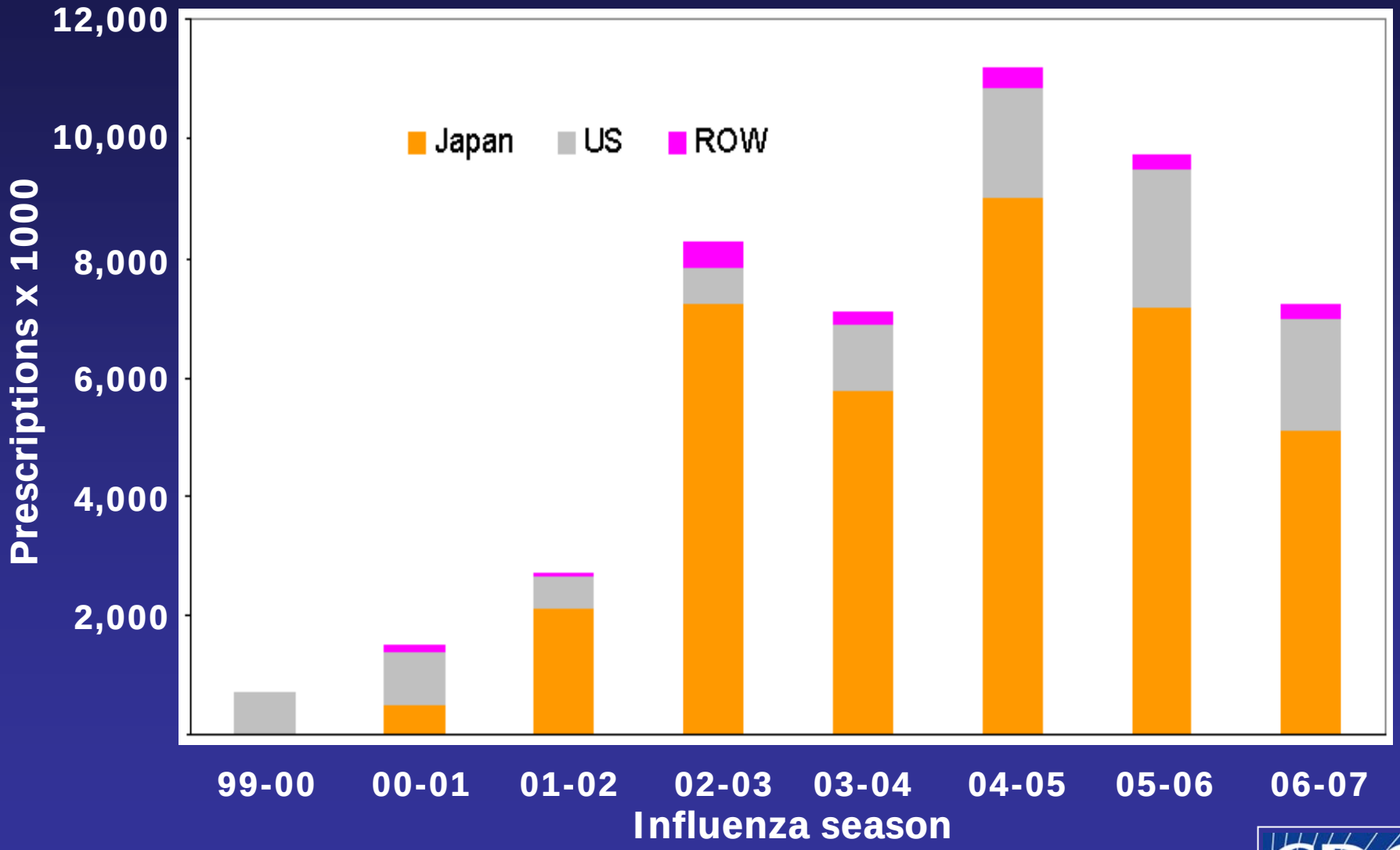
Japan: 2%

## European Influenza Surveillance: Oseltamivir-resistance in H1N1 (%)




May 2008*

# Global Oseltamivir Use




Cumulatively 48.9 million patients up to Sept 07

Source : IMS Rx data (quarterly), Japan, USA, Germany, France ONLY.

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## Frequency of resistance was not related to in-country use of oseltamivir

- Up to 67% resistance in Europe, where little oseltamivir is used
- ~2% H1N1 resistance in Japan, which has highest *per capita* oseltamivir use

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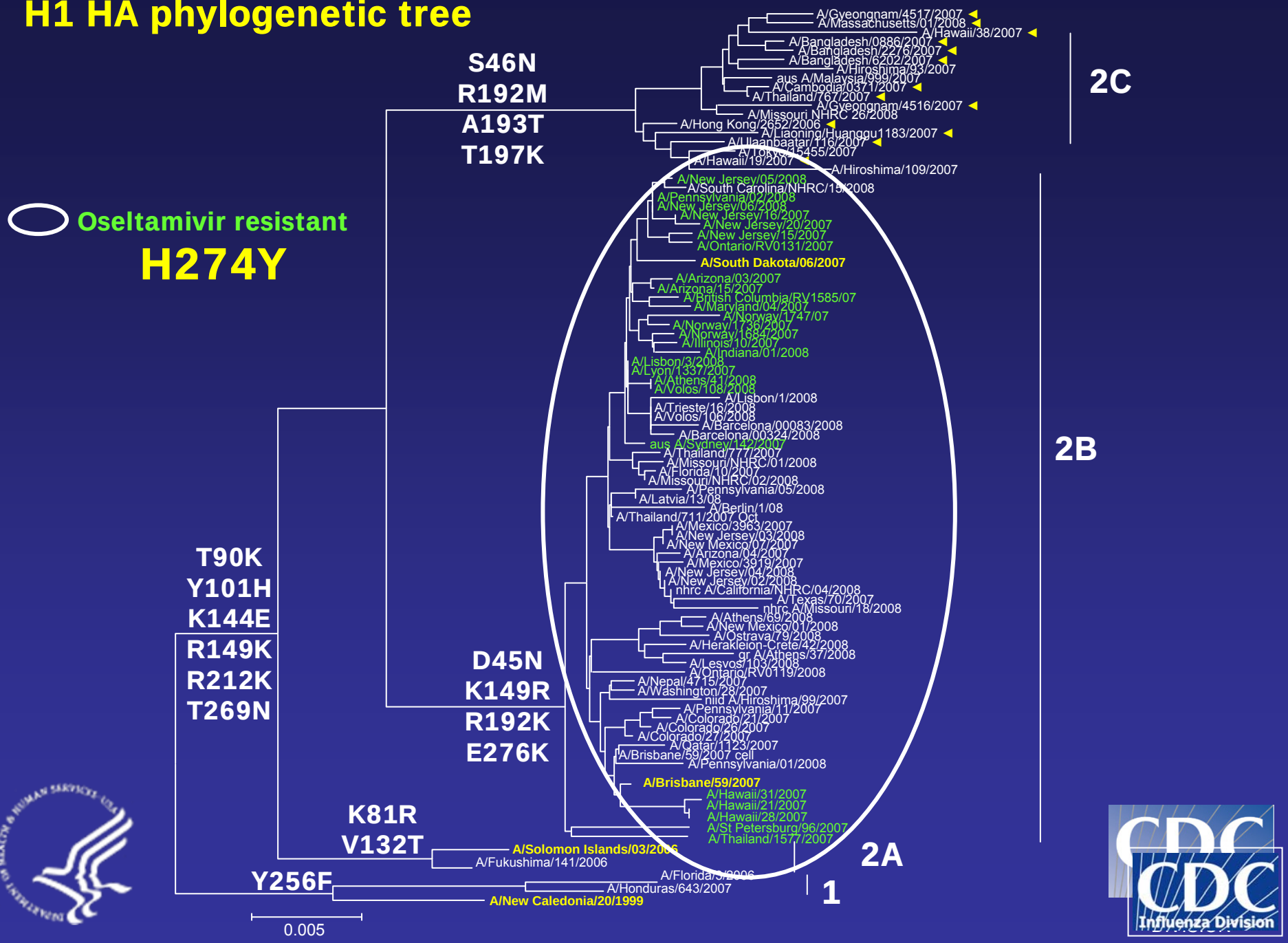
# H1 HA phylogenetic tree



# Oseltamivir Resistance in U.S. H1N1 Isolates, 2007-08

- U.S., 2007-08 season*
  - Among 99 patients with influenza caused by oseltamivir-resistant influenza A (H1N1)
    - None took oseltamivir prior to testing
    - None had household contacts taking oseltamivir prior to their onset of illness
    - No travelers
  - When compared to illnesses caused by oseltamivir-sensitive influenza A (H1N1)viruses,
    - Similar clinical illness
    - Similar severity of illness
    - Similar risk groups affected

- EU, 2007-08 season **
  - Oseltamivir use not the cause of increases in resistance
  - Clinical characteristics same as for infection with oseltamivir-sensitive viruses

* Dharan et al, CDC IDSA 2008

**ECDC 28 Aug 2008. http://ecdc.europa.eu/en/Health_topics/influenza/antivirals.aspx

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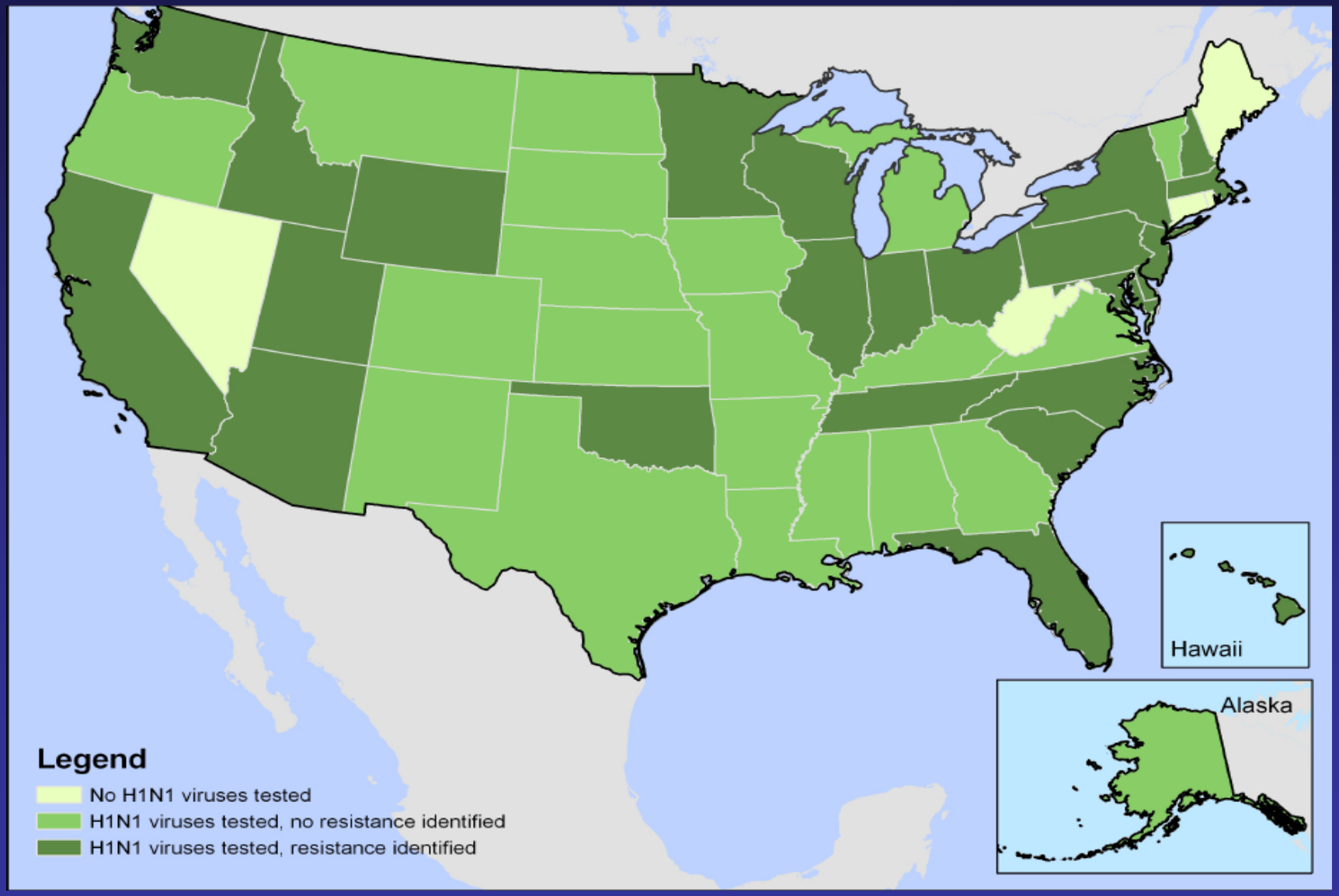
# Geographic Distribution of Identified Oseltamivir-Resistant Influenza A (H1N1) Cases (N=123)



# Influenza A(H1N1) Virus Resistance to Oseltamivir

| Country | 10/01/07 – 03/31/08 | | 04/01/08 – 10/13/08 | |
|---|---|---|---|---|
| | N | % | N | % |
| Austria | 164 | 7 % | - | - |
| France | 496 | 47 % | - | - |
| Germany | 505 | 13 % | - | - |
| Italy | 106 | 1 % | - | - |
| Luxemburg | 227 | 26 % | - | - |
| Netherlands | 170 | 27 % | 2 | 50 % |
| Norway | 265 | 67 % | 8 | 75 % |
| Russian Federation | 128 | 45 % | 20 | 45 % |
| Spain | 106 | 2 % | - | - |
| United Kingdom | 346 | 11 % | 1 | 100 % |
| **Europe Total** | **3158** | **25 %** | **31** | **55 %** |
| China | 4 | 0 % | - | - |
| Hong Kong SAR | 666 | 12 % | 583 | 17 % |
| Japan | 1652 | 3 % | 61 | 0 % |
| Philippines | 37 | 0 % | 11 | 91 % |
| Rep. of Korea | 99 | 0 % | - | - |
| Singapore | 7 | 0 % | 6 | 50 % |
| **Asia Total** | **2496** | **5 %** | **681** | **16 %** |

# Influenza A(H1N1) Virus Resistance to Oseltamivir

| Country | 10/01/07 – 03/31/08 | | 04/01/08 – 10/13/08 | |
|---|---|---|---|---|
| | N | % | N | % |
| Australia | 164 | 7 % | 59 | 80 % |
| New Caledonia | - | - | 7 | 100 % |
| New Zealand | 88 | 0 % | 2 | 100 % |
| **Oceania Total** | **187** | **1.5 %** | **31** | **82 %** |
| Algeria | 2 | 0 % | 13 | 31 % |
| Ghana | - | - | 13 | 38 % |
| Senegal | 16 | % | 10 | 100 % |
| South Africa | - | - | 225 | 100 % |
| **Africa Total** | **93** | **3 %** | **290** | **88 %** |
| Argentina | - | - | 33 | 45 % |
| Brazil | 16 | 0 % | 12 | 0 % |
| Chile | 5 | 0 % | 79 | 13 % |
| Canada | 508 | 26 % | 2 | 50 % |
| USA | 1026 | 12 % | 7 | 43 % |
| **Americas Total** | **1584** | **17 %** | **200** | **29 %** |

**Global Total: ~16 %**

# Neuraminidase Inhibitors and A(H5N1) Viruses

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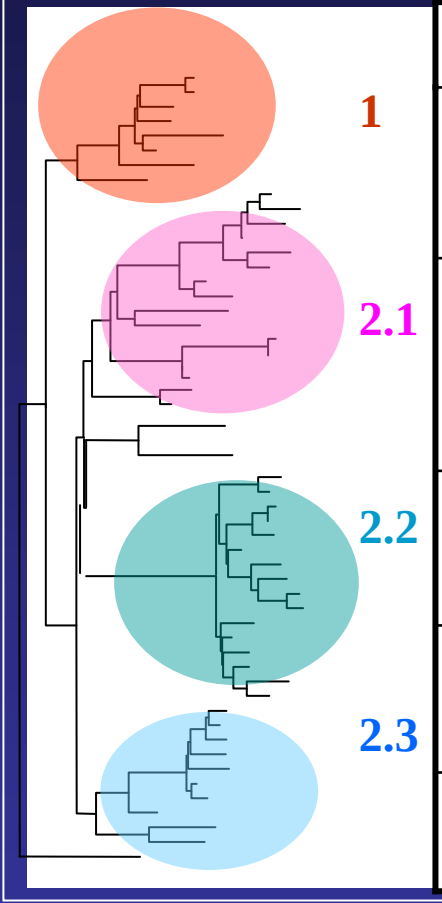
# H5N1 Virus Susceptibility to NAIs: Genetic and Drug Specific Differences (Fluorometric NAI Assay)




| | Oseltamivir ($IC_{50}$ , nM) | Zanamivir ($IC_{50}$, nM) |
|---|---|---|
| n=40 | 0.02 - 0.8 (~ 40-fold) | 0.5 - 1.7 (~3-fold) |
| n=37 | 2.3 - 21.0 (~ 10-fold) | 0.8– 9.3 (~12-fold) |
| n=34 | 0.9 - 8.4 (~10-fold) | 0.4 - 9.0 (~21-fold) |
| n=146 | 0.2 – 73.0 (~ 430-fold) | 0.3 – 3.0 (~10-fold) |
| N=257 | 0.02- 73.0 (>3,500-fold) | 0.3 – 9.3 (~30-fold) |

**Sensitive human A(H1N1) virus: oseltamivir -2.4nM; zanamivir-3.2nM.**

n – number of viruses tested per clade;

$IC_{50}$ – concentration of drug that reduces NA activity by 50 %

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# NA Sequence and Susceptibility to Oseltamivir (Fluorometric NAI Assay)

| Clade | NA mutation | Isolate | Treatment | $IC_{50}$, nM | ~ Fold increase* |
|---|---|---|---|---|---|
| 1 | H-274-Y | Human | + | 800 | 3,200 |
| | N-294-S | Human | + | 3 | 12 |
| 2.2 | N-294-S | Human | - | 138 | 92 |
| | I-222-M | Human | - | 56 | 23 |
| 2.3 | I-222-L | Avian | - | 73 | 52 |
| | K-150-N | Avian | - | 54 | 39 |
| | I-117-V | Human and avian | - | 25 | 4 |

* Fold- compare to the most closely related strain w/o NA mutation

Certain mutations (eg, N294S) confer clade-specific resistance;
Some mutations (eg, 117, 150, 294) lie outside the active site

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# Assessment of Fitness of Oseltamivir Resistant H5N1 Viruses

- In ferret model (*Le, Nature, 2005*):
  - Clade 1 oseltamivir-resistant mutant H274Y
  - Viable
  - 10-fold lower lung titers compared to wild type

- In murine model *(Yen, JV, 2007):*
  - Clade 1 virus RG-generated with H274Y or N294S mutation
  - Both caused lethal infection
  - Unaltered infectivity

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# Antiviral Resistance, Current Status

- Oseltamivir-resistance in influenza A(H1N1) viruses only
  - No oseltamivir resistance among influenza A(H3N2) and B viruses
- All oseltamivir-resistant A (H1N1) viruses had same mutation in the neuraminidase gene (H274Y)
- Oseltamivir-resistant influenza A(H1N1) viruses tested were sensitive to rimantidine and amantadine
  - ~10% of oseltamivir-sensitive influenza A(H1N1) viruses were resistant to adamantanes
- An estimated 2.1% of all influenza A and B viruses in circulation in the U.S. were resistant to oseltamivir
- All influenza viruses tested were sensitive to zanamivir
- Adamantane resistance among other influenza viruses is very high
  - >99% of influenza A H3N2 viruses tested
  - Influenza B viruses are not sensitive to adamantanes

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# Key Considerations at Start of 2008-09 Influenza Season in the United States

- Oseltamivir-resistant influenza A(H1N1) viruses are likely to circulate in the U.S.
- Overall prevalence of oseltamivir resistance in the U.S. during 2008-09 season is difficult to predict but will depend on
  - Prevalence of resistance among H1N1 viruses
  - Prevalence of H1N1 among circulating viruses
- Virulence of oseltamivir-resistant H1N1 viruses does not appear to be different
- Few options for changing antiviral recommendations
  - Limited number of alternative antivirals
  - Limited uses for alternative antivirals (zanamivir, adamantanes)
- There were no changes in recommendations from ECDC, WHO, medical professional groups (e.g., IDSA, AAP, etc.)

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