IDAHO BUREAU OF LABORATORIES

CLINICAL FORUM

Volume 3, Issue 1
Spring 2010

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IDAHO DEPARTMENT OF HEALTH & WELFARE

Detection of Oseltamivir Resistance in Novel H1N1 Influenza Strains at IBL

Amanda J. Bruesch, MS

In April of 2009 the emergence of a novel strain of influenza A (H1N1) was detected in the US and Mexico. As we are all too well aware, the virus quickly spread throughout the nation overwhelming typical seasonal strains of influenza and exhibited rapid human-to-human transmission[1]. In June the WHO declared an influenza pandemic and the virus was named influenza A pandemic (H1N1) 2009 virus.

Initial characterizations of the virus indicated that it contained a mutation that made it resistant to treatment with adamantane antiviral medications[1]. However, neuraminidase inhibitors (oseltamivir and zanamivir) were an effective choice for treatment as no resistance had been detected in the circulating virus. In cooperation with CDC, states were sending a representative sample of their influenza A pandemic (H1N1) 2009 viruses to CDC for additional characterization and surveillance for antiviral resistance mutations. Not long after the declaration of the pandemic, a few strains of the virus were determined to have a mutation in their neuraminidase gene that resulted in oseltamivir being ineffective against the virus; zanamivir was still an effective drug against this virus.

In an effort to expand our capabilities and corroborate CDC's findings of antiviral resistance in Idaho influenza A pandemic (H1N1) 2009 virus strains, we used DNA sequencing to detect the mutation that confers antiviral resistance. The neuraminidase gene of several strains of seasonal influenza A H1N1 and the pandemic (H1N1) 2009 virus was amplified by PCR. The amplified product was then sequenced using our ABI310 genetic analyzer. The wild type (sensitive to oseltamivir) DNA sequence should have the 3-nucleotide codon C-A-C corresponding to a histidine residue as the 275th amino acid in the sequence. The mutation is a transition of the first base (and possibly the last base) in the codon to T-A-C or T-A-T that corresponds to a tyrosine residue as the 275th amino acid. This mutation results in resistance to oseltamivir. As you can see in Figure 1 on page 3, we detected the wild type sequence in all of our pandemic (H1N1) 2009 virus strains and the mutation in only one seasonal influenza A H1N1 virus.

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In This Issue

Celebrate National Medical Laboratory Professionals Week April 18-24, 2010

Arsenic Testing Improvements Coming Soon

Dan Rousselle, Hank Huang,
and Christopher Ball, PhD

A growing concern in Idaho is elevated arsenic levels in ground water. Arsenic (chemical symbol As) is a toxic and carcinogenic trace metal that occurs naturally in the earth's crust, air, water and plants. Despite its toxicity, arsenic is used as an industrial wood preservative and in the manufacturing of semi-conductors, paints/dyes, agricultural chemicals, drugs, and soaps. In addition to anthropogenic sources, such as fires, illegal disposal, mining, and smelting, arsenic is also naturally released into the environment through rock erosion and volcanic activity.

Two different categories of arsenic compounds (or species) exist: inorganic arsenic species and organic arsenic species. If arsenic combines with chlorine, sulfur, and oxygen it is classified as inorganic arsenic. If it combines with carbon and hydrogen it is an organic species. Five of the most common arsenic species are Arsenious acid (AsIII), Arsenic acid (AsV), Monomethylarsonate (MMA), Dimethylarsinate (DMA), and Arsenobetaine (AsB). The inorganic species are AsIII and AsV. The organic species, MMA, DMA, and AsB, are commonly found in seafood and seaweed. The toxicity of arsenic is highly dependant on its species. The toxicity of the inorganic species compared to organic species is very different. AsIII and AsV exhibit high toxicity while MMA, DMA, and AsB exhibit moderate-to-low toxicity. The toxicity and mobility of arsenic in the environment is not well understood, which is prompting the development of new environmental regulations designed to specifically monitor individual arsenic species in water.

Scientists from the Environmental Analysis and Chemical Threat sections are collaborating to validate methods for the speciation of arsenic in both water and urine. Arsenic speciation requires an extremely sophisticated instrument (a High Pressure Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometer or HPLC-ICP-MS), which is not widely available in the private sector. This instrument is extremely sensitive and can detect all five of the arsenic species listed above at concentrations as low as 250 parts per trillion.

When the method validation is completed, the Bureau will be the only laboratory in the state capable of arsenic speciation in either water or urine samples. Not only will this new testing allow public health officials to determine which arsenic species present in Idaho water but they may be able to assess the health impacts of consuming this water. Most importantly, arsenic speciation data will assist with the proper remediation of arsenic in water supplies and protect the health of Idahoans who live in exposed areas.

For more information on Arsenic visit the following websites:

http://www.deq.state.id.us/WATER/assist_citizen_comm/drinking_water/arsenic.cfm
http://www.cdc.gov/ncidod/dpd/healthywater/factsheets/arsenic.htm
http://www.bt.cdc.gov/agent/arsenic/
http://www.atsdr.cdc.gov/toxprofiles/tp2.html

Bats of Idaho

E P N M Y O T I S A W C N E R I P M A V
M L S O Y C H N I L O G O H E D I U Q G
O Y L C C O R N H N Q P I S P S S T W I
S N E E A T R A S B E P T E S I C U S B
Q O F R R O U E N O I T A C O L O H C E
U L Y I F T R R D K R B N W L B Q D L W
I O L I Q V S T N K U S I F V F M T Q Z
T C L Q A V D I G A X I L B T Y T L Y T
O A V T D W C R P C L L L P E I W M V V
C R I K P O R C J I D V O E L M Z X G E
B O A M F O I O O W P E P U N J W B C H
N Y Q C L E E K D P Y R Z V A I G G O I
W X G N L U B K P F A F B S C U M E E K
W I X Z F A F O G Y R L Q P L P B K Y O
L Y M P I J C E Z I F D L E J Y F C K W
S H F O L R J L N F Q Z D I U H E K Q V
P H P H M H X G M P B N G U D E M W O E
W U X A R N E Q P D W N L H K C C P H N
B C W T R D P H U W H R M E I A K B B X
V Q C Z J G Z E I V X P X B R H C Y A P

BIG
CALCAR
CALIFORNIA
COLONY
CONSERVATION
ECHOLOCATION
EPTESICUS
FRINGED
HOARY
KEEL
LITTLE
MINE
MOSQUITO
MYOTIS
NOCTURNAL
PALLID
PIPISTRELLE
POLLINATION
SILVER
VAMPIRE

answers on last page

IBL Employee News!

Michael Stevenson, PhD, joined the IBL staff on January 11, 2010, as Chemical Threat Laboratory Coordinator. Previously employed in Idaho Department of Agriculture's Feed and Fertilizer Lab, Michael graduated from Butler University and earned his Doctor of Philosophy in Chemistry from Stanford University in 1993. Michael replaced Ian Elder, who left IBL for the Consumer Product Safety Commission in August. We welcome Michael to our Laboratory Preparedness Team!

Carole Morgan, Laboratory Training Coordinator, retired at the end of December. Carole had worked in various positions at the lab for over 33 years! We sent her off with a retirement party in December just before leaving on a trip to Hawaii. Carole is definitely missed around the lab.

In November of 2009, IBL's Microbiology Section welcomed Joanna Lewis. Joanna has a bachelor's degree in biology from the College of Idaho and was previously employed as a plant researcher with J.R. Simplot Company. Joanna performs an array of Virology and DNA probe assays.

Vivian Lockary, MT, MPH, replaced Carole as IBL's new Health Education Specialist. Many of you already know Vivian from her work in the TB and Bacteriology labs over the last ten years. In her new position on the Laboratory Preparedness Team, she will serve as a general liaison between IBL, sentinel labs, and public health districts for laboratory and response needs.

Detection of Oseltamivir Resistance

Expanding our role in the influenza antiviral resistance surveillance program will allow us to provide data to CDC to assist them in their efforts to monitor influenza antiviral resistance across the nation.

References

[1] Mandelboim M, Hindyieh M, Segman-Meningher T, Mendelson E. Possible transmission of pandemic (HIN1) 2009 virus with oseltamivir resistance [letter]. Emerg Infect Dis. 2010 May; [Epub ahead of print]

[2] Chen H, Cheung CL, Tai H, Zhao P, Chan JFW, Cheng VCC, et al. Oseltamivir-resistant influenza A pandemic (H1N1) 2009 virus, Hong Kong, China. Emerg Infect Dis [serial on the Internet]. 2009 Dec. Available from http://www.cdc.gov/EID/content/15/12/1970.htm

(continued from page 1)

Figure 1. Sample ID numbers, viral strain identification, and partial nucleic acid sequence for 8 influenza A H1N1 viruses sequenced at the Idaho Bureau of Laboratories. The highlighted bases show the wild-type sequence (blue) and the mutation (red) that results in resistance of the virus to treatment with oseltamivir (Tamiflu).

C090700388	Novel A H1N1	.....TATCACTAT.....
C090801895	Novel A H1N1	.....TATCACTAT.....
C091000283	Novel A H1N1	.....TATCACTAT.....
C091201161	Novel A H1N1	.....TATCACTAT.....
C091201453	Novel A H1N1	.....TATCACTAT.....
C090402018	Novel A H1N1	.....TATCACTAT.....
C090600149	Novel A H1N1	.....TATCACTAT.....
C090200144	Seasonal A H1N1	.....TTTTATTAT.....

UPCOMING TELECONFERENCES

May 4th, 11:00
APHL presents
Intestinal Flagellates and Ampicomplexans

June 8th, 11:00
APHL presents
Dengue Fever

July 20th, 12:30
TNT presents
Clostridium difficile testing

Contact Dave Eisentrager to be added to our teleconference notification list.
Eisentra@dhw.idaho.gov

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TB LABORATORY ISSUES
and
A TB CONTROLLER'S PERSPECTIVE

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Solution to Bats of Idaho Puzzle
(Over,Down,Direction)

BIG(20,4,N) CALCAR(7,15,NW)
CALIFORNIA(1,10,NE) COLONY(2,8,N)
CONSERVATION(12,1,SW) ECHOLOCATION(20,5,W)
EPTESICUS(11,4,E) FRINGED(12,13,SW)
HOARY(7,2,SW) KEEL(8,12,W)
LITTLE(15,10,NE) MINE(17,13,NW)
MOSQUITO(1,2,S) MYOTIS(4,1,E)
NOCTURNAL(3,1,SE) PALLID(10,12,SE)
PIPISTRELLE(11,11,NW) POLLINATION(13,11,N)
SILVER(12,7,S) VAMPIRE(20,1,W)

SAVE THE DATE!

The Idaho Bureau of Laboratories (IBL) has scheduled a Bioterrorism Workshop for **Thursday, June 24th** from 8 am until 5 pm. This workshop will review the Laboratory Response Network in Idaho, laboratory safety issues applicable to Category A and B agents, handling of Select Agents, and laboratory diagnostics for rule-in/rule-out of Select Agents. The afternoon will take participants through a wet workshop to include laboratory cultures, microscopic viewing, and biochemical tests for some of the Select Agents. A tour of IBL facilities and familiarization with other IBL programs will be included as time permits.

IBL has limited funding available for transportation and lodging for this workshop.

If interested in attending, please contact Vivian Lockary at 208.334.2235 ext 258 or
Lockaryv@dhw.idaho.gov