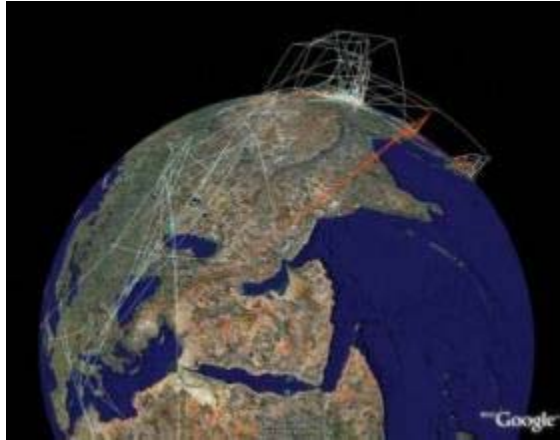


Study: Excessive use of antiviral drugs could aid deadly flu

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Researchers using Google Earth technology are able to visually chart individual outbreaks of the avian flu as it has spread outward from China over the past decade, including gene mutations that are causing a resistance to a major class of antiviral drugs. Credit: Google Earth, University of Colorado

Influenza's ability to resist the effects of cheap and popular antiviral agents in Asia and Russia should serve as a cautionary tale about U.S. plans to use the antiviral Tamiflu in the event of widespread avian flu infection in humans, scientists say.

Researchers analyzed almost 700 genome sequences of avian influenza strains to document where and when the virus developed resistance to a class of antiviral drugs called adamantanes and how far resistant strains spread. The analysis suggests that widespread antiviral drug use can accelerate the evolution of drug resistance in viruses, and that resistant strains can emerge and spread rapidly.

The results should serve as a warning to those who consider Tamiflu the next great antiviral medication, the researchers say. Stockpiling Tamiflu has become a standard part of many government, business and health organization plans to prepare for a long-feared pandemic flu outbreak, especially in the event that avian flu mutates enough to infect and be easily transmitted among humans.

"We can't necessarily say what we've seen in adamantanes is predictive of what will happen with Tamiflu. But in the larger dynamic, perhaps it serves as a cautionary tale," said Daniel Janies, senior author of the study and an associate professor of biomedical informatics at Ohio State University.

"Fighting infection is an arms race, and if we're not smart about how we use our arms and understand the evolutionary implications, then we will have ongoing and accelerating problems with drug-resistant microorganisms."

Resistance to adamantanes among strains of seasonal influenza spiked in Asia in 2002, and by 2006 the agents were considered virtually worthless worldwide as a treatment for the flu because more than 90 percent of the strains had developed a resistance to the drugs.

With that knowledge, Janies and colleagues analyzed hundreds of avian flu genomes isolated from avian, feline and human hosts between 1996 and 2007. They found that about one-third of those samples carried mutations enabling the virus strains to resist the effects of adamantane drugs.

The researchers also looked at resistance to oseltamivir-based agents (Tamiflu is the brand name for oseltamivir phosphate), but found that fewer than 1 percent of all of the samples were resistant to that class of drugs. Different classes of antivirals target influenza in different ways in the hosts' cells.

Janies and researchers from the University of Colorado and Kansas State University also designed a four-dimensional interactive map that traces the resistant avian flu lineages, showing over time where they originated and where they moved, mostly across Asia, but also to one European site in Belgium. The map is projected onto a virtual globe using Google Earth and can be downloaded at: http://supramap.osu.edu/supramap/files/h5n1_677.kmz.

The study is published online in the journal *Infection, Genetics and Evolution*.

So far, avian flu, the H5N1 strain of the influenza A virus, has been restricted to fewer than 400 human cases worldwide, but the virus's presence in birds has led to culling of large populations of infected species. Experts believe that to date, the avian flu can be transmitted to humans only from diseased birds. But the 63-percent death rate among the humans who had the virus has led to global concerns that if H5N1 were to become highly transmissible among humans, it could start an influenza pandemic.

Janies and colleagues obtained 676 whole genomes of influenza A/H5N1 available in Genbank, a public database of sequences supported by the National Institutes of Health, as of June 2007. They then used powerful supercomputers to analyze these genomes and their various mutations.

Adamantanes fight influenza by inhibiting the function of a protein called the membrane ion channel, or the M2 protein. According to the computational comparison of the avian flu genomes, upwards of one-third of the strains contained a key mutation that changed the M2 protein in a way that allowed the virus to escape the inhibiting effects of adamantanes. To evade adamantanes, mutations can occur at several positions on the protein, suggesting that influenza can evolve in many ways to resist the drug.

The researchers also were able to demonstrate that the resistance developed as a result of natural selection, because the avian flu virus strains experienced mutations that changed the M2 protein to evade the drug more often than one would expect by chance. Sometimes, dramatic changes to the genetic code occur when diverse strains of viruses shuffle whole genes among themselves in a process called reassortment. The analysis determined that any reassortment that occurred in the H5N1 strains studied did not lead to drug resistance.

The study also showed that the mutation-mediated cases of drug resistance didn't start in just one strain of avian flu. One resistant strain originated in China and spread through Southeast Asia, while another strain that was originally susceptible to adamantanes spread to Indonesia and then independently developed resistance in that country. The Google Earth map offers a vivid visualization of exactly where in the world these resistant lineages originated and where they are spreading.

At the height of their popularity in China and Russia, adamantanes were added to over-the-counter cold medicines and were also given to animals in some agricultural settings.

"We don't have hard data on how it was used or whether it was appropriately or inappropriately used, but in general, people are putting a lot of

antimicrobials into the environment now," Janies said. "When people do that, they change the selective landscape. The virus would rather remain in its wild type form, but that one gets killed by the drug. So according to the survival of the fittest, a slightly modified virus can spread by escaping the effects of the drug."

Researchers believe that Tamiflu has not been used widely anywhere in the world except Japan, and no pattern of resistance similar to that seen for adamantanes has emerged. However, recent reports have suggested a spike of resistance to oseltamivir in strains of seasonal influenza have occurred in Northern Europe and Canada. In analyzing the avian flu genomes, the researchers looked for mutations that would show the virus's ability to resist the oseltamivir class of drugs to which Tamiflu belongs. These drugs fight flu by inhibiting the neuraminidase protein in the virus.

"Resistance to Tamiflu was not nearly as widespread as is resistance to adamantanes," Janies said. "But based on our results, we know resistance to Tamiflu can occur spontaneously in nature, we know it can occur in patients, and we know Tamiflu is widely used in Japan. We should continue to watch for resistance, and use this adamantane history as a warning."

A critical part of any genome comparison is assembling supercomputers that allow researchers to put complex data into context.

"Genomes are represented as raw, partially annotated strings of letters. Each genome on its own doesn't tell you much because all you see is a single state. What we need to see is change over time to find the evolutionary history. That requires computational power to match like regions of the genome, put the data into context and see the trajectory of the change," Janies said.

The result is called a phylogenetic tree that documents the shared mutations. Phylogenetics is the study of the evolutionary relationships among various biological species believed to have a common ancestor. In this analysis, the phylogenetic tree is projected into Google Earth and animated to show when mutations emerged and where drug-resistant avian flu strains are traveling.

Key to any ongoing tracking of antiviral drug resistance will be the broad availability of genomic data, Janies said. The technology exists to do the job, but worldwide cooperation in data sharing is still a work in progress. "Not all viruses that are isolated are sequenced, and not all viral genomes that have been sequenced are shared," he said. With this publication, Janies and colleagues have done some sharing of their own, establishing a service for other researchers at <http://supramap.osu.edu>. "Anyone can go there, upload genomes, and our computers will calculate a tree for them and give them both the tree and that tree data mapped into the earth. We're rolling out our methods and making our supercomputer available for anyone in the world to do this kind of work," Janies said.

Source: Ohio State University