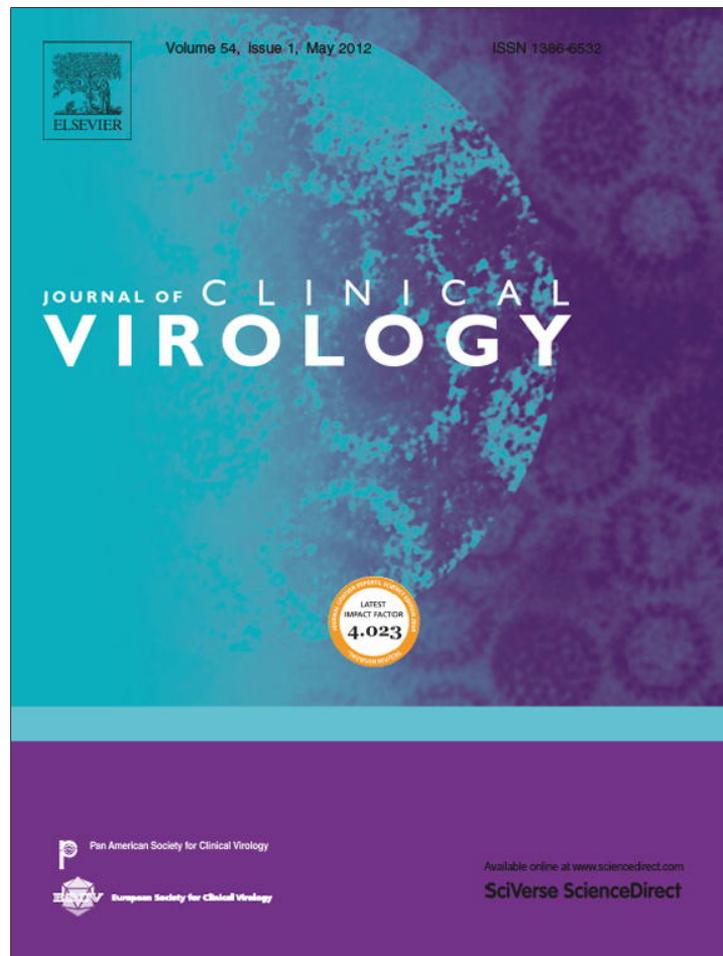


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Short communication

Pacific region influenza surveillance for oseltamivir resistance

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ABSTRACT

Background: Hawaii and the United States-affiliated Pacific islands (USAPI) host over 8 million travelers annually, most of whom originate in Asia, Australia, and the Americas where prevalence of oseltamivir resistance in 2009 pandemic influenza A (H1N1) has been reported to be 2.5–3.5%.

Objective: To survey a collection of samples from Hawaii and the USAPI that had tested positive for the 2009 pandemic influenza A (H1N1) virus by RTI-PCR to assess whether antiviral resistance emerged in these island communities during the 2009 H1N1 pandemic.

Study design: We examined RNA extracted from Hawaiian and USAPI cases for the neuraminidase H275Y mutation associated with oseltamivir resistance by pyrosequencing.

Results: Two hundred and sixty-three (263) 2009 pandemic influenza A (H1N1) positive specimens were tested and 263/263 (100%) were shown to lack the mutation most commonly associated with oseltamivir resistance.

Conclusions: There was no evidence of oseltamivir resistant A(H1N1)pdm09 virus during the 2009 pandemic in the Pacific islands despite considerable travel exposure. Geographic isolation, the lack of a “second wave” of pandemic influenza, judicious antiviral use, aggressive vaccination, and below average tourism due to the global economic crisis may have been contributing factors. Continued surveillance and vigilance is necessary to monitor unpredictable influenza activity.

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1. Background

Pacific islands represent travel crossroads between Asia, Australia, and the Americas. Over 6.5 million people visit Hawaii annually and another 1.65 million travel to the United States-affiliated Pacific islands (USAPI), which include U.S. territories (Guam, American Samoa, and Commonwealth of the Northern Mariana Islands) and freely associated independent nations (Federated States of Micronesia, Republic of the Marshall Islands, and Republic of Palau). Travel statistics show that the USAPI enjoy a crossover of visitors from North America and Asia (Supplemental Table 1).^{1–6} Hawaii visitors originate predominantly from the U.S. mainland and Canada (~66%), and Japan (~18%).⁷

Typically influenza infects 5–20% of the U.S. population causing approximately 200,000 hospitalizations and 36,000 deaths.⁸ Unique geographical location and global traffic patterns through the Pacific compelled the Hawaii Department of Health, together

with key partners such as the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), the Pacific Island Health Officers Association (PIHOA), and a local commercial laboratory (Diagnostic Laboratory Services) to establish a network to conduct surveillance in the USAPI, which is a very diverse and difficult demographic to survey.

There are currently two drug classes approved by the FDA to treat influenza.⁹ In 2006, the CDC advised against the use of adamantanes due to high levels of resistance in seasonal H3N2 and H1N1.^{10,11} The neuraminidase (NA) inhibitors (oseltamivir, zanamivir) interfere with the release of new virus from infected cells,^{9,13} and have become the class of choice for both prophylaxis and treatment.^{9,14,15} While both influenza A and B viruses remain susceptible to zanamivir, resistance to oseltamivir emerged in 10.9% of U.S. seasonal Influenza A H1N1 during the 2007–2008 season,¹² and quickly escalated to near 100% in the first half of the 2008–2009 season.^{16–18} A few of the 2009 H1N1 strains detected early in the pandemic contained the same H275Y mutation in the neuraminidase gene that conferred high level resistance to oseltamivir in seasonal influenza, and more recent resistance activity has been reported.^{19–21} Having already lost the adamantane drug class, it was important to monitor for NA inhibitor resistance in the new H1N1 strain.

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2. Objective

We analyzed representative 2009 pandemic influenza A (H1N1) samples from Hawaii and the USAPI, including those from patients who had oseltamivir treatment.

3. Study design

3.1. Surveillance specimens from Hawaii and the USAPI

The 2009 pandemic influenza A (H1N1) positive specimens ($n=263$) were selected from those submitted over a 26-month period (Supplemental Table 2) to the Hawaii State Laboratories Division from in-state and from collaborating regions of the USAPI. All specimens had positive PCR results with cycle threshold (Ct) values ≤ 30 for Influenza A, H1, and 2009 pdm H1N1 targets. All samples and controls were run in duplicate. Of the 109 specimens from Hawaii, 42 were prescribed oseltamivir; an exposure risk associated with emergence of resistance.

3.2. Nucleic acid extraction and amplification

Manual nucleic acid extraction was performed using QIAamp Viral RNA Mini kit (Qiagen, Valencia, CA). Optionally, the automated MagNA Pure Compact (Roche Applied Science, Indianapolis, IN) or MagNA Pure LC (Roche) was used. Primers were provided by CDC for both the M2 region and the NA histidine (wildtype) to tyrosine (resistant) mutation at the H275Y codon. Extracted RNA was amplified using biotinylated primers on a Veriti thermal cycler (Applied Biosystems, Carlsbad, CA),²² and amplicon was purified according to CDC protocol.²³

3.3. Pyrosequencing

Using the PyroMark™ vacuum prep tool and work station (Qiagen), amplicons were washed in a series of denaturing and purification agents resulting in the isolation of single-stranded DNA. The ssDNA was sequenced on the PyroMark™ Q96 ID (Qiagen) using the SQA protocol, analyzed using IdentiFire software (Qiagen), and compared to a library provided by CDC containing both the wildtype and resistant nucleic acid. Sequencing required a software-generated score of ≥ 80 and a pyrogram characterized by quality peaks, low background “noise”, and rated acceptable by the PyroMark™ software.

4. Results

4.1. Analysis of Hawaii and USAPI samples

Preliminary testing of early A(H1N1)pdm09 cases demonstrated expected uniform resistance to adamantanes and susceptibility to neuraminidase inhibitors (data not shown). None of the 263 specimens contained the H275Y mutation, regardless of time or origin of collection. Hawaii specimens, many of which were selected because oseltamivir treatment was indicated on submission forms ($n=42$) were also negative for this mutation.

5. Discussion

Immunization remains the primary defense against influenza; however, NA inhibitors such as oseltamivir have become important tools in both the treatment and prophylaxis of this disease. Both the World Health Organization (WHO) and CDC recommend early antiviral treatment in patients that are at increased risk for serious complication or who show signs of progressive illness.²⁴

According to WHO, there have been at least 596 cases of oseltamivir resistant influenza associated with 2009 pdm H1N1.²⁵ Most of these patients were either immunosuppressed or had a history of oseltamivir treatment^{25,26}; however recent clusters and sustained transmission in New South Wales, Australia may signal significant change.^{20,21} Previous verification of expected adamantane resistance demonstrated testing for M2 mutations was not necessary.²⁷

The USAPI is a diverse demographic for which there were very little data. Oseltamivir use was unavailable.²⁸ Influenza test results, when available, were limited to rapid antigen, which has significant analytical limitations compared to PCR,²⁹ although some jurisdictions occasionally send specimens to the WHO laboratory in Melbourne, Australia.²⁶ A Pacific Island surveillance system was developed in response to the pandemic, and the data indicated the prevalence of oseltamivir resistance in A(H1N1)pdm09 strain was zero, which was surprising because Hawaii accounted for over 94% of all drug-resistant seasonal H1 in the U.S., going into in the 2007–2008 season.³⁰ Although we were able to identify oseltamivir-exposed patients by reviewing submission documents, there were limitations. Specimens tended to be collected the day treatment began in Hawaii, and oseltamivir use was not available for the USAPI. This limited our ability to assess any impact of local antiviral drug pressure. Despite current oseltamivir susceptibility, resistance in surrounding regions including potentially foreshadowing clusters in Australia, resident and visitor travel patterns, and the explosive emergence of H275Y-associated resistance in seasonal influenza H1 underscore the importance of surveillance in this region.

There may be several explanations for the absence of this mutation in our study set. Although we tested all positive specimens from submitting USAPI laboratories, there was not a systematic or consistent approach to sampling by providers, which could have introduced bias. We also suspect there was limited availability or use of oseltamivir in the Pacific. Decreased travel to these geographically isolated destinations because of the pandemic and poor economic conditions may have reduced exposure risk. In Hawaii, a statewide emphasis on vaccination included the third year of an innovative DOH “Stop Flu at School” program that provides free vaccinations to all public and private school children at their schools probably contributed to the absence of a “second wave” of 2009 pdm H1N1 in the Pacific.

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Competing interests

The authors have no competing interests.

Ethical approval

This was an epidemiological study for surveillance purposes only.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2012.01.007.

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