

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Mai LQ, Wertheim HFL, Duong TN, et al. A community cluster of oseltamivir-resistant cases of 2009 H1N1 influenza. *N Engl J Med* 2010;362:86-7. DOI: 10.1056/NEJMc0910448.

## **A Community Cluster of Oseltamivir-Resistant Cases of 2009 H1N1 Influenza**

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N Eng J Med, 2010;362

### **Supplementary appendix**

#### **Background**

Influenza A/H1N1 2009 was first detected in Vietnam in late May 2009 and widespread community transmission has subsequently developed. At the time the cluster occurred, the Ministry of Health guidance was to test all suspected cases of H1N1 2009 by RT-PCR and to admit all confirmed cases to a health care facility and treat with oseltamivir phosphate 75mg twice daily for at least five days. Oseltamivir has not been widely used for prophylaxis in the community in Vietnam.

#### **Cluster detection**

On the 9<sup>th</sup> September 2009 the Vietnam National Influenza Center at the National Institute for Hygiene and Epidemiology (NIHE) sequenced a batch of 85 H1N1 2009 viruses to look for resistance mutations, including S31N in the M2 protein conferring resistance to the M2 inhibitors (adamantanes) and H275Y in the neuraminidase that confers resistance to oseltamivir. All 85 viruses contained the S31N mutation and three contained the H275Y mutation. The three oseltamivir resistant strains prompted an immediate retrospective public health investigation that entailed medical note review, tracing and interviews of the three cases and a review of all pandemic H1N1 case reports for an epidemiological link to the three cases.

The investigation revealed that the three cases formed part of a cluster of seven 2009 H1N1 cases all linked to a 42-hour train journey between Ho Chi Minh City and Hanoi in July [Figure 1]. During the trip ten students, who were seated near to each other but did not know each another prior to the journey, socialized together. The carriage was not air-conditioned and contained 48 hard seats, most of which were occupied throughout the journey [Figure 2]. One of the students left the train after 34 hours, one left after 37 hours (Case D), and the other eight continued to the final destination of Hanoi. Eight of the ten students could be traced through 2009 H1N1 case records or through email or mobile phone numbers exchanged during the trip. Six of the traced students had symptomatic H1N1 2009 infection and two reported no illness. Serology was not done on the two asymptomatic students. The two untraceable members of the group of students were reported to have appeared completely well throughout the entire trip. It was not possible to trace the other occupants of the carriage as passenger names and seat numbers are not recorded. The seventh case (Case G) was seated in a different carriage to the group of ten students and had no identifiable contact with them.





### **Additional laboratory results**

The mean IC<sub>50</sub> of 12 other unrelated 2009 H1N1 isolates from Vietnam without the H275Y substitution was 4.27 Nm (range 1.04-13.91).

Approximately 420 nucleotides of the neuraminidase gene were sequenced for each of the three cultured virus and the homology of these was 99%. The HA sequences of the three viruses were also highly homologous to other published H1N1 2009 HA sequences.

### **Methods**

Nose swabs and/or throat swabs were collected from patients and tested at the molecular diagnostic laboratory of NIITD by rRT PCR according to the protocol developed by the Center for Disease Control (CDC), USA. Separate clinical samples were sent to the National Influenza Centre of the National Institute of Hygiene and Epidemiology (NIHE) Hanoi Vietnam for independent confirmation using RT-PCR and viral culture. Both molecular diagnostic laboratories use unidirectional workflow to prevent contamination and participate in external quality assessments for influenza molecular diagnostics.

For virus isolation, clinical specimens were inoculated onto Madin-Darby Canine Kidney (MDCK) cells in biosafety level 3 laboratories at NIHE. Virus isolates were identified by haemagglutination inhibition using ferret reference anti-serum supplied by WHO (raised against California H1N1/04/2009) and RT-PCR. In vitro susceptibility of virus isolates to oseltamivir carboxylate was

determined using the fluorogenic substrate MUNANA as previously described <sup>15</sup>. Susceptibility testing for zanamivir was not performed. A total of 15 virus isolates were tested by neuraminidase inhibition assay: three isolates from the described cases, plus another 12 unrelated 2009 H1N1 strains that did not possess the H275Y substitution (seven from Hanoi and five from Ho Chi Minh City).

At NIITD the H275Y mutation was detected using an in-house rRT-PCR assay. At NIHE, neuraminidase sequencing to assess mutations coding for oseltamivir resistance was performed on RNA extracted from virus isolates and from purified RT-PCR products from clinical specimens. For sequencing of viral RNA from culture, the following primers were used to amplify a 1134 base-pair fragment of the neuraminidase gene: NA-318F: TGT AAA ACG ACG GCC AGT TAC ACA AAA GAC AAY AGC; NA-1452B: CAG GAA ACA GCT ATG ACC AGT AGA AAC AAG GAG. For direct sequencing of viral RNA from clinical samples, the following primers were used to amplify a 493 base-pair fragment of the neuraminidase gene: NA-780F: GGG GAA GAT TGT YAA ATC AGT YGA; NA 1273 : CWA CCCA GAA RCA AGG TCT TAT G.

Purified PCR products and viral RNA were sequenced using BigDye Terminator version 3.1 Cycle Sequencing Kit on an ABI 3100 Genetic Analyzer (Applied Biosystems, CA, USA) following the manufacturer's instructions. Sequence fragments were assembled and edited using Lasergene v8 (DNASTar Inc, USA).