

## Evidence of Person-to-Person Transmission of Oseltamivir-Resistant Pandemic Influenza A(H1N1) 2009 Virus in a Hematology Unit

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**We describe the first confirmed person-to-person transmission of oseltamivir-resistant pandemic influenza A(H1N1) 2009 virus that occurred in a hematology unit in the United Kingdom. Eleven cases of (H1N1) 2009 virus infection were identified, of which, ten were related as shown by sequence analysis of the hemagglutinin and neuraminidase genes. H275Y analysis demonstrated that 8 of 10 case patients had oseltamivir-resistant virus, with 4 of 8 case patients infected by direct transmission of resistant virus. Zanamivir should be considered as first-line therapy for influenza in patients with lymphopenic hematological conditions and uptake of influenza vaccination encouraged to further reduce the number of susceptible individuals.**

Pandemic influenza A(H1N1) 2009 virus emerged in Mexico during April 2009 and has caused 2 successive pandemic waves [1]. Analysis of the (H1N1) 2009 virus showed that it was

inherently resistant to the adamantane group of antivirals due to a serine to asparagine mutation at amino acid 31 (S31N) in the M2 ion channel [2]. Unlike the previously circulating seasonal influenza A(H1N1) virus, which is fully resistant to oseltamivir due to the presence of the H275Y mutation in the neuraminidase (NA) gene [3], the (H1N1) 2009 virus was shown to be susceptible to both oseltamivir and zanamivir [2].

However, by October 2009, the sporadic emergence of oseltamivir-resistant (OR) (H1N1) 2009 virus was reported in immunocompromised patients who were receiving oseltamivir therapy and in some individuals who had received oseltamivir prophylaxis [4]. Fewer than 60 isolates of OR-(H1N1) 2009 had been reported to the World Health Organization by the end of October 2009, and, of these, only 3 isolates had been identified in the United Kingdom. There were no confirmed reports of person-to-person transmission of OR-(H1N1) 2009 virus except for 1 incident involving 2 cases of resistant virus in adolescents at a summer camp in the United States, where evidence of spread was inconclusive [5]. We describe the emergence of OR-(H1N1) 2009 virus during a nosocomial outbreak on a hematology unit between October and November 2009 with epidemiological and molecular evidence of person-to-person transmission.

### METHODS

**Setting and patients.** During October 2009, an outbreak of OR-(H1N1) 2009 virus began in an adult hematology unit at the University Hospital of Wales (UHW), Cardiff, United Kingdom. The hematology unit provides hematology services to a local population of 770, 000 and hematopoietic stem cell transplant (HSCT) service for Wales (population 3 million). The unit has a general hematology section for nontransplant recipients (17 beds) and a dedicated HSCT section for patients immediately after transplantation (10 beds). The HSCT section has its own positive pressure, high efficiency particulate (HEPA) filtered air supply. Elective admissions are for planned chemotherapy, transplantation, or central line insertion, whereas emergency admissions are mostly associated with neutropenic sepsis or chemotherapy complications.

Upper respiratory tract samples were collected from inpatients presenting with an influenza-like illness (ILI), defined by the Health Protection Agency (HPA) as a fever  $>38^{\circ}\text{C}$  with 2 or more of the following: cough, coryza, sore throat, headache, myalgia, diarrhea, and from patients with an unresolving pyrexia. Patients who received mechanical ventilation had

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diagnostic bronchoalveolar lavages (BALs) performed. Samples were obtained from case patients every 3–5 days after treatment until 2 sequential test results were influenza A negative. Patients who continued to have symptoms or who became symptomatic were retested regardless of previous polymerase chain reaction (PCR) results. All patients in contact with confirmed case patients were monitored for symptoms with a low threshold of suspicion to ensure early sampling.

Retrospective review of admission records was undertaken by the attending physician for each of the case patients to determine clinical characteristics, date of admission, and length of time on the unit (Table 1). Lymphocyte counts were recorded (reference range, 1.0–4.0  $10^9$  cells/L) and compared for each of the case patients during viral excretion and post viral clearance. Statistical analysis was undertaken using the Mann-Whitney 2-tailed nonparametric test.

**Community surveillance and controls.** Positive (H1N1) 2009 virus isolates from across Wales were tested for the H275Y mutation by pyrosequencing to determine whether OR-(H1N1) 2009 virus was circulating in the wider community [6]. These data were used to determine whether the emergence and transmission of OR-(H1N1) 2009 virus in the UHW was an isolated event in Wales.

**Outbreak management.** Early cases in the outbreak were managed following standard outbreak interventions, including isolating and cohorting of case patients and the reinforcement of the use of personal protective equipment by health care workers (HCWs) when working with symptomatic patients. HCWs on the unit were offered both seasonal and pandemic influenza vaccination.

Additional control measures were initiated when later cases of OR-(H1N1) 2009 virus were identified. Elective admissions were delayed, and the HSCT unit was used as an isolation facility. Prophylaxis was stopped, and zanamivir was used as dual therapy with oseltamivir. Patients who could not tolerate inhaled zanamivir or who were receiving mechanical ventilation were prescribed intravenous zanamivir on a named-patient basis. Seasonal and pandemic vaccination was extended to include all patients and close contacts. Finally, screening of all inpatients on the unit was undertaken to locate additional cases; this was repeated 72 h later to show no further transmission events had occurred.

**Molecular diagnostics.** Generic influenza A testing was performed by real-time reverse-transcription PCR (RT-PCR) targeting the matrix gene. To ensure sample quality and lack of inhibition, an assay targeting human RNaseP was used; a sample with a crossing threshold (ct) value of <40 in the influenza A assay as described in the Centers for Disease Control and Prevention (CDC) protocol indicated a positive test result for influenza A [7]. Influenza A–positive samples were subtyped using a specific (H1N1) 2009 duplex real-time RT-PCR assay designed by the Health Protection Agency

(HPA), Centre for Infections (Cfi) (Colindale, London, United Kingdom) [8].

**Antiviral susceptibility testing.** Positive samples from case patients who did not clear virus despite treatment and from cases whose first diagnosis occurred after evidence of viral resistance was found on the unit were forwarded to the respiratory virus unit at Cfi for pyrosequencing analysis for the H275Y mutation using (H1N1) 2009 specific primers. When virus could be isolated in cell culture, phenotypic testing for oseltamivir and zanamivir was undertaken [6].

**Sequencing and phylogenetic analysis.** Amplification and sequencing of the complete coding regions of the haemagglutinin (HA) and NA genes was performed directly from original respiratory material with a 2-step RT-PCR using Superscript III RT reverse transcriptase and Platinum Pfx polymerase (Invitrogen Ltd), following manufacturer's instructions. The HA gene was amplified in 2 overlapping fragments (1.2–1.0 kb), whereas NA was amplified in a single fragment (1.4 kb). Primers are available upon request. Sequencing was performed on a 48-capillary ABI 3730 Genetic Analyser (Applied Biosystems). Raw sequencing data were edited and assembled using Sequencher software (version 4.9).

Nucleotide sequences were aligned, trimmed to include coding regions, and concatenated in the order HA-NA. Before phylogenetic analysis, the triplet coding for position 275 associated with drug resistance was deleted from all NA sequences. A maximum likelihood (ML) phylogenetic tree of the concatenated HA-NA segments was inferred using PAUP software package, version 4.0 (Swofford DL). The best-fit model of nucleotide substitution was identified using Modeltest [9] as the HKY85+ $\Gamma$  model, with parameters estimated from the empirical data. ML trees were determined through an heuristic search. Bootstrap analysis was performed through neighbor joining algorithm with 1000 replicates, incorporating the ML substitution model previously determined. Accession numbers and standard strain names are provided in Suppl Table 1.

## RESULTS

**Case identification.** From 29 October through 25 November 2009, 11 cases of (H1N1) 2009 virus were found in the hematology unit (defined as an in-patient in the hematology unit with a virological diagnosis of [H1N1] 2009). Epidemiological analysis suggested an outbreak of (H1N1) 2009 virus had occurred because the patients involved could be linked in time and place. The outbreak affected male patients with underlying hematological malignancy, and sequence analysis of the isolates showed that 10 cases were virologically linked (Table 1 and Figure 1). The probable index case of the outbreak (case 1) was identified retrospectively on the basis of the HA and NA sequencing of this virus.

**Table 1. Clinical and Virological Background of the 10 Cases involved in the Oseltamivir-Resistant (H1N1) 2009 Virus Outbreak, University Hospital of Wales, Cardiff, United Kingdom**

Case	Age	Underlying Condition	Admission	Reason for admission	Length of admission	Date of first Influenza PCR Positive	Date of sample positive with H275Y mutation	Minimum time of viral shedding	Treatment	Other information
1	19	Acute Lymphoblastic leukemia	10/18/09	ILI	<1 day	10/18 (Oseltamivir sensitive)	N/A	N/A	5 days oseltamivir (75 mg/BD)	Admitted for sampling Recovered
2	26	Acute myeloid leukemia	10/11/09	Fever	>40 days (transferred to ICU 11/05)	10/29 (Oseltamivir sensitive)	11/04 (70% H275Y) (result available for clinician 11/12)	30 days	5 days oseltamivir (75 mg/BD), 4 days oseltamivir (150 mg/BD), 10 days intravenous zanamivir	Recovered
3	52	Mantle cell lymphoma	10/25/09	Induction chemotherapy	7 days readmitted 11/09 for 6 days	10/30 (Oseltamivir sensitive)	11/05 (86% H275Y)(result available for clinician 11/12)	12 days	5 days oseltamivir (75 mg/BD), stopped treatment 1 day, 10 days oseltamivir (150 mg/BD) + inhaled zanamivir	Recovered
4	56	Multiple myeloma	10/28/09	Consolidation chemotherapy	8 days	11/02 (Oseltamivir sensitive)	No evidence of H275Y mutation in repeat samples	<5 days	5 days oseltamivir (75 mg/BD)	Earlier sample 10/30 negative Recovered
5	48	Multiple myeloma	10/29/09	Fever	25 days	11/07 (Oseltamivir resistant)	11/07 (100% H275Y) (result available for clinician 11/19)	23 days	3 days Oseltamivir (75 mg/BD), 9 days oseltamivir (150 mg/BD), 1 day oseltamivir (150 mg/BD) + inhaled zanamivir, 4 days inhaled zanamivir	Recovered
6	27	Non-Hodgkin lymphoma	10/25/09	Induction chemotherapy	24 days	11/09 (oseltamivir sensitive), Negative PCR 11/17	Viral rebound 11/19 (100% H275Y)(result available for clinician 11/26)	10 days	10 days Oseltamivir (150 mg/BD)	Recovered
7	48	Burkitt's lymphoma	11/02/09	Consolidation chemotherapy	9 days readmitted 11/13 for >14 days	11/09 (Oseltamivir resistant)	11/09 (100% H275Y)(result available for clinician 11/19)	20 days	5 days Oseltamivir (150 mg/BD), 3 days oseltamivir (150 mg/BD) + inhaled zanamivir, 10 days intravenous zanamivir	Recovered

8	45	Acute myeloid leukemia	10/24/09	Induction chemotherapy	25 days	11/09 (Osetamivir resistant)	11/09 (100% H275Y) (result available for clinician 11/19)	6 days	10 days oseltamivir (150 mg/BD)	Earlier sample 11/02 negative Recovered
9	74	Acute myeloid leukemia	10/26/09	Perianal abscess	11 days readmitted 11/16	11/17 (Osetamivir resistant)	11/17 (100% H275Y) (result available for clinician 11/19)	22 days	2 days oseltamivir (150 mg/BD), 8 day oseltamivir (150 mg/BD) + inhaled zanamivir 2 days intravenous zanamivir	Readmitted with ILI Recovered
10	68	Chronic lymphocytic leukemia	11/05/09	Expressive dysphasia, poor coordination	>25 days	11/20 (Osetamivir resistant)	11/20 (100% H275Y) (result available 11/26)	10 days	Osetamivir prophylaxis from 11/10, 10 days Osetamivir (150 mg/BD) + inhaled zanamivir	Earlier sample 11/07 negative Recovered

**NOTE.** ILI, influenza-like illness.

The 11th case patient was admitted in late October 2009 but was ruled out of the outbreak by sequence analysis. He was symptomatic and had been admitted directly into isolation where he was managed for his entire admission. No evidence of onward transmission of his virus was found, suggesting appropriate infection control measures were being undertaken. Overall, outbreak management was challenging because of the difficulty in clinically diagnosing influenza in this patient cohort using standard definitions of ILI. Mild symptoms and asymptomatic illness were main features of this outbreak.

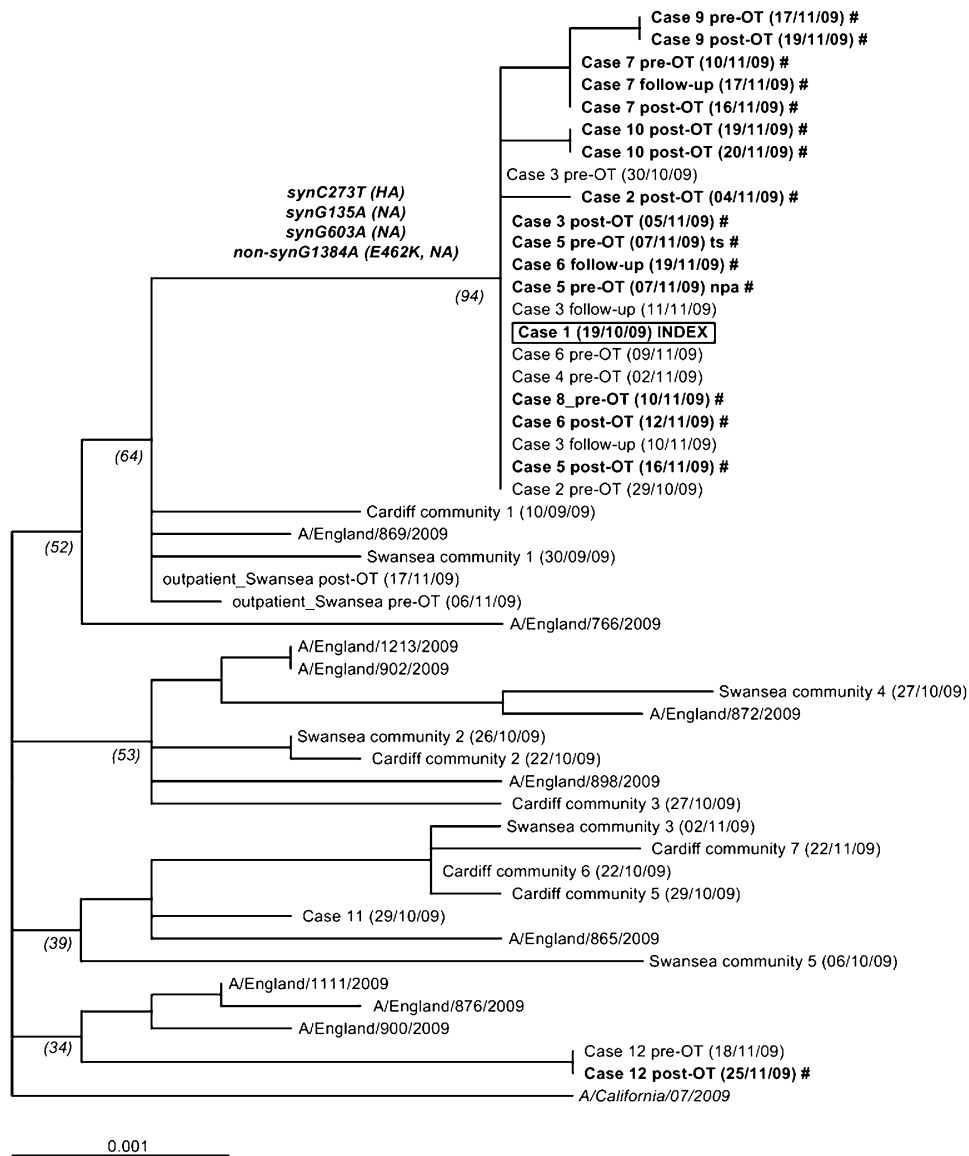
During the first 2 weeks of the outbreak, 6 HCWs on the unit were absent from work with a multitude of different symptoms, including lethargy, diarrhea, and coryza. No respiratory sampling of HCWs had been undertaken because symptoms were generally mild and nonspecific.

**Antiviral susceptibility findings.** Eight of the 10 outbreak case patients had (H1N1) 2009 virus expressing the H275Y mutation (Table 1). Where phenotypic testing was performed, the presence of this mutation correlated with high-level oseltamivir resistance (200–600-fold increase in oseltamivir IC<sub>50</sub>). Three of 8 case patients developed resistant virus while receiving treatment. Pretreatment samples from these patients showed sensitive virus by pyrosequencing and phenotypic assays analysis. Cases 2 and 3 developed a mixed majority populations of H275Y (Table 1), which did not alter on repeat testing. No other case patients in the unit with OR-(H1N1) 2009 were shown to have a similar mixed population. Case 6 had viral rebound with OR-(H1N1) 2009 that was fully resistant to oseltamivir. Case 10 was on oseltamivir prophylaxis due to being in contact with 2 cases, and this possibly drove the emergence of OR-(H1N1) 2009 virus. No other patients had prior exposure to oseltamivir, suggesting that person-to-person transmission of oseltamivir-resistant virus had occurred.

**Prophylaxis and treatment.** Treatment and prophylaxis used in the outbreak is summarized in Table 1. At the start of the outbreak, all case patients were treated with 5 days of standard dose oseltamivir (75 mg/BD). Subsequently, all case patients were treated with 10 days double-dose oseltamivir (150 mg/twice daily) and inhaled or intravenous zanamivir added if resistant virus was suspected. Oseltamivir prophylaxis was used sparingly and was stopped when it became apparent that the outbreak involved an OR-(H1N1) 2009 virus.

**Viral excretion and immune status.** OR-(H1N1) 2009 virus was excreted by case patients for a mean of 17 days (range, 6–30 days). Prolonged positivity was seen in patients in whom lymphocyte counts were  $<0.5 \times 10^9$  cells/L, with a median of  $0.27 \times 10^9$  cells/L compared with  $0.65 \times 10^9$  cells/L when virus was not detected ( $P < 0.001$ ).

**Sequence analysis results.** Phylogenetic analysis of the concatenated HA and NA genes from the outbreak influenza viruses showed that they clustered together in a separate branch from other (H1N1) 2009 viruses isolated in Wales and the



**Figure 1.** Maximum-likelihood tree of the nucleotide coding region of the concatenated HA and NA genes of pandemic H1N1 2009 influenza viruses from the outbreak and the community in Wales and the United Kingdom. Tree was rooted using A/California/07/2009 as out-group. Branch lengths are drawn to scale. Oseltamivir-resistant viruses are in bold marked with #. Bootstrap values are displayed in brackets below the nodes. Signature mutations are annotated in bold italics and refer to changes seen in the nucleotide sequence of the HA and NA gene using as reference the sequence of A/California/07/2009. OT = oseltamivir treatment.

United Kingdom during the same period (October – November 2009) (Figure 1). This cluster was characterized by a bootstrap value of 94% and the presence of 4 nucleotide substitutions: 1 synonymous change in the HA gene (C273T), 2 synonymous changes in the NA gene (G603A, T645C), and a nonsynonymous mutation also in NA (G1384A) leading to an amino acid replacement (E462K). After deletion of codon position 275, all the NA sequences from this cluster were shown to be identical. Given the higher variable nature of the HA gene, a few additional single mutations had accumulated in all the viruses sampled from case patients 9 (G751A), 7 (A330G), and 10

(G1035A). Also, posttreatment sample from case patient 2, taken 5 days after the pretreatment sample, had accumulated single substitution A716G. To further confirm the distinctive feature of the 4 mutations characterizing the outbreak cluster, a thorough search through all the sequences deposited in public influenza sequencing databases (NCBI, GISAID) was carried out, revealing that these mutations were present in <1% of global sequences, none of them from the United Kingdom, and not concomitantly seen in any NA global sequence deposited to date. This striking finding strongly supports our hypothesis of transmission of OR viruses among the outbreak patients.

No evidence of the H275Y mutation was found in any other (H1N1) 2009 virus isolate collected over the same period as the outbreak from Wales. One further hematology patient (case 12, Figure 1) later developed a minority mixed population of OR-(H1N1) 2009 virus on treatment, but this virus was shown to be genetically distinct from the outbreak cluster.

## DISCUSSION

This is the first confirmed outbreak to our knowledge of OR-(H1N1) 2009 virus with person-to-person transmission, as demonstrated by the presence of OR virus in pretreatment and posttreatment samples of 2 patients. Subsequent genetic analysis of the HA and NA genes proved that the same virus was involved in the outbreak.

It occurred during the peak of the second wave of the (H1N1) 2009 pandemic in Wales, when 60% of community surveillance samples tested positive for (H1N1) 2009 virus [10]. (H1N1) 2009 virus was introduced to the hematology unit from the community with oseltamivir resistance being driven by treatment in 2 lymphopenic patients with onward transmission of OR-(H1N1) 2009 virus to other patients on the unit. Case patients were all men, and their admissions on the ward overlapped.

Treatment guidelines for hematology patients with (H1N1) 2009 virus infections were revised in light of this outbreak. Dual treatment with oseltamivir and zanamivir was used when resistant virus was suspected or confirmed. Although all of the cases recovered from influenza using this approach, recent data suggest there is possibly no synergy to be gained from this approach [11]. Zanamivir alone therefore would be preferable as a frontline treatment in particularly high-risk groups. Prophylaxis should be used with caution in patients with lymphopenia, because they may have asymptomatic infection with prolonged viral excretion; both of which are significant factors in the emergence of resistance [12]. Viral clearance in this outbreak was associated with immune reconstitution, supporting previous findings [12, 13]. Case 6 transiently had a lymphocyte count above  $0.5 \times 10^9$  cells/L and became PCR negative, suggesting that a combination of antivirals and a degree of immune reconstitution temporarily suppressed viral replication. Viral rebound may have occurred because of the presence of a minority population of OR-(H1N1) 2009 virus that replicated despite the presence of oseltamivir [6]. Drug resistance should therefore always be considered in any immunosuppressed patient who does not clear virus.

Transmission of infection on hematology units might be reduced by protective isolation of neutropenic patients using single cubicles, but this approach is controversial. Although some studies show reduced infection rates, none have been able to demonstrate reduced mortality [14]. Patients treated in isolation were shown to have a significant psychological burden,

including increased levels of insomnia and depression [15]. An alternative to isolation is increasing vigilance for the introduction of respiratory infections into areas where immunocompromised patients are cared for, together with good infection control procedures to prevent transmission events.

There were no deaths and only limited morbidity associated with (H1N1) 2009 infection regardless of oseltamivir susceptibility in this outbreak. Despite the ease of transmission of the OR-(H1N1) 2009 virus on the unit, the mild symptoms associated with this outbreak led to difficulties in determining when a case patient acquired their infection, making the production of a meaningful outbreak curve impossible. Because screening of the unit did not occur until late into the outbreak, it is possible that an individual with mild symptoms or asymptomatic shedding played a role in the continued transmission of the OR-(H1N1) 2009 virus.

Pandemic influenza vaccine was available in the United Kingdom in late October 2009; when the outbreak was identified, vaccination of hematology patients, their families, and hospital staff was undertaken. Vaccination against influenza remains the best way of preventing infection in these patients, and therefore, vaccine uptake must be strongly encouraged to reduce the pool of susceptible individuals prior to the start of each influenza season.

## Supplementary Data

Supplementary data are available at [http://www.oxfordjournals.org/our\\_journals/jid/](http://www.oxfordjournals.org/our_journals/jid/) online. Supplementary Table 1 contains GenBank accession numbers of nucleotide sequences of pandemic influenza (H1N1) 2009 used for phylogenetic analysis.

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