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Original Article

An outbreak of the 2009 influenza a (H1N1) virus in a children's hospital

Allison Bearden,[a] Thomas C. Friedrich,[b] Tony L. Goldberg,[b] Barbara Byrne,[c] Carol Spiegel,[a,d] Peter Schult,[e] Nasia Safdar,[a]

[a]Sections of Infectious Diseases, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. [b]Department of Pathobiological Sciences, University of Wisconsin School of Veterinary Medicine, Madison, WI, USA. [c]Department of Nursing, University of Wisconsin Hospital and Clinics, Madison, WI, USA. [d]Pathology and Laboratory Medicine, Madison, WI, USA. [e]Division of Health State Lab of Hygiene, Madison, WI, USA.

Correspondence: Nasia Safdar, Section of Infectious Diseases, Department of Medicine, University of Wisconsin Madison, MFCB 5221, 1685 Highland Avenue, Madison, WI 53705, USA. E-mail: ns2@medicine.wisc.edu

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Context Preventing nosocomial transmission of influenza is essential to reduce the morbidity and mortality associated with this infection. In October 2009, an outbreak of the 2009 influenza A (H1N1) virus occurred in a hematology ward of a children's hospital over a 21-day period and involved two patients and four healthcare workers.

Objective To investigate nosocomial transmission of the 2009 influenza A (H1N1) virus in patients and healthcare workers.

Design, setting, and participants An outbreak investigation was initiated in response to suspected nosocomial transmission of the 2009 influenza A (H1N1) virus during the peak of the 2009 pandemic. Cases were confirmed using a polymerase chain reaction (PCR) test specific for the 2009 H1N1 influenza A virus. Viruses isolated from nasopharyngeal swabs were genetically characterized using Sanger sequencing of uncloned ''bulk'' PCR products.

Main outcome measures Virus sequencing to investigate nosocomial transmission.

Results Two immunocompromised patients and four healthcare workers were found to be part of a nosocomial outbreak of the 2009 influenza A (H1N1) virus. One immunocompromised patient had a second episode of clinical influenza infection after isolation precautions had been discontinued, resulting in additional exposures. Strain-specific PCR showed that all cases were caused by infection of the 2009 H1N1 virus. Sequencing of viral genes encoding hemagglutinin and polymerase basic subunit 2 (PB2) revealed that all viruses isolated were genetically identical at these loci, including the two episodes occurring in the same immunocompromised patient.

Conclusions Prompt institution of isolation precautions is essential in preventing nosocomial outbreaks of the 2009 novel influenza A (H1N1) virus. Our data suggest that isolation precautions may need to be continued for a prolonged period of time in immunocompromised patients with influenza infection.

Keywords Influenza, nosocomial, outbreak.

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Introduction

The recent pandemic of the 2009 influenza A (H1N1) virus presented significant challenges to healthcare institutions with large numbers of patients requiring hospitalization.[1–3] Although the virus proved to cause mild, self-limited disease in most cases, risk for severe disease, including death, was much higher in certain groups, including those with underlying illness or immune compromise.[1] Nosocomial transmission of influenza has been well described, but the duration of time that hospitalized patients need to stay in isolation has not been well characterized, and prolonged shedding of virus in hospitalized patients with the 2009 Influenza A (H1N1) infection has been described.[4]

Infection control recommendations from the Centers for Disease Control and Prevention included the use of N-95 respirators for healthcare workers caring for patients with suspected pandemic influenza, in addition to eye protection, gowns, and gloves (enhanced respiratory precautions). These recommendations represented a departure from the droplet precautions used for typical seasonal influenza. Institutions faced challenges in timely and continued healthcare worker (HCW) compliance with the use of these enhanced isolation precautions.[5] We report an outbreak of the 2009 influenza A (H1N1) virus that occurred in a children's hospital and involved transmission of infection from an immunocompromised patient to healthcare workers and another patient.

Methods

In October 2009, suspected transmission of the 2009 influenza A (H1N1) virus from a patient to a HCW prompted an investigation on the hematology–oncology floor of a children's hospital, with identification of additional cases. In each suspected case, a nasopharyngeal swab was obtained within 1–2 days of onset of symptoms and tested for influenza A (H1N1) by PCR at our institution (GenProbe/Prodesse, San Diego, CA, USA) and confirmed as the 2009 Influenza A (H1N1) by the Wisconsin State Laboratory of Hygiene. To confirm nosocomial transmission, medical records of contact patients were reviewed to determine underlying co-morbid illnesses, treatment, timing of institution of isolation precautions, and outcomes. Testing was based on clinical symptoms and continued until no further symptomatic patients or healthcare workers were identified.

Sequencing of H1N1 viral RNA

To sequence hemagglutinin (HA) and PB2 genes, viral RNA (vRNA) was isolated from nasopharyngeal swabs from all four subjects, using the MinElute virus spin kit (Qiagen, Germantown, MD, USA). Sequence gaps in the samples from the second timepoint from Patient no. 2 were filled using a third-passage virus stock grown from the original nasal wash. Sequence of this expanded virus matched available contemporaneous *ex vivo* virus sequences (data not shown). vRNA from the *in vitro*-expanded stock was amplified using Qiagen's One-Step RT-PCR kit with PB2 and HA gene-specific primer sets developed by the WHO Collaborating Center for Influenza at the Centers for Disease Control and Prevention (CDC; primer sequences and protocol published at: http://www.who.int/csr/resources/publications/swineflu/sequencing_primers/en/index.html).

vRNA from patient samples was amplified using a nested approach: first, cDNA was generated using Invitrogen's SuperScript™ (Carlsbad, CA, USA) III First-Strand Synthesis kit with primer 5′ NCR 3′ (5′-AGCGAAAGCAGG-3′). Each RT reaction was followed by two 45-cycle PCR. The first PCR was performed using BioRad's (Hercules, CA, USA) iProof High-Fidelity DNA Polymerase, primers MBtuni-13 and MBtuni-12a (MBtuni-13 – 5′-ACGCG TGATCAGTAGAAACAAGG-3′ and MBtuni-12a – 5′-ACG CGTGATCAGCGAAAGCAGG-3′; originally described by Zhou *et al.*,[6] and the following cycling conditions: 98°C for 30 seconds, 45 cycles of 98°C for 15 seconds, 62°C for 30 seconds, 72°C for 2 minute, and a final extension time of 10 minute at 72°C. The second PCR was performed using Qiagen's HotStarTaq polymerase and the WHO PB2 and HA gene-specific primer sets. The cycling conditions for the second PCR were as follows: 95°C for 15 minute, 45 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 40 seconds, and a final extension time of 10 minute at 72°C. Both strands of each amplicon were sequenced on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were assembled using CodonCode Aligner version 3·5·6 (CodonCode Corporation, Dedham, MA, USA) and analyzed in MacVector 11·1·1 trial version (Accelrys, San Diego, CA, USA). Phylogenetic analyses were performed using mega5 software (Tamura *et al.*, 2011, Mol Biol Evol). Trees were constructed using the neighbor-joining method (Saitou and Nei, 1987, Mol Biol Evol, 4, 406–425) with a Tamura/Nei maximum composite likelihood distance correction. Accession numbers for these sequences are pending.

Ethical considerations

This investigation was initiated in response to a nosocomial outbreak of the 2009 novel influenza A (H1N1) virus and was conducted using standard infection control practices. Confidentiality of patient information, in accordance with HIPAA guidelines, was maintained at all times during the investigation. In preparation for publication, institutional review board consultation was obtained and verbal permission obtained from the patients' legal guardians.

Results

Table 1 shows the clinical characteristics of the patients. We did not collect data on the healthcare workers involved other than treatment and outcomes. The timeline of the outbreak is shown in Figure 1. The presumed index patient (Patient no. 1) was a 6-year-old immunocompromised boy who presented to an outpatient clinic with respiratory symptoms. Influenza was suspected, a nasopharyngeal swab was obtained and the decision for hospitalization was made later that evening when at home, the patient's fever and symptoms worsened. The patient was hospitalized on the pediatric hematology–oncology unit, but was inadvertently not placed in isolation immediately. Isolation was instituted upon confirmation of the diagnosis a day later.

Two days following admission of the index case, a healthcare worker (HCW no. 1) who took care of Patient no. 1 at the time of admission contracted the 2009 H1N1 influenza A virus and developed symptoms while at work. HCW no. 1 also cared for a 2-year-old immunocompromised child. This child subsequently developed H1N1 infection (Patient no. 2). Patient no. 2 had no contact with visitors nor any sick contacts. Two additional staff members (HCW no. 2 and no. 3) who were involved in the care of Patient no. 2 also developed influenza-like illness while at work, which was subsequently confirmed (HCW no. 2) or presumed (HCW no. 3) to be H1N1 infection. HCW no. 3 was presumptively treated without microbiologic confirmation. The patients

Table 1. Clinical characteristics of the patients involved in a nosocomial outbreak of the 2009 influenza A (H1N1) virus

	Patient no. 1	Patient no. 2
Sex	Male	Male
Age (years)	6	2
Medical history	Sickle cell anemia, asthma	Hepatoblastoma
Immunosuppression	Prednisone burst (15 mg × 5 days)	Chemotherapy
Reason for admission	Fever, cough	Diagnosis and treatment of hepatoblastoma
Duration of fever	3 days	Episode 1: 3 days Episode 2: 4 days
Treatment	Oseltamivir	Oseltamivir (both episodes)
Chest X-ray	No acute disease	No acute disease
Presentation	Fever, cough, wheezing, congestion, headache, myalgias	Fever, fussiness, congestion
Outcome	Discharged home on hospital day 2	Second episode occurred with initiation of chemotherapy (presumed reactivation), recovered

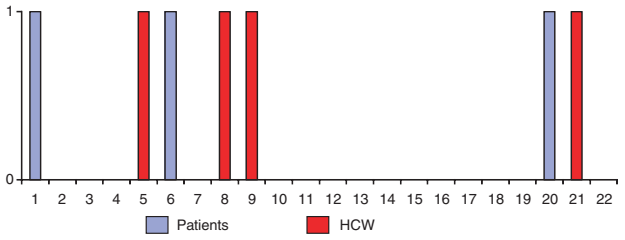

Figure 1. Epidemiologic curve. *X*-axis: day of outbreak, *Y*-axis: number of individuals infected per day. HCW, Healthcare worker.

were present on the same inpatient unit at the same time, but did not share rooms.

Details of clinical presentation and clinical course of patients

Patient no. 1 had a history of sickle cell anemia complicated by previous stroke, asthma with a concurrent prednisone treatment burst at the time of admission (15 mg × 5 days), and was being treated for suppurative otitis media with oral antibiotics and otic antibiotic drops. On the day before admission, the patient presented to the pediatric hematology clinic complaining of 1 day of fever to 103°F/39·4°C, abdominal pain, headache, body pain, cough, wheezing, and several days of nasal congestion. A chest radiograph showed no abnormalities. A nasopharyngeal swab for viral culture and H1N1 PCR testing was obtained, and he was started on oseltamivir and ceftriaxone (one dose) and given a previously scheduled blood transfusion. The patient returned home, but the fever worsened to 104°F/40°C (axillary), and the patient was admitted. After admission, all symptoms improved, the patient was afebrile by hospital day no. 2 and was discharged home approximately 48 hours after admission and completed 5 days of therapy.

Patient no. 2 was a 2-year-old boy, present on the inpatient pediatric hematology–oncology floor for over a month, receiving inpatient care for hepatoblastoma and associated complications. This patient was likely exposed to the 2009 influenza A (H1N1) virus by HCW no. 1, on day 4 of the outbreak, and became febrile on day 6. Staff and family noted increased fussiness and a runny nose prior to development of fever, but otherwise no new symptoms. Nasopharyngeal swabs were obtained within 24 hours of the onset of fever, and oseltamivir therapy and isolation precautions were initiated. The patient initially did well, was without fever after 48 hours, and isolation precautions were discontinued after 7 days (on day 13 of the outbreak). On day 13 of the outbreak, Patient no. 2 began a new cycle of chemotherapy and developed new fevers beginning on day 20 of the outbreak with no new symptoms noted other than nasal congestion on exam. Testing for respiratory viruses, including H1N1, was performed that same day, isolation and treatment with oseltamivir were reinitiated, and the patient gradually improved with resolution of fever after 4 days.

Control measures

In response to the outbreak, emphasis was placed on enhanced respiratory precautions. The nursing staff were cohorted and vaccinated with inactivated pandemic H1N1 vaccine. Other exposed patients, many of whom were immunocompromised, received oseltamivir prophylaxis. The prophylactic treatment was well tolerated, and no additional patients developed symptoms.

Initially, the outbreak appeared to be successfully contained and Patient no. 2, who had recovered well, was taken out of isolation precautions after 7 days. Approximately 1 week after the discontinuation of isolation precautions (14 days following the initial positive test), Patient

no. 2 underwent chemotherapy, developed fever, and a second nasopharyngeal swab tested positive for Influenza A (the 2009 pandemic H1N1 virus) by RT-PCR. Testing for other respiratory viruses was negative. A chest radiograph was normal. The patient remained hospitalized throughout this course of events. While it is possible that the second episode represented new nosocomial acquisition, given lack of evidence of ongoing transmission, reactivation is a more likely explanation of the patient's recurrent infection. A healthcare worker (HCW no. 4) caring for Patient no. 2 between episodes, while no isolation precautions were in place, subsequently developed H1N1 infection as well. Observations to determine HCW compliance with isolation precautions were undertaken during the period of the outbreak and increased over the course of a few days from 80% to 98%. However, observations were not undertaken routinely prior to the outbreak.

Clinical course of the healthcare workers

Three of the four healthcare workers tested positive for H1N1 at onset of symptoms – one was treated presumptively and did not undergo testing. All four healthcare workers were treated with oseltamivir for 5 days and responded to treatment. They returned to work 1 week after treatment was initiated. A follow-up nasopharyngeal swab was not required or obtained, but all were free of symptoms at the time of returning to work.

Viruses isolated from patients and healthcare workers were genetically identical

To determine whether these epidemiologically linked cases of influenza were initiated by a single source patient, we performed genetic analyses on viruses isolated from nasopharyngeal swabs taken from both patients and from the two healthcare workers who were tested. We first isolated influenza vRNA from each sample and determined its concentration using a quantitative reverse-transcription (QRT-) PCR assay. The concentration of viral vRNA ranged from 70 000 to 51 million copies/ml.

Because of the relatively low concentration of vRNA in Patient no. 2's samples, we chose to focus our genetic analysis on two of the eight segments of the influenza virus RNA genome. The highly variable HA gene encodes the viral attachment protein and is the major target of virus-specific antibodies, which can drive diversification of virus sequences.[7] We reasoned that if the nosocomial outbreak was initiated by more than one virus, then sequence differences distinguishing the strains would be most apparent in HA.

We also analyzed the basic polymerase subunit 2 (PB2) gene, which is more tightly conserved than HA, but encodes well-characterized pathogenicity determinants.[8] We were able to sequence full-length HA and PB2 genes directly from all available samples except for Patient no. 2's second sample; sequence data from this sample was, therefore, derived from an *in vitro*-expanded virus stock. The HA and PB2 segments of viruses isolated from all patients were completely identical to each other (Figure S1). blast database searches revealed no previously identified influenza viruses that matched these sequences exactly at the nucleotide level, but both the HA and PB2 genes in viruses isolated from these patients showed 99% identity to contemporaneous viruses isolated in Southeastern Wisconsin (http://www.ncbi.nlm.nih.gov/nuccore). To estimate the genetic distance of viruses in this outbreak from the contemporaneous virus population, we performed a phylogenetic analysis of HA and PB2 genes using the vaccine strain A/California/07/2009 (CA07; H1N1), isolated early in the pandemic, as an outgroup (Figure S2). Although the topologies of the HA and PB2 trees differed slightly, in both trees, the UW Hospital isolates clustered together, as expected for identical sequences. Indeed, the CA07 sequences, isolated in April 2009, clustered closely together with Southeastern Wisconsin isolates from September to December 2009, suggesting that all these viruses are closely related. Our results are consistent with the conclusion that Patient no. 1 had a community-acquired infection with 2009 influenza A (H1N1) virus and was the sole source of this nosocomial outbreak.

Discussion

The arrival of the 2009 novel influenza A (H1N1) pandemic posed several challenges to healthcare institutions.[3,9–15] More transmissible than seasonal influenza,[16] the large numbers of patients requiring hospitalization and ICU care strained available resources.[17,18] The addition of the N-95 respirator recommendation for healthcare workers to the usual droplet precautions employed for seasonal influenza also posed challenges in terms of tolerability and compliance.[19] This was particularly important when caring for small children in whom the potential for transmission to other individuals is heightened because of close contact, and patient compliance with a mask to contain secretions is highly variable. We found, as anticipated, that nosocomial cases of infection with the 2009 influenza A (H1N1) virus occurred with great ease in the absence of prompt implementation and strict adherence to isolation precautions. Although nosocomial transmission of influenza has been well described, not many studies have undertaken strain sequencing to confirm circulation of the same strain. These data were instrumental in motivating staff to improve adherence to influenza precautions.

There is considerable variation in the preparedness of healthcare facilities for the detection and containment of influenza. In a survey of Infectious Diseases Society of America, Emerging Infections Network members, Ortiz

et al.[20] found that only 10% of EIN respondents indicated that their principal hospital had a written policy to screen patients with febrile respiratory illnesses for influenza during the winter months. Overall, 35% of the EIN respondents indicated that their principal hospital had a written policy for controlling outbreaks. Of these policies, 22% reported the use of droplet precautions for confirmed cases, 23% undertook cohorting of patients, and 13% employed chemoprophylaxis for patients or staff.

The HA and PB2 segments of viruses isolated in this study were genetically identical and clustered together in phylogenetic trees of closely related viruses circulating contemporaneously in the same geographic area. This finding suggests nosocomial transmission of the same virus strain among patients and healthcare workers. It is important to note, however, that Sanger sequencing cannot reliably detect minor population variants present at <20–25% of viral sequences.[21,22] In contrast, a recent study of a 2009 H1N1 influenza infection clusters showed that "next-generation" sequencing methods can detect oseltamivir resistance mutations present in 9% of sequences in a clinical isolate.[22] Similarly, pyrosequencing of an oseltamivir-resistant H1N1 isolate revealed the presence of the canonical neuraminidase H275Y substitution in 19% of virus sequences, which Sanger sequencing could not detect.[23] Indeed, it appears likely that "next-generation" sequencing will reveal levels of intrahost influenza virus diversity that cannot be detected by conventional Sanger sequencing.[24] Thus, it is possible that the composition of virus populations in each patient and HCW in this study differed, and we cannot exclude the possibility that healthcare workers could have acquired very similar viruses from the community and not all transmissions in this cluster were nosocomial. However, we can conclude that the dominant virus populations (75–100% of viruses) in each subject in this cluster were completely identical. Moreover, no other completely identical sequences were detected in a survey of viruses circulating contemporaneously in Southeastern Wisconsin. The most parsimonious explanation of these data is that this cluster represents nosocomial transmission of a single virus "swarm" among patients and healthcare workers.

Our containment of the outbreak emphasizes several important points. A stringent policy should be in place to screen patients for respiratory symptoms at the point of entry into the healthcare institution and appropriate precautions should be undertaken immediately. In addition, our results and those of others suggest that reactivation and prolonged shedding occurs commonly in immunocompromised patients, especially children and the elderly, and isolation precautions should be prolonged, probably for the duration of hospitalization, far beyond the typical requirement for immunocompetent patients.[25–27] In this respect, it is perhaps surprising that the HA and PB2 sequences of the viruses isolated from Patient no. 2's first and second episodes of illness were identical. Such cases of prolonged virus shedding are likely to be particularly conducive to the emergence of immune-adapted virus variants, although few studies have examined this possibility in humans. Finally, cohorting of staff and the judicious use of chemoprophylaxis have been found to effectively interrupt influenza transmission and should be employed in outbreak situations.[18]

Our study has several limitations. Given the small numbers of affected patients and healthcare workers, we cannot draw conclusions regarding the route of transmission and the relative importance of each (airborne, droplet, fomites, or all). We did not undertake susceptibility testing of the virus. However, all the affected individuals responded to oseltamivir, and resistance to oseltamivir has been reported only rarely for the 2009 Influenza H1N1 strain, whereas resistance is common among recently circulating seasonal H1N1 strains.[28] Also, we did not sequence the entire genome of the virus isolated from these individuals, or use "next-generation" sequencing approaches, so it is possible that these viruses could harbor undetected sequence differences. Indeed, it is perhaps surprising that no sequence differences, even in HA, were seen after what were likely several serial passages, or after prolonged shedding in an immunocompromised host. This observation is in contrast to another recent study of a hospital outbreak in which a single amino acid substitution was detected in HA in the virus isolated from at least one patient.[29] Finally, we undertook several concurrent interventions to contain the outbreak, and it is not possible to arrive at conclusions regarding the utility of each individual intervention.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Results of sequencing data show that the HA and PB2 segments of viruses isolated from patients and healthcare workers in this study were completely identical to each other.

Figure S2. Phylogenetic analysis of HA and PBP2 genes.

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