

Transmission and Effect of Multiple Clusters of Seasonal Influenza in a Swiss Geriatric Hospital

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OBJECTIVES: To investigate a nosocomial outbreak of influenza.

DESIGN: Prospective outbreak investigation with active case finding and molecular typing.

SETTING: A large academic geriatric hospital in Switzerland.

PARTICIPANTS: Elderly hospitalized adults.

MEASUREMENTS: Based on syndromic surveillance, a nosocomial influenza outbreak was suspected in February 2012. All suspected cases were screened for respiratory viruses using real-time reverse transcription polymerase chain reaction of nasopharyngeal swabs. Infection control procedures (droplet precautions with single room isolation whenever possible) were implemented for all suspected or confirmed cases. Specimens positive for influenza viruses were processed and sequenced whenever possible to track transmission dynamics.

RESULTS: Respiratory samples from 155 suspected cases were analyzed during the outbreak period, of which 69 (44%) were positive for influenza virus, 26 (17%) were positive for other respiratory viruses, and 60 (39%) were negative. Three other cases fulfilled clinical criteria for influenza infection but were not sampled, and one individual was admitted with an already positive test, resulting in a total of 73 influenza cases, of which 62 (85%) were classified as nosocomial. Five distinct clusters of nosocomial transmission were identified using viral sequencing, with epidemiologically unexpected in-hospital transmission dynamics. Seven of 23 patients who experienced influenza complications died. Sixteen healthcare workers experi-

enced an influenza-like illness (overall vaccination rate, 36%).

CONCLUSION: Nosocomial influenza transmission caused more secondary cases than repeated community importation during this polyclonal outbreak. Molecular tools revealed complex transmission dynamics. Low healthcare worker vaccination rates and gaps in recommended infection control procedures are likely to have contributed to nosocomial spread of influenza, which remains a potentially life-threatening disease in elderly adults. *J Am Geriatr Soc* 63:739–744, 2015.

Key words: transmission; elderly; viral disease; health-care-associated infection; genome sequencing

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Elderly persons, especially those with underlying comorbidities, are at risk of developing complications after infections caused by influenza viruses.^{1,2} Influenza infection is of particular concern in confined environments with a high proportion of debilitated individuals, such as geriatric hospitals and long-term care facilities, where attack rates can reach 60%.^{3,4} Outbreaks of seasonal influenza are frequent in these settings, resulting in high morbidity and mortality of affected individuals.^{5,6}

An annual vaccination policy aimed at reaching as many older individuals as possible in the community, the application of strict infection control measures in hospitals, and yearly vaccination of healthcare workers (HCWs) have all been suggested as measures to reduce the burden of nosocomial influenza in elderly adults,^{7–9} but the results of these strategies are often disappointing.¹⁰ Inadequate immune response, a low proportion of vaccinated individuals, and the possible antigenic drift of circulating influenza viruses may impair the protective effect of influenza vaccination in elderly adults.^{11,12} The fact that isolation in single rooms or cohorting of infected patients is often not feasible further complicates control-

ling influenza in geriatric settings. In addition, adherence to specific infection control procedures tends to be lower in the geriatric setting, because collaboration of patients with regard to basic hygiene measures is difficult to achieve.¹³

There have been only a few reports about the epidemiology of seasonal influenza outbreaks in acute-care geriatric hospitals that also include a detailed molecular characterization of the circulating strains. Here, a large nosocomial outbreak of influenza A/H3N2 that occurred in a large geriatric hospital in Switzerland during the winter season 2011 to 2012 is described and a detailed virological and epidemiological analysis provided.

METHODS

Setting

The geriatric hospital in Geneva is a 294 bed-facility of the University of Geneva Hospitals system providing acute and intermediate care for approximately 4,000 individuals annually. The hospital is divided into 18 wards on five floors, with pairs of wards sharing the same corridor and some common rooms (e.g., television room) but with different teams of HCWs. Patients are hospitalized in the respective wards and floors based on the principal admission diagnosis and comorbidities (e.g., cognitive disorders, first and third floors; osteoporosis and orthopedic rehabilitation, ground floor; general internal medicine, second and fourth floors).

Infection Control Measures

During the winter season 2011 to 2012, the following infection control measures were recommended during the influenza season (as defined by the national influenza surveillance center located in Geneva), as previously described:¹⁴

For HCWs:

Zoning, which is restricted areas of care where HCWs have to be vaccinated or wear masks.

Information on the epidemic provided to HCWs by the infection control nurse in charge of the geriatric hospital.

For patients:

Droplet precautions for symptomatic patients pending virology results and until clearance of symptoms.

Separation of more than 150 cm between beds plus drawn curtains in multibed rooms.

Patient education for use of alcohol-based hand rubs and wearing of protective mask outside rooms.

No new admissions to wards with attack rates of 30% or greater.

For visitors:

Zoning application to visitors, regardless of symptoms. Alcohol-based hand rubbing for visitors plus protective mask in case of influenza-like symptoms, with information desks with alcohol-based hand-rubs, disposable masks, and posters provided at the entrance of the restricted areas.

Additional recommendations for physicians:

Test every patient with symptoms of an influenza-like syndrome.

Start oseltamivir treatment in each case with severe symptoms or in patients at high risk for complications.

All procedures and policies were made available online (vigigerme.hug-ge.ch).

Case Definitions and Surveillance

A case was defined as a patient presenting with influenza-like symptoms (defined as temperature $>37.8^{\circ}\text{C}$, with cough or sore throat¹⁵) with a nasopharyngeal swab positive for influenza virus A or B using real-time reverse transcription (rRT) polymerase chain reaction (PCR) or an epidemiological link with a proven case (hospitalization in the same room) in absence of other reasons for the symptoms. A case was defined as nosocomial for patients admitted to the hospital for reasons other than acute respiratory infection and in whom respiratory symptoms developed more than 72 hours after admission with a positive PCR result.¹⁶ An infection control nurse systematically tracked cases, encouraging virological sampling in each suspected influenza case. All positive cases were followed until discharge or death, and medical complications (e.g., pneumonia, respiratory failure, need for supportive measures, death) were recorded. The number of HCWs declaring influenza-like symptoms or leave of absence for the same reason was also documented, but pharyngeal swabbing was not performed in symptomatic HCWs.

VIROLOGICAL EXAMINATION AND PHYLOGENETIC TREE ANALYSIS

All suspected influenza cases were screened for respiratory viruses using nasopharyngeal rRT-PCR, as previously described.¹⁷ For molecular characterization of influenza-positive cases, 35 HA gene sequences underwent phylogenetic analysis. The goal was to characterize the different genetic groups of the circulating strains in the hospital and determine the putative common origin (clusters) to better understand nosocomial transmission dynamics. Viral specimens with a cycle value at threshold of <30 were processed and submitted to HA1 gene sequencing. Because of economic constraints, only one gene was determined using sequencing analysis. Primer sequences and PCR conditions were applied according to the standard operating procedures of the World Health Organization Collaborating Centre at the National Institute for Medical Research (London, UK). Ribonucleic acid sequences for the HA1 gene were assembled using a desktop software application framework for the organization and analysis of biological data, with a focus on molecular sequences and related data types (MUSCLE program, Geneious, Biomatters, Auckland, New Zealand).¹⁸ Overall, 986 nucleotides of the HA1 gene were extracted using a computer program that eliminates poorly aligned positions and divergent regions of an alignment of deoxyribonucleic acid or protein sequences (gBlocks, EMBL, Heidelberg, Germany)¹⁹ and maximum-likelihood trees were estimated using a software that estimates maximum likelihood phylogenies from

alignments of nucleotide or amino acid sequences (PhyML, South of France Bioinformatics Platform, Montpellier, France).²⁰ One thousand bootstrap replicates were performed using the general time-reversible model for correcting nucleotide substitution rates. HA1 sequences of 21 influenza viruses detected in the Swiss community using the Swiss Influenza Surveillance network were introduced in the phylogenetic analysis. All trees were rooted on the influenza A/Perth/16/2009 (H3N2) strain.

RESULTS

Epidemiological Investigation

Between February 3 and April 2, 2012, nasopharyngeal specimens were sampled from 155 suspected cases at the geriatric hospital and processed for virological investigation. Sixty samples proved negative (38%), whereas 95 were positive for respiratory viruses (62%), 69 of which were positive for influenza A/H3N2. The remaining were positive for *Coronavirus* (n = 14), *Metapneumovirus* (n = 6), *Picornavirus* (n = 3), and respiratory syncytial virus (n = 3). Influenza A (H1N1)pdm09 and influenza B viruses were not detected. One patient who tested positive had been sampled at the emergency department of the general hospital the day before the admission to the geriatric institution, and three were not sampled but fulfilled the clinical criteria for influenza infection, having clinical symptoms and signs of influenza and residing in close contact (same room) to patients with confirmed infection. Overall, 73 cases fulfilled the case definition for influenza infection (mean age 85.3, 62% female). Eleven (15%) had a positive nasopharyngeal swab within 72 hours after admission and were considered community-acquired. Sixty-two (85%) were considered healthcare associated, with an attack rate ranging between 12% and 34% in different wards.

The epidemic curve with weekly cases stratified according to floor level is shown in Figure 1. The first and third floors had the highest numbers of cases because of the lack of efficient single room isolation and droplet pre-

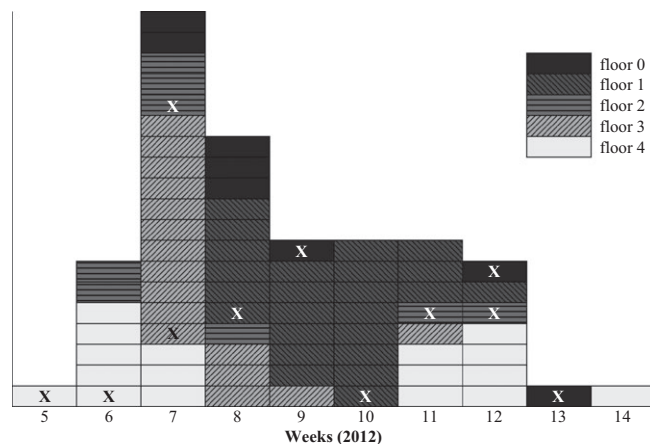


Figure 1. Weekly attack rate of patients affected by the influenza epidemic from February 3 to April 2, 2012, according to floor (n = 73). Each box represents one case. Boxes marked with X indicate community-acquired cases (influenza onset <72 hours after admission).

cautions. The ground, second, and fourth floors had fewer cases because of better isolation and cohorting practices. In particular, on the ground floor, most of the patients were bedridden for orthopedic problems or rehabilitation, without the ability to leave their rooms, whereas on the first floor, patients hospitalized in the ward for Alzheimer's disease and severe cognitive impairment were frequently unable to adhere to the implemented infection control procedures. A random audit of HCW adherence to advocated control measures revealed that only 25 of 48 observed HCWs were fully adherent (nurses, 63%; nursing aides, 58%; others, 20%).

Molecular Investigation

The HA1 gene sequence was obtained from 35 available influenza A (H3N2) genomes that underwent phylogenetic analysis. Viral sequences were genetically grouped into five clusters of various case sizes, named arbitrarily A to E, with 99% nucleotide homology shared in the same cluster (Figure 2). The overall transmission dynamics and clustering of cases is shown in Figure 3. Based on the phylogenetic analysis and distribution of cases, it was observed that Cluster A was limited to the first floor, except for one case on the third floor, Cluster B occurred in parallel to Cluster A and remained completely confined to the first floor, Cluster C started the outbreak and exclusively circulated between the third and fourth floors, and Cluster D was observed for only 2 weeks on the second and third floors and then disappeared. Cluster E was the smallest identified cluster and affected only three patients on the ground floor and another on the fourth floor a few weeks later.

The five clusters were distributed in three previously described genetic groups (GG 3B, 5, and 6). Clusters A and B consisted of six strains harboring the N145S, A198S, V223I, and N312S mutations specific to the previously described clade 3B. Clusters C, D, and E, of 11, seven, and four strains, respectively, had different mutations than the previously described D53N, Y94H, I230V and E280A mutations specific to GG 5 and GG 6 (Figure 2). Cluster C strains had the GG 5-specific mutation K2A. Cluster D and E strains had the GG 6-specific mutation S199A. The influenza A/Geneva/6036720/2012 virus has some mutations common with Clusters C and D but remained distinct from all previously described clades (Figure 2). Different origins of variants described using phylogenetic analysis demonstrated multiple introductions of community strains, refuting the hypothesis of a monoclonal, long-lasting outbreak by a single influenza strain transmitted within the institution and supporting the model of multiple virus importations leading to several nosocomial clusters limited in their duration and spread.

CLINICAL FEATURES AND VACCINATION STATUS

The median time from hospital admission to onset of influenza symptoms or virologically confirmed influenza was 21 days (interquartile range 17–50). Forty-three patients (40%) were given oseltamivir, and four (9%) of these were treated more than 72 hours after the onset of symptoms.

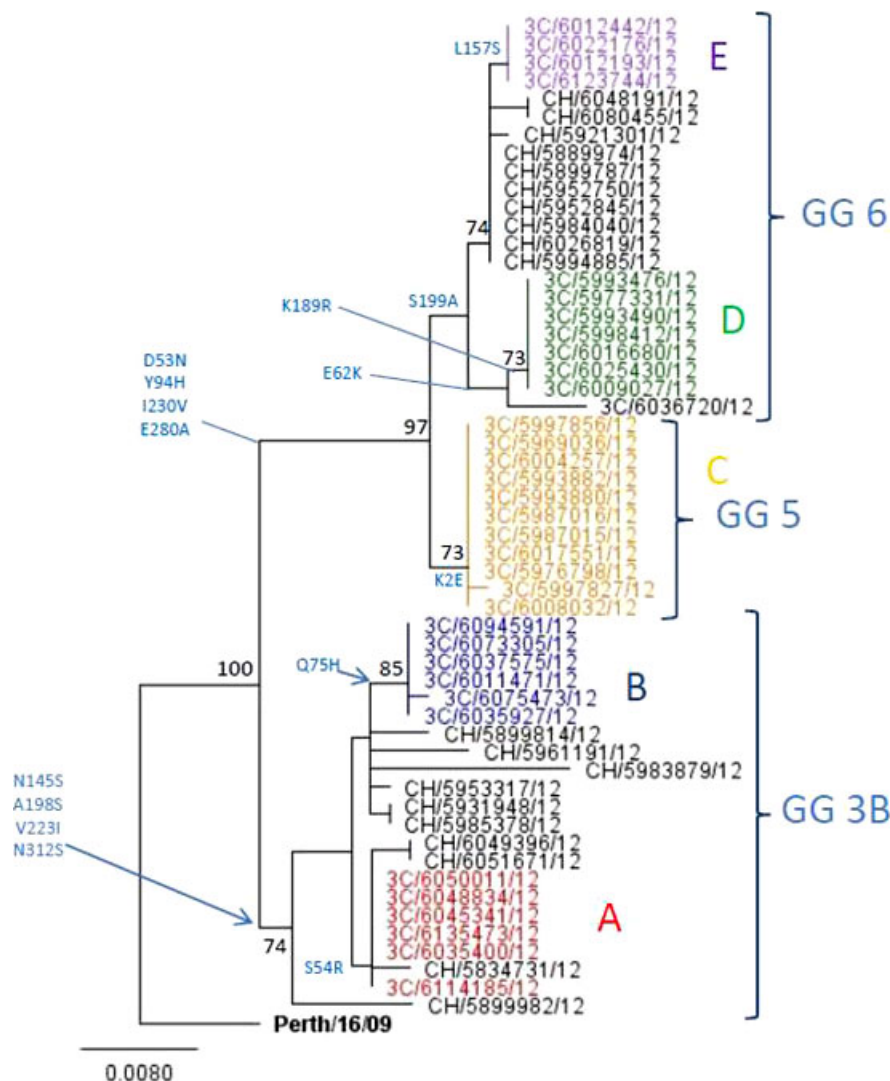


Figure 2. Phylogenetic tree analysis of HA gene sequence. Influenza A (H3N2) strains detected in the geriatric hospital (beginning with 3C, colored), in the community (beginning with CH, black), and reference strains (bold) are represented in the tree. Bootstrap values obtained with 1,000 replicates and higher than 70% are mentioned. Clusters A (red), B (blue), C (yellow), D (green), and E (purple) are labeled accordingly. The three genetic groups have also been labeled GG5, GG6, and GG3B. Mutations observed on HA amino acid sequence have been noted on the tree embranchments.

Preemptive oseltamivir treatment was given to two patients only, who were considered at high risk and resided in the same room with two proven cases.

Of 73 patients with infection, 23 (32%) experienced medical complications attributable to influenza. The most frequent complications were exacerbation of chronic pulmonary disease, secondary bacterial pneumonia, and requirement of enhanced care due to worsening of clinical status. Thirteen of these patients had been given oseltamivir appropriately. Seven patients died because of influenza-related complications (3/7 treated with oseltamivir).

Data regarding previous influenza vaccination of admitted patients were scanty and difficult to retrieve. On a random basis, the vaccination status of 13 patients in one ward, 10 of whom had been vaccinated at the beginning of the winter season, was checked.

Sixteen HCWs experienced an influenza-like illness during the outbreak period, three of whom had been

vaccinated. The number of sick HCWs stratified according to floor is shown in Figure 3. The influenza attack and vaccination rates for all HCWs working at the geriatric hospital during the winter season 2011 to 12 were 6.3% and 36%, respectively.

DISCUSSION

A large-scale outbreak of seasonal influenza in an academic geriatric hospital in Switzerland was traced. The principal findings of this investigation were that several concomitant factors may have contributed to multiple iterative clusters of nosocomial transmission (e.g., low HCW vaccination rates, suboptimal adherence to infection control practices); molecular tools substantially helped to understand transmission patterns and suggested multiple introductions of seasonal influenza A (H3N2) strains of different genetic groups from the community; and

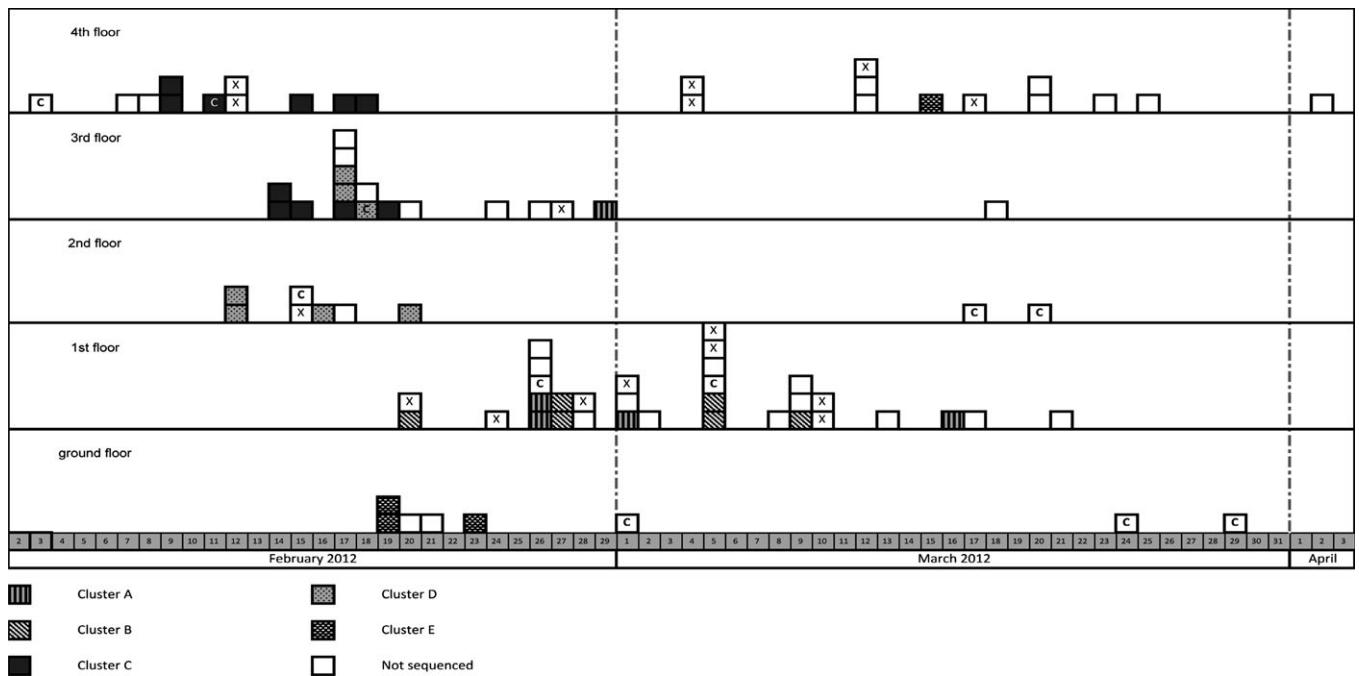


Figure 3. Viral transmission dynamics and clustering of suspected and confirmed influenza cases (patients and healthcare workers) according to hospital floor. White boxes represent influenza cases that were not available for molecular typing. C = community-acquired influenza case; X = healthcare worker with influenza-like illness.

one-third of patients experienced medical complications attributable to nosocomial influenza, with a case-fatality rate of 10%.

Several nosocomial influenza outbreaks have been reported in the medical literature. Seasonal influenza A strains circulating also in the surrounding communities caused most of these outbreaks. An analysis of three influenza outbreaks that occurred in nursing homes in the Spanish Navarra region found that influenza virus A (H3N2) was involved in all three outbreaks and that the genotyped strains were characterized as A/Stockholm/18/2011(H3N2), coinciding with the strain most frequently found in the general population during that season,²¹ although no advanced molecular analysis was performed, and it was therefore impossible to track the transmission dynamics within those institutions. The current findings are in concordance with details from a previous outbreak that was sustained by multiple entries of different viral clusters from the community.²² Likewise, a recent investigation of an influenza outbreak in a French hospital showed several clusters in patients and HCWs, confirming the current study’s findings that distinct influenza strains may circulate simultaneously in geriatric healthcare institutions.²³

This study has important implications for hospital epidemiologists and clinicians. During the early phase of an institutional influenza outbreak, high adherence to infection control measures is mandatory to contain further spread of influenza, but as observed on some floors of this geriatric hospital, several factors may contribute to viral spread: the multiple comorbidities of frail patients, the difficulties in effectively geographically isolating cases because patients share dining or common rooms as the only means of social interaction, the difficulties for many of those patients in

adhering to personal hygiene recommendations because of cognitive limitations, and the potential weakness of the immune response to influenza vaccination in elderly adults. In addition, asymptomatic cases (patients and HCWs) may be important vectors of influenza transmission. However, a recent meta-analysis failed to establish firm evidence that preventive strategies help contain the spread of influenza infection within institutions for elderly adults.²⁴ This lack of evidence to support any single preventive measure against influenza transmission in hospitalized elderly adults is due to the paucity of high-quality clinical trials and related methodological challenges, including molecular diagnostics and clustering effects.

The present outbreak investigation confirms the usefulness of molecular typing techniques to elucidate the epidemiology of influenza cross-transmission. Initially, it was hypothesized that a single strain would have affected the whole institution during the entire outbreak period; to the contrary, phylogenetic analysis revealed five different clusters of influenza viruses circulating on specific floors, linked to close contacts of index patients. This result suggests multiple introductions into the geriatric institution of influenza A (H3N2) variants of different genetic groups circulating in the community.

Elderly persons are at high risk of developing serious complications and functional decline after influenza infection. Vaccination has been advocated as the cornerstone to reduce influenza complications and deaths in elderly adults.²⁵ Older adults should benefit from vaccination, as well as close family contacts and HCWs.²⁶ Although definite evidence about the effectiveness of influenza vaccination of HCWs for reducing nosocomial spread is lacking,²⁷ several studies and a recent meta-analysis have suggested a benefit to residents of nursing homes when staff had high

vaccination rates.^{8,28} These findings indicate that the low vaccination rate of HCWs in the current authors' institution might have played an important role in sustaining the outbreak by fostering nosocomial circulation of influenza.

Oseltamivir prophylaxis in case of documented exposure may be considered for high-risk patients, but its effectiveness could not be evaluated because of the small number of patients who received antiviral prophylaxis in this study. Antiviral treatment was given to 60% of patients with influenza, roughly half the patients who subsequently experienced clinical complications. Three of the seven patients who died had received oseltamivir.

Some limitations of this study merit consideration. First, there is potential detection bias because the viral load in samples from elderly adults is lower than from younger adults, although detection of respiratory viruses using rRT-PCR in nasopharyngeal samples is a highly sensitive method, especially for influenza viruses.²⁹ Second, HCWs or visitors were not tested using pharyngeal swabbing because of logistical barriers and ethical concerns. This lack of virological confirmation may have inflated the number of HCWs with suspected influenza. Third, not all symptomatic patients were immediately swabbed, especially if they were hospitalized at the start of the outbreak in rooms distant from the index cases. Furthermore, not all influenza virus genomes were sequenced, so the overall number of cases could have been underdetected, or there could have been misclassification of community- and healthcare-acquired cases. Finally, it was not possible to verify the influenza vaccination status of most patients, so vaccine effectiveness could not be evaluated in this population.

There are few reports about the epidemiology of influenza transmission in geriatric hospitals. The present report demonstrates that large nosocomial outbreaks may occur within these institutions despite vaccination campaigns and efforts to improve preventive measures. It also shows that influenza remains a serious threat for institutionalized elderly people and highlights the need for better early syndromic surveillance and proactive infection control.

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REFERENCES

- Self WH, Griffin MR, Zhu Y et al. The high burden of pneumonia on US emergency departments during the 2009 influenza pandemic. *J Infect* 2014;68:156–164.
- Memoli MJ, Athota R, Reed S et al. The natural history of influenza infection in the severely immunocompromised vs nonimmunocompromised hosts. *Clin Infect Dis* 2014;58:214–224.
- Patriarca PA, Arden NH, Koplan JP et al. Prevention and control of type A influenza infections in nursing homes. Benefits and costs of four approaches using vaccination and amantadine. *Ann Intern Med* 1987;107:732–740.
- Libow LS, Neufeld RR, Olson E et al. Sequential outbreak of influenza A and B in a nursing home: Efficacy of vaccine and amantadine. *J Am Geriatr Soc* 1996;44:1153–1157.
- Brinkhof MW, Spoerri A, Birrer A et al. Influenza-attributable mortality among the elderly in Switzerland. *Swiss Med Wkly* 2006;136:302–309.
- Jhung MA, D'Mello T, Perez A et al. Hospital-onset influenza hospitalizations—United States, 2010–2011. *Am J Infect Control* 2014;42:7–11.
- Darvishian M, Gefenaite G, Turner RM et al. After adjusting for bias in meta-analysis seasonal influenza vaccine remains effective in community-dwelling elderly. *J Clin Epidemiol* 2014;67:734–744.
- Ahmed F, Lindley MC, Allred N et al. Effect of influenza vaccination of healthcare personnel on morbidity and mortality among patients: Systematic review and grading of evidence. *Clin Infect Dis* 2014;58:50–57.
- Rainwater-Lovett K, Chun K, Lessler J. Influenza outbreak control practices and the effectiveness of interventions in long-term care facilities: A systematic review. *Influenza Other Respir Viruses* 2014;8:74–82.
- Harbarth S, Siegrist CA, Schira JC et al. Influenza immunization: Improving compliance of healthcare workers. *Infect Control Hosp Epidemiol* 1998;19:337–342.
- Maltezou HC, Poland GA. Vaccination policies for healthcare workers in Europe. *Vaccine* 2014;32:4876–4880.
- Dolan GP, Harris RC, Clarkson M et al. Vaccination of healthcare workers to protect patients at increased risk of acute respiratory disease: Summary of a systematic review. *Influenza Other Respir Viruses* 2013;7(Suppl 2):93–96.
- Finnie TJ, Copley VR, Hall IM et al. An analysis of influenza outbreaks in institutions and enclosed societies. *Epidemiol Infect* 2014;142:107–113.
- Iten A, Siegrist CA, Kaiser L et al. Prévention de la grippe dans les hôpitaux: Un défi à relever. *Bulletin de l'Office Fédéral de la Santé Publique* 2012;41:696–698.
- Monto AS, Gravenstein S, Elliott M et al. Clinical signs and symptoms predicting influenza infection. *Arch Intern Med* 2000;160:3243–3247.
- Enstone JE, Myles PR, Openshaw PJ et al. Nosocomial pandemic (H1N1) 2009, United Kingdom, 2009–2010. *Emerg Infect Dis* 2011;17:592–598.
- Ambrosioni J, Bridevaux PO, Wagner G et al. Epidemiology of viral respiratory infections in a tertiary care centre in the era of molecular diagnosis, Geneva, Switzerland, 2011–2012. *Clin Microbiol Infect* 2014;20:O578–O584.
- Edgar RC. MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 2004;5:113.
- Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000;17:540–552.
- Guindon S, Dufayard JF, Lefort V et al. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst Biol* 2010;59:307–321.
- Castilla J, Cia F, Zubicoa J et al. Influenza outbreaks in nursing homes with high vaccination coverage in Navarre, Spain, 2011/12. *Euro Surveill* 2012;17. pii: 20141. PMID: 22516002.
- Jonges M, Rahamat-Langendoen J, Meijer A et al. Sequence-based identification and characterization of nosocomial influenza A(H1N1) pdm09 virus infections. *J Hosp Infect* 2012;82:187–193.
- Eibach D, Casalegno JS, Bouscambert M et al. Routes of transmission during a nosocomial influenza A(H3N2) outbreak among geriatric patients and healthcare workers. *J Hosp Infect* 2014;86:188–193.
- Thomas RE, Jefferson T, Lasserson TJ. Influenza vaccination for healthcare workers who care for people aged 60 or older living in long-term care institutions. *Cochrane Database Syst Rev* 2013;7:CD005187.
- McElhaney JE, Beran J, Devaster JM et al. AS03-adjuvanted versus non-adjuvanted trivalent influenza vaccine against seasonal influenza in elderly people: A phase 3 randomised trial. *Lancet Infect Dis* 2013;13:485–496.
- Fiore AE, Uyeki TM, Broder K et al. Prevention and control of influenza with vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Recomm Rep* 2010;59:1–62.
- Beyer WE, McElhaney J, Smith DJ et al. Cochrane re-arranged: Support for policies to vaccinate elderly people against influenza. *Vaccine* 2013;31:6030–6033.
- Carman WF, Elder AG, Wallace LA et al. Effects of influenza vaccination of health-care workers on mortality of elderly people in long-term care: A randomised controlled trial. *Lancet* 2000;355:93–97.
- Sethi S. Molecular diagnosis of respiratory tract infection in acute exacerbations of chronic obstructive pulmonary disease. *Clin Infect Dis* 2011;52 (Suppl 4):S290–S295.