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Epidemiology and molecular characterization of co-circulating influenza A/H3N2 virus variants in children: Houston, Texas, 1997–8

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SUMMARY

Co-circulating variants of influenza A/H3N2 viruses in children were studied in Houston, Texas between October 1997 and March 1998 to assess the effects of a new variant strain on the severity of clinical illness. Influenza A virus was isolated from the nasal wash or nasal aspirate specimens collected from children at two tertiary care hospitals, and 271 isolates were available for variant-specific subtyping using RT–PCR and restriction fragment length polymorphism (RFLP) analysis. We classified 124 (46%) influenza viruses as A/H3N2/Wuhan/359/95-like and 137 (50%) as A/H3N2/Sydney/05/97-like. Ten (4%) virus isolates could not be classified. Ill contacts in the household were reported more frequently in patients infected with A/Sydney-like viruses than in those infected with A/Wuhan-like viruses (85% *vs.* 71%, respectively, $P = 0{\cdot}02$). There were no differences in other demographic variables among children infected with these strains. This study found no increase in illness severity in children infected with a newly emerging strain.

INTRODUCTION

Influenza A viruses account for substantial excess morbidity and mortality each year, especially among persons of all ages with certain underlying conditions [1–8]. Emergence of novel influenza A virus variants may cause severe epidemics in populations lacking prior immunity [9–12]. The transmission of avian influenza A (H5N1) to human contacts in 1997 served as a startling reminder that pandemic influenza is a continuous threat to public health [13].

In recent decades, the influenza A virus subtype most commonly associated with epidemic influenza has been influenza A/H3N2 [14, 15]. Variants of influenza A/H3N2 viruses result from mutations in the haemagglutinin (HA) and neuraminidase (NA) genes that result in minor structural changes in these viral surface proteins. Influenza A/Sydney/05/97, a novel variant of influenza A/H3N2, was detected in the United States in June, 1997 and co-circulated with the existing influenza A/H3N2/Wuhan/359/95-like viruses, becoming predominant by the latter part of

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the 1997–8 influenza season [16]. This occurrence presented us with the unique opportunity to estimate the prevalence of each co-circulating virus variant during the influenza season, and to assess the effects of the new variant on the severity of clinical illness in children.

The present study sought to test the hypotheses that children who became infected with one of the co-circulating variants of influenza A/H3N2 virus during the 1997–8 influenza season differed in regard to demographic characteristics, and that infection with the novel strain, A/Sydney/05/97 (A/Sydney), resulted in more severe illness than the existing strain, A/Wuhan/359/95 (A/Wuhan).

METHODS

Population and study period

Data and samples were collected from children diagnosed with influenza A virus infection at Texas Children's Hospital or Ben Taub General Hospital in Houston, Texas between 14 October 1997 and 18 March 1998.

Specimen collection

Influenza A viruses were isolated from nasal wash or nasal aspirate specimens submitted to the Diagnostic Virology Laboratory at Texas Children's Hospital, Houston, Texas, for detection of respiratory viruses by routine culture or rapid antigen detection tests. Specimen collection and isolation of influenza A viruses were carried out by methods described previously [17, 18]. Isolates identified as influenza A viruses were harvested from cell cultures, labelled, and stored at −70 °C until RT–PCR analysis. Cell culture harvests not yielding a detectable product after the RT–PCR assay (described below) were inoculated onto monolayers of rhesus monkey kidney cells and virus was harvested as described previously [19]. The RT–PCR assay was then repeated using the harvests from these cultures.

RT–PCR assay

Extraction of viral RNA from cell culture harvests of influenza A viruses was performed using a modification [17] of the method described by Boom et al. [20]. Viral nucleic acids were then amplified by using a modification of previously described RT–PCR assays [19, 21]. Briefly, cDNA synthesis was performed by adding 15 μl of purified viral nucleic acids to a reaction mix containing 10 mM Tris hydrochloride [pH 8·3], 50 mM potassium chloride, 1·5 mM magnesium chloride, 3·3 μM of the primer HA282P (5′-CAGCAACTGTTACCCTTA-3′), 667 μM deoxynucleoside triphosphates, 20 unit Rnasin (Promega, Madison, WI) and 5 units AMV reverse transcriptase (Life Sciences, St. Petersburg, FL) in a final volume of 30 μl. The RT mix was incubated for 1 h at 43 °C; 70 μl of PCR mix was added to the RT mix to yield a solution containing 10 mM Tris hydrochloride (pH 8·3), 50 mM potassium chloride, 1·5 mM magnesium, 1 μM of each of the primers HA282P and HA507N (5′-TGGCATAGTCACTGGCAG-3′), 200 μM deoxynucleoside triphosphates and 5 units of *Taq* polymerase (Perkin Elmer, Norwalk, CT). The mixture was overlaid with mineral oil and amplified using a PTC-100 thermal cycler, in which the cDNA underwent initial heat denaturation at 94 °C for 5 min, followed by 40 cycles of heat denaturation at 52 °C for 1·5 min and primer extension at 72 °C for 1 min, and a final primer extension step at 72 °C for 5 min.

Restriction fragment length polymorphism (RFLP) assay

An RFLP assay was designed to distinguish A/Sydney/5/97-like strains from A/Wuhan/359/95-like strains. A multiple sequence alignment of A/Wuhan-like (GenBank® Accession numbers AF008722, AF038268) and A/Sydney-like (AF096306 to AF096316) strains was performed using CLUSTALW, and a *Hin*dIII restriction site unique to A/Sydney-like viruses and a *Bst*F5I restriction site unique to A/Wuhan-like viruses were identified in the amplified portion of the haemagglutinin gene; 1 μl (10 units) of *Hin*dIII (Boehringer Mannheim) and 1 μl (10 units) of *Bst*F5I (New England Biolabs, Beverly, MA) were added to two separate 10 μl samples of the amplicon, and the mixtures were incubated for 2 h at 37 °C and for 1 h at 65 °C, respectively. After the restriction endonuclease digestion, the PCR products were analysed by agarose gel electrophoresis using 1·5 SeaKem™ agarose as described previously [22]. Amplicons from A/Wuhan-like viruses yielded a single band 226 base pairs in length after restriction with *Hin*dIII and two bands 121 and 105 in length after restriction with *Bst*F5I. The restriction pattern from A/Sydney-like viruses resulted in two distinct bands 138 and 88 bp in length following digestion

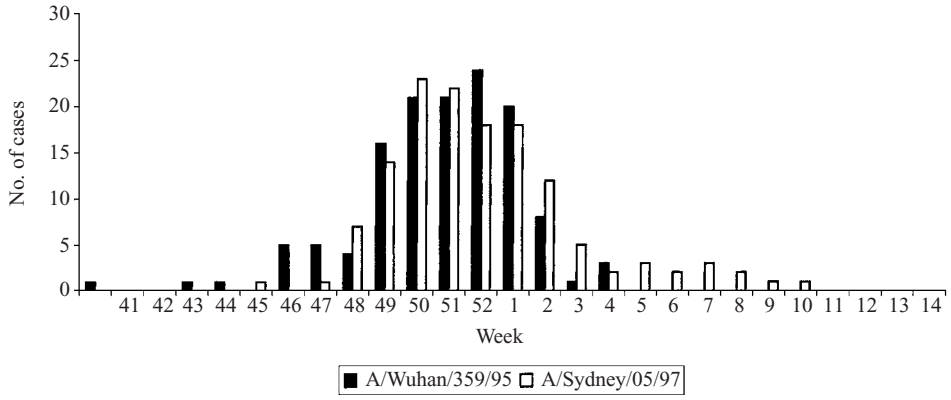

Fig. 1. Distribution of influenza A virus variants by week: Houston, 1997–8.

with *Hin*dIII and a single band 226 bp in length after digestion with *Bst*F5I. The banding patterns were identified and photographed using a UV transilluminator after staining the gel with ethidium bromide. The performance characteristics of the assay were confirmed using reference strains for A/Wuhan/359/95 and A/Sydney/05/97 and with clinical isolates that were characterized antigenically by the Centers for Disease Control and Prevention, Atlanta, GA, USA.

Demographic and clinical factors

Demographic and clinical data were collected from patient medical records by one of the investigators (F. M. M.) using a standard form (see Appendix). The following demographic factors were evaluated as potential risk factors for infection by each virus variant: age (0–<6 months, 6 months to <5 years or ⩾5 years), gender, ethnic origin (white, black, Hispanic or other), source of insurance (none, Medicaid or private insurance), and health status (presence or absence of at least one chronic underlying condition, including prematurity, pulmonary disease, cardiovascular disease, neuromuscular disease, AIDS, malignancy, immunotherapy and congenital anomalies).

In order to compare clinical characteristics of infection by each virus variant, the severity of illness was determined for each patient. A hierarchy of severity was constructed that treated lower respiratory illness (LRI) as the most severe outcome. LRI was defined as laryngotracheobronchitis, bronchiolitis or pneumonia diagnosed clinically and/or radiographically by the treating physician and listed as a discharge diagnosis. Otitis media (OM) or sinusitis (exclusive of LRI) was considered the second most severe outcome, and upper respiratory illness (URI), exclusive of LRI and OM, the least severe outcome. Each patient's influenza illness was assigned to one of these mutually exclusive categories. In addition, data were collected on the frequency of reported presenting symptoms, courses of antibiotics and antiviral medications prescribed at the time of diagnosis, and the occurrence of influenza-related complications including oxygen requirement, the need for mechanical ventilation, treatment with bronchodilators, dehydration and secondary bacterial infections. Finally, each patient was classified as ambulatory or inpatient, based on whether or not the influenza illness resulted in hospitalization.

Statistical analysis

Chi-square values were computed from multiway contingency tables to compare distributions of demographic variables and characteristics of influenza illnesses among those infected with each influenza virus variant (A/Wuhan-like or A/Sydney-like). Univariate Odds Ratios were calculated to evaluate the dependence of infection with influenza A/Wuhan or A/Sydney on the independent demographic variables (age, gender, ethnicity, source of insurance and health status). Initially, all variables were included in the multivariate model. Variables not associated with either virus variant were removed by backward-stepwise elimination to obtain the most parsimonious, biologically plausible model [23]. Multivariate proportional odds ratios were then computed for the variables remaining in the model. In all cases, results were considered significant when $P<0{\cdot}05$. All statistical analyses were performed with Epi Info 6, 1997 (Centers for Disease Control and Prevention, USA) and STATA 6.0, 1999 for PC (STATA Corp., College Station, TX, USA).

Table 1. *Demographic factors associated with influenza A viruses in children: Houston, 1997–8**

	Total No. (%)†	A/Wuhan/359/95 No. (%)	A/Sydney/05/97 No. (%)
Total	261 (100)	124 (100)	137 (100)
Gender			
Male	166 (63·6)	84 (67·7)	82 (59·9)
Female	95 (36·4)	40 (32·3)	55 (40·1)
Age‡			
<6 mo	98 (38·3)	50 (41·0)	48 (35·8)
6 mo–<5 yr	117 (45·7)	58 (47·5)	59 (44·0)
⩾5 yr	41 (16·0)	14 (11·5)	27 (20·2)
Ethnic Origin§			
White	51 (22·3)	24 (22·9)	27 (22·0)
Black	74 (32·5)	36 (34·3)	38 (30·9)
Hispanic	99 (43·4)	42 (40·0)	57 (46·3)
Other	4 (1·8)	3 (2·9)	1 (<1·0)
Insurance¶			
Medicaid	108 (47·8)	50 (48·1)	58 (47·5)
Private	88 (38·9)	42 (40·4)	46 (37·7)
None/self	30 (13·3)	12 (11·5)	18 (14·8)
Health status‖			
Previously healthy	157 (69·2)	72 (69·2)	85 (69·1)
At least one underlying condition	70 (30·8)	32 (30·8)	38 (30·9)
Exposures			
Daycare or school	53 (39·0)	17 (30·4)	36 (45·0)
Siblings	121 (68·4)	50 (62·5)	71 (74·0)
Ill contacts	139 (79·0)	57 (71·3)	82 (85·4)

* Chi-square tests were used to compare values in each group. With the exception of ill contacts in the household ($P=0·02$), no other demographic variable was significantly associated with infection by either virus variant.

† Percentages based on the total number of patients for whom information on each demographic variable was available (No. does not equal 261 for all variables). Due to rounding, percentages may not sum to 100.

‡ Age unknown for 5 patients.

§ Ethnic origin unknown for 33 patients.

¶ Insurance status unknown for 35 patients.

‖ Health status unknown for 34 patients.

RESULTS

Influenza A virus isolates collected from 271 patients at two large tertiary care hospitals between October 1997 and March 1998 were available for variant specific subtyping. Of these, 10 (4%) could not be classified as either variant because they either failed to amplify any detectable product by RT–PCR ($n=7$), or did not give a conclusive result by RFLP analysis ($n=3$). Insufficient quantities of the virus harvests were left over to allow further evaluation of these specimens to determine the reasons for the observed results. Of the remaining 261 influenza A/H3N2 viruses, 124 (48%) were classified as A/Wuhan/359/95-like and 137 (52%) were classified as A/Sydney/05/97-like. Influenza activity peaked in Houston during the week ending 27 December (Fig. 1). A χ^2 test indicated that the proportion of influenza A infections attributable to A/Sydney/05/97-like viruses increased during the period between 14 October 1997 and 18 March 1998, while the proportion due to A/Wuhan/359/95-like viruses decreased ($P=0·001$).

Patients infected with A/Wuhan-like virus were similar to those infected with A/Sydney-like virus with respect to gender, ethnic origin and insurance status (Table 1). We did not observe any association

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Table 2. *Prevalence of underlying conditions by variant-specific subtype: Houston, 1997–8**

	Total		A/Wuhan/359/95		A/Sydney/05/97	
	No.	Prevalence	No.	Prevalence	No.	Prevalence
Total†	226	1·00	104	1·00	122	1·00
At least one underlying condition	70	0·31	32	0·31	38	0·31
Prematurity	21	0·09	12	0·12	9	0·07
Pulmonary disease	28	0·12	9	0·09	19	0·16
Cardiovascular disease	14	0·07	7	0·06	7	0·06
Neuromuscular disease	13	0·06	6	0·06	7	0·06
Secondary						
Immunodeficiency‡	6	0·03	5	0·06	1	0·01
Concomitant infection§	19	0·08	9	0·08	10	0·09
Congenital anomaly¶	9	0·04	6	0·06	3	0·02
Other conditions‖	8	0·04	4	0·04	4	0·03

* None of the selected underlying conditions was significantly associated with infection by either virus variant.
† Includes only those patients for whom clinical records were available ($n=226$).
‡ Malignancy + immunotherapy ($n=5$), AIDS (1).
§ Bacteraemia (1), RSV (13), hepatitis A (1), adenovirus (1), conjunctivitis (1), picornavirus (1), bacterial infection (1).
¶ Treacher Collins syndrome (1), Down syndrome (5), duplicate ureter system (1), Williams syndrome (1), cleft palate (1).
‖ Obesity, sleep apnea (1), diabetes (1), haemoglobinopathy (6).

between age and infection with either virus variant. No association was observed either between daycare and school attendance or the presence of siblings in the household with either virus variant. The proportion of patients infected with influenza A/Sydney-like viruses who reported ill contacts (children or adults) in the household (85%) was significantly higher than the proportion of patients infected with influenza A/Wuhan virus reporting ill household contacts (71%) ($P=0·02$).

The prevalence of selected underlying medical conditions among those infected with each virus variant is shown in Table 2. Of 226 children for whom clinical records were available, 70 (31%) reported at least one chronic underlying medical condition. The prevalence of each underlying condition was similar among patients infected with either virus variant. Adjusting for age had no effect on this relationship.

The distribution of illness severity among subjects with each influenza A virus variant, as well as the prevalence of resulting symptoms and complications, is presented in Table 3. Of the 226 patients with available clinical information, 73 (32%) had lower respiratory illnesses, 22 (10%) had otitis media, exclusive of LRI, and 131 (58%) had upper respiratory illnesses, exclusive of LRI and OM. The severity of illness was similar for patients infected with influenza A/Wuhan-like virus and those infected with A/Sydney-like virus. Further analysis revealed no significant association between variant and severity of illness for any age group, nor between age and severity (data not shown). The prevalence of various symptoms and prescriptions for antibiotics and antiviral medications were also found to be independent of virus variant. Among the influenza-related complications examined, the need for mechanical ventilation was not significantly related to virus variant, but was associated with the presence of underlying medical conditions. Of the 70 patients with at least one underlying condition, 7 (10%) required mechanical ventilation, *vs.* only 3/157 (2%) patients with no underlying conditions ($P=0·005$) (data not shown).

Need for hospitalization was not associated with age or with virus variant. However, 70% of patients with at least one underlying medical condition required hospitalization compared with only 40% of previously healthy individuals ($P<0·001$) (Table 4).

After adjusting for selected demographic characteristics (age, health status and ill contacts in the household), multivariate regression analysis showed that the presence of ill contacts in the household was more strongly associated with infection by A/Sydney-like virus than with A/Wuhan-like virus (Table 5). Proportional odds ratios computed after adjusting for age and ill contacts were not markedly different from univariate estimates, indicating that the confounding effects of these covariates are likely to be small.

Table 3. *Prevalence of clinical factors by variant-specific subtype: Houston, 1997–8**

	Total		A/Wuhan/359/95		A/Sydney/05/97	
	No.	Prevalence	No.	Prevalence	No.	Prevalence
Total†	226	1·00	104	1·00	122	1·00
Severity of illness						
LRI‡	73	0·32	34	0·33	39	0·32
Otitis media	22	0·10	7	0·07	15	0·12
URI	131	0·58	63	0·61	68	0·56
Symptoms						
Fever	214	0·95	103	0·95	111	0·94
Rhinhorrea/congestion	158	0·70	73	0·68	85	0·72
Cough	160	0·71	78	0·72	82	0·69
Dyspnoea	41	0·18	19	0·18	22	0·19
Vomiting/diarrhea	60	0·27	28	0·26	32	0·27
Decreased oral intake	31	0·14	14	0·13	17	0·14
Other§	62	0·27	28	0·26	34	0·29
Medications prescribed						
Antibiotics	110	0·49	51	0·47	59	0·50
Antivirals	34	0·15	19	0·18	15	0·13
Hospitalization¶						
Ambulatory	118	0·51	53	0·50	65	0·52
Admission	112	0·50	53	0·50	59	0·48
Complications						
O_2 requirement	31	0·14	14	0·14	17	0·14
Mechanical ventilation	10	0·04	2	0·02	8	0·07
Secondary						
Bacterial infection	3	0·01	2	0·02	1	0·02
Inhalation therapy	54	0·24	23	0·21	31	0·26
Dehydration	12	0·05	5	0·05	7	0·06
Other‖	5	0·02	2	0·02	3	0·03

URI, upper respiratory illness; LRI, lower respiratory illness.

* None of the selected clinical factors was significantly associated with infection by either virus variant.

† Includes only those patients for whom clinical records were available ($n=226$).

‡ Includes 1 patient with febrile seizure disorder and 1 patient with myocarditis.

§ Includes myalgia, headache, irritability, febrile seizures, abdominal pain, chest pain, eye discharge, syncope and rash.

¶ Hospitalization status was known for 6 patients despite the unavailability of their clinical records.

‖ Mesenteric adenitis + acute sinusitis (1 case), myocarditis (1), syncope + hypertension (1), thrombocytopenia (1), haemodialysis (1).

DISCUSSION

The present study examined factors related to influenza illness at two levels. Demographic variables such as age, gender, insurance status, ethnicity, health status and exposures such as siblings or ill contacts in the household and daycare or school attendance were tested as predictors of infection by A/Sydney-like or A/Wuhan-like viruses. We then studied the relationship between infection by each virus variant and resulting clinical outcomes; specifically, severity of illness, frequency of hospitalization and occurrence of influenza-related complications.

Distribution of influenza A virus variants by week between 14 October 1997 and 18 March 1998 was consistent with surveillance reports published during the same time period, which confirmed that the novel influenza A/H3N2 variant, A/Sydney/05/97, co-circulated in the United States with A/Wuhan/359/95 during the 1997–8 influenza season, and was predominant during the latter part of that season [13,16].

The presence of ill contacts in the household was reported more frequently by patients infected with influenza A/Sydney-like virus than by those with influenza A/Wuhan-like virus infection. This difference

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Table 4. *Prevalence of chronic underlying conditions among patients with selected demographic factors and clinical outcomes: Houston, 1997–8*

	Chronic underlying conditions		
	At least one No. (%)*	None No. (%)*	*P* value
Total	70 (30·8)	157 (69·2)	
Age			
<6 mo	15 (21·7)	68 (43·3)	<0·001
6 mo–<5 yr	33 (47·8)	75 (47·8)	
⩾5 yr	21 (30·4)	14 (8·9)	
Requiring hospitalization			
Total	49 (70·0)	62 (39·5)	
Age			
<6 mo	9 (18·8)	32 (51·6)	<0·001
6 mo–<5 yr	24 (50·0)	25 (40·3)	
⩾5 yr	15 (31·3)	5 (8·1)	
Severity of illness			
LRI	30 (44·1)	43 (27·4)	0·047
Otitis media	5 (7·4)	17 (10·8)	
URI	33 (48·5)	97 (61·8)	

URI, upper respiratory illness; LRI, lower respiratory illness.
* Represents the proportion of patients with and without chronic underlying conditions fitting the demographic criteria or experiencing the clinical outcome.

Table 5. *Estimated effects of demographic factors on infection by influenza A viruses in children: Houston, 1997–8*

	No. of cases (%)		Unadjusted		Adjusted*	
Covariate	A/Wuhan	A/Sydney	OR†	95% CI	OR†	95% CI
Age						
<6 mo	50 (41·0)	48 (35·8)	1·0		1·0	
6 mo–<5 yr	58 (47·5)	59 (44·0)	1·1	(0·6–1·8)	1·4	(0·7–2·8)
>5 yr	14 (11·5)	27 (20·2)	2·0	(0·9–4·2)	2·4	(0·8–7·0)
Underlying conditions						
None	72 (69·2)	85 (69·1)	1·0		1·0	
At least one	32 (30·8)	38 (30·9)	1·0	(0·6–1·8)	1·1	(0·5–2·3)
Exposures						
Ill household contacts	57 (71·3)	82 (85·4)	2·4	(1·1–4·9)	2·6	(1·2–5·7)

* Adjusted for the effects of age, chronic underlying medical conditions and ill household contacts.
† Proportional odds of infection by A/Sydney using infection by A/Wuhan as the referent.

may be related to prior immunity to influenza A viruses, as described previously [24, 25]. It is possible that family members of patients infected with A/Wuhan were more likely to have prior immunity to this virus variant than family members of patients infected with the new virus variant, influenza A/Sydney. However, in a previous study it was found that natural infection by A/Wuhan-like virus in children resulted in cross protection against subsequent infection by A/Sydney-like virus [26].

No significant association was observed between age and infection by either virus variant. It was expected that children age ⩾5 years with apparent influenza illness would more likely be infected with

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A/Sydney-like virus than with A/Wuhan-like virus, to which they were more likely to have prior immunity. A greater proportion of children in this age group were infected with A/Sydney compared to other age groups, but the difference was not significant ($P = 0{\cdot}16$). Because children age $\geqslant 5$ years represented only 16% of the total sample, we may have had insufficient power to detect a difference in the prevalence of each virus variant within this age group.

Similarly, it was expected that a novel variant of influenza A/H3N2 virus such as A/Sydney/05/97 would cause more severe illness than A/Wuhan-like viruses, due to lower levels of immunity to the novel virus variant. However, the severity of illness experienced by those infected with each virus variant was similar. Adjusting for age had no effect on this relationship, indicating that illness severity was not associated with the variant strain for any age group.

The occurrence of influenza-related complications was similarly distributed among individuals infected with each virus variant. Although no association was found between age and severity of illness (Table 2), children $\geqslant 5$ years of age were significantly more likely to have at least one chronic underlying medical condition than younger children ($P < 0{\cdot}001$) (Table 4). Patients with at least one chronic underlying condition were more likely to have a more severe illness (LRI and OM > URI) than patients without underlying conditions ($P = 0{\cdot}05$).

The proportion of subjects requiring hospital admission was similar for those infected with each virus variant ($P = 0{\cdot}71$). We sought to determine whether the novel variant A/Sydney-like virus was more likely than A/Wuhan-like virus to result in proportionately more hospitalizations for certain age groups. The proportion of patients with each variant requiring hospitalization was similar in all age groups. Adjusted for health status, however, children age $\geqslant 6$ months with at least one underlying condition were hospitalized more frequently than children of the same age with no underlying conditions ($P < 0{\cdot}001$) (Table 4). This is consistent with previous studies, which have demonstrated an association between chronic underlying conditions and increased risk of hospitalization and serious complications resulting from respiratory virus infections [16, 27, 28].

By previous estimates, attack rates of influenza among children during epidemics have been near 30–40% [29], with hospitalization rates that can be as high as 2–7/1000 children <2 years of age. Because our sample was limited to patients who sought medical treatment for influenza-related illnesses at a large tertiary care hospital, patients were included with more severe illness; therefore, they probably had less preexisting immunity to either of the variants than others in their age range despite the fact that H3N2 viruses had circulated regularly during the previous years. Children in our sample were also more likely to have high-risk conditions, possibly overestimating the severity and occurrence of influenza related complications experienced by the general population. Although nearly one third of the patients in our study had at least one underlying condition, information on current influenza immunization status was not collected prospectively. However, in a similar study of a the impact of influenza in children at our hospital, the rate of vaccination was recently found to be less than 5% in children for whom the vaccine would have been recommended [30]. Individuals age $\geqslant 6$ months with underlying conditions should be offered annual influenza immunization [8, 31–34]. Low (<30%) rates of immunization of children at risk have also been described by other investigators [35].

Our results indicate that a newly emerging variant of influenza A/H3N2, A/Sydney/05/97, was not associated with increased illness severity among previously healthy children and children with underlying conditions during the winter of 1997–8. Our use of RT–PCR–RFLP demonstrates that a large number of isolates can be screened and characterized rapidly and at a lower cost than direct sequencing of PCR products if restriction sites can be identified to distinguish variants. In this study, *Hin*dIII and *Bst*F5I restriction sites unique to A/Sydney-like and A/Wuhan-like viruses, respectively, allowed distinction of variants that shared 97–8% identity in the nucleotide sequence of the haemagglutinin genes. Similar studies have been performed by other investigators to identify virus subtype and variants, including distinguishing A/Wuhan-like viruses from A/Sydney-like viruses [36–40]. Classification of influenza A viruses by variant could be especially useful at the start of an influenza epidemic when it could provide timely information about antigenic changes to influenza viruses. Routine use of variant-specific typing could help meet the US Public Health Service's goals of enhancing current surveillance efforts and improving pandemic preparedness [22, 41, 42].

APPENDIX

Data collection form

IRB: H-520 **Study Number**:_____

INFLUENZA STUDY
DATA COLLECTION FORM

DEMOGRAPHIC INFORMATION

Medical Record Number: _____

Emergency department visit: ☐ Yes ☐ No
Date of visit: ____/____/____

Hospital admission: ☐ Yes ☐ No
Date of admission: _____/_____/_____ Date of discharge: _____/_____/_____
Location: ☐Ward ☐PICU ☐PCU ☐NICU Other: _____
Disposition: 1. Discharged home Total Hospital days: _____
2. Death Total Hospital charges: _____
3. Other: _____

Nosocomial infection: ☐ Yes ☐ No
Influenza immunization: ☐ Yes ☐ No Date: ____/_____/_____

Date of birth : _____/_____/_____ Age:_____ Gender: ☐M ☐F
Race: C=Caucasian, B=Black, H=Hispanic, A=Asian, O=Other
Origin: Zip code of residence:_____ County: _____
Source of insurance: ☐ Medicaid ☐ Private ☐ None

CLINICAL INFORMATION

Discharge Diagnosis: _____
_____
_____

Influenza illness:
☐ Upper respiratory tract
☐ Lower respiratory tract
☐ Otitis media
☐ Other _____

Symptoms: Days of illness prior to admission _____
☐ Fever
☐ Rhinorrea/congestion
☐ Cough
☐ Shortness of breath/difficulty breathing
☐ Vomiting/diarrhea
☐ Other _____

Underlying condition:
☐ No underlying conditions
☐ Prematurity
☐ BPD
☐ Asthma
☐ Other lung disease _____
☐ Cardiovascular disease _____
☐ Neuromuscular disease _____
☐ Primary immunodeficiency _____
☐ Secondary immunodeficiency: ☐AIDS
☐Cancer _____
☐ Transplant recipient Organ:_____
☐ Immunosupressive therapy _____
☐ Concomitant infection _____

Exposures: Daycare attendance: ☐Y ☐N
Duration:_____
Number of siblings:_____
Ill contacts in household: ☐Y ☐N

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Complications: ☐ Oxygen requirement
☐ Intubation/mechanical ventilation
☐ Secondary bacterial infection: _____
☐ Other _____

LABORATORY EVALUATION:

Chest X Ray: ☐ Normal
☐ Abnormal : ☐ Pneumonia ☐ Bronchiolitis ☐ Atelectasis ☐'Viral'
☐ Other _____
Rapid influenza ☐ Positive ☐ Negative
Influenza culture ☐ Positive ☐ Negative Source _____
Other virus Cx ☐ Positive ☐ Negative Virus/Source _____
Rapid RSV ☐ Positive ☐ Negative
Bacterial cultures

Blood	CSF	Respiratory	Urine	Stool	Other

ANTIBIOTICS/ANTIVIRALS ☐ Yes ☐ No

Drug	Dose	Duration

PATHOLOGY

Cause of death as per autopsy report

_____
_____
_____

COMMENTS

_____
_____
_____

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