

# Surveillance of 16 Respiratory Viruses in Patients With Influenza-Like Illness in Nanjing, China

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Much less is known about the etiology of influenza-like illness in China. A continuous surveillance of 16 respiratory viruses was conducted from November 2010 to October 2011 of outpatients with influenza-like illness in Nanjing, China. The two largest general hospitals and a provincial virus laboratory in Nanjing participated in this study. Throat swabs were collected from outpatients during medical visits for influenza-like illness and were tested for 16 respiratory viruses using PCR. Three hundred seventeen viruses were detected in samples from 246 (50.6%) patients with influenza-like illness. The viruses found mostly commonly were influenza, rhinovirus, hCoV HKU1, and adenovirus. The identification rates of respiratory viruses differed significantly among different sampling seasons ( $P = 0.0002$ ). The rates of influenza A and hCoV HKU1 were much higher during the influenza-like illness winter peak than during the influenza-like illness summer peak and other months. Co-infections were detected in 57 (11.7%) patients and were found most commonly in adults older than 60 years. RSV was detected in 5.9% and 2.6% of patients who were 0–5 and 6–15 years old, respectively, but was not detected in other age groups. This study confirmed that multiple respiratory viruses may circulate concurrently in the population and account for a large proportion of influenza-like illness. In addition to influenza virus, hCoV HKU1 may be associated with the influenza-like illness winter peak in Nanjing, China. *J. Med. Virol.* 84:1980–1984, 2012. © 2012 Wiley Periodicals, Inc.

**KEY WORDS:** respiratory viruses; influenza-like illness; surveillance; outpatients

## INTRODUCTION

Acute respiratory infections impose a significant burden of both morbidity and mortality worldwide.

The morbidity from acute respiratory infections is roughly the same in developing as in developed countries, but the associated mortality is many times higher in developing countries [Leowski, 1986]. Influenza-like illness accounts for about 62% of acute respiratory infections [Heijnen et al., 1999]. Viral pathogens are the most significant contributors [Laguna-Torres et al., 2011]. Most of the data on the epidemiology of the etiologic agents of influenza-like illness come from more developed regions of the world, including the United States and Europe. In the case of influenza-like illness, influenza viruses are detected commonly [Puzelli et al., 2006], followed by parainfluenza viruses, respiratory syncytial viruses, and adenoviruses [Heijnen et al., 1999; Gwaltney, 2002]. However, much less is known concerning the etiology of influenza-like illness besides influenza viruses in China. The objective of this study was to identify the viruses associated with influenza-like illness in Nanjing, China.

## MATERIALS AND METHODS

### Study Design and Inclusion Criteria

The two largest general hospitals and a provincial virus laboratory in Nanjing participated in this study. Throat swabs were collected from outpatients during medical visits for influenza-like illness with the criteria that the time interval between the onset of symptom and the hospital visit must be less than 72 hr. Five to 15 swabs were collected each week per hospital depending on the numbers of influenza-like illness visits. Influenza-like illness was defined as a sudden onset of fever of 38°C or above, with cough

The authors have declared that no competing interests exist.

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Accepted 11 July 2012

DOI 10.1002/jmv.23401

Published online in Wiley Online Library (wileyonlinelibrary.com).

or pharyngitis. The study was conducted from November 2010 through October 2011 and was approved by the Ethics Committee of Jiangsu Provincial Center for Disease Control and Prevention. Written informed consent was obtained from all participants and their parents (for individuals <18 years).

### Samples and Data Collection

Throat swabs were taken from individuals with influenza-like illness. The nurses, who were trained by Jiangsu Provincial Center for Disease Control and Prevention, were in charge of swabbing. The swabs were sent in 3 ml of viral transport medium to the virus laboratory within 48 hr after collection and were stored in  $-70^{\circ}\text{C}$ . Detailed demographic and clinical data were obtained from the patients.

### Nucleic Acid Extraction and Detection by PCR

The total nucleic acids were extracted and eluted automatically using the MagMAX Viral Nucleic Acid Isolation kits (Austin, TX) and MagMAX Express -24 (Applied Biosystems, Austin, TX), according to the manufacturer's instructions. Sixteen respiratory viruses were tested, that is, influenza viruses (types A, B, and C), parainfluenza viruses (PIV, types I, II, III, and IV), human coronaviruses (hCoV, types 229E, OC43, NL63, and HKU1), rhinovirus (RV), human metapneumovirus (hMPV), respiratory syncytial virus (RSV), adenovirus (AdV), and human bocavirus (hBoV). Thermocycling RT-PCR conditions for RNA viruses were as follows:  $50^{\circ}\text{C}$  for 15 min, 10 min at  $95^{\circ}\text{C}$ , and 40 cycles of  $95^{\circ}\text{C}$  for 15 sec and  $60^{\circ}\text{C}$  for 45 sec, while PCR conditions for DNA viruses were  $50^{\circ}\text{C}$  for 2 min, 10 min at  $95^{\circ}\text{C}$ , and 40 cycles of  $95^{\circ}\text{C}$  for 15 sec and  $60^{\circ}\text{C}$  for 1 min. The fluorescent (RT-) PCR was performed using Fluorescence (RT-) PCR Detection kits (Nanjing, Jiangsu Province, China).

### Statistical Analyses

According to the surveillance data of influenza-like illness conducted in the two general hospitals simultaneously, January was defined as influenza-like illness winter peak and June–August was defined as influenza-like illness summer peak. As in January, the proportion of influenza-like illness in outpatients was 3.5%, in June, July, and August the proportions were all above 2% (i.e., 2.1% for June, 2.2% for July, and 2% for August) while in other months the proportions were all below 2%. The proportions and identification rates of respiratory viruses were compared among different groups. The proportions/rates were compared using Pearson's chi-squared test or continuity correction chi-squared test/Kruskal–Wallis test. The 95% confidence intervals (CIs) of identification rates were calculated using binomial exact test. All tests were two-tailed; statistical significance was set at  $P \leq 0.05$ . All the statistical analyses were performed with Statistical Analysis System Software (9.1.3; SAS Institute, Cary, NC).

## RESULTS

In all, 490 samples were collected, of which 99.2% (486) were PCR results. One or more viruses were detected in throat swabs from 246 (50.6%; 95% CI, 46.1–55.2%) patients with influenza-like illness. The viruses detected most frequently were influenza A in 111 (22.8% of the total specimens) patients, influenza B in 37 (7.6%) patients, influenza C in 30 (6.2%) patients, and RV in 29 (6.0%) patients. All other viruses were found to be less than 5%: hCoV HKU1 in 24 (4.9%) patients, AdV in 23 (4.7%) patients, hCoV 229E in 22 (4.5%) patients, hMPV in 13 (2.7%) patients, hCoV OC43 in 10 (2.1%) patients, hBoV in 6 (1.2%) patients, PIV I and III in 3 (0.6%) patients each, and PIV II, IV and RSV in 2 (0.4%) patients each. More than one virus was detected in 57 (11.7%; 95% CI, 9.0–14.9%) patients. Co-infection with two viruses was detected in 44 (9.1%) patients. Co-infection with three viruses was detected in 12 (2.5%) patients and co-infection with four viruses was detected in 1 (0.2%) patient (Table I). The most frequently detected viruses in co-infection patients were influenza A (77.2%), hCoV 229E (29.8%), AdV (26.3%), hCoV HKU1 (22.8%), RV and hMPV (14%), and influenza C (12.3%).

Samples were divided into three groups according to their sampling dates, that is, samples collected during January (influenza-like illness winter peak), during June–August (influenza-like illness summer peak), and during other months. The identification rates of respiratory viruses differed significantly among groups. The overall identification rates were

TABLE I. Viral Etiology of the Outpatients With Influenza-Like Illness

Viral etiology	Frequency (N)	Percent of patients (%)
Influenza virus		
Type A	111	22.84
Type B	37	7.61
Type C	30	6.17
Parainfluenza		
Type I	3	0.62
Type II	2	0.41
Type III	3	0.62
Type IV	2	0.41
Rhinovirus	29	5.97
Respiratory Syncytial virus	2	0.41
Human metapneumovirus	13	2.67
Human coronavirus		
Type 229E	22	4.53
Type NL63	0	0
Type OC43	10	2.06
Type HKU1	24	4.94
Bocavirus	6	1.23
Adenovirus	23	4.73
Co-infection		
0	240	49.38
1	189	38.89
2	44	9.05
3	12	2.47
4	1	0.21

67.4%, 32.6%, and 52.9% during influenza-like illness winter peak, influenza-like illness summer peak and other months, respectively ( $P = 0.0002$ ). The rate during summer peak was significantly lower than that during the winter peak and during other months ( $P = 0.0002$  and  $0.0007$ ). The difference in identification rate between winter peak and other months was not significant ( $P = 0.07$ ). However, the detected types of respiratory viruses were quite different between these two groups. During the influenza-like illness winter peak, the identification rates of influenza A (48.8%) and hCoV HKU1 (18.6%) were much higher than that during other months (24.9% and 4.2%,  $P = 0.0009$  and  $0.0005$ ; Fig. 1).

The overall identification rates of respiratory viruses did not differ significantly across age groups ( $P = 0.96$ ). Co-infections were found most commonly in adults older than 60 years. In this age group, 21.1% and 40.0% of swabs which were positive for respiratory viruses were co-infected. However, the co-infection rates were not significantly different across age groups ( $P = 0.14$ ; Table II). RSV was detected in 5.9% and 2.6% of patients with ages of 0–5 and 6–15, respectively, but was not detected in other age groups.

## DISCUSSION

Respiratory diseases were the leading cause of years of life lost and the third leading cause of years with disability in China [Zhou et al., 2011]. And influenza-like illness placed a substantial health and economic burden, the individual cost per episode of influenza-like illness represented approximately 20% of monthly per-capita income of residents [Guo et al., 2011]. Nevertheless, much less is known about the etiology of influenza-like illness in China. In the present study, a continuous surveillance of 16 respiratory viruses of

patients with influenza-like illness was conducted in Nanjing, China from November 2010 to October 2011.

Falchi et al. [2011] carried out a study over 5 weeks between January 25, 2010 and February 27, 2010 in France and found 58% samples of influenza-like illness patients positive for one or more respiratory viruses. Hasman et al. [2009] conducted a study during two successive winters, 1998–1999 and 1999–2000 in the USA, founding 68% of the samples positive for at least one virus. These two studies only collected respiratory specimens during winter. In contrast, the present study was conducted continuously from November 2010 through October 2011, including not only epidemic but also non-epidemic seasons of influenza-like illness. In this study, 50.6% (95% CI, 46.1–55.2%) of influenza-like illness patients were found positive for at least one respiratory virus. And the proportion was much higher in January (67.4%) than in June–August (32.6%), which was consistent with the findings of Brittain-Long et al. [2012], indicating that respiratory viruses were more likely associated with influenza-like illness peak in winter rather than peak in summer. Influenza-like illness peak in summer may be due to other respiratory pathogens including bacteria, chlamydia, or mycoplasma. During non-epidemic seasons of influenza-like illness, the detected rates of respiratory viruses were not significantly lower than in influenza-like illness winter peak. However, the identification rates of influenza A and hCoV HKU1 were significantly lower than in the winter peak. It is reasonable to infer that influenza A and hCoV HKU1 may account for the influenza-like illness winter peak. In addition to the sampling periods, detection method, and case definition could also lead to the differences of identification rates among studies. Laguna-Torres et al. [2011] employed virus isolation instead of PCR to identify pathogens and

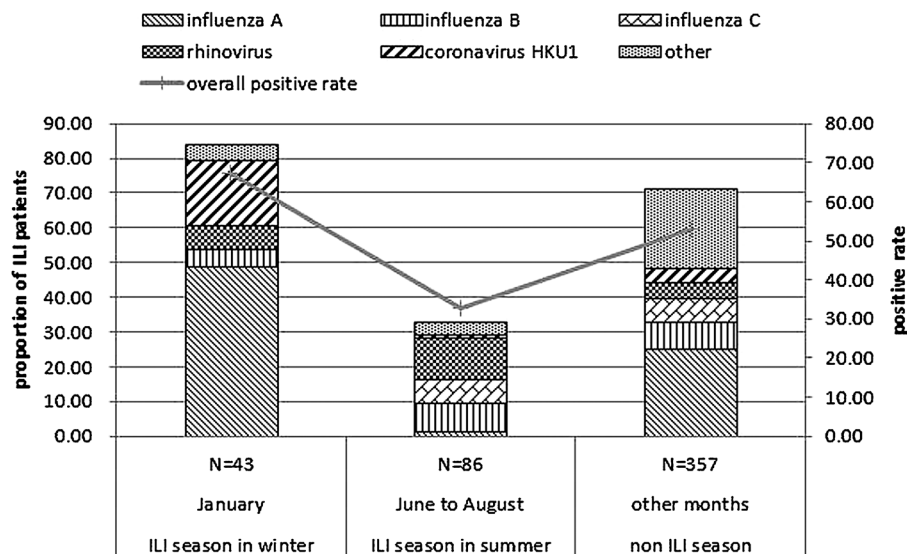


Fig. 1. Viral etiology of ILI patients and overall positive rates of respiratory viruses by ILI seasons.

TABLE II. Overall Identification Rates of Respiratory Viruses and Co-Infections by Age Groups

Age group (years)	Samples (N)	Overall identification rate of respiratory viruses (%)	Percent of co-infections of all samples (%)	Percent of co-infections of positive samples (%)
0–5	17	52.94	5.88	11.11
6–15	39	51.28	12.82	25.00
16–25	87	55.17	14.95	27.08
26–60	267	50.56	9.74	19.26
>60	57	52.63	21.05	40.00
		$P = 0.965^a$	$P = 0.140^b$	$P = 0.136^b$

<sup>a</sup>Pearson's chi-squared.

<sup>b</sup>Kruskal–Wallis test.

Rezza et al. [2006] included milder febrile cases into analyses. The positive rates of their findings, 24.7% and 43.9%, were both much lower than other studies.

The respiratory viruses found most commonly were quite different among studies. In France, the respiratory viruses detected most frequently were hMPV and RSV [Falchi et al., 2011]. In the USA, influenza A infections were found most commonly, followed by RSV and PIV-IV [Hasman et al., 2009]. In Central American countries, the viruses found most commonly were influenza A, RSV, AdV, and PIV [Laguna-Torres et al., 2011]. In Italia and Belgium, the sequences were influenza, AdV, PIV [Rezza et al., 2006] and influenza, RV, RSV [Hombrouck et al., 2012], respectively. In this study, the viruses detected most frequently were influenza, RV and hCoV HKU1 overall. Influenza A was found most commonly during the influenza-like illness winter peak and rhinovirus was found most commonly during influenza-like illness summer peak. Sampling seasons, ages of patients and virus types for test may contribute to the differences [Alonso et al., 2012]. Such as, hCoV HKU1 was only tested in the present study. It was not recognized as the principal respiratory virus of influenza-like illness in other studies. Interestingly, RSV was seldom detected in this study. RSV is the most common cause of lower respiratory tract disease and the leading cause of hospital admission among young children worldwide [Ou et al., 2009; Ranmuthugala et al., 2011; Stockman et al., 2012]. When more children under the age of 5 and more patients required hospitalization were included into analyses, the identification rates of RSV increased significantly [Hasman et al., 2009; Falchi et al., 2011; Laguna-Torres et al., 2011]. In the present study on outpatients with influenza-like illness, RSV was common in age groups of 0–5 and 6–15 but was not detected in other age groups, which may be attributed to the developing immune state and vulnerability to infections [Raboni et al., 2011].

As reported elsewhere [Heikkinen et al., 2008], co-infections were found relatively commonly in this study (11.7%) and the proportion was in line with the findings of Hasman et al. [2009] (11%). Previous studies reported that co-infections were associated with more severe signs than mono-infections [Wolf et al., 2006; Frobert et al., 2011]. Adults older than 60 years

were found more likely to be co-infected than other age groups in the present study, however, the subsequent clinical conditions of influenza-like illness patients were not obtained and thus the association between co-infections and severe signs cannot be analyzed.

In conclusion, this study confirms that multiple respiratory viruses may circulate concurrently among the population and account for a large proportion of influenza-like illness. In addition to influenza A, hCoV HKU1 may be associated with the influenza-like illness winter peak in Nanjing, China, 2011. Although the present study covered not only the influenza-like illness epidemic season but also the non-epidemic season, the circulating pathogen profile can vary significantly from year to year for both influenza and other viruses. Surveillance over many years would give a better picture of this variation.

## ACKNOWLEDGMENTS

The authors would like to thank the staff of the Jiangsu Province Hospital and Second Affiliated Hospital of Nanjing Medical University for their assistance with throat swabs acquisition and information collection.

## REFERENCES

- Alonso WJ, Laranjeira BJ, Pereira SA, Florencio CM, Moreno EC, Miller MA, Giglio R, Schuck-Paim C, Moura FE. 2012. Comparative dynamics, morbidity and mortality burden of pediatric viral respiratory infections in an equatorial city. *Pediatr Infect Dis J* 31:e9–14.
- Brittain-Long R, Andersson LM, Olofsson S, Lindh M, Westin J. 2012. Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reaction assay. *Scand J Infect Dis* 44:9–17.
- Falchi A, Turbelin C, Andreoletti L, Arena C, Blanchon T, Bonmarin I, Hanslik T, Leruez-Ville M, De Lamballerie X, Carrat F. 2011. Nationwide surveillance of 18 respiratory viruses in patients with influenza-like illnesses: A pilot feasibility study in the French Sentinel network. *J Med Virol* 83:1451–1457.
- Frobert E, Escuret V, Javouhey E, Casalegno JS, Bouscambert-Duchamp M, Moulinier C, Gillet Y, Lina B, Floret D, Morfin F. 2011. Respiratory viruses in children admitted to hospital intensive care units: Evaluating the CLART<sup>®</sup> Pneumovir DNA array. *J Med Virol* 83:150–155.
- Guo RN, Zheng HZ, Li JS, Sun LM, Li LH, Lin JY, He JF. 2011. A population-based study on incidence and economic burden of influenza-like illness in south China, 2007. *Public Health* 125:389–395.

- Gwaltney JM. 2002. Clinical significance and pathogenesis of viral respiratory infections. *Am J Med* 112:13S–18S.
- Hasman H, Pachucki CT, Unal A, Nguyen D, Devlin T, Peeples ME, Kwilas SA. 2009. Aetiology of influenza-like illness in adults includes parainfluenzavirus type 4. *J Med Microbiol* 58:408–413.
- Heijnen ML, Dorigo-Zetsma JW, Bartelds AI, Wilbrink B, Sprenger MJ. 1999. Surveillance of respiratory pathogens and influenza-like illnesses in general practices—The Netherlands, winter 1997–1998. *Euro Surveill* 4:81–84.
- Heikkinen T, Osterback R, Peltola V, Jartti T, Vainionpää R. 2008. Human metapneumovirus infections in children. *Emerg Infect Dis* 14:101–106.
- Hombrouck A, Sabbe M, Van Casteren V, Wullaume F, Hue D, Reynders M, Gérard C, Brochier B, Van Eldere J, Van Ranst M, Thomas I. 2012. Viral aetiology of influenza-like illness in Belgium during the influenza A(H1N1)2009 pandemic. *Eur J Clin Microbiol Infect Dis* 31:999–1007.
- Laguna-Torres VA, Sánchez-Largaespada JF, Lorenzana I, Forshey B, Aguilar P, Jimenez M, Parrales E, Rodriguez F, García J, Jimenez I, Rivera M, Perez J, Sovero M, Rios J, Gamero ME, Halsey ES, Kochel TJ. 2011. Influenza and other respiratory viruses in three Central American countries. *Influenza Other Respi Viruses* 5:123–134.
- Leowski J. 1986. Mortality from acute respiratory infections in children under 5 years of age: Global estimates. *World Health Stat Q* 39:138–144.
- Ou SY, Lin GY, Wu Y, Lu XD, Lin CX, Zhou RB. 2009. Viral pathogens of acute lower respiratory tract infection in hospitalized children from East Guangdong of China. *Zhongguo Dang Dai Er Ke Za Zhi* 11:203–206.
- Puzelli S, Boros S, Affinito C, Calzoletti L, Facchini M, Danaya RT, Owen IL, Pozio E, Rezza G, Donatelli I. 2006. Prevalence of antibodies against A and B influenza viruses in South-Western Papua New Guinea. *J Med Virol* 78:820–824.
- Raboni SM, Stella V, Cruz CR, França JB, Moreira S, Gonçalves L, Nogueira MB, Vidal LR, Almeida SM, Debur MC, Carraro H, Jr., dos Santos CN. 2011. Laboratory diagnosis, epidemiology, and clinical outcomes of pandemic influenza A and community respiratory viral infections in southern Brazil. *J Clin Microbiol* 49:1287–1293.
- Ranmuthugala G, Brown L, Lidbury BA. 2011. Respiratory syncytial virus—The unrecognised cause of health and economic burden among young children in Australia. *Commun Dis Intell* 35:177–184.
- Rezza G, Valdarchi C, Puzelli S, Ciotti M, Farchi F, Fabiani C, Calzoletti L, Donatelli I, Perno CF. 2006. Respiratory viruses and influenza-like illness: A survey in the area of Rome, winter 2004–2005. *Euro Surveill* 11:251–253.
- Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. 2012. Respiratory syncytial virus-associated hospitalizations among infants and young children in the United States, 1997–2006. *Pediatr Infect Dis J* 31:5–9.
- Wolf DG, Greenberg D, Kalkstein D, Shemer-Avni Y, Givon-Lavi N, Saleh N, Goldberg MD, Dagan R. 2006. Comparison of human metapneumovirus, respiratory syncytial virus and influenza A virus lower respiratory tract infections in hospitalized young children. *Pediatr Infect Dis J* 25:320–324.
- Zhou SC, Cai L, Wang J, Cui SG, Chai Y, Liu B, Wan CH. 2011. Measuring the burden of disease using disability-adjusted life years in Shilin County of Yunnan Province, China. *Environ Health Prev Med* 16:148–154.