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Prevalence of human respiratory viruses in adults with acute respiratory tract infections in Beijing, 2005–2007

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Abstract

To determine the aetiological role and epidemiological profile of common respiratory viruses in adults with acute respiratory tract infections (ARTIs), a 2-year study was conducted in Beijing, China, from May 2005 to July 2007. Nose and throat swab samples from 5808 ARTI patients were analysed by PCR methods for common respiratory viruses, including influenza viruses (IFVs) A, B, and C, parainfluenza viruses (PIVs) 1–4, enteroviruses (EVs), human rhinoviruses (HRVs), respiratory syncytial virus (RSV), human metapneumovirus (HMPV), human coronaviruses (HCoVs) OC43, 229E, NL63, and HKU1, and adenoviruses (ADVs). Viral pathogens were detected in 34.6% of patient samples, and 1.6% of the patients tested positive for more than one virus. IFVs (19.3%) were the dominant agents detected, followed by HRVs (6.5%), PIVs (4.3%), EVs (3.2%), and HCoVs (1.1%). ADVs, RSV and HMPV were also detected (<1%). The viral detection rates differed significantly between infections of the lower and upper respiratory tracts in the sample population: PIVs, the second most commonly detected viral agents in lower acute respiratory tract infections (LRTIs), were more prevalent than in upper acute respiratory tract infections, indicating that the pathogenic role of PIVs in LRTIs should be investigated. Currently, this study is the largest-scale investigation of respiratory virus infections in China with multiple agent detection, providing baseline data for further studies of respiratory virus infections in adults with ARTIs.

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Introduction

Viruses are among the major causes of acute respiratory tract illnesses (ARTIs) throughout the world. The most common viruses responsible for ARTIs include influenza viruses (IFVs), respiratory syncytial virus (RSV), parainfluenza viruses (PIVs) 1–4, enteroviruses (EVs), human rhinoviruses (HRVs), adenoviruses (ADVs), and human coronaviruses (HCoVs) 229E and OC43 [1–4]. Improvements in molecular detection techniques have resulted in the recent identification of several new respiratory viruses [5]. Recently discovered viruses such as human metapneumovirus (HMPV), novel strains of coronaviruses (SARS-CoV, HCoV-NL63, and HKU1), human bocavirus and novel polyomaviruses (WU and KI) have been detected around the world [5–14]. The illness and mortality due to respiratory viruses have made viral ARTIs a top priority in the global health challenge. As vaccination is currently unavailable for most of these viruses, it is necessary to monitor epidemic patterns and investigate the spread of respiratory virus infections to efficiently identify, control and prevent future epidemics.

Viral emergence varies from season to season, year to year, and region to region [15]. The risk of respiratory virus infection correlates with age, pre-existing medical condition, and immune status [16,17]. Most studies of respiratory viral activity patterns have focused on children, and large-scale epidemiological investigations of respiratory virus infections in adults have rarely been conducted. In an attempt to

characterize the respiratory virus infections and to provide insights into the aetiology and clinical associations of respiratory viruses in adult ARTIs, a 2-year study was conducted with adults suspected of having ARTIs in Beijing, China.

Materials and Methods

Patients and clinical specimens

Recruitment of patients took place from May 2005 to July 2007 at the Fever Outpatient Clinic Department (FOCD) of the Peking Union Medical College Hospital (PUMCH), Beijing, China. To include the potential viral ARTIs and to exclude typical bacterial infections, patients enrolled in the study were selected by physicians according to the following criteria: ≥14 years of age, with respiratory symptoms such as cough or wheezing, acute fever (body temperature ≥38°C), and normal or low leukocyte count, with or without radiological pulmonary abnormalities. Nose and throat swabs were collected from each patient, and the two swabs were pooled in one tube containing virus transport medium (VTM; Copan, Brescia, Italy). A total of 5808 patient samples (i.e. *c.* one-third of the patients who visited the FOCD of PUMCH during the study period) were collected and tested.

Nucleic acid extraction

Total nucleic acids (DNA and RNA) were extracted from 200 μL of each specimen (VTM) using the NucliSens easyMAG apparatus (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions [18].

Molecular detection of respiratory viruses

The presence of RSV, IFVs A, B, and C, PIVs 1–4, HRVs, EVs, HCoVs (229E, OC43, NL63, and HKU1), HMPV and ADVs was determined by PCR assays, as previously reported [1,19–22], and the details of these assays are summarized in Table 1. Briefly, two multiplex nested RT-PCRs were used for the simultaneous detection of PIVs 1–4, EVs, and HRVs, as well as IFVs A, B, and C, and RSVs A, and B. In addition, two one-step RT-PCRs were used to detect HCoVs and HMPV. ADVs were detected by one-step PCR. The sensitivity of the PCR systems was determined by using cloned, amplified products of each type of virus. Blank VTM was used as a negative control, and 100 copies of invariant β-actin gene were added to lysis buffer as internal controls to exclude inhibitors for nucleic acid extraction and PCR. Each RT-PCR amplification was performed using the SuperScript II One-Step RT-PCR Platinum Taq kit (Invitrogen), and ExTaq DNA polymerase (Takara) was used for PCR assays. PCR products were analysed by electrophoresis in 2% agarose gel containing ethidium bromide. Of the PCR-positive samples, half were randomly selected for verification by PCR product sequencing.

Statistical analysis

Comparison among groups was performed using a chi-square test with a significance level of $p < 0.05$.

Results

Overall detection of respiratory viruses

The investigation was performed on working days from May 2005 to July 2007, with the exception of July and November 2005, when clinical samples were not collected. Between 11 and 54 (average 33) patients per day were diagnosed with ARTIs at the FOCD, PUMCH during the study period. On average, ten patients with ARTIs were selected each day, according to the criteria described in Materials and Methods, providing 5808 patients in total. Patient ages ranged between 14 and 97 years (median, 30 years; mean, 35.7 years). Specimens were collected from both females (3137, 54.0%) and males (2671, 46.0%). The sensitivity of detection of viruses was as follows: 1–10 molecules for PIVs 2 and 4, EVs, HRVs, IFVs A, B, and C, RSVs A and B, HMPV and ADVs; and 10–100 molecules for PIVs 1 and 3, and HCoVs.

Respiratory samples from 2010 (34.6%) patients were found to be positive for at least one virus, and those from 3798 (65.4%) patients were negative for all respiratory viruses tested. Pathogens in such patients as these need to be further investigated. The monthly detection rates of respiratory viruses ranged from 12.7% to 69.8% of patients tested (Fig. 1). There were no significant differences between viruses infecting men and women (data not shown). All of the common respiratory viruses were detected. As expected, IFV infection was dominant, being detected in 1119 (19.3%) patients. The detection rate of HRVs was 6.5%, and those of other viruses were as follows: PIVs, 4.3%; EVs, 3.2%; and HCoVs, 1.1%. ADVs, RSV and HMPV were rarely detected, displaying positivity rates of <1% (Table 2).

Seasonality of respiratory virus infection

IFV A was detected at the highest frequency and throughout the study period. Two major peaks were observed: in a period from July to September 2006, and in a period from December 2006 to February 2007. Three minor IFV A peaks were observed in a period between August and September 2005, in December 2005, and in March 2006 (Fig. 2a). Unlike IFV A, IFV B is active in spring in Beijing. Apart from two distinct peaks in March and April of 2006