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ARTICLE

Detection of three human adenovirus species in adults with acute respiratory infection in China

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Abstract Human adenoviruses (HAdVs) are recognized as causal agents in a wide range of human diseases. However, researchers lack sufficient data on the exact HAdV species and serotypes associated with adult acute respiratory tract infections (ARTIs). To detect and characterize HAdV infections in adults in China, clinical specimens were collected from 10,310 adults with ARTIs from May 2005 to July 2010. The partial HAdV hexon gene was amplified by polymerase chain reaction (PCR), sequenced, and phylogenetically analyzed. HAdVs were detected in 86 samples (0.8%), of which 67 (77.9%) were species B (HAdV-3, -7, -11, and -14), 7 (8.1%) were species C (HAdV-1, -2, and -6), and 12 (14%) were species E (HAdV-4). HAdV-3 was the most frequently detected serotype (41/86, 47.7%), followed by HAdV-7 (13/86, 15.1%), HAdV-4 (12/86, 14.0%), and HAdV-11 (11/86, 12.8%). Patients 14–25 years old (60.5%) exhibited a higher rate of adenovirus detection than older patients. Co-infections with other respiratory viruses were observed in samples positive for HAdV species B and E. Human rhinovirus was the most commonly found virus in patients with HAdV infection. These findings provide baseline data for the surveillance and control of HAdV infection in China.

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Introduction

Human adenoviruses (HAdVs) play important roles in a wide spectrum of clinical diseases, including respiratory disorders, gastroenteritis, pharyngitis, keratoconjunctivitis, meningoencephalitis, acute hemorrhagic cystitis, and hepatitis [1]. Fifty-six human serotypes have been identified based on serum neutralization assays [2] and classified into six species (HAdV-A to HAdV-F), according to the sequence homology of genomic DNA and biological properties [3].

Adenoviruses are non-enveloped, icosahedral viruses with linear double-stranded DNA genomes that are 26 to 45 kb in length and characterized by an inverted terminal repeat (ITR) [4]. Because HAdV genomes are stable and genetic recombinations are rare, the species and serotype can be deduced from polymerase chain reaction (PCR) assay or sequence data [5].

Different HAdV species are associated with different diseases. HAdV species B, C, and E are mainly associated with respiratory infections (HAdV-B-3, -7, -11, -14, -16, -21, -34, and -35; HAdV-C-1, -2, -5, and -6; HAdV-E-4) [6–8]. Species C is primarily responsible for the respiratory tract infections in children, whereas species B and E are more likely to infect healthy adults [9]. However, incidences of adenovirus-associated illnesses are higher among children

than among adults. As such, in China, the impact of HAdVs on respiratory infection is well characterized in children [10–15] but not in adults.

The identification of the predominant HAdV species and serotypes that impact adult ARTIs is important because clinical manifestations of adenovirus-induced respiratory illnesses range from non-symptomatic to severe, life-threatening disease, depending on the infecting serotype, patient age, and other factors [16]. In this study, the prevalence of HAdV infections in adults with ARTIs was evaluated from 2005 to 2010 in Beijing, China, using phylogenetic analysis.

Materials and methods

Clinical specimens

Nasal and throat swabs were collected from 10,310 adult patients who visited the Fever Outpatient Clinic at Peking Union Medical College Hospital (PUMCH) from May 2005 to July 2010, except during July and November of 2005. Of the patients, 92.7% were from Beijing, 1.5% were from Hebei province, adjacent to Beijing, and 5.8% were from other provinces of north China, including Shangdong, Liaoning, Henan, and Jilin province. The patient recruitment followed the criteria described elsewhere [17]. All selected cases were ≥14 years old, had fever with body temperature ≥38°C, acute respiratory symptoms, and normal or low leukocyte count. One nasal swab and one throat swab were taken from the same patient simultaneously and were combined in a tube containing 3 ml of viral transportation medium (VTM). The VTM samples were stored at −80°C prior to use.

The study was approved by the ethical review committees of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences, and Peking Union Medical College. Informed consent was obtained from each participant who provided specimens.

Clinical data

The symptoms, history of illness, and results of clinical examination and laboratory tests were recorded using a standard data collection form at the time of sampling. The specimens and clinical data of all HAdV-positive adults were reviewed from the medical records.

Detection of HAdVs

Viral nucleic acids were extracted from clinical samples using the NucliSens easyMAG system (bioMérieux, Marcy l'Etoile, France). HAdVs were detected by PCR analysis (HAdV screening assay) using primers targeting nucleotides (nt) 21–322 of the hexon gene to amplify a 301 base pair (bp) fragment, as described elsewhere [18]. All PCR products were cloned into the pGEM-T Easy Vector (Promega, Madison, WI, USA) and verified by sequence analysis. To verify the results of the HAdV screening assay, 50 samples that were positive according to the screening PCR assay were randomly selected and tested in a second PCR assay targeting the E1A gene and another fragment of the hexon gene. The PCR assays were performed as previously described [7]. Briefly, specimens identified as positive by the HAdV screening assay were subjected to a multiplex PCR assay for species B, C, and E, targeting nt 1042 to 1358, nt 354 to 844, and nt 707 to 929, respectively, of the E1A gene with predicted product sizes of 317 bp, 491 bp, and 222 bp, respectively. The positive samples of species B were subsequently verified using a multiplex PCR assay for serotypes HAdV-3, -7, and -21, targeting nt 796 to 1297, nt 500 to 810, and nt 547 to 783, respectively, of the hexon gene fragment, to produce amplicons of 502 bp, 311 bp, and 237 bp, respectively. The samples positive for HAdV-14, which belongs to species B, were further confirmed using primers that target nt 921 to 1245 of the Ad14 hexon gene to generate a 324-bp product.

For all of the screened specimens, additional viral infections, including influenza viruses (A, B, and C), human parainfluenza virus 1–4, human rhinovirus, respiratory syncytial virus, enterovirus, human coronaviruses (229E, NL63, HKU1, and OC43), and human metapneumovirus, were screened as previously described [17].

Phylogenetic analysis

Multiple sequence alignments and neighbor-joining trees with 1,000 bootstrap replicates were generated based on the nucleotide sequences of the PCR products using MEGA 4.0 software [19].

Statistical analysis

Age, laboratory parameters, clinical features, and the detection rate of HAdV infection were evaluated using the χ^2-test or Fisher's exact test for categorical variables and Student's *t*-test for continuous variables. A p-value<0.05 was considered to be significant.

Nucleotide sequence accession numbers

Nucleotide sequences obtained in this study have been deposited in GenBank under accession numbers JF712937–JF713022.

Results

Prevalence of HAdV species and serotypes

A total of 10,310 patients (4,746 males and 5,564 females) were recruited in this study. The age of these patients ranged from 14 to 97 years (median 30 years; mean 35.2 years). HAdVs were detected in 86 (0.8%) patients, ranging in age from 14 to 76 years (median 23 years; mean 25 years). The frequency of HAdV infection did not differ significantly ($\chi^2=0.1$, $p>0.05$) between male (0.4%) and female patients (0.4%).

Additional respiratory pathogens were identified in 9 (10.4%) of the HAdV-positive patients. Human rhinovirus, influenza B virus, human parainfluenza virus 3, enterovirus, and respiratory syncytial virus were detected with HAdV-3, HAdV-4, HAdV-7, and HAdV-14. One patient was positive for human rhinovirus, human parainfluenza virus 3, and enterovirus simultaneously. For the other eight patients, six were co-detected with human rhinovirus, one with respiratory syncytial virus, and one with human influenza B virus, respectively (Tables 1 and 2). We did not observe a specific relationship between the HAdV species and the co-infecting viruses.

Based on the sequence and phylogenetic analysis of the amplicons generated by the screening PCR assay, three HAdV species (B, C, and E) were detected. In total, 67 (77.9%) patients were positive for species B (HAdV-3, -7, -11, and -14), 7 (8.1%) for species C (HAdV-1, -2, and -6), and 12 (14%) for species E (HAdV-4). HAdV-3 was the most frequently detected serotype (41/86, 47.7%) in this study, followed by HAdV-7 (13/86, 15.1%), HAdV-4 (12/86, 14.0%), and HAdV-11 (11/86, 12.8%). HAdV-1, HAdV-2, HAdV-6 (all belonging to species C), and HAdV-14 (subspecies B2) were occasionally detected during the study period (Fig. 1).

To verify the results of the screening PCR assay, 50 positive specimens were randomly subjected to a second round of PCR assays targeting E1A and a different fragment of the hexon gene. The results were consistent between each detection assay. Of the 50 specimens selected for verification, 47 (94%) were positive according to the second PCR assay. The results of the phylogenetic analysis are also consistent with the results of the screening assay (data not shown). These data indicate that the results of our HAdV detection assay are reliable.

Phylogenetic and variation analysis

The phylogeny and variation of HAdV strains were analyzed based on the partial sequences of the hexon gene

Table 1 Distribution of the human adenovirus (HAdV) species versus patient characteristics and clinical symptoms

Parameters	HAdV species		
	Species B	Species C	Species E
No. of patients	67	7	12
Age range (years)	15–76	17–40	17–53
Median/mean age (months)	22/24.3	29/29.6	25/28
Male/female	35/32	2/5	4/8
Clinical manifestations (%)			
Fever	67 (100)	7 (100)	12 (100)
Cough	33 (49.3)	3 (42.9)	5 (41.7)
Sputum production	21 (31.3)	0	4 (33.3)
Headache	55 (82.1)	6 (85.7)	10 (83.3)
Muscle pain	46 (68.7)	6 (85.7)	9 (75)
Sore throat	58 (86.6)	7 (100)	10 (83.3)
Chills	49 (73.1)	5 (71.4)	9 (75)
Rhinorrhea	12 (17.9)	2 (28.6)	4 (33.3)
Sneezing	9 (13.4)	1 (14.3)	3 (25)
Peripheral blood tests			
Mean leukocyte count ($\times10^9$/L)	7.91±1.6	7.76±1.2	7.17±1.6
Co-infection in samples (%)[a]	9 (13.4)	0 (0)	2 (16.7)
Human rhinovirus	6 (8.9)	0 (0)	1 (8.3)
Human parainfluenza virus 3	1 (1.5)	0 (0)	0 (0)
Influenza B viruses	1(1.5)	0 (0)	0 (0)
Enterovirus	1(1.5)	0 (0)	0 (0)
Respiratory syncytial virus	0 (0)	0 (0)	1 (8.3)

[a]One sample was co-infected with HAdV-3, human rhinovirus, human parainfluenza virus 3, and enterovirus

Table 2 The co-infected respiratory viruses with HAdV species and serotypes

Species and serotype	Co-infection in adenovirus-positive samples					Total
	Human rhinovirus	Human parainfluenza virus	Influenza viruses	Enterovirus	Respiratory syncytial virus	
Species B						
HAdV-3	5 (5.8)[a]	1 (1.2)	0 (0)	1 (1.2)	0 (0)	5 (5.8)[b]
HAdV-7	1 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.2)
HAdV-11	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
HAdV-14	0 (0)	0 (0)	1 (1.2)	0 (0)	0 (0)	1 (1.2)
Species C						
HAdV-1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
HAdV-2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
HAdV-6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0
Species E						
HAdV-4	1 (1.2)	0 (0)	0 (0)	0 (0)	1 (1.2)	2 (2.3)
Total	7 (8.1)	1 (1.2)	1 (1.2)	1 (1.2)	1 (1.2)	9 (10.5)[b]

[a] The numbers in parentheses indicate the percentages of co-detection in the adenovirus-positive samples

[b] One sample was co-infected with HAdV-3, human rhinovirus, human parainfluenza virus 3, and enterovirus

(Fig. 1). The HAdV-3 and HAdV-7 strains exhibited 96–99% and 94.4–98.7% nucleotide identity, respectively, to other Chinese strains identified primarily in children (GenBank accession number DQ099432 for HAdV-3 and GU230898 for HAdV-7). The HAdV-4 strains exhibited 97.3–99.7% nucleotide identity to the vaccine strain (GenBank accession number AF065063). The HAdV-11 strains had 97.3–98.3% nucleotide identity to a strain responsible for an outbreak in Singapore (GenBank accession number FJ607012). Multiple-alignment analysis based on sequences identified in this study showed that the nucleotide and amino acid identity of the partial hexon region were 77.1–100% and 82.8–100% among strains of different species, respectively. Among the species B1 strains in this study, the nucleotide identity ranged from 94.4 to 100%, with an amino acid identity of 96.0–100%, whereas the nucleotide identity of the species B2 strains ranged from 97.0 to 99.7%, with an amino acid identity of 98.0–100%. Species C had 94.0–98.7% and 98.0–100%, species E had 98.3–99.7% and 97.0–100% nucleotide and amino acid identity, respectively.

Clinical and epidemiological features of HAdV infections

The yearly occurrence of HAdV infections varied between species (Fig. 2). We detected species B yearly, with the highest rates in February 2008. Species C was not detected in 2005 and 2010. Species E was not detected in 2009 and was the only HAdV species detected in December 2005, March 2006, and December 2007. However, a difference in the monthly distribution between serotypes HAdV-3, -7, and -11 of species B was observed. HAdV-7 and HAdV-11 were found mainly in February–June and August, whereas HAdV-3 showed no obvious variation in the seasonal distribution. Moreover, the incidences of HAdV-4 were higher in March–July and December. Because the detection rates for HAdV-1, -2, and -6 (species C) are low, it is difficult to draw meaningful conclusions from the seasonal distribution. The monthly distribution of adenovirus serotypes is similar to that reported by Cooper et al. [20]. Such variations were not observed for co-infecting viruses (data not shown).

HAdV infections varied by age group (χ^2=30.509, $p<$ 0.01). The rate of HAdV detection was highest in patients aged 14–25 years (52/86, 60.5%), followed by the 26–65 years old age group (33/86, 38.4%). Unexpectedly, only one patient (1/86, 1.1%) more than 65 years old was positive for HAdV. In addition, HAdV was not detected in patients 56–65 years of age (Table 3).

All of the 86 HAdV-positive patients were diagnosed with upper respiratory tract infections. The clinical signs and symptoms of HAdV-positive patients included fever (100%), sore throat (88.4%), headache (83.7%), chills (74.4%), muscle pain (72.1%), cough (45.3%), sputum production (29.1%), rhinorrhea (19.8%), and sneezing (15.1%). The maximum body temperature (mean±standard deviation [SD]) was similar between patients with different HAdV infections: species B (39.0±0.56°C), species C (39.0±0.83°C), and species E (38.9±0.26°C).

Clinical presentations did not differ significantly between patients infected with different HAdV species or those infected with single HAdV infection and co-infection with other respiratory viruses (data not shown).

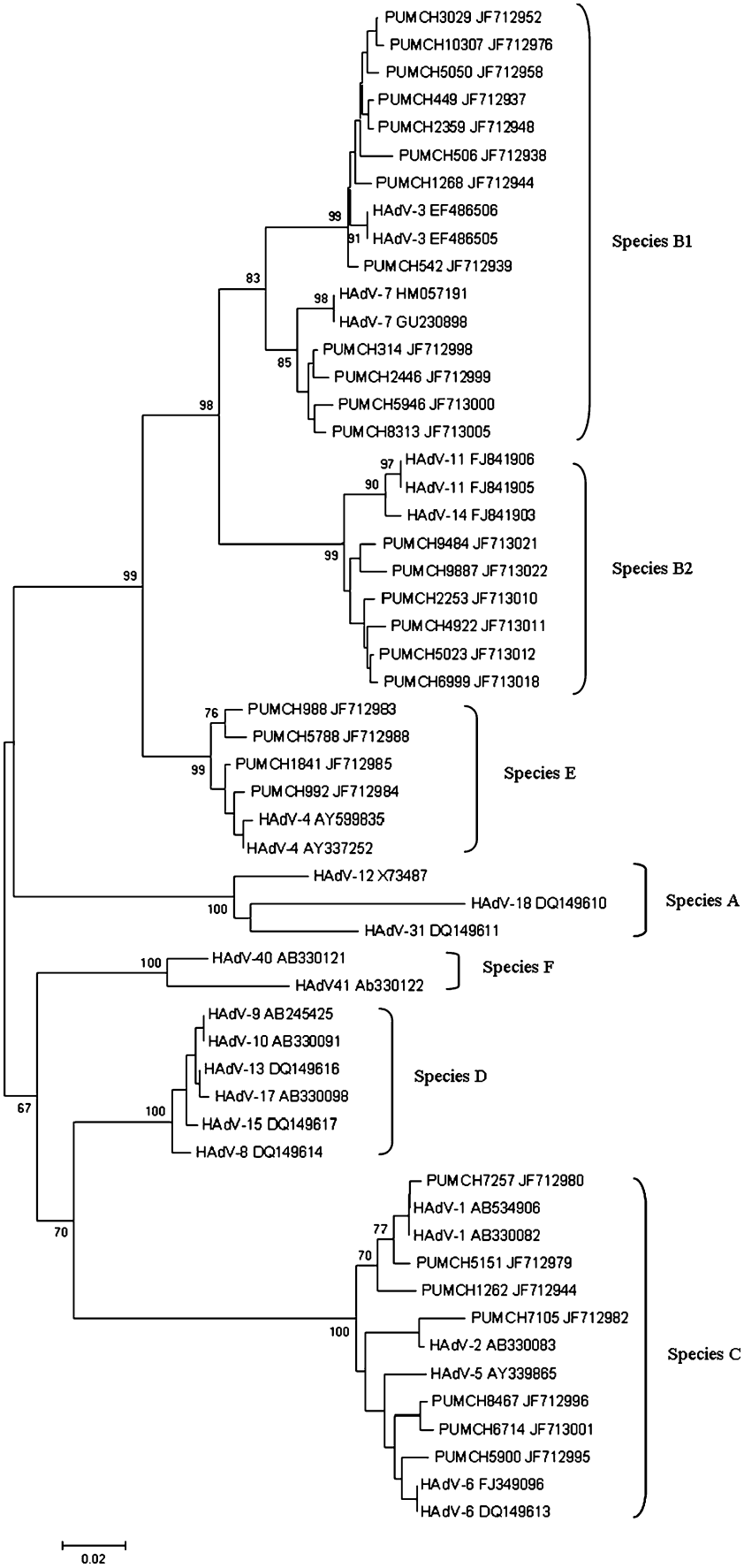

Fig. 1 Phylogenetic analysis of human adenovirus (HAdV) species based on the partial sequence of the hexon gene. The phylogenetic tree with 1,000 bootstrap replicates was generated using the Clustal W and MegAlign programs in the MEGA 4.0 software package based on the nucleotide sequences of the 29 randomly selected representatives from the 86 HAdV-positive samples. Each strain from this study is indicated by a specific identification code (PUMCH) followed by the patient number and marked with GenBank accession numbers

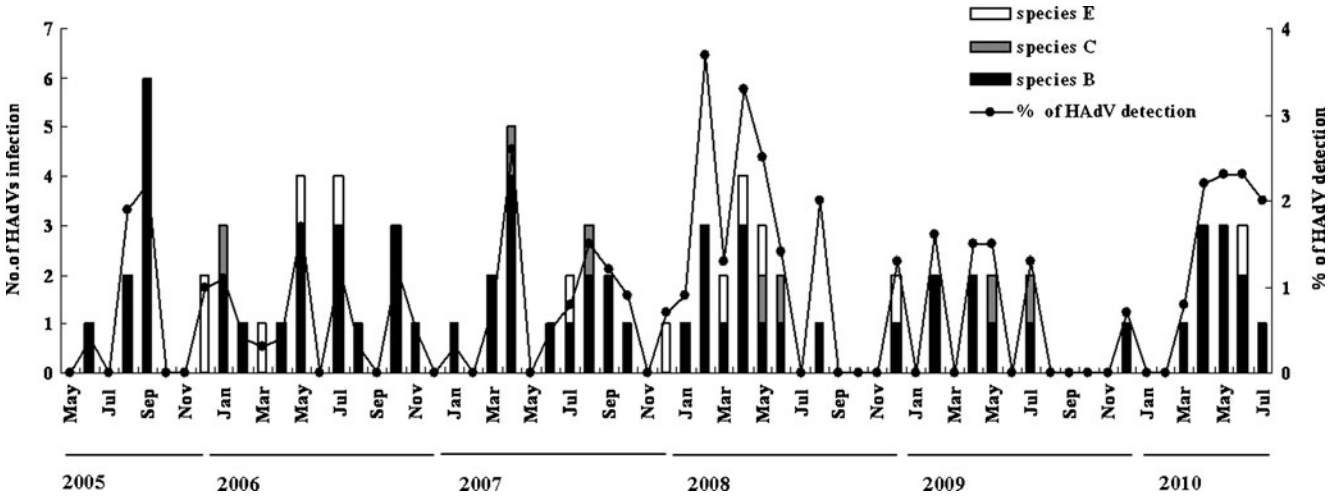

Fig. 2 Number and detection rate of HAdV in respiratory samples. The bar graph indicates the number of species B-, species C-, and species E-positive cases. The line graph indicates the total detection rate of HAdV by month

Discussion

To understand the prevalence of HAdV and its impact on adults, a retrospective study was conducted on adults with ARTIs in Beijing, China, over a 5-year period (from May 2005 to July 2010). Although HAdV infections are less common in adults than other respiratory virus infections, such as influenza viruses, human rhinoviruses, human parainfluenza viruses, enteroviruses, and human coronaviruses [17], they can cause fatal respiratory tract infections in affected patients [5, 21]. Moreover, if one extrapolates from the observed HAdV infection rate to the population as a whole, 0.8% could translate to a significant number of HAdV infections related to ARTIs in China on a yearly basis. Our data underscore the important role of HAdV in adult ARTIs in China.

The 0.8% detection rate of HAdV in this study is lower than the 2.0 to 9.14% reported in several previously published studies [12, 22, 23]. This disparity cannot be attributed to the detection method used, as our PCR experiments which target a different hexon gene fragment and a different gene (E1A) are consistent with our PCR screening results. This disparity may be attributed to: (1) differences in the age of the study population—the subjects recruited in this study were adults, whereas those in other studies were mainly children [12, 22, 23]. Childhood exposure to some adenovirus serotypes may generate protective immunity in response to the same serotypes, which could lead to the low infection rates observed in adults; (2) the method of sample collection—adenoviruses were detected from nasal and throat swabs of upper respiratory tract infections in this study, whereas those in other studies were recovered from nasal aspirate [23] or autopsied pulmonary tissue of pediatric fatal pneumonia [12]; (3) the impact of geographical location. The majority of patients (92.7%) recruited in this study came from Beijing. The geographical location can influence the prevalence of respiratory viruses [24, 25].

Species B, specifically, serotypes HAdV-3 and HAdV-7, were the most common HAdVs detected in Beijing from 2005 to 2010. Our results are similar to those of a previous study among children in China [10], in which HAdV-3 and HAdV-7 were the most identified serotypes. These results are also consistent with data from the United Kingdom

Table 3 Age distribution of HAdV infection in adults with acute respiratory tract infections (ARTIs)

	≤25 years	26–65 years	>65 years	Total
No. tested	3,414	6,177	719	10,310
Species B/co-infection	44 (51.2)/5 (5.8)[a]	22 (25.6)/2 (2.3)	1 (1.2)/0 (0)	67 (77.9)/7 (8.1)
Species C/co-infection	2 (2.3)/0 (0)	5 (5.8)/0 (0)	0 (0)/0 (0)	7 (8.1)/0 (2.3
Species E/co-infection	6 (7.0)/2 (0.02)	6 (7.0)/0 (0)	0 (0)/0 (0)	12 (14)/2 (2.3)
Total (%)[b]	52 (60.4)/7 (8.1)	33 (38.4)/2 (2.3)	1 (1.2)/0 (0)	86 (100)/9 (10.5)

[a] The numbers in parentheses indicate the percentages of samples positive for human adenovirus

[b] $\chi^2 = 30.509$, $p < 0.01$

[20], Canada [26], Korea [23], and Taiwan, China [27], in which species B was the most commonly identified HAdV. However, species C, specifically, HAdV-1 and HAdV-2, has been reported to be more common in Malaysia [22], and species E (HAdV-4) in the US military [7, 16]. Differences in geography, recruited population, and study period may account for the data variations from different studies, and further investigation is needed in order to characterize HAdV infections in detail throughout the country.

Fatal HAdV-14 infections have gained attention recently [5, 28]. We detected HAdV-14 in two patients enrolled in this study. However, we did not observe a very acute and severe infection by HAdV-14. The main symptoms of the two HAdV-14 patients were fever (lasting for two days and one day, respectively), cough, sputum production, headache, sore throat, chills, and sneezing, similar to infection by the other HAdV serotypes.

HAdV-3 (belonging to species B) showed no obvious variation in the seasonal distribution in this study, whereas the incidence of two other species B serotypes, HAdV-7 and HAdV-11, mainly occurred in the spring and summer (February–June and August). The seasonal distribution of HAdV species B is consistent with that of a previous report from the United Kingdom [20]. HAdV-4 (belonging to species E) infection was more prevalent in the spring and summer (March–July) and peaked in December, which is also similar to that in the United Kingdom [20]. However, the seasonality of HAdV-4 was slightly different from Shinozaki's study, in which HAdV-4 was the most prevalent in July and August [29]. This disparity may be due to the fact that most of the HAdV-4 isolates were from conjunctivitis cases in that study, whereas our samples were taken from patients with ARTIs. However, it is difficult to draw conclusions about the seasonal distribution of HAdVs because of the limited number of positive cases.

The phylogenetics of HAdVs was analyzed based on the partial sequences of the hexon gene (301 bp). Nucleotide and amino acid variations among the different HAdV species varied up to a maximum of 22.9 and 17.2%, respectively. However, the variation of the nucleotide and amino acid sequences in the same region among the same species is low. To evaluate the real level of variation among HAdV species and serotypes, full-length genome or open reading frame (ORF) sequencing is necessary.

In conclusion, this report documents the prevalence and clinical characteristics of HAdV infection in a Chinese adult population with ARTIs from 2005 to 2010. The study identified species B (HAdV-3, -7, -11, and -14), C (HAdV-1, -2, and -6), and E (HAdV-4) from respiratory samples of adults with ARTIs in Beijing. Our results show that human adenovirus B and HAdV-3 are the most frequently detected species and serotype, respectively. Although our findings may not completely reflect the infection of HAdVs circulating in China due to the limited number of HAdV-positive samples and geographical scale, they improve our understanding of the pathogenesis and disease burden of HAdVs in adults.

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