

## Original Article

# Molecular epidemiology of respiratory adenovirus detection in hospitalized children in Shenzhen, China

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**Abstract:** Objective: This study aimed to understand the molecular type of respiratory adenovirus in hospitalized children in Shenzhen and the relation to clinical diagnoses. Methods: Nasopharyngeal swab specimens were obtained from 6,479 hospitalized children younger than 14 years with respiratory tract diseases in Shenzhen Children's Hospital from December 2012 to November 2013. Nasopharyngeal swabs were routine examined by direct immunofluorescence assay to detect respiratory agents including seven respiratory viruses. Multiplex PCR of adenovirus types 3, 7, 11 and 21 in a single tube based on the sequence of the encoding gene for hexon was used to type for adenovirus positive specimens. For those strains that could not be typed by multiplex PCR, the gene fragment was amplified by a universal primer pair for all adenovirus types and the PCR products were sequenced directly. Results: A total of 1,066 of 6,479 (16.45%) specimens were positive for at least one of the seven viruses and 228 of 6,479 (3.52%) specimens were positive for adenovirus. 86.4% of children with adenovirus infection occurred less than 5 years of age and just over half of the children (54.4%) less than two years old. There was no significant difference in infection rates between males and females. AdV3 (46.3%) and AdV7 (36.3%) were the genotypes most commonly found followed by AdV1 (6.0%), AdV4 (5.0%), AdV2 (3.0%), AdV6 (1.5%), AdV5 (1.5%) and AdV3/7 (0.5%). No type 11, type 21, and other types of adenovirus were detected. Seven children had type 3 or type 7 and one had type 3/7 mixed infection in 15 severe pneumonia cases. Conclusions: Our study demonstrated that respiratory adenovirus infection is an important cause of hospitalizations in children in Shenzhen, China. Types 3 and 7 were the most common followed by types 1 and 4. AdV3 and AdV7 were similarly contributed to the severe cases.

**Keywords:** Adenovirus, hospitalized children, respiratory tract infection, types monitoring

## Introduction

Acute respiratory tract infections (ARTIs) are a major threat in children, with more than 90% of these cases due to viral infection [1]. Human adenovirus (HAdV) is responsible for approximately 3% to 8% of reported childhood viral respiratory infections. HAdV infections can occur endemically throughout the year or in epidemics [2].

To date, HAdV consists of seven species (A-G) and more than 60 types within these species have been identified. Respiratory viral infection is caused mainly by species B, C and E [3]. The severity of respiratory tract infections is different for each type of HAdV. Children infected with serotype AdV7 (subgroup B) are commonly prone to respiratory tract infections [4]. The major types of HAdV causing respiratory tract

infections change over time in different countries and regions. Therefore, monitoring the types of HAdV causing respiratory tract infections in certain regions is important for understanding the prevalence or probably finding new adenovirus type in certain period. However, the molecular epidemiology of HAdV and the relation to clinical diagnoses in Shenzhen remain unknown.

Species B (AdV types 3 and 7) is the most frequent HAdV causing respiratory tract infection in China, but there are obvious differences in various regions [5-8]. Shenzhen Children's Hospital is the referral centre of Shenzhen and the surrounding area, admit all respiratory tract infection patients younger than 14 years. To analyze the prevalence and types of HAdV among children hospitalized for respiratory tract infections and HAdV infection with the

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**Table 1.** Characteristics of total hospitalized children and children diagnosed with adenovirus infection

Patient characteristic	Total hospitalized children	Children diagnosed with adenovirus	P value
Average age (Month) ± SD	22.7±28.5	30.3±24.7	0.038
Male/female ratio	1.81:1	2.04:1	0.130
No. (%) of children with diagnosis of:			
Pneumonia	3906 (60.3)	127 (55.7)	0.165
Bronchitis	1219 (18.8)	35 (15.4)	0.187
Acute upper respiratory tract infection	902 (13.9)	42 (18.4)	0.055
Tonsillitis	257 (4.0)	20 (8.8)	0.001
Conjunctivitis pharynx	88 (1.4)	4 (1.8)	0.613
Others	105 (1.6)	0 (0)	0.001

Applied by Independent samples t-test or fisher's exact test.

**Table 2.** Oligonucleotide primers for PCR amplification of adenovirus general hexon gene and types 3, 7, 11 and 21

Types	Primer	Sequence (5'→3')	Amplicon size (bp)
General	G-F	TTCCCCATGGCICAYAACAC	482
	G-R	CCCTGGTAKCCRATRTTGTA	
3	3-F	GGTAGAGATGCTGTTGCAGGA	502
	3-R	CCCATCCATTAGTGTTCATCGGT	
7	7-F	GGAAAGACATTACTGCAGACA	311
	7-R	AATTCAGGCGAAAAAGCGTCA	
11	11-F	AAATCAACTGGTGAGGAACA	880
	11-R	CGCATTGTCTCCATTGGAAC	
21	21-F	GAAATTACAGACGGCGAAGCC	237
	21-R	AACCTGCTGGTTTTCGCGTTG	

relation to clinical features in Shenzhen, China, consecutive hospitalized children were systematically tested and types of HAdV were identified from December 2012 to November 2013. Additionally, the age-related and season patterns of respiratory adenovirus infection were also described.

## Material and methods

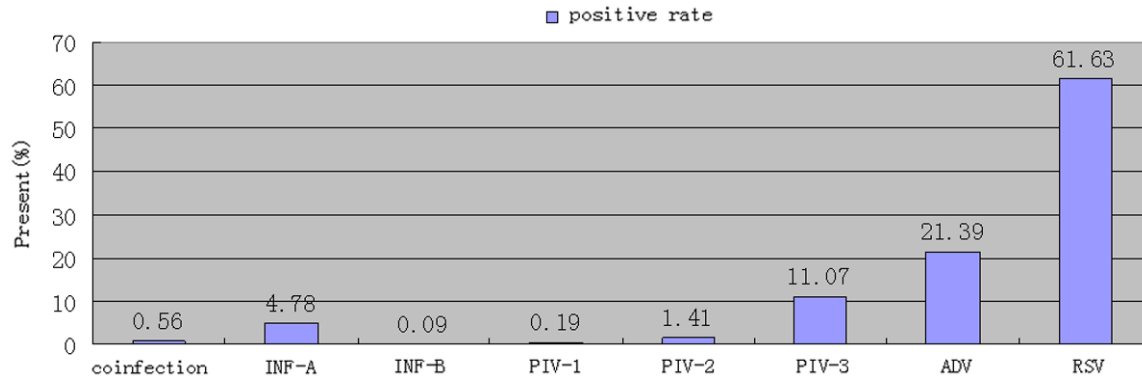
6,479 consecutive hospitalized children, younger than 14 years, with respiratory tract infection on admission in Shenzhen Children's Hospital were enrolled from December 2012 to November 2013 (**Table 1**). Respiratory tract infections diagnosed by clinical diagnosis guidelines were included pneumonia, bronchitis, tonsillitis, acute upper respiratory tract infection, pharynx, etc. Severe adenovirus infections had lower respiratory tract infec-

tions, which manifested as lobar or segmental pneumonia and pleural effusion [9].

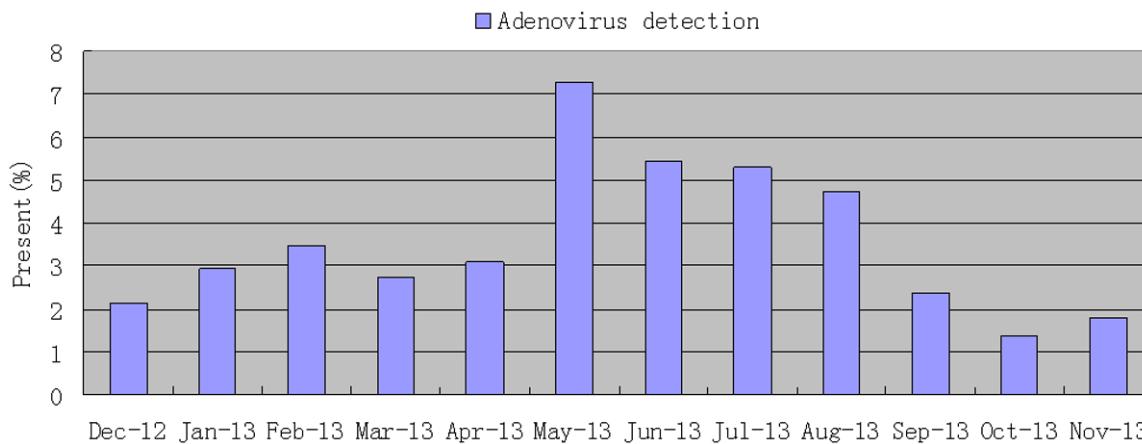
Nasopharyngeal swab samples obtained from all consecutive hospitalized children. All samples were routine tested for seven respiratory viruses (including respiratory syncytial virus, adenovirus, influenza virus types A/B and parainfluenza virus types 1, 2, and 3) with direct immunofluorescence assay kits (Diagnostic Hybrids, Inc. USA) according to the manufacturer's protocol. The samples that were positive for HAdV were selected for molecular analysis and stored at -70°C.

Each stored positive HAdV sample had deoxyribonucleic acid (DNA) extracted with an AxyPrep Humoral virus DNA and RNA extraction kit (AXYGEN, USA), according to the manufacturer's instructions. Firstly, four primer pairs (**Table 2**) located in the hexon gene of HAdV were specifically designed for amplifying types 3, 7, 11 and 21 and were used for multiplex PCR in a single tube [7]. The products from this multiplex PCR were 502 bp (for type 3), 311 bp (for type 7), 880 bp (for type 11), and 237 bp (for type 21). The type of HAdV was determined after agarose electrophoresis analysis of the PCR products. Then the positive HAdV samples that were negative for types 3, 7, 11, and 21 had their general hexon gene amplified according to Wanhong Xu's study of 2000 [10]. The PCR products were then sent to the Invitrogen Biotechnology Company (Shanghai, China) for sequencing carried out by conventional Sanger sequencing. The nucleotide sequences of the hexon gene from this study were submitted to

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**Figure 1.** Distribution of detection of seven human respiratory viruses for one year.



**Figure 2.** Adenovirus detection in hospitalized patients between December 2012 and November 2013, distributed by month.

the GenBank database compared with reference strains.

### Statistical analysis

SPSS version 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Chi-squared and Fisher's exact tests were employed. *P*-values < 0.05 were considered statistically significant.

## Results

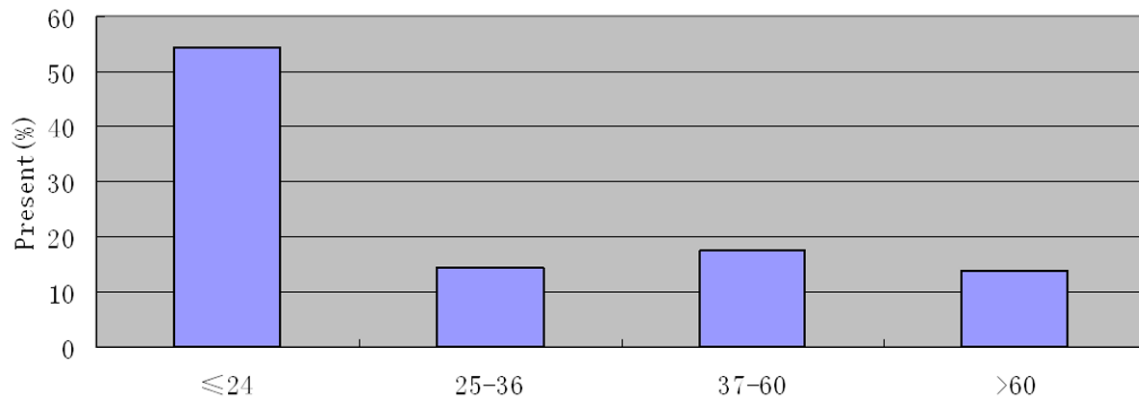
### Patient characteristics

6479 consecutive hospitalized children with respiratory tract infection were enrolled from December 2012 to November 2013. The ages of the children ranged from 10 days to 14 years. The vast majority of patients (89.9%) were ≤ 5 years old. The ratio of male to female was 1.81:1.

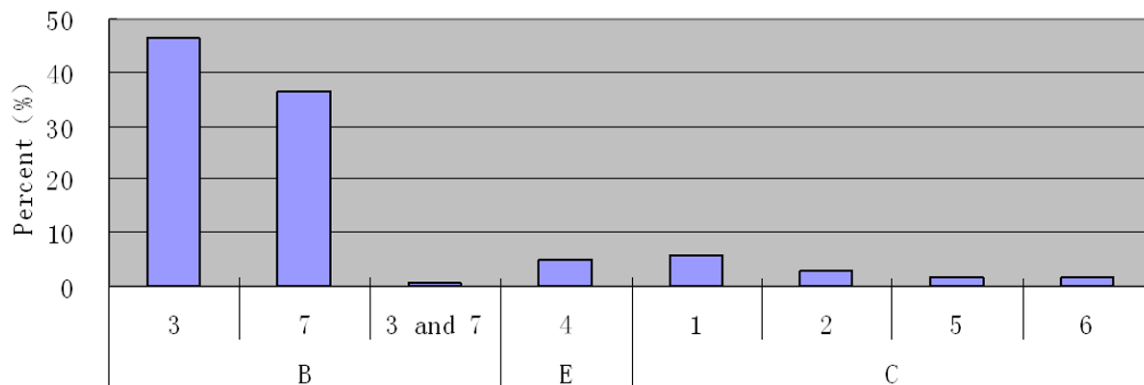
### Detection and prevalence of HAdV and other respiratory viruses in hospitalized children

A total of 1,066 of 6,479 (16.45%) specimens were positive for the seven viruses. The RSV was detected in 657 of 1,066 (61.63%) samples, HAdV was detected in 228 (21.39%) samples, PIV-III was detected in 118 (11.07%) samples, FLU-A was detected in 51 (4.78%) samples, PIV-II was detected in 15 (1.41%) samples and PIV-I and FLU-B were detected in 2 and 1 samples, respectively (**Figure 1**). Co-infections with two respiratory viruses were only detected in six of the samples and there was no detection of co-infections of more than two respiratory viruses. The total positive detection rate for HAdV was 3.52% (228/6479). HAdV infection occurred throughout the year and peaked from May to August (**Figure 2**). Only two HAdV samples showed coinfection with other respiratory viruses; i.e., FLU-A and FLU-B.

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**Figure 3.** Age distribution of 201 hospitalized patients diagnosed with adenovirus infection from December 2012 to November 2013.



**Figure 4.** Species and types distribution of 201 hospitalized patients diagnosed with adenovirus Infection.

### *The characteristics of age and gender in the infected children*

The median age of HAdV infected patients was 22.5 months (min = 1 month, max = 14 years). Children 0-2 years old represented the majority of infected HAdV cases at 54.4% (127/228) and children under the age of five years account for 86.4% (197/228) (**Figure 3**). Of the HAdV positive cases in 6,479 children with respiratory tract infection, 3.6% (150/6479) were males and 3.4% (78/6479) were females. There was no significant difference in the infection rates between males and females ( $\chi^2 = 0.201$ ,  $P = 0.654$ ).

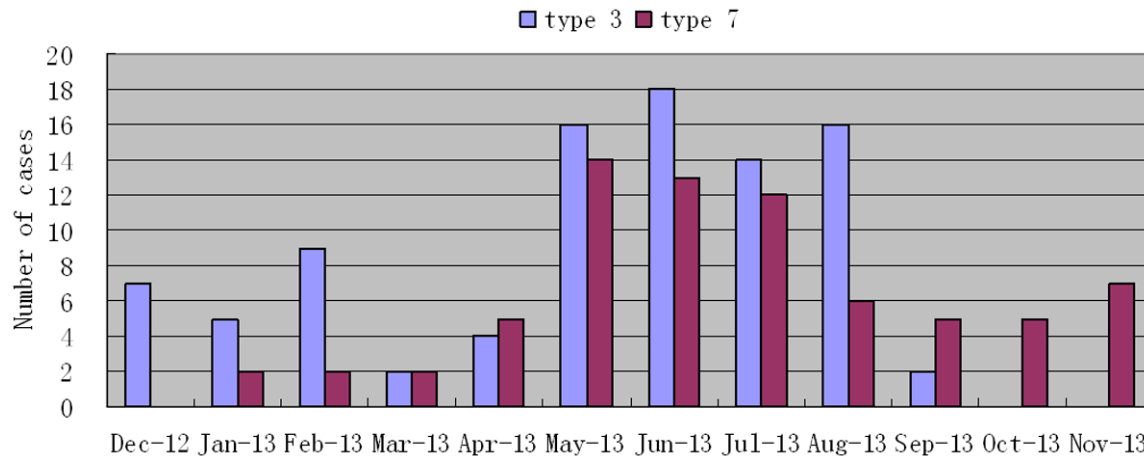
### *Molecular characterization and prevalence of HAdV*

We used the sequencing method outlined in the Materials and Methods section to type 201

of the 228 (88.2%) adenovirus positive samples (27 samples were not stored in daily detection). Multi-PCR identified 167 and sequencing identified 34 of the viruses. AdV3 (93) was the most frequently identified, followed by AdV7 (73), AdV1 (12), AdV4 (10), AdV2 (6), AdV5 (3), AdV6 (3), and 1 was co-infected with AdV3 and AdV7. No type 11, type 21, or other types were detected (**Figure 4**). AdV3 and AdV7 were the major types found in hospitalized children in Shenzhen, followed by AdV1 and AdV4.

HAdV genotype prevalence fluctuated according to analysis of AdV3 and AdV7 detection during the study year. AdV3 was the major virus detected from December to February of the next year and June to August, while AdV7 was the major virus detected from September to November. The AdV3 and AdV7 detection rates were not significantly different from March to May (**Figure 5**).

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**Figure 5.** Detection of adenovirus identified as type 3 or type 7, distributed by month.

### *The relation between ADV infection and clinical diagnoses*

Of 228 hospitalized patients who were HAdV positive, 127 patients had pneumonia, 15 of the cases were severe. Seven children had type 3 or type 7 and one had type 3/7 mixed infection in 15 severe cases. 35 had bronchitis, 20 had tonsillitis with an upper respiratory tract infection, 42 had acute upper respiratory tract infection, and four had a conjunctivitis pharynx.

### **Discussion**

In this study, the positive rate for one or two of the seven respiratory viruses was 16.5% (1,066/6,479) in hospitalized children in Shenzhen, China. The positive rate for HAdV was 3.52% (228/6,479) and two children were co-infected, one with Flu-A and the other with Flu-B. The rate of HAdV detection in our study was lower than that reported recently in Lanzhou (6.33%), Guangzhou (4.88%), Taiwan, Israel and South Korea, but it was higher than the rate in Beijing (1.69%) and Suzhou (1.54%) [5-7, 11-14]. The differences among the prevalence rates may be due to geographic location.

Adenovirus infections can occur endemically or as outbreaks, especially where there is a concentration of military personnel or in cities. There are differences in virus types in different populations and regions. For example, adenovirus type 4 infection is most common in the US military [15], type 14 outbreaks were found in schools in China [16], and type 3 outbreaks

were found in Guangzhou, China [17]. AdV3 was previously the predominant genotype for respiratory ADV infection in many regions, especially in children. In our study, nearly half of the adenovirus infections were type 3 (46.3%), which was followed by type 7 (36.3%), type 1 (6.0%), type 4 (5.0%), type 2 (3.0%), type 6 (1.5%), and type 5 (1.5%). One hospitalized child was co-infected with AdV3 and AdV7. AdV3 and AdV7 were the genotypes that were mostly commonly detected, which was consistent with the results from Beijing [7], while these two genotypes were not the most common in Guangzhou [6], Hong Kong [18] and Israel [11], which had predominant genotypes of AdV1 and AdV2 or AdV2 and AdV3. This may be related to the method used for detection and whether the population was close to Beijing. The Guangzhou and Hong Kong studies had fewer cases and Israel is a completely region with a different ethnic composition. AdV4 reached 5.0% in our study and appeared only in Guangzhou and Hong Kong [6, 18], which are southern cities in China. Other areas of Beijing, Lanzhou, and Nanjing had no positive cases [5, 7, 8]. AdV11 was not detected in Shenzhen, China, but appeared in Beijing, Lanzhou, and Nanjing [5, 7, 8], which indicated that the adenovirus infection in children was region-specific in China. AdV7 infection is most frequently reported elsewhere and may be related to severe infections in children, including in Beijing. However, in our study, AdV3 and AdV7 were related to severe infections. Shenzhen may possibly be the site of virulent variations of AdV3, which leads to severe pneumonia and hospitalization of children.

Adenoviruses are known to typically infect young children with immunological underdevelopment and in immunocompromised populations. In our study, over half of the patients (54.4%) with adenovirus infection were less than two years old, and the majority of patients (86.4%) with adenovirus infection were less than five years old. These results are consistent with studies from Lanzhou, Korea, and Israel [5, 11, 13]. There were no significant differences in the HAdV infection ratio of males (3.6%) and females (3.4%) in our study. This was in contrast to the tendency toward male predominance in previously reported studies of HAdV infection. The difference may be due to patient populations, methodology, or geographic location. In this study, the most common clinical diagnosis was pneumonia and bronchitis (71.1%), indicating the adenovirus mainly caused lower respiratory tract infections in children. There were a lower proportion of acute upper respiratory infections, such as tonsillitis, which is consistent with the results from Beijing and Lanzhou.

The present study enabled the construction of a general picture of the common adenovirus genotypes in hospitalized children in Shenzhen, China. We showed that HAdV is a prevalent viral agent in hospitalized children in Shenzhen and subgroup B types 3 and 7 were related to severe infections.

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## Disclosure of conflict of interest

None.

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