

# Molecular Typing of Human Adenovirus Infection among Hospitalized Patients with Respiratory Tract Infections in Guangzhou, China

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## Research Article

**Keywords:** adenovirus, PCR, genotyping molecular epidemiology, respiratory diseases

**Posted Date:** April 8th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-401554/v1>

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**Version of Record:** A version of this preprint was published at BMC Infectious Diseases on August 3rd, 2021. See the published version at <https://doi.org/10.1186/s12879-021-06412-0>.

# Abstract

**Background** Human Adenoviruses (HAdVs) cause a wide array of illnesses in all age groups. They particularly cause frequent morbidity among children. In China, human adenovirus types 3, 4, 7, 11, 14, 21, and 55 have caused at least seven outbreaks since 2000. However, limited studies are available regarding the epidemiological patterns and diversity of HAdVs types among hospitalized patients with respiratory tract infections (RTIs).

**Methods** To understand the epidemiology and type distribution of HAdV infections associated with RTIs in China, nasal swab (NS) clinical samples were collected from 4129 patients in a Guangzhou hospital between August 2017 and October 2019. PCR, sequencing, and phylogenetic analysis were performed on these specimens to identify HAdV virus types.

**Results** HAdV was detected in 99 (2.4%) of the 4,129 NS specimens, with the highest HAdV prevalence (6.3%) found in children between the ages of 5 and 10 years. Among HAdV-positive specimens, the most prevalent genotypes identified were HAdV-B3 (55.6%) and HAdV-B7 (25.3%). The most common symptoms in the HAdV-infected patients were fever (100%), cough (80.8%) and rhinorrhea (71.8%). HAdV infections were detected throughout the year with a relatively higher prevalence in summer.

**Conclusion** All ages suffer adenovirus infections, but young children are at greatest risk. These study data demonstrate that at least three species of HAdVs (species B, C, and E) are circulating in Guangzhou City, China. As antiviral therapies and type-specific vaccines become available, such epidemiological data will be useful in guiding therapy and public health interventions.

## Background

Human adenoviruses (HAdV) are non-enveloped double-stranded DNA viruses. They belong to the family *Adenoviridae*. Approximately 103 subtypes of HAdV have been documented to date. They are organized into seven species (A-G) [1, 2]. HAdV is a highly contagious pathogen that causes various clinical illnesses, including upper and lower respiratory infections, bronchitis, pneumonia, conjunctivitis, and acute gastroenteritis [3]. All age groups of people are susceptible to HAdV infections, while children, immunocompromised patients, cardiovascular disease patients, and military trainees are at a higher risk for developing severe disease [3–5]. It has been reported that HAdV is responsible for at least 5–10% of pediatric and 1–7% of adult respiratory tract infections (RTIs) [6]. HAdV infections have a worldwide distribution, but the distribution of adenovirus types often differs by geographical region or human population. Among all HAdVs, type 3, 4, 7, 14, 21, and 55 often cause severe infections and have been linked to outbreaks globally [7–11]. HAdV-associated outbreaks are more likely to occur in closed and crowded conditions, such as schools, hospitals, or military recruits [7, 9].

In recent years, an increasing number of HAdV outbreaks have been reported in China. An acute respiratory infection (ARI) outbreak caused by a re-emergent isolate of HAdV55 occurred in Shanxi Province in 2006 [12]. An outbreak of febrile respiratory illness caused by HAdV-14p1 occurred in Gansu

Province in 2011 [13]. Two outbreaks of acute respiratory diseases caused by HAdV-7 were detected in military training camps, one in Hubei Province and another in Shaanxi Province between 2012 and 2013 [7]. HAdV-B3 was also frequently reported as the common cause of epidemic ARI outbreaks in China [14]. However, limited data are available regarding the epidemiological and clinical features of HAdV in hospitalized patients. This study we sought to determine the prevalence and types of HAdVs among hospitalized patients with RTIs in Guangzhou, China. Such epidemiological data are useful to health professionals regarding decisions in employing appropriate therapy and adopting effective prevention strategies for adenovirus control.

## Materials And Methods

### Case Definition

A total of 4,129 nasal swab (NS) specimens were previously collected from patients with RTIs at the Second Affiliated Hospital of Guangzhou University of Chinese Medicine between August 2017 and October 2019. These patients were recruited for participation in the hospital's study if they met the following inclusion criteria: (i) had a fever (temperature  $\geq 37.3^{\circ}\text{C}$ , measured at the hospital) with the apparent respiratory symptom(s); (ii) did not have the bacterial infection (white blood cell count  $> 12 \times 10^9/\text{L}$ , or PCT  $> 0.5\text{ng}/\text{L}$ ). Individuals who met the inclusion criteria were consented (parental consent required for adolescents under the age of 18) and invited to complete a brief questionnaire about their demographics and symptoms.

### Subject Sample Collection

Each participant permitted the collection of one NS specimen (the swab stayed in the nose for 15 seconds), which was placed in viral transport media (COPAN Diagnostics Inc, Italy), stored in the icebox and then transported to the hospital's laboratory. Specimens were preserved at  $-80^{\circ}\text{C}$  until further processing.

At the hospital's laboratory, all clinical NS specimens were first screened with the commercially available Immunofluorescence assay (respiratory viral panel 1 screening and identification kit, Light Diagnostic<sup>TM</sup>, Chemicon International Inc, Temecula, USA) targeting adenovirus, influenza A and B virus, parainfluenza 1–3, and respiratory syncytial virus (RSV). A total of 117 HAdV-positive specimens were detected. Later these HAdV-positive specimens were sent to the Duke Kunshan University (DKU) One Health Research Laboratory for further study.

### Detection of HAdV with Real-Time PCR

At the DKU One Health Research Laboratory, DNA was extracted from HAdV-positive specimens and eluted in 60 $\mu\text{l}$  of elution buffer using a QIAamp MinElute Virus Spin Kit (Cat. No.57704, Qiagen Inc., Hilden, Germany). Extracted DNA was then tested for adenovirus by real-time reverse transcriptase-polymerase chain reaction (qPCR) assays using a BioRad SsoAdvanced Universal Probes Supermix (BioRad Laboratories, Richmond, CA) as previously described [15] on a Mic qPCR Cycler (BioMolecular Systems, El Cajon, CA). Positive and negative template controls were included in each qPCR run. The

qPCR cycling was run as follows: 90°C for 2 min, 95°C for 3 min, followed by 40 cycles of 95°C for 15s, and 60°C for 30s. Samples with quantification cycles (Cq) values < 38 were considered positive for adenovirus.

Nested PCR targeting the HAdV hexon gene's hyper-variable regions 1–6 (HVR1-6) was performed for genotyping (predicted amplicon size: 764–896 base pairs (bp) [16]. The outer primers used in the first-round amplification were AdhexF1 (5'-TGTAACGACGGCCAGT-TICTTTGACATCGIGGIGTICTIGA-3') and AdhexR1 (5'-CTGTTCIACIGCCTGRTTCCACA-3'). The inner primers used in a second PCR were AdhexF2 (5'-GGYCCYAGYTTAARCCCTAYTC-3') and AdhexR2 (5'-GGTTCTGTCCCCAGAGARTCIAGCA-3'). Nested PCR was conducted in a 50µl volume comprising 5µl of 10 \* EXTaq buffer, 0.2µl (50µM) of each primer, 1.0µl of dNTP Mix, 1.5µl of 50mM MgCl<sub>2</sub>, 0.2µl of Taq DNA Polymerase, 0.5µl of first nested-PCR product, and 41.4µl of double-distilled water. Cycling conditions were employed as follows: 94°C for 2 minutes, followed by 36 cycles at 94°C for 1 min, 45°C for 1 min, and 72°C for 2 min, and a 5 min extension at 72°C. PCR products were analyzed on 2% agarose gels and sent for sequencing.

### **Sequence and Phylogenetic Analysis**

Hexon gene nucleotide sequences from the specimens collected in this study were aligned with multiple reference strains available in GenBank (Supplementary Table 1) using ClustalW. The phylogenetic tree was constructed by the neighbor-joining method with bootstrap analysis (n = 1,000) by MEGA7.0 software.

### **Statistical Analysis**

STATA version 15.0 (StataCorp LP, College Station, TX) was used for statistically analysis. Chi-square or Fisher's exact tests were used to examine risk factor associations with HAdV molecular detections

## **Results**

### **Demographic Data of the Hospitalized Patients**

The 99 HAdV-positive patients identified in this study included 66 (63.67%) males and 33 (33.33%) females. Their median age was 4 years, range 2 months to 63 years. The HAdV-positive patients were distributed across age groups: Age groups <5, 5-10, 10-19, and ≥19 years, accounted for 3.8%, 6.3%, 0.6%, 0.16% of positives, respectively (Table 1). A significant difference in the number of HAdV-positive patients among the different age groups was observed with the highest prevalence of HAdV infections being found among children who were between 5 and 10 years (6.3%), followed by those under five years (3.8%). There was no significant difference in HAdV-positivity observed between males and females.

### **Clinical Feature of HAdV-Infected Patients**

Among 99 HAdV-positive patients, two patients had missing clinical information and were not included in Table 2. Clinical diagnosis included pneumonia (38.1%), acute bronchitis (24.7%), acute upper respiratory tract infections (AURTI) (17.5%), acute tonsillitis (3.2%), and gastroenteritis (4.1%). It is worth noting that

approximately 79% of pneumonia patients were found among children under ten years in age. The most prevalent clinical signs were fever (99.0%), cough (82.5%), rhinorrhea (69.1%), and expectoration (62.9%), while the other clinical presentation were sore throat (19.6%) and diarrhea (8.2%). No HAdV-associated deaths were reported.

## **Seasonal Distribution of the HAdV Infections**

In China, 12 months were classified into four seasons, including Spring (March to May), Summer (June to August), Autumn (September to November), and Winter (December to February). The number of HAdV-positive patients from August 2017 to October 2019 detected in different seasons are depicted in Figure 1. HAdV infections were detected during each season. However, the highest numbers of HAdV positive patients were found in the summer in both 2018 and 2019 with 22 and 17 HAdV patients, respectively.

## **Molecular Characterization of HAdV**

### **qPCR Results**

Among the 117 immunofluorescence-positive HAdV specimens, 103 (88.0%) specimens were detected as HAdV-positive with qPCR at DKU One Health Research Laboratory.

### **Sequence and Phylogenetic Analysis**

To further analyze the HAdV genotype, the hexon gene from 103 HAdV positive specimens was amplified by conventional PCR. Ninety-nine (96.1%) specimens were successfully genotyped. Eight out of 99 sequences selected as representative sequences (see details in supplementary material) were further aligned with other reference strains (partial Hexon gene) (Figure 2). The phylogenetic analysis indicated that 84 patients belonged to species B, 7 patients belonged to species C, and 8 patients belonged to species E (Table 3). Within the HAdV-B species, type 3, 7, and 55 were identified. It is worth noting that all the HAdV-B strains identified in this study were highly identical to strains detected in Shenzhen and Jiangxi City, suggesting the genetic conservation of some HAdV-B viruses in different areas of China. Additionally, HAdV-B3 and HAdV-B7 were the most prevalent types in this study. Within the HAdV-C species, types 1, 2 and 5 were identified. All the HAdV-C species generated from this study seem to be more closely related to HAdV-C viruses circulated in neighboring countries. For example, the HAdV-C1 strain in this study was nearly identical to the strain CAU230/AdV/KOR/2016 found in South Korea. The HAdV-C5 strain identified in this study was identical to a Japanese HAdV virus. Study findings indicated that Species B, C, and E (at least seven subtypes) circulated simultaneously in Guangzhou in 2017-2019.

### **Clinical Characteristics of HAdV Genotypes**

HAdV genotypes differed in their characteristics (Table 3). The most common clinical manifestations of all HAdV genotypes were fever (100%), cough (50 - 100%), and rhinorrhea (50-100%). The comparison among the seven HAdV types revealed that HAdV-B7 caused more severe diarrhea than other HAdV types. The majority (94.6%) of pneumonia patients were found to be associated with HAdV-B species (including

type 3, 7, and 55). Unexpectedly, three out of four gastroenteritis patients were found in patients who were infected with HAdV-B7.

## Discussion

A recent multicenter, prospective registry study found that adenovirus was the third-leading cause of viral infection among community-acquired pneumonia (CAP) patients in China, after influenza viruses and respiratory syncytial virus [17]. An increasing number of HAdV outbreaks have been reported in China in recent years [7, 12, 14, 18]. But because there is no national surveillance system for HAdV in China and there is often no difference in symptom clustering between viral and bacterial infections, the diagnosis of HAdV is often underestimated [2].

The present study recorded the epidemiological distribution of circulating HAdV strains among hospitalized patients with respiratory tract infections (RTIs) between 2017 and 2019 in Guangzhou City, China. In this study, the overall positive rate of HAdV was 2.4%, which is consistent with the positive rate (2.0%-6.1%) found in hospitalized patients with acute viral respiratory infection in other recent reports [3, 19–21]. However, two previous studies conducted in the Northern part of China showed a higher HAdV prevalence (10.4%-20.1%) than our study [22, 23]. These findings demonstrate that HAdV prevalence may differ by geographic locations. Such differences in HAdV prevalence could be influenced by a number of factors, including sample type, small sample size, sampling period, study duration, and patients' demographic information.

Regarding patient demographic data, there was no significant difference observed in HAdV detections between genders in this study. This is consistent with the findings of previous studies conducted in China [3, 20]. However, this was in contrast to some previous reports that male children were more likely to be infected with HAdV [24, 25]. The highest HAdV-positive rate was observed among children between the ages of 5 and 10 years, which is similar to the findings of a recent study in the same city [3]. However, most previous reports found that the HAdV infections occurred more often among children under five years of age [19, 26–28].

Our study revealed that although HAdV infections were detected throughout the year, the prevalence peaked in the summer. This is consistent with findings of Chen et al [28] during 2012–2013. But it was not consistent with that of studies conducted in Northern China [22] and Mexico [29]. In Tanzania and Switzerland, HAdV infections were observed during all seasons of the year with no clear seasonality demonstrated [21, 27]. These difference in HAdV prevalence between seasons are interesting and bear future study as seasonal risk could influence future employment of HAdV vaccines which are in development in China [30].

In this study, seven HAdV types were identified: HAdV-B3, HAdV-B7, HAdV-B55, HAdV-C1, HAdV-C2, HAdV-C5, and HAdV-E4. Among these types, HAdV-B3 and HAdV-B7 were most prevalent which is consistent with other reports in Asia [26, 31, 32].

The majority (94.6%) of HAdV types detected in this study were of species B. HAdV-B (e.g., 3, 7, and 55) has been continuously reported to be associated with more severe acute respiratory disease than other HAdVs species [2, 14, 18]. Only seven patients were found to have HAdV-C and only eight patients were found to have HAdV-E infection during the study period.

Previously, three types of HAdV-C (e.g., 1, 2, and 5) were identified in China, although studies of HAdV-C species have been limited [33]. HAdV-C species viruses were identified as the primary pathogens responsible for respiratory tract infections among hospitalized children, particularly among infants under two years of age [33]. Our study is consistent with this in that more than half of HAdV-C-positive patients were observed in children less than two years old. Previous research suggests that recombination events are commonly observed among HAdV-C types [33]. As recombinant HAdV strains have caused epidemics, it seems prudent to monitor for changes in HAdV-C types in China.

Although our study provides crucial molecular evidence regarding the epidemiology and clinical features of HAdV infections in Guangzhou, China, it has several limitations. First, we did not study outpatients and they could have had a different distribution of HAdV types. Second, study samples were first identified using a commercial assay which is not thought to be as sensitive as the qPCR we employed. Hence, we likely only captured data on the specimens with higher viral titers. Thus, the true prevalence of HAdV among hospitalized patients may have been higher and the distribution of HAdV types different. Third, we were unable to rule out a number of possible viral coinfections that were not surveilled for with the commercial product (e.g., parainfluenza 4, rhinoviruses, enteroviruses, etc.). Hence, some of the clinical findings we observed could be due coinfections with viruses other HAdV.

At present, there are no specific antiviral drugs or vaccine available in China for the treatment or prevention of HAdV infections. However, effective antivirals [34, 35] and vaccines [30, 36] seem to be on the near horizon. Hence, conducting type-specific surveillance for HAdV is an important need for China. Nationwide, periodic HAdV surveillance could alert Chinese public health officials of the emergence of pre-pandemic or particularly virulent strains and help them mitigate the threat. For instance, currently, there seems to be a worldwide increase in the threat of HAdV type four which could be mitigated with the Teva, Inc. HAdV four live vaccine [37].

## **Declarations**

### **Ethics Statement**

This study was approved by the Ethics Committee of The Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Reference B2017-032-01). Individual written informed consent was obtained from the patients or patients' parents or guardians.

### **Acknowledgements**

This study was supported by funding from The Second Affiliated Hospital of Guangzhou University of Chinese Medicine. Funding included the specific research fund for TCM Science and technology of Guangdong Provincial Hospital of Chinese Medicine (No. YN2015MS09 and YN2016QN10) and major national science and technology projects (2017ZX10305501-006). We thank many professionals who collected the achieved human specimens.

### Conflict of Interest

The authors declare that they have no conflicts of interest.

### Author Contribution

X.W. conducted laboratory analysis and drafted the manuscript. D.W attended to field work and revised the manuscript. S.U. guided the work and revised the manuscript. Q.S. and L.Q. identified the HAdV positive specimens using immunofluorescence assays and collected clinical information from patients. Y.L guided the field work in Guangzhou and attended to draft the manuscript. G.C.C conceived the study, guided the work, and revised the manuscript.

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## Tables

**Table 1.** Human adenovirus (HAdVs) infections among 99 patients hospitalized with respiratory tract infections in Guangzhou, China between August 2017 and October 2019. Patients were enrolled from the Second Affiliated Hospital of Guangzhou University of Chinese Medicine.

Characteristics	Number of Patients*	Number of patients positive for HAdVs	Percentage of patients positive for HAdV	P-value
Age (years)				0
<5 years	1485	57	3.80%	
5 -<10 years	600	38	6.30%	
10-<19 years	169	1	0.60%	
≥19 years	1855	3	0.20%	
Gender				0.107
Male	2416	66	2.70%	
Female	1693	33	2.00%	

Note: \* Of the 4129 patients, 20 patients had information missing on age, gender, and clinical information, and were thus excluded from the analysis.

**Table 2.** Clinical characteristics of patients who had adenovirus (HAdVs)-positive nasal swab specimens.

Characteristics	HAdV positive (%) (N=97)
<b>Diagnosis</b>	
Pneumonia	37 (38.1)
Acute bronchitis	24 (24.7)
Acute tonsillitis	3 (3.2)
AURTI	17 (17.5)
Gastroenteritis	4 (4.1)
<b>Symptoms</b>	
Fever	96 (99.0)
Cough	80 (82.5)
Rhinorrhea	67 (69.1)
Expectoration	61 (62.9)
Sore throat	19 (19.6)
Diarrhea	8 (8.2)
<b>Prognosis</b>	
Totally recovery	97 (100)
Death	0 (0)

**Table 3.** Clinical and laboratory characteristics of patients who had a human adenovirus (HAdVs)-positive nasal swab specimen, by HAdV type.

Characteristics	HAdV B3* (n=53)	HAdV B7 (n=25)	HAdV B55 (n=4)	HAdV E4 (n=8)	HAdV C1 (n=2)	HAdV C2 (n=3)	HAdV C5 (n=2)
<b>Symptoms</b>							
Fever	53 (100.0)	24 (100.0)	4 (100.0)	8 (100.0)	2 (100.0)	3 (100.0)	2 (100.0)
Cough	45 (84.9.4)	21 (84.0)	4 (100.0)	4 (50.0)	2 (100.0)	2 (66.7)	2 (100.0)
Rhinorrhea	38 (71.7)	15 (60.0)	4 (100.0)	6 (75.0)	1 (50.0)	2 (66.7)	2 (100.0)
Sore throat	9 (17.0)	5 (20.0)	1 (25.0)	3 (37.5)	1 (50.0)	0 (0.0)	0 (0.0)
Expectoration	36 (67.9)	13 (52.0)	3 (75.0)	3 (37.5)	2 (100.0)	2 (66.7)	2 (100.0)
Diarrhea	2(3.8)	5 (20.0.)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
<b>Diagnosis</b>							
Acute Tonsillitis	2 (3.8)	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
URTI	8 (15.1)	4 (15.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
Acute Bronchitis	12 (22.6)	7 (28.0)	1 (25.0)	2 (25.0)	1 (50.0)	1 (33.3)	0 (0.0)
Pneumonia	21 (39.6)	12 (48)	2 (50.0)	1 (12.5)	1 (50.0)	0 (0.0)	0 (0.0)
Gastroenteritis	0 (0)	3 (12)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)

Notes: 1) The total number of HAdV-B3 positive specimens is 55, but two patients with information miss on clinical features were not described. 2) URTI (upper respiratory tract infection); HAdV (human adenovirus)

## Figures

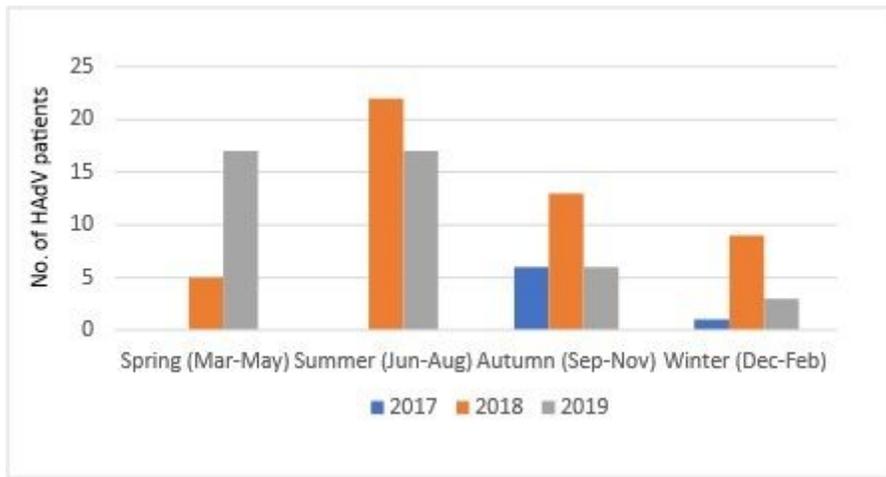
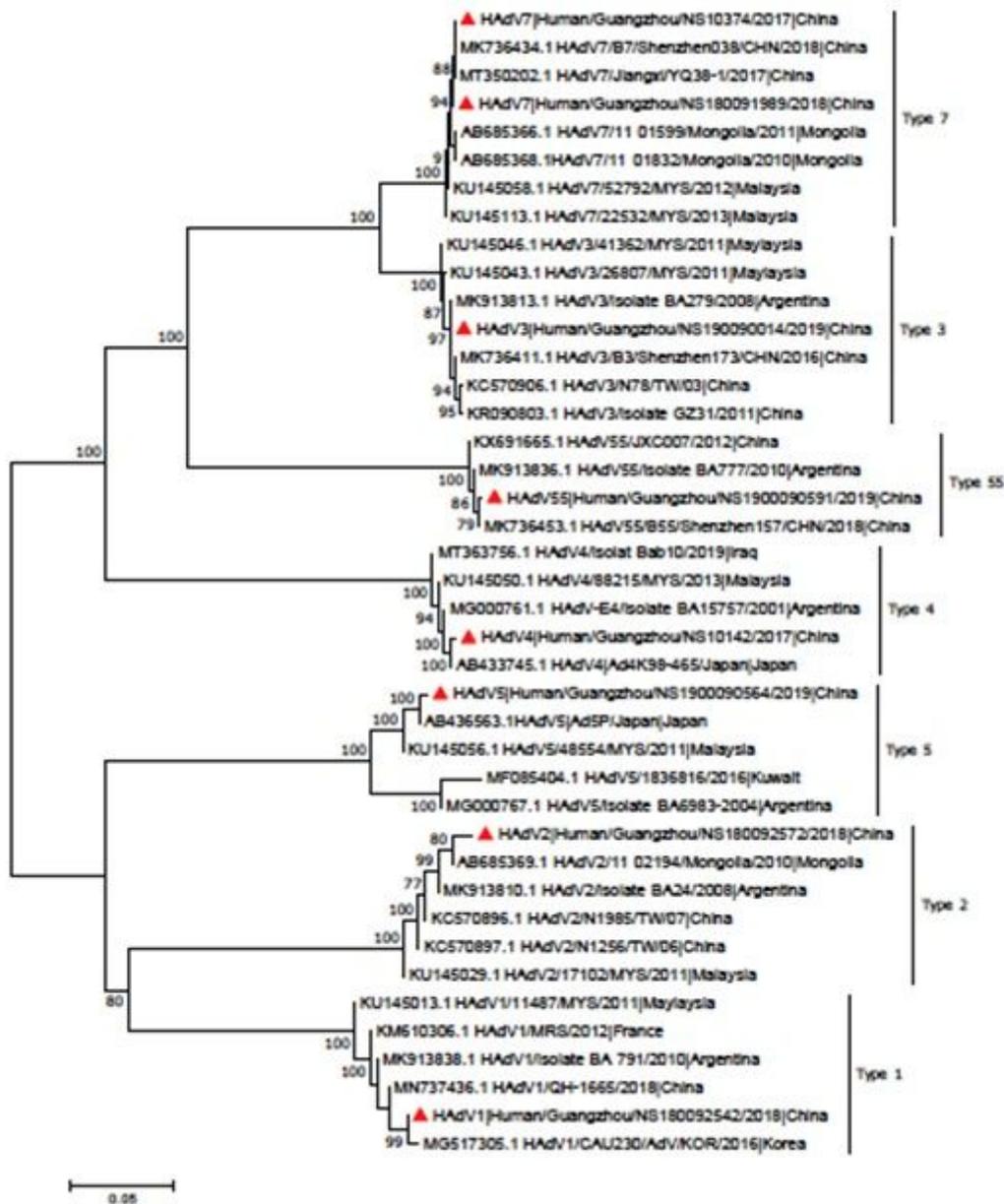


Figure 1

HAdV patients detected in different season



## Figure 2

Phylogenetic analysis of the hexon gene of HAdV strains identified in patients hospitalized with respiratory track infections in Guangzhou between 2017 and 2019. The representative strains detected in this study are marked with red solid triangles. Other reference strains were gathered from previous publications (Supplementary Table1). The phylogenetic tree was constructed with a neighbor-joining tree method and p-distance model using MEGA version 7 (<http://www.megasoftware.net>). Bootstrap values were calculated on 1000 replicates, and values <70% are not shown.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [BMCSupplementaryMaterialXWGCG202104.docx](#)