Sporadic and Severe Lower Respiratory Illness-related Human Adenovirus Type 21 Infection in Southern China

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Research Article

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Abstract

Background: Human adenovirus type 21 (HAdV-21) is an important member of HAdV species B, but our understanding of this type is limited.

Methods: We screened HAdV and 17 other common respiratory pathogens for 1,704 pediatric patients (≤14 years old) hospitalized with acute respiratory illness in Guangzhou, China in 2019. HAdV-21 infections were further confirmed by molecular typing from HAdV-positive patients, and their clinical manifestations, genomes, infectivity and pathogenicity *in vitro* were analyzed.

Results: 151 of 1,704 cases (8.9%) were positive for HAdV, making it the third most frequently detected pathogen. Two sporadic HAdV-21 infections were identified in June and September. Both HAdV-21-positive patients presented with severe lower respiratory illness and had similar initial symptoms at onset of illness. The genome structure of HAdV-21 was found to be similar to that of other members of HAdV species B. The phylogenetic analysis showed that it was closely related to HAdV-B21 strain BB/201903 (MN686206) isolated in Bengbu, China in 2019, suggesting the possibility of the same source, and attention need to be paid to its prevention and control. *In vitro*, the infectivity and pathogenicity of HAdV-21 were lower than the main epidemic types 7 and 3. Plaques formed by HAdV-21, -7, and -3 were significantly different in shape and size ($p < 0.05$), with plaques formed by HAdV-21 being the smallest and with poorly defined edges. There was no significant difference between the plaques of the HAdV-21 isolates ($p > 0.05$).

Conclusions: This study provides an important reference for the in-depth understanding of the epidemiology and pathogenicity of HAdV-21, and suggests the necessity of HAdV-21 research, prevention and control.

Introduction

Human adenoviruses (HAdVs) are non-enveloped, double-stranded DNA viruses of the family *Adenoviridae*. More than 100 genotypes of HAdVs have been identified, which are classified into seven species (A–G) (1, 2). HAdVs are associated with a broad spectrum of clinical diseases, such as acute respiratory illness (ARI), conjunctivitis, gastrointestinal infections, and obesity (2–5). Members of species B are known to cause human diseases, with HAdV types 3, 7, 14, and 55 being the most common causes of respiratory disease outbreaks (6).

HAdV-21 is a member of species B, and was first isolated in 1956 from a 1-year-old child with trachoma and conjunctivitis in Saudi Arabia (7). HAdV-21 was later found to be associated with a variety of diseases, including ARI (8–12). Severe pneumonia, myocarditis, flaccid paralysis, and even fatal infections in both pediatric and adult patients have been reported (13–16). The circulation of ARI-associated HAdV-21 has been reported among military recruits and civilians in several developed countries (8, 17–19), and caused nosocomial infections in lung transplant patients at a large tertiary care hospital (20). However, data on HAdV-21 are limited as HAdV-21 infections have rarely been reported in

regions outside North America and Europe and are especially rare in China (21). To better understand the epidemiology of HAdV-21, infection data from different regions are of great importance.

In this study, we analyzed the distribution of respiratory pathogens in 1,704 pediatric patients with ARI in Guangzhou, China, in 2019. Two patients with HAdV-21 displaying severe lower respiratory illness (LRI) were identified. The clinical features of these infected patients were assessed, and the genomic characteristics and cyto-pathogenicity of HAdV-21 in vitro were analyzed.

Materials And Methods

Respiratory sample collection

Respiratory samples, including throat swabs, sputum, and bronchoalveolar lavage fluid, from pediatric patients (≤ 14 years old) hospitalized with ARI were collected for routine screening of respiratory viruses *Mycoplasma pneumoniae* (MP) and *Chlamydophila pneumoniae* (CP) in accordance with established clinical protocols at the First Affiliated Hospital of Guangzhou Medical University between January and December 2019 (22). The samples were refrigerated at 2–8°C in viral transport medium, transported on ice to the State Key Laboratory of Respiratory Diseases, and analyzed immediately or stored at − 80°C before analysis, as previously described (23). The patients' clinical presentations were collected from the medical records.

Screening for HAdV and common respiratory pathogens

Respiratory pathogen screening was conducted to detect HAdV and 17 other common respiratory pathogens, including influenza A virus (infA), influenza B virus (infB), respiratory syncytial virus (RSV), parainfluenza virus types 1–4 (PIV1–4), human metapneumovirus (HMPV), human rhinovirus (HRV), enterovirus (EV), four types of coronaviruses (HCoV-229E, -OC43, -NL63, and -HKU1), human bocavirus (HBoV), MP, and CP simultaneously using TaqMan real-time quantitative polymerase chain reaction (qPCR), as previously reported (24).

HAdV-21 identification

HAdV-positive samples were subjected to further molecular typing for HAdV-3, -7, -14, -21, -55, -C5, and -E4 using TaqMan qPCR. The specific primers, which probed the hexon or fiber genes of the different HAdV types, are shown in Table S1. Probe qPCR Mix (TaKaRa, Dalian, China) was used according to the manufacturer's protocol. Clinical characteristics, treatments, and outcomes of the HAdV-21-positive patients were collected retrospectively.

Cells, HAdV stocks, and HAdV-21-positive sample culture

A549 cells were cultured in Dulbecco's minimum essential medium (DMEM) (Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin (Gibco) at 37°C and 5% (v/v) CO_2. HAdV-21-positive samples were cultured in the A549 cells at 37°C and 5% CO_2 and maintained under standard conditions in DMEM supplemented with 2% (v/v) FBS and 100 U/mL penicillin-streptomycin.

Inoculated cells were monitored daily for the cytopathic effect (CPE) and were harvested at almost full CPE. HAdV-3-Guangzhou01 (accession no. DQ099432), HAdV-7-CQ1198 (accession no. JX625134), and HAdV-21 reference stain AV-1645 (ATCC, accession no. AY601633) were used simultaneously for analysis of the cyto-pathogenicity of the HAdV-21 isolates. HAdV-3-Guangzhou01 and HAdV-7-CQ1198, from the State Key Laboratory of Respiratory Diseases, were collected from patients with severe pneumonia in Guangzhou in 2005 and Chongqing in 2010, respectively. HAdV-21-AV-1645, which was first isolated in 1956 from a 1-year-old child with trachoma and conjunctivitis in Saudi Arabia (7), was kindly provided by Prof. Chenyang Li (Hecin Scientific, Guangzhou, China).

HAdV-21 genome sequencing and annotation

HAdV-21-positive samples were cultured and harvested. Viral genomic DNA was extracted using a TaKaRa Mini BEST Viral RNA/DNA Extraction Kit Ver.5.0 (TaKaRa) according to the manufacturer's instructions. Next-generation sequencing was conducted with a Illumina NovaSeq 6000 sequencer following a protocol from Synbio-Technologies (paired-end, 2 × 150 bp). The complete genome of HAdV-21 was assembled using CLC Genomics Workbench 11.0. The complete genomes of the HAdV-21 isolates were annotated based on the annotation of HAdV-21 strain BB/201903 (accession no. MN686206). Complete genome sequences were logged in the GenBank database.

Phylogenetic analysis and HAdV sequences used

Phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA) version 5.05 (25). Phylogenetic trees were constructed by the Neighbor-joining (NJ) method with 1,000 bootstrap replicates and default settings for all other parameters. HAdV sequences of the penton base, hexon, and fiber genes, and the genomes for phylogenetic analyses retrieved from GenBank are summarized in Table 1.

Table 1
Sequences of adenovirus species A–G used for phylogenetic analysis

Type	Strain	Country	Year isolated	GenBank accession number
HAdV-B21	GZ06109#	China	2019	MW091531
	GZ09107#	China	2019	MW151243
	BB/201903	China	2019	MN686206
	AV-1645	Saudi Arabia	1956	AY601633
	OHT-006	USA	2016	MF502426
	LRTI-1	Germany	2005	KF577595
	LRTI-8	Germany	2016	KY307858
	PEL0066	USS	2005	KJ364587
	NHRC 64589	USA	2007	KJ364582
	NHRC 71227	USA	2005	KJ364584
	NHRC 52331	USA	2006	KJ364581
	NHRC 44288	USA	2006	KJ364576
	NHRC 32389	USA	2005	KJ364573
	NHRC 91447	USA	2007	KJ364590
	LRTI-3	Germany	2010	KF577593
	LRTI-5	Germany	2012	KF802425
	LRTI-5	Germany	2012	KF577597
	LRTI-4	Germany	2012	KF938575
	LRTI-6	Germany	2013	KF577598
	LRTI-7	Switzerland	2013	KY307857
	Sibu-97	Malaysia	1997	KY307860
	VRDL T87-0342	USA	1987	KJ364591
	LRTI-9	Switzerland	2016	KY307859
	LRTI-2	Germany	2008	KF802426
	CDC RU8176	USA	1978	KJ364577

#The HAdV-B21 isolated in this study.

Type	Strain	Country	Year isolated	GenBank accession number
	NHRC 10030	USA	1998	KJ364586
	CDC V1375E	USA	1984	KJ364579
	NHRC 20007	USA	1998	KJ364580
	NHRC 63218	USA	2006	KJ364575
	NHRC 71139	USA	2004	KJ364583
	NHRC 71252	USA	2005	KJ364585
	NHRC 5	USA	1996	KJ364578
	CDC V2148A	USA	1988	KJ364588
	VRDL T97-1745	USA	1997	KJ364589
	NHRC 32493	USA	2005	KJ364574
	VRDL T98-1269	USA	1998	KJ364592
	GER	Germany		KF528688
SAdV-21	Bertha	USA	1954	AC_000010
HAdV-B3	GB	USA	1953	AY599834
HAdV-B7	Gomen	USA	1952	AY594255
HAdV-B11	Slobitski	USA	1956	NC_011202
HAdV-B14	de Wit	Netherlands	1955	AY803294
HAdV-B55	BJ01	China	2011	JX491639
HAdV-B34	Compton	USA	1972	AY737797
HAdV-B35	Holden	USA	1973	AY128640
HAdV-B16	ch. 79	USA	1955	AY601636
HAdV-B50	Wan	USA	1988	AY737798
HAdV-B66	AY128640	Argentina	1987	JN860676
HAdV-B68	Arg 827/04	Argentina	2004	JN860678
HAdV-A12	Huie	USA	1954	AC_000005
HAdV-C1	Adenoid 71	USA	1953	AF534906
HAdV-D9	Hicks	USA	1954	AJ854486

[#]The HAdV-B21 isolated in this study.

Type	Strain	Country	Year isolated	GenBank accession number
HAdV-E4	RI-67	USA	1952	AY594253
HAdV-F40	Dugan	Netherlands	1979	NC_001454
HAdV-G52	T03-2244	USA	2003	DQ923122
[#]The HAdV-B21 isolated in this study.				

Viral plaque formation assay

A549 cells were seeded into 6-well culture plates and incubated overnight to form dense monolayers with more than 90% confluence. After removal of the growth media, the cultures were inoculated with 0.4 mL of 10-fold serial dilutions of the viral stocks and incubated for 1 h at 37°C with rocking every 15 min. The viral inocula were removed by aspiration and 3 mL DMEM-agarose mulch (2% SeaPlaque GTG-agarose [Lonza] mixed 1:1 with 2× DMEM medium containing 4% FBS) was added to each well. The agarose was allowed to solidify at room temperature (20–26°C). Plaque plates were incubated at 37°C and 5% CO_2 for a total of 13 days, with 1.5 mL/well of DMEM-agarose mulch supplementation after 4 and 8 days. The plates were stained with 2 mL/well 20% ethanol, 2% paraformaldehyde, and 1% crystal violet overnight at room temperature. The diameters of the plaques were measured with the assistance of the VisionWorks software package.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL). Differences between groups were calculated using the *t*-test and Mann–Whitney *U* test. A p-value < 0.05 (two-tailed) was considered statistically significant.

Results

Overall respiratory pathogen infection

Samples from 1,704 pediatric patients hospitalized with ARI were collected, with 845 (49.6%) patients infected with one or more of the pathogens of interest. Of these, 151 patients (8.9%) were identified with HAdV infections, which was the third most frequently detected pathogen in this study (Fig. 1).

HAdV-21 identification

Two of the 151 (1.3%) HAdV-positive patients, GZ06109 and GZ09107, were diagnosed with HAdV-21 in June and September 2019. In the other 149 patients, HAdV-3 (47.0%, 71/151), HAdV-7 (46.4%, 70/151), HAdV-4 (4.0%, 6/151), and HAdV-55 (1.3%, 2/151) were detected, while HAdV-5 and HAdV-14 were not.

Clinical characteristics of the HAdV-21-positive patients

Data on the clinical characteristics, treatments, and outcomes of the two HAdV-21-positive patients were collected (Table 2). Both patients had similar initial symptoms of fatigue at the onset of the disease, and both were diagnosed with severe LRI by radiologic testing. Patient GZ06109 also had sepsis, although no microbes were found by blood culture. The indexes of white cell count, procalcitonin (PCT), C-reactive protein (CRP), aspartate aminotransferase (AST), and D-dimer exceeded the upper limits of the normal ranges for both patients. Levels of creatine kinase (CK) and lactate dehydrogenase (LDH) were abnormal and differed between the two patients. While the patients had similar disease durations, patient GZ09107, who had severe pneumonia in both lungs, was hospitalized for longer (9 days) than patient GZ06109 (5 days; Table 2).

Table 2
Clinical characteristics, treatments, and outcomes of the two patients infected with HAdV-21

Characteristic	HAdV-21-positive patient	
	GZ06109	GZ09107
Clinical characteristic		
Gender	Male	Female
Age, year	4.6	1
Existing chronic disease	Rhinitis	Negative
Initial symptom	Repeated coughing and fever	Repeated coughing and fever
The highest temperature, ^{o}C	40.5	40.2
Sputum production	Yes	Yes
Shortness of breath	No	Yes
Fatigue	Yes	Yes
Radiologic findings		
	Pneumonia	Pneumonia on both lungs
Laboratory findings		
The blood oxygen saturation under inhalation, %	99	88–90
Bacteria or fungus culture	Negative	Negative
White-cell count, $\times 10^9$/L	15.3, ↑	10.3, ↑
Lymphocyte count, $\times 10^9$/L	3.6	3.4
Platelet count, $\times 10^9$/L	342	332
Hemoglobin, g/L	101	104

Normal index range of test items: white cell count, 4–10 × 10^9/L; lymphocyte count, 0.9–5.2 × 10^9/L; platelet count, 100–400 × 10^9/L; hemoglobin, 120–150 g/L; procalcitonin, 0–0.05 ng/mL; alanine aminotransferase, 5–40 U/L; C-reactive protein, 0–0.6 mg/dL; aspartate aminotransferase, 5–40 U/L; creatine kinase, 10–190 U/L; lactate dehydrogenase, 109–255 U/L; D-dimer, 68–494 ng/mL. "↑", exceeding the upper limit of the normal range.

Characteristic	HAdV-21-positive patient	
	GZ06109	GZ09107
Procalcitonin, ng/mL	1.47, ↑	0.2, ↑
Alanine aminotransferase, U/L	15	12
C-reactive protein, mg/dL	11.57, ↑	1.02, ↑
Aspartate aminotransferase, U/L	51.1, ↑	53, ↑
Creatine kinase, U/L	1152, ↑	86
Lactate dehydrogenase, U/L	215	642, ↑
D-dimer, ng/mL	509, ↑	1268, ↑
Diagnosis of physician		
	Pneumonia and sepsis	Severe pneumonia
Treatments		
Symptomatic treatment	Anti-infection, anti-inflammation, intravenous fluid therapy, atomization inhalation treatment	Anti-infection, anti-inflammation, intravenous fluid therapy
Intravenous immune globulin	No	Yes
Mechanical ventilation	No	Yes
Clinical outcomes-recovery duration		
Total disease duration, day	20	19
Length of hospital stay, day	5	9

Normal index range of test items: white cell count, 4–10 × 10^9/L; lymphocyte count, 0.9–5.2 × 10^9/L; platelet count, 100–400 × 10^9/L; hemoglobin, 120–150 g/L; procalcitonin, 0–0.05 ng/mL; alanine aminotransferase, 5–40 U/L; C-reactive protein, 0–0.6 mg/dL; aspartate aminotransferase, 5–40 U/L; creatine kinase, 10–190 U/L; lactate dehydrogenase, 109–255 U/L; D-dimer, 68–494 ng/mL. “↑”, exceeding the upper limit of the normal range.

Genome features of HAdV-21 isolates

HAdV-21 isolates GZ06109 and GZ09107 were sequenced, annotated, and uploaded to the GenBank database with accession numbers MW091531 and MW151243, respectively. The identified genomes were 35,362 and 35,365 bp in length for GZ06109 and GZ09107, respectively, and had similar genomic organization and transcription maps (shown for strain GZ09107 in Fig. 2).

Phylogenetic features of the HAdV-21 isolates

Phylogenetic analysis of the genomes and three structural protein sequences showed that HAdV-B21 members are closely related to each other, forming a clade (Fig. 3). The percent identities of the genomes and three capsid protein genes of the two HAdV-21 isolates identified here were compared with the most closely related strain, HAdV-21-BB/201903 (98.82–100%), and the first strain to be isolated, HAdV-21-AV-1645 (98.38–99.47%; Table 3). Compared with HAdV-21-BB/201903, GZ06109 contained one insertion mutation in the penton base (GCG, alanine) and two synonymous mutations in the non-coding region. No non-synonymous mutations were found in strain GZ09107 (Fig. 4).

Table 3
Percent identities of genomes and capsid protein sequences of the isolated strains GZ06109 and GZ09107 with representative HAdV-21 strains

Nucleic acid	GZ06109				GZ07109			
	Genome	Hexon	Penton base	Fiber	Genome	Hexon	Penton base	Fiber
BB/201903[a]	99.97	100	98.82	100	99.97	100	100	100
AV-1645[b]	98.86	99.16	99.28	99.38	98.86	99.16	99.47	99.38

[a]The most closely related strain, HAdV-21a/MN686206/CHN/BB/201903/2019;

[b]The first HAdV-21 strain to be isolated, HAdV-B21/AY601633/Saudi Arabia/AV-1645/1956.

Cyto-pathogenicity of the HAdV-21 isolates

Cyto-pathogenicity analyses of HAdV-21 strains GZ06109, GZ09107, AV-1645, HAdV-3-Guangzhou01, and HAdV-7-CQ1198 were performed using the plaque formation assay (Fig. 5). Plaques formed by HAdV-7-CQ1198 and HAdV-3-Guangzhou01 were bigger and had clearer boundaries than those from the HAdV-21 strains ($p < 0.001$; Fig. 5A). Plaque sizes were significantly different among the three HAdV types ($p < 0.05$), but not among the three HAdV-21 strains (Fig. 5B).

Discussion

Among the more than 100 types and seven species (A–G) of HAdV, species B is of particular importance in ARI (http://hadvwg.gmu.edu/). Although HAdV-21 is an important member of HAdV species B, our understanding of this type is inadequate. From 2017 to 2019, HAdV strains were prevalent in China,

causing outbreaks of severe pneumonia in children (http://www.chinacdc.cn/) (26). In this study, we analyzed the epidemiologic characteristics, genome features, and cyto-pathogenicity of HAdV-21

Of the 1,704 participants in this study, 8.9% of the patients were infected with HAdV, making it the third most prevalent pathogen detected (Fig. 1). This positive rate was higher than that reported for previous years in this region (5%, 213/4242) (22). The main HAdV types detected were type 7 (46.4%) and 3 (47.0%), which is largely consistent with previous reports (22, 26, 27). Two sporadic cases of HAdV-21, GZ06109 and GZ09107, were identified in June and September. The low prevalence of HAdV-21 may signify low immunity against this type in the general population, increasing its potential to cause an epidemic.

Both HAdV-21-positive patients presented with severe LRI (Table 2), which highlights the need for increased awareness of HAdV-21 infections. The HAdV-21 infections had similar initial symptoms at onset of illness, and multiple indexes exceeded the normal ranges, such as white cell count, PCT, CRP, AST, and D-Dimer. These factors may help physicians judge and screen for this pathogen (Table 2), although definitive diagnosis will require laboratory screening as many respiratory viruses have similar manifestations. Because there are no specific treatments against adenoviruses, only symptomatic treatment can be used at present. Previous studies have shown that HAdV-21 can cause nosocomial infections in immune-compromised patients (20), highlighting the importance of prevention and control of this type.

To improve our understanding of HAdV-21, we cultured the two HAdV-21 isolates, GZ06109 and GZ09107. Sequencing and annotation of their genomes revealed similar structures to other members of HAdV species B (28) (Fig. 2). By comparing and analyzing the genome sequences of HAdV-21 and other HAdV species (Table 1), we found that the HAdV-21 genome is comparatively stable and constitutes a clade (29) (Fig. 3). The two HAdV-21 strains in this study had highest genome identity (99.97%) with strain HAdV-21-BB/201903 (accession no. MN686206), which was isolated in Bengbu, China in 2019, and 98.86% similarity with the first strain to be isolated, HAdV-21-AV-1645 (Table 3). In terms of the main structural protein genes, GZ09107 had 100% similarity to the Bengbu strain, with only two synonymous mutations (T antigen and 20k protein) and two non-coding region mutations (Fig. 4). Compared with the Bengbu strain, GZ06109 had 100% sequence identity for the fiber and hexon genes (Table 3) and an alanine insertion in the penton base (Fig. 4). This suggests that HAdV-21 isolates prevalent in China have a high degree of kinship and are from the same source, although there is insufficient data to identify the potential source.

To analyze the pathogenicity of HAdV-21, plaque formation assays with the two HAdV-21 isolates in this study, reference HAdV-21-AV-1645, and severe pneumonia-related HAdV-3-Guangzhou01 and HAdV-7-CQ1198 were conducted (Fig. 5). Although the plaques formed by the three HAdV-21 strains showed similar characteristics (Fig. 5A) and were similar in size ($p > 0.05$; Fig. 5B), they were significantly smaller ($p < 0.001$) than plaques from HAdV-7 and HAdV-3, and had poorly defined edges. It was also found that plaques formed by HAdV-7 were significantly larger than those of HAdV-3 ($p < 0.05$). These plaque

features indicate that HAdV-21 < HAdV-3 < HAdV-7 with regard to virulence and infectivity. Compared with clinical research reports, the prevalence and pathogenicity of HAdV-7 and HAdV-3 are largely consistent with this result (30). There are too few reports on HAdV-21 to determine its overall pathogenic characteristics; thus, more research on this type is urgently needed.

The main limitation of this study is that selection bias may have occurred, because the sample comes from one hospital, and there is a lack of samples from outpatient clinics and healthy people. This may lead to deviations in the understanding of HAdV-21 infection, especially the epidemiological characteristics.

Conclusions

In this study, we investigated HAdV-21 infections in Guangzhou, China, and identified two patients infected with severe LRI-related HAdV-21 strains. Genomic analysis showed that the Chinese isolates of HAdV-21 showed a high degree of similarity, suggesting that attention should be paid to its prevention and control. Analyses of the clinical characteristics, genome structures, and cyto-pathogenicity of these strains revealed important information that will contribute to a deeper understanding of HAdV-21.

Abbreviations

HAdV: Human adenovirus

ARI: acute respiratory illness

LRI: lower respiratory illness

infA: influenza A virus

infB: influenza B virus

RSV: respiratory syncytial virus

PIV: parainfluenza virus

HMPV: human metapneumovirus

HRV: human rhinovirus

EV: enterovirus

HCoV: human coronaviruses

HBoV: human bocavirus

MP: *Mycoplasma pneumoniae*

CP: *Chlamydophila pneumoniae*

qPCR: quantitative polymerase chain reaction

DMEM: Dulbecco's minimum essential medium

FBS: fetal bovine serum

CPE: cytopathic effect

MEGA: Molecular Evolutionary Genetics Analysis

NJ: Neighbor-joining

PCT: procalcitonin

CRP: C-reactive protein

AST: aspartate aminotransferase

CK: creatine kinase

LDH: lactate dehydrogenase

Declarations

Ethics approval and consent to participate

The First Affiliated Hospital of Guangzhou Medical University Ethics Committee approved the involvement of human subjects in this study. Next of kin, caretakers, or guardians gave signed informed consent for participation in the study on behalf of the minors/children.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: WKL, QL, XGT, and RZ; Methodology: WKL, LZ, YC, and RZ; Formal analysis: WKL, LZ, YC, QL, XGT, and RZ; Investigation: WKL, LZ, YC, YSQ, YQW, DX, SJG, XL, and JD; Resources: DHC, QL; Writing – Original Draft Preparation: WKL, LZ, YC, and QL; Writing – Review & Editing: XGT and RZ; Funding Acquisition: WKL, RZ, and QL. All authors have read and agreed on the final manuscript.

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Figures

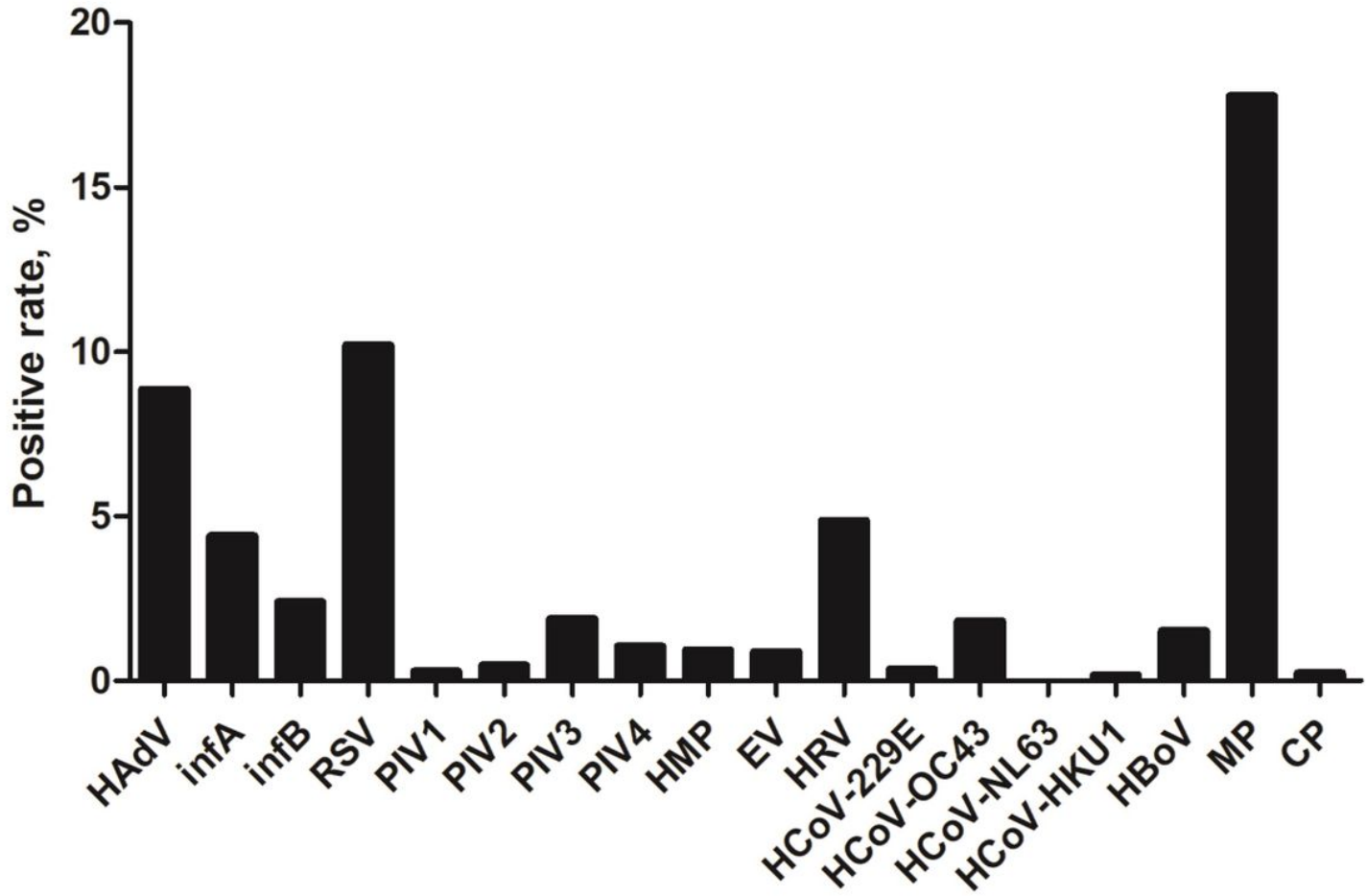

Figure 1

Distribution of respiratory pathogens in 1,704 children hospitalized with acute respiratory illness in Guangzhou, China in 2019

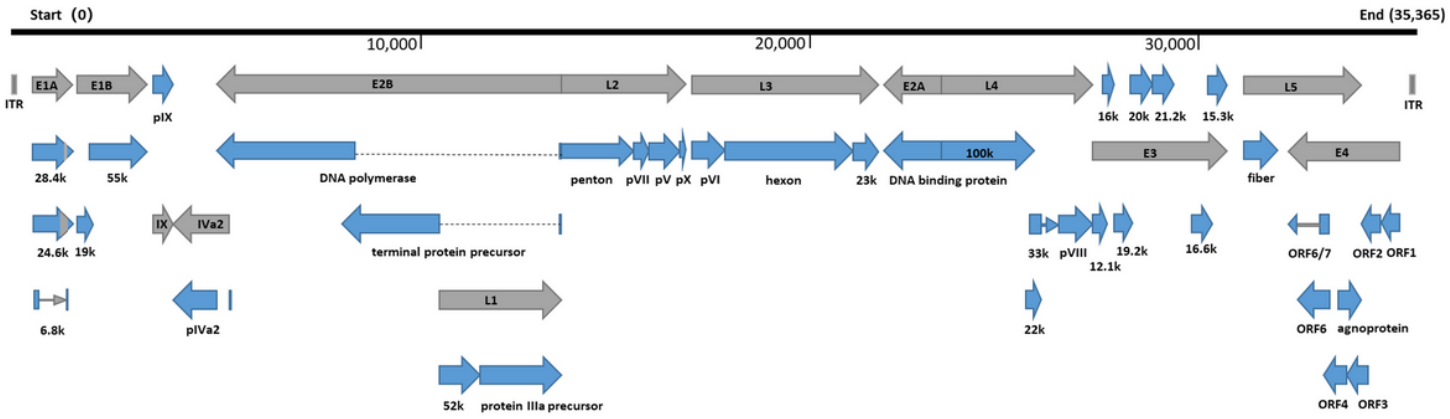

Figure 2

Transcriptional map and genome organization of HAdV-21 strain GZ09107 The genome is indicated by the black horizontal line marked at 10,000 bp intervals. The transcription units are designated by gray arrows, while blue arrows designate coding regions. Arrows reflect the transcriptional orientation of the coding transcripts.

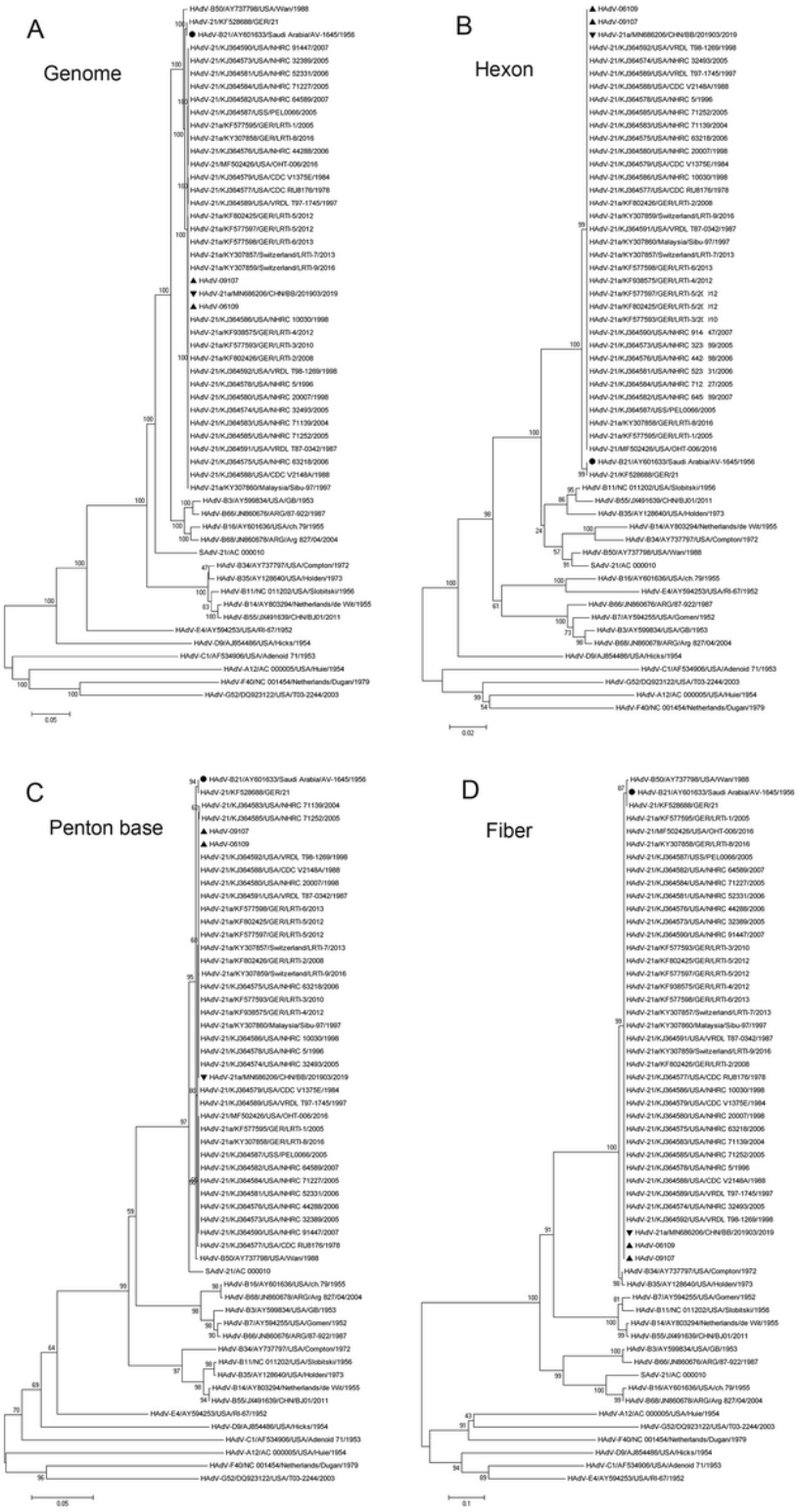

Figure 3

Phylogenetic analysis of HAdV-21 strains GZ06109 and GZ09107 The nucleotide sequences of the whole genome (A), hexon (B), penton base (C), and fiber (D) genes of the HAdV strains were analyzed for their phylogenetic relationships using the Neighbor-Joining method with 1,000 bootstrap replicates implemented in the MEGA 5.0 software package. For reference, taxon names include the genome type, corresponding GenBank accession number, country of isolation, strain name, and year of isolation. The two HAdV-21 strains isolated in this study are marked with "▲"; "▼", strain isolated from Bangbu (BB/201903), China in 2019; "●", reference standard HAdV-21 isolated in Saudi Arabia in 1945.

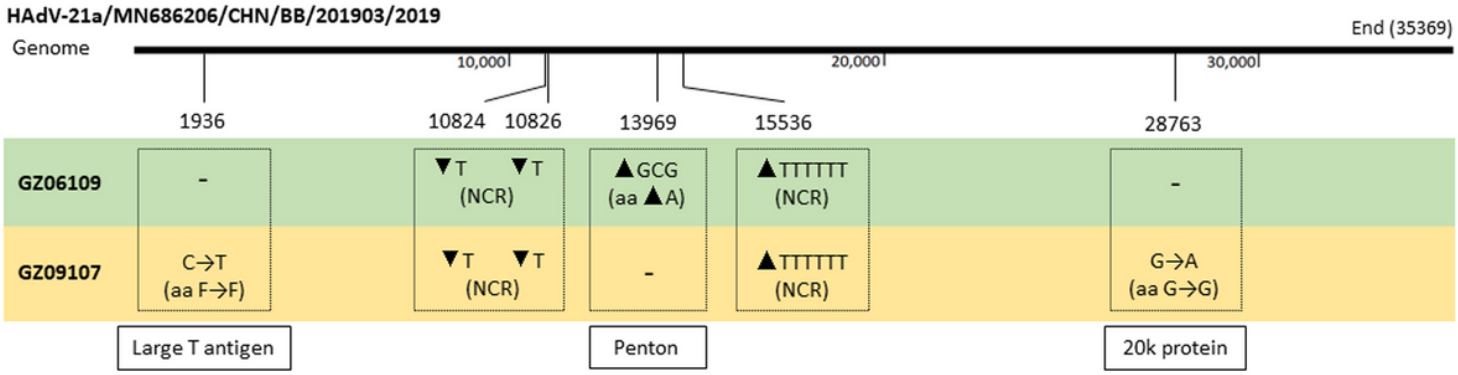

Figure 4

Comparative genomics analysis of the two HAdV-21 isolates and reference strain BB/201903 Nucleic acid and amino acid sequence changes are noted, along with their genome locations and coding consequences. The reference genome of strain HAdV-21 BB/201903 is indicated by the black horizontal line marked at 10,000 bp intervals. NCR, non-coding region; ▼, insertion; ▲, deletion; –, no change.

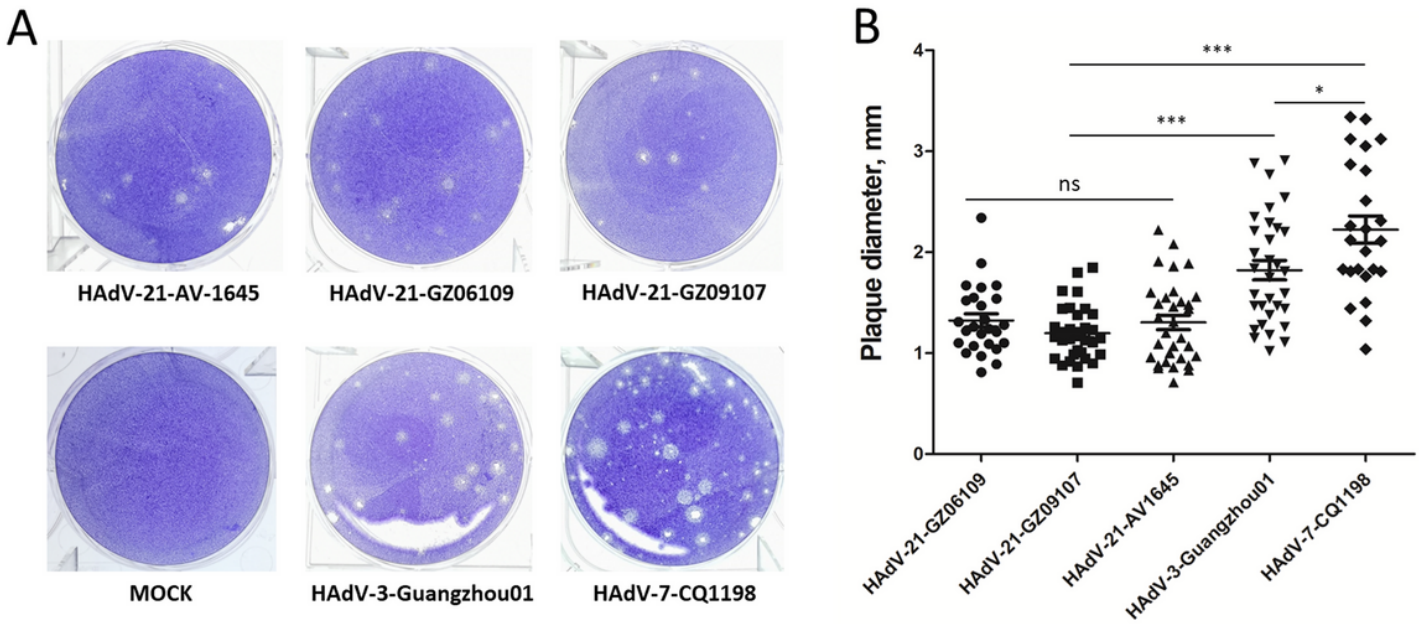

Figure 5

Plaque formation and size distribution of the HAdV-21 isolates and references HAdV-21-AV-1645, HAdV-3-Guangzhou01, and HAdV-7-CQ1198 (A) Plaque formation. (B) Size distribution. Plaque plates were incubated and stained with crystal violet for a total of 13 days in 6-well culture plates. ns, not significant; *, $p < 0.05$; ***, $p < 0.001$.

Supplementary Files

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

- TableS1.docx