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ORIGINAL RESEARCH

Genotypes and Phylogenetic Analysis of Human Adenovirus in Hospitalized Pneumonia and Influenza-Like Illness Patients in Jiangsu Province, China (2013-2021)

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Background: Human adenovirus (HAdV) is common pathogens that cause various respiratory diseases. The genetic diversity of viruses caused by recombination is considered to be the main source of emerging outbreaks. The aim of this study is to explore the evolutionary relationship and recombination events of HAdV genome in respiratory tract infections in Jiangsu Province.

Methods: Whole-genome sequencing (WGS) technology was used to sequence 66 patients with HAdV infection (37 patients with influenza-like illness (ILI) and 29 hospitalized patients with pneumonia) from Jiangsu Province. Epidemiological analysis was performed on hospitalized pneumonia and ILI patients infected with HAdV. Subsequently, phylogenetic, recombination, and nucleotide and amino acid identity analyses were performed.

Results: Epidemiological analysis of patients undergoing WGS showed that 75.7% of ILI patients were infected with the HAdVB strain and 69.0% of hospitalized pneumonia patients were infected with the HAdVC strain. Moreover, the hospitalized pneumonia and ILI patients infected with HAdV were different in region and time. The strains of HAdVB3 and HAdVB7 genotypes were mainly infected in 2015 and 2017, and the strains of HAdVC1 and HAdVC2 genotypes were mainly infected in 2020. The results of histogram analysis showed that the HAdV strain mainly infected children under 5 years old. In addition, 36 novel recombinant strains were identified. The discovery of these recombinant strains may contribute to understanding the epidemiology of HAdV and research on related vaccines. Furthermore, the percentage of nucleotide and amino acid identities revealed a high level of genetic conservation within isolates from HAdVB3, HAdVB7, HAdVC1, HAdVC2 and HAdVC5 genotypes.

Conclusion: The WGS analysis reveals the evolutionary relationships and recombination events of HAdV strains in Jiangsu Province, which is helpful to deepen the understanding of HAdV epidemiology and evolution. In addition, it provides a basis for the formulation of public health strategies in Jiangsu Province.

Keywords: human adenovirus, whole genome sequencing, phylogenetic analysis, recombination

Introduction

Human adenovirus (HAdV) is common pathogens that cause various respiratory, eye, and gastrointestinal diseases.[1] HAdV is a large, complex, non-enveloped double-stranded DNA virus belonging to the genus *Mastadenovirus*, family *Adenoviridae*.[2,3]

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Hexon, Penton and Fiber are the major capsid proteins of HAdV that mediate virus-host cell interactions.[4] The HAdV is divided into 7 species A-G,[5] and 113 HAdV genotypes have been assigned according to the classification criteria of the Human Adenovirus Working Group (http://hadvwg.gmu.edu/). The symptoms and disease manifestations of patients with HAdV infection are related to the species and site of HAdV infection. Species HAdVA, HAdVF, and HAdVG are associated with gastrointestinal infections, species HAdVB, HAdVC, and HAdVE are associated with adenovirus related respiratory infections, and species HAdVD mainly targets the conjunctiva.[6,7] HAdV is a highly infectious agent. The transmission modes of adenovirus are various, and the main transmission mode is respiratory transmission.[8]

HAdV are prevalent in the human population and constitute a significant burden of respiratory diseases worldwide. HAdVB and HAdVC were the most common HAdV genotypes in children with acute respiratory diseases. Moreover, compared to HAdVB patients, HAdVC patients are more likely to be younger, hospitalized, and have a higher frequency of seizures.[9] Severe pneumonia and acute respiratory distress syndrome caused by HAdVB21 infection are devastating diseases that can rapidly develop into multiple organ failure and death.[10,11] HAdVE4 is the only type in species HAdVE thus far isolated from humans, and associated with respiratory tract and ocular infections.[12] The analysis of patients with severe respiratory infection in Guangdong Province that HAdV was the third most frequently detected pathogen, of which HAdVB3 and HAdVB7 were the main epidemic types.[13] Therefore, it is essential to investigate the molecular epidemiological characteristics of HAdV in respiratory tract infections.

The HAdV genome is unstable because it is influenced by genetic drift caused by base insertion, substitution, and deletion, as well as genomic recombination between at least two virus strains.[14] Recombination is the main driver of HAdV evolution and the emergence of new pathogenic strains.[15,16] Influenza and pneumonia are common respiratory diseases that seriously affect human health.[17,18] Influenza-like illness (ILI) and pneumonia can be caused by a variety of viral and bacterial pathogens, including adenovirus.[19–21] In this study, whole-genome sequencing (WGS) technology was used to sequence 66 patients with HAdV infection (37 patients with ILI and 29 hospitalized patients with pneumonia) from Jiangsu Province. Subsequently, the evolutionary relationships and potential recombination events of the strains were determined through molecular typing and genomic characterization. This study will help to deepen the understanding of HAdV epidemiology and evolution, and provide a basis for the development of effective vaccine and the formulation of public health strategy in Jiangsu Province.

Materials and Methods

Samples

ILI was defined as fever (body temperature ≥38°C) with cough or sore throat. Hospitalized pneumonia cases should simultaneously meet the following two requirements: 1) Patients who require hospitalization and are diagnosed with "pneumonia" or "lung infection"; 2) In the early stages of the disease, the total number of white blood cells is normal or reduced, or lymphocytes is normal or reduced. Throat swab samples were collected from different sentinel hospitals for respiratory multi-pathogen surveillance in Jiangsu Province (Nanjing, Suzhou, Taizhou, and Xuzhou). The sampling period was 2013–2021. Samples that cannot be detected in time are stored at −70 °C ultra-low temperature refrigerator. From 2013–2021, a total of 4200 patients with ILI were tested, including 206 HAdV positive patients (4.9% positivity rate); and 4253 patients with hospitalized pneumonia were tested, including 76 HAdV positive patients (1.8% positivity rate). Only 66 patient samples (37 patients with ILI and 29 hospitalized patients with pneumonia) successfully isolated HAdV in 282 adenovirus positive patient samples. Therefore, samples from these 66 patients were selected for subsequent sequencing analysis. In addition, variance analysis and chi-square test were used to analyze the sample information of these 66 sequencing samples. This study was approved by the ethics committee of Jiangsu Provincial Center for Disease Control and Prevention (JSJK2022-BO16-02). All participants were informed as to the purpose of this study, and that this study complied with the Declaration of Helsinki. The informed consent was obtained from the all participants.

Nucleic Acid Extraction and Detection of HAdV

The qEx-DNA/RNA viral nucleic acid extraction or purification kit (Xi'an Tianlong Technology Co., LTD., China) was used to extract total viral DNA according to the instructions. The extracted nucleic acid was transferred to a 1.5 mL eppendorf (EP) tube and stored at −70 °C for future use. Adenovirus fluorescent quantitative PCR detection kit (commercial kit produced by

Jiangsu Shuoshi Biotechnology Co., LTD., China) was used to detect the extracted nucleic acid by real-time quantitative polymerase chain reaction (RT-PCR), and the cycle threshold (Ct) value was recorded. Usually, samples with Ct value less than 30 are separated and cultured. The specific detection method was carried out according to the instructions.

HAdV Cultivation and Amplification

Human epidermoid larynx carcinoma cell line (Hep-2, item number FH0119) were purchased from Shanghai Fuheng Biotechnology Co., LTD. Hep-2 with good growth status (high cell transparency, few intracellular particles, clear cell membrane, normal cell proliferation rate, etc.) was selected to inoculate HAdV throat swab samples for HAdV isolation. Inoculate 200ul per well. Cytopathic effect (CPE) of HAdV mainly showed that the cells became round, the refraction was increased, and the diseased cells gathered into grape clusters. Cells were observed daily after inoculation. When CPE reached 75%~100%, the viral nucleic acid was extracted using the qEx-DNA/RNA viral nucleic acid extraction or purification kit and detected using the respiratory adenovirus nucleic acid detection kit. Subsequently, Hexon gene was amplified by PCR using HAdV universal primers[22] (AdhexF1: 5′-TICTTTGACATICGIGGIGTICTIGA-3′, AdhexR1: 5′-CTGTCIACIGC CTGRTTCCACA-3′; AdhexF2: 5′-GGYCCYAGYTTYAARCCCTAYTC-3′, AdhexR2: 5′-GGTTCTGTCICCCAG AGARTCIAGCA-3′) for initial genotype screening. Capillary electrophoresis was used to confirm whether the amplification was successful. Then, the successfully validated amplification products were subjected to first-generation sequencing to obtain preliminary typing data for HAdV.

Whole Genome Sequencing of HAdV

The ULSEN Ultra-sensitive Adenovirus whole genome capture kit (Beijing Micro Future Technology Co., LTD., China) was used for whole-genome specific amplification of the extracted total viral DNA. The Agencourt AMPure XP kit (Beckman Coulter, USA) was used to purify the amplified products. The Nextera XT Library Prep Kit (Illumina, USA) was used to construct DNA sequencing library according to the instructions. The Illumina NextSeq2000 sequencer was used for whole-genome deep sequencing of HAdV. CLC Genomic Workbench 12.0 software was used to assemble the sequencing raw FASTQ data and splice the raw nucleotide sequences. Then, the spliced sequences were BLAST in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank) of the United States to obtain HAdV infection types, and the full length of the Hexon, Penton base and Fiber genes were intercepted.

Phylogenetic Analysis

HAdV reference sequences AC_000017 (HAdVC1), AC_000007 (HAdVC2), AC_000008 (HAdVC5), HQ413315 (HAdVC6), HQ003817 (HAdVC57), MH121097 (HAdVC89), KR699642 (HAdVC2), MF315028 (HAdVC2), MF315029 (HAdVC2), MH183293 (HAdVC1), MN737436 (HAdVC1), MK165452 (HAdVC2), MK165453 (HAdVC2), MN628614 (HAdVC1), MN628615 (HAdVC), NC_011203 (HAdVB3), AC_000018 (HAdVB7), NC_011202 (HAdVB11), AY803294 (HAdVB14), AY601636 (HAdVB16), AY601633 (HAdVB21), AY737797 (HAdVB34), AC_000019 (HAdVB35), AY737798 (HAdVB50), FJ643676 (HAdVB55), JX423386 (HAdVB66), JN860678 (HAdVB68), LC177352 (HAdVB79), JX491639 (HAdVB55), NC_003266 (HAdVE4), KY996446 (HAdVE4a), AY487947 (HAdVE4p), AY601636 (HAdVB16), and FJ025923 (SAdV26) were obtained from the GeneBank database. Phylogenetic analyses were performed in MEGA 11.0 using the Neighbor-joining method and the maximum composite likelihood model. The robustness of the phylogenetic trees was statistically evaluated by bootstrap test with 1000 replicates. MAFFT 7.520 and default parameters were used to perform multiple sequence alignment of HAdV sequences from patients with HAdV reference sequences.

Recombination Analysis and Nucleotide and Amino Acid Identity Analysis

Potential genomic recombination events were identified according to the phylogenetic results of MEGA 11.0. SimPlot 3.5.1 was used to further validate potential recombination events. Bootscan analysis in SimPlot 3.5.1 was performed with default parameters. Parameters are as follows: window size of 2000 bp, step size of 100 bp, gap strip, 100 replicates, Kimura (2-parameter), and Neighbor-Joining method. In addition, the BioAider_v1.527 software and its default parameters were used to calculate the percentage of nucleotide and amino acid identity among different sample genomes.

Results

Epidemiological Analysis of HAdV in Patients

All 66 sequencing samples in this study were from Jiangsu Province (31 patients from Suzhou, 18 patients from Xuzhou, 14 patients from Nanjing, and 3 patients from Taizhou) (Figure 1A and Table 1). The detailed information of 66 patients with HAdV infection is shown in Table S1. Most of the hospitalized pneumonia patients infected with HAdV were from Suzhou (31.0%) and Xuzhou (62.1%), while most of the ILI patients infected with HAdV were from Nanjing (37.8%) and Suzhou (59.5%) (Table 1). From the perspective of gender distribution, 18 out of 37 ILI patients were female, and 12 out of 29 hospitalized pneumonia patients were female, indicating that these two respiratory diseases have no gender preference (P=0.734) (Table 1). From 2013 to 2021, the number of HAdV infections was constantly changing. Among them, ILI patients infected with HAdV were more in 2015, while hospitalized pneumonia patients infected with HAdV were more in 2020 (Figure 1B and Table 1). Moreover, the adenovirus strains infected between ILI patients and hospitalized pneumonia patients were also significantly different (Table 1). Of the ILI patients that were positive for HAdV, the majority (n=28) were infected with HAdVB strains. Of the hospitalized pneumonia patients that were positive for HAdV, the majority (n=20) were infected with HAdVC strains. The strains of HAdVB3 and HAdVB7 genotypes were mainly infected in 2015 and 2017, and the strains of HAdVC1 and HAdVC2 genotypes were mainly infected in 2020 (Figure 1C). In addition, the results of histogram analysis showed that the HAdV strain mainly infected children under 5 years old (Figure 1D).

HAdV Genotyping and Whole-Genome Phylogenetic Analysis

In this study, 66 newly obtained HAdV strains were divided into 6 HAdV genotypes. The whole-genome phylogenetic trees that were constructed for genotyping enabled the classification of the 6 genotypes as follows: HAdVB3 (n=22), HAdVB7 (n=15), HAdVC1 (n=12), HAdVC2 (n=11), HAdVC5 (n=5), and HAdVE4 (n=1) (Figure 2). All sequences

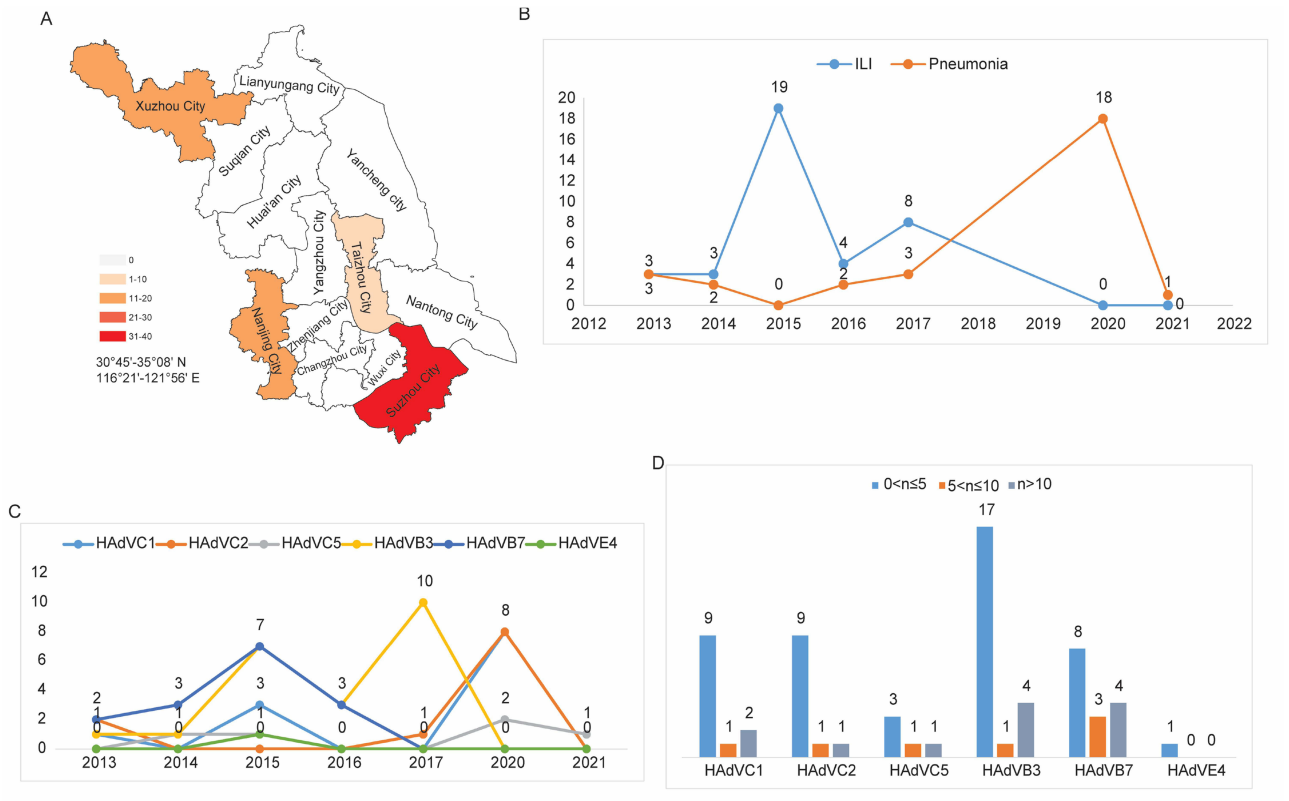

Figure 1 The regional distribution, year, and genotypes of infection of HAdV infected patients in this study. (**A**) The regional distribution of HAdV infected patients in this study; (**B**) The year of infection of HAdV infected patients in this study; (**C**) The genotype distribution for different years; (**D**) The genotype distribution for different ages.

Table 1 Sample Information Statistics of HAdV Infected Sequencing Patients in This Study

	ILI	Pneumonia	P test
N	37	29	
Gender= Female (%)	18 (48.6)	12 (41.4)	0.734
Year (%)			<0.001
2013	3 (8.1)	3 (10.3)	
2014	3 (8.1)	2 (6.9)	
2015	19 (51.4)	0 (0.0)	
2016	4 (10.8)	2 (6.9)	
2017	8 (21.6)	3 (10.3)	
2020	0 (0.0)	18 (62.1)	
2021	0 (0.0)	1 (3.4)	
Region (%)			<0.001
Nanjing City	14 (37.8)	0 (0.0)	
Suzhou City	22 (59.5)	9 (31.0)	
Taizhou City	1 (2.7)	2 (6.9)	
Xuzhou City	0 (0.0)	18 (62.1)	
CT value (mean (SD))	12.76 (1.04)	12.21 (0.82)	0.023
Subgenus (%)			0.001
HAdVB	28 (75.7)	9 (31.0)	
HAdVC	8 (21.6)	20 (69.0)	
HAdVE	1 (2.7)	0 (0.0)	
Serotype (%)			0.009
HAdVC1	4 (10.8)	8 (27.6)	
HAdVC2	3 (8.1)	8 (27.6)	
HAdVB3	17 (45.9)	5 (17.2)	
HAdVE4	1 (2.7)	0 (0.0)	
HAdVC5	1 (2.7)	4 (13.8)	
HAdVB7	11 (29.7)	4 (13.8)	

Abbreviations: HadV, human adenovirus; ILI, influenza-like illness; Ct, cycle threshold.

belonging to the same genotype clustered together. Despite the limited sample size, multiple genotypes were detected in this study, which implied the diversity of HAdV in Jiangsu Province.

Phylogenetic and Recombination Analysis of HAdVB Species

In this study, 37 newly obtained HAdV strains were classified as HAdVB, including 22 HAdVB3 strains and 15 HAdVB7 strains. The newly discovered HAdVB3 strain was closely related to NC_011203 and JX423386, while HAdVB7 strain was closely related to AC_000018 (Figure 3A). The phylogenetic analysis of hexon, penton and fiber genes once again confirmed the clustering results (Figure 3B-D). All 22 newly discovered HAdVB3 strains were recombinant strains of HAdVB3 (NC_011203) and HAdVB66 (JX423386) (Supplementary Figure 1). Moreover, the newly discovered HAdVB7 strains isolated a from hospitalized pneumonia patient JSSZ1306_S13 in Suzhou in 2013 were recombinant strains of HAdVB7 (AC_000018) and HAdVB66 (JX423386) (Supplementary Figure 1).

Phylogenetic and Recombination Analysis of HAdVC Species

In this study, 28 newly obtained HAdV strains were classified as HAdVC, including 12 HAdVC1 strains, 11 HAdVC2 strains and 5 HAdVC5 strains. Phylogenetic analysis showed that JSXZ2002_S51, JSXZ2012_S58, JSXZ2014_S59, JSXZ2017_S62, JSXZ2018_S63, JSXZ2020_S64, JSXZ2021_S65, JSXZ2022_S66 and JSSZ1302_S73 were clustered with CBJ113 (KR699642), while JSXZ2001_S50, JSXZ2005_S54, JSXZ2015_S60 and JSNJ1519_S70 were clustered with Shanghai HAdV-C1 strain (MH183293.1_SH2016) (Figure 4A). The phylogenetic analysis of hexon, penton and

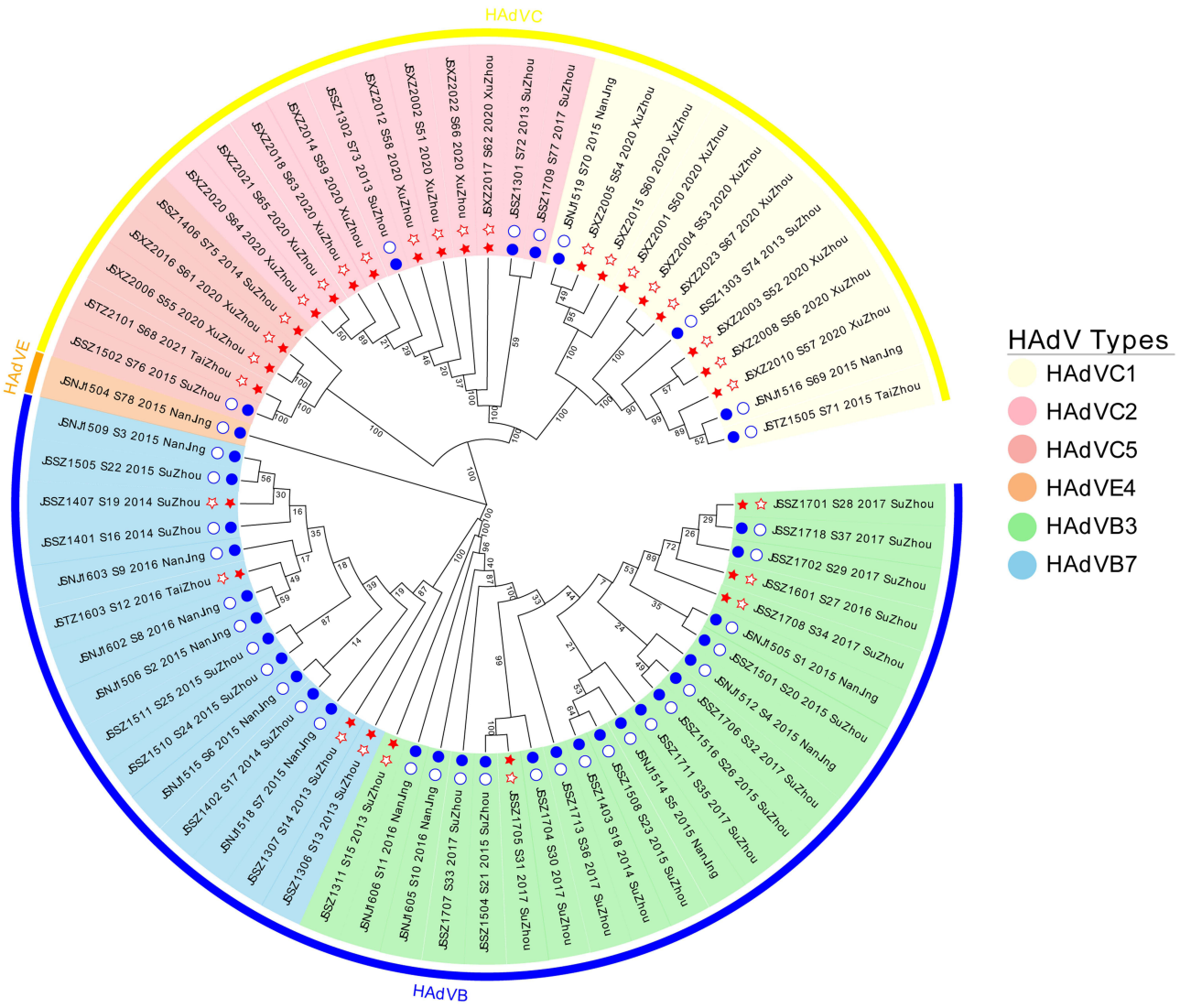

Figure 2 Phylogenetic analysis of all HAdV strains detected in this study based on WGS data. Red stars represent hospitalized pneumonia patients, and blue circles represent ILI patients.

fiber genes once again confirmed the clustering results (Figure 4B-D). Recombination analysis showed that JSXZ2001_S50, JSXZ2002_S51, JSXZ2005_S54, JSXZ2012_S58, JSXZ2014_S59, JSXZ2015_S60, JSXZ2017_S62, JSXZ2018_S63, JSXZ2020_S64, JSXZ2021_S65, JSXZ2022_S66, JSNJ1519_S70 and JSSZ1302_S73 were also associated with multiple recombination events (Supplementary Figure 2).

Phylogenetic and Recombination Analysis of HAdVE Species

In this study, only one newly obtained HAdV strain was HAdVE (HAdVE4 subtype). Phylogenetic analysis showed that the whole genome of the newly obtained HAdVE strain was located in E4, and the bootstrap value was 100% (Figure 5A). In addition, the newly obtained HAdVE strain was more closely related to HAdVE4a strain evolution. The phylogenetic analysis of hexon, penton and fiber genes once again confirmed the clustering results (Figure 5B-D).

Nucleotide and Amino Acid Identity Analysis

The BioAider_v1.527 software was used to calculate the percentage of nucleotide and amino acid. The percentage of nucleotide and amino acid identities revealed a high level of genetic conservation within isolates from HAdVB3, HAdVB7, HAdVC1,

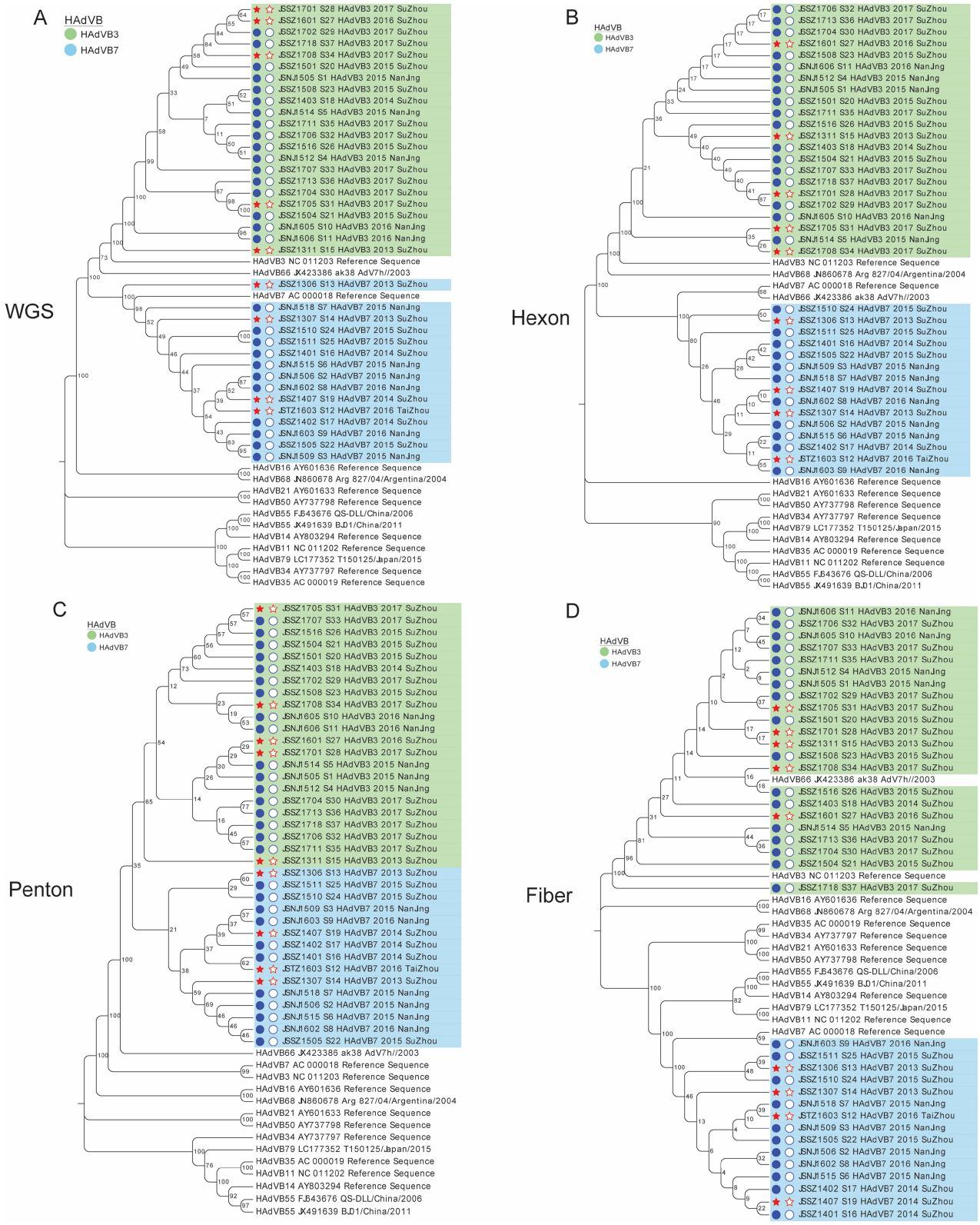

Figure 3 Phylogenetic analysis of HAdVB. (**A**) The phylogenetic analysis of whole genome; (**B**) The phylogenetic analyses of hexon genes from species HAdVB; (**C**) The phylogenetic analyses of penton genes from species HAdVB; (**D**) The phylogenetic analyses of fiber genes from species HAdVB. Red stars represent hospitalized pneumonia patients, and blue circles represent ILI patients.

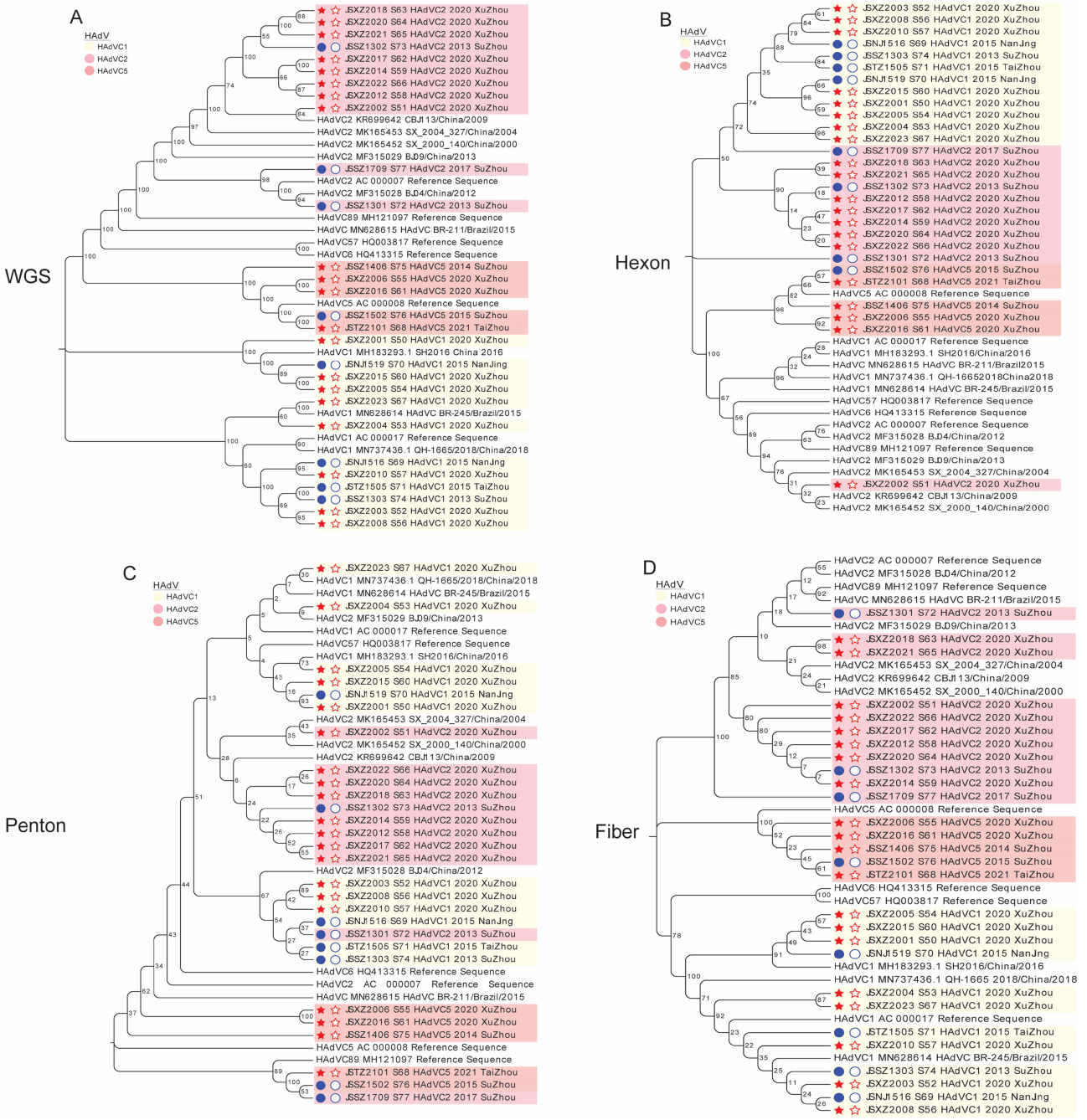

Figure 4 Phylogenetic analysis of HAdVC. (**A**) The phylogenetic analysis of whole genome; (**B**) The phylogenetic analyses of hexon genes from species HAdVC; (**C**) The phylogenetic analyses of penton genes from species HAdVC; (**D**) The phylogenetic analyses of fiber genes from species HAdVC. Red stars represent hospitalized pneumonia patients, and blue circles represent ILI patients.

HAdVC2 and HAdVC5 genotypes. The nucleotide identities ranges of HAdVB3, HAdVB7, HAdVC1, HAdVC2 and HAdVC5 were 93.76%-98.17%, 90.25%-98.53%, 81.49%-96.78%, 73.43%-99.59% and 88.59%-97.63%, respectively. The amino acid identities ranges of HAdVB3, HAdVB7, HAdVC1, HAdVC2 and HAdVC5 were 93.38%-98.06%, 89.67%-98.29%, 79.42%-96.45%, 71.84%-99.40% and 88.37%-97.35%, respectively. Moreover, the nucleotide and amino acid identities of the newly discovered recombinant strains and the reference strains were also analyzed. The nucleotide identities of the newly discovered 22 HAdVB3 recombinant strains with the reference strain NC_011203 were greater than 90%, and the nucleotide identities with the reference strain JX423386 were greater than 89%. They also had high amino acid identities (greater than 89%) with the reference

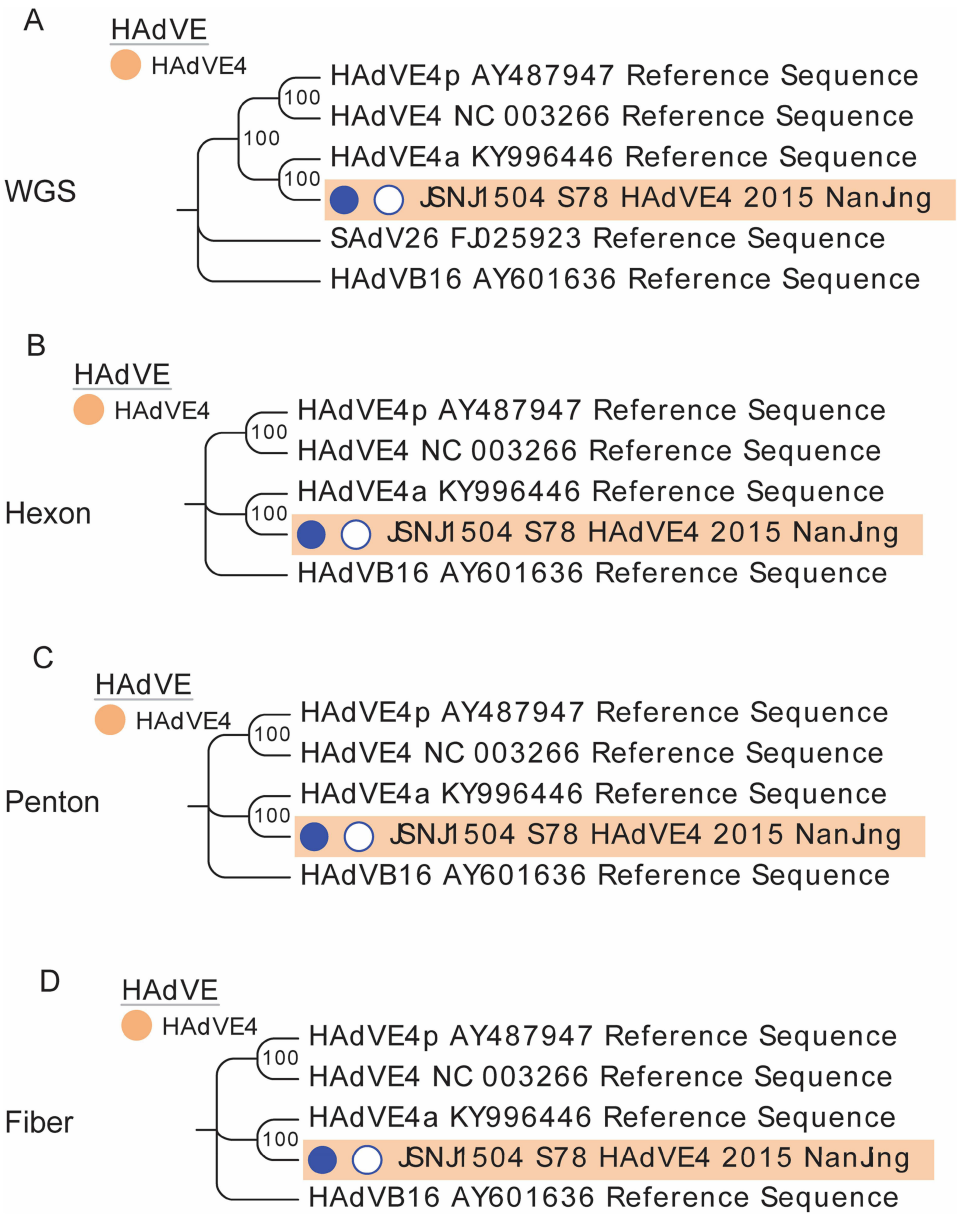

Figure 5 Phylogenetic analysis of HAdVE. (**A**) The phylogenetic analysis of whole genome; (**B**) The phylogenetic analyses of hexon genes from species HAdVE; (**C**) The phylogenetic analyses of penton genes from species HAdVE; (**D**) The phylogenetic analyses of fiber genes from species HAdVE. Blue circles represent ILI patients.

strains NC_011203 and JX423386. The nucleotide identities of the recombinant strain JSSZ1306_S13 and the reference strains AC_000018 and JX423386 were 88.48% and 87.86%, respectively. The amino acid identities with the reference strains AC_000018 and JX423386 were 87.70% and 86.64%. The nucleotide and amino acid identities of newly discovered recombinant strains JSXZ2001_S50, JSXZ2005_S54, JSXZ2015_S60 and JSNJ1519_S70 with reference strain SH2016 were all greater than 88%. The nucleotide and amino acid identities of newly discovered recombinant strains JSXZ2002_S51, JSXZ2012_S58, JSXZ2014_S59, JSXZ2017_S62, JSXZ2018_S63, JSXZ2020_S64, JSXZ2021_S65, JSXZ2022_S66 and JSSZ1302_S73 with reference strain CBJ113 were all greater than 97%. In addition, it was found that the nucleotide and amino acid identities of HAdVE4 (JSNJ1504_S78) and HAdVE4a were greater than 96% and 95%, respectively.

Discussion

The genetic diversity of viruses caused by recombination is considered to be the main source of emerging outbreaks.[23] Emerging pathogens pose a major threat to global public health. Adenovirus is a commonly used carrier for delivering exogenous genes or vaccine antigens, and has been widely used in gene therapy and vaccine development.[24] Moreover, HAdV is common pathogens that cause various respiratory, eye, and gastrointestinal diseases.[1] Therefore, exploring the evolutionary relationship and recombination events of HAdV genomes is helpful for the treatment of diseases, and is also crucial for constructing adenovirus vectors for downstream clinical applications. HAdVB, HAdVC and HAdVE are often associated with respiratory infections.[9,25] In mainland China, HAdV accounts for 5.8%-13% of patients with acute respiratory infections. Moreover, children under 5 years of age are most susceptible to HAdV infection.[26] The type of HAdV infection varies with age, setting and season.[27] Herein, WGS technology was used to sequence 66 patients with HAdV infection (37 patients with ILI and 29 hospitalized patients with pneumonia) from Jiangsu Province. The epidemiological analysis of hospitalized pneumonia and ILI patients infected with HAdV showed that different genotypes of HAdV had different infection preferences. ILI patients were mainly infected with HAdVB strains, and hospitalized pneumonia patients were mainly infected with HAdVC strains. From the perspective of gender distribution, there is no gender preference for ILI and hospitalized pneumonia patients infected with HAdV. It has been previously reported that circulating HAdV may differ in time and geography.[25] Herein, the hospitalized pneumonia and ILI patients infected with HAdV were also different in region and time. Most of the hospitalized pneumonia patients infected with HAdV were from Suzhou and Xuzhou, while most of the ILI patients infected with HAdV were from Nanjing and Suzhou. ILI patients infected with HAdV were more in 2015, while hospitalized pneumonia patients infected with HAdV were more in 2020. Moreover, the results of this study also showed that the HAdV strain mainly infects children under 5 years of age, which is consistent with previous report.[26]

In this study, 66 newly obtained HAdV strains were divided into 6 genotypes (HAdVB3, HAdVB7, HAdVC1, HAdVC2, HAdVC5, and HAdVE4). Among these genotypes, HAdVB3 and HAdVB7 were predominant which is consistent with other reports from Asia.[28–30] Acute respiratory diseases caused by HAdVB3 and HAdVB7 has been prevalent and caused outbreaks in Asia, Europe, and North America.[31–34] The infections caused by HAdVB3 and HAdVB7 in individuals with normal immune function are usually mild and self-limited, and are the main types of lower respiratory diseases in children under 5 years old.[35] Compared with HAdVB3, patients with HAdVB7 infection have a longer duration of fever, more severe shortness of breath, dyspnea, incidence of toxic encephalopathy, and respiratory failure.[35–37] Due to the continuous emergence of new recombinant adenovirus strains, HAdV surveillance has also attracted more and more attention. Herein, all 22 newly discovered HAdVB3 strains were recombinant strains of HAdVB3 (NC_011203) and HAdVB66 (JX423386). In addition, the newly discovered HAdVB7 strains isolated a from hospitalized pneumonia patient JSSZ1306_S13 in Suzhou in 2013 were recombinant strains of HAdVB7 (AC_000018) and HAdVB66 (JX423386). The discovery of these recombinant strains may contribute to understanding the epidemiology of HAdV and research on related vaccines.

Infection with the HAdVC is highly prevalent and may be asymptomatic or mild and self-limiting, but may cause severe effects in immunocompromised hosts.[38,39] After the initial infection, HAdVC can establish latent infections and persist in lymphocytes for a long time. Therefore, asymptomatic individuals can transmit infectious viruses in feces for many years.[40–42] CBJ113 strain is a new subtype of HAdVC that is prevalent in China. The CBJ113 genome has an intra-subtype recombinant structure and includes gene regions mainly originating from HAdVC1 and HAdVC2.[43] The three hexon sequences of HAdV isolated from children with respiratory tract infection in Beijing, China were in the same branch with CBJ113 strain, and they showed maximum homology with CBJ113 strain.[44] SH2016 strain is a novel recombinant HAdVC strain isolated from children with acute respiratory infection in Shanghai, China.[45] It is caused by recombination of two HAdV genotypes, HAdVC1 and HAdVC2, which frequently cause respiratory infections.[46,47] In this study, the newly discovered recombinant strains JSXZ2002_S51, JSXZ2012_S58, JSXZ2014_S59, JSXZ2017_S62, JSXZ2018_S63, JSXZ2020_S64, JSXZ2021_S65, JSXZ2022_S66 and JSSZ1302_S73 were clustered with CBJ113, while JSXZ2001_S50, JSXZ2005_S54, JSXZ2015_S60 and JSNJ1519_S70 were clustered with SH2016. Moreover, these newly discovered recombinant strains also exhibit high nucleotide and amino acid identity with CBJ113 and SH2016. Therefore, we propose that these recombinant strains are potential new genotypes of HAdVC species, which may be pathogens causing respiratory infections.

HAdVE4 has been previously classified as two separate evolutionary lineages, prototype (p)-like and a-like, and the mutation rate of a-like was higher, indicating that it has a wider host range and stronger transmission ability.[16,48] HAdVE4 caused febrile acute respiratory disease outbreaks in the military and are also circulating in civilian population. Patients infected with HAdVE4 are at increased risk of developing severe disease.[49] The genome of the early prevalent HAdVE4 strain was produced by the recombination of the simian adenovirus E26 (SAdVE26) genome and the hexon gene of HAdVB16.[50] Therefore, FJ025923 (SAdV26) and AY601636 (HAdVB16) were also included in the reference sequence in this study. Herein, only one newly obtained HAdV strain (JSNJ1504_S78) was HAdVE. The whole genome of the newly obtained HAdVE strain was located in E4, and the bootstrap value was 100%. It was more closely related to HAdVE4a strain evolution. Moreover, it was also found that the nucleotide and amino acid identities of JSNJ1504_S78 and HAdVE4a were greater than 96% and 95%, respectively. This implies that this strain may also have a wide host range and a strong ability to spread.

HAdV is a highly infectious agent, and its main mode of transmission is respiratory transmission.[8] Good living environment and habits can help prevent HAdV infection. Children are the main population infected with HAdV. Therefore, childcare institutions and schools should conduct timely disinfection, ventilation, and ensure that children maintain good personal hygiene. During the high incidence season of HAdV infection, medical institutions should strengthen infection control measures to prevent cross infection in children, the elderly, and other patients with weak immune abilities. Overcrowding promotes the spread of HAdV, therefore basic environmental hygiene measures should be improved. In addition, scientists, scholars, the pharmaceutical industry, international organizations, and government agencies are working together to continuously develop new vaccines.

In conclusion, our study revealed the evolutionary relationship and potential recombination events of HAdV strains infected with two respiratory diseases, pneumonia and ILI, in Jiangsu Province based on WGS. Meanwhile, it also provides a basis for understanding the epidemiology and related risks of HAdV in Jiangsu Province. In addition, it provides a basis for the formulation of public health strategies in Jiangsu Province. The most important limitation of this study is the small sample size and sample collection range. Therefore, the range of sample collection and sample size should be expanded for further study.

Data Sharing Statement

All the raw data were submitted into the BioProject database with the accession number PRJNA1108873.

Ethics Approval and Consent to Participate

This study was approved by the ethics committee of Jiangsu Provincial Center for Disease Control and Prevention (JSJK2022-BO16-02). All participants were informed as to the purpose of this study, and that this study complied with the Declaration of Helsinki. The informed consent was obtained from the all participants.

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Disclosure

The authors declare that they have no competing interests.

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