BMC Genomics

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Epidemiological characteristics and genomic analysis of respiratory adenovirus in Jining City from February 2023 to July 2024

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Abstract

Background Human adenovirus (HAdV) infection can cause high fever, pneumonia, and even death, posing a serious threat to human health. This study analyzes the epidemiological characteristics and genetic features of respiratory adenovirus in Jining City from February 2023 to July 2024.

Methods From February 2023 to July 2024, 3,947 throat swab specimens were collected from influenza-like illness cases at Jining First People's Hospital and Rencheng District Maternal and Child Health Hospital. Real-time fluorescent quantitative PCR was used to detect HAdV nucleic acid. Whole-genome sequencing was performed on HAdV-positive samples. Phylogenetic trees were constructed for the whole genome, Penton base gene, Hexon gene, and Fiber gene of HAdV, and protein variation sites were analyzed.

Results The HAdV positivity rate in influenza-like cases in Jining City from February 2023 to July 2024 was 3.42% (135/3947), with higher positivity rates in younger age groups. HAdV positivity rates were higher between December 2023 and July 2024, while lower between January 2023 and November 2023. Whole-genome sequencing was performed on 47 HAdV samples, revealing 38 cases of HAdV-B and 9 cases of HAdV-C, with no HAdV-E detected. HAdV-B and HAdV-C co-circulated in Jining City from February to December 2023, while HAdV-B predominated from January to July 2024. All 38 HAdV-B sequences from Jining City are HAdV-3. In the phylogenetic trees of the whole genome, Penton base gene, Hexon gene, and Fiber gene of HAdV-B, all 38 HAdV-B strains from Jining City are located in the same evolutionary branch as the Chinese HAdV-3 candidate vaccine strain Guangzhou01. Compared to Guangzhou01, the RGD motif in Penton base and antigenic epitopes in Hexon of the 38 HAdV-B strains from Jining remained unchanged. Among the 9 HAdV-C sequences from Jining, 4 were identified as HAdV-104 and 5 as HAdV-108. The RGD motif in Penton base protein and receptor-binding sites in Fiber protein remained unchanged in all 9 HAdV-C sequences.

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Conclusions From February 2023 to July 2024, HAdV in Jining City primarily circulated among younger age groups in influenza-like illness cases, with no apparent seasonal pattern. Enhanced surveillance of HAdV and accelerated development and marketing of HAdV vaccines are necessary.

Keywords Influenza-like illness, Human adenovirus, Whole-genome sequencing, Antigenic epitopes

Introduction

Human adenovirus (HAdV) is a major pathogen responsible for 5%– 10% of respiratory infections in children and 1%– 7% in adults [1]. HAdV infection can cause fever, cough, pneumonia, and even death, with the probability of pneumonia in children infected with HAdV reaching up to 20% [1–3]. All populations are susceptible to respiratory adenovirus, showing sporadic infections throughout the year, with an incubation period of 5.6 days [4].

Adenovirus is a non-enveloped, icosahedral capsid double-stranded DNA virus with a genome length ranging from 34 to more than 37 kb, capable of encoding over 40 proteins [5, 6]. HAdV contains three major capsid proteins: Penton base, Hexon, and Fiber [7, 8]. The interaction between the RGD (Arg-Gly-Asp) motif of Penton base and host cell integrins promotes endocytosis [9]. Hexon is a type-specific and species-specific antigen that is highly sensitive to immune selection pressure [10–12]. Fiber contains the virus's receptor-binding site and plays a crucial role in viral attachment and entry [13].

HAdV can be classified into seven species (A to G), with species B, C, and E primarily causing respiratory infections [5, 14]. Based on the nucleotide sequences of Penton base, Hexon, and Fiber regions, HAdV is divided into different subtypes. As of Octorber 2024, HAdV has been classified into 116 genetic subtypes (http://hadvwg.gmu.edu/). Currently, only the United States has a bivalent HAdV-4 and HAdV-7 vaccine used in the military [15]. Although no vaccine for respiratory adenovirus is available in China, research and development of HAdV vaccines are ongoing. A research team from Guangzhou Medical University has constructed a trivalent candidate vaccine for HAdV-3, HAdV-7, and HAdV-55, which can induce antibodies against these three types in mice [16]. Additionally, the treatment of HAdV infection is mainly symptomatic, with no effective specific drugs targeting HAdV, which affects both prevention and treatment of HAdV infections [17].

Jining is a large city in Shandong Province with a permanent population of 8.3579 million. It has a warm temperate monsoon climate with cold winters and hot summers, and distinct seasons. Some studies have reported on the epidemiological characteristics of influenza viruses and the novel coronavirus [18–20]. To understand the epidemiological characteristics and genomic features of adenovirus, samples from February 2023 to July 2024 were analyzed to reveal the pathogen's characteristics. This study promotes active HAdV epidemic monitoring, diagnosis, and early warning for clinical management, providing technical support for future interventions.

Materials and methods

Study participants

From February 2023 to July 2024, throat swab specimens were collected from influenza-like illness cases (fever with temperature ≥ 38 °C, accompanied by cough or sore throat) within 3 days of onset at Jining First People's Hospital and Jining Rencheng District Maternal and Child Health Hospital. Samples were collected using virus sampling tubes (MT0901) produced by Youkang Biotechnology (Beijing) Co., Ltd. A total of 3,947 samples were collected, including 3,576 from outpatient cases and 371 from inpatient cases.

Nucleic acid detection

200 μL of throat swab specimen was extracted using an automatic nucleic acid extraction instrument (SSNP-9600A) and nucleic acid extraction and purification reagent (SDKF60101) produced by Jiangsu Shuoshi Biotechnology Co., Ltd. HAdV nucleic acid detection was performed using an adenovirus nucleic acid detection kit (fluorescence PCR method, YJC10117N) produced by Jiangsu Shuoshi Biotechnology Co., Ltd. The system configuration for the real-time fluorescence quantitative PCR was 12.5 μL of the nucleic acid amplification reagent from the kit, 7.5 μL of the adenovirus detection reagent, and 5 μL of nucleic acid. The reaction conditions were: 95 °C for 5 min; 95 °C for 10 s, 58 °C for 30 s, 45 cycles. The fluorescence channel for detection was FAM, and a Ct value < 37 with an S-shaped curve was considered positive. The minimum detection limit of the kit was 500 copies/mL.

Whole genome sequencing

Forty-eight HAdV-positive samples with CT values ≤ 30 were selected for whole genome sequencing. The ULSEN® ultra-sensitive adenovirus whole genome capture kit (batch number: B-170930) produced by Beijing Weiwei Technology Co., Ltd. was used for capture and

amplification. The reaction system was configured as follows: A-Mix 25 μL, B1/B2/C1/C2/E1/E2-Mix 5 μL, template nucleic acid 15 μL, water 5 μL. Reaction conditions: 98 °C for 30 s; 98 °C for 10 s, 63 °C for 30 s, 72 °C for 45 s, 35 cycles; 72 °C for 2 min. The resulting products were purified using the AMpure XP nucleic acid purification kit (Beckman Coulter, A63880). Nucleic acid quantification was performed using a Qubit 3 fluorometer (Invitrogen). DNA fragmentation was carried out using the Illumina Nextera XT DNA library preparation kit (USA, batch number: 15032350). The Nextera XT Index Kit v2 Set A (Illumina, USA, catalog number 15052163) was used for adapter ligation. Reaction conditions: 72 °C for 3 min; 95 °C for 30 s; 95 °C for 10 s, 55 °C for 30 s, 72 °C for 30 s, 12 cycles; 72 °C for 5 min. After purification, whole genome sequencing was performed using the Illumina NextSeq 2000 sequencing platform and NextSeqTM 1000/2000 P1 reagent Cartridge 300 cycles (Illumina, batch number: 20049920). The data presented in the study are deposited in the National Microbiology Data Center (NMDC), accession number list in Supplementary Table S1.

Sequence alignment and analysis

QIAGEN CLC Genomics Workbench 24 was used for sequence assembly and analysis of the sequencing data. The sequence assembly was performed based on reference sequences. HAdV-B was assembled using Human adenovirus B1 (NC_011203.1), and HAdV-C was assembled using Human adenovirus type 1 (AC_000017.1). The low coverage definition threshold was set to 30. The obtained HAdV whole genome sequences were compared and analyzed using BLAST in the NCBI database. Whole genome sequences of the Chinese species B candidate vaccine strain Guangzhou01 (GenBank: DQ099432.4), the American species B type 7 vaccine strain Gomen (GenBank: AY594255.1), and recently prevalent HAdV strains, including clearly identified HAdV-3, HAdV-104, and HAdV-108 sequences, were downloaded from the GenBank database. Sequence alignment was performed using MEGA 7.0.14 software, and the phylogenetic dataset was reduced using Treemmer software [21, 22] and phylogenetic trees for the whole genome, Penton base gene, Hexon gene, and Fiber gene were constructed using the maximum-likelihood method with 1000 bootstrap replicates. The phylogenetic tree was refined using FigTree v1.4.4 for visualization. MEGA 7.0.14 software and MegAlign of DNASTAR 7.0.1 software were used to analyze the similarity of HAdV genes and encoded proteins. MEGA 7.0.14 software was used to analyze amino acid variation sites in Penton base, Hexon, and Fiber proteins.

Statistical analysis

Statistical analysis was performed using SPSS 25.0 software. The 95% Confidence Interval (95% CI) was used to evaluate the stability and overall representativeness of the rate. Chi-square tests were used for inter-group comparisons, with $P < 0.05$ considered statistically significant.

Results

Epidemiological characteristics of HAdV

Among 3,947 influenza-like illness specimens collected from February 2023 to July 2024, 135 cases were positive for HAdV, with a positivity rate of 3.42% (135/3947). The infection rates varied across different age groups, with higher rates observed in the ≥5- <15 years group (7.63%, 78/1022), the ≥1- <5 years group (5.27%, 40/759), and the <1 year group (3.52%, 4/123). Lower rates were found in the ≥15- <25 years group (0.81%, 4/494), the ≥25- <60 years group (0.82%, 9/1093), and the ≥60 years group (0%, 0/454). The differences in HAdV positivity rates among age groups were statistically significant ($\chi2 = 111.41$, $P < 0.001$) (Table S2, Fig. 1).

The HAdV positivity rate for males was 3.51% (68/1940) and for females was 3.34% (67/2007), with no statistically significant difference between genders ($\chi2 = 0.083$, $P = 0.77$). The positivity rate for outpatients was 3.24% (116/3576) and for inpatients was 5.12% (19/371), with no statistically significant difference between these two groups ($\chi2 = 3.59$, $P = 0.06$) (Table S2, Fig. 1).

In Jining City, Higher positivity rates (6.54%, 102/1559) were observed between December 2023 and July 2024, while lower rates (1.38%, 33/2388) were seen between January 2023 and November 2023. The adenovirus positivity rate from December 2023 to July 2024 (6.54%, 102/1559) was significantly different from the adenovirus positivity rate from December 2023 to November 2023 (1.38%, 33/2388) ($\chi2 = 76.05$, $P < 0.001$). The differences in HAdV positivity rates among different months were statistically significant ($\chi2 = 103.67$, $P < 0.001$) (Table S3 and Fig. 1).

This study also tested for infections with Influenza virus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), and Respiratory Syncytial Virus (RSV). Among the 135 HAdV-positive samples, 1 was co-infected with A(H1N1)pdm09 influenza virus, 7 with influenza A(H3N2) virus, 5 with influenza B/Victoria virus, 5 with SARS-CoV-2, and 4 with RSV.

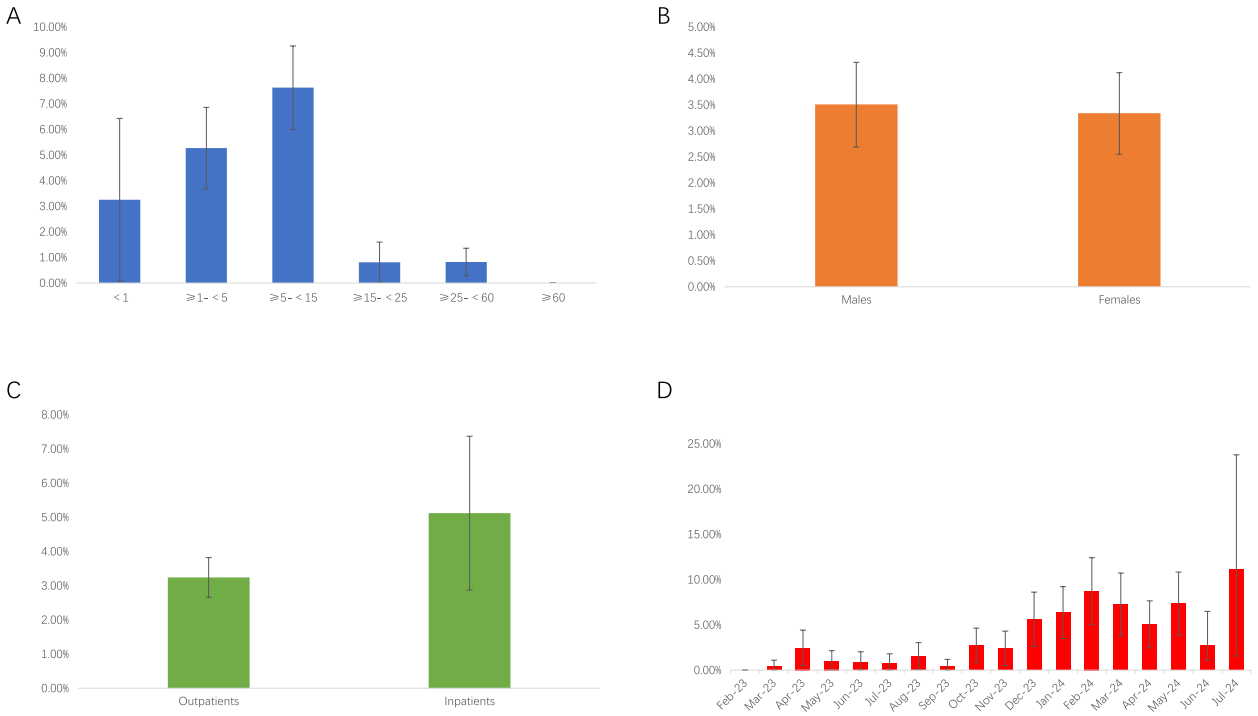

Fig. 1 HAdV Epidemiological Characteristics. **A** Adenovirus positivity rate across different age groups. The x-axis represents the age, while the y-axis indicates the positivity rates. The black line represents the 95% confidence interval. **B** Adenovirus positivity rate by gender. The x-axis represents the gender, while the y-axis indicates the positivity rates. The black line represents the 95% confidence interval. **C** Adenovirus positivity rate in inpatients and outpatients. The x-axis represents the Inpatient/outpatient, while the y-axis indicates the positivity rates. The black line represents the 95% confidence interval. **D** Adenovirus positivity rate by month. The x-axis represents the months, while the y-axis indicates the positivity rates. The black line represents the 95% confidence interval

HAdV whole genome sequence analysis and subgenus analysis

A total of 48 samples underwent whole-genome sequencing, resulting in complete genome sequences for 47 samples, with a sequencing success rate of 97.92%. The 47 HAdV specimens produced genome sequences ranging from 34,390 bp to 35,537 bp. The reads mapped for the 47 sequences ranged from 2,133,524 to 5,151,593, with a median coverage ranging from 2,872 to 9,019. (Table S4 and Figure S1). Among the 47 infected individuals, 22 were male and 25 were female; 13 were aged ≥1- <5 years, 32 were ≥5- <15 years, and 2 were ≥25- <60 years. Of these, 20 cases were from 2023 and 27 from 2024.

Using NCBI BLAST to analyze the obtained whole genome sequences, as well as the Penton base gene, Hexon gene, and Fiber gene sequences, it was found that among the 47 HAdV specimens, 38 were HAdV-B, 9 were HAdV-C, and none were HAdV-E. The 21 sequences from 2023 included 13 HAdV-B and 8 HAdV-C; the 26 sequences from 2024 included 25 HAdV-B and 1 HAdV-C. All 38 HAdV-B sequences were of the HAdV-3 genotype. Among the 9 HAdV-C cases, 4 sequences were HAdV-104, and 5 were HAdV-108.

Similarity analysis

The nucleotide similarity among the 47 HAdV whole genome sequences from Jining City ranged from 66.87% to 99.99%. Specifically, the similarity among the 38 HAdV-B whole genome sequences from Jining City was between 99.58% and 99.99%, while the similarity among the 9 HAdV-C whole genome sequences ranged from 97.56% to 99.94%. When comparing the 38 HAdV-B sequences from Jining City with the Chinese HAdV-B candidate vaccine strain Guangzhou01 (HAdV-3), the whole genome sequence similarity ranged from 99.08% to 99.45%, and the Hexon gene nucleotide sequence similarity was between 99.01% and 99.93%. In comparison with the American HAdV-B vaccine strain Gomen (HAdV-7), the whole genome sequence similarity of the 38 HAdV-B sequences from Jining City ranged from 97.05% to 97.37%, while the Hexon gene nucleotide sequence similarity was between 96.01% and 96.69%. For detailed information (Table 1).

Phylogenetic analysis

Using the gene sequences of 38 HAdV-B strains from Jining City, along with the U.S. HAdV- 7 vaccine strain

Table 1 Similarity analysis

Comparison	Whole genome nucleotide	Penton base		Hexon		Fiber	
		Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid
Comparison among 47 HAdV sequences from Jining City	66.87%– 99.99%	74.92%– 100%	73.78%– 100%	75.01%– 100%	78.27%– 100%	64.22%– 100%	40.80–100%
Comparison among 38 HAdV-B sequences from Jining City	99.57%– 99.99%	99.88%– 100%	99.63%– 100%	99.05%– 100%	99.79%– 100%	99.90%– 100%	99.69%– 100%
Comparison between 38 HAdV-B sequences from Jining City and HAdV-B candidate vaccine strain Guangzhou01	99.08%– 99.45	99.69%– 99.82%	99.26%– 99.63%	99.01%– 99.93%	99.68%– 99.89%	99.79%– 99.90%	99.37%– 99.69%
Comparison between 38 HAdV-B from Jining City and American HAdV-B HAdV- 7 vaccine strain Gomen	97.05%– 97.37%	99.33%– 99.45%	99.26%– 99.63%	96.01%– 96.69%	96.84%– 97.06%	66.11%– 66.21%	46.69%– 47.22%
Comparison among 9 HAdV-C sequences from Jining City	97.85%– 99.94%	99.24%– 100%	99.24%– 100%	91.47%– 100%	97.50%– 100%	99.86%– 100%	99.79%– 100%

Gomen, the Chinese HAdV-3 candidate vaccine strain Guangzhou01, and the prevalent HAdV- 3 and HAdV-7 sequences from recent years, phylogenetic trees were constructed for the whole genome, Penton base gene, Hexon gene, and Fiber gene of HAdV-B. In the whole-genome phylogenetic tree of HAdV-B, all 38 HAdV-B gene sequences from Jining City are in the same evolutionary branch as the Chinese HAdV-3 candidate vaccine strain Guangzhou01. The Penton base gene of HAdV-3 and HAdV-7 belong to genotypes P3 and P7, respectively; the Hexon gene to genotypes H3 and H7; and the Fiber gene to genotypes F3 and F7. In the Penton base gene, Hexon gene, and Fiber gene phylogenetic trees, the 38 HAdV-B gene sequences from Jining City are also in the same evolutionary branch as the Chinese HAdV-3 candidate vaccine strain Guangzhou01 (see Fig. 2, Figures S2–4).

Using the gene sequences of 9 HAdV-C strains from Jining City and the prevalent HAdV- 104 and HAdV- 108 sequences from recent years, phylogenetic trees were constructed for the whole genome, Penton base gene, Hexon gene, and Fiber gene of HAdV-C. In the whole-genome phylogenetic tree of HAdV-C, five sequences from Jining City fall within the HAdV- 108 branch, while four fall within the HAdV-104 branch. The Penton base gene of both HAdV-104 and HAdV-108 belongs to genotype P1, and the Fiber gene belongs to genotype F2. In the Penton base gene and Fiber gene phylogenetic trees, the 9 HAdV-C gene sequences show high homology. The Hexon gene of HAdV-104 and HAdV-108 belongs to genotypes H1 and H2, respectively. In the Hexon gene phylogenetic tree, the five HAdV-108 sequences and four HAdV-104 sequences from Jining City are located in distinct evolutionary branches, with all five HAdV-108 sequences clustered in the same branch and all four HAdV-104 sequences clustered in another branch (see Fig. 3, Figures S5–7).

Analysis of amino acid variations

Penton base, Hexon, and Fiber are the primary structural proteins of Human Adenovirus (HAdV), playing crucial roles in the viral infection process [7, 8]. Compared to the Chinese candidate vaccine strain Guangzhou01, the Penton base protein of the 38 HAdV-B strains from Jining City shows a total of 4 amino acid mutations and 1 insertion mutation; the Hexon protein shows a total of 3 amino acid mutations; and the Fiber protein shows a total of 2 amino acid mutations. In 5 sequences, an insertion mutation at position P17 in the Penton base protein results in the amino acids at positions 14–19 changing from PEGPPP to PEGPPPP. The five Jining sequences with insertion mutations (Jining/22, Jining/29, Jining/30,

Fig. 2 Phylogenetic analysis of the whole genome of HAdV-B in Jining City. An evolutionary tree was constructed using MEGA 7.0.14 software with the maximum-likelihood method and 1000 bootstrap replicates. "■"represents the Chinese HAdV-3 candidate vaccine strain Guangzhou01, "●" represents the American HAdV-7 vaccine strain Gomen. Red represents the HAdV-3 phylogenetic branch, blue represents the HAdV-7 phylogenetic branch, and yellow represents 38 HAdV-B sequences from Jining City

Fig. 3 Phylogenetic analysis of the whole genome of HAdV-C in Jining City. An evolutionary tree was constructed using MEGA 7.0.14 software with the maximum-likelihood method and 1000 bootstrap replicates. Red represents the HAdV-108 phylogenetic branch, blue represents the HAdV-104 phylogenetic branch, green represents the 5 HAdV-108 sequences from Jining City, and brown represents the 4 HAdV-104 sequences from Jining City

Jining/31, Jining/37) form a distinct branch in the HAdV-3 whole-genome phylogenetic tree.

The Arg-Gly-Asp (RGD) motif in the Penton base protein, which binds to host cell integrins and plays a key role in HAdV endocytosis and endosomal release [9, 23], remained unchanged in all 38 HAdV-B sequences from Jining City. Hexon contains serotype-specific neutralizing epitopes that induce the host to produce serotype-specific antibodies. The serotype-specific neutralizing epitopes of HAdV-3 Hexon mainly include amino acid residues 136–151, 164–187, 241–255, 265–284, and 422–437 [10, 11]. Compared to the Chinese candidate vaccine strain Guangzhou01, no changes were observed in the Hexon antigenic epitopes of the 38 HAdV-B sequences from Jining City. In comparison with the vaccine strain Gomen, the 38 HAdV-B Hexon proteins from Jining City exhibited mutations AGEER139 - 143 TNRDN, E168Q, ADN175 - 177 TTE, 244 T insertion, D247G, E264D, 266 V insertion, D267G, FS269 - 270LA, S421 V, T423 insertion, 424 - 425DA insertion, and D430 A within the serotype-specific neutralizing epitopes (Table 2 and Fig. 4).

Table 2 Analysis of amino acid mutations in 38 HAdV-B Strains from Jining City

Name	Amino Acid Mutation Sites Compared to Guangzhou01
Penton base	P17 insert (5), V159 A (3), M204I (38), G378S (38), A462 T (34)
Hexon	S731 T (1), T740S (1), T862M (38)
Fiber	D72 N(18),I286M(38)

The Fiber protein is crucial for the binding of HAdV to host receptors. Key binding sites on the HAdV- 3 Fiber protein are located at amino acids 187–200, 244–256, and 281–294 [13]. Compared to the Chinese candidate vaccine strain Guangzhou01, all 38 HAdV-B sequences from Jining City have an I286M mutation in the receptor-binding region.

Among the 9 HAdV-C sequences from Jining City, the Penton base protein showed 9 amino acid differences, the Hexon protein exhibited 26 amino acid differences,

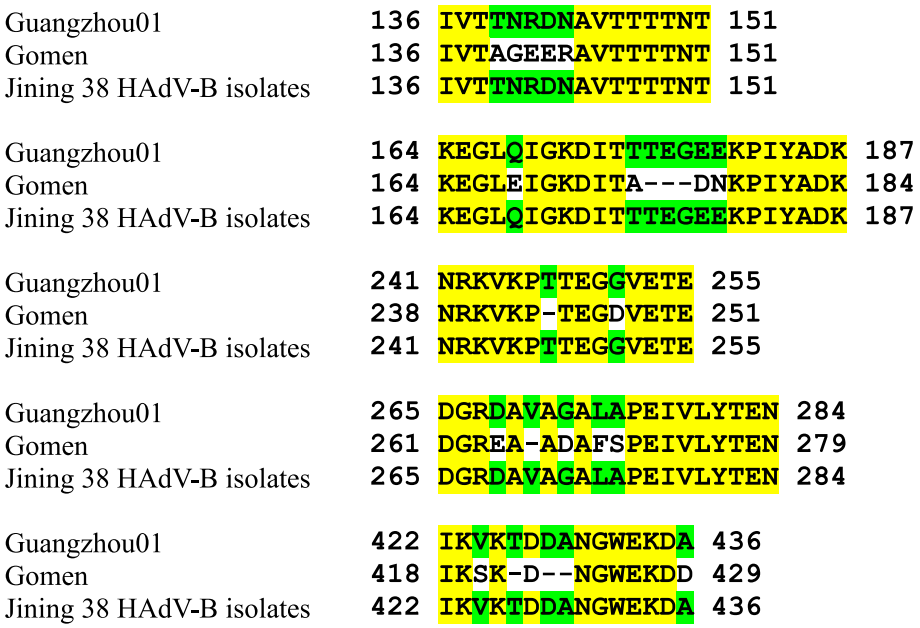

Fig. 4 Analysis of variations in HAdV-B Hexon protein serotype-specific neutralizing epitopes in Jining City. "■" represents the Hexon protein serotype-specific neutralizing epitope sequences that are completely identical among the 38 HAdV-B strains from Jining, Guangzhou01, and Gomen. "■" represents the Hexon protein serotype-specific neutralizing epitope sequences that are identical between the 38 HAdV-B strains from Jining and Guangzhou01, but different from the Gomen sequence

and Fiber displayed 11 amino acid differences. The RGD motif in the Penton base protein remained unchanged in all 9 HAdV-C sequences from Jining City. Although there were numerous amino acid differences in the Hexon protein among the 9 HAdV-C sequences from Jining City, only 3 amino acid differences were observed among the 4 HAdV-104 sequences, and only 2 amino acid differences were found among the 5 HAdV-108 sequences (Table 3).

Discussion

The prevalence trends of HAdV vary across different regions in China. In Yunnan Province, located in southern China, HAdV positivity rates in respiratory specimens peak in June and November [24], while in Guangzhou City, also in southern China, the highest rates occur in July–August [25]. In contrast, Jilin Province in northern China showed higher rates in December 2018, January and June 2019, but lower rates in December 2019 and January 2020 [26]. This study, conducted in Jining City in northern China from February 2023 to July 2024, yielded results similar to those in Jilin Province, with higher positivity rates in July and February 2024, but lower rates in July and February 2023. The adenovirus positivity rate was higher from December 2023 to July 2024, while the positivity rate was lower from December 2023 to November 2023.。 This suggests that HAdV prevalence in some northern Chinese regions may exhibit a complex pattern.

In Jining City, children under 15 years old showed higher positivity rates, with the highest rate of 7.63% (78/1022) in the 5–15 age group. In contrast, individuals over 15 years old had lower rates, with the lowest rate of 0% (0/454) in those aged 60 and above. These results indicate that children under 15 are at high risk for HAdV infection in Jining City, emphasizing the need for enhanced scientific prevention and control measures for this age group.

The prevalent HAdV genotypes differ across various regions in China. From 2010 to 2021, HAdV-3 and HAdV-7 were predominant in Guangzhou [25], while HAdV-41 was the main type in Yunnan Province from 2015 to 2021 [24]. In Jiangsu Province, HAdV-3, HAdV-7, HAdV-1, HAdV-2, and HAdV-5 were the main types from 2013 to 2021 [27]. HAdV outbreaks in China from 2009 to 2020 were primarily caused by HAdV-3, HAdV-4, HAdV-7, and HAdV-55 [1]. This study, through whole-genome sequencing of 47 HAdV strains from Jining City, identified 38 HAdV-3, 4 HAdV-104, and 5 HAdV-108 strains, indicating that HAdV-3, HAdV-104, and HAdV-108 are the main circulating types in Jining.

HAdV- 3 is widely prevalent in China [1, 25, 27]. The HAdV-104 genotype was first discovered in Guangdong Province, China, in 2017 [28]. However, no new HAdV-C104 genome updates or infection reports have been added to the NCBI database since then. This study's discovery of 4 new HAdV-104 strains further confirms the

Table 3 Analysis of amino acid variations in 9 HAdV-C Strains from Jining City

Name	Penton base									Hexon																										Fiber
	2	10	30	47	50	81	368	386	567	218	227	229	231	255	256	312	317	319	331	440	455	456	457	460	472	500	508	512	675	691	734	736	829	858	869	103
Jining/01/2023/104P1H1F2	R	G	S	R	G	S	A	T	H	V	E	T	S	P	F	I	S	T	A	G	N	D	D	A	L	T	P	S	V	A	A	T	D	F	D	A
Jining/02/2023/104P1H1F2	R	G	S	A	G	S	T	T	R	V	E	T	S	P	F	I	S	T	A	G	N	D	D	A	L	T	P	S	V	A	A	T	D	F	D	A
Jining/03/2023/104P1H1F2	R	G	S	R	G	S	A	T	R	V	E	T	S	P	F	I	S	T	A	G	N	D	D	A	L	T	P	T	V	A	A	T	D	F	D	A
Jining/36/2024/104P1H1F2	R	G	S	R	G	S	T	T	R	V	E	T	S	N	I	I	S	T	A	G	N	D	D	A	L	T	P	S	V	A	A	T	D	F	D	S
Jining/04/2023/108P1H2F2	R	G	S	A	E	S	A	S	R	I	D	N	A	P	F	L	G	G	S	A	D	E	T	T	M	N	D	T	V	S	A	T	E	V	D	A
Jining/05/2023/108P1H2F2	R	G	Y	R	G	S	A	S	R	I	D	N	A	P	F	L	G	G	S	A	D	E	T	T	M	N	D	T	V	A	S	M	E	V	E	A
Jining/07/2023/108P1H2F2	R	G	S	R	G	Y	A	S	R	I	D	N	A	P	F	L	G	G	S	A	D	E	T	T	M	N	D	T	V	A	A	T	E	V	D	A
Jining/10/2023/108P1H2F2	R	G	S	R	G	S	A	S	R	I	D	N	A	P	F	L	G	G	S	A	D	E	T	T	M	N	D	T	V	A	A	T	E	V	D	A
Jining/15/2023/108P1H2F2	P	R	S	A	E	S	A	S	R	I	D	N	A	P	F	L	G	G	S	A	D	E	T	T	M	N	D	T	I	S	A	T	E	V	D	A

existence of HAdV-104 and enriches the global HAdV-104 whole-genome sequence characteristics, suggesting that HAdV-C104 may be more common than previously thought, but potentially underreported due to limited HAdV sequencing studies.

The HAdV-C108 genotype was first reported in China in 2013 [29, 30], followed by reports from Russia and the United States [31, 32]. Bradford A. Becken et al. found that HAdV-C108 may have been circulating in the United States as early as 2009, based on analysis of recently uploaded whole-genome sequences of clinically isolated HAdV strains of unreported origin in GenBank [32]. The discovery of 5 HAdV-108 strains in this study further demonstrates the widespread presence of HAdV-108.

The 38 HAdV-B whole-genome sequences, Penton base gene sequences, and Fiber gene sequences obtained in this study showed high similarity to the Chinese HAdV-3 candidate vaccine strain Guangzhou01, clustering in the same evolutionary branch. In contrast, they showed lower similarity to the American HAdV-7 vaccine strain Gomen, appearing in different evolutionary branches. The 9 HAdV-C Hexon sequences showed relatively low similarity. In the HAdV-C whole-genome phylogenetic tree, the 5 HAdV-108 sequences from Jining City were closely related, as were the 4 HAdV-104 sequences.

The interaction between HAdV Penton base and host integrins can activate PI3K, which promotes the assembly of the actin cytoskeleton and leads to HAdV endocytosis [33–35]. Proteins containing the PXXPXXP motif (where P represents proline and X represents any amino acid) may interact with PI3K [36–38]. In this study, 5 of the 38 HAdV-B sequences from Jining City showed a mutation in the Penton base protein at amino acid positions 14–19, changing from PEGPPP to PEGPPPP. This creates a new PXXPXXP motif, which may enhance the interaction between Penton base and PI3K, potentially affecting PI3K activation and HAdV endocytosis.

Hexon is the protein that induces serotype-specific antibodies in the host [10–12]. This study found that the Hexon antigenic epitopes of the 38 HAdV-B sequences from Jining City showed no mutations compared to the Chinese candidate vaccine strain Guangzhou01, suggesting that this vaccine strain may provide good immune protection against HAdV-B in Jining City. However, compared to the American vaccine strain Gomen, all 5 antigenic epitopes of the 38 HAdV-B sequences from Jining City showed numerous mutations, indicating that the Gomen strain may offer poor immune protection against HAdV-B in Jining City. Among the 9 HAdV-C sequences from Jining City, there were significant amino acid differences in Hexon, but fewer differences among the 4 HAdV-104 sequences and the 5 HAdV-108 sequences. This suggests that there may be substantial amino acid differences in Hexon between HAdV-104 and HAdV-108. However, as HAdV-104 and HAdV-108 are recently discovered HAdV types with no studies on their Hexon antigenic epitopes, it is currently impossible to determine whether the differences in Hexon between HAdV-104 and HAdV-108 in Jining City are located in the antigenic epitopes.

In conclusion, this study analyzed the prevalence and genetic characteristics of HAdV in influenza-like illness cases in Jining City, China, from February 2023 to July 2024. The results identified children under 15 as the key population for HAdV prevention and control, showed no seasonal pattern in HAdV prevalence, and revealed the co-circulation of different HAdV types. These findings underscore the need for long-term, continuous HAdV surveillance and the development of vaccines for different types.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11558-1.

Supplementary Material 1: Figure S1. Adenovirus sequencing coverage, with blue representing coverage.

Supplementary Material 2: Figure S2. Phylogenetic analysis of the HAdV-B Penton base gene in Jining City.

Supplementary Material 3: Figure S3. Phylogenetic analysis of the HAdV-B Hexon gene in Jining City.

Supplementary Material 4: Figure S4. Phylogenetic tree analysis of the HAdV-B Fiber gene in Jining City.

Supplementary Material 5: Figure S5. Phylogenetic analysis of the HAdV-C Penton base gene in Jining City. An evolutionary tree was constructed using MEGA 7.0.14 software with the maximum-likelihood method and 1000 bootstrap replicates. Green represents the 5 HAdV-108 sequences from Jining City, and brown represents the 4 HAdV-104 sequences from Jining City.

Supplementary Material 6: Figure S6. Phylogenetic analysis of the HAdV-C Hexon gene in Jining City. An evolutionary tree was constructed using MEGA 7.0.14 software with the maximum-likelihood method and 1000 bootstrap replicates. Red represents the H2 evolutionary branch, blue represents the H1 evolutionary branch, green represents the 5 HAdV-108 sequences from Jining City, and brown represents the 4 HAdV-104 sequences from Jining City.

Supplementary Material 7: Figure S7. Phylogenetic analysis of the HAdV-C Fiber gene in Jining City. An evolutionary tree was constructed using MEGA 7.0.14 software with the maximum-likelihood method and 1000 bootstrap replicates. Green represents the 5 HAdV-108 sequences from Jining City, and brown represents the 4 HAdV-104 sequences from Jining City.

Supplementary Material 8.

Supplementary Material 9.

Supplementary Material 10.

Supplementary Material 11.

Acknowledgements
Not applicable.

Authors' contributions
SY, BJ, BJ conceived and designed the experiments. HD, CC, TS performed the experiments. HD, YJ, XW, YJ, YY, BJ, BJ analyzed the data. HD, CC, TS, XS, YJ, XW, YJ, YY, SH, BJ, BJ, SY interpreted the data. SY, BJ, BJ wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding
This work was supported by Science and Technology Development Funds for Shandong Medical and Health (202112060725 and 202412060875), Shandong Province Medical Staff Science and Technology Innovation Program (Project No.: SDYWZGKCJHLH202467) and Key Research and Development Funds for Jining Medical and Health (2021018 and 2023YXNS026).

Data availability
Data is provided within the supplementary information files.

Declarations

Ethics approval and consent to participate
The studies involving human participants were conducted in accordance with the Declaration of Helsinki and were reviewed and approved by the Ethics Committee at Jining Center for Disease Control and Prevention (SWS20240331). The patients/participants provided their written informed consent to participate in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 10 October 2024 Accepted: 1 April 2025
Published online: 14 April 2025

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