An Outbreak of Acute Respiratory Illness at a Training Base in Beijing, China, Due to Human Adenovirus Type B55

CURRENT STATUS: UNDER REVIEW

BMC Infectious Diseases  □ BMC Series

Guilan Lu
Beijing Center for Disease Prevention and Control

Xiaomin Peng
Beijing Center for Disease Control and Prevention

Renqing Li
Beijing Center for Disease Prevention and Control

Yimeng Liu
Shanxi Center for Disease Control and Prevention

Zhanguo Wu
Daxing District Center for Disease Prevention and Control, Beijing

Xifeng Wang
Daxing District Center for Disease Prevention and Control, Beijing

Daitao Zhang
Beijing Center for Disease Prevention and Control

Jiachen Zhao
Beijing Center for Disease Prevention and Control

Ying Sun
Beijing Center for Disease Prevention and Control

Li Zhang
Beijing Center for Disease Prevention and Control

Peng Yang
Beijing Center for Disease Prevention and Control and Prevention

Quanyi Wang
DOI: 10.21203/rs.2.18498/v1

SUBJECT AREAS
Infectious Diseases

KEYWORDS
Outbreak, Human adenovirus, Acute Respiratory Illness, Phylogenetic Analysis, Whole Genome Sequencing
Abstract
Background: An outbreak of acute respiratory illness (ARI) occurred at a training base in Beijing. A total of 12 students were diagnosed with symptoms of ARI from August 26 to August 30, 2015. The cause of the ARI outbreak was investigated.

Methods: In partnership with the local center for disease control, we conducted an epidemiological investigation on the outbreak, collecting a total of twelve pharyngeal swab specimens as well as patient demographic for the affected patients. We used multiplex real-time PCR to screen for sixteen common respiratory viruses in these samples. To isolate the causative virus, we inoculated Hep-2 cells with the human adenovirus (HAdV)-positive samples and then carried out sequencing and phylogenetic analysis of the hexon, fiber and penton genes of the isolated adenoviruses. In addition, we analyzed the whole genome of one strain isolated from the index case to identify single-nucleotide substitutions.

Results: We identified ten HAdV-positive students by multiplex real-time PCR. None of the students were co-infected with other viruses. We successfully isolated seven strains from the pharyngeal swab specimens. Genetic analysis showed that the coding sequences of the hexon, fiber, and penton genes obtained from those seven HAdV strains were identical, suggesting that they represented seven isolates of a single virus strain. One HAdV isolate obtained from the index case, BJDX-01-2015, was selected for whole genome analysis. From this isolate, we obtained a 34,774-nucleotide sequence. Phylogenetic analysis showed that the genome of BJDX-01-2015 clustered with HAdV-B55 and had 99.97% identity with human adenovirus 55 isolate HAdV-B/CHN/BJ01/2011/55 (GenBank accession no. JX491639).

Conclusions: We identified HAdV-B55 as the culprit of the ARI outbreak. This was the first reported outbreak in Beijing due to HAdV-B55 virus. Continuous surveillance of respiratory adenoviruses is urgently needed for understanding the epidemiological and evolutionary features of HAdV-B55 and could also find value in an epidemiological modeling approach.

Background
Human adenoviruses (HAdVs) cause a wide variety of clinical manifestations, including respiratory
tract illnesses, gastroenteritis, kerato-conjunctivitis, acute hemorrhagic cystitis, nephritis, hepatitis, and encephalitis [1-3]. HAdVs are responsible for 2–5% of all respiratory illnesses and for 4–10% of pneumonias in children [4-5]. Most HAdV infections are mild, self-limiting, and indistinguishable from other viral infections. However, the diseases caused by HAdVs can be severe or even fatal and can result in substantial morbidity [6-7]. Outbreaks of HAdV-associated acute respiratory infection (ARI) usually occur in healthy children or in adults in enclosed or crowded settings [1, 8].

HAdV was first reported as a viral pathogen in 1953 [6]. Since this initial identification, HAdVs have been classified into seven species (A to G), and the Human Adenovirus Working Group has identified 90 HAdV types as of July 2018 (http://hadvwg.gmu.edu/) [7–12]. Adenoviruses are non-enveloped icosahedral particles that contain genomes of linear double-stranded DNA with sizes ranging from 26 to 45 kb. The genomes of all adenoviruses have a similar genomic structure that is characterized by inverted terminal repeat sequences (LTR) with sizes ranging from 36 to over 200 bp [12-13].

Species of HAdV display various tissue tropisms that correlate with the different clinical symptoms of infection [14]. HAdV species A has often been associated with the gastrointestinal tract, whereas species B (HAdV-3, 7, 14, and 55), C (HAdV-1, 2, 5, and 6) and E (HAdV-4) are the causative pathogens of respiratory tract infections. Species D commonly causes adenoviral keratoconjunctivitis. Species F variants, including HAdV-F40 and-F41, and species G variant HAdV-G52 are mainly associated with gastrointestinal tract infections [2].

In China, HAdV-B3 and HAdV-B7, two subtypes of species B, are common causes of epidemic ARI outbreaks [15-18]. In 2006, an outbreak of acute respiratory tract infection occurred in Qishan, Shaanxi Province, China. A re-emergent isolate of HAdV-B55 (QS-DLL), originally described as HAdV-11a and fully characterized in 2009, was found to be the cause of this outbreak [19]. This re-emergent HAdV-B55 was shown to have evolved from a hexon recombination between HAdV-B11 and HAdV-B14 [9, 19]. HAdV-B55 has been associated with several respiratory infection outbreaks and is known to be responsible for severe respiratory diseases [9, 19–22].

Here we describe an outbreak of ARI caused by HAdV-B55 at a training base in the Daxing District of
Beijing, China. To help identify the causative pathogen, we collected pharyngeal swab specimens from the affected students and carried out molecular detection and typing, phylogenetic analysis, and whole-genome sequencing. This is the first reported outbreak of ARI in Beijing due to HAdV-B55.

Methods

The training base where the HAdV-B55 outbreak took place

On August 31, 2015, local public health authorities were informed about an outbreak of ARI among young students at a training base located in the Daxing District of Beijing. The training base consisted of two three-floor buildings for teaching and three three-floor dormitories with eight persons to a room. The training base recruits only male middle school graduates. Approximately 3,000 students majoring in Math, Chinese, and English were enrolled in a total of sixty classes. The training base employs 100 full time staff members.

Epidemiological investigation

On August 26, 2015, one student reported having symptoms of an ARI and had a body temperature of 38.4°C. For the purposes of our analysis, we defined ARI cases as individuals with a body temperature over 38.0°C and with at least one symptom of a respiratory tract infection, such as cough or sore throat. By August 30, a total of 12 ARI patients from the same class were reported by the local hospital. On August 31, the Daxing District Center for Disease Prevention and Control (CDC) began an epidemiological investigation, collecting demographic, clinical and laboratory data. Under the guidance of the CDC, the training base took precautionary measures, including quarantining the affected students, carrying out a routine cleaning and disinfection of living quarters, and morning body temperature checks. No further new cases were reported by September 08, 2015.

Patients and samples

Pharyngeal swab samples were obtained from each of the twelve students infected in this ARI outbreak. The specimens were collected in 3 mL vials containing transport medium and quickly transported on ice to the laboratory of the Daxing District CDC. The specimens were stored at -80°C until further use. Patient information and laboratory results are shown in Table 1.

Respiratory virus detection
Nucleic acid was extracted from 140 μL of each of the clinical samples using QIAamp Viral RNA mini Kits (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Pharyngeal swab specimens were analyzed by real-time PCR multiplex assay using commercial kits (Uninovo Biological Technology, Zhenjiang, China) to screen for 16 common respiratory pathogens as described by Shi W et al.[23]. The 16 assayed pathogens are influenza viruses A (H3), pandemic influenza viruses A (H1N1), influenza viruses A and B (Flu A and B), parainfluenza viruses 1, 2, 3, and 4 (PIV1, 2, 3, and 4), human metapneumovirus (hMPV), human bocavirus (HBoV), human coronavirus OC43/NL63, 229E/HKU1, human respiratory syncytial virus (HRSV), human rhinovirus (HRhV) and HAdV.

**HAdV isolation and typing**

Hep-2 cells were inoculated with HAdV PCR-positive specimens and cultured in high-glucose Dulbecco’s Modified Eagles Medium (Gibco, NY, USA) containing 2% fetus bovine serum (Gibco, NY, USA), 100 U/mL penicillin (Gibco, NY, USA) and 100 mg/mL streptomycin (Gibco, NY, USA) at 37°C in a 5% CO₂ incubator for one week following standard protocols [19]. The culture medium was harvested until cytopathic effects (CPE) were observed. Cultures with CPE were screened for specific HAdVs as described by Kim C et al. [24].

Molecular typing for HAdVs was also performed via conventional PCR using specific primers targeting the complete coding sequences of hexon, fiber and penton genes [25]. Viral DNA was extracted from cultured medium with QIAamp RNA mini kits (Qiagen, Hilden, Germany) according to the manufacturer’s instructions [26]. Conventional PCR was conducted using high-fidelity DNA polymerase (Takara, Dalian, China) according to the manufacturer’s instructions. The hexon, fiber, and penton genes of HAdV were amplified as described previously [25]. For the hexon and penton genes, the PCR protocol was: 94°C for 5 min, followed by 35 cycles of 50 sec at 94°C, 50 sec at 55°C, 3 min at 72°C, and a final extension step of 72°C for 10 min. The PCR protocol for amplification of fiber gene fragments was identical, with the exception of the annealing temperature, which was 52°C instead of 55°C. The amplified PCR products were excised from agarose gels, purified using an Axyprep DNA gel extraction kit (Axygen, Hangzhou, China), and bi-directionally sequenced using the Sanger sequencing method by Invitrogen Biotechnology Co. Ltd (Invitrogen, Beijing, China) with an
ABI 3730 DNA Analyzer (Applied Biosystems, Austin, TX, USA).

**Whole-genome sequencing**

To further analyze mutations in the genome sequences of the viruses isolated in this ARI outbreak, the whole genome of one isolate from the index case was sequenced using the Sanger method after PCR amplification of targeted 1-2 kb segments that covered the entire genome with overlapping sequences of about 200 bp. The 5′ and 3′ LTR of the genome were amplified and cloned into a plasmid T-vector and then sequenced. A set of 47 pairs of primers was designed in-house to amplify the whole genome according to the reference sequence (GenBank accession no. FJ643676) and then used for separate PCRs. All primer sequences are available upon request.

Whole-genome sequencing segments were amplified using high-fidelity polymerase (Takara, Dalian, China) using 1.0 mM of each primer. PCRs were carried out using a BioRad thermocycler (Applied Biosystems, Austin, TX, USA) with the following protocol: 94°C for 5 min, followed by 35 cycles of 50 sec at 94°C, 50 sec at appropriate annealing temperature for separate primers, 3 min at 72°C, and a final extension step of 72°C for 10 min.

The amplified segments were purified and bi-directionally sequenced. Gaps and ambiguous sequences were PCR-amplified using different primers and re-sequenced. DNA sequence fragments were assembled using the SeqMan program implemented in DNASTAR Lasergene 7.0 (DNASTAR, Inc. Madison, WI.) into a single contig. The whole genomic sequence determined in this study was deposited in GenBank with accession numbers (GenBank accession number MK886831).

**Sequence alignment and phylogenetic analysis**

Nucleotide sequence homologies were identified using the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/). Multiple nucleotide sequence alignments were performed using the ClustalW program implemented in BioEdit. Comparisons of the whole genome sequence of the BJDX-01-2015 virus strain with those of other types of HAdVs were generated using CLC Genomics Workbench (Qiagen, Hilden, Germany).

Phylogenetic trees were constructed using the neighbor-joining method with the Kimura-2-parameters in the MEGA program (Version 5.05). One thousand bootstrap replications were used for distance
estimation. Bootstrap values greater than 70% are shown for selected nodes Figures 2 (A) to 2 (D).
Whole-genome sequences and hexon, fiber, and penton gene sequences from other HAdVs were downloaded from GenBank.

Results
Epidemiology

Outbreak description
On August 26, 2015, a 15-year old male student developed a case of ARI, with a peak body temperature of 38.4 °C. By August 31, a total of 12 male students were infected. No females were infected. The mean age of the infected students was 15.4 years (median, 15 years; range, 14–17 years). The distribution of daily cases is shown in Fig. 1. Reported students were from the same class but living in six different dormitory rooms. The outbreak lasted for six days.

Clinical symptoms of the infected students
The clinical symptoms of the infected students are described in Table 1. All 12 infected individuals had a fever, while 9 (75%) of them had a sore throat and 7 (58.33%) had a headache. Other symptoms reported by the patients were cough, body ache, stuffy nose, and diarrhea. Laboratory diagnosis showed that all affected students had normal white blood counts. Most of the infected students were treated in outpatient clinics; only one patient, who had pneumonia, was hospitalized. The index case (ID No.1) was diagnosed as having an upper respiratory infection with a peak temperature of 38.4 °C, a percentage of neutrophils of 72.2% (normal range, 50–70%), percentage of lymphocytes of 22.2% (normal range, 20–40%), and white blood cell count measuring 7.8 × 10^9/L (normal range, 4.0–10.0 × 10^9/L).

The hospitalized student (ID No.6) had a peak temperature of 40.0 °C, accompanied by a cough, headache, sore throat, and diarrhea. He had a neutrophil percentage of 78.6% (normal range, 50–70%), a percentage of lymphocytes of 17.8% (normal range, 20–40%), and a white blood cell count of 5.04 × 10^9/L (normal range, 4.0–10.0 × 10^9/L). A chest X-Ray showed patchy shadows on the right lung of the hospitalized student.

Etiological detection of the outbreak
Multiplex-PCR detection

A total of 12 respiratory samples were obtained from the 12 students. Ten specimens were shown to be HAdV-positive using multiplex-PCR (Uninovo Biological Technology, Jiangshu, China). None of the 10 HAdV-positive patients were co-infected with other respiratory viruses.

Virus isolation and HAdV typing

To isolate viruses, we inoculated Hep-2 cells with the HAdV-positive samples, then amplified the HAdV hexon gene using in-house primers. This approach isolated seven HAdV virus strains [24]. Using typing primers, which allow for the determination of viral type, we sequenced the hexon, fiber, and penton genes in the seven HAdV strains [25].

The hexon, fiber, and penton sequences from the seven HAdV isolates were 100% identical, suggesting that this outbreak was caused by a single viral strain. We next compared the hexon, fiber, and penton sequences the viral strain to other sequences in GenBank. A BLAST sequence analysis revealed that the hexon, fiber, and penton genes were 99.6%, 100%, and 100% identical to their counterparts in the HAdV-B55 reference strain isolate China/QS-DLL (Genbank accession number FJ643676), respectively. One of the seven isolates (named as BJDX-01-2015) was selected for further whole genomic sequence studies.

Whole genome sequencing

To understand the genetic mutations occurred during this ARI outbreak, we sequenced the full genome of one isolate, BJDX-01-2015. We assembled the complete genome using DNASTAR Lasergene 7.0 (DNASTAR Lasergene 7.0 (DNASTAR, Inc. Madison, WI ), and deposited the sequence in GenBank (GenBank accession number MK886831). The complete genome of BJDX-01-2015 was 34,774 nucleotides in length and had a 137-bp inverted terminal repeat sequence in the 5′- and 3′-untranslated regions (5′-UTRs and 3′-UTRs).

Phylogenetic analysis of the HAdV-B55 strain

To investigate the genetic relationships between isolate BJDX-01-2015 and other HAdV strains, we constructed phylogenetic trees (Figs. 2A to 2D) using the neighbor-joining method based on the complete hexon, fiber, and penton gene sequences of strain BJDX-01-2015 and their counterparts in
other HAdV strains representing HAdV species A to G.

All phylogenetic trees demonstrated that the strain BJDX-01-2015 isolate in this outbreak clustered with HAdV-B55. The BJDX-01-2015 hexon gene also clustered with HAdV-B11 (GenBank accession number AF532578), while the fiber and penton genes and full BJDX-01-2015 genome clustered with HAdV-B14 (GenBank accession number AY803294).

Whole-genome alignment and mutation analysis

We next carried out whole genome nucleotide identity analysis for BJDX-01-2015, HAdV-B55, HAdV-B11, and HAdV-B14. BJDX-01-2015 was 99.97% identical to the CHN/BJ01/2011 (GenBank accession number JX491639) strain with 11 differential nucleotides, and 99.89% identical to the strain QS-DLL (GenBank accession number FJ643674) with 38 differential nucleotides. BJDX-01-2015 shared 97.62% of its genome with HAdV-B11 (GenBank accession number AF532578), and 98.89% and 98.88% with two HAdV-B14 strains (GenBank accession number AY803294 and JX892927), (Fig. 3).

We then generated alignments between the genome sequence of strain BJDX-01-2015 and those of strains HAdV-B55, HAdV-B11 and HAdV-B14. Compared to strain CHN/BJ01/2011 (Genbank accession number JX491639), which was isolated from a single patient with severe pneumonia infected in Beijing with HAdV-B55, most of the mutations in BJDX-01-2015 were found in the coding region of protein VI (Fig. 4A), where we found seven nucleotide changes and six amino acid substitutions. We also found different numbers of poly “T” and poly “A” tracts (Fig. 4B).

Discussion

This study describes an ARI outbreak with 12 students infected at a training base in the Daxing District of Beijing, China, in August, 2015. Based on epidemiological and laboratory investigation, we confirmed that the etiologic pathogen of this ARI outbreak was HAdV-B55.

HAdVs have been associated with previous outbreaks of ARI. In the United States, HAdV-B3 and HAdV-B7 are a frequent cause of outbreaks [27]. In Asia, the prevalence of HAdVs in patients with ARIs has ranged from 0.8% to 11.30% [28-32]. Recently, Guo et al. (2012) reported that HAdV-B3, HAdV-B7, HAdV-B11, and HAdV-B14 were the most frequently detected virus strains among patients with acute adult adenovirus infections in Beijing from May 2005 to July 2010 [28].
In recent years, HAdV-B55 has received additional attention. HAdV-B55 is a recombinant virus, and it is associated with more severe acute respiratory diseases than other types of HAdVs [33]. HAdV-B55 was originally described as HAdV-B11a based on earlier putative, sporadic occurrences in Spain (1969) [34], Turkey (2004) [35], and Singapore (2005) [36]. HAdV-B55 was also identified as a re-emergent acute respiratory disease pathogen after a recent outbreak in Qishan County, Shaanxi Province, China in 2006 [9, 19]. Subsequent analysis revealed that HAdV-B55 consists of a HAdV-B14 backbone and a partial HAdV-B11 hexon gene. This re-emergent virus exhibited a neutralizing antigen epitope of HAdV-B11 and the pathogenic properties of HAdV-14. Therefore, this virus was renamed HAdV-B55 [9, 37].

In China, an increasing number of outbreaks of HAdV-B55 have been reported since March 2006 [19]. Epidemic outbreaks of HAdV-B55 occurred in military camps, schools, and even hospitals in Hebei Province (February, 2012) [38], Tianjin City (January, 2013) [21], Beijing (2015) (this study), Guangzhou City (2016) [22]. Tibet, and Sichuan and Yunnan Provinces (2016) [39].

In the 2006 HAdV-B55 outbreak in China, a total of 254 patients were infected, and one died [19]. Although the virus spread quickly in the 2015 outbreak reported here, only 12 individuals were infected, including one student who developed pneumonia and was hospitalized. This suggests that the HAdV-B55 strain that caused this outbreak is not as virulent as those reported previously [19, 33], yet the strain should still be considered an urgent public health threat with need to take measures to contain or control it in order to prevent epidemics.

Unlike other outbreaks caused by HAdVs in the northern regions of China [19, 21, 38], which occurred in the winter or spring, this outbreak in Beijing took place at the end of summer. In other studies by Yu J et al. and Liu T et al., although infections of HAdVs occur throughout the year, HAdV outbreak prevalence often peaks in winter and spring in the north of China, and in summer and spring in the south of China [40, 41]. It is possible that HAdV outbreaks differ in their seasonality based on relative humidity and temperature. The fact that the HAdV outbreak identified in our study occurred in summer is thus significant as it could indicate this HAdV-B55 virus strain may have a better capability
to circulate in the reservoir under warm temperature.

The virus strain BJDX-01-2015 isolated in this study shared 100% sequence identity with the hexon, fiber and penton genes in the HAdV-B55 strain CHN-BJ01-2011 (Genbank accession number JX491639) that was isolated from a severe pneumonia case in Beijing in 2011 [42, 43]. However, the genome of BJDX-01-2015 is most similar to that of CHN-BJ01-2011, leading to milder ARIs. These findings imply that severe ARIs were not only caused by HAdV-B55 virus strains themselves, but are also correlated with the hosts, e.g. the age of individuals, their general state of health, and any underlying diseases or additional infections.

In this outbreak, we also found that both the index case and the hospitalized case correlated with an increase in neutropils and/or a decrease in lymphocytes in the peripheral blood. Studies on other respiratory viruses, e.g. influenza virus, respiratory synsytial virus, or human rhinovirus have shown that an increase of neutrophils has a significant role in limiting virus replications[44, 45]. Thus, the increase in neutrophils we observed here may also have limited the virus we identified appears to be associated with milder clinical symptoms in this outbreak.

Based on the genome alignment analysis, most of the mutations in BJDX-01-2015 were observed in the coding regions of protein VI. During virus replication of HAdVs, protein VI functions as an adaptor to shuttle the hexon protein to the nucleus, where virus assembly occurs [46]. Whether these variations affect the virulence of BJDX-01-2015 and are responsible for the milder clinical symptoms observed in this study requires further investigation. We also found that BJDX-01-2015 has different numbers of poly “T” and poly “A” tracts compared to the HAdV-B55, HAdV-B11, and HAdV-B14 strains. The role that poly “T” and poly “A” tracts play in the evolution of HAdVs also remains unclear and requires further research.

The increasing frequency of ARI outbreaks due to HAdV-B55 suggests that this re-emergent virus poses a serious threat to public health. It is therefore urgent that the local CDC improve epidemiological and virological surveillance of HAdV-B55.

Conclusions

We identified HAdV-B55 as the culprit of a recent localized ARI outbreak. This incident was the first
reported outbreak in Beijing that can be attributed to this re-emergent virus. Continuous surveillance of respiratory adenoviruses is an urgent need for understanding the epidemiological and evolutionary features of HAdV-B55 and could also find value in an epidemiological modeling approach.

Abbreviations

PCR: Polymerase chain reaction; ARI: Acute respiratory illness; HAdV: Human adenovirus; LTR: Inverted terminal repeat; CDC: Center for Disease Prevention and Control; UTR: Untranslated region.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Beijing Center for Disease Prevention and Control. Prior written consent form was obtained for all individuals who participated in this study.

Consent for publication

The parents of the patients had given their consent for publication of clinical details and written informed consent was obtained.

Availability of data and materials

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

Competing interests

The authors have no conflicting interests.

Funding

This study was supported in part by the Research on Early Detection, Genetic Evolution and Risk Assessment for Novel Influenza Virus by Capital's Funds for Health Improvement and Research (2018-1-1012).

Authors’ contributions

All authors made significant contributions to the data, analysis and draft of this manuscript and approved the final version submitted. Guilan Lu wrote the manuscript. Guilan Lu, Xiaoming Peng and Renqing Li performed genomic analysis. Yimeng Liu, Daitao Zhang, and Jiachen Zhao conducted the diagnostic experiments. Zhanguo Wu, Xifeng Wang, Ying Sun, and Li Zhang participated in collecting
information of this outbreak. Peng Yang and Quanyi Wang helped review this manuscript.

Acknowledgements

The authors thank all colleagues in the Beijing CDC for their help in molecular detection and whole-genome analysis. They also thank the technicians of the Daxing CDC of Beijing for their excellent technical assistance with the epidemiological investigation and with the initial laboratory diagnosis.

References

1. Lynch JP 3rd, Kajon AE. Adenovirus: Epidemiology, Global Spread of Novel Serotypes, and Advances in Treatment and Prevention. Semin Respir Crit Care Med. 2016, 37 (4): 586-602.

2. Jones MS 2nd, Harrach B, Ganac RD, Gozum MM, Dela Cruz WP, Riedel B, et al. New adenovirus species found in a patient presenting with gastroenteritis. J Virol. 2007, 81 (11): 5978-84.

3. Engelmann I, Madisch I, Pommer H, Heim A. An outbreak of epidemic keratoconjunctivitis caused by a new intermediate adenovirus 22/H8 identified by molecular typing. Clin Infect Dis. 2006, 43 (7): e64-6.

4. Alharbi S, Van Caeseele P, Consunji-Araneta R, Zoubeidi T, Fanella S, Souid AK, et al. Epidemiology of severe pediatric adenovirus lower respiratory tract infections in Manitoba, Canada, 1991-2005. BMC Infect Dis. 2012, 12: 55.

5. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, et al. Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. N Engl J Med. 2015, 373 (5): 415-27.

6. Rowe WP, Huebner RJ, Gilmore LK, Parrott RH, Ward TG. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. Proc Soc Exp Biol Med. 1953, 84 (3): 570-3.

7. Ghebremedhin B. Human adenovirus: Viral pathogen with increasing importance. Eur J Microbiol Immunol (Bp). 2014, 4 (1): 26-33.
8. Kajon AE, Lamson DM, St George K. Emergence and re-emergence of respiratory adenoviruses in the United States. Curr Opin Virol. 2019, 14 (34): 63-9.

9. Walsh MP, Seto J, Jones MS, Chodosh J, Xu W, Seto D. Computational analysis identifies human adenovirus type 55 as a re-emergent acute respiratory disease pathogen. J Clin Microbiol. 2010, 48 (3): 991-3.

10. Walsh MP, Seto J, Liu EB, Dehghan S, Hudson NR, Lukashev AN, Ivanova O, Chodosh J, Dyer DW, Jones MS, Seto D. Computational analysis of two species C human adenoviruses provides evidence of a novel virus. J Clin Microbiol. 2011, 49 (10): 3482-90.

11. Matsushima Y, Shimizu H, Kano A, Nakajima E, Ishimaru Y, Dey SK, et al. Novel human adenovirus strain, Bangladesh. Emerg Infect Dis. 2012, 18 (5): 846-8.

12. Davison AJ, Benko M, Harrach B. Genetic content and evolution of adenoviruses. J Gen Virol. 2003, 84 (Pt 11): 2895-908.

13. Saha B, Wong CM, Parks RJ. The adenovirus genome contributes to the structural stability of the virion. Viruses. 2014, 6 (9): 3563-83.

14. Tebruegge M, Curtis N. Adenovirus: an overview for pediatric infectious diseases specialists. Pediatr Infect Dis J. 2012, 31 (6): 626-7.

15. Wo Y, Lu QB, Huang DD, Li XK, Guo CT, Wang HY, et al. Epidemical features of HAdV-3 and HAdV-7 in pediatric pneumonia in Chongqing, China. Arch Virol. 2015, 160 (3): 633-8.

16. Xie L, Yu XF, Sun Z, Yang XH, Huang RJ, Wang J, et al. Two adenovirus serotype 3 outbreaks associated with febrile respiratory disease and pharyngoconjunctival fever in children under 15 years of age in Hangzhou, China, during 2011. J Clin Microbiol. 2012, 50 (6): 1879-88.

17. Tang L, Wang L, Tan X, Xu W. Adenovirus serotype 7 associated with a severe lower
16

respiratory tract disease outbreak in infants in Shaanxi Province, China. Virol J. 2011, 8: 23.

18. Cheng J, Qi X, Chen D, Xu X, Wang G, Dai Y, et al. Epidemiology and transmission characteristics of human adenovirus type 7 caused acute respiratory disease outbreak in military trainees in East China. Am J Transl Res. 2016, 8 (5): 2331-42.

19. Zhu Z, Zhang Y, Xu S, et al. Outbreak of acute respiratory disease in China caused by B2 species of adenovirus type 11. J Clin Microbiol. 2009, 47 (3): 697-703.

20. Salama M, Amitai Z, Amir N, Gottesman-Yekutieli T, Sherbany H, Drori Y, et al. Outbreak of adenovirus type 55 infection in Israel. J Clin Virol. 2016, 78: 31-5.

21. Li X, Kong M, Su X, Zou M, Guo L, Dong X, et al. An outbreak of acute respiratory disease in China caused by human adenovirus type B55 in a physical training facility. Int J Infect Dis. 2014, 28: 117-22.

22. Yi L, Zou L, Lu J, Kang M, Song Y, Su J, et al. A cluster of adenovirus type B55 infection in a neurosurgical inpatient department of a general hospital in Guangdong, China. Influenza Other Respir Viruses. 2017, 11 (4): 328-36.

23. Shi W, Cui S, Gong C, Zhang T, Yu X, Li A, et al. Prevalence of human parainfluenza virus in patients with acute respiratory tract infections in Beijing, 2011-2014. Influenza Other Respir Viruses. 2015, 9(6): 305-7.

24. Kim C, Ahmed JA, EidexRB, Nyoka R, Waiboci LW, Erdman D, et al. Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. PLoS One. 2011, 6 (6): e21610.

25. Chen M, Zhu Z, Huang F, Liu D, Zhang T, Ying D, et al. Adenoviruses associated with acute respiratory diseases reported in Beijing from 2011 to 2013. PLoS One. 2015, 10 (3): e0121375.
26. Gao Z, Liu B, Yan H, Li W, Jia L, Tian Y, et al. Norovirus outbreaks in Beijing, China, from 2014 to 2017. J Infect. 2019, S0163-4453 (19) 30163-X.

27. James L, Vernon MO, Jones RC, Stewart A, Lu X, Zollar LM, et al. Outbreak of human adenovirus type 3 infection in a pediatric long-term care facility--Illinois, 2005. Clin Infect Dis. 2007, 45 (4): 416-20.

28. Guo L, Gonzalez R, Zhou H, Wu C, Vernet G, Wang Z, Wang J. Detection of three human adenovirus species in adults with acute respiratory infection in China. Eur J Clin Microbiol Infect Dis. 2012, 31 (6): 1051-8.

29. Ou ZY, Zeng QY, Wang FH, Xia HM, Lu JP, Xia JQ, et al. Retrospective study of adenovirus in autopsied pulmonary tissue of pediatric fatal pneumonia in South China. BMC Infect Dis. 2008, 8: 122.

30. Abd-Jamil J, Teoh BT, Hassan EH, Roslan N, Abubakar S. Molecular identification of adenovirus causing respiratory tract infection in pediatric patients at the University of Malaya Medical Center. BMC Pediatr. 2010, 10: 46.

31. Hong JY, Lee HJ, Piedra PA, Choi EH, Park KH, Koh YY, et al. Lower respiratory tract infections due to adenovirus in hospitalized Korean children: epidemiology, clinical features, and prognosis. Clin Infect Dis. 2001, 32 (10): 1423-9.

32. Yu X, Lu R, Wang Z, Zhu N, Wang W, Julian D, et al. Etiology and clinical characterization of respiratory virus infections in adult patients attending an emergency department in Beijing. PLoS One. 2012, 7 (2): e32174.

33. Cao B, Huang GH, Pu ZH, Qu JX, Yu XM, Zhu Z, et al. Emergence of community-acquired adenovirus type 55 as a cause of community-onset pneumonia. Chest. 2014, 145 (1): 79-86.

34. Hierholzer JC, Pumarola A, Rodriguez-Torres A, Beltran M. Occurrence of respiratory illness due to an atypical strain of adenovirus type 11 during a large outbreak in
Spanish military recruits. Am J Epidemiol. 1974, 99 (6): 434-42.

35. Chmielewicz B, Benzler J, Pauli G, Krause G, Bergmann F, Schweiger B. Respiratory disease caused by a species B2 adenovirus in a military camp in Turkey. J Med Virol. 2005, 77 (2): 232-7.

36. Kajon AE, Dickson LM, Metzgar D, Houn HS, Lee V, Tan BH. Outbreak of febrile respiratory illness associated with adenovirus 11a infection in a Singapore military training cAMP. J Clin Microbiol. 2010 Apr;48(4):1438-41.

37. Seto D, Jones MS, Dyer DW, Chodosh J. Characterizing, typing, and naming human adenovirus type 55 in the era of whole genome data. J Clin Virol. 2013, 58 (4): 741-2.

38. Lu QB, Tong YG, Wo Y, Wang HY, Liu EM, Gray GC, et al. Epidemiology of human adenovirus and molecular characterization of human adenovirus 55 in China, 2009-2012. Influenza Other Respir Viruses. 2014, 8 (3): 302-8.

39. Wang W, Liu Y, Zhou Y, Gu L, Zhang L, Zhang X, et al. Whole-genome analyses of human adenovirus type 55 emerged in Tibet, Sichuan and Yunnan in China, in 2016. PLoS One. 2017, 12 (12): e0189625.

40. Yu J, Xie Z, Zhang T, Lu Y, Fan H, Yang D, et al. Comparison of the prevalence of respiratory viruses in patients with acute respiratory infections at different hospital settings in North China, 2012-2015. BMC Infect Dis. 2018, 18(1):72.

41. Liu T, Li Z, Zhang S, Song S, Julong W, Lin Y, et al. Viral Etiology of acute respiratory tract infections in hospitalized children and adults in Shandong Province, China. Virol J. 2015, 12:168.

42. Gu L, Liu Z, Li X, Qu J, Guan W, Liu Y, Song S, Yu X, et al. Severe community-acquired pneumonia caused by adenovirus type 11 in immunocompetent adults in Beijing. J Clin Virol. 2012, 54 (4): 295-301.

43. Zhang Q, Seto D, Cao B, Zhao S, Wan C. Genome sequence of human adenovirus type
55, a re-emergent acute respiratory disease pathogen in China. J Virol. 2012, 86 (22): 12441-2.

44. Tate MD, Brooks AG, Reading PC. The role of neutrophils in the upper and lower respiratory tract during influenza virus infection of mice. Respir Res. 2008, 9:57.

45. Bataki EL, Evans GS, Everard ML. Respiratory syncytial virus and neutrophil activation. Clin Exp Immunol. 2005, 140(3):470-7.

46. Moyer CL, Besser ES, Nemerow GR. A single maturation cleavage site in adenovirus impacts cell entry and capsid assembly. J Virol. 2015, 90(1):521-32.

Table

| ID No. | Gender | Age | Onset time (m/d/y) | Specimen Collection time (m/d/y) | Body temperature °C | Cough | Sore throat | Body aches |
|-------|--------|-----|-------------------|----------------------------------|----------------------|-------|-------------|------------|
| 1     | male   | 15  | 08/26/2015        | 08/31/2015                       | 38.4                 | -     | +           | +          |
| 2     | male   | 16  | 08/26/2015        | 08/31/2015                       | 39.0                 | -     | -           | +          |
| 3     | male   | 15  | 08/27/2015        | 08/31/2015                       | 38.5                 | +     | -           | -          |
| 4     | male   | 15  | 08/28/2015        | 08/31/2015                       | 39.5                 | -     | +           | -          |
| 5     | male   | 16  | 08/28/2015        | 08/31/2015                       | 39.6                 | -     | +           | +          |
| 6     | male   | 16  | 08/29/2015        | 08/31/2015                       | 40.0                 | +     | +           | -          |
| 7     | male   | 15  | 08/29/2015        | 08/31/2015                       | 38.8                 | -     | +           | -          |
| 8     | male   | 14  | 08/30/2015        | 08/31/2015                       | 38.2                 | -     | +           | -          |
| 9     | male   | 16  | 08/30/2015        | 08/31/2015                       | 39.4                 | -     | -           | +          |
| 10    | male   | 15  | 08/30/2015        | 08/31/2015                       | 39.0                 | -     | +           | -          |
| 11    | male   | 17  | 08/30/2015        | 08/31/2015                       | 38.6                 | -     | +           | +          |
| 12    | male   | 15  | 08/29/2015        | 08/31/2015                       | 39.6                 | -     | +           | -          |

ID No., identification number; +, yes; -, no; /, not tested.

Figures
Case distribution during a 2015 outbreak of HAdV-B55 in Beijing. Left Y-axis, number of cases; right Y-axis, onset date of cases.
Figure 2

Based on the complete sequences of (A) hexon, (B) fiber, (C) penton and (D) complete genome, the phylogenetic trees were constructed using the neighbor-joining method with 1,000 bootstrap replicates using MEGA 5.0. Bootstrap-supported values greater than 70% are indicated at each node. The scale bar indicates the number of nucleotide substitutions per site. The species of adenovirus is specified in the parentheses at the end of strain information. ●, isolate from this outbreak; ■, HAdV-B55 CHN/Bj/2011 strain; ○, QS-DLL strain; ▲, HAdV-B11 isolates; ▼, HAdV-B14 isolates.

Based on the complete sequences of (A) hexon, (B) fiber, (C) penton and (D) complete genome, the phylogenetic trees were constructed using the neighbor-joining method with 1,000 bootstrap replicates using MEGA 5.0. Bootstrap-supported values greater than 70% are indicated at each node. The scale bar indicates the number of nucleotide substitutions per site. The species of adenovirus is specified in the parentheses at the end of strain information. ●, isolate from this outbreak; ■, HAdV-B55 CHN/Bj/2011 strain; ○, QS-DLL strain; ▲, HAdV-B11 isolates; ▼, HAdV-B14 isolates.
Percent identity and differences in the genome of BJDX-01-2015 relative to strains of HAdV-B55, HAdV-B11 and HAdV-B14. The numbers above the white grids represent the percent similarity, while those below the grids represent difference values calculated by CLC Genomic Workbench.

![Comparison of genomes](image)

|         | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| BJDX-01-2015 | 100 | 99.97 | 99.90 | 99.89 | 99.88 | 99.85 | 99.82 | 99.87 | 99.84 | 98.98 | 98.88 |
| JX491639     | 2   | 99.97 | 99.90 | 99.89 | 99.88 | 99.85 | 99.82 | 99.87 | 99.84 | 98.98 | 98.88 |
| FJ643676     | 3   | 99.87 | 99.89 | 99.88 | 99.85 | 99.82 | 99.87 | 99.84 | 99.81 | 98.98 | 98.88 |
| JX123028     | 4   | 99.84 | 99.87 | 99.88 | 99.85 | 99.82 | 99.87 | 99.84 | 99.81 | 98.98 | 98.88 |
| KJ883521     | 5   | 99.82 | 99.87 | 99.85 | 99.82 | 99.87 | 99.84 | 99.81 | 98.98 | 98.88 | 98.81 |
| KJ883521     | 6   | 99.81 | 99.84 | 99.82 | 99.81 | 99.84 | 99.82 | 99.81 | 98.98 | 98.88 | 98.81 |
| KY002688     | 7   | 99.81 | 99.84 | 99.82 | 99.81 | 99.84 | 99.82 | 99.81 | 98.98 | 98.88 | 98.81 |
| AF532578     | 8   | 99.68 | 99.81 | 99.80 | 99.68 | 99.79 | 99.76 | 99.74 | 98.98 | 98.88 | 98.81 |
| AY803294     | 9   | 99.41 | 99.54 | 99.67 | 99.54 | 99.67 | 99.54 | 99.41 | 98.65 | 98.58 | 98.51 |
| JX892927     | 10  | 99.40 | 99.53 | 99.66 | 99.53 | 99.66 | 99.53 | 99.40 | 98.64 | 98.57 | 98.50 |
Figure 4

(A). Alignment analysis on the CDS of protein VI among HAdVs. ‘.’ represents identical bases for sequence alignment. (B). Different numbers of poly “T” and poly “A” tracts were observed in the aligned genomes of the HAdV-B55, HAdV-B11, and HAdV-B14 strains. ‘.’ and ‘-’ represent identical bases and deletions for sequence alignment, respectively.