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BRIEF REPORT

Adenovirus Type 21 Outbreak Among Lung Transplant Patients at a Large Tertiary Care Hospital

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Here we summarize an April 2016, 7-patient cluster of human adenovirus (HAdV) infections in a cardiothoracic surgery intensive care unit. We show that the patients were infected with a single HAdV21b type. Rapid HAdV typing diagnostics and effective antiviral interventions are needed for immunocompromised patients suffering from HAdV infections.

Keywords. adenovirus; epidemiology; lung transplant; outbreak.

Human adenoviruses (HAdVs) were first identified in 1953, and since then, 8 species (A–G) have been identified comprising at least 68 unique types [1, 2]. The viruses have been shown to be associated with a variety of clinical manifestations, including febrile respiratory illness, gastroenteritis, cystitis, and encephalitis [2, 3]. Because HAdV diseases are highly contagious and the virus can remain viable outside the host for several days, they have the potential to cause local outbreaks in both immune-competent and immune-deficient populations [3]. Severe HAdV infections are prevalent in patients receiving stem cell or organ transplants as a result of their suppressed immune systems [3, 4]. Lung transplantation patients are particularly susceptible to HAdV infections, which can lead to severe pneumonia and mortality [4].

When HAdV outbreaks occur in a clinical setting, it is very important to investigate so that future outbreaks might be prevented. One seeks to understand if the outbreak is due to a single clone or multiple virus strains and if the virus or viruses were initially acquired from the community or nosocomially. Among transplant or other immunosuppressed patients, one also seeks to learn if the index patient may have experienced viremia from his or her latent infection or from transplanted tissue [5].

In this report, we summarize our investigation regarding a 7-patient cluster of infections in a cardiothoracic intensive care unit at Duke University Hospital (a 950-bed tertiary care hospital in Durham, NC) during a 3-week period in April 2016.

METHODS

A case of HAdV infection in this cluster was defined as a patient admitted to the cardiothoracic surgery intensive care unit whose specimens were positive for HAdV B/E species (eSensor Respiratory Viral Panel, GenMark Diagnostics, Carlsbad, CA) over a 3-week time span in the spring of 2016. An epidemiologic investigation was conducted to identify potential common exposures among cases, including ward locations, procedures, medication, and staff. Bronchoscopes used for sample collection were identified, and their cleaning and high-level disinfection procedures were assessed. Finally, staff were interviewed to identify concerns related to adherence of infection prevention policies and environmental cleaning.

Bronchoalveolar lavage (BAL) samples were collected from all patients post-transplant. BAL samples from each patient in the outbreak had been preserved at –80°C, were de-identified, and were sent to the Duke One Health Research Laboratory for additional molecular characterization. Viral DNA was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN, Inc., Valencia, CA). Conventional polymerase chain reaction (PCR) amplification was carried out on extracted DNA using primers that target hypervariable hexon regions (nucleotide positions 18 784–19 161 compared with the complete reference genome KF577595), as previously described [6–8]. If initial PCR amplification did not result in a relatively clear band of expected molecular weight, it was considered unsuccessful. Six of the samples were unsuccessful for fiber, and 4 of the samples were unsuccessful for hexon. We then attempted to propagate an aliquot of original lavage samples in A549 cells (ATCC, Manassas, VA) for 3 to 7 days or until a cytopathic effect (CPE) was detected. From the supernatant, extracted DNA was again examined by HAdV hexon gene PCR assays. PCR products were then purified and sequenced using Sanger sequencing (Eton Bioscience, Inc., Raleigh, NC). All viral sequences were edited and assembled using Geneious R9.0.3 (Biomatters, Ltd.). A total of 6 new hexon partial genome sequences were generated from this study and deposited in GenBank (accession numbers:

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MH371128-MH371133) (Table 1). In addition, a next-generation sequencing (NGS) method was used to obtain the complete HAdV genome of the first index patient OHT-006 using the Illumina MiSeq platform (Illumina, Inc., San Diego, CA) and a Kapa Hyper prep kit with a 300-bp insert for library preparation. The quality of the NGS reads was then checked using FastQC, followed by trimming using Trimmomatic and assembling in Geneious. The novel HAdV21 genome was deposited in GenBank (accession number: MF502426).

Phylogenetic analyses were performed using hexon gene and full genome data sets. To reconstruct the hexon gene phylogeny, the final data set consisted of 176 hexon sequences, including global sequences (representing species B, D, and E) downloaded from NCBI GenBank. Maximum likelihood (ML) phylogeny was reconstructed using RAxML, v. 8.0.14, as implemented in Geneious, and branch support was assessed using 1000 nonparametric bootstrap (BS) replicates. Additionally, 2 full genome data sets of global HAdV species B (147 sequences) and HAdV21 (32 sequences) were used to reconstruct maximum likelihood phylogenies, as described above.

RESULTS

Seven patients met the HAdV infection case definition: 6 were recent lung transplant recipients, and 1 was on extracorporeal membrane oxygenation support for acute respiratory distress syndrome. Among the lung transplant patients, the median time from transplant to positive culture (range) was 3 (1–13) days. No specific medical personnel or bronchoscopes were implicated in the investigation. Six of the 7 patients had relatively few signs or symptoms and fully recovered without treatment or sequelae. One patient with symptoms was given brincidofovir therapy and also fully recovered. A number of mitigation strategies were implemented in the unit once the HAdV outbreak was identified. They included a mandatory mask policy for all staff and visitors to the unit, restriction of visitors in the unit, cohorting of infected patients, use of gowns and gloves for care of immunocompromised patients, and enhanced environmental cleaning. A single additional case (BAL specimen was not available for typing) occurred 10 days after implementation of the above measures, with no further cases occurring thereafter. The average length of stay for lung transplant patients in 2016 was 37.2 days, with length of stay during this HAdV outbreak ranging from 13 to 109 days (Table 1). The 30-day survival of all of the patients found to have HAdV infection was 100%.

Our hexon phylogeny (Supplementary Figure 1) of HAdV revealed that the cluster's novel viruses belonged to human HAdV species B, and they were clearly well-nested within the strongly supported monophyletic B21 lineage (BS ML, 99%). The hexon gene of the 7 novel viruses shared a high level of genetic similarities (nucleotide sequence identities, 97.5%–99.9%) with the global HAdV21a and HAdV21b strains that were circulating during 1978–2016 [9]. In addition, the virus strains exhibited 99.2%–100% nucleotide similarities among the 7 lung transplant patients, suggesting that the HAdV21 outbreak likely emerged from a single source, possibly from the first index case. Although we obtained only 1 complete genome from the index patient, our full genome HAdV21 phylogeny (Supplementary Figure 2) revealed that this isolate was most closely related to HAdV21b straoms (BS ML, 100%), which are co-circulating to date. The index virus also shared a high level of genetic similarities with other HAdV21b strains (nucleotide sequence identities, 99.91%–99.99%).

DISCUSSION

Among the HAdV strains associated with respiratory disease, HAdV21 infections are relatively uncommon but have sporadically caused outbreaks [1, 3]. In our 2004–2006 US national study of clinical HAdV infections from 22 laboratories, we found a relatively low prevalence of HAdV21 (2.1%) among 2189 typed HAdV specimens, an increased risk (odds ratio, 7.6; 95% confidence interval, 2.6–22.3) of severe disease compared with the more common HAdV3 infections, and an increasing trend of HAdV21 detections over time [10]. In a 2015 report, Kajon et al. reviewed the molecular epidemiology of HAdV21 strains infecting military personnel from 1997 to 2011, also documenting recent increases in detection [9].

Table 1. Characteristics of 7 HAdV-B-Positive Lung Transplant Patients During an Adenovirus Outbreak at Duke University Hospital in April 2016

			Hexon Gene	
Patients' Specimens	Day of Outbreak Adenovirus First Detected	Length of Hospital Stay, d	GenBank Accession Number	Type
OHT-006[a]	0	109	MF502426	Ad21b
OHT-004	7	—	MH371131	Ad21b
OHT-005	13	72	MH371132	Ad21b
OHT-001	19	13	MH371128	—
OHT-002	19	16	MH371129	Ad21b
OHT-007	19	109	MH371133	Ad21b
OHT-003	20	—	MH371130	Ad21b

Hexon and full genome sequence data indicated that the cluster of cases was due to a single type of HAdV-21b (also, see Supplementary Figures 1–3).

Abbreviation: HAdV, human adenovirus.

[a]Only the index case yielded a full genome sequence.

The different genotypes of HAdV21 strains may also be changing in prevalence over time. Kajon et al. used restriction enzyme digests to study 173 HAdV21s detected among US military personnel [9]. In this work, the authors identified recent clusters of what they called HAdV21a and HAdV21b strains, which differ from the prototypic HAdV21 strain (first detected in Saudi Arabia in 1956), which is now designated HAdV21p. Kajon et al. identified 8 HAdV21 variants and noted a switch in the most prevalent strains among US military personnel from HAdV21a to HAdV21b in 2007. HAdV21a genotype viruses have been reported to be associated with neuropathic and cardiopathic disease in Malaysia (1997) and severe pneumonia and deaths in Germany (2005–2013) [11, 12].

To our knowledge, this is the first HAdV21 outbreak associated with immunocompromised patients in a US cardiothoracic surgery intensive care unit. We detected HAdV21b infection in at least 7 patients within a short period of time. We showed that the HAdV21 strains from the patients were closely related to recently circulating HAdV21b strains. Although HAdV21b was detected in these patients, they did not all present with serious clinical symptoms. Other research suggests that up to 78% of lung transplant patients are asymptomatic at the time of viral detection, which explains the relatively few symptoms of disease in study patients [13].

Fortunately, effective infection control interventions likely stopped the spread of HAdV21 in this outbreak. Were such measures not conducted, HAdV strains could have spread much more widely among this large hospital's immunocompromised population. The outbreak underscores how rapid HAdV typing diagnostics and effective antiviral interventions are needed for immunocompromised patients suffering from HAdV infections.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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