**Background**

- Human adenoviruses (HAdV) are comprised of over 51 recognized serotypes, divided taxonomically into 7 species (A-G).
- Most reported respiratory illnesses associated with HAdV are caused by a limited number of types: 1, 2, and 5 in young children; 3, 4, and 7 in older children and adults.
- HAdV type 14 (HAdV14) is a species B, first identified in the 1950's and is rarely detected.
- Respiratory samples are submitted to the Wadsworth Center NYSDOH for surveillance.

**Methods**

**Samples**
- Respiratory samples were collected from patients presenting with ILI between October 2014 - May 2015.

**Laboratory Testing**
- Samples were inoculated into MDCK, prRMK, A549, and Caco-2 cells for respiratory virus screening.
- Cultures with CPE indicative of HAdV were confirmed by an IFA specific for HAdV.
- Additionally, samples were extracted on the bioMerieux easyMAG (350 µl eluted to 110 µl).
- Nucleic acid was tested for influenza virus and if the sample was collected from a patient in Tompkins County, HAdV real-time PCR was performed.
- Samples testing positive for HAdV were further serotyped by sequencing and BLAST analysis of partial hexon and fiber gene sequences.

**RFLP and WGS**
- Intracellular genomic HAdV DNA was isolated and used in both restriction enzyme analysis (REA) and whole genome sequencing (WGS).
- For WGS, library preparation was performed using the Nextera XT and sequenced using the paired-end sequencing on the Illumina MiSeq with the 500 cycle v2 kit.
- Resulting sequences were de novo assembled with SPAdes 3.5 and remapped to consensus sequences using Genious Pro 10.
- De novo assembled sequences were virtually digested (vRFLP), to determine the HAdV genotype type of the co-infected HAdV14 and HAdV4 samples.

**Phylogenetic Analysis**
- WGS for HAdV 14 were aligned and a tree created using the Maximum Likelihood method based on the Kimura 2 parameter model in MEGA 6.

**Results**

- In total 13 specimens were positive for HAdV:
  - 8-HAdV14, 2-HAdV1, 1-HAdV2, and 1-HAdV4/HAdV14 co-infection.
- Cultured isolates were obtained from 2-HAdV14 specimens and the HAdV4/14 co-infection.
  - A fourth NYS HAdV14 was identified by sequence analysis of the hexon gene.
- Two of the HAdV14 isolates were determined to be HAdV14p1 by REA.
- WGS demonstrated the NYS HAdV14 samples to be phylogenetically similar to the 2007 Lackland Airforce base isolate, and 100% identical to each other.
- The HAdV4/14 co-infection was identified as HAdV4s1 and HAdV14p1, by use of virtual REA of the de novo assembled sequences.
- All recent isolates (2006-2015) are less than 100 bp different compared to the reference strain, AY803294 de Wf (Figure 3).
- The variability between all recent HAdV14p1 isolates (2006-2015) are between 0-28 bp (Figure 3).

**Conclusion**

- During the 2014-15 influenza season, samples submitted from multiple colleges in NYS tested positive for HAdV.
- HAdV14 was identified only in specimens received from one college in Tompkins County.
- Clinicians and laboratories should be aware of HAdV as a cause of ILI and of the potential for HAdV14 to be present.
- Clinicians and laboratories should be prepared to identify cases of infection by this virus and not prescribe unnecessary antiviral therapy.
- The occurrence of respiratory HAdV infection and outbreaks are common in military recruits training centers among non-vaccinated trainees.
- The military recruit environment and college student settings are very similar.
- A major concern in the college setting is the possibility of failure to detect a virulent strain early enough for effective intervention.
- Without active surveillance for HAdV and respiratory pathogens other than influenza, outbreaks may go undetected, leading to rapid spread and subsequent difficult containment.
- The ability of WGS to separately and correctly identify the components of the mixed HAdV infection demonstrated the power of the technology and of the tools used in the analysis.

**Acknowledgements**

The authors thank Susan Core at the Lovelace Respiratory Research Institute for technical assistance, the General Virology Laboratory at the Wadsworth Center for performing the initial testing of samples and the CDC for partial funding of the surveillance network that funded collection of the original samples (CDC Cooperative agreement U01/CC028671).

**References Cited**