Journal of Clinical Virology 104 (2018) 16–22

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Molecular epidemiology of human adenovirus infections in Denmark, 2011–2016

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ARTICLE INFO

Keywords:
Human adenovirus
Molecular epidemiology
Phylogeny
Respiratory virus
Gastroenteritis virus

ABSTRACT

Background: Human adenoviruses (HAdVs) can cause respiratory tract infections, conjunctivitis, diarrhoea and outbreaks have been reported. However, little is known about the disease burden and the molecular epidemiology of HAdV.
Objectives: To retrospectively perform a molecular characterization of HAdV positive samples received at Statens Serum Institut during the period 2011–2016 and to compare this with demographic information, geographic location, sample collection date and type and co-infection with other viral pathogens.
Study design: 152 HAdV positive samples were genotyped by Sanger sequencing of a fragment of the *hexon* gene using published primers along with a newly developed primer set for enhanced genotyping of HAdV D. Phylogenetic analysis was used for genotyping and genotypes were compared with epidemiological information. In addition, HAdV burden and co-infection was evaluated for samples tested in laboratory analysis packages.
Results: Six out of seven HAdV species were identified and represented by 13 types. Young children (< 5 years old) were more likely to be positive for HAdV and co-infections with other gastrointestinal or respiratory viruses were common. Possible outbreaks of ocular infections due to HAdV D could not be confirmed.
Conclusion: A diverse set of HAdV species were circulating in Denmark in the study period and although possible transmission clusters were identified, this could not be verified with current genotyping methods Young children were commonly affected by HAdV infection and co-infections with other viral pathogens were frequent suggesting a possible underestimation of the real HAdV burden.

1. Background

Human adenoviruses (HAdVs) consist of seven different species (A to G) subdivided into 67 HAdV types [1,2]. HAdV typing is performed by PCR amplification and sequencing of a hypervariable region of the *hexon* gene, coding for one of three HAdV capsid proteins. HAdV can cause a wide range of diseases, namely febrile respiratory tract infections, conjunctivitis, diarrhoea and more rarely haemorrhagic cystitis and meningitis. Recent studies identified HAdV among 6% to 20% of hospitalised or emergency room admitted children [3–6] presenting with lower respiratory tract infections and 10% and 23% of children admitted with acute gastroenteritis [7,8]. HAdV species have been associated with different clinical manifestations [9]: gastroenteritis with HAdV species F, keratoconjunctivitis with species D and respiratory disease with species C and E. Species A and B (type 1) have been associated with gastroenteritis, respiratory infections, and keratoconjunctivitis. In particular, HAdV-B7 has been associated with severe respiratory disease [5,10].

HAdV transmission occurs either through aerosols, respiratory secretions, person-to-person contact, contaminated fomites, or through the faecal-oral route. Non-symptomatic infected individuals can keep emitting viral particles in the environment for weeks through their feces, notably species B and C [11–13]. HAdVs are non-enveloped viruses, which make them more resistant to lipid disinfectants and provide them with a prolonged capacity to survive in the environment [14,15]. All these factors contribute to the outbreak potential of HAdVs. The potential for outbreaks of respiratory disease has led to the development of a vaccine to be used among US military recruits [16,17].

Although the proportion of HAdV infections among children < 5 years hospitalised for gastroenteritis was estimated to be 11.2% in a survey conducted over a 12-month period in Denmark [7], no systematic surveillance of HAdV infections or typing exist currently in Denmark.

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https://doi.org/10.1016/j.jcv.2018.04.012
Received 3 January 2018; Received in revised form 9 April 2018; Accepted 17 April 2018

2. Objectives

Our objectives were to report demographic characteristics of HAdV cases identified in Denmark over the 2011–2016 period, to report the association between HAdV species and sample types (as a proxy for the type of infection) and to estimate the prevalence of HAdV infections in samples simultaneously analysed for a broad range of viruses in respiratory and gastrointestinal multiplex assays. The genotyping analysis of HAdV positive samples were used to: i) characterize which HAdV species and types circulated in Denmark in study period and ii) to investigate whether phylogenetic, geographical and temporal clusters occurred in the study period and whether the current methods for HAdV genotyping might be sufficient for outbreak investigations. Together, this knowledge was used as an evidence-based platform for an evaluation of the added value of a laboratory-based surveillance system for HAdV.

3. Study design

3.1. Patient samples

Feces, eye or respiratory samples received at the Statens Serum Institut for primary (real time PCR targeting F/non-F species in feces or non-feces, respectively) differential diagnosis of HAdV infections [18,19] between January 2011 and September 2016 were retrospectively selected for this study. Type of sample material, demographic information (age and sex) and geographical location were available from most samples. One sample per patient was genotyped.

3.2. Genotyping of HAdV and phylogenetic analyses

Nucleic acids were extracted from samples using the MagNA Pure LC Total Nucleic Acid Isolation Kit on the MagNa Pure 96/32 (Roche Diagnostics). A fragment of the *hexon* gene was amplified using AmpliTaq DNA (Applied Biosystems™) and the following nested-PCR primers: PCR1-F 5′-GATGCCGCAGTGGKCKTACATG-3′, PCR1-R 5′-GCTTACAAYTCNCTSGCT-3′, and PCR2-F 5′-GACGCYTCGGAGTACCTGAG-3′ [13], PCR2-R 5′- GGCYAGCACNTACTTTGACATYCG −3′. Cycling conditions were as follows for both nested PCR reactions: initial denaturation at 95 °C for 3 min followed by 40 cycles of: 20 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C. PCR products were purified using *exo*-SAP IT (GE Healthcare, Buckinghamshire, UK) prior to Sanger sequencing. Sequences were assembled in BioNumerics v7.6 (Applied Maths, Belgium) and Geneious and subsequently aligned against HAdV reference using MAFFT [20]. Phylogenetic trees were constructed in MEGA6 [21] using the Neighbor Joining method (Jukes Cantor model, using the 'partial deletion' option for handling gaps and 1000 bootstrap replicates). Species identification was based on the phylogenetic analysis.

Further subtyping of samples belonging to species D was performed with a nested-PCR with primers amplifying a larger fragment of the *hexon* gene: PCR1-D-F 5′-TACAAGGCGCGMTTCA-3′, PCR1-D-R 5′-CAGGTTGGCCTGVAGGTT-3′, PCR2-D-F 5′-ACAACCGGGTGCTAGACATGG-3′, PCR2-D-R 5′-TCVACHGCAGARTTCCACAT-3′. The following cycling conditions were used: initial denatureation at 95 °C for 3 min followed by 40 cycles of: 10 s at 95 °C, 10 s annealing at 55 °C (PCR1) or 61 °C (PCR2), 80 s at (PCR1) or 60 s (PCR2) at 72 °C (PCR1). Sequences were analysed as described above. All sequences were deposited in GenBank under the accession numbers MG199243 to MG199441.

4. Results

4.1. HAdV infection and patient sex and age

During the study period (2011-16), HAdV was identified in 664 samples out of ~13,000 clinical samples screened for HAdV. 594 HAdV positive samples out of 12934 samples (Table 1) were included in the study (no more than one sample per patient). Feces (45%) and respiratory specimen (28%) were the most common sample types. No difference in sex distribution was observed between HAdV positive and negative individuals across all sample types, except for a higher proportion of HAdV positive eye samples from males (Table 1). The median age of HAdV positive individuals was lower than that of HAdV negative individuals across the different sample types. In particular, the median age of HAdV positive patients was 1.5 years for those who had a feces specimen tested and 3.5 years old for those who had a respiratory specimen tested. Overall children < 5 years old were 5 (95% CI = 4.3-7.6) and 15 (95% CI = 7.0-33.9) times more likely to be tested positive for HAdV, in respiratory and feces samples respectively, than other individuals in this sample set.

4.2. HAdV species and types and associations with sample material type

257 samples from 257 patients were available for genotyping and a HAdV species was identified in 152 samples through phylogenetic analyses (Fig. 1).

An association between HAdV species and sample types was observed (Table 2): HAdV species D, E and B were most frequently identified from eye specimens and species C from respiratory specimens ($p < 0.001$ for all). Species F was only detected once in a respiratory sample, species G was not identified in this sample set, and species A in one respiratory sample only. As expected from the analysis of the population affected by HAdV presented above and the distribution of HAdV species among sample types, the median age of infected individuals was different ($p < 0.001$). However, it cannot be excluded that some species were not identified due to the exclusion of negative samples in the initial diagnostics (See 3.1).

4.3. HAdV prevalence in analysis packages

We then estimated the prevalence of HAdV infections and compared it to that of other viruses in our sample set. For this analysis, we limited the sample set to the samples which were tested simultaneously in laboratory analysis packages including both HAdV and a defined set of respiratory and gastrointestinal viruses. This approach was taken in order to limit potential biases (overestimation of HAdV prevalence) in samples that would have been tested only for HAdV. The sample set comprised 5858 feces samples and 1784 respiratory samples. In respiratory specimens, the package consisted of Rhinovirus, Enterovirus, Influenza, Respiratory Syncytial Virus (RSV), Parainfluenza, Metapneumovirus, Coronavirus and Parechovirus. In the feces samples, viruses included in the laboratory analysis package were Norovirus GGI and GGII, Rotavirus, Sapovirus and Astrovirus. Overall the estimated prevalence of HAdV infections in the selected sample set was low at 0.9% (n = 54) and 3.5% (n = 62) among feces and respiratory specimens, respectively. Norovirus was the most frequently detected viral pathogen in feces specimens and Rhinovirus-Enterovirus in respiratory specimens (Table 3a and b). In 16 feces samples (28%), HAdV was found together with one or more other virus (Norovirus, n = 6, Astrovirus, n = 5, Rotavirus, n = 3 and Sapovirus, n = 1), whereas samples containing HAdV alone accounted for 72%. In most HAdV positive respiratory samples (n = 38, 61%), a co-infection was found (Rhinovirus-Enterovirus, n = 27, RSV, n = 9, Coronavirus, n = 6, Parainfluenza virus, n = 3, Influenza, n = 2, Parechovirus, n = 1, Metapneumovirus, n = 1).

4.4. HAdV types circulating in Denmark in the 2011–2016 period and geographical clusters

Through analysis of the short *hexon* gene fragment, it was possible to assign 95 of 102 (93%) samples into 13 different HAdV types (Figs. 1 and 2). The frequency of each type as well as sample types screened are indicated in Table 4. Isolates belonging to species B were observed in all