

Molecular detection of human adenovirus among hospitalized patients with lower respiratory tract infection in Hospital Sungai Buloh, Malaysia

Musa, S.N.¹, Idris, S.², Lee, Y.L.³ and Sekawi, Z.^{1*}

¹Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia

²Pathology Department, Sungai Buloh Hospital, 47000 Sungai Buloh, Selangor, Malaysia

³Department of Paediatrics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author e-mail: zamberi@upm.edu.my

Received 23 September 2017; received in revised form 5 February 2018; accepted 7 February 2018

Abstract. Human adenovirus (HAdV) is one of the common pathogens that are responsible for a wide variety of infectious diseases. There are about 54 different adenovirus serotypes that are responsible for respiratory infections in humans. The prevalence of lower respiratory tract infection (LRTI) – associated with HAdV varies throughout different regions. The prevalence of HAdV in Malaysia is rarely investigated and reported despite severity of infection worldwide. This study was undertaken to identify the HAdV types associated with lower respiratory tract infection (LRTI) in Hospital Sungai Buloh, Malaysia between April 2013 until January 2014, a total of 210 specimens were collected from patients hospitalized with LRTI. Human adenovirus was detected using polymerase chain reaction (PCR). The positive products were sequenced and phylogenetic analysis of the virus was performed. Eighteen of 210 specimens (8.57%) were positive with HAdV infection. Based on the phylogenetic analysis study, HAdV-7 strains were the most common serotype with 11 cases, followed by HAdV-1, HAdV-2 and HAdV-4 with 2 cases each and one case of HAdV-5. The HAdV strains in this study were closely related to strains in Singapore and India. In this study, HAdV infection from LRTI patients in Hospital Sungai Buloh Malaysia were caused by different types of adenovirus mainly HAdV-7. This study will become a reference for further epidemiological study in this country.

INTRODUCTION

Lower respiratory tract infections (LRTI) are common infectious diseases worldwide affecting both children and adults (Caroll, 2002). Viral respiratory tract infections (RTI) remain a leading cause of illness, despite the control of many infectious diseases in this current time (Stockton *et al.*, 2002). HAdV are common pathogens that have the potential to cause opportunistic infections with significant morbidity and mortality especially immunocompromised hosts (Florescu *et al.*, 2013).

Outbreaks of acute respiratory illness, including pneumonia, caused by adenovirus serotypes 3, 4, 7, 14 and 21 are common among military recruits, and fatal outcomes have occasionally been reported in US (Kolavic-Gray *et al.*, 2002). In Malaysia, infections rate of HAdV occurs in children with prevalence range from 1% to 6% (Abd-Jamil *et al.*, 2010; Ng *et al.*, 2015). The clinical presentations of HAdV infections vary with virus serotypes, depending on age and immune status of the host. Serotypes 1–5, 7 and 21 were associated with upper respiratory infection and pneumonia, while

serotypes 11, 34 and 35 are associated with hemorrhagic cystitis and interstitial nephritis. Other HAdV such as, serotypes 40 and 41 were commonly associated with infantile gastroenteritis, while serotypes 8, 19 and 37 with epidemic keratoconjunctivitis. HAdV were found to cause problems mostly in children followed by adults (Lessler *et al.*, 2009). Data on HAdV prevalence in Southeast Asia especially Malaysia is still lacking (Ng *et al.*, 2015). Thus, this study was undertaken to identify the types of HAdV associated with lower respiratory tract infections (LRTI) in Hospital Sungai Buloh, Malaysia.

MATERIALS AND METHODS

Specimen collection

Respiratory specimens were collected from patients hospitalized with symptoms of LRTI from April 2013 to January 2014. The specimens included nasopharyngeal aspirates, tracheal aspirates or sputum. The specimens were kept in viral transport medium (VTM), [Sigma Virocult (Sigma, UK)], labeled and transported to virology laboratory in Universiti Putra Malaysia.

Specimen processing

The collected specimens were vortexed and filtered through 0.22 µm syringe filter. The filtrate was then kept in 2 ml screw-capped tube and stored in -80°C until further analysis.

Viral DNA extraction

Viral DNA was extracted using QIAamp MinElute Spin Kit (Qiagen, Germany). Extraction procedures were performed according to the manufacturer's instructions. Extracted DNA was kept in -80°C for further analysis.

PCR for Adenovirus and sequencing

HAdV hexon gene hyper-variable regions 1-6 (HVR1-6) were used for detection and typing as described by Lu and Erdman (2006). Primer set of HAdVF1 (5'-TICTTT GACATICGIGGIGTICTIGA-3') HAdVR1 (5'-CTGTCLACIGCCTGRTTCCACA-3') was used for the first PCR. The cycling parameters consisted of an initial denaturation step of

10 min at 94°C, followed by 35 cycles including denaturation at 94°C for 50 sec, annealing at 44°C for 30 sec, and elongation at 72°C for 45 sec, with a final elongation at 72°C for 10 min.

In nested PCR of HAdV, reaction was performed by using internal primers HAdVF2 (5'-GGYCCYAGYTTYAARCCCTAYTC-3') and HAdVR2 (5'-GGTTCCTGTCICCCAGAGAR TCIAGGCA-3'). The cycling parameters consisted of an initial denaturation step of 10 min at 94°C, followed by 35 cycles including of denaturation at 94°C for 50 sec, annealing at 52°C for 30 sec, and elongation at 72°C for 45 sec, with a final elongation at 72°C for 10 min.

PCR products were detected by using 1.5% (w/v) agarose gel in electrophoresis with 1X TAE buffer. QIAquick gel purification kit (QIAGEN, Germany) was used to purify the products. The purified products were sequenced with ABI PRISM 3730xl DNA sequencer (Applied Biosystem, USA), which was commercially sequenced (1st BASE, Malaysia).

Phylogenetic and data analysis

HAdV sequences were trimmed and by using ClustalW, multiple sequence alignments were performed with reference of homologous sequences that are available in GenBank. Phylogenetic tree of HAdV was constructed using neighbor-joining method with maximum likelihood in MEGA7. Data analysis was performed on demographic, clinical signs and symptoms, laboratory findings and hospitalization status descriptively.

RESULTS

A total of 210 samples were collected from April 2013 until January 2014. A total 131 (62.4%) male and 79 (37.6%) female patients were involved. Among 210 specimens, 18 (8.57%) of them were HAdV PCR positive. HAdV was detected from ten (55.6%) male and eight (44.4%) female patients. The age range of the patients was divided into seven groups respectively as shown in Table 1.

Table 1. Age group of HAdV positive samples (n=18)

Age group	Value, n (%)
≤ 1 year	6(33.3)
> 1 year to 4 years	9(50.0)
> 4 years to 7 years	1(5.6)
> 7 years to 18 years	–
> 18 years to 50 years	1(5.6)
> 50 years to 65 years	1(5.6)
> 65 years	–

Among the symptoms for LRTI, cough was the most common symptom with 16 cases (88.9%) followed by fever, 14 cases (77.8%), and breathing difficulties with 10 cases (55.6%) while wheezing being the less common symptom that appears only in one case (5.6%). Hospital diagnosis that was mainly made for HAdV PCR positive samples was pneumonia with seven cases (38.9%), followed by bronchopneumonia, bronchiolitis and viral fever with two cases (11.1%) respectively. Other diagnosis of HAdV infection was Acute Exacerbation of Bronchiole Asthma (AEBA), sepsis and viral-induced wheeze with one case (5.6%) each.

In this study, 11 samples were likely to be in HAdV-B group, five samples from HAdV-C and another two samples in HAdV-E as shown in Figure 1. Further analysis reveals that the samples in HAdV-B were HAdV-7 while the five samples from HAdV-C group were HAdV-1, HAdV-2 and HAdV-5 respectively (Figures 2 and 3). Two other samples were related to HAdV-E which probable to be HAdV-4. In this study, positive samples were compared with samples from Malaysia, Singapore, South Korea, India, China and USA. Demographically, HAdV strains in this study were most closely related with strains originated from Singapore in 2013 and one strain from India in 2013 with 11 samples. Other positive samples showed relatedness to strains from India in 2013 as shown in Figure 4.

DISCUSSION

This study showed HAdV with 8% prevalence rate. This rate is similar to previous studies conducted using the same method in Cuba and Brazil with 8% and 8.2% prevalence respectively (Belsy *et al.*, 2009; Moura *et al.*, 2007). The prevalence of HAdV infection detected by PCR worldwide range from 2–10%, depending on types of test used (Ng *et al.*, 2015; Tsou *et al.*, 2012). Result presented the mixture of HAdV serotypes; which were HAdV-B serotype 7; HAdV-C serotypes 1, 2, 5 and HAdV-E serotype 4. This finding supported by previous study that showed respiratory diseases were commonly associated with HAdV-B (serotypes 3 and 7), HAdV-C (serotypes 1, 2 and 5), and HAdV-E (serotype 4) viruses (Jin *et al.*, 2013).

The findings of this study exhibited that pneumonia was the most common diagnosis made which agrees with previous research that stated pneumonia and bronchiolitis as the most common finding (Sung *et al.*, 2011; Van Woensel *et al.*, 2003). This study demonstrated that cough was the most common symptoms followed by fever and breathing difficulties. This was supported by studies done previously in Malaysia (Yusof *et al.*, 2012) and Thailand (Naorat *et al.*, 2013) that rule out the same major symptoms that are cough, difficulties in breathing, fever and runny nose that presented HAdV LRTI.

HAdV-B specifically type 7 was the most commonly detected which are similar to other studies previously done in South Korea with HAdV-7 present in 41% of the cases (Hong *et al.*, 2001). A similar study using molecular method at the Institute of Medical Research (IMR), Malaysia with samples collected from police training center showed that HAdV-7 was responsible for the deadly outbreak which killed three trainees in 2011 (Yusof *et al.*, 2012). Geographically, the sequences of HAdV in this study were highly related to HAdV isolated from an outbreak that occurred in Singapore military camp during the same research time frame in 2013 (Ng *et al.*, 2015). The sequences in this study were also found

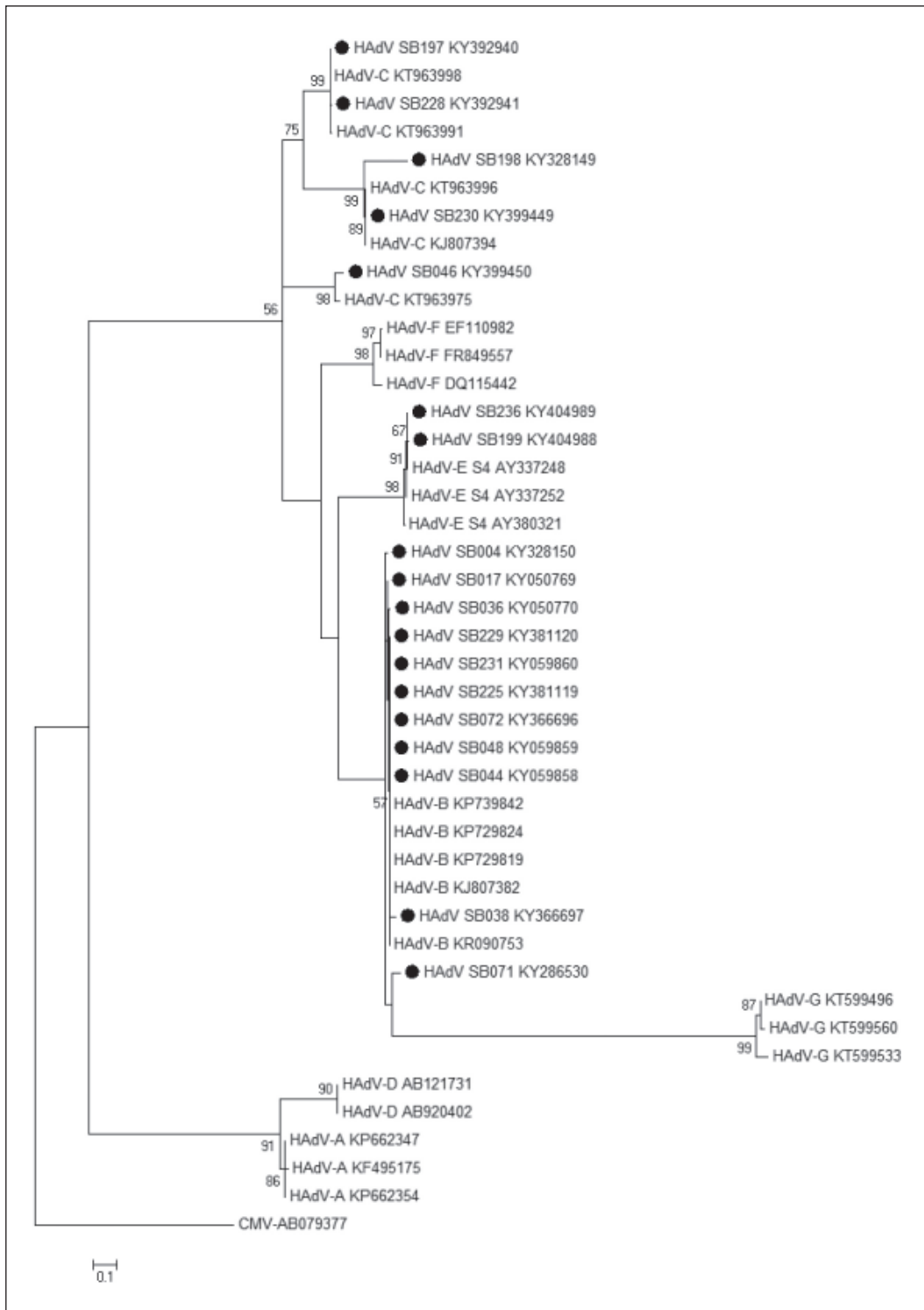


Figure 1. Phylogenetic tree of HAAdV hexon gene isolates based on its genotype group. The ● indicated HAAdV strains in this study.

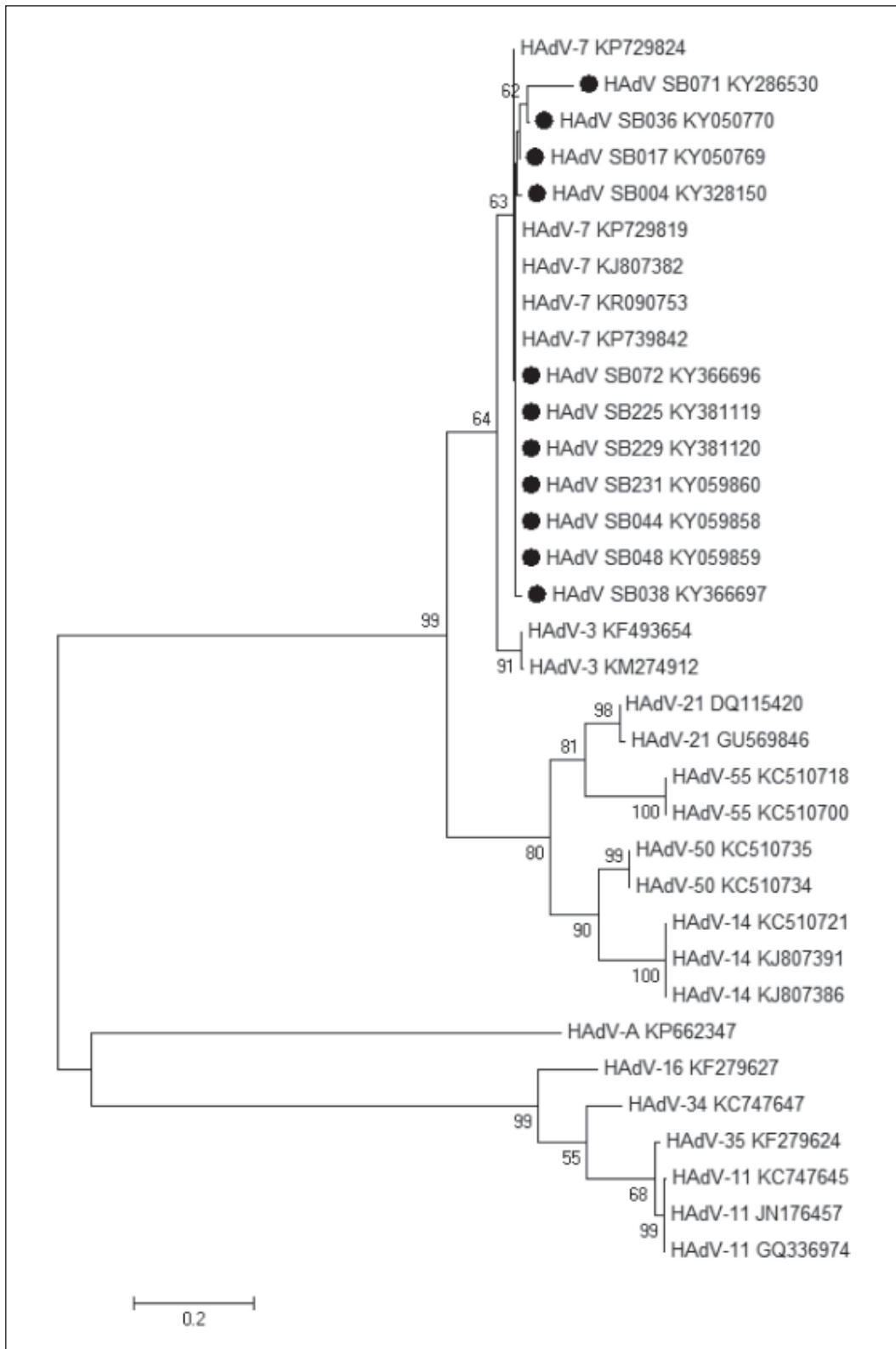


Figure 2. Phylogenetic tree of group HAAdV-B hexon gene isolates based on its strains. The ● indicated HAAdV strains in this study.

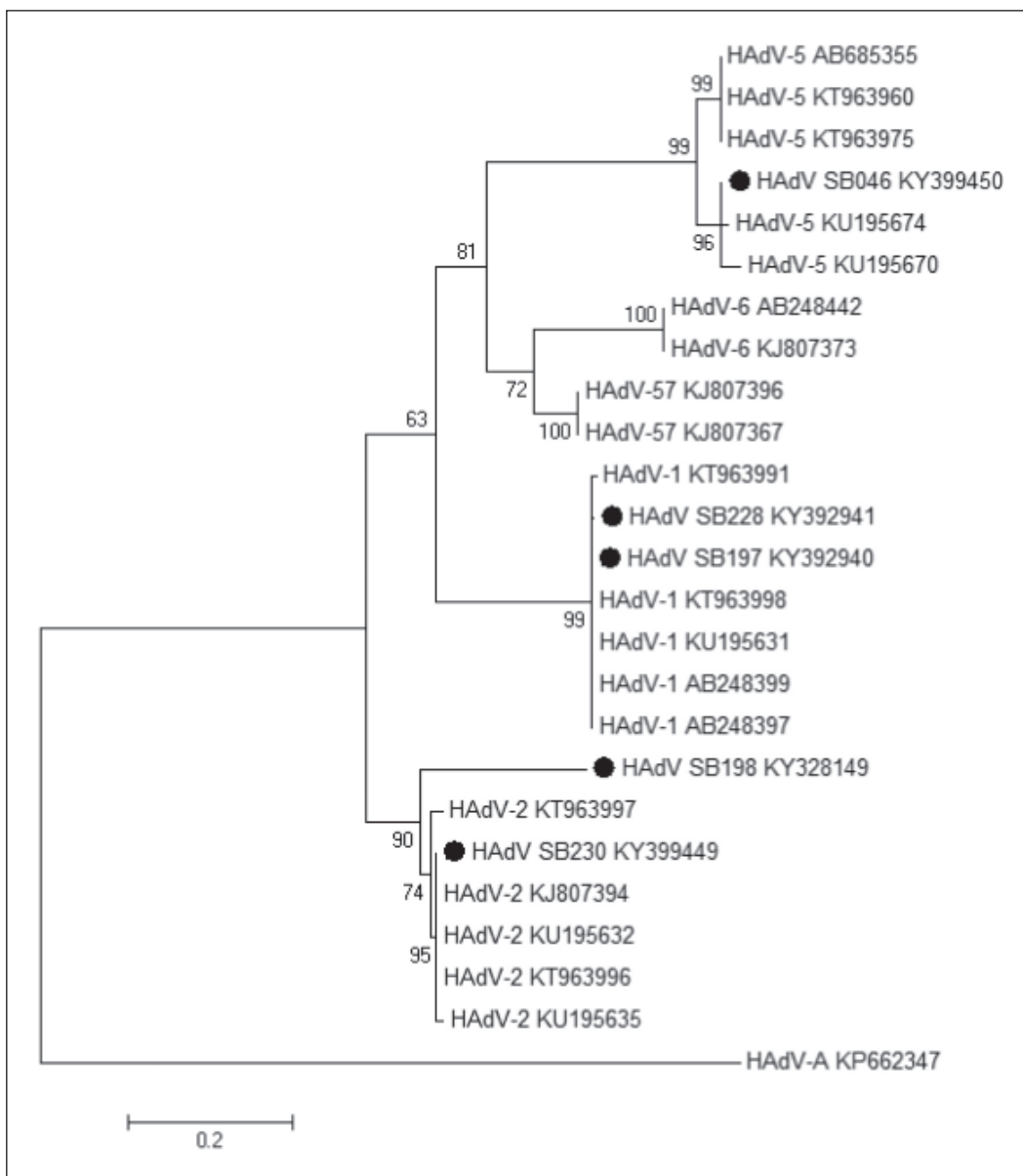


Figure 3. Phylogenetic tree of group HAAdV-C hexon gene isolates based on its strains. The ● indicated HAAdV strains in this study.

to be related with a study in Southwest India that were done during the same time interval, suggesting possible outbreak of HAAdV-7 that required in-depth future research (Akhil *et al.*, 2016).

In conclusion, this prevalence of HAAdV-related LRTI in Hospital Sungai Buloh was mainly contributed by HAAdV-7 and the strain revealed to be closely related to those found

in Singapore and India. This study adds to the limited data and provides as reference on prevalence and incidence of HAAdV in Malaysia.

Acknowledgements. We would like to thank Dr. Salmah Idris and staffs of Pathology Department, Hospital Sungai Buloh for their cooperation and help in this study.

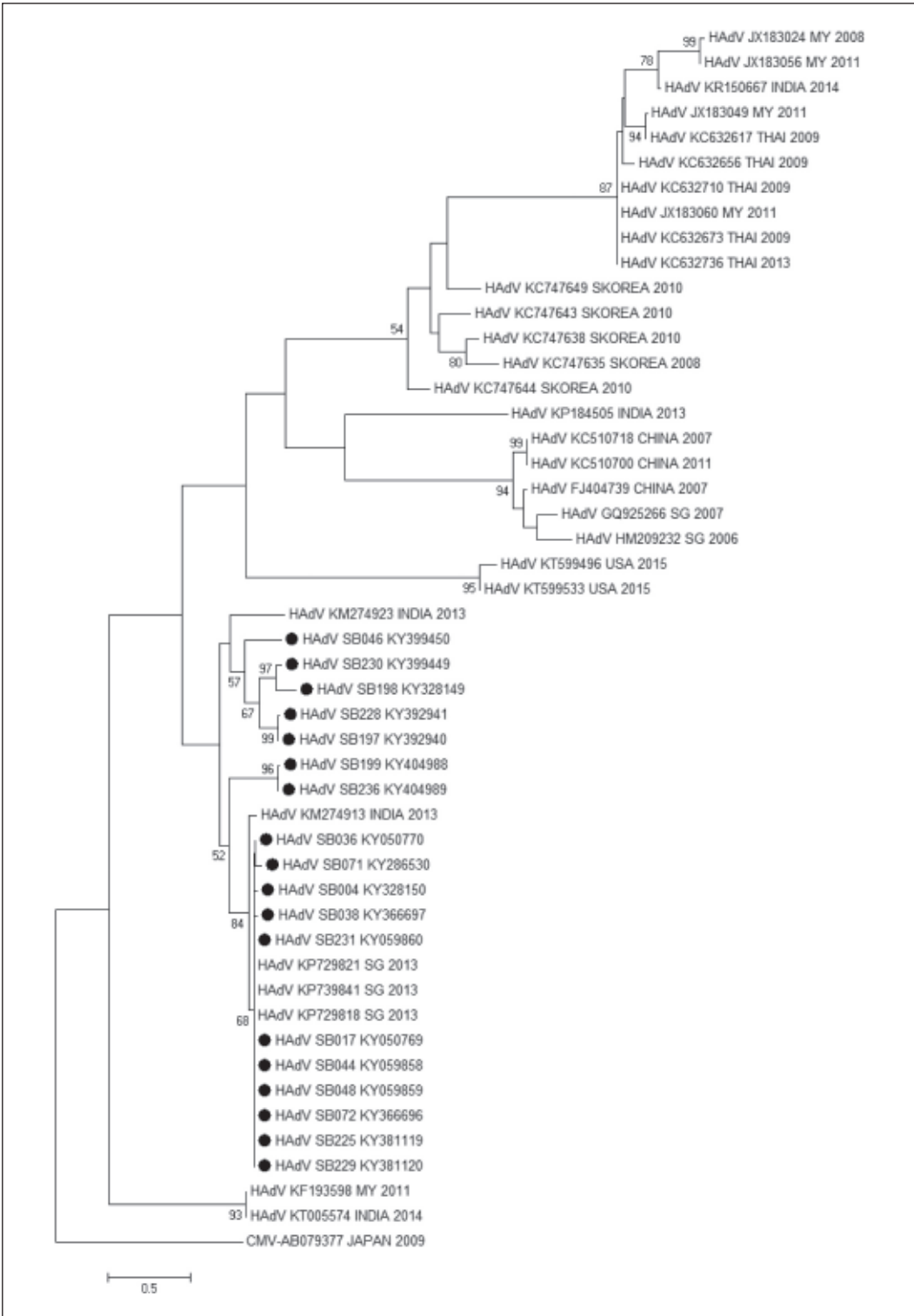


Figure 4. Phylogenetic tree of HAdV hexon gene isolates based on its demographic distribution. The • indicated HAdV strains in this study.

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