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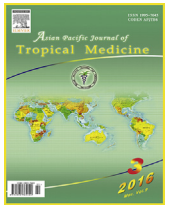

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Echovirus serotypes circulating in Malaysia from 2002 to 2013

Jeyanthi Suppiah*, T.S. Saraswathy, K. Amry, Apandi Yusof, Zainah Saat

Virology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia

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ABSTRACT

Objective: To identify the circulating serotypes of human echovirus in Malaysia from 2002 to 2013.
Methods: A total of 31 retrospective samples from non-polio acute flacid paralysis, hand-food-and-mouth disease, viral meningitis and enterovirus cases were subjected to amplification of partial *VP1* gene by RT-PCR.
Results: Sequencing and phylogenetic analysis of the partial sequences identified presence of human echovirus and human coxsackie viruses. It was found that echovirus 11 was the commonly circulating serotype followed by echovirus 6, echovirus 7, echovirus 3, echovirus 9, echovirus 30 and echovirus 1 in decreasing order. Additionally two types of human coxsackie virus isolates were detected which were coxsackie A24 and B3.
Conclusions: From the findings, there is a possibility that echovirus 11 is the predominant serotype among Malaysian patients with echovirus infection. However, a larger sample size will yield a more confident result to support this evidence.

1. Introduction

The echovirus, being the largest subgroup of Enteroviruses in the *Picornaviridae* family, consists of 32 serotypes. Echovirus is accounted for causing a huge spectrum of human diseases from asymptomatic or acute febrile illness in infants and young children to fatal encephalitis, aplastic anemia and pulmonary hypertension [1]. It is also reported to be the most common cause of aseptic meningitis [2].

Laboratory diagnosis for echovirus was initially focused on classical method which required viral isolation from cell culture of clinical specimens. However, this method has its limitation whereby not all serotypes were able to grow in cell cultures. To date, molecular assays particularly RT-PCR are being widely used [3–5] as it provided a more sensitive, rapid and accurate platform for characterization and serotyping of echovirus.

The *VP1* gene was often targeted for serotyping of non-polio enteroviruses as it codes for the major antigenic sites and most type-specific neutralization determinants [6]. Partial sequence analysis of *VP1* gene served as tool of identification of echovirus serotypes, thus discriminating it from other non-polio enteroviruses. Therefore, using this method, we report the molecular epidemiology of echovirus serotypes circulating in Malaysia from 2002 to 2013.

2. Materials and methods

2.1. Samples

A total of 31 patient samples were obtained from the Virology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia over a period of 12 years (2002–2013). Samples were selected from non-polio acute flacid paralysis cases (60.0%), hand-food-and-mouth disease cases (3.3%), viral meningitis (26.7%) and enterovirus cases (13.3%) The samples were previously confirmed as echovirus-positive by culture (86.7%) and pan-enterovirus positive (13.3%) by PCR. The sources of sample procurement include cerebrospinal fluid, stool, throat swab, rectal swab and lung fluid.

2.2. RNA isolation

Viral RNA isolation from virus-infected culture supernatant was performed using QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The isolation procedure was based on spin-column method. The final

*Corresponding author: Jeyanthi Suppiah, Virology Unit, Institute for Medical Research, Jln Pahang, 50588 Kuala Lumpur, Malaysia.
Tel: +60 326162674
E-mail: jeyanthi@imr.gov.my

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elution volume of 50 μL containing viral RNA from each sample was stored at −20 °C for long-term usage.

2.3. VP1 gene partial amplification

Isolated RNA's were subjected to RT-PCR amplification of partial *VP1* gene by using published primers [7]. All amplification reactions were carried out in a 96-well Thermal Cycler (Bio Rad, USA). PCR was undertaken at 50 °C for 30 min, 94 °C for 3 min followed by thermocycling for 35 cycles at 94 °C, 30 s; 42 °C, 30 s and 60 °C, 30 s and a final incubation at 72 °C for 5 min. PCR reaction was composed of 12.5 μL of 2× MyFi RT-PCR Mix (Bioline, USA), 1.0 μL of each oligonucleotides (10 μM), 1.0 μL of Rnase Inhibitor, 0.5 μL of RT enzyme, 5 μL of extracted RNA and 4.0 μL of sterile distilled water.

2.4. Post PCR purification and sequencing

A 15 μL aliquot of each PCR reaction was analyzed on 2% agarose by gel electrophoresis and viewed under UV illumination. The agarose was pre-stained with Red Safe Dye (Intron Biotech, Korea). The corresponding amplicons were extracted from the agarose gel and purified using Gel Extraction Kit (Qiagen, USA) according to the manufacturer's instruction. Final elution contained 35 μL of purified PCR amplicons from which 5 μL was reanalyzed on 2% agarose gel to confirm that the purification step was performed precisely. All purified PCR were

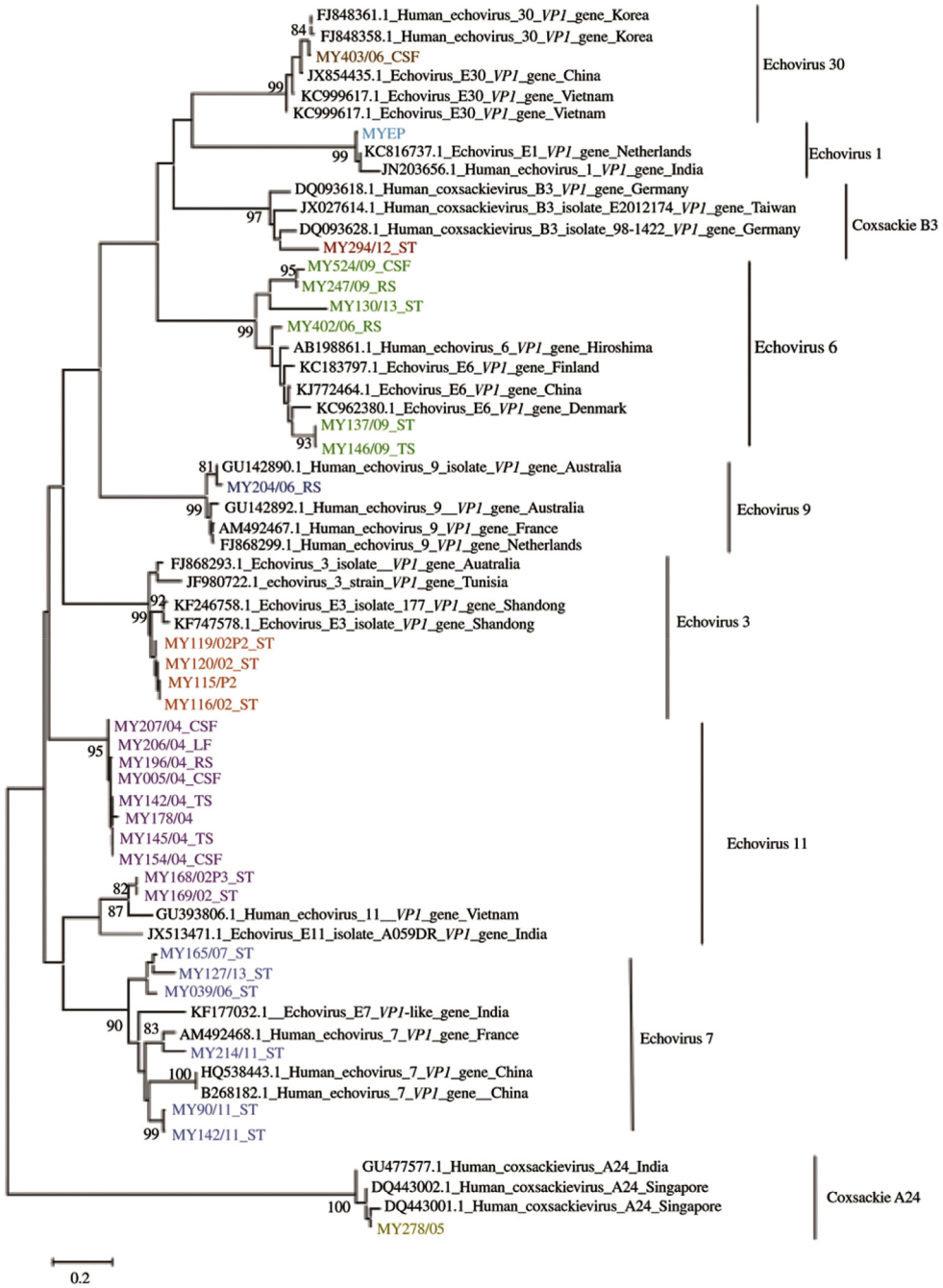

Figure 1. Phylogram of partial *VP1* sequences of human echovirus and human coxsackievirus isolates.
Malaysian isolates were labeled as MY and color coded. RS = rectal swab; ST = stool; CSF = cerebrospinal fluid; TS = throat swab; LF = lung fluid.