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Molecular epidemiology of chikungunya strains in Singapore in 2008

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Background

Strain characterization and genotyping of chikungunya virus (CHIKV) using sequencing for epidemiological study.

Methods

Blood samples were inoculated into *A. albopictus* C6/36 cells and Vero cells. Positive cultures for CHIKV were confirmed by RT-PCR targeting the gene that codes for CHIKV viral envelope protein E2. Viral RNA was extracted from the supernatant of viral infected Vero cells using QIAamp Viral RNA Mini Kit (Qiagen). RT-PCR was performed using a one-step RT-PCR kit (SuperScript™ III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase, Invitrogen). Primers 9648F, 10403R, 10145F, 11158R, 10959F and 11690R were used to amplify and sequence the E1 gene. DNA fragments were purified using QIAquick PCR Purification Kit (Qiagen) and sequenced using the same primers. The sequences obtained were aligned using ClustalX programme. The phylogenetic tree was constructed based on the 1,044-nt region within the E1 gene from codons 91-438 by neighbor-joining method with Phylip, Version 3.5 and the statistical significance estimated by bootstrap analysis using 1,000 pseudoreplicate data sets.

Results

Three CHIKV strains were successfully isolated. These samples were all collected in August 08. The molecular epidemiological study revealed that all viruses were related to East, Central, and South Africa (ECSA) phylogroup. All isolates had alanine replaced by valine at aa residue 226 (A226V) of the E1 gene. Besides the nonsynonymous mutation, these isolates possessed 2 nucleotide mutations, C300T and A363G of the E1 gene. One of the isolates showed another synonymous mutations at nucleotide position 1030 (A1030G) of the E1 gene.

Discussion

Phylogenetic analysis suggests that the circulating chikungunya strains in Singapore in August 08 belong to the genogroup ECSA, which has caused large CHIKV outbreaks in several countries worldwide and beyond the African continent, like in the Indian Ocean Islands and India during 2005-2006. C300T, A363G and C677T (A226V) found in all CHIKV isolates in Singapore were also identified in CHIKV strains isolated in Malaysia from April to December 2008. Notably, C300T was unique to CHIKV strains isolated in Malaysia. Our study suggests that the concurrent chikungunya outbreaks in Singapore and Malaysia, where two countries are of close geographical proximity, were interconnected.

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