

First Complete Genome Sequence of a Chikungunya Virus Strain Isolated from a Patient Diagnosed with Dengue Virus Infection in Malaysia

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Here, we report the complete genome sequence of a chikungunya virus coinfection strain isolated from a dengue virus serotype 2-infected patient in Malaysia. This coinfection strain was determined to be of the Asian genotype and contains a novel insertion in the *nsP3* gene.

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Chikungunya virus (CHIKV) is an alphavirus in the family of *Togaviridae* and was first isolated in Tanzania in 1952 (1). CHIKV outbreaks in Africa and Asia have been reported since the 1960s, and reemergences of CHIKV have been observed (2). Global expansion of CHIKV outbreaks has broadened due to infected travelers, with the first known autochthonous CHIKV infection case in the Western Hemisphere reported in 2013 (3). Due to global expansion and reemergences of CHIKV outbreaks, CHIKV is recognized as an emerging epidemic-prone pathogen by the public health community (4). CHIKV infection has similar clinical manifestations to a few other mosquito-borne global health threat pathogens, particularly flaviviruses, which have a similar *Aedes* mosquito vector, e.g., dengue virus (DENV) (4). Due to the similar transmitting vector and geographical distribution of outbreaks, coinfection of DENV and CHIKV has emerged as a global threat, as coinfections usually remain undiagnosed (5, 6). Although coinfections of DENV and CHIKV were reported in India, Sri Lanka, Malaysia, Gabon, and Taiwan (7-12), to date, there is no complete genome sequence of CHIKV isolated from a coinfecting patient with DENV. Here, we report the complete genome sequence of a CHIKV isolated from serum of a patient coinfecting with DENV-2, in Selangor, Malaysia, in 2009.

Strain MUM001-2009-Selangor was isolated through *in vitro* limiting-dilution-plaque purification in Vero cells. The viral RNA was extracted from the virus supernatant using GENEzol reagent (Geneaid), and the cDNA was synthesized using Moloney murine leukemia virus (M-MLV) reverse transcriptase and random primers. Second-strand synthesis was performed using the NEB 2nd-strand synthesis kit (NEB, Ipswich, MA). The purified double-stranded DNA was subsequently prepped using Nextera XT (Illumina, San Diego, CA) and sequenced on the MiSeq (2 × 150 bp setting) located at the Monash University Malaysia Genomics Facility, Selangor, Malaysia. The draft genome was assembled using IDBA_UD (13) and subsequently gap filled using a conventional primer walking strategy.

The complete genome of CHIKV consists of 11,900 nucleo-

tides (nt), containing two open reading frames (ORFs) coding for the typical structural and nonstructural proteins.

The preliminary phylogenetic analysis based on the neighbor-joining method showed that strain MUM001-2009-Selangor formed a monophyletic clustering (data not shown) with strains from the Asian genotype with close similarity (99%) strain MY/06/37348 (accession no. FN295483.3) and MY/06/37350 (accession no. FN295484.2) isolated from Malaysia. Intriguingly, a unique amino acid insertion in the *nsP3* gene was observed in this isolate in positions 376 to 451. This insertion consists of a duplicated sequence of part of the N-terminal domain of *nsP3*. Deletions of seven amino acids at this similar position starting from amino acid (aa) 376 were observed initially in a few of the Malaysian isolates, later in Indonesian isolates, and isolates currently circulating in the Western Hemisphere (14, 15). *nsP3* is involved in the negative-strand RNA synthesis, and the essential role remains enigmatic.

Accession number(s). The complete genome of coinfecting chikungunya virus, Asian genotype, has been deposited in GenBank under the accession no. **KX168429**.

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