First complete genome sequence of circulating dengue virus serotype 3 in Jeddah, Saudi Arabia

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Abstract

Here we report the first full-length genome sequence of dengue virus serotype 3 (DENV-3) from a strain isolated from a patient in Jeddah, Saudi Arabia, in 2014. The genome consists of 10 635 bp and shows close similarity to circulating genotype III isolates from Singapore, suggesting possible importation, most probably during religious pilgrimages to Saudi Arabia.

Keywords: Dengue virus, DENV, full genome, genotypes, Saudi Arabia
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Dengue virus (DENV) is an arbovirus transmitted primarily by *Aedes aegypti* and *Ae. albopictus* mosquitos. It causes a disease ranging from acute dengue fever (DF) to severe dengue haemorrhagic fever (DHF) or dengue shock syndrome [1]. Dengue is endemic in ~100 countries in tropical and subtropical regions of the world; current estimates suggest that ~40% of the world's population are at high risk of dengue infection, and there are 50–100 million annual DF cases, resulting in 500 000 DHF and 22 000 deaths (https://www.cdc.gov/dengue/epidemiology/index.html). There are four antigenically and genetically distinct DENV serotypes (DENV-1 to -4) which are further classified into different genotypes genetically.

DENV-3 has been classified into five different genotypes [2], with genotypes I, II and III being responsible for most DENV-3 infections and DHF outbreaks compared to genotypes IV and V [2–4]. DENV-3 has been circulating and causing several outbreaks in the western region of Saudi Arabia (the cities of Jeddah and Makkah) since its first isolation, in 1997 in Jeddah, until now, although with lower prevalence compared to DENV-1 and DENV-2 [5–10]. However, there has been no report of any DENV-3 full genome sequence from Saudi Arabia. Therefore, in this study, we report the first full genome of DENV-3 strain isolated from Jeddah, Saudi Arabia.

Serum and plasma samples were obtained from a patient suspected to have DF at King Abdulaziz University Hospital in 2014, with ethical approval obtained from the Unit of Biomedical Ethics (approval 19-14). The serum sample was found positive for anti-dengue IgM but not IgG antibodies, suggesting an acute primary DENV infection, which was confirmed by real-time RT-PCR using the plasma sample as previously described [11]. The full genome sequence of the DENV-3-Jeddah-2014 isolate was then obtained as previously described [12]. The sequenced length of this isolate was 10 635 bp with an open reading frame coding for 3390 aa.

Phylogenetic analyses based on full genome and complete envelope gene of this strain and isolates representing diverse geographical locations were conducted using the maximum

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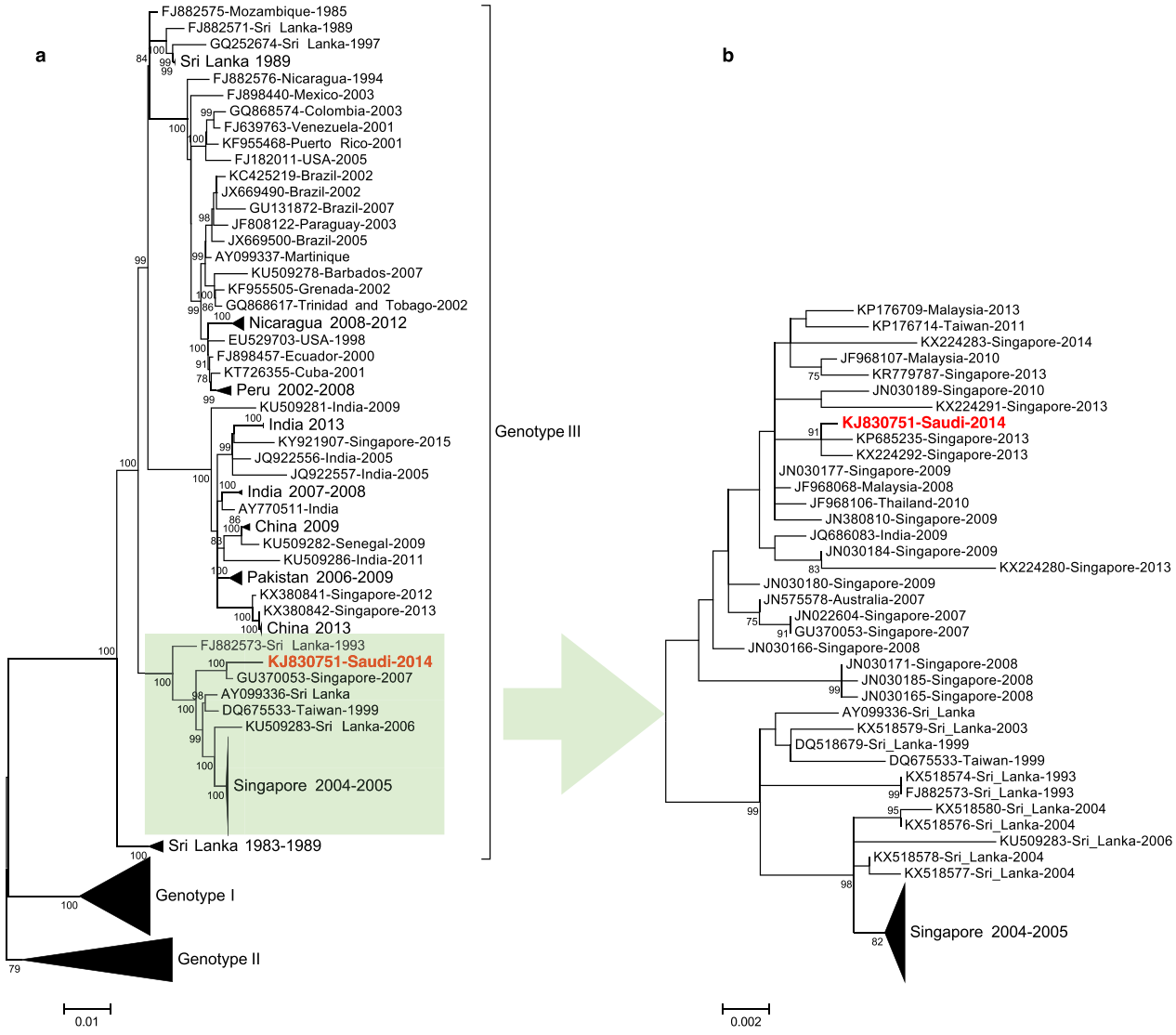

FIG. 1. Phylogenetic trees of dengue virus serotype 3 (DENV-3). (a) Phylogenetic tree based on full genome sequences of DENV-3. (b) Phylogenetic tree based on complete E gene sequences of DENV-3. Phylogenetic trees were constructed using maximum likelihood method based on best-fit model of nucleotide substitution and bootstrapping of 1000 replicates in MEGA6 software. DENV-3 from this study indicated by red box.

likelihood method in MEGA6 software [13]. Full genome analysis showed close clustering of the DENV-3-Jeddah-2014 isolate with genotype III isolates collected between 2004 and 2007 from Sri Lanka and Singapore, with highest similarity to strain GU370053, which was isolated in 2007 from Singapore (Fig. 1(a)). However, because of the temporal difference between the DENV-3-Jeddah-2014 isolate and closely related strains, as well as the limited number of available full genome sequences, a phylogenetic tree based on the envelope (E) gene was constructed using all available E gene sequences in the GenBank database within the clade of interest (Fig. 1(b)). This analysis revealed that the DENV-3-Jeddah-2014 isolate has a close relationship with recent DENV-3 strains from Singapore obtained between 2013 and 2014 (KP685235 and KX224292) (Fig. 1(b)). These results suggest that the DENV-3-Jeddah-2014 strain might have been introduced from Singapore to Jeddah, most probably during religious pilgrimages (Hajj and Umrah).

To our knowledge this is the first report of the DENV-3 full genome from Saudi Arabia. It should help in the study of the evolution of DENV-3 in the region. However, more studies and sequences are required to clearly monitor dengue importation into Saudi Arabia.

Nucleotide sequence accession number

The virus genome sequence described here has been deposited in the GenBank database as accession number KJ830751.

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Conflict of Interest

None declared.

References

[1] Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. Curr Opin Infect Dis 2006;19:429–36.
[2] Aquino VH, Amarilla AA, Alfonso HL, Batista WC, Figueiredo LT. New genotype of dengue type 3 virus circulating in Brazil and Colombia showed a close relationship to old Asian viruses. PLoS One 2009;4, e7299.
[3] Araújo JM, Nogueira RM, Schatzmayr HG, Zanotto PM, Bello G. Phylogeography and evolutionary history of dengue virus type 3. Infect Genet Evol 2009;9:716–25.
[4] King CC, Chao DY, Chien LJ, Chang GJ, Lin TH, Wu YC, et al. Comparative analysis of full genomic sequences among different genotypes of dengue virus type 3. Virol J 2008;5:63.
[5] Zaki A, Perera D, Jahan SS, Cardosa MJ. Phylogeny of dengue viruses circulating in Jeddah, Saudi Arabia: 1994 to 2006. Trop Med Int Health 2008;13:584–92.
[6] Ahmed MM. Clinical profile of dengue fever infection in King Abdul Aziz University Hospital Saudi Arabia. J Infect Dev Ctries 2010;4:503–10.
[7] Khan NA, Azhar EI, El-Fiky S, Madani HH, Abuljadial MA, Ashshi AM, et al. Clinical profile and outcome of hospitalized patients during first outbreak of dengue in Makkah, Saudi Arabia. Acta Tropica 2008;105:39–44.
[8] Organji SR, Abulreesh HH, Osman GEH. Circulation of dengue virus serotypes in the city of Makkah, Saudi Arabia, as determined by reverse transcription polymerase chain reaction. Can J Infect Dis Med Microbiol 2017;2017, 1646701.
[9] Al-Saeed MS, El-Kafrawy SA, Farraj SA, Al-Subhi TL, Othman NA, Alsultan A, et al. Phylogenetic characterization of circulating dengue and Alkhurma hemorrhagic fever viruses in western Saudi Arabia and lack of evidence of Zika virus in the region: a retrospective study, 2010–2015. J Med Virol 2017;89:1339–46.
[10] Ashshi AM. The prevalence of dengue virus serotypes in asymptomatic blood donors reveals the emergence of serotype 4 in Saudi Arabia. Virol J 2017;14:107.
[11] Drosten C, Göttig S, Schilling S, Asper M, Panning M, Schmitz H, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. J Clin Microbiol 2002;40:2323–30.
[12] Christenbury JG, Aw PP, Ong SH, Schreiber MJ, Chow A, Gubler DJ, et al. A method for full genome sequencing of all four serotypes of the dengue virus. J Virol Methods 2010;169:202–6.
[13] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30:2725–9.