



Complete Coding Sequences of Dengue Virus Type 2 Strains from Febrile Patients Seen in Malindi District Hospital, Kenya, during the 2017 Dengue Fever Outbreak

Kimita Gathii,^a Josphat N. Nyataya,^a Beth K. Mutai,^a George Awinda,^a  John N. Waitumbi^a

^aWalter Reed Project/Kenya Medical Research Institute, Basic Science Laboratory, Kisumu, Kenya

ABSTRACT We report here 10 complete polyprotein-coding sequences of dengue virus type 2 strains isolated from febrile patients who presented at Malindi District Hospital, Kenya, during a recent dengue fever outbreak. Phylogenetically, all the strains belonged to clonal serotype 2 of the Cosmopolitan genotype.

Dengue virus (DENV), a mosquito-borne flavivirus, is the etiological agent of dengue fever (DF) (1). DENV is delineated into four serotypes (DENV-1 through DENV-4) that are further graded into the following distinct genotypes: Asian I (AI), Asian II (AII), Cosmopolitan (C), American (AM), Asian/American (AA), and Sylvatic (S) (2, 3). Due to its ease of transmission and frequent occurrence, the DENV-2 genotypes are under changing evolutionary pressure, leading to the emergence of new lineages (4).

The past 5 decades have seen an unprecedented increase in the number of DF outbreaks, the majority of which have occurred in East Africa (5). In Kenya, the first documented outbreak was in 1982 in Malindi, Mombasa, and like in most outbreaks, it was due to DENV-II. Subsequent outbreaks have occurred sporadically, mostly in the northeastern and coastal regions of Kenya (5). Although multiple complete genome sequences of DENV strains are available, the African strains are underrepresented.

Ten serum samples collected from Malindi District Hospital, under an approved febrile illness surveillance protocol (WRAIR 1402/KEMRI 1282), tested positive for DENV-2 with the EasyScreen flavivirus PCR typing kit (Genetic Signatures, Australia). Total RNA was extracted from the serum samples using Direct-zol miniprep kit (Zymo Research) and used for cDNA synthesis by sequence-independent single-primer amplification (6), followed by cDNA amplification with MyTaq DNA polymerase (Bioline, MA). Sequence libraries were prepared with the Nextera XT kit (Illumina) and sequenced on the MiSeq platform (Illumina). Sequence data were assembled and annotated using the DENV-2 reference genome (NCBI reference sequence number NC001474) using CLC Genomics Workbench version 8.5.1 (Qiagen), and phylogenies were inferred in MEGA version 7.

Ten complete polyprotein-coding sequences (10,176 bp) were assembled. The open reading frames for all sequences encoded a DENV-2 polyprotein consisting of three structural proteins, capsid (100 amino acids [aa]), membrane glycoprotein precursor (166 aa), and envelope protein (495 aa), and seven nonstructural proteins, the NS1 (352 aa), NS2A (218 aa), NS2B (130 aa), NS3 (618 aa), NS4A (127 aa), NS4B (248 aa), and NS5 (900 aa). With all 10 samples, the closest polyprotein gene homologues (10,176 bp, 98% nucleotide identity) were with DENV-2 strains of the Cosmopolitan genotype (GenBank accession numbers KY427084 and JX475906, isolated from India in 2010 and 2009, respectively). Intrasequence comparison of the 10 samples using the complete polyproteins revealed few variations in nucleotides (<0.01%), 14 of which were nonsynonymous. Phylogenetically, the 10 study strains were clonally derived and clustered

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with the Cosmopolitan DENV-2 genotypes. We conclude that the 10 DENV strains are of clonal derivation. More work is required to understand the molecular differences between DENV strains that are detected routinely and those detected during outbreaks.

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Accession number(s). All sequences from this work have been deposited in GenBank under the accession numbers [MG779194](#) to [MG779203](#).

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J.N.W. designed the study. K.G., B.K.M., J.N.N., and G.A. processed the samples and performed lab experiments. K.G. processed the data and drafted the manuscript. B.K.M., J.N.N., and J.N.W. revised the manuscript. All authors read and approved the manuscript.

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