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Short communication

Zika virus complete genome from Salvador, Bahia, Brazil

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ABSTRACT

In May 2015 the first autochthonous Zika virus infection was reported in Brazil. Rapid and urgent measures are needed to contain the ongoing outbreak. Here we report the full-length ZIKV coding sequence from Bahia. Genetic analysis of outbreak sequences will be essential for characterizing the diversity of circulating strains, identifying hotspots of virus transmission and guiding public health control. Rapid and urgent measures are needed to contain the ongoing outbreak.

1. Introduction

Zika virus (ZIKV) is an emerging arthropod born Flavirus with a positive-sense, single-stranded RNA genome about 10,794-nt in length (Kuno et al., 2007). Its natural transmission cycle involves primarily *Aedes* mosquito species including *Aedes aegypti* and *Aedes albopictus*, which are commonly found in tropical and sub-tropical areas of the globe (Mourão et al., 2012). Since its first isolation in Uganda 1947, sporadic isolations have occurred from humans and a variety of mosquito species in both Africa and Asia (Duffy et al., 2009). However in 2007 a Zika fever epidemic took place in the Yap Island, Micronesia and a pediatric case of ZIKV infection was also reported in Cambodia demonstrating not only that the virus could cause outbreaks in human populations but also that its geographic distribution was expanding (Grad et al., 2014; Faye et al., 2014). In May 2015 the first autochthonous ZIKV infection was reported in Brazil. Worryingly, by January 2016, more than 17,000 suspected cases had been notified in Salvador, the capital city of the state of Bahia (Zanluca et al., 2015). To date Bahia is the most affected region with the greatest number of ZIKV notifications in America. Rapid and urgent measures are needed to contain the ongoing outbreak. Here we report the full-length ZIKV coding sequence from Bahia. Genetic analysis of outbreak sequences will be essential for characterizing the diversity of circulating strains, identifying hotspots of virus transmission and guiding public health control. Rapid and urgent measures are needed to contain the ongoing outbreak.

2. Materials and methods

On 30th June 2015, a 52 year-old previously healthy woman presented with moderate arthralgia and conjunctival hyperemia to Santa Isabel Hospital in Salvador. The following day (day 2 of symptoms) the patient developed fever (38 °C), pruritus and bilateral swelling in knees, ankles, wrists and hands. During an outpatient visit on the 1st of July 2015, the medical doctor identified a maculopapular rash on the patient's face, abdomen and thighs and blood samples were collected. Painkillers and dexchlorpheniramine were prescribed to control

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symptoms. The fever lasted only one day. Arthralgia worsened on days 3 and 4 and the patient developed an inability to walk. Improvement of symptoms occurred on day 5, with total recovery on day 6. Because the course of ZIKV infection is frequently asymptomatic (Zanluca et al., 2015) and the clinical presentation may be mistaken for dengue virus (DENV) or chikungunya virus (CHIKV) infection, both of which co-circulate in Bahia (Rodrigues Faria et al., 2016), molecular detection was undertaken using RT-PCR and the patient tested positive for ZIKV and negative for both CHIKV and DENV.

2.1. Virus isolation and RNA isolation and genome sequencing

A serum sample of the patient was subjected to virus isolation attempt in *A. albopictus* C6/36 cell cultures as previously described (Zamree et al., 2005) however no positive result was observed for ZIKV. Total RNA was isolated direct from the clinical sample (serum) by using the Qiamp Viral RNA Minikit (Qiagen), and first subject to RTqPCR for genome detection (Lanciotti et al., 2008). After confirmation of presence of ZIKV genome in the RNA, viral genomes were recovered using both Next Generation Sequencing platforms, Ion Torrent PGM (Life Technologies) and GSFLX 454 (Roche, Life Sciences) and the ion semiconducting and pyrosequencing methods, respectively.

Raw reads were combined and assembled using Mira v4.0 software and sequences were inspected for quality, coverage and confidence by the Geneious v.7 software (Kearse et al., 2012). The ZIKV genome (10,648 bp) from Salvador Bahia collected on 01 July 2015 was deposited in the GenBank, accession number KU707826.

2.2. Phylogenetic analysis

In order to identify the origin of the ZIKV genome from Salvador, we performed a maximum likelihood (ML) phylogenetic analysis using ZIKV whole genome sequences (19 sequences) published in peer-reviewed journals for which sampling year and geographic location is available. Sequences were codon aligned using Clustal X and manually edited by Bioedit as already described (Hall, 1999) and sub-genomic regions were identified.

The ML tree reconstructed from the complete coding region, generated with the HKY + I + G substitution nucleotide model by using Phyml v 3.0 (Guindon et al., 2003). The evolutionary model was chosen as the best-fitting nucleotide substitution model in accordance with the results of the hierarchical likelihood ratio test (HLRT) implemented in Modeltest software version 3.7 (Posada et al., 2004). The statistical robustness and reliability of the branching order within the phylogenetic trees was confirmed by the bootstrap analysis and considering as significant statistical support a bootstrap value >90%.

2.3. Evolutionary analysis

In order to identify if specific amino-acids were positively selected in the ZIKV viral genome, we performed a site-specific positive selection analysis.

The non-synonymous (dN) and synonymous (dS) rates (ω) per codon site was estimated by the ML approach implemented in the program HyPhy assuming a significance level of 1% ($\alpha = 0.01$) to infer the selection pressures acting on the Envelope, NS3 and NS5 gene (Pond et al., 2005) of ZIKV. In the HyPhy output, values of ω are

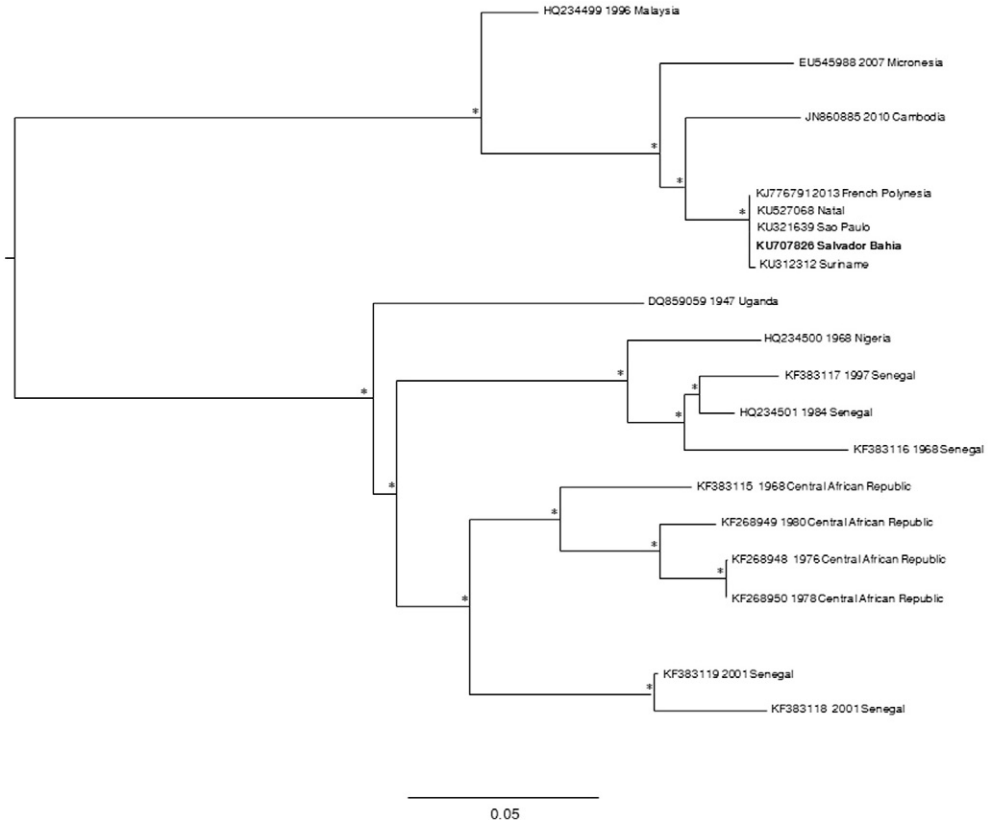

Fig. 1. Phylogenetic tree of ZIKV full-length coding sequences. Phylogenetic analysis of the complete genome sequences of Zika Virus. The tree was mid-point rooted. Scale bar is in units of nucleotide substitutions per site. Asterisks represent bootstrap values >90%. The GenBank accession number, year of isolation, and country of origin of each isolate are indicated on the tips of the tree for all strains except for those identified in 2015 and 2016. ZIKV strain from Salvador Bahia, Brazil (KU707826), was obtained in this study is highlighted in bold.