

Report of East-Central South African Chikungunya virus genotype during the 2016 outbreak in the Alagoas State, Brazil

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ABSTRACT

Chikungunya virus (CHIKV) causes a self-limiting disease characterized by the onset of fever, skin rash and persistent arthralgia. In the last decade, it has emerged as a serious public health problem causing several outbreaks around the world. Here, we report the CHIKV genotype characterization during the 2016 CHIKV outbreak in Alagoas State, Brazil. Partial E1 sequence from CHIKV-positive samples coming from different cities of Alagoas were submitted to DNA sequencing followed by phylogenetic analysis thus characterizing the virus genotype. The circulating CHIKV virus in Alagoas during 2016 outbreak belongs to the East-Central South African genotype. In this way, virus genotyping to monitoring the spread of CHIKV is needed to continued surveillance supporting the development of prevention strategies, mainly in endemic areas of mosquitoes and arboviruses co-circulation.

KEYWORDS: Chikungunya virus. ECSA genotype. Phylogenetic analysis. Brazil.

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Chikungunya virus (CHIKV) is an emergent arthropod-borne virus belonging to the *Togaviridae* family transmitted mainly by mosquitoes of *Aedes* genus which was first isolated in the 1950s during an outbreak in the Tanzania^{1,2}. This arbovirus causes a self-limiting disease known as chikungunya fever (CHIKF) characterized by the onset of fever, skin rash and persistent severe arthralgia. In the last decade, CHIKF has been re-emerged as a serious public health problem causing several outbreaks around the world³⁻⁹. Nowadays, CHIKV genotypes have been classified as Asian, East-Central South African (ECSA) or West African according to genomic comparison with phylogenetic analysis from original reported regions^{10,11}. The first case of virus autochthonous transmission in the Americas was identified in Caribbean island of Saint Martin in 2013, and the CHIKV Asian genotype was detected in that outbreak¹². The first ECSA genotype infection in the Americas was reported in 2014 in the northeast Brazil (Bahia State)¹³. Interestingly, the same CHIKV genotype was detected in the *Ae. aegypti* mosquito two years later in Sergipe State, concurring with an increase of CHIKF cases¹⁴. In this scenario, there was a large concern about the outbreak reported in Alagoas State in 2016, which is bordered by both States, Sergipe and Bahia. In Brazil, two different CHIKV genotypes were notified in different regions (Asian genotype in the north and southeast and ECSA genotype in the northeast), emerging the necessity of continuous virus surveillance, mainly in areas of high risk which have the endemic vector as well as co-circulation of Dengue and Zika virus¹⁵. In the following two years a huge increase in the number of CHIKF was reported in Brazil, reaching 272 thousand probable cases in 2016 and 196 deaths were associated with this infection. The northeast

region of Brazil had the major number of reported cases, with more than 86% of total notifications and an incidence rate of 415.7 cases/100,000 inhabitants (55.7% positive cases) according to the epidemiological report from the Brazilian Ministry of Health¹⁵. However, the role of distinct genotypes in the disease severity, prognosis and chronicity are still not well understood. In addition, it is known that the identification of mutations in CHIKV has impact in epidemiological surveillance related to virus infectivity and vector specificity¹⁶.

In 2016, an increase incidence of CHIKF was reported in Brazil, mainly in the northeast region. A huge increase in the number of cases was reported in the Alagoas State in the same year, with the incidence rising from 41.5 in the previous year to 514.8 cases/100,000 inhabitants, according to the epidemiological report of the Brazilian Ministry of Health. In this context, the objective of this study was to identify the potential CHIKV genotype during the 2016 outbreak in the Alagoas State, northeast Brazil.

This study was approved by the CNS (National Council of Health of Brazil) resolution N° 466/12 from the Universidade Federal de Alagoas Research Ethical Committee (CEP C.A.A.E. 59229716.9.0000.5013).

Patients' blood samples with suspicion of CHIKV infection during 2016 were collected at several public health centers in the Alagoas State, Brazil, and then tested in the Laboratório Central de Saúde Pública de Alagoas (LACEN/AL) for genome virus detection by quantitative reverse transcription polymerase chain reaction (qRT-PCR) using the specific primer set for the nonstructural protein NSP4, as previously described¹⁷. In this way, 496 CHIKV-positive samples were detected from a total of 791 samples tested, reaching an infection rate of 62.7%. The peak of CHIKV positive samples occurred during the rainy months (Figure 1A), which increases the mosquito population and virus transmission rate. In this present study, eleven positive samples collected from February to August 2016 during the CHIKF outbreak in different cities of the Alagoas State (Figure 1B) were submitted to phylogenetic analysis.

The viral RNA was extracted from serum of positive samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The reverse transcription was done with ImProm-II Reverse Transcription System (Promega, Madison, USA) using the specific primer for the E1 region of CHIK/E1-C (5'-GCCTTTGTACACCACGATT) according the manufacturer's recommendations. Then, cDNA was PCR amplified with Pfu DNA Polymerase (Promega, Madison, USA) and both primers: CHIK/E1-S (5'-TACCCATTCATGTGGGGC) and CHIK/E1-C (5'-GCCTTTGTACACCACGATT)¹⁸. PCR amplicon of

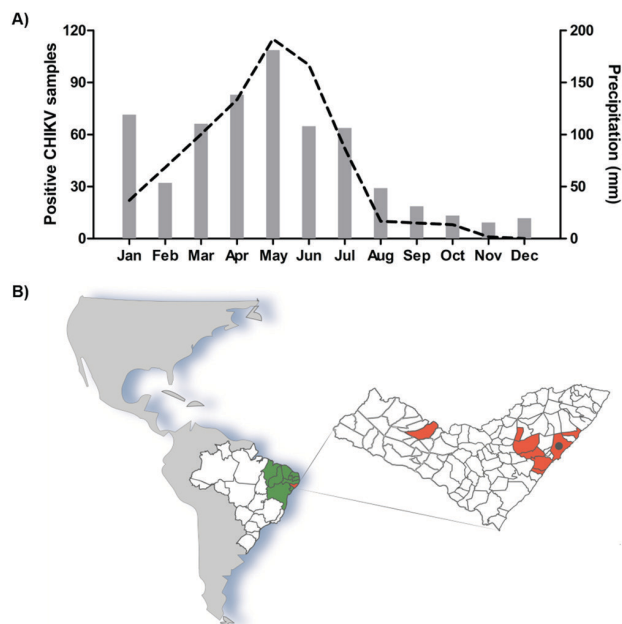


Figure 1 - CHIKV positive samples during the 2016 outbreak in the Alagoas State. A) Number of CHIKV positive samples detected by qRT-PCR at the LACEN/AL (left axis) and rainfall precipitation of Alagoas State (right axis) from January to December 2016; B) Alagoas State map located in the northeast of Brazil, showing cities with positive CHIKV samples used in the genotype identification (n=11) colored in red. The capital city Maceio is represented by the black dot

294 bp was purified using the High Pure PCR Product Purification Kit (Roche Diagnostics, Indianapolis, USA) and DNA sequencing reactions were performed in both directions using the BigDye Terminator Cycle Sequencing Ready Reaction on an ABI-Prism 3500 Genetic Analyzer (both from Applied Biosystems, California, USA). For the phylogenetic tree based on the E1 partial sequence, the evolutionary history was inferred using the UPGMA method with a bootstrap of 500 replications and the evolutionary distances were computed using the Kimura 2-parameter method in MEGA7 software. All CHIKV E1 partial genome sequences identified in this study were deposited in NCBI GenBank: accession numbers from MF589165 to MF589175.

Eleven CHIKV-positive samples used for molecular characterization comprised eight Alagoas cities (Figure 1B) and were grouped into ECSA genotype clade in the phylogenetic analysis (Figure 2). Interestingly, the nucleotide comparative alignment shows that Alagoas samples were identical to ECSA strains reported in Bahia (2014) and Sergipe (2016)^{13,14}, which border Alagoas State.

To evaluate the sequence identity among samples obtained from different locations in the Alagoas State, a nucleotide alignment was performed comparing these sequences with the first CHIKV strain isolated in Tanzania

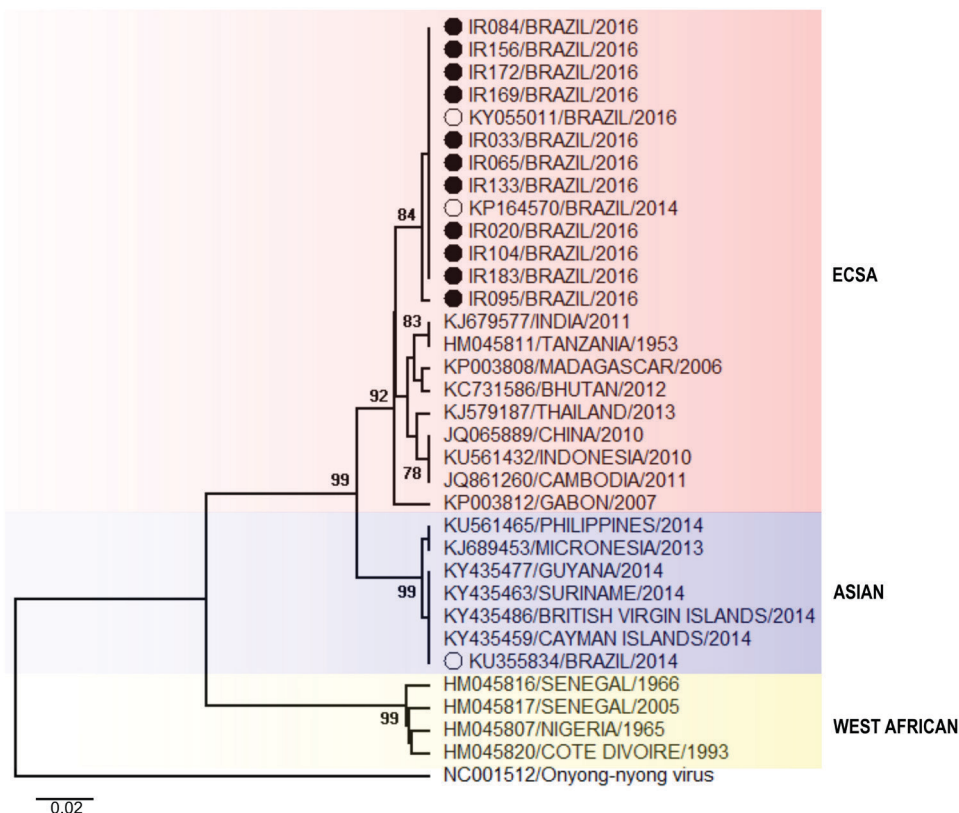


Figure 2 - Phylogenetic analysis of CHIKV genotype circulating during the 2016 outbreak in the Alagoas State. The CHIKV sequences from Alagoas analyzed are represented by black circles (n=11). CHIKV strains previously detected in Brazil are marked by blank circles and all sequences used in the analysis were named with GenBank accession number/Country/Year. The O'nyong nyong virus was used as outgroup

in 1953 and other CHIKV strains from different countries worldwide deposited in GenBank in the last years (Figure 3). As shown in Figure 3, the partial E1 gene sequence of Alagoas CHIKV are identical to each other, except for the single nucleotide substitution at position 10477 (T>A), present in the IR095 sample (MF589169). Interestingly, several nucleotide mutations could be detected in all samples compared to the Tanzania strain (10249C>A, 10297T>C and 10420A>T). Although several nucleotide mutations were detected, no amino acid substitutions were observed in the E1 region analyzed in the present study.

In conclusion, this study reports the ECSA genotype during the 2016 CHIKV outbreak in the Alagoas State, Brazil, highlighting the need for monitoring the spread of different genotypes of this emergent virus in the Americas.

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CONFLICT OF INTERESTS

None.

AUTHORS' CONTRIBUTIONS

ELLT, ISBT, LA and EJB designed the study, analyzed the data and wrote the manuscript. ELLT, ISBT and ECS performed the experiments. JM and MCL processed the samples by qRT-PCR. EJB and AAB were responsible for funding acquisition. All authors reviewed the manuscript.



Figure 3 - Nucleotide alignment of Alagoas CHIKV partial E1 sequences compared to CHIKV strains from several worldwide countries. Nucleotide sequences from Alagoas CHIKV samples (black circles) were compared to ECSA genotypes from different outbreaks previously reported in the world and the first CHIKV strain isolated from Tanzania in 1953. The Brazilian CHIKV sequences previously reported in the Sergipe, Bahia and Rio de Janeiro States outbreaks are marked with blank circles

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