ORINOQUIA
REVISTA
PUBLICACIÓN DE LA DIRECCIÓN GENERAL DE INVESTIGACIONES • ISSN: 0121-3709 (IMPRESA) • ISSN(E): 2011-2629 • VOL. 27 NÚMERO 2 • AÑO 2023

Genetic characterization of the complete genome of a strain of Chikungunya virus circulating in Brazil

Caracterización genética del genoma completo de una cepa del virus Chikungunya circulante en Brasil

Caracterização genética do genoma completo de uma cepa do vírus Chikungunya que circula no Brasil

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Artículo de investigación
Recibido: 26 de septiembre de 2023
Aceptado: 18 de noviembre de 2023

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ABSTRACT

The Chikungunya virus (CHIKV) is a single-stranded positive-sense RNA virus that belongs to the Alphavirus genus of the Togaviridae family. It is primarily transmitted by Aedes aegypti and albopictus mosquitoes. Its genome encodes four non-structural proteins (NSP 1-4) and three structural proteins (C, E1, and E2). Four lineages of this virus have been identified, namely the West African, East African, Central and South African (ECSA), Asian (AL), and Indian Ocean Lineages (IOL). CHIKV is an endemic arbovirus circulating in 51 countries in the Americas. Clinical manifestations attributed to it include high fever, rash, myalgia, and episodes of arthralgia, which subsequently lead to chronic pain and disability, especially in the joints. Sequencing the complete genome of the Chikungunya virus is essential to understand its biology, evolution, and spread and to develop effective strategies for prevention, diagnosis, and treatment. This information is crucial for combating the disease and minimizing its impact on public health. For these reasons, the complete genome of the Chikungunya virus strain br33, identified in the northeastern city of Recife, in the state of Pernambuco, Brazil, was sequenced. The genome has a size of 11,601 nucleotides and

Como Citar (Norma Vancouver): Villarreal-Julio RG, Yepes-Blandón JA. Genetic characterization of the complete genome of a strain of Chikungunya virus circulating in Brazil. Orinoquia, 2023;27(2):e-789 https://doi.org/10.22579/20112629.789

contains coding regions for two polyproteins. A phylogenetic analysis indicates that the recent Brazilian strain of CHIKV belongs to the East, Central, and South African lineage (ECSA). This phylogenetic identification is important because this particular genotype has been associated with greater damage and clinical severity. Until 2016, the CHIKV virus was directly associated with travel, and its transmission was limited. Subsequently, the largest outbreak occurred in the state associated with the introduction of a new ECSA lineage, as identified in this study. It is highly likely that new CHIKV outbreaks will occur in the near future due to the abundance of competent vectors in Brazil and a susceptible population, exposing more than 11 million inhabitants to an increasing risk of infection.

Keywords: Emerging alphaviruses, Chikungunya fever, Genome, Sequencing

RESUMEN

El virus Chikungunya (CHIKV) es un virus de ARN monocatenario de sentido positivo que pertenece al género Alphavirus de la familia Togaviridae. Se transmite principalmente por mosquitos *Aedes aegypti* y *albopictus*. Su genoma codifica cuatro proteínas no estructurales (NSP 1-4) y tres proteínas estructurales (C, E1 y E2). Se han identificado cuatro linajes de este virus que son los linajes de África occidental, África oriental, central y sudafricana (ECSA), asiático (AL) y del océano Índico (IOL).1. CHIKV es un arbovirus endémico circulante en 51 países de las Américas. Las manifestaciones clínicas que se le atribuyen son; fiebre alta, erupción cutánea, mialgia y episodios de artralgia, que en consecuencia provocan dolor crónico y discapacidad, especialmente en articulaciones. La secuenciación del genoma completo del virus del Chikungunya es esencial para comprender su biología, evolución y propagación, y para desarrollar estrategias efectivas de prevención, diagnóstico y tratamiento. Esta información es fundamental para combatir la enfermedad y minimizar su impacto en la salud pública. Por esas razones se secuenció el genoma completo del virus Chikungunya br33, identificada en la ciudad nororiental de Recife, en el estado de Pernambuco, Brasil. El genoma tiene un tamaño de 11601 nucleótidos y fragmentos que codifican para dos poliproteínas.

Se realizó un análisis filogénico que indica que la reciente cepa brasileña del CHIKV pertenece al linaje del este, centro y sur de África (ECSA). Dicha identificación filogenética es importante porque este genotipo en particular ha sido asociado a mayor daño y severidad clínica.

Hasta 2016, el virus CHIKV estaba asociadas directamente a viajes y la transmisión era limitada. Posteriormente se produjo el brote más grande en el estado asociado con la introducción de un nuevo linaje ECSA como el indetificado en este estudio. Es muy probable que se produzcan nuevos brotes de CHIKV en un futuro cercano debido a la abundancia de vectores competentes en brazil y a una población susceptible, exponiendo a más de 11 millones de habitantes a un riesgo de infección cada vez mayor.

Palabras clave: Alfavirus emergentes, Fiebre Chikungunya, Genoma, Secuenciación

RESUMO

O vírus Chikungunya (CHIKV) é um vírus de RNA de sentido positivo de cadeia simples que pertence ao gênero Alphavirus da família Togaviridae. É transmitido principalmente por mosquitos Aedes aegypti e albopictus. Seu genoma codifica quatro proteínas não estruturais (NSP 1-4) e três proteínas estruturais (C, E1 e E2). Quatro linhagens desse vírus foram identificadas, a saber: as linhagens da África Ocidental, da África Oriental, da África Central e Sul (ECSA), Asiática (AL) e do Oceano Índico (IOL). O CHIKV é um arbovírus endêmico que circula em 51 países das Américas. As manifestações clínicas atribuídas a ele incluem febre alta, erupção cutânea, mialgia e episódios de artralgia, que, subsequentemente, levam a dores crônicas e incapacidade, especialmente nas articulações. A sequenciação completa do genoma do vírus Chikungunya é essencial para compreender sua biologia, evolução e propagação, além de desenvolver estratégias eficazes de prevenção, diagnóstico e tratamento. Essas informações são cruciais para combater a doença e minimizar seu impacto na saúde pública. Por essas razões, o genoma completo da cepa do vírus Chikungunya br33, identificada na cidade nordestina de Recife, no estado de Pernambuco, Brasil, foi sequenciado. O genoma tem um tamanho de 11.601 nucleotídeos e contém regiões de codificação para duas poliproteínas. Uma análise filogenética indica que a recente cepa brasileira do CHIKV pertence à linhagem da África Oriental, Central e Sul (ECSA). Essa identificação filogenética é importante porque esse genótipo em particular tem sido associado a maiores danos e gravidade clínica. Até 2016, o vírus CHIKV estava diretamente associado a viagens, e sua transmissão era limitada. Posteriormente, ocorreu o maior surto no estado associado à introdução de uma nova linhagem ECSA, como identificada neste estudo. É altamente provável que novos surtos de CHIKV ocorram em um futuro próximo devido à abundância de vetores competentes no Brasil e a uma população suscetível, expondo mais de 11 milhões de habitantes a um risco crescente de infecção.

Palavras chave: Vírus alfa emergentes, febre de Chikungunya, genoma, sequenciamento

INTRODUCTION

Chikungunya virus (CHIKV) is an alphavirus that produces a reemerging infection transmitted to humans by the mosquito vectors *Aedes aegypti* and *Ae. Albopictus* (Carrillo et al., 2023; Nunes et al., 2015)*an outbreak of Chikungunya virus (CHIKV*. CHIKV infection is a significant public health issue in tropical and subtropical regions. An infection by CHIKV is characterized by an acute fever, rash, and arthralgia, most commonly known as joint pain, often accompanied by headache, swelling of the joints, and conjunctivitis (Hakim & Aman, 2022; Nunes et al., 2015).

As far as we know, three different CHIKV phylogenetic groups with differing antigenic properties have been identified: the East, Central, and South African (ECSA) genotypes. The first cases of autochthonous CHIKV in Brazil were confirmed in the city of Oiapoque, in the state of Amapá, back in September 2014 Where two genotypes of CHIKV were identified, ECSA and Asian (Alguridi et al., 2023; Nunes et al., 2015; Volk et al., 2010)

In this document, we present the complete genomic sequence of the BR33 genotype ECSA isolated on March 3, 2016, from a pregnant patient in Brazil, in the city of Recife, state of Pernambuco, initially suspected of being infected with the Zika

virus. The patient was initially diagnosed with CHIKV through PCR via reverse transcription (RT-PCR), amplifying a section of the gene E1 designed and standardized by the Gehrke Laboratory at the Massachusetts Institute of Technology (MIT).

METHODS

The CHIKV strain was propagated in Vero-E6 cells (Vero) that were maintained in minimal essential medium (MEM) (Sigma), supplemented with 5% to 10% fetal bovine serum and antibiotics at 37° in 5% CO2. C6/36 cells were maintained in Leibovitz's L-15 medium (Invitrogen, Carlsbad, California, USA) supplemented with 10% fetal bovine serum, antibiotics, and 1% TPB (Sigma, St. Louis, Missouri, USA) at 32°C. collected 7 days after infection or after displaying cytopathogenic effect (Ang et al., 2016; Miller et al., 2018). All experiments were carried out in a biosafety level 3 laboratory. RNA extraction was performed using QIAamp Viral RNA mini kit from Qiagen, following instructional directions. Novo sequencing of the entire genome was performed in an Illumina HiSeq 2500 system (Conteville et al., 2016; Xf et al., 2023) using the Trinity RNA seq assembly method (Haas et al., 2013; Hartline et al., 2023), and version r2013-02-25. Sequencing and assembly were undertaken at the Massachusetts Institute of Technology (MIT) facilities.

Characterization was performed using the ViPR database (www.viprbrc.org). The function was generated by the InterPro program (http://www.ebi.ac.uk/interpro/protein/A0A192GR82). Phylogenetic analysis was carried out using the software Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 (7-17) with the neighbor-joining tree method.

RESULTS

The complete genome sequence was called Chikungunya virus isolate BR33, and it was submitted to the NCBI GenBank, with the accession number: KX228391.1.

The genome has a size of 11 601 nucleotides, and two open reading frames that encode for two polyprotein precursors evidenced, these are the structural and non-structural polyprotein of Chikungunya. The first is a nonstructural polyprotein ranging from nucleotide position 80 to 7504. The product is called CHIKVgp1, and its ID is ANK58564.1 (Fig 1). The second structural polyprotein ranges from nucleotide position 7570 to 11 316, the product is called CHIKVgp2, and its ID is ANK58565.1 (Table 1). Functional and structural characteristics are described in detail (Fig 1-4). In addition, the phylogenetic classification of Chikungunya virus isolate BR33 was performed (Fig 5).

Table 1. Physical and functional characteristics of annotation and search of CHIKV BR33.

a.

General Info	
Genome ID	371.244.891
Genome Name	Chikungunya virus BR33
Taxonomy Info	
Taxon ID	37124
Superkingdom	Viruses
Kingdom	Orthornavirae
Phylum	Kitrinoviricota
Class	Alsuviricetes
Order	Martellivirales
Family	Togaviridae
Genus	Alphavirus
Species	Chikungunya virus
Status	
Genome Status	Complete
Type Info	
Strain	BR33
Database Cross Reference	
Completion Date	6/20/2016
Genbank Accessions	KX228391
Sequence Info	
Sequencing Platform	Illumina
Assembly Method	trinityrnaseq v. r2013-02-25
Genome Statistics	
Chromosomes	1
Contigs	1
Genome Length	11601
GC Content	5.027.153
Contig L50	1
Contig N50	11601

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DOI: https://doi.org/ 10.22579/20112629.789

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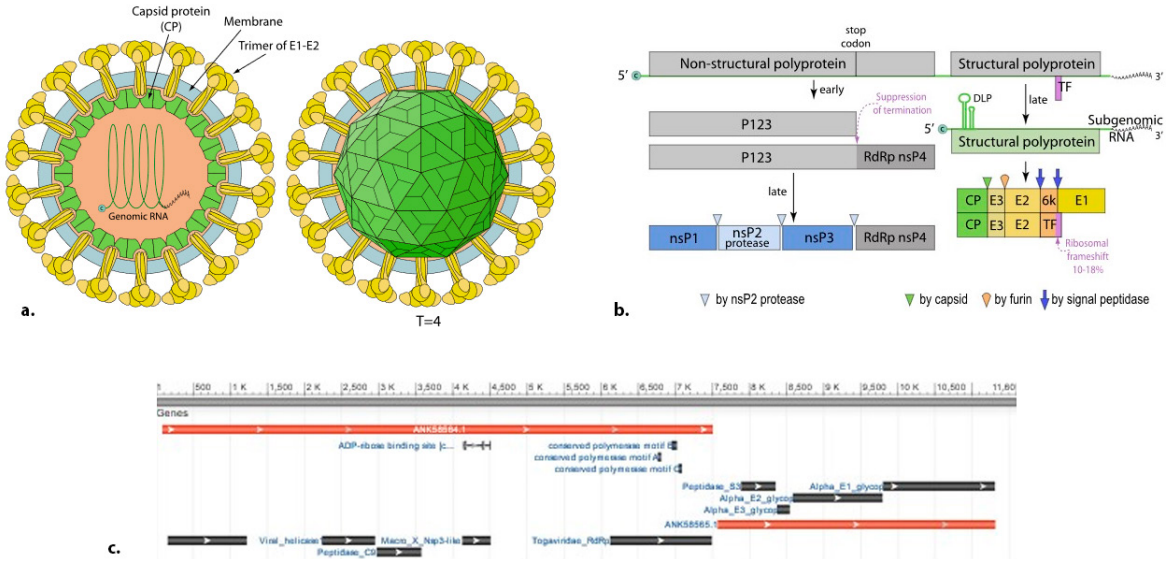
Annotation Statistics	
CDS	2
CDS Ratio	0.17239892
Genome Quality	
	None available
Isolate Info	
Collection Date	3/03/2016
Collection Year	2016
Isolation Country	Brazil
Geographic Group	South America
Geographic Location	Brazil: Pernambuco
Host Info	
Host Name	Homo sapiens
Host Common Name	Human
Host Group	Human

b.

Source	CDS Region in Nucleotide	Protein	Name	Organism
INSDC	KX228391.1 80-7504 (+)	ANK58564.1	CHIKVgp1	Chikungunya virus
INSDC	KY704954.1 75-7499 (+)	ASM47579.1	nonstructural polyprotein	Chikungunya virus
INSDC	MH000700.1 57-7481 (+)	QBM78314.1	nonstructural protein	Chikungunya virus
INSDC	MH000703.1 80-7504 (+)	QBM78318.1	nonstructural protein	Chikungunya virus
INSDC	MH000704.1 80-7504 (+)	QBM78320.1	nonstructural protein	Chikungunya virus
INSDC	MH000705.1 80-7504 (+)	QBM78322.1	nonstructural protein	Chikungunya virus
INSDC	MH000706.1 19-7443 (+)	QBM78324.1	nonstructural protein	Chikungunya virus

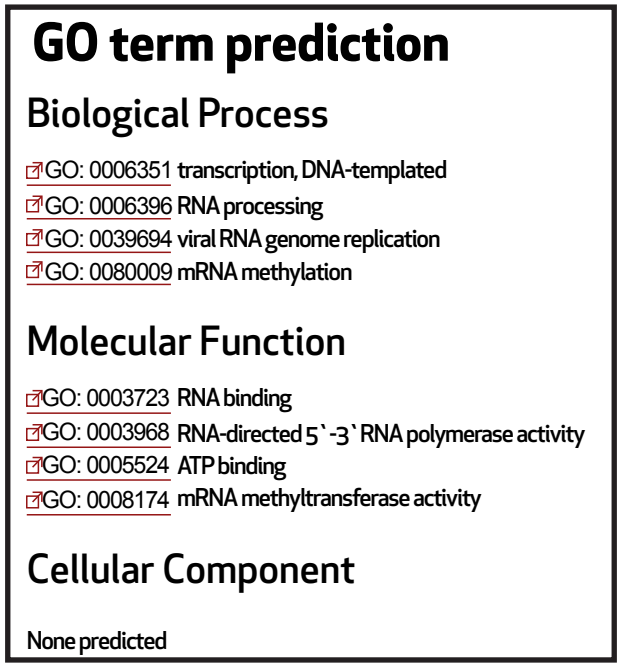
Figure 1.



Structural (a) and Genetic characteristics (b,c) of CHIKV BR33. Organization of the CHIKV Genome and Gene Products: The genomic organization of the chikungunya virus (CHIKV) resembles that of other alphaviruses. It consists of two open reading frames (ORFs), both flanked by 5′ cap structures and a 3′ poly(A) tail. The regions proximal to the 5′ and 3′ ends of the CHIKV genome contain nontranslated regions (NTR), with the junction region (J) also being noncoding.

The 5′ ORF is translated directly from the genomic RNA and encodes four nonstructural proteins (nsP1, nsP2, nsP3, and nsP4). In contrast, the 3′ ORF is translated from a subgenomic 26S RNA and codes for several structural proteins, including the capsid protein (C), two surface envelope glycoproteins (E1 and E2), and two small peptides known as E3 and 6k. These non-structural and structural proteins, namely nsP1 to nsP4, and C, E1, E2, E3, and 6k, are produced through proteolytic cleavage of polyprotein precursors. This genomic organization and the subsequent protein synthesis are fundamental aspects of the CHIKV life cycle and play a crucial role in its pathogenicity and interactions with the host. https://viralzone.expasy.org/625?outline=all_by_species

Figure 2.



Gene Ontology Classification of protein CHIKgp1, divided into functional biological, molecular, and cellular components. Gene Ontology (GO): Gene Ontology (GO) stands as a cornerstone in the realm of biological information by offering precise definitions of protein functions. GO is an organized and regulated lexicon consisting of terms known as GO terms. It's categorized into three distinct and non-overlapping ontologies: Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). The structure of GO is represented as a Directed Acyclic Graph (DAG), where terms take the form of nodes, and relationships among terms form the edges. This framework offers greater flexibility than a traditional hierarchy, as each term can have multiple connections to broader parent terms as well as more specific child terms (du Plessis et al., 2011). Genes or proteins become associated with GO terms through an annotation process, which serves as a linkage. Each GO annotation has an attributed source and database entry. These sources can range from literature references to database references and computational evidence. Every biological molecule is linked to the most specific set of terms that accurately depict its functional attributes. Consequently, if a biological molecule is associated with a particular term, it will be inherently connected to all the parent terms within that term's hierarchical structure (du Plessis et al., 2011). This comprehensive system enables the precise classification of biological functions and greatly aids in the understanding of gene and protein functionality.

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Figure 3.

Genomic Annotation*[1]

CDS	CDS Start	CDS End	CDS Length (nt)	Protein Lenght (aa)	Codon Start	View Sequence and Design Primers	Source
CHIKVgp2	7570	11316	3747	11248	1	CDS | Protein	GenBank

Isoelectric Point/Molecular Weight (SOP)

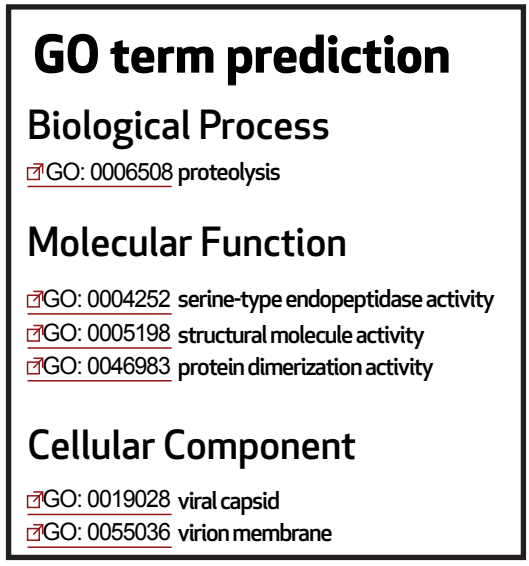
Isoelectric pt	Molecular Weught	Evidence Code
8.7	138351.1	RCA

Other Domains/Motifs (SOP)

Domain/Motif	Start	End	Program
transmembrane	733	755	tmhmm
transmembrane	690	7112	tmhmm
transmembrane	794	816	tmhmm
low_complexity	780	791	seg
transmembrane	1223	1245	tmhmm
transmembrane	765	7787	tmhmm
low_complexity	57	99	seg
low_complexity	20	44	seg
low_complexity	693	707	seg
low_complexity	1224	1241	seg

Functional features of annotating and searching for domains of the CHIKgp2 protein. Sequence annotations provide detailed information about specific regions or features within a protein sequence. These annotations encompass a wide range of elements, including post-translational modifications, binding sites, enzyme active sites, local secondary structures, and other characteristics that are either reported in the cited references or predicted. Additionally, any discrepancies or conflicts in the sequence information between different references are also documented in this manner.

Figure 4.



Name	GO ID	Annotation Source	Evidence
Biological Process			
virion attachment to host cell surface receptor	GO: 0019062	UniProtKB	- N/A -
Molecular Function			
serine - type endopeptidase activity	GO: 0004252	UniProtKB	IEA
structural molecule activity	GO: 0005198	UniProtKB	IEA
Cellular Component			
host cell membrane	GO: 0033644	UniProtKB	IEA Interpro
integral to membrane	GO: 0016021	UniProtKB	IEA
viral capsid	GO: 0019028	UniProtKB	IEA
virion membrane	GO: 0055036	UniProtKB	IEA UniProtKB - SubCell

Gene Ontology Classification of protein CHIKgp2, divided into functional biological, molecular, and cellular components.

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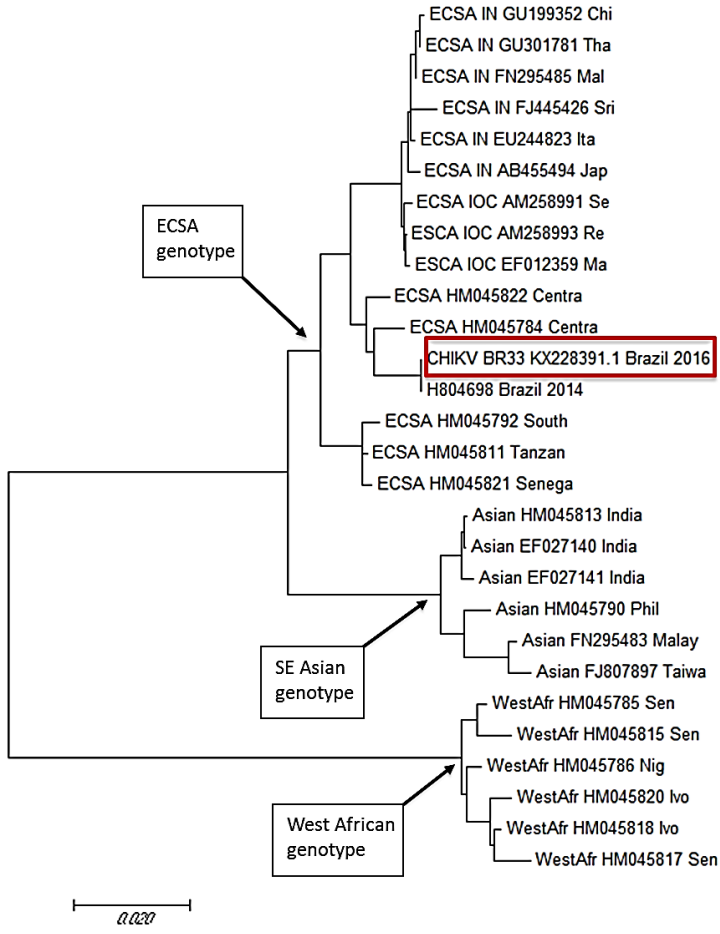
Figure 5.



Phylogenetic Analysis Details (Serotype). Bayesian filament of CHIKV BR33 including the three genotypes: West African (WA), East-Central-South Africa (ECSA) and South East (SE) Asian. The red box shows CHIKV BR33.

Results showed that the isolated CHIKV BR33 strain belongs to the genotype ECSA (Fig 5). A query search using the nucleotide search tool, BLASTn (Huh, J. E. et al 2021), revealed that the strain analyzed is closely related to the CHIKV virus strain BHI3734/H804698, with the GenBank accession number: KP164568.1 it was isolated in Brazil from a patient from Feira de Santana-BA and was submitted to the NCBI by the Center for Technological Innovation, Evandro Chagas Institute. It has a size of 11812 base pairs and phylogenetically belongs to the ECSA genotype. Other strains isolated by the same research group in 2015, in Feira de Santana, a municipality in the state of Bahia, also located in the northeastern region of Brazil (Nunes et al., 2015), showed a 99% similarity regarding nucleotide identity.

DISCUSSION

The detection of this CHIKV strain serves as compelling evidence of its current circulation within Brazil, giving rise to significant implications for both virus dissemination and public health. Key

considerations involve the transmission dynamics: Chikungunya, primarily transmitted by Aedes aegypti and Aedes albopictus mosquitoes, is facilitated by the prevalence of these vectors in Brazil and other tropical and subtropical regions. The introduction of an African strain augments the virus's genetic diversity, potentially affecting its capacity to infect and spread among humans. This diverse genetic landscape poses a challenge in managing the virus, as infected individuals become reservoirs for mosquito-borne transmission, elevating the risk of localized outbreaks, particularly in densely populated urban areas (Hakim & Aman, 2022; Nunes et al., 2015).. The genetic diversity also influences immune responses and vaccine efficacy, with varying susceptibility and disease severity among individuals. Moreover, different strains may exhibit differential responses to existing treatments, complicating the development of effective therapies. In terms of public health, the presence of an African Chikungunya strain underscores the critical need for vector control measures, encompassing mosquito breeding site elimination and public education on mosquito bite prevention. These measures are paramount in curtailing virus spread and averting potential outbreaks. Vigilant epidemiological surveillance is essential, especially in areas where the African strain is prevalent, enabling early and effective responses to contain virus transmission. In summary, the introduction of an African Chikungunya strain in Brazil presents additional challenges to public health and disease control. Addressing virus spread and genetic diversity necessitates a coordinated response at local, national, and international levels, emphasizing continual monitoring, research, and collaborative efforts to tackle this global health concern (Hakim & Aman, 2022; Nunes et al., 2015)..

The detection of this specific Chikungunya strain in Brazil warrants a deeper exploration of its clinical implications, particularly regarding its virulence and its association with the severity of the disease. Understanding the virulence of this strain is crucial, as it can significantly impact the clinical outcomes in infected individuals. Virulence refers to the ability of the virus to cause disease and the severity of that disease. Investigating whether this strain exhibits enhanced virulence compared to other Chikungunya strains is essential for predicting the potential impact on public health. Furthermore, assessing the relationship between this strain and the severity of the disease is pivotal. Some Chikungunya strains have been associated with more severe clinical manifestations, such as a higher incidence of severe joint pain, arthritis, and neurological complications. If this African strain shows a stronger correlation with severe disease, it could have important implications for healthcare systems and clinical management strategies in regions affected by the virus. In addition, the genetic diversity of the virus could potentially influence the effectiveness of diagnostic tests, treatment approaches, and vaccine development. A comprehensive study of the specific genetic characteristics of this strain is warranted to evaluate these aspects thoroughly. By delving deeper into the clinical implications, we can gain valuable insights into the potential impact of this African Chikungunya strain on the health of affected populations and, consequently, inform more targeted and effective public health interventions and clinical management strategies (Lima-Camara, 2016; Zerfu et al., 2023).

No vaccines are currently available for use as a prophylactic method, and no effective antiviral drugs are available for the treatment of a CHIKV infection. Thus, evidence such as the presented in this document is crucial for the continuous alert and vigilance of national and international disease control agencies, for the prevention of new cases that could collapse the health services during simultaneous explosive epidemics circulating in the country (Lima-Camara, 2016; Zerfu et al., 2023). It is important to identify phylogenetically the circulating genotype in Brazil's outbreak, because the presence of particular genotypes has been associated with more dangerous and severe clinical manifestations, mortality, high pathogenicity, and even an increase of mosquito infectivity, followed

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by Asian strains (Hakim & Aman, 2022; Kumar et al., 2014; Spicher et al., 2021).

The present study helps to better understand the pathophysiology and molecular aspects of the disease by achieving a complete genetic and functional description of the virus and its structural proteins.

CONCLUSION

As far as we know, this is the first report of a complete genome of CHIKV, isolated in Brazil, in 2016. The sequence described, the additional phylogenetic analyzes of these genomes, and the other sequences will be detailed in future publications. Sequencing the complete genome of the Chikungunya virus is of paramount importance for several reasons. Firstly, it allows the identification of different strains and mutations of the virus. This is crucial for comprehending the genetic diversity of the virus and its capacity for evolution, enabling effective surveillance of mutations, which is vital for predicting the virus's spread and developing more effective prevention and treatment strategies. Furthermore, genomic sequencing facilitates diagnosis and the development of specific tests. These tests play a fundamental role in detecting the presence of the virus in infected patients, enabling swift and precise decision-making regarding control measures. Additionally, this genomic information is essential in designing vaccines against Chikungunya. A detailed understanding of the viral genome allows the creation of vaccines capable of eliciting effective and specific immune responses against the virus. Genomic sequencing also provides valuable insights into epidemiological studies and understanding the virus's spread in different regions, which is essential for the implementation of control and prevention measures, especially in areas where the virus is endemic. Knowledge of the Chikungunya virus's genetics is crucial in the development of effective antiviral treatments. These medications can target specific components of the viral genome to inhibit its replication. Finally, genomic sequencing allows us to monitor the potential emergence of resistance to antiviral medications used in Chikungunya treatment, which is essential for adjusting treatments and ensuring their efficacy over time. In summary, sequencing the Chikungunya virus genome is a critical component in the fight against this disease and in safeguarding public health.

ACKNOWLEDGEMENTS

The authors particularly acknowledge to Dr. Irene Bosch at the Columbia Mailman School of Public Health, New York, NY, USA and Dr. Lee Gehrke, director of Gehrke Laboratory at Harvard Medical School (HMS), Boston, Massachusetts, USA and Massachusetts Institute of Technology (MIT), Cambridge, MA, USA for the academic formation, methodological advice and equipment loan for the realization of this research.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

RVJ and JYB designed the study; collected and prepared the materials; performed the experiments; performed data analyses; drafted and wrote the manuscript. All authors read, edited, and approved the final draft.

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