IDCases 38 (2024) e02087

Contents lists available at ScienceDirect

IDCases

journal homepage: www.elsevier.com/locate/idcases

Case report

Genetic characterization and phylogenetic analysis of *Chikungunya Virus*: A case from Jeddah during the COVID-19 Pandemic

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ARTICLE INFO

Keywords:
Chikungunya
ECSA
E1: K211E
E1-I317V
E2: V264A

ABSTRACT

Chikungunya fever is an arboviral disease caused by the *Chikungunya virus* (CHIKV), which is classified into three genotypes, namely Asian, West African, and East/Central/South African (ECSA). Due to the frequency and severity of CHIKV outbreaks, it is crucial to genetically characterize the virus, especially in non-endemic regions. This report describes a case and genome characterization of CHIKV from a case that was detected in Jeddah (in 2021) during the COVID-19 pandemic. CHIKV was identified in a 32-year-old male patient in Jeddah, originally from India, who presented with fever, myalgia, malaise, and fatigue and was initially suspected of having dengue. The patient had no history of travel in the fortnight prior to his presentation. Treatment included paracetamol, saline, and vitamin C, which is important for the host immune response and detoxification of viral products. The genome of CHIKV was sequenced using various techniques and bioinformatics tools. Amino acid mutations were studied. Phylogenetic analysis showed that the CHIKV strain detected in 2021 was genetically distinct from those reported in 2018. The 2021 virus shared ancestry with CHIKV strains reported in India. This strain possessed E1-K211E, E2-V264A, and E1-I317V mutations. Novel substitutions were identified. The CHIKV re-emergence in 2021 in Jeddah belonged to the Indian subcontinent/Southeast Asia clade of ECSA. More molecular epidemiological information is needed to better understand and evaluate the prevalence of CHIKV in Saudi Arabia.

Introduction

Chikungunya fever (CHIK) is an arboviral disease caused by the *Chikungunya virus* (CHIKV), which is transmitted to humans by *Aedes albopictus* and *Aedes aegypti* mosquitoes [1]. CHIKV infections manifest as arthralgia, fever, and rash. CHIKV belongs to the *Alphavirus* genus within the *Togaviridae* family. Its 11–12 kb positive-sense RNA genome consists of two open reading frames, which encode non-structural proteins (nsP1, nsP 2, nsP 3, and nsP 4) and structural proteins (Capsid, E3, E2, 6 K, and E1) as well as 5′ and 3′ untranslated regions [2]. Jeddah is home to several species of *Aedes* mosquitoes, which are significant vectors for a variety of arboviral diseases, including dengue, chikungunya, and yellow fever. Among the most prevalent species in this area is *Aedes aegypti*, a highly efficient vector known for its ability to breed in small, stagnant water containers found in urban and peri-urban environments. This species is particularly well-adapted to thrive in

Abbreviation: CHIKV, Chikungunya Virus; ECSA, East/Central/South African; IE clade, Indian subcontinent/Eastern Africa clade; IS clade, the Indian subcontinent/Southeast Asia clade; nsP, non-structural proteins; tMRCA, the time to the most recent common ancestor.

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https://doi.org/10.1016/j.idcr.2024.e02087
Received 24 August 2024; Received in revised form 22 September 2024; Accepted 10 October 2024
Available online 11 October 2024

Jeddah's humid climate and rapid urbanization [3]. CHIKV is classified into three main genotypes, namely Asian, West African and East/-Central/South African (ECSA) genotypes [4]. It is thought that the distribution of CHIKV across countries is attributed to infected travelers returning from regions with a high prevalence of the disease [1]. CHIKV has spread widely since 2004, resulting in extensive and prolonged outbreaks throughout Asia and Africa [5]. CHIKV infection leads to significant morbidity, which considerably lowering the quality of life [6]. The severe arthralgia and myalgia associated with *chikungunya* fever can last for years and have a significant negative impact on health and economic output [7]. Although CHIK is not typically life-threatening, severe cases have been reported [8]. Since there are currently no specific antiviral treatments or vaccinations for CHIKV, surveillance and vector control are crucial to disease management [7]. Importantly, due to the frequency and severity of CHIKV outbreaks, it is crucial to characterize and understand the biology of the virus [9]. In Saudi Arabia, few CHIK cases have been reported [10,11] and characterized recently [12]. However, CHIKV is not endemic to the country, therefore the virus origin should be understood. Also, limited studies have been performed in the country. We are reporting a case of Chikungunya from a patient presenting with dengue-like symptoms in Jeddah (a non-endemic region) during the COVID-19 pandemic in 2021. The patient lacked a history of travel abroad. Despite having no recent travel history, the patient's residence in a neighborhood with a significant population originating from the Indian subcontinent raises the possibility of local transmission. Given the presence of *Aedes* mosquitoes in the area, this suggests that the virus could have been introduced by other individuals returning from endemic regions, facilitating local spread within the community. Genome characterization of CHIKV was conducted. This report also compares data from the virus that re-emerged in 2018 with data obtained from the current case (2021). The outcomes of this study may be useful for vector borne diseases preparedness.

Case

A 32-year-old male patient in Jeddah and originally from India who was initially suspected of having dengue infection during the COVID-19 pandemic in 2021. Jeddah, Saudi Arabia, has experienced a significant increase in dengue fever cases in recent years. Factors such as rapid urbanization, increased international travel, and favorable climatic conditions, particularly the presence of *Aedes aegypti* mosquitoes, have contributed to the spread of dengue in the region. These conditions made it reasonable to initially suspect dengue in this patient, given the high prevalence of the disease in Jeddah [13]. The patient presented with fever (39.8 °C), myalgia, malaise, and fatigue. He had no history of travel abroad in the two weeks prior to his presentation. Meanwhile, the patient lived in a neighborhood with many residents from the Indian subcontinent. Laboratory investigations of the patient's serum for dengue (PCR, IgM, and NS1 antigen) were negative. According to our lab policy, all dengue-negative samples should be further investigated for other viruses including *Alkhumra virus*, *Chikungunya Virus*, and *Rift Valley fever Virus*. However, due to the COVID-19 pandemic and occupancy of lab facilities, these tests were not conducted immediately. The sample was tested retrospectively in the middle of 2023, several months after the patient's initial presentation in December 2021, and was confirmed as positive for CHIKV by real-time PCR using RealStar Chikungunya RT-PCR kit (Altona Diagnostics–GmbH, Hamburg). The genome of CHIKV was sequenced utilizing various techniques and bioinformatics tools, and the sequence has been submitted to GenBank under the accession number OR626603. Supportive management was provided using analgesics, saline, and vitamin C.

As the case is reported from non-endemic area, it is important to conduct phylogenetic analysis. The viral RNA was extracted from 400 µL of patient serum using an EZ1 instrument (Qiagen diagnostics-Germany). RNA was eluted in 60 µL, of which 5 µL was used to synthesize cDNA utilizing Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics–Germany). The cDNA was then used for PCR amplification using a FastStart DNA Master HybProbe master mix kit from (Roche Diagnostics–Germany) and 22 sets of primers [12] to generate the near-complete genome of CHIKV. Bi-directional Sanger sequencing was conducted on 22 PCR amplicons. The raw sequence data (11,282 nucleotides) was analyzed. Multiple sequence alignment and phylogenetic analysis were conducted using the BEAST 1.10.4 software suite. The alignment included a global set of CHIKV sequences retrieved from GenBank. This set was selected by filtering out redundant sequences (threshold of 99.5 % similarity) from all complete envelope gene sequences (n = 1342) available in NCBI. CHIKV sequences reported in Saudi Arabia in 2018 were also included. The parameters included a GTR+G4 +I substitution model and a relaxed random clock with a Bayesian skyline tree prior. The MCMC chain was run for 10 million iterations. The tree converged with ESS of 765 (joint). Any ESS value > 200 indicates strong statistical support for convergence. Time-scaled analysis was performed using BEAST software. Furthermore, the details of the amino acid substitutions were assessed via alignment of CHIKV amino acids in this report with the closest Indian isolate (KY057363.1), known isolate (HQ456251.1) as a reference of ECSA in addition to two CHIKV sequences (HF-777 and HF-793) identified in Jeddah during 2018.

Discussion

Numerous CHIKV outbreaks have been documented over the past 50 years at an irregular interval of 2–20 years between outbreaks in both Asia and Africa [1]. In Saudi Arabia, CHIKV was first reported in 2011 in Jeddah in a 55-year-old female patient who presented with fever, generalized arthralgia, skin rash, vomiting, and abdominal pain. A limited molecular investigation of the E1 gene of CHIKV obtained from this case showed that the virus belonged to the wild-type CHIKV lineage without the E1-A226V substitution [10]. Further study of CHIKV that re-emerged during 2018–2019 in Jeddah demonstrated that the strains belonged to the IE clade of ECSA genotype. Furthermore, genetic characterization and phylogenetic analyses based on near-complete genome sequencing showed that those 2018 viruses clustered with isolates from Kenya (Mombasa), which experienced an outbreak during 2017–2018. CHIKV strains detected during 2018 did not possess E1-A226V, but had E1-L136F, E1-K211E, E1-I317V, E2-M74I, E2-A76T, E2-V264A, and C-N79S substitutions, further supporting the notion that they were imported from Kenya [12].

In the present case, the patient was treated with vitamin C, analgesics (paracetamol) and saline. Ascorbic acid has antiviral action and is crucial in enhancing the host's antiviral immune response. Vitamin C can deactivate DNA and RNA viruses. Furthermore, viral products that cause inflammation and pain can be detoxified by Vitamin C [14]. Given that the patient had no recent travel history and resided in a neighborhood with a significant population from the Indian subcontinent, it is plausible that local *Aedes* mosquitoes, particularly *Aedes aegypti*, may have acquired and transmitted the virus. The presence of *Aedes aegypti* in Jeddah, which is known to be an efficient vector for arboviral diseases such as dengue and chikungunya, suggests the possibility of local transmission. This scenario highlights the importance of ongoing mosquito surveillance and control efforts in non-endemic regions, where the introduction of the virus by infected individuals could lead to sustained transmission within the local mosquito population. We conducted near-complete genome sequencing of CHIKV. The phylogenetic analysis presented herein (Fig. 1) showed that the study sequence (2021) also belonged to the ECSA genotype but was genetically distinct from ECSA strains reported in Saudi Arabia during 2018 – 2019. The study sequence shared close ancestry with sequences reported earlier in Asia from 2016 to 2019. The current sequence (HF-442 - accession number OR626603) showed the closest ancestry to a sequence from India reported in 2016 (accession number KY057363). The tMRCA (the time to the most recent

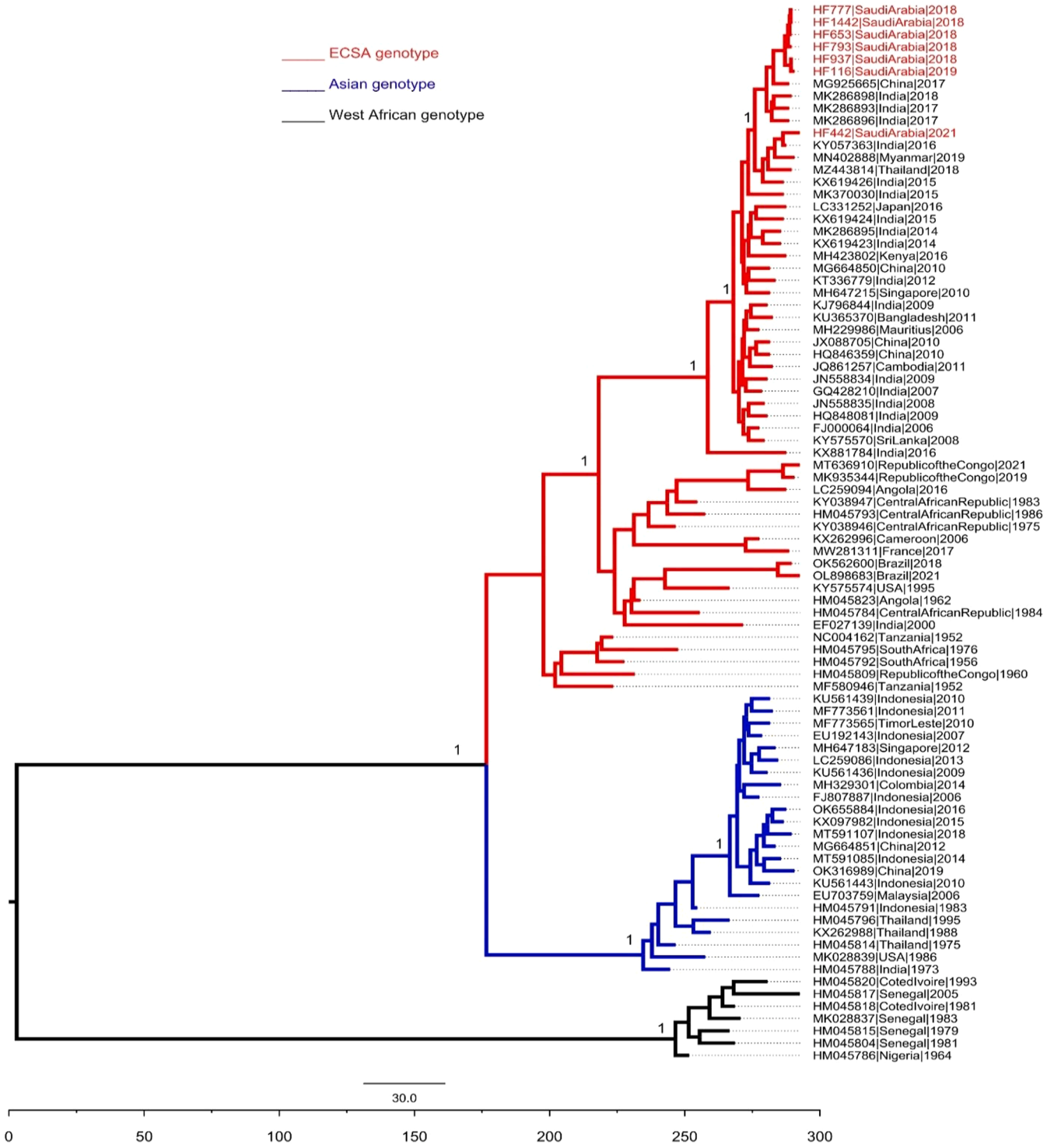

Fig. 1. : Phylogenetic tree of CHIKV from Jeddah during 2021 (HF-442, accession number OR626603). Genotypes of CHIKV are shown in different colors (East/Central/South African – Red; Asian – Blue and West African – Black). Taxa names of the study sequence and CHIKV strains reported in Saudi Arabia during 2018–2019 are shown in red. The numbers on the tree nodes are posterior probability values.

common ancestor) analysis suggested that the virus in the current case (HF-442) originated from a common ancestor in 2015, coinciding with the presence of closely related strains in India in 2016. These findings are consistent with a study conducted in Pakistan during the 2016–2017 outbreak, which also identified CHIKV strains belonging to the ECSA genotype and possessing a close genetic relationship with a strain circulating in India (KY057363) [4]. This further supports the notion of a regional transmission network.

It has been demonstrated that adaptive mutations in viral lineages contribute to the transmission potential and fitness of CHIKV in various hosts and vectors [4]. A widely cited example is the acquisition of the E1-A226V substitution by the 2004–2009 pandemic lineage that caused epidemics across Asia. The epidemic success of this lineage was attributed to its adaptation to *Aedes albopictus* primarily driven by the E1-A226V mutation, which enhanced its transmissibility by *Aedes albopictus* [2]. Since 2016, a CHIKV lineage that does not possess

E1-A226V, but has additional mutations (E1-K211E, E2-V264A, and E1-I317V) has spread broadly to numerous areas. This lineage demonstrated enhanced fitness in *Aedes aegypti* [15]. Two sub-lineages, namely the Indian subcontinent/Eastern Africa clade (IE clade) and the Indian subcontinent/Southeast Asia clade (IS clade), emerged within this lineage. The IE clade was characterized by the E2-A76T and C-N79S substitutions, while the IS clade had the nsP2-E145D and nsP4-S55N substitutions [2]. In the CHIKV 2021 isolate, two significant E1 mutations, E1-K211E and E1-I317V, were detected. The CHIKV 2021 strain lacks the E2-A76T and the C-N79S substitution. However, nsP4 showed the nsP4-S55N mutation. Therefore, the CHIKV 2021 isolate can be classified among the IS clade. Notably, some novel substitutions were identified in the 2021 sequence including E1-A249T, E2-M366T, C-A147V, nsP4-R82S, nsP3-V58I and nsP3-Q328H. Although the effects of these substitutions have not been studied, they might open a window for future work to evaluate their significance.

The 2021 CHIKV strain exhibited mutations that have been experimentally shown to be more adapted to *Aedes aegypti* than *Aedes albopictus*. *Aedes* mosquito populations are abundant in the western region of Saudi Arabia, where Jeddah and Makkah are located, as well as in southwestern areas like Jazan. Moreover, millions of travelers from over 180 countries, including expatriate workers, tourists, and Hajj and Umrah pilgrims, travel to the western part of Saudi Arabia [12]. Dengue fever, which is also transmitted by *Aedes* mosquitoes, has been a major public health concern in these areas [16]. Therefore, repeated importations of CHIKV into areas inhabited by *Aedes* mosquitoes significantly increase the risk of sustained virus transmission, leading to outbreaks. The vector control program in Saudi Arabia has historically been very effective in preventing the spread of *Aedes* mosquitoes and arboviral diseases such as dengue. However, dedication and community involvement in control measures are crucial for the sustained control of vector populations [12]. Hence, further effort and continuous future studies should be conducted. This case highlights the need for extensive epidemiological surveillance in the region to better understand local transmission dynamics of arboviruses like chikungunya. Increased monitoring of mosquito populations and viral activity is crucial for early detection and prevention of outbreaks.

Conclusion

The CHIKV detected in Jeddah in 2021 belonged to the IS clade of the ECSA genotype. Genetic characterization and phylogenetic analyses supported a shared ancestry between the studied sequence and CHIKV reported in the Indian subcontinent since 2016. Based on the case history, given the presence of *Aedes* mosquitoes in the area, the virus could have been introduced by individuals from the neighborhood returning from endemic regions, facilitating local transmission. More molecular epidemiological information is needed to better understand and evaluate the prevalence of CHIKV in Saudi Arabia. Constant monitoring of mosquito-transmitted diseases, particularly imported ones, might be useful for the preparedness and proactive control of vector-borne diseases to avoid the occurrence of autochthonous outbreaks.

Ethics approval

This work was conducted from data and remaining sample obtained for virological diagnostic purposes. It was ethically approved by the Ethics Committee of the Research and Studies Department at Jeddah Health Affairs in 2021 (1474). The work was performed in compliance with relevant Jeddah Health Affairs guidelines.

Funding

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Consent to participate

The patient in this case is an adult, and a verbal consent was obtained. No images or identifying information were used in this report.

CRediT authorship contribution statement

Hapuarachchige Chanditha Hapuarachchi: Writing – review & editing, Visualization, Formal analysis. **Ali A. Alzahrani:** Writing – review & editing, Formal analysis, Data curation. **Safar A Almalki:** Investigation, Formal analysis. **Faisal A. Alzahrani:** Supervision, Methodology, Conceptualization. **Waleed S. Alsalem:** Writing – review & editing, Formal analysis. **Hisham N. Altayb:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Eitezaz A Zaki:** Validation, Methodology, Investigation. **Hassan Ibrahim Alguridi:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The team acknowledges Dr. Kamal Alshaeb from Biochemistry Department (King Abdulaziz University) for his support. Also, thank the Ethics Committee of the Research and Studies Department at Jeddah Health Affairs. Additionally, we would like to thank Jeddah Regional Laboratory (Molecular Biology), Mr. Alhussian Hejazi and Dr. Ioanna Skountzou.

Data Availability

Raw data are available upon reasonable request from the corresponding author.

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