

Full Length Research Paper

***In silico* analysis of Chikungunya virus (CHIKV), a mosquito-borne alphavirus**

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Chikungunya virus (CHIKV) is a mosquito-borne alpha virus, which belongs to the family, *Togaviridae*. This virus is known to cause an acute onset of high fever, severe arthralgia and rash, and is usually accompanied by headache and severe joint pain. The present study aimed to construct an updated phylogenetic tree of currently published data and perform a phylogeographic analysis of Chikungunya virus obtained during different outbreak in the last five years after the re-emerging of chikungunya virus to get further insight into the epidemiology and transmission of CHIKV. In this study, twenty two sequences from the E1 envelope glycoprotein gene were aligned using ClustalW software program. A phylogenetic tree was constructed by using MEGA 5 software version 6, to determine the phylogenetic relationships of CHIKV during different outbreak recently in Yemen, Italy, Philippines, India and Africa. An updated phylogenetic tree was constructed, the results obtained suggested that CHIKV strains isolated recently in the Eastern Mediterranean Region share high similarity with chikungunya virus isolated in Tanzania in 1953.

Key words: Chikungunya fever, epidemiology, outbreaks, phylogenetic tree.

INTRODUCTION

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus, that belongs to the family of *Togaviridae* (Schuffenecker et al., 2006), this virus is known to cause an acute onset of high fever, severe arthralgia and rash, and is usually accompanied by headache and severe joint pain (McGill, 1995; Adebajo, 1996; Mourya et al., 2006; Ligon, 2006; Yazdani et al., 2007; Leparac-Goffart et al., 2014). CHIKV is principally transmitted to humans via the bite of an infected anthropophilic vector *Aedes*

aegypti and *Aedes albopictus* (Centers for Disease Control and Prevention, 2011).

CHIKV is a spherical, enveloped, positive-strand RNA virus (Higashi et al., 1967; Simizu et al., 1984) with a genome of 12 kb, CHIKV genome contains two ORFs, which encodes for structural and non-structural polyproteins (Khan et al., 2002). Until now, four genotypes of CHIKV have been reported (Weaver, 2014), The Est Central South African (ECSA) genotype, West

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African genotype, Asian genotype and Indian Ocean Linage (IOL) genotype.

CHIKV was first reported as a human pathogen in 1952 in Africa, when the virus was isolated by Ross from a serum of human during an epidemic in Tanzania (Lumbsden, 1952; Ross, 1956). In 1958, numerous cases of chikungunya fever have been also identified in several countries in Asia. The significant outbreaks occurred between the 1960s and 1973 in Bangkok and India (Nimmannitya et al., 1964; Shah et al., 1964; Padbidri et al., 1979; Jupp et al., 1988). Interestingly, the re-emergence of the virus has been reported between the 1960s and 1990s in several African countries such as Uganda, Zimbabwe, Senegal, Cameroon and Guinea (Williams et al., 1965; Halstead, 1969; Padbidri, 1979; Jupp, 1988; Lanciotti, 1998). In 2005, several cases of chikungunya fever were reported in La Reunion island, interestingly, the number of people infected have increased in 2006, more than 266,000 cases were documented (Chretien et al., 2007; Cire La Reunion-Mayotte, 2006). Numerous scientists suggested that the virus was introduced into La Reunion Island because of the movement of people from the islands of the Indian Ocean to this island. In addition, other researchers suggested that CHIKV was transmitted by *Aedes albopictus* and not via *Aedes aegypti* due to the limited numbers of *A. aegypti* on La Reunion Island (Reiter et al., 2006).

In 2007, chikungunya virus was detected in Italy for the first time, which means that the virus has been introduced into Europe causing a new outbreak, this finding suggested that CHIKV can move and affect new ecological niches in Europe and other countries such as Australia and countries in the Western Hemisphere (Rezza et al., 2007; Staples et al., 2009).

In January 2011, CHIKV was detected for the first time in the Eastern Mediterranean Region of the World Health Organization when the Ministry of public health and population of Yemen reported several numbers of Dengue-like illnesses in AL-Hudaydah governorate in Yemen. Since, numerous researches have been carried out to investigate the origin of this outbreak. Unfortunately, the epicenter of this outbreak is still unknown; however, this outbreak was completely curtailed (Malik et al., 2014).

In 2012, another study was performed in Yemen to investigate the co-circulation of Dengue and CHIKV. In this study, the sera of 400 patients with dengue-like illness symptoms were studied using immunological and molecular technique. Among the 400 patients, 116 (29%) were positive for dengue virus, whereas 49 (12%) were positive for CHIKV (Rezza et al., 2014) the results obtained demonstrated that mosquito-borne infections in Yemen represent a serious public health threat.

In 2015, the complete genome of CHIKV was sequenced by Fahmy et al. (2015); this virus was isolated from an *A. aegypti* mosquito during the outbreak in

Yemen in 2011. In this work, genome analysis showed that CHIKV isolate represent significant similarity with the Indian oceans strains (Fahmy et al., 2015).

Recently, another outbreak occurred in 2012 in the Philippines (Tan et al., 2015). In this study, scientists studied the phylogenetic relationship of CHIKV isolate obtained during the Philippines outbreak with numerous Chikungunya viruses sequences isolated from different regions in China, Micronesia and Caribbean. Interestingly, the results obtained suggested independent emergence of CHIKV in the Philippines, which then extend into China, Micronesia and the Caribbean region. Few years later, CHIKV has re-emerged in 2014 causing new outbreak in Puerto Rico and Brazil (Chiu et al., 2015; Nunes et al., 2015).

Objective

The present study aimed to construct an updated phylogenetic tree of currently published data and perform a phylogeographic analysis of CHIKV obtained during different outbreak in the last five years after the re-emergence chikungunya virus to get further insight into the epidemiology and transmission of CHIKV.

MATERIALS AND METHODS

Collection of E1 gene sequences

Twenty two sequences from the E1 envelope glycoprotein gene were collected and retrieved from the National Center for Biotechnology Information (NCBI) available (<https://www.ncbi.nlm.nih.gov/>). These twenty two sequences were published recently after the re-emerging of CHIKV in Europe, Asia, Africa, as well as the Eastern Mediterranean Region of the World Health Organization. The retrieved sequences were from Yemen outbreak- KJ742803- KJ742804- KJ742805- KJ742806- KJ742807- KJ742808- KJ742809 (Rezza et al., 2014), Italy outbreak- KM267638 (Rossini et al., 2016), Philippines outbreak- KM014692- KM014693- KM014694- KM014695- KM014696 (Yoon et al., 2015), Philippines outbreak- KP276677 (Velasco et al., 2015), India outbreak- KX358423- KX358422- KX358421- KX358419- KX358417- KX358410- KX358408 deposit in GenBank by Khan and Ray (unpublished) and from Tanzania outbreak- AF192905 deposit in GenBank by Logue and Atkins (unpublished).

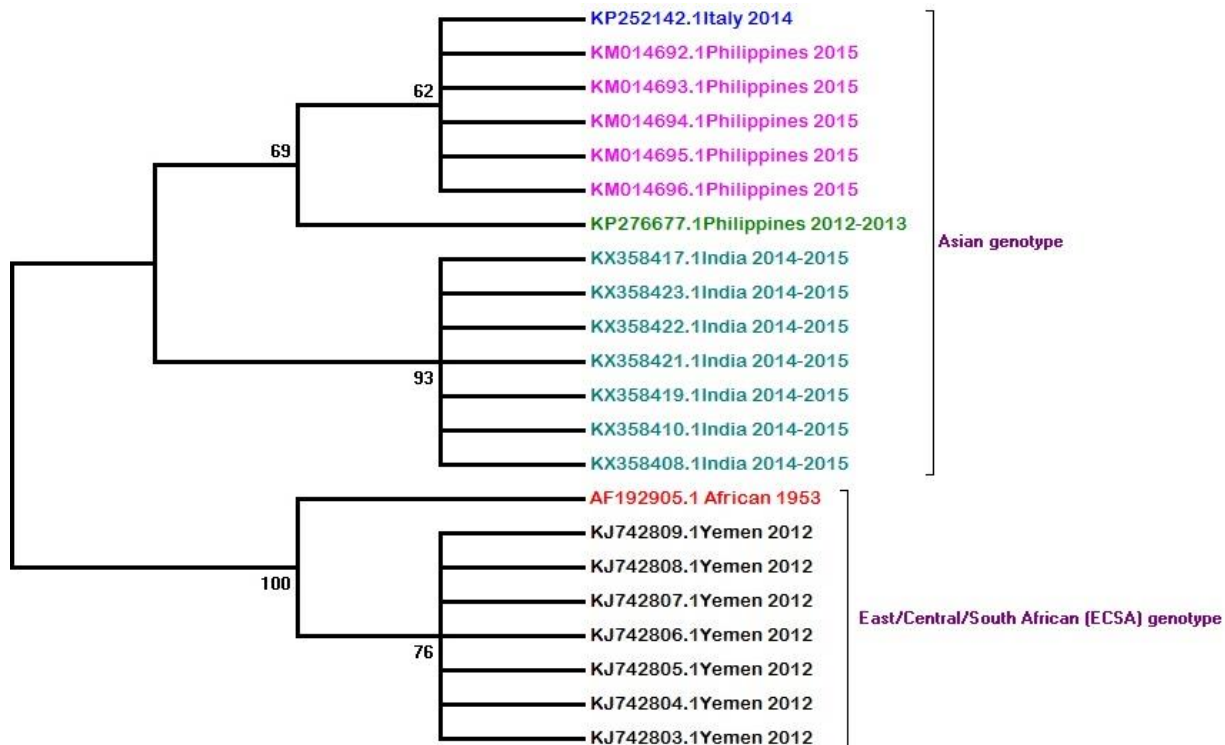
Phylogenetic tree and sequences analysis

The 22 sequences were aligned using ClustalW software program (<http://www.ebi.ac.uk/clustalw2/>). Phylogenetic trees was constructed by using MEGA 5 software version 6 (Tamura et al., 2011), to determine the phylogenetic relationships.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 2.14740026 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969) and are in the units of the number of base substitutions per site. All

Table 1. Localization and sample size of chikungunya virus.

Organism	State	Sample size
Chikungunya Virus	Yemen	7
Chikungunya Virus	Italy	1
Chikungunya Virus	Philippines	6
Chikungunya Virus	India	7
Chikungunya Virus	Tanzania	1

**Figure 1.** Evolutionary relationships among twenty two sequences of chikungunya virus obtained during different outbreak that occurred in the last five years based on the neighbor-joining (NJ) algorithm. Bootstrap values are marked on the branches

positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

RESULTS

In this study, phylogenetic analysis were performed and an updated phylogenetic tree was constructed comparing twenty two sequences of CHIKV strains from Yemen, Italy, Philippines, India and Africa. All sequences were published recently and were obtained during different outbreak in the last five years after the re-emerging of CHIKV. Information regarding sample size and localities are listed in the Table 1.

The phylogenetic tree was constructed by using

neighborjoining (NJ) algorithm (Figure 1). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

The results obtained from this model indicate that among 22 CHIKV obtained from different states, CHIKV strains isolated during the outbreak in Yemen in 2011 show close relationship and form one group. Interestingly, this group share high similarity with CHIKV isolated in Tanzania in 1953.

In addition, among the six sequences obtained during Philippines outbreak, five sequences showed a close relationship with CHIKV isolated in Italy in 2014 and formed one group. On the contrary, CHIKV isolated in Philippines in 2012 was disclosed to this group. Sequence

analysis of CHIKV strains isolated during the outbreak in India in 2014 and 2015 demonstrated that the seven sequences share high similarity and form one group. In addition, this group show close relationship with the group of CHIKV isolated recently in Philippines.

DISCUSSION

CHIKV is known to cause an acute onset of high fever, severe arthralgia and rash, and is usually accompanied by headache and severe joint pain. This virus was first reported as a human pathogen in 1952 in Africa (Lumsden, 1952; Ross, 1956). Interestingly, Chikungunya virus has re-emerged recently in new areas, and numerous outbreaks occurred in different states in Europe, Asia, America and Africa. These mosquito-borne infections represent a serious public health threat. CHIKV is principally transmitted to humans via the bite of an infected anthropophilic vector *A. aegypti* and *A. albopictus* (Centers for Disease Control and Prevention, 2011).

Unfortunately, until now, there is no vaccine for CHIKV. The control of the disease mainly remains dependent on the control of the vector. Furthermore, many researchers have demonstrated that CHIKV transmission is mediated by vectors that can colonize new geographical area due to its capacity to acclimatize to different climates. This can explain why the rate of infection has recently increased dramatically especially in tropical countries. In addition, the return of peoples from affected areas is also one of several reasons that explain the detection of CHIKV outside tropical countries (Presti et al., 2014).

In the present work, an updated phylogenetic tree was provided, the results demonstrated that CHIKV strains isolated recently in the Eastern Mediterranean Region share high similarity with chikungunya virus isolated in Tanzania in 1953.

Unfortunately, the epicenter of many outbreaks is still unknown, however, some of these outbreaks were completely contained. To conclude, phylogenetic analyses of virus sequences are important tools to get more insight into the epidemiology and transmission of CHIKV. Moreover, several phylogeographic studies are needed to know and determine the epicenter of many outbreaks that occurred recently.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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