Molecular characterization of chikungunya virus causing the 2017 outbreak in Dhaka, Bangladesh

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Abstract

Chikungunya viruses from the 2017 outbreak in Dhaka, Bangladesh, were analysed phylogenetically. *E1* sequences from 21 strains belonged to the Indian Ocean clade of the East/Central/South African (ECSA) genotype, forming a novel cluster with latest South Asian strains. They lacked the A226V substitution.

Keywords: Bangladesh, chikungunya virus, Dhaka, genotype, molecular epidemiology
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Chikungunya virus (CHIKV), one of the notable causes of reemerging mosquito-borne infectious diseases, is often associated with acute febrile and polyarthritis illness. On the basis of the sequence of *E1* viral structural glycoprotein, CHIKV strains have been classified into three genotypes: West African, Asian and East/Central/South African (ECSA) [1]. Global chikungunya outbreaks have occurred since 2005 via spread of CHIKV belonging to the ECSA lineage, which acquired a mutation in *E1* (A226V) conferring increased virus adaptation and replication ability in *Aedes albopictus* [1,2]. In Bangladesh, the first chikungunya outbreak was identified in the northwest area in 2008 [3]. Thereafter, serologic evidence has confirmed occurrence of outbreaks and sporadic cases reported in Dhaka and rural areas [4,5]. However, CHIKV in Bangladesh has not yet been genetically analysed, except for strain Bangladesh/0810aTw, which was identified in an imported case from Bangladesh to Taiwan in 2008 [6]. Here we report the first molecular characterization of autochthonous CHIKV in Bangladesh.

A major outbreak of chikungunya occurred in Dhaka, Bangladesh, from April to September, 2017, resulting in more than 13 000 clinically confirmed cases [7]. Serum samples were collected from patients in Dhaka with sudden onset of high fever of unknown origin (temperature $\geq$38.5°C lasting $\leq$7 days), with or without joint pain and rash, at the National Institute of Preventive and Social Medicine from July to August 2017. Among the 83 samples collected, CHIKV was detected in 71 samples by multiplex reverse transcription PCR [8]. Nucleotide sequences of the *E1* gene (1317 bp) were determined for 21 CHIKV-positive samples by reverse transcription PCR and direct sequencing, and were deposited in GenBank under accession numbers MG697262 to MG697282.

Phylogenetic analysis of the *E1* gene using MEGA6 software revealed that the 21 CHIKV strains belonged to the Indian Ocean clade of the ECSA genotype, forming a novel cluster with viruses in India, Pakistan, Australia, Hong Kong and Italy in 2016 and 2017 (2016–2017 South Asian cluster) (Fig. 1). The *E1* gene of all 21 CHIKV samples showed high sequence identity with each other (99.6–100%) but showed slightly lower identity (98.7–98.9%) to the strain Bangladesh/0810aTw, which had valine at position 226. Deduced amino acid sequences of the whole region of the *E1* glycoprotein (439 aa in length) from the 21 CHIKV were mostly identical except for four positions (amino acids 20, 316, 400 and 437), and all the strains had alanine at positions 98 and 226 (Supplementary Fig. S1). Substitutions M269V and D284E, which had been described as molecular signatures of the Indian Ocean CHIKV outbreak [9,10], were detected in all the strains. Compared to *E1* sequences of the same cluster, half of the 2017 Dhaka outbreak strains had unique substitution N20S, and additional amino acid differences (positions 316, 400 and 437) were detected in two strains (BD1501 and BD1541) (Supplementary Figs S1 and S2).

CHIKV from the 2017 outbreak in Dhaka was found to be genetically distinct from the strain Bangladesh/0810aTw, lacking A226V substitution. The Dhaka outbreak strains constitute a new cluster within the Indian Ocean clade, associated with *E1* sequence diversity, suggesting that they are novel variants within the ECSA genotype. Further genetic and epidemiologic study are necessary in Bangladesh to monitor the spread of the variant CHIKV and to define molecular characteristics relevant to large outbreaks in Dhaka.

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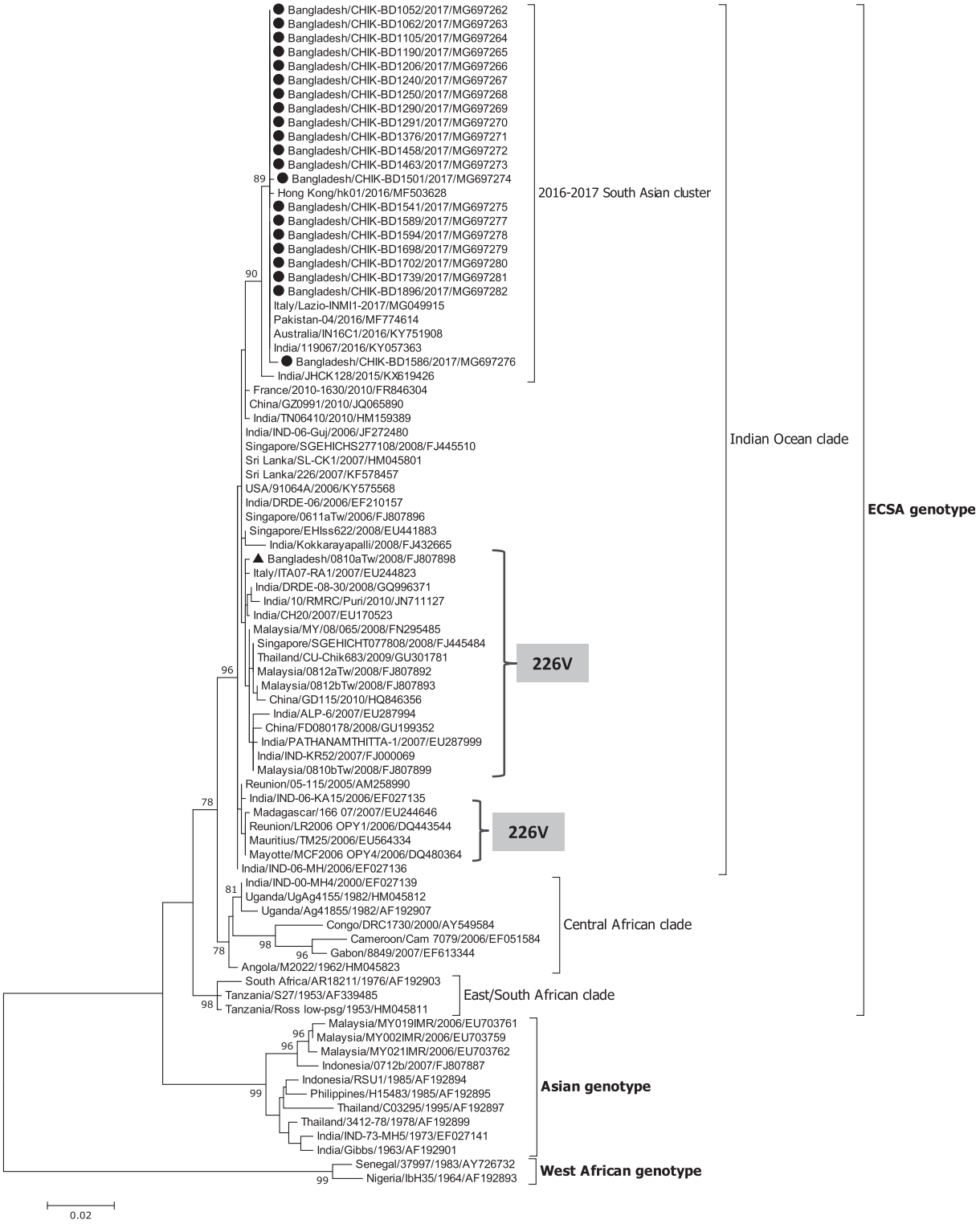

FIG. 1. Phylogenetic dendrogram of chikungunya virus *E1* gene generated among strains from 2017 outbreak in Dhaka, Bangladesh (solid circle) and other diverse geographical locations, constructed by maximum likelihood method using MEGA6. Tree was statistically supported by bootstrapping with 1000 replicates, and genetic distances were calculated by Kimura two-parameter model. Variation scale is provided at bottom. Percentage bootstrap support is indicated by values at each node (values <75 are omitted). Triangle indicates strain Bangladesh/0810aTw, detected in Taiwan in case imported from Bangladesh in 2008. Three major genotypes, clades in East/Central/South African (ECSA) genotype, 2016–2017 South Asian cluster are shown at right. Virus strains with A226V mutation are indicated as 226V.

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nmni.2018.03.007.

References

[1] Thiberville SD, Moyen N, Dupuis-Maguiraga L, Nougairede A, Gould EA, Roques P, et al. Chikungunya fever: epidemiology, clinical syndrome, pathogenesis and therapy. Antivir Res 2013;99:345–70.

[2] Tsetsarkin KA, Chen R, Sherman MB, Weaver SC. Chikungunya virus: evolution and genetic determinants of emergence. Curr Opin Virol 2011;1:310–7.

[3] icddr. b. First identified outbreak of chikungunya in Bangladesh, 2008. Health Sci Bull 2009;7:1–11. Available at: http://dspace.icddrb.org/jspui/bitstream/123456789/4865/1/2009-ICDDRBHealthScienceBulletin-Vol7%281%29-English.pdf.

[4] Khatun S, Chakraborty A, Rahman M, Nasreen Banu N, Rahman MM, Hasan SM, et al. An outbreak of chikungunya in rural Bangladesh, 2011. PLoS Negl Trop Dis 2015;9:e0003907.

[5] Rahim MA, Uddin KN. Chikungunya: an emerging viral infection with varied clinical presentations in Bangladesh: reports of seven cases. BMC Res Notes 2017;10:410.

[6] Huang JH, Yang CF, Su CL, Chang SF, Cheng CH, Yu SK, et al. Imported chikungunya virus strains, Taiwan, 2006–2009. Emerg Infect Dis 2009;15:1854–6.

[7] Kabir I, Dhimal M, Müller R, Banik S, Haque U. The 2017 Dhaka chikungunya outbreak. Lancet Infect Dis 2017;17:1118.

[8] Dash PK, Parida M, Santhosh SR, Saxena P, Srivastava A, Neeraja M, et al. Development and evaluation of a 1-step duplex reverse transcription polymerase chain reaction for differential diagnosis of chikungunya and dengue infection. Diagn Microbiol Infect Dis 2008;62:52–7.

[9] Singh RK, Tiwari S, Mishra VK, Tiwari R, Dhole TN. Molecular epidemiology of chikungunya virus: mutation in *E1* gene region. J Virol Methods 2012;185:213–20.

[10] Bordi L, Carletti F, Lalle E, Colavita F, Meschi S, Di Caro A, et al. Molecular characterization of autochthonous chikungunya cluster in Latium region, Italy. Emerg Infect Dis 2018;24:178–80.