**The reappearance of Chikungunya virus in Bangladesh, 2024**

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**Abstract:**

**Objectives:** We investigated a new Chikungunya virus (CHIKV) outbreak in Dhaka and nearby areas in 2024, examining its epidemiology, clinical features, and genomic characteristics.

**Methods:** The Institute of Epidemiology, Disease Control and Research enrolled suspected cases from October 19 to December 31, 2024. RT-PCR was performed and positive cases were followed up via telephone between days 21 and 28. The E1 gene of 12 samples was sequenced.

**Results:** Of 394 enrolled patients, 138 were CHIKV-positive, mostly male (64.5%) and over 30 years old (83.3%), with 98.6% residing in Dhaka. Common symptoms included fever (100%), arthralgia (97.8%), myalgia (83.2%), and headache (65.0%). While no fatalities recorded, 14.5% required hospitalization with an average stay of 5.9 days, and patients lost an average 10.5 workdays. Disease severity correlated with age ≥30 years (IRR: 1.14, 95% CI: 1.02–1.28). At 28 days, 81% of 58 follow-up patients had persistent symptoms. Sequence analysis identified an E1-K211E substitution, with phylogenetics revealing a distinct ECSA sub-lineage compared to 2017 strains.

**Conclusions:** CHIKV is likely to re-emerge in Bangladesh amid the ongoing severe dengue outbreak, posing a risk of a major outbreak soon. Strengthening efforts to control *Aedes* mosquitoes is essential for managing arboviruses.

**Introduction:**

Chikungunya virus (CHIKV) is a member of the Alphavirus genus of the family Togaviridae transmitted by *Aedes* mosquitoes, primarily *Ae. aegypti* and *Ae. albopictus* [1]. CHIKV was first identified in Tanzania in 1950s [2] and initially caused sporadic outbreaks in Africa and Asia until 2004 [3], when a significant outbreak in Kenya in 2004 marked the beginning of a resurgence of CHIKV, resulting in extensive spread to the Indian Ocean islands, including the Comoros, Seychelles, Mauritius, and the French territories of Mayotte and La Réunion [3]. The epidemiology and transmission patterns of CHIKV shifted notably during the 2005–2006 outbreaks in La Réunion, where *Aedes albopictus* mosquitoes were identified as the primary vector [3,4]. The global spread of CHIKV has been partially attributed to its adaptation to this mosquito species, facilitated by a mutation in the envelope protein 1 (E1) gene resulting in the substitution of E1-A226V [3]. This mutation enhanced the ability of *Aedes albopictus* mosquitoes to transmit the virus to humans [3]. After this adaptation, CHIKV has transmitted to more than 100 countries worldwide between 2014 and 2019 [1]. The CHIKV infects approximately 3 million people annually, with an estimated 1.3 to 2.7 billion people currently residing in areas at risk of CHIKV transmission [5].

Chikungunya virus was first reported in Bangladesh in December 2008 in two adjacent north-western districts, Rajshahi and Chapai Nawabganj. Subsequently, outbreaks were reported in 2009, 2011 and 2012 [6,7]. In 2017, Bangladesh experienced the largest CHIKV outbreak with 13,176 clinically confirmed cases in 17 of 64 districts of the country [8]. A modelling study predicted a peak prevalence of 47 cases per 1,000 people in Dhaka city during the 2017 outbreak [9]. These estimates are significantly higher than the official report of 13,176 total cases between April and September [9]. The study also estimated a very high basic reproduction number (*R0*) of CHIKV (4.20) during the 2017 outbreak [9]. Nationwide surveillance conducted between 2015 and 2016 reported a seroprevalence of 2.4% and predicted 4.99 million people to be infected with CHIKV before the 2017 major outbreak in Bangladesh [10]. However, after 2017, CHIKV had almost disappeared from Bangladesh with a few sporadic cases detected in the country. This study reports an outbreak of CHIKV in Dhaka and its surrounding areas in the late 2024, detailing the clinical & epidemiological features of the outbreak as well as genomic characteristics of the virus.

**Methods:**

**Epidemiological Data collection**

During the third week of October 2024, a Chikungunya outbreak in Dhaka city was detected by the Institute of Epidemiology, Disease Control and Research (IEDCR) through the event-based surveillance system. In response to the outbreak, being the mandated institute for outbreak investigation, control and response in Bangladesh, IEDCR established a sample collection booth to facilitate testing for suspected Chikungunya cases referred by the physicians. A suspected Chikungunya case was defined as any individual presenting with fever and arthralgia/arthritis not attributable to other medical conditions. Data were collected using a pre-designed questionnaire, and informed consent was obtained from all participants during sample collection to include them in the study. A total of 394 suspected cases were enrolled during the period of 19 October to 31 December 2024.

**Sample collection**

Following aseptic procedures, 3–4 mL of blood was collected from each participant into tubes containing a blood clot activator. The serum was then separated and stored at 4°C refrigerator at IEDCR until further testing could be performed. Positive serum samples were stored at -800C for further analysis.

**RT-PCR testing**

Viral RNA was extracted from 140 µL of serum using the QIAamp Viral RNA Mini Kit (QIAGEN, Germany) following the manufacturer's instructions. The RNA was purified and eluted in a final volume of 30 µL.

Real-time reverse transcription PCR (RT-PCR) was performed using the Genesig Dengue, Zika, Chikungunya multiplex RT-PCR Kit (Primer Design, UK) on an ABI QuantStudio 5 thermal cycler. The thermal cycling protocol included the following steps: Reverse Transcription at 550C for 10 mins, enzyme activation at 950C for 2 mins followed by 50 cycles of denaturation at 950C for 10 seconds and annealing & extension at 600C for 1 min. Fluorescence data were collected during the extension phase through the VIC (DENV), FAM (ZIKV), Cy5 (CHIKV), and ROX (internal control) channels. Post-PCR analysis involved evaluating amplification curves on a linear scale. Baseline thresholds were manually set for each run. Amplification curves with a cycle threshold (CT) value of <50 was considered as positive. Amplification of the internal control (IC) confirmed the absence of PCR reaction inhibition.

**E1 Gene Sequencing**

A total of 12 samples with cycle threshold (*CT*) values of <27 was selected for sequencing of the E1 gene using the Oxford Nanopore Sequencing technology. Viral RNA was extracted from 140 µL of serum using QIAamp Viral RNA mini-Kit (QIAGEN, Germany), converted into first-strand cDNA using LunaScript RT SuperMix (New England Biolabs, USA) according to the manufacturer’s instruction. The cDNA was amplified with Q5 Hot start High fidelity 2X Master mix (New England Biolabs, USA) using the 3 sets of primers described previously [11]. The thermal cycling protocol include: Initial denaturation at 980C for 30 seconds followed by 35 cycles of denaturation at 980C for 10 seconds, annealing at 700C/700C/680C (for primer 19, 20 & 21) for 30 seconds, extension at 720C for 30 seconds and a final extension step at 720C for 2 mins. The primers produced a set of 3 overlapping amplicons of 756, 1014 and 839 bp size. The amplicons were normalized and pooled per sample in equimolar amount and cleaned with AMPure XP beads (Beckman Coulter, USA). The samples were end-repaired with NEBNext Ultra II end repair/dA-tailing module (New England Biolabs, USA), followed by barcoding with EXP-NBD104 (Oxford Nanopore Technologies, UK). The barcoded amplicons were normalized, pooled, and cleaned with AMPure XP beads, followed by final Library preparation with Ligation Sequencing Kit LSK109 (Oxford Nanopore Technologies, UK). A total of 20 fmol of the library was loaded and sequenced in a standard flow cell FLO-MIN106 (version 9.4.1) for 6 hours. MinKNOW v22.12.5 was used for base calling (high accuracy mode) and demultiplexing of raw reads. Minimum read quality was set to 9 for read filtering. The library generated 27195 reads that passed the quality filter. Mean read length was 853 and mean read quality was 11.2. The read length N50 was 860.

The read quality of fastq files was assessed with NanoPlot v1.42 [12]. Read Mapping and alignment were done with minimap2 [13] using the [**NC\_004162**](https://www.ncbi.nlm.nih.gov/nuccore/NC_004162.2) as reference. BAM files were sorted and indexed with samtools v1.19.2 [14]. The consensus sequence was generated with medaka v1.11.3 [15]. The sequences were submitted in the NCBI GenBank with accession no. [**PQ963011**](https://www.ncbi.nlm.nih.gov/nuccore/PQ963011) to [**PQ963022**](https://www.ncbi.nlm.nih.gov/nuccore/PQ963022) and in the EpiArboTM database of GISAID [16] with accession no. **EPI\_ISL\_19683650** to **EPI\_ISL\_19683661**. Genotype was assigned using the Chikungunya typing tool hosted at Genome Detective website [17].

**Phylogenetic analysis**

E1 gene sequences of human Chikungunya viruses circulating in Asia were retrieved from the GISAID database. Sequences with long stretches of ‘N’ and without complete collection date was excluded. A time-resolved maximum likelihood (ML) phylogenetic tree was constructed, refined, and annotated using the Nextstrain tool **Augur** [18–21]. The tree was exported and visualized using **Auspice** [18]. The final dataset included 286 E1 genes of human Chikungunya virus from 13 Asian countries.

**Statistical analysis**

We examined the associations of outcomes variable, disease severity and with different independent variables using modified Poisson regression model. A generalized estimating equation-modified Poisson regression approach with a robust error variance option was employed to directly assess risk ratios (RRs) accompanied by 95% confidence intervals (CIs) for significance testing. Data analysis was performed using the latest version of R software [22].

**Results:**

We tested a total of 394 suspected Chikungunya patients, of which 138 (35%) were confirmed positive for CHIKV**.** Additionally, 11 samples (2.8%) tested positive for Dengue virus (DENV). No co-infection with both CHIKV and DENV was detected in any of the positive cases. Among the 138 CHIKV-positive cases, the majority were male (n = 89, 64.5%) and aged ≥30 years (n = 115, 83.3%). Two of the positive case had travelled internationally within two weeks prior to symptom onset. The most common clinical symptoms included fever (100%), arthralgia (97.8%), myalgia (83.2%) and headache (64.9%). Over 47% of patients (n = 65) had at least one comorbidity, with hypertension (26.1%, n = 36) and diabetes mellitus (26.1%, n = 36) being the most prevalent. Most patients were recruited during December (n=75), while the rest were in November (n=58) and October (n=5) **(Figure 1)**.

Most of the patients were recruited from Dhaka City (72 from Dhaka South City Corporation and 64 from Dhaka North City Corporation) while only two were from outside Dhaka City, one being in Narayanganj district and another one in Keraniganj, a subdistrict of Dhaka **(Figure 2).** The maximum distance between the two geographically separated cases was 12.21 kilometers.

We followed up with 58 patients (42.0%) to assess their health outcomes between 21 and 28 days after the initial illness. No patient died, however, 47 out of 58 patients (81.0%) reported persistent symptoms during the follow-up period, while only 7 patients (12.1%) had fully recovered. The most common persistent symptoms were joint pain (96.0%), fatigue (29.4%) and joint swelling (19.6%). On average, patients lost 10.5 working days (range: 3–60 days) due to CHIKV infection. Considering the daily per capita income of USD 6.98 in Bangladesh [23], the disease caused an average household income loss of USD 73.3 per patient.

Among the 138 CHIKV-positive cases 20 (14.5%) patients require hospitalization with a mean duration of 5.9 days of hospital stay. The mean (range) age of the hospitalized patients was 52 (15-78) years. The most common comorbidity of the patients who required hospitalization was diabetes mellitus (45%), hypertension (45%), followed by ischemic heart disease (25%). The hospitalization was associated with the older age group (≥30 years) (Incidence rate ratio (IRR): 1.14, 95% Confidence interval: 1.02-1.28), and any comorbidity (IRR: 1.04, 95% CI: 0.92-1.19).

The sequencing library generated 27,195 reads that passed the quality filter. The mean read length was 853 and the mean read quality was 11.2. The read length N50 was 860. The average depth of coverage ranges from 413x – 1250x. The 12 E1 gene sequences in this study has a length of 1510 bp. They exhibit >99% nucleotide similarity among themselves. Compared to the reference sequence[**NC\_004162**](https://www.ncbi.nlm.nih.gov/nuccore/NC_004162.2),the sequences shared 97% - 97.2% identity at the nucleotide level and 98.2% - 98.6% identity at the amino acid level. All samples were assigned to the East-Central-South-African (ECSA) genotype. Important amino acid mutations were found in the sequences D284E, I55V, K211E, M269V, V322A & V367A. Sequencing findings and related metadata are summarized in **Table 2.**

Phylogenetic analysis showed, all the sequenced cases were closely related and form a cluster within the previously circulating Indian Ocean Lineage (IOL) of the ECSA genotype **(Figure 3).** This cluster is related more distantly to the previously circulating Bangladeshi strains. The analyses indicate that their most recent common ancestor has evolved from the previously circulating strains in the country during the early part of 2019 (CI: 2018-03-04, 2020-11-04). The phylogeography map indicates a few events of viral exchange with the neighbouring countries, India, Thailand, and China **(Figure 4).**

**Discussion:**

The re-emergence of the CHIKV in Dhaka, Bangladesh, came after a near disappearance of the virus following the 2017 outbreak highlighting a serious concern, especially in the context of the ongoing and large-scale dengue epidemic in Bangladesh, which continues to place significant strain on public health systems [24,25]. Although this study is limited to a single centre and may be subject to bias, the clinical characteristics observed are consistent with previous reports of CHIKV infections, with fever, arthralgia, myalgia, and headache being the predominant symptoms [1]. Importantly, majority of the patients who were followed up reported persistent symptoms at 28 days follow-up, underscoring the potential long-term health impact of CHIKV infections, even in the absence of fatalities.

No co-infection with CHIKV and DENV was detected in our study, although such co-infections are not uncommon, as reported in several studies [26]. The clinical impact of CHIKV and DENV co-infection remains controversial; while some studies have reported more severe clinical manifestations and longer hospital stays in co-infected patients, others have found no significant difference [26]. Given the overlapping symptoms of both diseases, misdiagnosis is likely if diagnostic testing targets only one virus, particularly in regions where both CHIKV and DENV are endemic.

The study also offers valuable insights into disease severity, revealing that it is higher in individuals over 30 years old and those with multiple comorbidities. The hospitalization of 14.5% of patients and the resulting loss of workdays further highlight the significant social and economic burden of the outbreak. This is especially concerning given the fact that, the healthcare systems are already strained by the ongoing dengue crisis [24].

The phylogenetic analysis revealed the evolution and emergence of a new sub-lineage within the previously circulating ECSA genotype of the virus in Bangladesh. These outbreak strains are closely related and their most recent common ancestor likely evolved during early 2019, from the previously circulating local strains rather than being introduced from external sources. Notably, the sequences in this study lack the E1-A226V substitution but carry the E1-K211E. Chikungunya viruses sequenced during the 2017 outbreak in Bangladesh were also found to harbour this substitution [27]. This mutation was first detected in Chikungunya viruses causing outbreak in India during 2016 and spread extensively onwards to the countries in the region including Bangladesh, Pakistan, Maldives, Myanmar, Thailand and also to the African countries like Kenya [28]. Previous studies have shown that the E1-K211E substitution enhances the virus's fitness in *Aedes aegypti* mosquitoes resulting in increased replication and transmission of the virus in mosquito contributing to the major outbreaks [29]. Given the abundance of this mosquito species in urban areas of Bangladesh [30], this finding raises serious concerns about the potential for large-scale outbreaks in major cities across the country.

Bangladesh provides a highly conducive environment for *Aedes* mosquito breeding, driven by factors such as rapid urbanization, extended rainfall, and widespread presence of mosquito breeding sites [31]. Additionally, the country’s temperature remains highly favourable for Aedes mosquitoes for approximately nine out of the twelve months each year [31]. The basic reproduction number (*R0*) for Chikungunya virus has been estimated at 3.4 globally [32] and as high as 4.2 during the 2017 outbreak in Bangladesh [9] suggesting the potential for a large scale CHIKV outbreak in the near future possibly in 2025 or 2026. In 2023, Bangladesh experienced the largest dengue outbreak in its history, leading to a national crisis involving shortages of intravenous saline solutions and hospital beds [24]. In this context, the present study underscores the need for a comprehensive arboviral surveillance in the country. The study also serves as a crucial alert to prepare for a potential large-scale outbreak of CHIKV in near future.

**Conflicts of interest**

The authors declare no conflict of interest.

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**Ethics statement**

This study is a part of the response to chikungunya outbreaks in Bangladesh, 2024 and thus exempted from the formal ethical application. There are no identifiable individual-level data, and ethical approval is not required.

**Author Contributions**

Conceptualization: AON, and NH. Data curation: AON, MNH. Formal Analysis: AON, MNH, NH. Writing original draft: NH & AON. Supervision: TS and ANA. Writing, review, and editing: IM, OQ, MRH, MHK, SS, JF, KTPP, MR, MR, ANA, TS.

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**Figures:**

**Figure 1**: The daily recruited confirmed Chikungunya cases at the Institute of Epidemiology, Disease Control and Research (IEDCR), Bangladesh between 16 October and 31 December 2024.

A graph of different types of data

Description automatically generated with medium confidence

**Figure 2:** The geographical location of Chikungunya cases in Bangladesh between 16 October 2024 and 31 December 2024.

A map of patients with locations

AI-generated content may be incorrect.

**Figure 3:** Time-scaled phylogeny of Chikungunya viruses circulating in Asia showing 286 genomes sampled between June 2006 and November 2024. The colour of the tips indicated the host country of the taxa. Branch colour indicated the inferred ancestral geographic location of the descendants.Genotype was indicated adjacent to the key branches. The number above the black arrow denoted the inferred year of introduction of the currently circulating lineage in Bangladesh. The green triangle indicates the sequenced samples in this study. Numbers in the X axis represent the time in the unit of year.



**Figure 4:** Geographical transmission map of Chikungunya viruses in Asia showing the regional movement of viruses. The placement of the circles (demes) in the map is according to the sampling location. The size of the demes indicates the number of sequences sampled from a specific country. Colours of the demes are according to the Countries indicated in the legend. The shape & colour of the transmission lines denotes the direction of the virus movement.

A map of the world

AI-generated content may be incorrect.

**Table 1:** Factors associated with hospitalization and persistent symptoms at 28 days follow-up of Chikungunya-positive patients in Bangladesh between 16 October and 31 December 2024, using a Modified Poisson regression model

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Hospitalization** | | **Persistent symptoms at 28-day follow-up** | |
|  |  | **IRR (95% CI)** | **P-value** | **IRR (95% CI)** | **P-value** |
| **Age groups** | <30 | Reference |  | Reference |  |
|  | ≥30 | 1.02 (0.92 - 1.12) | 0.741 | 1.18 (0.97 – 1.43) | 0.092 |
| **Sex** | Female | Reference |  | Reference |  |
|  | Male | 1.06 (0.94- 1.19) | 0.331 | 0.92 (0.78 - 1.10) | 0.359 |
| **Respiratory Disease** | No | Reference |  | Reference |  |
|  | Yes | 1.10 (0.91 - 1.34) | 0.321 | 1.08 (0.87 - 1.34) | 0.505 |
| **Cardiometabolic Disease** | No | Reference |  | Reference |  |
|  | Yes | 1.28 (1.11 - 1.47) | 0.005 | 1.05 (0.88 - 1.24) | 0.613 |
| **Chronic Disease** | No | Reference |  | Reference |  |
|  | Yes | 1.44 (1.04 - 1.99) | 0.029 | 1.30 (0.91 - 1.86) | 0.152 |
| **Symptoms** | <=4 | Reference |  | Reference |  |
|  | >4 | 1.14 (1.02 - 1.27) | 0.020 | 1.19 (1.01 - 1.39) | 0.036 |



**Table 2:** Sequencing findings and related metadata of 12 Chikungunya Viruses from 2024 Outbreak

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Serial** | **Sequence ID** | **Collection Date (YYYY-MM-DD)** | **Geographical Location** | **Sex** | **Age (years)** | **Patient status during Sample collection** | **Accession (GISAID/**  **GenBank)** | **Sequence Length** | **Sequencing Depth (×)** | **Genotype** | **Mutations** |
| 1 | **OIS23-288** | 2024-11-07 | Dhaka | F | 49 | Live | EPI\_ISL\_19683650/ [PQ963011](https://www.ncbi.nlm.nih.gov/nuccore/PQ963011) | 1510 | 987 | ECSA | D284E, I55V, K211E, M269V, V322A |
| 2 | **OIS23-299** | 2024-11-10 | Dhaka | M | 48 | Live | EPI\_ISL\_19683651/ [PQ963012](https://www.ncbi.nlm.nih.gov/nuccore/PQ963012) | 1510 | 1147 | ECSA | D284E, I55V, K211E, M269V |
| 3 | **OIS23-306** | 2024-11-12 | Dhaka | F | 32 | Live | EPI\_ISL\_19683652/ [PQ963013](https://www.ncbi.nlm.nih.gov/nuccore/PQ963013) | 1510 | 771 | ECSA | D284E, I55V, K211E, M269V |
| 4 | **OIS23-309** | 2024-11-12 | Dhaka | M | 32 | Live | EPI\_ISL\_19683653/ [PQ963014](https://www.ncbi.nlm.nih.gov/nuccore/PQ963014) | 1510 | 413 | ECSA | D284E, I55V, K211E, M269V, V322A |
| 5 | **OIS23-313** | 2024-11-13 | Dhaka | M | 60 | Live | EPI\_ISL\_19683654/ [PQ963015](https://www.ncbi.nlm.nih.gov/nuccore/PQ963015) | 1510 | 853 | ECSA | D284E, I55V, K211E, M269V, V322A |
| 6 | OIS23-347 | 2024-11-17 | Dhaka | F | 35 | Hospitalized | EPI\_ISL\_19683655/ [PQ963016](https://www.ncbi.nlm.nih.gov/nuccore/PQ963016) | 1510 | 1250 | ECSA | D284E, I55V, K211E, M269V, V322A |
| 7 | OIS23-351 | 2024-11-18 | Dhaka | F | 37 | Live | EPI\_ISL\_19683656/ [PQ963017](https://www.ncbi.nlm.nih.gov/nuccore/PQ963017) | 1510 | 975 | ECSA | D284E, I55V, K211E, M269V, V322A |
| 8 | OIS23-352 | 2024-11-18 | Dhaka | M | 30 | Live | EPI\_ISL\_19683657/ [PQ963018](https://www.ncbi.nlm.nih.gov/nuccore/PQ963018) | 1510 | 1021 | ECSA | D284E, I55V, K211E, M269V |
| 9 | OIS23-365 | 2024-11-19 | Dhaka | F | 31 | Live | EPI\_ISL\_19683658/ [PQ963019](https://www.ncbi.nlm.nih.gov/nuccore/PQ963019) | 1510 | 490 | ECSA | D284E, I55V, K211E, M269V, V322A |
| 10 | OIS23-373 | 2024-11-20 | Dhaka | M | 56 | Live | EPI\_ISL\_19683659/ [PQ963020](https://www.ncbi.nlm.nih.gov/nuccore/PQ963020) | 1510 | 499 | ECSA | D284E, I55V, K211E, M269V |
| 11 | OIS23-384 | 2024-11-21 | Dhaka | F | 48 | Live | EPI\_ISL\_19683660/ [PQ963021](https://www.ncbi.nlm.nih.gov/nuccore/PQ963021) | 1510 | 857 | ECSA | D284E, I55V, K211E, M269V, V367A |
| 12 | OIS23-403 | 2024-11-25 | Dhaka | M | 35 | Hospitalized | EPI\_ISL\_19683661/ [PQ963022](https://www.ncbi.nlm.nih.gov/nuccore/PQ963022) | 1510 | 709 | ECSA | D284E, I55V, K211E, M269V, V322A |