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An outbreak of acute hemorrhagic conjunctivitis due to Coxsackievirus A24 in a residential school, Naharlagun, Arunachal Pradesh: July 2023

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ABSTRACT

Purpose: An acute conjunctivitis outbreak was investigated at a residential school in Naharlagun, Arunachal Pradesh, Northeast India, in July 2023. We aimed to identify the etiological agent and assess any complications in follow-up cases.

Methods: We used a structured questionnaire to record clinical findings and followed up with cases one-month post-conjunctivitis. Sixty-one cases were examined and eight conjunctival and oropharyngeal swab samples were collected after obtaining informed consent from guardians/school authorities. We screened for 33 viral and bacterial pathogens using an IVD-approved Real-time PCR assay. Further, the samples were subjected to nucleic acid sequencing.

Results: Among 465 screened students and staff, 80 individuals (approximately 17.2%) showed acute hemorrhagic conjunctivitis symptoms among which 61 cases were available for clinical examination. We identified the Enterovirus responsible by targeted sequencing using next-generation sequencing. The etiological agent was found to be Coxsackievirus A24, a member of Enterovirus C, in seven out of eight samples subjected to sequencing. Common symptoms included conjunctival hyperemia and foreign body sensation (100%), bilateral eye involvement (73.8%), eye pain (70%), watery discharge (49.2%), and eyelid swelling (38%). Only 6.5% had purulent discharge. Most cases resolved within 5-6 days, with only 9.8% reporting abdominal symptoms post-conjunctivitis. No serious complications occurred within one month. Throat swabs aided in diagnosing enterovirus infections alongside eye swabs.

Conclusions: The outbreak of acute conjunctivitis was caused by Coxsackievirus A24, a member of Enterovirus C. Cases resolved spontaneously within 6-7 days, with no severe complications. Collecting oropharyngeal swabs alongside conjunctival swabs could improve enteroviral conjunctivitis diagnosis.

1. Introduction

Epidemic acute conjunctivitis, commonly known as “red eye” or “pink eye," is a highly contagious eye infection characterized by inflammation of the conjunctiva, the thin clear tissue covering the white part of the eye and the inner surface of the eyelids [1,2]. The condition

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can be caused by both viral and bacterial agents, each with distinct characteristics and implications for treatment and prevention.The leading cause of infectious conjunctivitis is viral conjunctivitis, often succeeded by bacterial conjunctivitis. On the other hand, allergic and toxin-induced conjunctivitis rank among the prevalent non-infectious causes [2]. Bacterial conjunctivitis is typically less common than viral conjunctivitis and is often caused by bacterial species such as *Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae,* and *Moraxella catarrhalis* [3]. Bacterial conjunctivitis tends to present with symptoms such as purulent discharge, redness, and eyelid swelling. Unlike viral conjunctivitis, bacterial conjunctivitis may respond well to antibiotic treatment.

Viruses are the most common causative agents of infectious conjunctivitis. The two primary groups of viruses associated with this condition are adenoviruses and enteroviruses [2]. Adenoviruses are responsible for a significant portion of conjunctivitis cases, causing a wide range of symptoms from mild irritation to more severe discomfort. Enteroviruses, particularly Enterovirus 70 (EV70) and Coxsackievirus A24 (CA24v), are also important viral agents responsible for conjunctivitis outbreaks [4]. CA24v presents as a non-enveloped, positive-stranded RNA virus containing a genome of approximately 7400 base pairs [5].

In India, instances of AHC outbreaks commonly coincide with the seasonal hot and humid weather prevailing in July to September. EV-70 and CA24v have historically been identified as the causative agents behind these epidemics, as documented in earlier reports from India [4, 6–8]. Notably, during earlier outbreaks, neurological manifestations have also been observed in association with EV-70 infections [9,10].

In July 2023, numerous instances of acute conjunctivitis were reported among both students and staff members of schools in Naharlagun, a town in the foothills of the eastern Himalayas in Arunachal Pradesh, Northeast India. Given the potential for an epidemic and the associated complications linked to the outbreak of conjunctivitis, an investigation was launched specifically targeting school children.

2. Methods

An investigation examined a sudden outbreak of acute conjunctivitis at a tribal Abotani residential school in Naharlagun, Arunachal Pradesh, Northeast India, during July 2023. A case investigation sheet was created based on initial cases, defining a 'suspect case' as Conjunctival Hyperemia with ocular symptoms among students and staff from July 1st to 27th. Among 465 screened individuals, 80 (17.2%) showed acute conjunctivitis symptoms. Of these, 61 consented to close examination by Ophthalmologists (See Fig-1). Data included symptom onset, clinical findings, and an inspection of the school campus. One month later, a follow-up was conducted to identify complications.

2.1. Sample collection

To determine the outbreak's cause, eye (conjunctival swab) and throat (oropharyngeal) swabs were collected from eight individuals with informed consent and sent to ICMR-RMRCNE, Dibrugarh, Assam for analysis following standard guidelines.

2.2. Bacterial culture and identification

The swabs were cultured aerobically in Blood agar, MacConkey agar, and Nutrient agar. Identification of the culture isolates was done conventionally by examining colony morphology, staining, and standard biochemical tests. A few of the isolates were subjected to identification and antimicrobial sensitivity testing using the Vitek 2 compact system (Biomerieux, France).

2.3. Screening with real-time reverse transcription PCR assay

In the reference laboratory, an IVD-approved Real-time RT-PCR assay was performed using a commercial IVD-approved kit (FTD Respiratory pathogens 33 assay, and Siemens Health care Pvt. Ltd, Mumbai, India) capable of detecting 20 viral and 13 bacterial pathogens within a period of 3-4 h as per manufacturer's protocol. The kit is based on real-

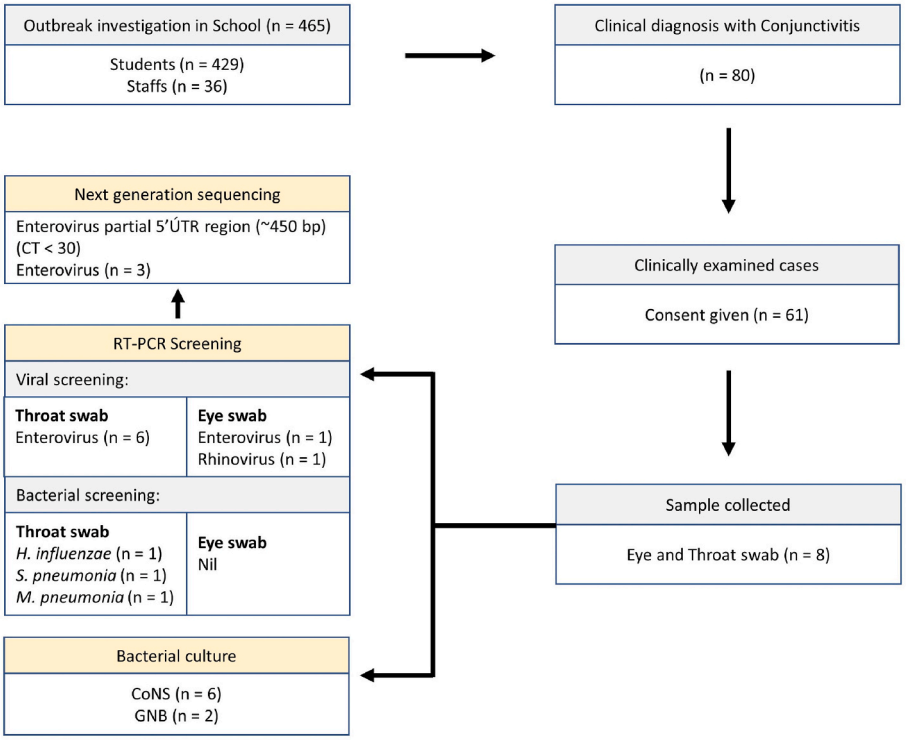

Fig-1. Workflow of the acute conjunctivitis outbreak investigation.

time RT-PCR TaqMan assay and enables the detection of respiratory viruses including human Adenovirus, Enterovirus, human Rhino virus, influenza A virus (IAV), influenza A(H1N1) virus (swine lineage), influenza B virus (IBV), influenza C virus (IVC), human coronaviruses (HCoV) NL63, 229E, OC43 and HKU1, human parainfluenza viruses (HPIV) 1, 2, 3 and 4, human metapneumoviruses (HMPV) A and B, human respiratory syncytial viruses (HRSV) A and B, human parechovirus (HPeV), human bocavirus (HBoV) along with other respiratory bacterial pathogens.

2.4. Conventional reverse transcription PCR (RT-PCR) and agarose gel-electrophoresis

Samples underwent conventional RT-PCR targeting a ~436 bp region in the 5′UTR of Enterovirus for amplification. This process involved reverse transcription PCR using pan-Enterovirus primers (FP: 5′-CAAGCACTTCTGTTTCCCCGG-3′, RP: 5′-ATTGTCACCATAAGCAGCCA-3′) described by Zoll et al., in 1992 [11]. A one-step RT-PCR was performed using the One-Step RT-PCR kit (cat No: 210212, Qiagen, USA). The thermal profile included 30 min of reverse transcription at 50 °C, followed by hot-start/inactivation of RT at 95 °C for 15 min. This was followed by 40 amplification cycles at 94 °C for 30s, 48 °C for 45s, and 72 °C for 45s, with a final extension at 72 °C for 10 min. Amplified products (5 μL) mixed with 6X loading dye (Promega, USA) were electrophoresed using 1.5% agarose gel electrophoresis (100 V for 1 h) stained with 10 μg/ml ethidium bromide (EtBr). Presence of Enterovirus was determined based on the expected product size of 436 bp. A positive control using archival enterovirus (Coxsackievirus A06) and a negative template control using nuclease-free water were included in the PCR reactions. A 100 bp DNA ladder marker was utilized to visualize and compare amplicon sizes.

Further, to rule out Adenovirus mediated conjunctivitis, all the samples were subjected to screening by conventional PCR targeting the human adenovirus hexon gene by using hexon specific primers (FP: 5′-TTCCCCATGGCICAYAACAC-3′, RP: 5′-CCCTGGTAKCCRATRTTGTA-3′) as described by Zheng et al., 2016 followed by gel electrophoresis to visualize the expected product length of 482 bp [12]. An adenovirus positive control and nuclease free water (NC) were used in PCR for quality assessment.

2.5. Nucleic acid sequencing

The sequencing procedure was performed utilizing the Illumina COVIDSeq Test kit and the enterovirus primers mentioned earlier targeting the 5′UTR region following the manufacturer's guidelines (Illumina, CA, USA). The sequencing was executed on an Illumina MiSeq platform with a 2 × 75 paired-end v3 Flow Cell. Analysis of NGS data was performed employing the Linux Command Line Interface (CLI). The reads were aligned to the reference genome (Enterovirus C accession No. NC_002058.3 and Coxsackievirus A24 accession No. DQ443001.1).

2.6. Evolutionary phylogenetics analysis

To determine the responsible enterovirus variant for the outbreak, we conducted an evolutionary phylogenetic analysis. This involved comparing partial 5′UTR sequences of the enterovirus with closely related reference sequences and those available in NCBI's GenBank. We selected the closest sequences using the Basic Local Alignment Search Tool (BLAST) on the NCBI website.

About 436 base pairs from the Enterovirus sequences' partial 5′UTR region were aligned with Enterovirus types A to F reference sequences using the ClustalW alignment program in MEGA X software. We selected the best nucleotide model using MEGA X's model test and used its parameters to construct an evolutionary phylogenetic tree via maximum likelihood. This tree-building process considered general time reversal, gamma distribution, and invariants for calculating rate differences among sites, with 500 bootstrap iterations to enhance robustness. Evolutionary divergence estimates between sequences were calculated using pair-wise distance with the Maximum Composite Likelihood model, and rate variation among sites was modelled with a gamma distribution. The analysis included 32 nucleotide sequences, and all these evolutionary analyses were conducted in MEGA X.

2.7. Complete sequencing of Coxsackievirus A24 using NGS

For further confirmation of the circulation of Coxsackievirus A24 in the region, all the acute conjunctivitis samples were processed for complete sequencing of Coxsackievirus A24 variant using the overlapping fragment primers (as presented in Supplementary Table 1) covering the full genome of the enteroviral strain (approximately 7.4 kb). The same methodology as described under section 2.5 and 2.6 has been employed for performing the sequencing reaction and post sequencing analysis followed by construction of the phylogenetic tree. The obtained sequences were further aligned with the reference sequences of complete genome of Coxsackievirus A24 from the year 2023 that was circulating in China and Pakistan (Coxsackievirus A24 isolate CA24v/China.Zhongshan/02/2023 with accession number: OR361388.1 and Coxsackievirus A24 isolate CA24v/NIH.PAK/01.2023 with accession number: OR633288.1). A total of 41 nucleotide sequences have been included for evolutionary analysis (nine partial nucleotide sequences of approximately 2000bp, one complete nucleotide sequence of 7.4 kb along with 31 complete reference sequences of Enterovirus A to F).

3. Results

The investigating team examined 61 cases of acute conjunctivitis from 465 school children and staff. The overall attack rate was 13.1 % (61/465), with attack rates of 12.8% (55/429) and 13.8% (5/36) in students and adults (staff) respectively.

3.1. Clinical presentations and complications

Fig. 1 illustrates a workflow detail. The subjects had a median age of 11 years (SD ± 6.65), ranging from 5 to 45 years. Among the conjunctivitis cases, 35 were females (57.3%) and 26 were males (42.7%). The most common symptoms were conjunctival hyperemia and foreign body sensation, observed in all cases. Eye pain was reported by 70%, eyelid swelling by 62.3%, watery discharge by 49.2%, itching by 27.9%, and prodromal symptoms like fever, headache, and malaise by 4.9%. A purulent eye discharge was seen in only 6.5% of cases. Bilateral eye involvement occurred in 73.8% (see Table 1). Importantly, all cases resolved within 5 to 6 days without complications.

Regarding the timeline, the first case at the school showed symptoms on July 16, 2023, with a gradual increase in cases, peaking on July 21, 2023, when 25 new cases were reported. The residential school's living

Table 1
Distribution of Clinical presentation among the cases of suspected acute hemorrhagic conjunctivitis (N = 61).

Sl. No	Clinical Presentation	No. of Cases (%)
1.	Conjunctival Hyperemia	61 (100 %)
	Unilateral	16 (26.2%)
	Bilateral	45 (73.7 %)
2.	Foreign Body Sensation	61 (100%)
3.	Pain	43 (70.5%)
4.	Swelling of Lid	38 (62.3%)
5.	Watery Discharge	30 (49.2%)
6.	Itching	17 (27.9%)
7.	Chemosis	13 (21.3%)
8.	Purulent discharge	04 (6.5%)
9.	Prodromal Symptoms (i.e. Fever, headache, malaise)	03 (4.9%)

conditions involved closely arranged dormitory beds, leading to inevitable close interpersonal contact. Occasional sharing of personal items like towels, beds, and pillows occurred, along with frequent handling of shared objects such as doorknobs, basin taps, and communal toilet facilities.

Out of the 61 students and staff examined, six individuals (9.8%) experienced fever, vomiting, diarrhea, and abdominal cramps during the fourth week of July, following the recovery from conjunctivitis cases.

3.2. Laboratory finding

Among the eight samples that underwent screening, the Real-time RT PCR assay targeting 33 viral and bacterial pathogens identified Enterovirus in seven cases (six in oropharyngeal swab and one in conjunctival swab) (see Fig-2). Additionally, one oropharyngeal swab had a co-infection of Enterovirus and Rhinovirus while the same qPCR assay detected *Haemophilus influenza*, *Streptococcus pneumoniae*, and *Mycoplasma pneumoniae* in three oropharyngeal swabs. However, no bacterial pathogens were detected in conjunctival swabs using the Real-time RT PCR assay. Bacteriological culture of the conjunctival samples revealed primarily bacterial commensals, such as coagulase-negative *Staphylococcus* in six samples. In two conjunctival swabs, non-fermenting gram-negative bacteria were detected through culture, and one sample identified *Acinetobacter Iwoffi*.

In order to determine the specific group and type of Enterovirus, conventional PCR was employed to amplify a partial 5' UTR region (approximately 436 base pairs) in the seven enterovirus-positive samples. Among these, three samples with a Real-time PCR Ct-value of less than 30 were successfully amplified. Subsequently, these three samples underwent nucleic acid sequencing using an NGS platform for sequence analysis. All three sequences (GenBank accession number: OR497189-OR497191) exhibited closest 97% nucleotide similarity with human Coxsackievirus A24, a member of the Enterovirus group C. Consequently, the outbreak of acute hemorrhagic conjunctivitis was definitively attributed to Coxsackievirus A24 within the Enterovirus C group. Further, it is essential to mention that the results obtained from FTD real time RT PCR assay and conventional Adenovirus PCR confirmed that the samples were negative for Adenovirus and positive for Enterovirus.

Additionally, phylogenetic analysis grouped the three sequences from the outbreak from Arunachal Pradesh along with the reference sequence of Enterovirus C with the closest sequence with Coxsackievirus A24 (DQ44301.1) strain collected in 2005 from Singapore and elsewhere. (See supplementary Figure 1). Pair-wise distance analysis revealed that Coxsackievirus A24 (DQ44301.1) strain had a pair-wise distance divergence of 4.3% from the outbreak strains of Arunachal Pradesh based on the partial 5′UTR region. Further, the outbreak strain showed an average p-distance of 20% from the reference poliovirus strain (NC_002058.3) and enterovirus D70 strain (MT081378.1).

Notably, complete sequencing of Coxsackievirus A24 was tried using targeted sequencing methodology. However, one Enterovirus positive sample from Nagaland could be successfully sequenced for whole genome (around 7.4 kb) and other samples including the Naharlagun, Arunachal Pradesh samples could be partially sequenced covering the protease (3C) and RdRp (3D) regions (around 1800-2300bp). The NGS analysis revealed 100% resemblance of the target sequences with the latest available sequences of Coxsackievirus A 24 in GenBank that were circulating in China and Pakistan in 2023 during the same study period (Coxsackievirus A24 isolate CA24v/China.Zhongshan/02/2023 with accession number: OR361388.1 and Coxsackievirus A24 isolate CA24v/

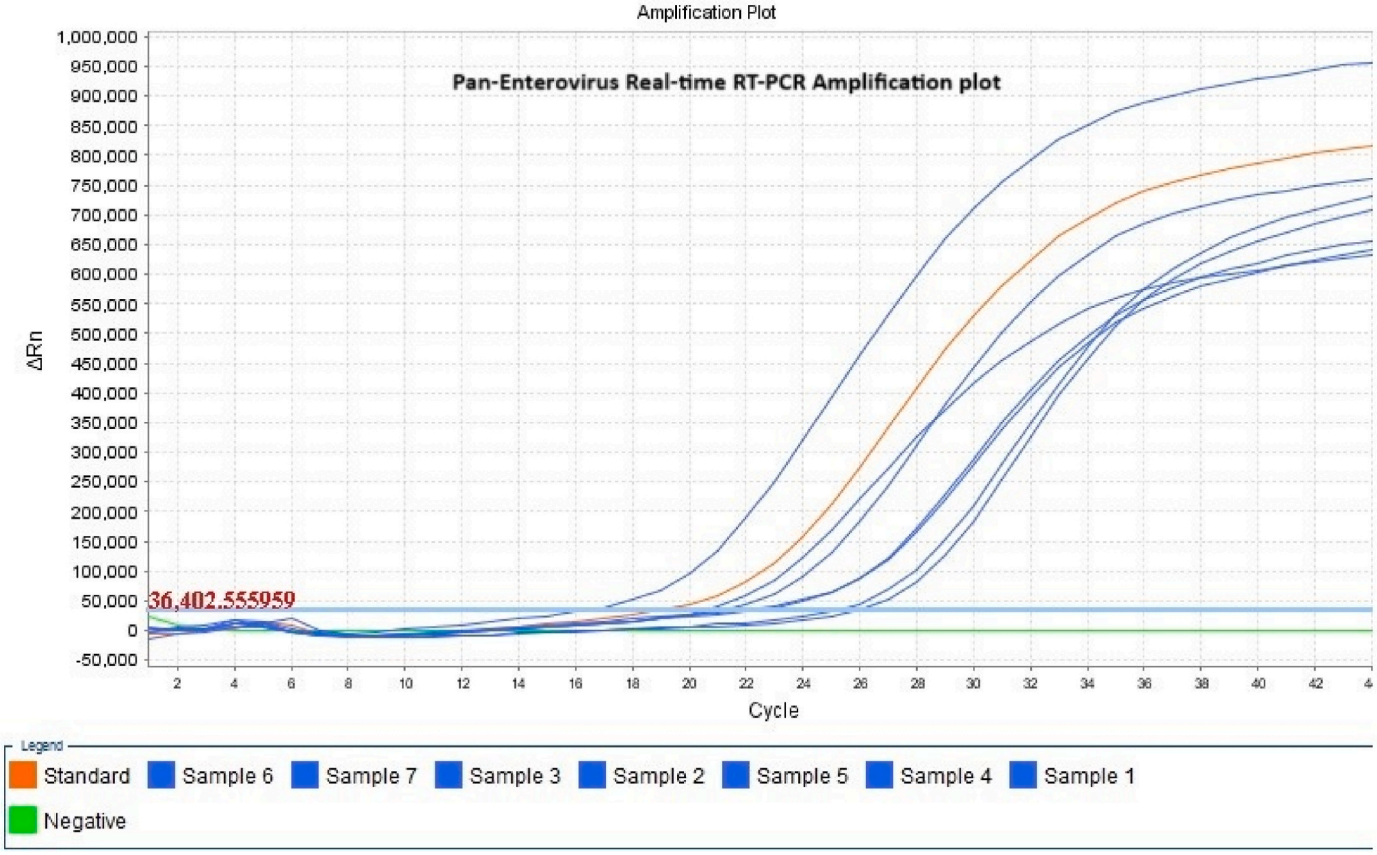

Fig-2. The amplification curve obtained through real-time RT-PCR assay for Pan-Enterovirus detection is shown. The positive control, represented by the red curve, consisted of an archival sample that tested positive for Coxsackievirus A06. In contrast, the enterovirus clinical samples collected during the outbreak are indicated in blue.

NIH.PAK/01.2023 with accession number: OR633288.1) confirming the circulation of the Coxsackievirus A24 in the Northeast region during the period. The newly obtained sequences have been submitted to NCBI GenBank (GenBank accession numbers: PP327391-PP327408).

From the evolutionary analysis, it was noted that all the sequences from the present study including the Naharlagun samples were clustered together with the reference sequences of Coxsackievirus A24 of Enterovirus C family showing 100% resemblance with Coxsackievirus A24 strain collected from China and Pakistan during 2023 as presented in Fig. 3 (accession numbers: OR361388.1 and OR633288.1).

4. Discussion

Conjunctivitis comprises a range of eye diseases marked by conjunctival inflammation, with around 80% of cases being viral, closely followed by bacterial infections. Non-infectious types include allergic, mechanical/irritative/toxic, and immune-mediated forms [14,15]. Viral conjunctivitis includes epidemic keratoconjunctivitis (EKC) and pharyngoconjunctival fever (PCF), both caused by adenovirus, while acute hemorrhagic conjunctivitis (AHC) is associated with enteroviruses like EV70 and CA24v [2,15]. Adenovirus primarily causes viral acute conjunctivitis, especially EKC, linked to adenovirus types 8, 37, 53, 54, 56, 64, and 85 [16]. However, PCR based initial screening data from the present study indicated that the samples were negative for adenovirus hence ruling out the possibility of adenovirus mediated epidemic keratoconjunctivitis. Enteroviruses, notably EV70 and CA24v, cause highly contagious epidemic AHC, leading to rapid community-wide outbreaks [2,4,14,15], characterized by watery discharge, photophobia, and foreign body sensation, with milder symptoms compared to EKC and subconjunctival hemorrhage as a distinguishing feature [15].

AHC is closely associated with human enterovirus groups A-D [17], with Coxsackievirus A24 (Enterovirus C) and Enterovirus-70 (Enterovirus D) being major culprits [18,19]. Both adenoviral and enteroviral conjunctivitis spread rapidly through hand-to-eye contact and contaminated items like towels and solutions. Challenges such as inaccurate diagnosis, self-medication, and delayed care hinder accurate epidemiological data collection on conjunctivitis [15,18].

Since its discovery in 1969, AHC has triggered global outbreaks, often following seasonal patterns [15,20]. India has experienced several AHC outbreaks in the past [4,21–25], including major ones in 1981 associated with Enterovirus 70, leading to poliomyelitis-like neurological complications [9,10,26]. In 2021, amid the COVID-19 pandemic in Madurai, India, Adenovirus caused most outpatient conjunctivitis cases (75%), followed by SARS-CoV-2 (11%) [27]. India is currently witnessing a surge in conjunctivitis cases [28].

Our study, utilizing Real-time PCR and targeted sequencing, confirmed that AHC at a residential school in Arunachal Pradesh was caused by Coxsackievirus A24 (CA24v), a member of Enterovirus group C. Phylogenetic analysis revealed the closest resemblance to Madagascar's 2011 and Singapore's 2005 CA24v strains (Accession numbers OK570202 and DQ44301.1). We focused on sequencing the non-coding 5′ untranslated region (5′ UTR) of the viral genome, which serves as an internal ribosome entry site (IRES), crucial for translating viral RNA into viral proteins [29]. Our contention on the fact that the present outbreak was associated with Coxsackievirus A24 was further supported by the

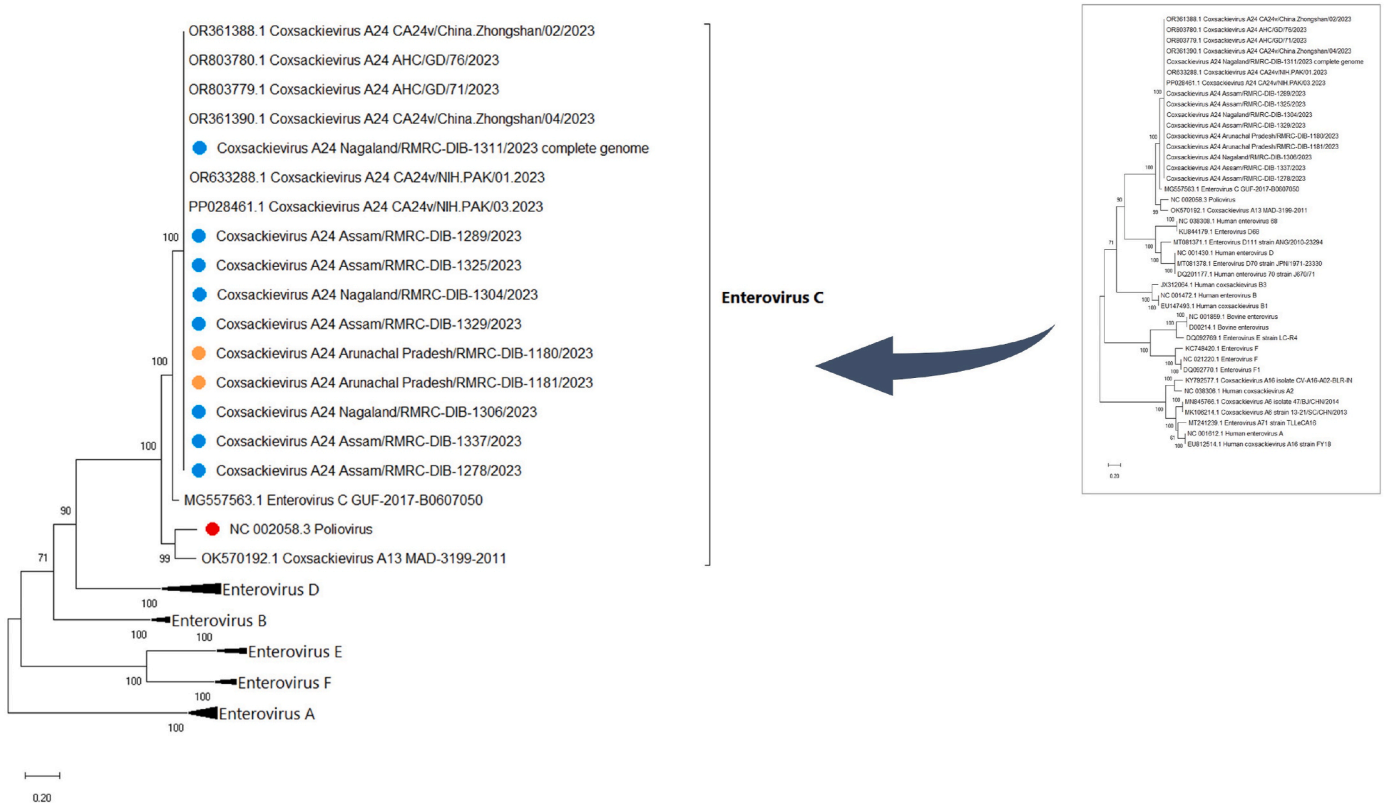

Fig-3. The Maximum Likelihood method and General Time Reversible model were used to infer the evolutionary tree [13]. This analysis involved 41 nucleotide sequences of which 31 nucleotide sequences are complete genome sequences of Enterovirus including reference sequences. Additionally, 10 nucleotide sequences have been taken from the present study (01 complete sequence, 07 partial sequences (~2000 bp) marked in blue and 02 partial sequences (~2000 bp) from Naharlagun marked in orange dot). A 500-bootstrap iterations were performed to make the tree robust. Evolutionary analyses were conducted in MEGA X. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Apart from Enterovirus C, other sequences of Enterovirus groups are collapsed.

complete sequencing data from our laboratory which revealed approximately 100% resemblance of the target sequences with the latest available complete sequences of Coxsackievirus A24 circulating in China (OR361388.1) and Pakistan (OR633288.1) during 2023 [30]. Notably, this highlights that Coxsackievirus A24 was responsible for the similar conjunctivitis outbreaks that occurred in these two countries. Typically, genotyping and molecular characterization of enterovirus variants rely on the 3C pro and VP1 genes due to their higher variability within the virus [31]. Previous evolutionary analysis based on the viral proteinase 3C region suggested that CA24v originated from a shared ancestor emerging in a single geographical location in 1963, approximately seven years before its initial isolation in Singapore. This analysis also demonstrated divergence among epidemic isolates from 1985 to 1989, including strains from Asia and Ghana, after 1981 [32]. So far, worldwide CA24v variants have been categorized into eight genotypes, labeled as G I through G VIII [20].

The exact cause of the ongoing AHC epidemic in India attributed to CA24v remains unclear. CA24v frequently undergoes rapid changes in its antigenic epitopes, precipitating sudden outbreaks. CA24v exhibits a high estimated evolutionary rate, approximately 3.0-3.7 × 10-3 substitutions per site per year, akin to other RNA viruses such as Influenza A [31].

Our findings align with prior Indian studies identifying CA24v-mediated AHC through typing and analysis [4,25]. Clinical symptoms, including subconjunctival hemorrhage, pain, and watery discharge, supported our results. Notably, 9.8% (6/61) of our study patients exhibited enteroviral infection characteristics like fever, vomiting, diarrhea, and abdominal cramps following conjunctivitis.

Among eight throat swab samples, 75% (6/8) were Enterovirus-positive, with *Haemophilus influenza* and *Streptococcus pneumoniae* detected once each. Detecting Enterovirus in throat swabs among conjunctivitis cases isn't unexpected due to nasolacrimal duct drainage into the upper respiratory system. Notably, the conjunctival swab-positive sample was collected on the day of symptom onset, while other eye swab negative but throat swab positive samples had symptoms 6 to 10 days earlier. This detection variation might relate to symptom onset duration. Early eye swab testing (within 2-3 days) could improve Enterovirus detection in conjunctival samples. Thus, for longer onset periods, a dual oropharyngeal and conjunctival swab could be more suitable than conjunctival swabs alone. Based on our study, dual sampling (throat and eye swab) could enhance Enterovirus-related conjunctivitis diagnosis. Our findings align with Kuo et al., 2010, who found Coxsackievirus A24 in throat and rectal swabs alongside conjunctival swabs, suggesting their suitability as sample types [33].

The current study had certain limitations that need to be acknowledged. These limitations encompass a limited number of representative samples and a focus on a single school. Moreover, a time gap of approximately 6-10 days between the onset of symptoms and the collection of samples potentially contributed to negative results in conjunctival swabs for enterovirus in the majority of cases.

In conclusion, the investigation determined that the outbreak of acute hemorrhagic conjunctivitis was attributed to Coxsackievirus A24, a member of Enterovirus C. Furthermore, it was observed that cases resolved spontaneously with supportive treatment within a span of 6 to 7 days, and no severe complications were documented, except for a small number of cases experiencing diarrhea 5 to 10 days after the conjunctivitis episodes. An interesting observation was that including oropharyngeal samples can increase the sensitivity of enteroviral conjunctivitis diagnosis, particularly in instances where there was a delay in collecting samples.

To prevent the spread of the conjunctivitis outbreak in a residential school, frequent hand washing and wearing of glasses should be implemented as compulsory practice for students and teachers infected with the pathogen. In addition, awareness programs should be organized for students, parents, and teachers of the school to educate them regarding the etiology and preventive measures of the disease [1]. Once detected, the patient should be immediately isolated from other individuals and sent to a primary health care facility for treatment.

Supplementary Figure 1: The Maximum Likelihood method and General Time Reversible model were used to infer the evolutionary tree [13]. The analysis included 32 nucleotide sequences (29 complete genomes of Enterovirus, including GenBank reference sequences, and three partial 5′UTR region sequences from this study marked with blue dots). A 500-bootstrap iterations was performed to make the tree robust. MEGA X conducted the evolutionary analyses, using a discrete Gamma distribution to account for rate differences among sites. The rate variation model allowed for some sites to remain evolutionarily invariable. The tree is drawn to scale, showing branch lengths as substitutions per site. Sequences from Enterovirus groups other than Enterovirus C, are collapsed.

Contribution

PB and BB drafted the original manuscript. PB, TG, YK, NE, MJ and LJ performed the outbreak investigation and clinical examinations. SJP performed the bacteriological test and culture. NS, AIS, and CB performed molecular tests including Sequencing by NGS. NS, BB and NKB did the literature search and performed partial drafting of the manuscript. BB and NKB performed the molecular analysis and overall review of the manuscript.

Source of funding

The financial support for conducting the laboratory investigations is provided by the Department of Health Research (DHR), Government of India, under the Scheme for Regional VRDL located in Dibrugarh, Assam.

Ethical statement

The Institution responsible for the outbreak investigation had Institutional ethical approval from ICMR-RMRC NE Dibrugarh under the DHR-funded Regional VRDL scheme which has an objective to study outbreaks and epidemics of viral infections in Northeast India.The institutional ethics committee of ICMR-RMRC Dibrugarh approves the schemevide letter No.RMRC/Dib/IEC (Human)/2019-20/135 date:12/08/2020.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. B. Borkakoty reports financial support was provided by Department of Health Research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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