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Short Report

A case report of the enterovirus-D68 associated severe acute respiratory illness in a pediatric case from India

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A B S T R A C T

Since, early 2000s, there have been several clusters of enterovirus-D68 (EV D68) associated respiratory illness reported from various countries. Recent largest and most wide-spread outbreak of EV-D68 associated severe acute respiratory illness (SARI) occurred in North America. Present report describes a case of EV-D68 associated severe acute respiratory illness from India with a whole genome sequence. The case was identified through retrospective analysis of Influenza SARI surveillance sample collected during September 2017 using Next Generation sequencing. EV D68 positive child aged two years and presented with asthma like symptoms for which he was admitted to ICU. The child tested negative for Influenza, RSV, Rhinovirus, PIV, HMPV and adenovirus, on real time RT-PCR. And on NGS full EV D68 genome was retrieved belonging to sub-clade B3. In ICU, child received anti-bacterial and anti-viral therapy. The child recovered without any sequelae and was discharged one week later. Present report highlights the importance of studying this emergent virus EV-D68 through prospective studies to understand the burden and epidemiological pattern in the country and its implications.

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Introduction

Enterovirus D68 (EV-D68) was first described in 1962 among four Californian children admitted in hospital with pneumonia [1]. After its discovery, EV-D68 detection has rarely been reported for causing any respiratory illness. But since the early 2000s, there have been several clusters of EV D68 associated respiratory illness reported from various countries [2]. In 2014 [August–October], the largest and most widespread outbreak of EV-D68 associated with severe acute respiratory illness (SARI) occurred in North America, affecting over 1153 people in 49 states and 14 deaths [3,4]. Similar reports of respiratory illness by EV-D68 have been documented in Europe, Thailand, China, New Zealand and several African countries [5]. Increasing number of EV-D68 associated severe respiratory infection cases and neurological complications has raised concern in the international community [6].

The EV-D68 case was identified through the retrospective analysis of respiratory samples collected under Influenza surveillance during 2017. The retrospective analysis was performed using Next Generation sequencing (NGS). Influenza surveillance network is operational through sentinel centers collecting samples from both acute respiratory illness (ARI) and SARI cases. Collected Nasal/throat swab are processed using real time RT-PCR for the panel of respiratory viruses viz Influenza A and B, Respiratory syncytial virus (RSV), Para-Influenza 1–4 (PIV), Adenovirus, Rhinovirus, human metapneumovirus (HMPV) and corona-virus. To discover the unknown RNA or DNA viral or bacterial agents from the test negative samples, a retrospective analysis/testing was performed using NGS on randomly selected 18 test negative samples (2% of the total negative samples) representative of the whole year i.e. two sample per month from Influenza season months which are February to April and July to September while one sample per month from rest of the non-season months.

Case summary

The identified EV-D68 positive case was from a SARI sample collected under Influenza surveillance during September 2017. The case is a two-year-old male child, resident of Purandhar village, Solapur, Maharashtra, India. At the time of admission, his main complaints were fever, sore throat, and breathlessness for which he was admitted in the pediatric intensive care unit (PICU) at a
tertiary care institute. Parents gave history of child having chills, nasal discharge and cough with sputum. There was no history of diarrhea or vomiting. On general examination, the child was conscious and cooperative; pallor was present, axillary temperature measured 38.8°C, respiratory rate was 40 per min, pulse rate 110/min, Body mass index and mid arm circumference was 15.6 kg/m² and 12.4 cm respectively. There was no evidence of cyanosis, icterus, clubbing or any significant lymphadenopathy. On respiratory system examination, chest in-drawing was present, focal chest signs showed grunting, wheezing and crepitation. No abnormalities were detected on systemic examination. Hematological analysis revealed decreased hemoglobin level (7.6 mg %) with raised White Blood cell (WBC) Count (13 thousand / micro liter) and platelet count was normal (387000/ micro liter). The X-ray chest suggested left lower lobe pneumonia with pleural effusion.

**Confirming diagnosis**

Nasal swab collected on the day of admission from the patient tested negative for Influenza, RSV, Rhinovirus, PIV, hMPV and adenovirus, on real time RT-PCR. RNA extraction was performed on the clinical samples using the QiAamp Viral RNA extraction kit (Qia-gen). RNA quantification, RNA depletion, library preparation, and RNA library quantification were performed [7]. The samples were loaded on the Illumina Miniseq platform for sequencing. The reads generated from the machine was analyzed using the CLC Genomics Workbench (CLC, version 10.1.1). De novo contigs of size greater than 1000 nucleotide were generated which were further identified by performing BLAST. The causative agent was identified to be Human EV-D68 after performing the BLAST search of 109 contigs. The complete genome of 7345 nucleotides was retrieved encoding a single open reading frame starting with 5' UTR and ending with 3' UTR along with a poly (A) tail. The accession number of the sequence submitted to the GenBank (MH330334) is publicly available.

**Phylogenetic analysis**

Evolutionary analysis for the retrieved Viral protein1 (VP1) gene was performed from the clinical specimen. Reference sequences

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**Fig. 1.** Phylogenetic tree of VP1 gene.
of different clades were downloaded from the GenBank database. These sequences were aligned using the ClustalW algorithm as implemented in MEGA software version 7.0 [8]. A maximum-likelihood method based phylogenetic tree was generated using the Tamura-3 parameter as the nucleotide substitution model with gamma distribution across sites. A bootstrap replication of 1000 replication was used for the statistical assessment of the generated tree. Phylogenetic analysis of the VP1 gene sequence demonstrated that it clustered with the B3 subclade (Fig. 1, Table 1). Report of severe neurological manifestation that includes acute flaccid paralysis has made this analysis intriguing [9].

Treatment received and outcome

Patient was started on broad-spectrum antibacterial and antiviral (oseltamivir) along with anti-pyretic. The child recovered without any sequelae and was discharged after one week of admission.

Discussion

Retrospective analysis using NGS of SARI sample revealed full genome of causative agent as Human EV-D68 in one of the test negative sample. EV-D68 positive child presented with asthma like symptoms. Similar presentation among pediatric age was observed in an EV-D68 outbreak in North America with clustering of cases from August to October months [3]. EV-D68 sub-clade B3 outbreak has been reported from Sweden, Italy and Netherlands [10]. In recent years, there has been significantly increased in EV-D68 associated respiratory illness from around the world [11]. Increased EV-D68 detection in recent years may be because of availability of EV D68 specific RT-PCR. However, the retrospective analysis done on the stored samples in China suggested that the EV D68 detection has been increased in recent years only [12]. The mechanism underlying the recent global increase in EV D68 has not been understood. EV-D68 presence has now been confirmed in India and is important as it holds a potential threat of outbreaks of respiratory or neurological illness in the country.

Conclusion

Present report highlights the importance of studying the virus through prospective systematic surveillance both in hospital and community settings to understand the impact and epidemiological pattern in the country.

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Competing interests

None declared.

Ethical approval

Not required.

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References

[1] Schieble JH, Fox VL, Lennette EH. A probable new human picornavirus associated with respiratory disease. Am J Epidemiol 1967;85:297–310.
[2] Centers for Disease Control and Prevention (CDC). Cluster of acute respiratory illness associated with human enterovirus 68-Asia, Europe and United States, 2008–2010. MMWR Morb Mortal Wkly Rep 2011;60:1301–4.
[3] Messacar K, Abzug MJ, Dominguez SR. 2014 outbreak of enterovirus D68 in North America. J Med Virol 2016;88:739–45.
[4] Enterovirus D68. Available online: https://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html. [Accessed 4 July 2018].
[5] Esposito S, Bosis S, Niesters H, Principi N. Enterovirus D68 infection. Viruses 2015;7:6043–50.
[6] Messacar K, Schreiner TL, Maloney JA, Wallace A, Ludke J, Oberste MS, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. Lancet 2015;385:1662–71.
[7] Yadav PD, Albariño CG, Nyayanit DA, Guerrero L, Jenks MH, Sarkale P, et al. Equine encephalitis virus in India. 2008. Emerg Infect Dis 2018;24(5):898–901.
[8] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol Biol Evol 2016;33(7):1870–4.
[9] Wei HY, Yeh T, Hsieh J, Lin I, Yang J. Updates on the molecular epidemiology of enterovirus D68 after installation of screening test among acute flaccid paralysis patients in Taiwan. J Microbiol Immunol Infect 2018;51(5):688–91.
[10] Cyril CY, Janice CL, Sridhar S, David CL, Luk S, Chan RH, et al. First report of a fatal case associated with EV-D68 infection in Hong Kong and emergence of an interclade recombinant in China revealed by genome analysis. Int J Mol Sci 2017;18:1065–81.
[11] Inamura T, Oshitani H. Global reemergence of enterovirus D68 as an important pathogen for acute respiratory infections. Rev. Med. Virol 2015;25:102–14.
[12] Xiang Z, Limin L, Lili R, Li Guo, Zhengde X, Chunyan L, et al. Seroepidemiology of enterovirus D68 infection in China. Emerg Microbes Infect 2017;6:e32.