EARLY MOLECULAR EVOLUTION TRACKING OF EMERGING ENTEROVIRUS D68 BY COMPLETE GENOME SEQUENCING

Giardina Federica[1], Pellegrinelli Laura[2], Ferrari Guglielmo[3], Mariani Benedetta Nicoletta[3], Seiti Arlinda[2], Galli Cristina[2], Pariani Elena[2], Piralla Antonio[3], Baldanti Fausto [1,3]

1. Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy;
2. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy;
3. Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

P247

BACKGROUND

Enterovirus D68 (EV-D68) belongs to the *Picornaviridae* family and it is associated with respiratory illness, ranging from mild to severe, especially in children with underlying respiratory conditions. Occasionally, EV-D68 infection could have neurological complications such as acute flaccid myelitis or acute flaccid paralysis (AFM/APM). So far, the emergence of new subclades and the phylogenetic analysis of EV-D68 are usually based on VP1 gene, which codes for one on the main proteins of viral capsid. Next Generation Sequencing technologies can allow a deeper analysis of EV-D68 complete genome and could identify molecular patterns eventually associated with severe respiratory or neurological syndromes. This study aimed at: I) designing a new PCR-based NGS method for EV-D68 whole genome sequencing; II) evaluating EV-D68 evolution in the 2022-2023 winter season along the entire genome.

METHODS

Respiratory samples were screened for EV-D68 using a real time-PCR assay targeting 5'UTR. New primers were designed for whole genome amplification; EV-D68 complete genome was divided into three segments and each segment was then amplified. Amplicons were quantified using fluorometric assays and then pooled together. Genomic libraries were prepared. The sequences obtained were analyzed with the bioinformatic platform INSaFLU.

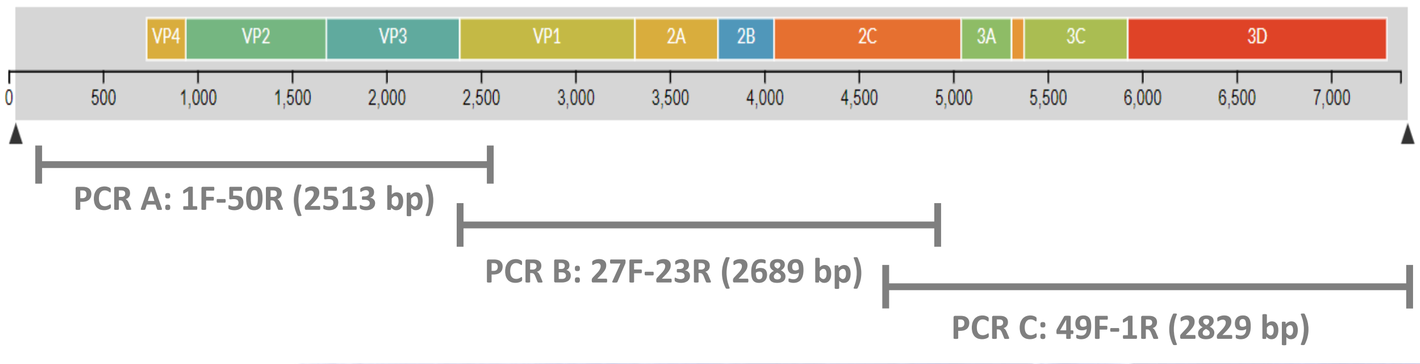

Figure 1. Schematic representation of EV-D68 genome and the three segments it was divided into for complete genome amplification (https://nextstrain.org/enterovirus/d68/genome)

RESULTS

Primers were designed using 906 EV-D68 complete genomes collected from online repositories. Sequences were sliced into 33-nt strings and then filtered to remove ambiguous positions. Sequences were trimmed to a melting temperature of 60°C, discarding all those mapping on human RNA. EV-D68 genome was divided into three segments (2513 bp, 2689 bp and 2829 bp) (Fig.1), overlapping by 334 and 344 nucleotides respectively.

To validate the method, a total of 1932 respiratory samples collected between June and December 2022 at Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, Pavia were screened for the presence of EV-D68 genome; among these, 37 (1.9%) were positive. Moreover, 52 additional EV-D68 positive samples, collected at the Biomedical Sciences for Health Department, University of Milan, were used. Complete genome amplification was performed on all EV-D68 positive samples (total number 89). Complete genome sequences were obtained for 65/89 (73%) of them, with a median length of 7349 nucleotides, ranging from 7347 to 7356 nucleotides. For those samples in which complete genome sequence was obtained, the median value of the cycle threshold for EV-D68 screening was 26.5 (range 18-36.5). After amplification, the three amplicons obtained for each sample were quantified, normalized at the same concentration and then pooled together. Final pool was quantified and the median value was 76.3 ng/µl, ranging from 2.9 to 134 ng/µl. The mean depth of coverage was 6650x (range 2815x-7560x). The median percentage of genome covered by at least 1-fold was 98.4% while the median percentage of genome covered by at least 10-fold was 98.3% (range 98.1%-98.5% and 77.6%-98.4%, respectively.

Table 1. Technical data of EV-D68 complete genome sequences obtained.

EV-D68 Subtype	Surveillance period	Median cycle theshold	Median Qubit quantification (ng/µl)	Median genome lenght	Mean depth of coverage	Mean percentage of size covered by at least 1-fold	Mean percentage of size covered by at least 10-fold
B3 (n=61)	2016-2022	26,5	77	7349	6650,8	98,4	98,3
A2 (n=4)	2018	29,7	57,5	7355	5150,3	98,4	94,9

Among all the EV-D68 strains sequenced, 61/65 (93.8%) belonged to B3 clade, 3/65 (4.6%) belonged to A2 clade. For one strain, typing was not successful. The great majority of strains belonged to one group (named Seq1 in Figure 2).

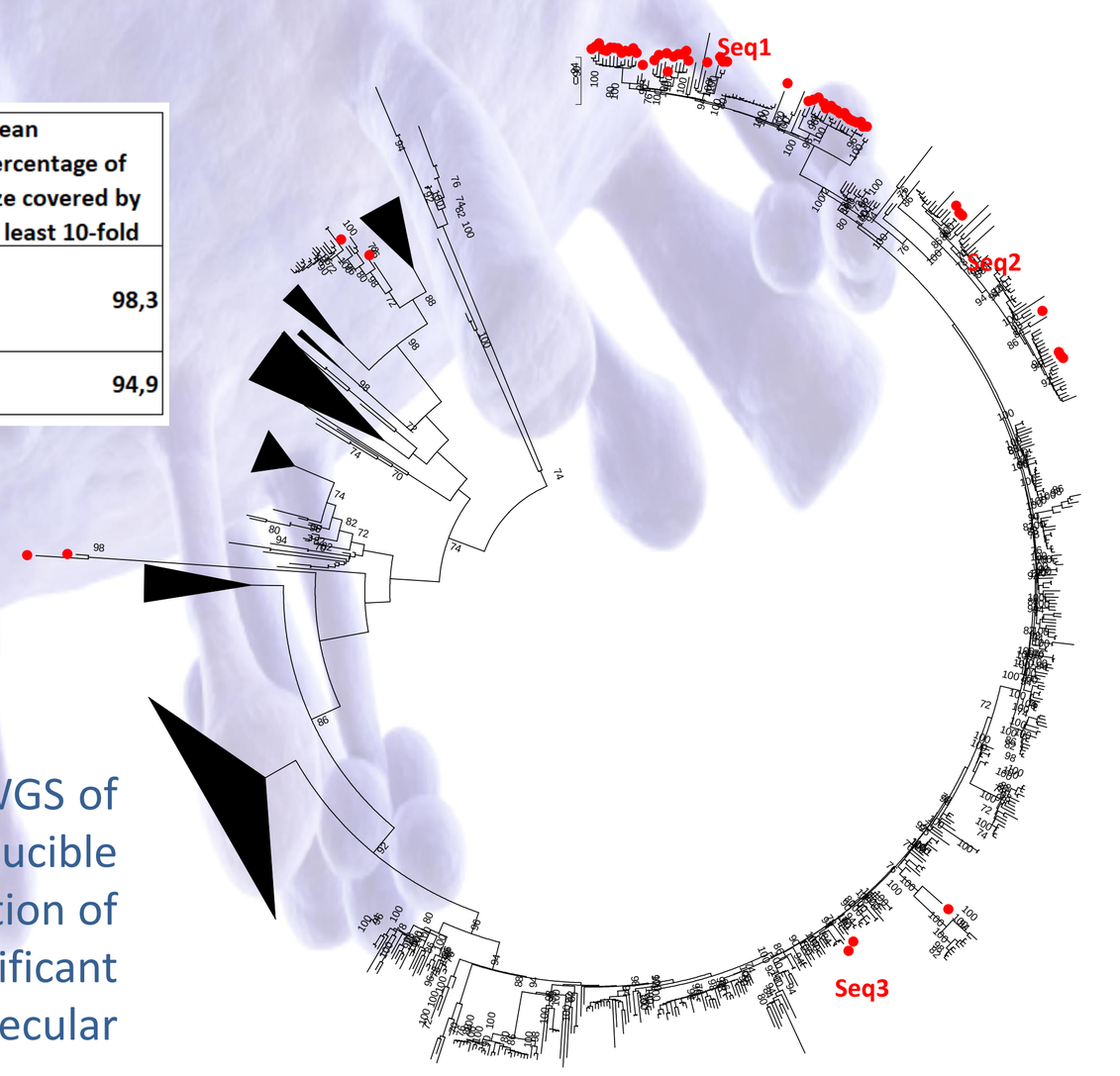

Figure 2. Phylogenetic tree of all EV-D68 strains included in this study.

CONCLUSIONS

The main aim of this study was to design and set up a protocol for WGS of EV-D68. Based on the obtained data, the WGS is robust and reproducible resulting in an easy-to-perform protocol helpful for tracing the evolution of EV-D68 in protein different from VP1. EV-D68 could become a significant pathogen for public health, and thus we need to be ready for molecular epidemiology surveillance.