Complete Genome Sequence of Human Coronavirus Strain 229E Isolated from Plasma Collected from a Haitian Child in 2016

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ABSTRACT   Human coronavirus strain 229E (HCoV-229E) and human alphaherpesvirus 1 were isolated from the plasma of a Haitian child in 2016 with suspected arbovirus diseases. To our knowledge, this is the first description of HCoV-229E in human plasma, which is the focus of this article.

Human coronavirus strain 229E (HCoV-229E), one of the causative agents of the common cold, is increasingly associated with more severe respiratory infections in children, elders, and individuals with underlying medical conditions (1–4). The virus also has neuroinvasive and neurotropic properties (5). The presence of HCoV-229E within clinical samples (e.g., nasal or throat swabs, nasopharyngeal aspirate, bronchoalveolar lavage, or saliva) is typically confirmed by molecular (e.g., reverse transcription-PCR [RT-PCR] targeting of virus-specific genes) or serological (immunofluorescence assay targeting viral antigen) methods (3, 6–8). Primary isolation of HCoV-229E in cell culture is technically challenging, and few have succeeded at isolating the virus from respiratory specimens (3, 6, 9, 10). The isolation of HCoV-229E from human plasma, to our knowledge, has never been reported.

During a suspected arbovirus outbreak in March 2016, HCoV-229E and human alphaherpesvirus 1 (information to be presented elsewhere) were isolated from the plasma of a child presenting with acute undifferentiated febrile illness at a school clinic in rural Haiti. The study protocol was approved by the University of Florida Institutional Review Board (IRB) and the Haitian National IRB, with written consent obtained from parents/guardians of all study participants. The patient's plasma tested negative for chikungunya, dengue, and zika genomic RNAs by RT-PCR (11). As the potential causative agent(s) was unknown, virus isolation was attempted in several human and animal cell lines. Virus-induced cytopathic effects (CPE) were observed in VERO E6, LLC-MK2, and MRC-5 by 14 days postinoculation of the cells. The CPE first consisted of either cell vacuolation or the formation of syncytia, followed by cell lysis or detachment of clumped cells from the cell monolayers, suggesting mixed infections. Infected cells and spent cell growth media were tested for the presence of viruses at various time points postinoculation using molecular assays. HCoV-229E viral genomic RNA (vRNA) was detected using a GenMark multiplex PCR eSensor XT-8 respiratory viral panel, and its identity was supported by the generation of correct-sized PCR amplicons using RT-PCR assays targeting HCoV-229E-specific genes (12–14). Whole-genome sequencing

of HCoV-229E vRNA from Vero E6 cells was accomplished by Sanger sequencing following previously described methods (15) to attain the consensus sequence. In parallel, next-generation sequencing (NGS) with a cDNA library prepared using the NEBNext Ultra RNA library prep kit, and sequencing performed using a version 3 chemistry 600-cycle kit on a MiSeq platform (Illumina), produced the same consensus sequence.

Whole-genome sequence analyses of the Haitian HCoV-229E isolate, designated strain 229E/Haiti-1/2016, revealed close genetic relatedness (>99% nucleotide identity) to several American HCoV-229E strains reported in 2015 (the GenBank accession numbers of the three HCoV-229E sequences with the highest nucleotide similarities as of 22 July 2017 are KY983587, KY684760, and KY967357). Compared to the American strains, the Haitian HCoV-229E genome has unique nucleotide polymorphisms that result in changes within the deduced amino acid sequences of the replicase polyprotein 1ab, spike, accessory, membrane, envelope, and nucleocapsid proteins.

Accession number(s). The complete genome sequence of HCoV-229E strain 229E/Haiti-1/2016 has been deposited in the GenBank database under accession number MF542265.

ACKNOWLEDGMENTS

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