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Genome Sequences of Rhinovirus Genotype C56 Detected in Three Patients with Acute Respiratory Illness, California, 2016 to 2017

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Abstract

We report here two genome sequences of a newly designated rhinovirus genotype, RV-C56, which were obtained from respiratory specimens of three patients with acute respiratory illness in 2016 and 2017. To our knowledge, these sequences represent the first near-complete genomes for RV-C56 strains.

Rhinovirus C (RV-C) is one of three rhinovirus species of the *Enterovirus* (EV) genus in the *Picornaviridae* family of nonenveloped, single-stranded, positive-sense RNA viruses. RV-C was first described in 2006 from patients with influenza-like illness (1, 2). RV-C has been associated with more severe respiratory illness than have RV-A and RV-B (3) and is also distinct from RV-A and RV-B in that RV-C is not culturable by conventional cell culture methods (4, 5).

The California Department of Public Health Viral and Rickettsial Disease Laboratory performs diagnostic testing for many respiratory viruses, including RVs and EVs, using real-time reverse transcription-PCR (RT-PCR) assays adapted from the U.S. Centers for Disease Control and Prevention protocols (6). Recently, we detected an increasing number of specimens that tested positive for both EV and RV by these assays. Subsequent viral protein 1 (VP1) and genomic investigations identified RV-C from some specimens, including a recent study of RV-C47 (7).

We generated complete protein-coding sequences (CDSs) of three RV-C strains. Nasopharyngeal swab specimens were clarified by centrifugation, 0.4-μm filtration, and nuclease treatment prior to extraction using the NucliSENS easyMAG system (bioMérieux, Durham, NC, USA). Extracted RNA was treated with DNase, followed by random RT and subsequent PCR (7, 8). Libraries were prepared using a Nextera XT kit (Illumina, San Diego, CA, USA) and sequenced (300-cycle paired-end run) on the MiSeq platform

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Data availability. The genome sequences of RV-C56 strains USA/2016/CA-RGDS-1002 and USA/2017/CA-RGDS-1003 have been deposited in GenBank under the accession numbers MG950178 and MG950179, respectively.

(Illumina). The next-generation sequencing (NGS) data were analyzed by an in-house NGS pipeline performing read quality control, *de novo* assembly, and BLAST analysis (9). Final consensus genomes were inspected and annotated using Geneious version 11. Average read coverage ranged from 352- to 1,863-fold for the three RV-C genomes.

The genome sequences were confirmed by the Picornaviridae Study Group of the International Committee for the Taxonomy of Viruses (10) as RV-C56. In one instance, twin 1-year-old siblings with a noticeable cough but no fever yielded nearly identical RV-C56 genomes, except for two synonymous nucleotide changes in VP3. We submitted one of the two sequences to GenBank as RV-C56 strain USA/2016/CA-RGDS-1002. We identified a second RV-C56 strain, USA/2017/CA-RGDS-1003, in a 31-year-old female with low-grade fever, shortness of breath, and productive cough who had returned from overseas travel approximately 1 day prior to the onset of the illness. The genome of RV-C56 strain USA/2017/CA-RGDS-1003 shared 99.3% nucleotide identity (NI) to strain USA/2016/CA-RGDS-1002.

No complete CDS of RV-C56 existed prior to this report. Both California RV-C56 strains share 96% NI to a partial capsid sequence reported from Japan in 2014 (GenBank accession number LC004772) (11). Both California RV-C56 strains shared <85% NI and <91% amino acid identity (AI) with VP1 genes from other RV-C genotypes. Compared to the closest full genomes of other RV-C types, the RV-C56 strain USA/2016/CA-RGDS-1002 sequence is missing 26 nucleotides (nt) in the 5= end and 11 nt in the 3= end. The rhinovirus polyprotein can be divided into one structural (P1-capsid) and two nonstructural (P2 and P3) regions. The polyprotein regions of the two RV-C56 genomes reported here all share <85% NI (P1, P2, and P3) and <92% AI (P1), <93% AI (P2), and <92% AI (P3) with other RV-C genotypes.

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