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Genome Sequence of Enterovirus D68 from St. Louis, Missouri, USA

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To the Editor: During the current (2014) enterovirus/rhinovirus season in the United States, enterovirus D68 (EV-D68) is circulating at an unprecedented level. As of October 6, 2014, the Centers for Disease Control and Prevention (CDC) had confirmed 594 cases of EV-D68 infection in 43 states and the District of Columbia (<http://www.cdc.gov/non-polio-enterovirus/outbreaks/EV-D68-outbreaks.html>); the actual number of cases was undoubtedly much higher. In mid-August, hospitals in Missouri and Illinois noticed an increased number of patients with severe respiratory illness (1). We observed this pattern at St. Louis Children's Hospital in St. Louis, Missouri.

Resources for studying this virus are limited. Before the current season, only 7 whole-genome sequences and 5 additional complete coding sequences of the virus were available. Therefore, determining whether there are genomic elements associated with rapid spread or severe and unusual disease was not possible.

To address these limitations, we determined the complete coding sequence of 1 strain from St. Louis by using high-throughput sequencing of nucleic acid from a clinical sample. To evaluate the sequence diversity in EV-D68 strains circulating in the St. Louis metropolitan area, we also generated partial-genome sequences from 8 more EV-D68-positive clinical samples from St. Louis. During the

preparation of this article, CDC generated and submitted to GenBank 7 complete or nearly complete genome sequences from viruses obtained from the Midwest. We documented the diversity of the sequences of strains from St. Louis and compared them to publicly available sequences.

The methods are described in brief here and in more detail in the online Technical Appendix (<http://wwwnc.cdc.gov/EID/article/21/1/14-1605-Techapp1.pdf>). This study was conducted under a protocol approved by the Human Research Protection Office of Washington University in St. Louis.

Patients were categorized retrospectively as having mild, moderate, or severe disease if they had been discharged home from the emergency unit, admitted to general wards, or admitted to the pediatric intensive care unit, respectively. Residual material from a subset of nasopharyngeal specimens positive for rhinovirus/enterovirus (tested by the BioFire FilmArray Respiratory Panel [BioFire Diagnostics, Salt Lake City, UT, USA] at the Clinical Virology Laboratory, St. Louis Children's Hospital) was selected for high-throughput sequencing. Total nucleic acid was extracted from clinical samples by using NucliSENS easyMAG (bioMérieux, Marcy l'Etoile, France) and used to make dual-indexed sequencing libraries. Enterovirus/rhinovirus sequences were enriched by using a NimbleGen custom sequence capture reagent (Roche/NimbleGen, Madison, WI, USA), which as of February 2014 was selective for all complete enterovirus and rhinovirus genomes in GenBank. Sequence data were generated on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA, USA). Sequences were assembled with IDBA-UD (2) and manually improved. The most contiguous genome was annotated by using VIGOR (3). Publicly available sequences were downloaded and compared by using the National Institute of Allergy and Infectious Diseases Virus Pathogen Resource (<http://www.viprbrc.org>) (4). Variants were identified by using VarScan (5). The sequence was deposited in GenBank under accession no. KM881710, BioProject PRJNA263037.

For 14 of the 17 samples, high-throughput sequencing data were interpretable (online Technical Appendix Table); for the other 3 samples, the number of virus sequence reads was too low to distinguish them from sample cross-talk, which occurs during high-throughput sequencing analysis (6). Of the 14 typed samples, EV-D68 sequences were detected in 7 of 10 samples from patients with severe disease, 2 of 2 with moderate disease, and 0 of 2 with mild disease. The complete coding sequence was assembled from sample EV-D68_STL_2014_12. The most closely related genomes from previous seasons were Thailand, CU134, and CU171 (7) (Figure, panel A). Several of the genome sequences obtained from Missouri strains from this season, which had been sequenced by CDC, were very similar to this genome sequence. Comparison of the virus protein 1 sequence with

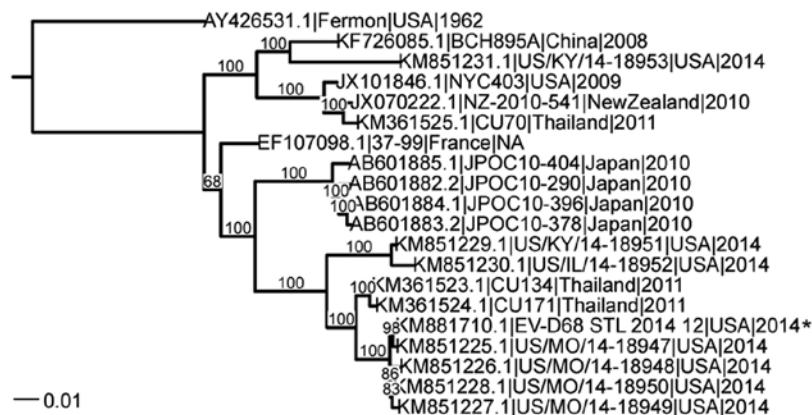
A**B**

Figure. Phylogenetic comparison of enterovirus D68 (EV-D68) obtained from St. Louis, Missouri, USA, in 2014, with other sequenced strains. The phylogenetic relationships of genome sequences (nucleotides) were estimated by using the maximum-likelihood method with RAxML (<http://www.viprbrc.org>) bootstrapped 100 times. A) Comparison of genome sequences for full-length and nearly full-length strains. B) Comparison of virus protein 1 sequences. Sequences were downloaded from ViPR (<http://www.viprbrc.org>) and supplemented with strains from the 2014 EV-D68 season. Sequences were clustered at 99% identity to obtain a representative set of sequences, which are shown. Bootstrap values are indicated on each tree. The strain from St. Louis is indicated by an asterisk on each tree. Scale bars indicate nucleotide substitutions per site.

that of publicly available sequences indicated that the strain from St. Louis and the strain from Missouri (CDC) cluster with virus strains identified in Europe and Asia within the past several years (Figure 1, panel B). The St. Louis virus shared 97%–99% aa sequence identity with all other sequenced strains. We observed little variation in the strains from St. Louis because they shared 98%–99% nt sequence identity (online Technical Appendix Figure).

We provide a genome sequence from the 2014 outbreak of EV-D68 infection in St. Louis, Missouri. This sequence seems to be highly representative of the strains circulating in St. Louis during this time because the other genomes we partially sequenced are very similar. To our knowledge, no amino acids have been associated with virulence or increased infectivity of EV-D68; therefore, we cannot associate the changes we observed in these genomes

to phenotypic traits. Because changes in the 5' untranslated region have the potential to affect the rate of replication (8–10), it is possible that minor genome changes are responsible for the rapid spread and high severity of disease in 2014. Correlation between clinical features of patients in conjunction with additional genomic analysis might provide further insight into the pathogenetic determinants of this strain. Therefore the genome sequence of EV-D68 determined from the 2014 outbreak in St. Louis, Missouri, provides a resource for tracking and genomic comparison of this rapidly spreading virus.

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Prisoners Treated for Hepatitis C with Protease Inhibitor, New York, USA, 2012

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To the Editor: Globally, epidemics of infection with hepatitis C virus (HCV) tend to be concentrated in correctional facilities, according to a review of worldwide literature during 1990–2012 (1,2). Nearly 1 in 3 persons infected with HCV in the United States spends at least part of each year in either a prison or jail (3). Proficiency by correctional health facilities in hepatitis C treatment by using the most effective agents may mitigate the predicted burden of end-stage liver disease and hepatocellular cancer in the coming years. The first wave of direct-acting agents against HCV had substantial side effects. Nonetheless, the New York Department of Corrections and Community Supervision (NYDOCCS) systematically approached the challenge of using these agents, and their experience serves as a lesson that prison medical services can overcome substantial barriers to care.

In 2011, NYDOCCS piloted a hepatitis C treatment program for HCV genotypes 1a and 1b, comprised of telaprevir, pegylated interferon, and ribavirin. Patients underwent extensive mandatory pretreatment screening for mental health issues, pregnancy, and cirrhosis by using the FibroSURE assay (LabCorp, Burlington, NC, USA) and Doppler sonogram of the portal vein. Eligibility requirements were a negative HIV test result, and liver fibrosis assessed either by liver biopsy or noninvasively, at METAVIR stage 2 or 3, or 4 (reference range 0–4) (4) with compensation. A patient in stage 1 was eligible if the patient had poor prognostic factors. An infectious disease consultant saw every patient; each patient received mandatory patient education. Mandatory conditions for patients participating in the program were to sign a consent

Genomic Characterization of Enterovirus D68 from St. Louis, Missouri, USA

Technical Appendix

Supplemental Methods

Genome sequencing: Total nucleic acid was extracted using the NucliSENS easyMAG instrument (bioMérieux, Marcy l'Etoile, France). DNase-treated samples were used to make cDNA as previously described (1,2). Dual-indexed sequencing libraries were constructed. Enterovirus/rhinovirus sequences were enriched by using a NimbleGen custom sequence capture reagent (Roche/NimbleGen, Madison, WI, USA) with probes targeting all complete enterovirus and rhinovirus genomes in GenBank as of February 2014. Paired-end, 100-base sequences were generated on the Illumina HiSeq 2500 platform in rapid mode (Illumina Inc., San Diego, CA, USA). Primer sequences were removed with Flexbar (3). Sequences were assembled with IDBA-UD (4). Data were visualized by using Tablet (5). The most contiguous sequence was manually evaluated and improved. The genome was annotated with VIGOR (6,7). Genome and VP1 sequences were compared by using the NIAID Virus Pathogen Resource (ViPR) (<http://www.viprbrc.org>) (8). In brief, VP1 sequences were downloaded from ViPR, and unique sequences that spanned a 723 base pair amplicon that was commonly deposited in the database were used (positions 2209–2931 in the St. Louis strain genome). Newly sequenced CDC strains were added to the datasets. VP1 sequences were clustered at 99% identity to obtain an easily visualized set of sequences. Nucleotide sequences were aligned with MUSCLE (9) and compared by the maximum-likelihood method by using RAxML (10) bootstrapped 100 times. Trees were visualized by using iTOL (11). Viruses were typed by aligning sequence reads to a complete set of viral reference genomes using BWA mem (12). Variants were identified by using VarScan (13). The sequence of the St.

Louis reference strain was deposited into GenBank, accession no. KM881710, BioProject PRJNA263037.

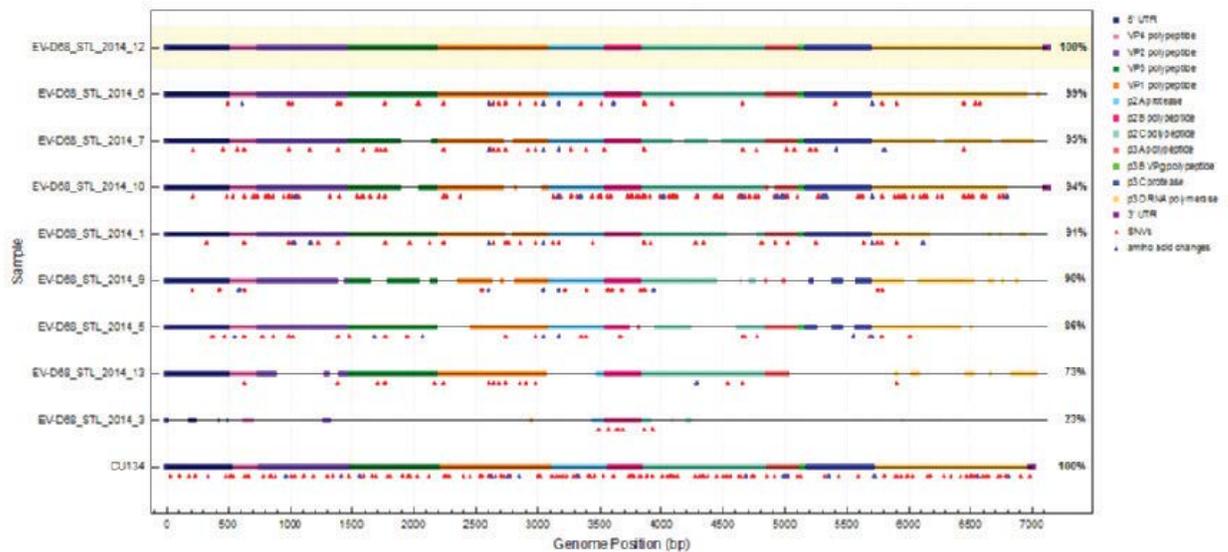
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Technical Appendix Table. Disease severity and virus type

Sample	Severity	Virus typing results
EV-D68_STL_2014_1	Severe	EV-D68
EV-D68_STL_2014_2	Severe	Rhinovirus C
EV-D68_STL_2014_3	Severe	EV-D68
EV-D68_STL_2014_4	Severe	Rhinovirus B
EV-D68_STL_2014_5	Severe	EV-D68
EV-D68_STL_2014_6	Severe	EV-D68
EV-D68_STL_2014_7	Severe	EV-D68
EV-D68_STL_2014_8	Severe	Rhinovirus C
EV-D68_STL_2014_9	Severe	EV-D68
EV-D68_STL_2014_10	Severe	EV-D68
EV-D68_STL_2014_11	Moderate	Not determined
EV-D68_STL_2014_12	Moderate	EV-D68
EV-D68_STL_2014_13	Moderate	EV-D68
EV-D68_STL_2014_14	Mild	Rhinoviruses A, C, and coxsackievirus
EV-D68_STL_2014_15	Mild	Not determined
EV-D68_STL_2014_16	Mild	Rhinovirus A
EV-D68_STL_2014_17	Mild	Not determined



Appendix Figure. Genomic comparison of St. Louis EV-D68 strains. Genomic differences between the 9 St. Louis strains and the most closely related sequence (CU134) from the public database are illustrated. Each genome is represented by a horizontal line. The genome positions (x-axis) are given relative to the St. Louis EV-D68_STL_2014_12 strain, which is the top genome in the figure. The genome features are indicated by colored bars across the genome and are described in detail in the key to the right of the figure. Genome features that are not represented in the sequence data from the sample (i.e., not covered by reads) are indicated by a thin black line and no colored bar. Total percentages of the genome that is represented in the sequencing data are shown to the right of each genome representation. Single nucleotide changes, compared with EV-D68_STL_2014_12, are shown by red triangles. Amino acid changes are indicated by blue triangles.