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Novel Human Enterovirus C Infection in Child with Community-acquired Pneumonia

To the Editor: Human enteroviruses (HEVs) are small, nonenveloped viruses with a positive-stranded RNA genome that includes regions P1, P2, and P3 (*1*). Most experts believe that HEV strains with >75% nt and >85% aa identity in complete or partial viral protein 1 (VP1) sequences should be considered the same type (*2*). However, more stringent criteria of 75% nt and 88% aa identity have been suggested for routine typing (*3*). We report the isolation and characterization of a novel HEV type within the species HEV-C (designated EV-C117 by the *Picornaviridae* Study Group, www.picornastudygroup.com/).

A 45-month-old boy was admitted to a hospital in Vilnius, Lithuania, in December 2010 after 1 day of high fever (temperature 40°C) with cough and a moderately increased respiratory rate (36 breaths/min). Decreased breath sounds were heard at the base of the left lung during auscultation, and a chest radiograph showed alveolar pneumonia with partial consolidation of the lower lobe of the left lung. The patient had a leukocyte count of 23,900 cells/mm³ and C-reactive protein level of 9.6 mg/dL. Blood culture results were negative for bacteria and fungi. The patient was treated with cefuroxime (500 mg every 8 h) for 7 days. Oxygen administration was not required because the saturation level of peripheral oxygen was always >97%. The patient was discharged in good clinical condition after 7 days and did not experience clinical problems in the following 4 weeks.

For research purposes, a nasopharyngeal sample was collected from the boy at hospital admission by using a flexible pernasal flocked swab; written informed consent was obtained from the parents. The swab was immediately placed in a minitube containing 1 mL of universal transport medium (UTM-RT Kit; Copan Italia, Brescia, Italy). The sample was stored at 4°C in the hospital laboratory before being sent to the central laboratory at the University of Milan, Italy in a refrigerated package. We extracted viral nucleic acids from the swab sample by using an automated extraction system (NucliSens easyMAG; Biomeriéux, Craponne, France), and we tested the extract for respiratory viruses by using the Respiratory Virus Panel (Fast assay (Luminex Molecular Diagnostics Inc., Toronto, Ontario, Canada) in accordance with the manufacturer's instructions (*4*).

The assay result was positive for bocavirus and enterovirus/rhinovirus, so we retested the sample to identify the rhinovirus. We performed real-time reverse transcription PCR by using the AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, Foster City, CA, USA) and primers and probe sequences reported by Lu et al. (*5*). Phylogenetic analysis of the VP4/VP2 region showed that some nucleotide sequences belonged to enterovirus species. We obtained a partial VP1 sequence by using the primers described by Nix et al. (*6*), and we obtained the remaining sequence of the VP1 capsid region by using in-house amplification and sequencing protocols (available upon request). The complete P1 sequence was submitted to the *Picornaviridae* Study Group, compared with other enterovirus sequences, and designated as a proposed new type of enterovirus, EV-C117 (GenBank accession no. JQ446368).

To obtain additional viral sequences, we analyzed and sequenced the complete P1 capsid region. This region was compared with the matching region of all of the complete enterovirus genomes available in the GenBank database (as of January 20, 2012). On the basis of the nucleotide sequences, we reconstructed a phylogenetic tree by using maximum likelihood methods with the Tamura 3-parameter model as the evolutionary model; rates among sites were heterogeneous, and gamma distribution was used for the relative rate (*7*). The closest genotypes were EV-C104 (GenBank accession no. EU840733) and EV-C109 (GenBank accession no. NC014336) (Figure). The VP4 genomic region had the greatest identity with other HEV strains; the VP1 coding region had the lowest identity.

We report the identification of a novel enterovirus (designated EV-C117) in a child hospitalized with community-acquired pneumonia in Vilnius, Lithuania. EV-C117 was detected in the child in association with bocavirus. Although it is not possible to say whether this new enterovirus was the etiologic cause of the disease, a close relationship has been found (mainly in children) between the development of severe lower respiratory tract infections requiring hospitalization and infections caused by EV-68 (*8*) and EV-C104 and EV-C109 (*9*), which are molecularly similar to EV-C117.
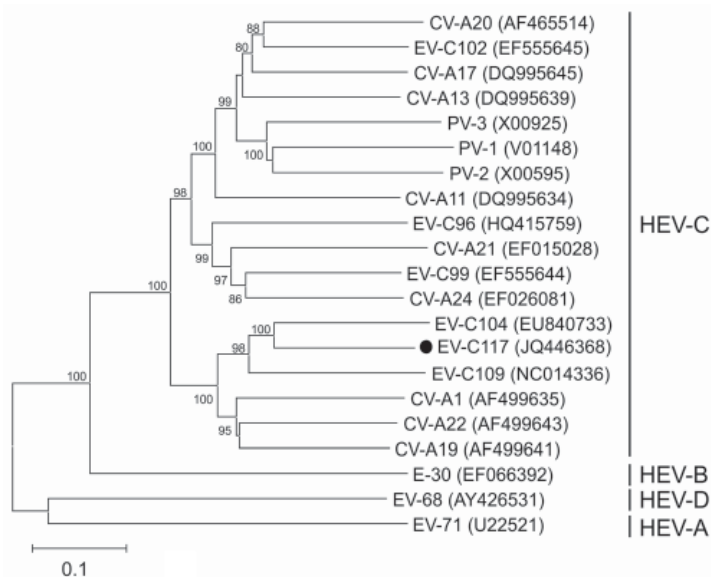
Figure. Phylogenetic relationships of human enterovirus C (HEV-C) and the new strain EV-C117 (dot), as determined on the basis of the complete capsid protein coding region sequences. The phylogeny of the nucleotide sequences was reconstructed by using maximum likelihood methods with the Tamura 3-parameter model as the evolutionary model rates among sites were heterogeneous, and gamma distribution was used for the relative rate (7). Branch support was assessed by means of bootstrap analyses of 1,000 replicates; a bootstrap value of 70% was used as the cutoff point for cluster analysis. Enterovirus strains EV-68 and EV-70 were used as the outgroup. Scale bar indicates nucleotide substitutions per site.

In addition, bocavirus is a frequently reported co-pathogen in children with community-acquired pneumonia. (10). No bacteria or fungi were observed in the blood culture. It is therefore reasonable to think that this new virus may have played a major role in the development of community-acquired pneumonia.

Our findings serve as a reminder that all HEV infections should be closely monitored; knowing the molecular characteristics of virus strains involved in lower respiratory tract infections will help determine appropriate prophylactic and therapeutic measures. However, further studies are needed to determine the tissue tropism and possible pathogenesis of EV-C117 in vivo, and epidemiologic studies are needed to clarify the circulation of this virus strain in countries other than Lithuania.

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Genome Sequencing of Pathogenic *Rhodococcus* spp.

To the Editor: Infections caused by non-equi *Rhodococcus* spp. are uncommon but can cause severe pneumonia and bloodstream infections with sepsis (*1–4*). Increasing prevalence of immune-compromising illnesses and use of immunosuppressive agents might contribute to re-emergence of these pathogens (*5,6*). Rhodococci infections may go undiagnosed or misclassified because of difficulties in laboratory identification, nomenclatural instability, and similarity of signs and symptoms to *Mycobacterium tuberculosis* infection (e.g., increasing cough, dyspnea, hemoptysis, and weight loss; failure to respond to broad-spectrum antimicrobial drugs; progressive cavitary lesion on repeated chest imaging).

A 73-year-old immunocompetent man with chronic obstructive pulmonary disease who lived on a ranch in the southwestern United States was evaluated for possible malignancy of the left lung. Computed tomography showed expansion of an upper lobe cavitary lesion, and he underwent a partial lobectomy. *Rhodococcus* spp. infection was suspected on the basis of the finding of the progressive lesion, salmon-pink colony growth on chocolate agar, and gram-positive coccobacilli on Gram stain. The isolate, R1101, could not be further identified by using commercial automated systems in the laboratory and so was subjected to genome study.

Alignment of 16S rRNA gene sequences showed that R1101 was most closely related to *R. rhodochrous*, with identities of 98.78%, 99.32%, and 99.86% to the 16S rRNA gene for type strains *R. rhodochrous* DSM43271, *R. pyridinivorans* PDB9, and *R. gordonae* W4937 (*7*), respectively. In contrast, sequence identity with *R. equi* type strain DSM20307 was 95.57%. Phylogenetic analysis with *Rhodococcus* 16S rRNA genes suggested that R1101 was a member of *R. rhodochrous* and evolutionarily separate from *R. equi* (Figure, panel A). A query against the National Center for Biotechnology Information nonredundant DNA database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) found 60 rhodococcal 16S rRNA sequences that are 99%–100% identical to R1101, which demonstrates worldwide distribution of these closely related strains in diverse environments.

Optical genome mapping (*8*) of R1101 generated a consensus full-length map showing a circular chromosome map of »4.32 Mb (± 5%) in length. The whole-genome restriction map for R1101 was compared with in silico *Nco*I restriction maps of 33 complete genome sequences from the family *Nocardiaceae*, retrieved from GenBank; these sequences included *R. equi*. No substantial similarity (alignment scores >15%) was detected between R1101 and these genomes. Thus, we determined that R1101 was not an *R. equi* isolate.

Pyrosequencing of the R1101 genome yielded an average coverage depth of 14-fold. The total length of 1,020 assembled de novo contigs was 4.65 Mb, with a G + C content of 68%. A total of 3,969 putative protein-encoding genes were identified. Whole-genome phylogeny was used to show evolutionary distances between R1101 and the sequenced *Rhodococcus* species (Figure, panel B). We sampled ≈50.4 kb of sequences, encoding 67 putative proteins shared among all selected rhodococci, to generate the
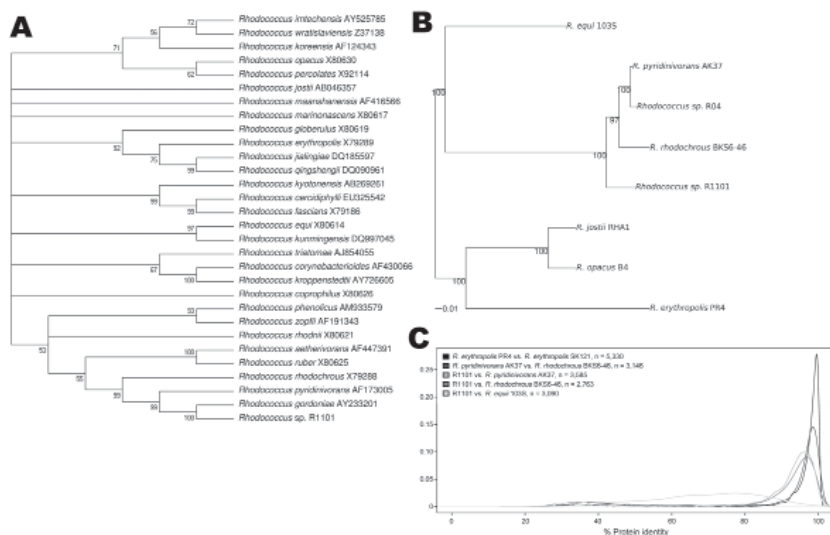

Figure. Phylogenetic and proteomic comparison of isolate R1101 and *Rhodococcus* spp. strains. A) 16S rRNA–based phylogenetic analysis. Complete or partial 16S rRNA gene sequences for 29 *Rhodococcus* spp. type strains were aligned with complete sequence of 16S rRNA gene for isolate R1101. GenBank accession numbers are provided. B) Genome-wide phylogenetic analysis. A sample of 67 protein-coding genes shared among genomes of isolate R1101 and 6 *Rhodococcus* spp. strains were analyzed. Scale bar indicates 0.01 nt substitutions per site. An expanded version of this figure including panel C is available online (wwwnc.cdc.gov/EID/article/18/11/12-0818-F1.htm).