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An outbreak of rhinovirus species C infection in a welfare facility in Fukuoka Prefecture, Japan

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Human rhinoviruses (HRV) are non-enveloped, single-stranded RNA viruses that belong to the genus *Enterovirus* of the family *Picornaviridae*. HRVs are among the most common and diverse respiratory pathogens in humans and are classified into three species: HRV-A, HRV-B, and HRV-C (1,2).

In January 2018, an outbreak of acute respiratory infection caused by HRV-C occurred in a welfare facility in Fukuoka Prefecture. Here, we report the epidemiological and laboratory findings of this outbreak.

On January 4, several elderly persons at a welfare facility that housed 45 residents and 46 staff members developed fever and respiratory symptoms. By January 18, a total of 43 persons [4 staff members (age range, 23–63 years) and 39 residents (age range, 66–98 years)] had developed symptoms. Of these 8 patients were hospitalized, 2 of whom died.

The information (age and sex) were collected from patients with respiratory symptoms. For laboratory diagnosis, respiratory specimens (via pharyngeal swab) were collected from 10 residents of the welfare facility (age range, 79–97 years) after obtaining verbal consent in compliance with the Act on Prevention of Infectious Diseases and Medical Care for Patients Suffering
Infectious Diseases (the Infectious Diseases Control Law). The clinical characteristics of the patients are presented in Table 1. These included fever, cough, and other signs of pneumonia. The pharyngeal swab samples were pretreated. Nucleic acids were extracted from the supernatant using the QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All samples tested negative for the following respiratory pathogens on one-step multiplex polymerase chain reaction (PCR) (3): HRV, human metapneumovirus, influenza virus, parainfluenza virus, respiratory syncytial virus, human coronavirus, enterovirus, human bocavirus, adenovirus, Chlamydophila pneumonia and Mycoplasma pneumonia. We further performed comprehensive pathogen detection using next-generation sequencing (NGS) (4). Metagenomic RNA-Seq performed for 10 pharyngeal specimens showed a partial sequence of 3D pol gene of HRV-C in one specimen. No other potential viral and bacterial sequences were detected.

Subsequently, we confirmed HRV-C using nested PCR, which has a high sensitivity and specificity. The primer sets (SRHI1: GCATCIGGYARYTCCACCACCANCC, SRHI2:
GGGACCAACTACTTTGGGTGTCGCTGTG, and Nestrhi1: ATGGGGNCWCANGTNCHANHCA) were derived from the 5’-UTR-VP4/VP2 region (5). Results of nested PCR were positive for HRV in 2 of the 10 samples. Purified positive PCR products were directly sequenced by a 3500 xl genetic analyzer (Applied Biosystems). The obtained sequence data (441bp) was deposited in GenBank (accession no. LC368821) and compared with 37 reference strains from the GenBank database. On the basis of phylogeny, the present strains were classified as HRV-C (Fig. 1). Furthermore, we designed specific primers for PCR based on the determined sequences of the 5’-UTR-VP4/VP2 region (346bp), and performed nested PCR using the following primers: RVC2018_15F, nucleotide position 602-622 in EF582385: (5’-TCAGTGTTGTCATCATGGGCG-3’), RVC2018_416R, nucleotide position 983-1003: (5’-ATCGATCGGTGGAAGTCTCAG-3’), RVC2018_35F, nucleotide position 622-640: (5’-GCCAGGGTTAGCAAACAGA-3’) and RVC2018_380R, nucleotide position 947-967: (5’-CCACAGCAGCAGCATCAGTAT-3’). Nested PCR was performed using the PrimeScript II High Fidelity One-Step RT-PCR Kit (Takara Bio, Inc.). The cycling conditions for first PCR were;
an initial cycle at 45°C for 15min and 94°C for 2min; followed by 40cycles at 98°C for 10s, 55°C for 15s and 68°C for 30s; and a final incubation at 68°C for 7min. The cycling conditions for nested PCR were; 40cycles at 98°C for 10s, 55°C for 15s and 68°C for 30s; and a final incubation at 68°C for 7min. HRV-C was detected in 8 of the 10 samples, and all 8 samples showed identical sequence homology.

These results indicated that the outbreak was caused by HRV-C infection. On January 31, pharyngeal swab samples were collected from 2 patients who exhibited persistent symptoms. These samples were tested by PCR and the results ruled out HRV-C infection. As no new cases were observed in the facility between January 25 and January 31, the case-free period was almost twice as long as the incubation period of HRV infection. Therefore, this outbreak was considered to have ended on February 1. Because pharyngeal swabs could not be collected from the 2 dead patients, a definitive confirmation of the cause of death could not be obtained.

There are a few recent reports of outbreaks of HRV infection in Japan. These reports pertained to outbreaks caused by HRV-A infection at welfare facilities in Toyama and Ibaraki prefecture in June 2016 (6,7). To our
knowledge, the present report documents a rare instance of an outbreak caused by HRV-C.

HRVs typically cause mild upper respiratory infection; however, these may cause both upper and lower respiratory illness including pneumonia (8) and asthma exacerbations (9) in both children and adults. Recent studies have reported that HRV-C was associated with LRI compared among populations such as children and asthmatic patients (10). However, the virus-specific effects and the prevalence of viruses in adult pneumonia remain largely unknown. This report suggests that HRV-C may cause outbreaks of respiratory infection among elderly residents in welfare facilities. Therefore, early control measures with use of disinfectants containing sodium hypochlorite are important in welfare facilities to thwart outbreaks caused by alcohol-resistant viruses such as HRV. Additionally, there is a need for intra-oral care to protect against co-infection with bacteria, because throat and nose membranes damaged by viruses are vulnerable to bacterial infection that may result in pneumonia.

NGS analysis helped predict the causative pathogen in the present outbreak. It is a rapid and comprehensive diagnostic procedure with a high sensitivity.
From this viewpoint, an NGS-based approach has great potential to detect causative pathogens of infectious diseases.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

References

1. Lee W-M, Kiesner C, Pappas T, et al. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory
illnesses in infants. Plos One. 2007; 2:e966


Figure legends

Table 1. Clinical characteristics of patients

Fig. 1. Phylogenetic tree was constructed from approximately 441 bp of 5′-UTR-VP4/VP2 region of HRV using MEGA7 (Molecular Evolutionary Genetics Analysis Version 7.0) software (http://www.megasoftware.net/) with the maximum likelihood method. Bootstrap analyses were performed with 1,000 resamplings of the data sets. Only significant bootstrap values (>70) were shown. The strain detected in this study is indicated by an asterisk.
<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Sex</th>
<th>Date of onset</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>F</td>
<td>6 Jan. 2018</td>
<td>fever (38.9°C), wheeze, pneumonia</td>
</tr>
<tr>
<td>2</td>
<td>82</td>
<td>F</td>
<td>7 Jan. 2018</td>
<td>fever (38°C), wheeze</td>
</tr>
<tr>
<td>3</td>
<td>91</td>
<td>F</td>
<td>7 Jan. 2018</td>
<td>fever (37.9°C), aspiration pneumonia</td>
</tr>
<tr>
<td>4</td>
<td>79</td>
<td>M</td>
<td>7 Jan. 2018</td>
<td>fever (39.4°C), cough, phlegm, stomachache, giddiness, pneumonia</td>
</tr>
<tr>
<td>5</td>
<td>86</td>
<td>F</td>
<td>9 Jan. 2018</td>
<td>fever (39.3°C), physical weariness</td>
</tr>
<tr>
<td>6</td>
<td>86</td>
<td>F</td>
<td>8 Jan. 2018</td>
<td>fever (37.7°C), cough, pneumonia</td>
</tr>
<tr>
<td>7</td>
<td>90</td>
<td>F</td>
<td>6 Jan. 2018</td>
<td>fever (37.9°C), cough, phlegm, pneumonia</td>
</tr>
<tr>
<td>8</td>
<td>92</td>
<td>F</td>
<td>7 Jan. 2018</td>
<td>fever (39.8°C), cardiac insufficiency, pneumonia</td>
</tr>
<tr>
<td>9</td>
<td>97</td>
<td>F</td>
<td>7 Jan. 2018</td>
<td>fever (38°C), cough, pneumonia</td>
</tr>
<tr>
<td>10</td>
<td>88</td>
<td>F</td>
<td>7 Jan. 2018</td>
<td>fever (38°C), cough, phlegm, pneumonia</td>
</tr>
</tbody>
</table>

M, male; F, female