because we included samples from children hospitalized with or without respiratory disease.

BLAST search (www.ncbi.nlm.nih.gov/blast/Blast.cgi) and phylogenetic analysis of amplicons from PCR and nested PCR indicated that samples were positive for HKU1 genotype B (samples BRA169, BRA90, and BRA99) or HKU1 genotype A (samples BRA09, BRA37, and BRA104). The sequences obtained in this study have been deposited in GenBank under accession nos. FJ931534.1 (BRA169), GU904424 (BRA37), GU904427 (BRA104), GU904423 (BRA09), GU904425 (BRA90), and GU904426 (BRA99).

This may be the oldest collection of human samples in which HKU1 has been detected. To our knowledge, the oldest previous sample positive for HCoV-HKU1 was detected in children in Finland during 1996–1998, without an exact date specified (7). Retrospective studies also have been conducted in the United States and Greece that showed the HKU1 virus in different countries in Europe and North America before its discovery (8,9). We have confirmed the circulation of HKU1 coronaviruses in children in Brazil in 1995.

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References


Macrolide Resistance–associated 23S rRNA Mutation in Mycoplasma genitalium, Japan

To the Editor: Mycoplasma genitalium is now recognized as a serious pathogen in sexually transmitted infections (1,2). Azithromycin regimens have been commonly used for treatment of M. genitalium infections (3). However, failure of azithromycin treatment has been reported in cases of M. genitalium–positive nongonococcal urethritis (NGU) (4,5), and macrolide-resistant strains of M. genitalium have been isolated from case-patients in Australia, Sweden, and Norway for whom azithromycin treatment has failed (4,5). In these strains, mutations in the 23S rRNA gene were associated with macrolide resistance, and mutations in ribosomal protein genes L4 and L22 were also found (5). Surveillance for antimicrobial resistance of M. genitalium is essential to identify antimicrobial resistant strains and to then determine appropriate treatment. Coculture of patient specimens with Vero cells has improved the primary isolation rate of M. genitalium from clinical specimens and offered some current clinical strains for antimicrobial drug susceptibility testing (6). To determine their antimicrobial susceptibilities, a molecular real-time PCR method has been developed (7,8). However, isolating M. genitalium from clinical specimens and antimicrobial drug susceptibility testing of clinical isolates remain labor-intensive, time-consuming tasks. In addition, no methods are available to directly determine antimicrobial drug susceptibilities of M. genitalium in clinical specimens. To monitor macrolide susceptibilities in clinical
strains of *M. genitalium* in Japan, therefore, we examined *M. genitalium* DNA found in the urine of men with NGU for the presence of macrolide resistance–associated mutations in the 23S rRNA gene and the ribosomal protein genes L4 and L22.

This retrospective study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Gifu, Japan. We collected pretreatment urine specimens from 308 men with NGU who had visited a urologic clinic (iClinic) in Sendai, Japan, during 2006 through 2008 and stored the specimens at −70°C. Each man gave informed consent. Twenty-five of 58 urine specimens confirmed to be positive for *M. genitalium* by PCR-based assay were randomly chosen for this study and subjected to DNA purification. The 23S rRNA gene and the ribosomal proteins genes L4 and L22 of *M. genitalium* were amplified from the purified DNA by PCR as reported previously and then sequenced (5).

In 1 specimen, we found an A-to-G transition at nucleotide position 2072 in the 23S rRNA gene of *M. genitalium*, corresponding to position 2059 in *Escherichia coli* (Table). An A2059 (*E. coli* numbering) residue in region V of the 23S rRNA gene is critical for the binding of macrolides (9). Mutations of A2058, A2059, and other 23S rRNA residues within the macrolide-binding site can confer a high-level resistance to macrolides in several bacterial species, including *M. genitalium* (5,9). Therefore, *M. genitalium* strains that harbor the A2059G (*E. coli* numbering) mutation in the 23S rRNA gene could be highly macrolide resistant. We also found a T-to-G transition at nucleotide position 2199 in the 23S rRNA gene of *M. genitalium*, corresponding to position 2185 in *E. coli*, in 3 specimens, but this mutation has not been associated with macrolide resistance in other bacterial species (9).

We found amino acid changes in L4 and L22 ribosomal proteins in *M. genitalium* in 9 specimens. L4 and L22 ribosomal proteins each have extended loops, which converge to form a narrowing in the exit tunnel adjacent to the macrolide-binding site (10). Therefore, macrolide resistance–associated missense mutations in L4 and L22 tend to be localized to Gln62–Gly66 in L4 and Arg88–Ala93 in L22 of *E. coli*, which are closest to the macrolide-binding site (10). All of the amino acid changes in L4 of *M. genitalium* found in this study corresponded to those at the downstream regions from Gln62–Gly66 in L4 of *E. coli*. Of the amino acid changes in L22 of *M. genitalium*, the only Gly93Glu change found in *M. genitalium* harboring the A2059G (*E. coli* numbering) mutation in the 23S rRNA gene was located within the region corresponding to Arg88–Ala93 in L22 of *E. coli*. In this strain, therefore, the Gly93Glu change in L22 might contribute to the increase of macrolide resistance. The patient with NGU, whose specimen exhibited this strain of *M. genitalium* that harbored both the A2059G (*E. coli* numbering) mutation in the 23S rRNA gene and in which the Gly93Glu change in L22 was detected, was given a single dose of 1 g azithromycin and was clinically cured of NGU. However, the present study suggests that *M. genitalium* strains with high-level macrolide resistance might have already emerged in clinical settings in Japan. The emergence and spread of such a clinical mutant could threaten the ability of macrolides to treat *M. genitalium* infections. We should continue monitoring macrolide resistance of *M. genitalium* clinical strains. The nonculture approach used in our study will be useful until culturing of mycoplasmas from clinical specimens and antimicrobial drug susceptibility testing can be performed easily in laboratories.

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**Table. Mutations in the 23S rRNA gene and amino acid changes in L4 and L22 ribosomal proteins of 25 Mycoplasma genitalium strains in the pretreatment urine specimens of men with nongonococcal urethritis, Japan**

<table>
<thead>
<tr>
<th>No. urine specimens</th>
<th>Mutation in the 23S rRNA gene*</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L4</td>
</tr>
<tr>
<td>1</td>
<td>A2059G</td>
<td>Gly93Glu/Asp109Glu</td>
</tr>
<tr>
<td>1</td>
<td>T2185G</td>
<td>Val84Gly</td>
</tr>
<tr>
<td>1</td>
<td>T2185G</td>
<td>GLu128Gly</td>
</tr>
<tr>
<td>2</td>
<td>T2185G</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>Pro81Ser</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>Tyr135Pro</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>Ser81Thr</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>Met82Lys</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>Asn112Asp</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>Arg114Lys</td>
</tr>
<tr>
<td>14</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

*Nucleotide position in the 23S rRNA gene is according to *Escherichia coli* numbering; –, identical to the type strain.*
Saffold Cardioviruses in Children with Diarrhea, Thailand

To the Editor: Cardioviruses currently consist of at least 3 viruses: Theiler murine encephalomyocarditis virus, encephalomyocarditis virus, and Saffold virus (SAFV) (1–4). Saffold cardiovirus in the family Picornaviridae was isolated and identified from feecal specimens of a child with fever of unknown origin in the United States (3).

Several reports have documented the presence of SAFV in fecal samples and respiratory secretions (5–10). However, it is not clear whether SAFV is associated with any disease, including gastroenteritis in humans, and epidemiologic data for SAFV are limited. We report an epidemiologic survey of SAFV in children hospitalized with diarrhea in Chiang Mai, Thailand.

A total of 150 fecal specimens were obtained from children hospitalized with acute gastroenteritis in Chiang Mai during January–December 2007. Patient ages ranged from >1 to 5 years. SAFV in fecal specimens was detected by using a nested PCR and primers specific for the virus 5′ untranslated region (7). A negative control was also included to monitor any contamination that might have occurred during the PCR.

SAFVs detected were further amplified by amplification of the viral protein (VP) 1 gene (6,9,10) and direct sequencing of the VP1 PCR amplicon by using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). VP1 sequence was compared with VP1 sequences of reference strains (Figure). In addition, the other 2 SAFVs identified in the present study (CMH038/2007 and CMH045/2007) were identical to each other and closely related to SAFV2 strains from China (BCHU79, BCHU353) (www.megasoftware.net). Nucleotide sequences of SAFV strains described were deposited in GenBank under accession nos. HQ668170–HQ668173.

Four (2.7%) of 150 specimens were positive for SAFV (CMH023/2007, CMH038/2007, CMH045/2007, and CMH143/2007). Two of these specimens (CMH023/2007 and CMH038/2007) were obtained in February 2007, one (CMH045/2007) in March 2007, and 1 (CMH143/2007) in November 2007. Co-infections with other viruses were detected in all 4 samples. Two specimens (CMH023/2007 and CMH045/2007), were co-infected with noroviruses GII/16 and GII/4 genotypes, respectively. One SAFV-positive sample (CMH038/2007) was co-infected with a group A rotavirus G1P[8] genotype, and another (CMH143/2007) was co-infected with human parechovirus.

All SAFV-positive specimens were further amplified for the VP1 gene to determine their phylogenetic lineages and genetic relationships with other SAFV reference strains. When we used 3 sets of primers used in other studies (6,9,10) for amplification of the VP1 gene, this gene was amplified only by the primer set reported by Itagaki et al. (10).

Analysis of partial VP1 sequences (369 nt) of 4 SAFV strains showed that strains CMH023/2007 and CMH143/2007 were highly conserved (nt sequence identities >97%). These 2 SAFV strains were most closely related to the prototype strain of SAFV1 (EF165067) isolated in the United States (nt sequence identity range 87.6%–88.9%) and SAFV strains from China (LZ50419, BCH895, GL311, and GL377). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA4 (www.megasoftware.net). Nucleotide sequences of SAFV strains described were deposited in GenBank under accession nos. HQ668170–HQ668173.

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