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.

This work was supported by the São Paulo Research Foundation and the Coordination for the Improvement of University Level Personnel.

Luiz G. Góes, Edison L. Durigon, Angélica A. Campos, Noely Hein, Saulo D. Passos, and José A. Jerez

Author affiliations: Universidade de São Paulo, São Paulo, Brazil (L.G. Góes, E.L. Durigon, A.A. Campos, N. Hein, J.A. Jerez); and Faculdade de Medicina de Jundiai, Jundiai, Brazil (S.D. Passos)

DOI: 10.3201/eid1706.101381

References

1. Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol. 2005;79:884–95. doi:10.1128/JVI.79.2.884-895.2005
2. Woo PC, Lau SK, Yip CC, Huang Y, Yuen K. More and more coronaviruses: human coronavirus HKU1 viruses. Viruses. 2009;1:57–71. doi:10.3390/v1010057
3. Albuquerque MC, Pena GP, Varella RB, Gallucci G, Erdman D, Santos N. Novel respiratory virus infections in children, Brazil. Emerg Infect Dis. 2009;15:806–8. doi:10.3201/eid1505.081603
4. Hein N. Epidemiological aspects of the respiratory viruses infection in a pediatric ward [dissertation] [in Portuguese]. São Paulo (Brazil): Universidade de São Paulo; 1997.
5. Canducci F, Debiaggi M, Sampaolo M, Marinozzi MC, Berre S, Terulla C, et al. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. J Med Virol. 2008;80:716–23. doi:10.1002/jmv.21108
6. Bellei N, Carraro E, Perosa A, Watanabe A, Arruda E, Granato C. Acute respiratory infection and influenza-like illness viral etiologies in Brazilian adults. J Med Virol. 2008;80:1824–7. doi:10.1002/jmv.21295
7. Ruohola A, Waris M, Allander T, Ziegler T, Heikkinen T, Ruuskanen O. Viral etiology of common cold in children, Finland. Emerg Infect Dis. 2009;15:344–6. doi:10.3201/eid1502.081468
8. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Coronavirus HKU1 infection in the United States. Emerg Infect Dis. 2006;12:775–9.
9. Papa A, Papadimitriou E, Luna LK, Al Masri M, Souliou E, Eboriadou M, et al. Coronaviruses in children, Greece. Emerg Infect Dis. 2007;13:947–9.

Address for correspondence: Edison L. Durigon, University of São Paulo, 1374 ICB-II, CEP: 05508-900, São Paulo, São Paulo, Brazil; email: eldurigo@usp.br

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.

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

No. urine specimens	Mutation in the 23S rRNA gene*	Amino acid change	
		L4	L22
1	A2059G	–	Gly93Glu/Asp109Glu
1	T2185G	Val84Gly	–
1	T2185G	GLu128Gly	–
2	T2185G	–	–
1	–	Pro81Ser	–
1	–	Tyr135Pro	–
1	–	–	Ser81Thr
1	–	–	Met82Lys
1	–	–	Asn112Asp
1	–	–	Arg114Lys
14	–	–	–

*Nucleotide position in the 23S rRNA gene is according to *Escherichia coli* numbering. –, identical to the type strain.

This study was supported in part by the Japan Society for the Promotion of Science, Japan, under a Grant-in-Aid for Scientific Research, (C) 22591788.

Yasushi Shimada, Takashi Deguchi, Keita Nakane, Mitsuru Yasuda, Shigeaki Yokoi, Shin-ichi Ito, Masahiro Nakano, Shin Ito, and Hiroaki Ishiko

Author affiliations: Gifu University, Gifu, Japan (Y. Shimada, T. Deguchi, K. Nakane, M. Yasuda, S. Yokoi, S.-I. Ito, M. Nakano); Mitsubishi Chemical Medience Corporation, Chiba, Japan (Y. Shimada, H. Ishiko); iClinic, Sendai, Japan (S. Ito)

DOI: 10.3201/eid1706.101055

References

1. Deguchi T, Maeda S. *Mycoplasma genitalium*: another important pathogen of nongonococcal urethritis. J Urol. 2002;167:1210–7. doi:10.1016/S0022-5347(05)65268-8
2. Jensen JS. *Mycoplasma genitalium*: the aetiological agent of urethritis and other sexually transmitted diseases. J Eur Acad Dermatol Venereol. 2004;18:1–11. doi:10.1111/j.1468-3083.2004.00923.x
3. Mena LA, Mroczkowski TF, Nsuami M, Martin DH. A randomized comparison of azithromycin and doxycycline for the treatment of *Mycoplasma genitalium*–positive urethritis in men. Clin Infect Dis. 2009;48:1649–54. doi:10.1086/599033
4. Bradshaw CS, Jensen JS, Tabrizi SN, Read TR, Garland SM, Hopkins CA, et al. Azithromycin failure in *Mycoplasma genitalium* urethritis. Emerg Infect Dis. 2006;12:1149–52.
5. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in *Mycoplasma genitalium*–positive patients with nongonococcal urethritis is associated with induced macrolide resistance. Clin Infect Dis. 2008;47:1546–53. doi:10.1086/593188
6. Jensen JS, Hansen HT, Lind K. Isolation of *Mycoplasma genitalium* strains from the male urethra. J Clin Microbiol. 1996;34:286–91.
7. Hamasuna R, Osada Y, Jensen JS. Antibiotic susceptibility testing of *Mycoplasma genitalium* by TaqMan 5′ nuclease real-time PCR. Antimicrob Agents Chemother. 2005;49:4993–8. doi:10.1128/AAC.49.12.4993-4998.2005
8. Hamasuna R, Jensen JS, Osada Y. Antimicrobial susceptibilities of *Mycoplasma genitalium* strains examined by broth dilution and quantitative PCR. Antimicrob Agents Chemother. 2009;53:4938–9.
9. Vester B, Douthwaite S. Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrob Agents Chemother. 2001;45:1–12. doi:10.1128/AAC.45.1.1-12.2001
10. Diner EJ, Hayes CS. Recombineering reveals a diverse collection of ribosomal proteins L4 and L22 that confer resistance to macrolide antibiotics. J Mol Biol. 2009;386:300–15. doi:10.1016/j.jmb.2008.12.064

Address for correspondence: Takashi Deguchi, Department of Urology, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu City, Gifu 501-1194, Japan; email: deguchit@gifu-u.ac.jp

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 fecal 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 analyzed 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 available in the National Center for Biotechnology Information (Bethesda, MD, USA). 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.

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 (CMH023/2007and 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) (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)