Novel Variant and Known Mutation in 23S rRNA Gene of *Mycoplasma pneumoniae*, Northern Vietnam, 2023

Dinh-Dung Nguyen,[1] Nhan Thi Ho,[1] Lynn G. Dover, Anh Hang Mai Vo, Ha Thi Thanh Ly, Phuong Mai Doan, Hang Thi Nguyen, Nguyen Thi Thao Luu, An Nhat Pham, Huyen Thi Thanh Tran

Author affiliations: Vinmec Healthcare System, Hanoi, Vietnam (D.-D. Nguyen, N.T. Ho, A.H.M. Vo, H.T.T. Ly, P.M. Doan, H.T. Nguyen, N.T.T. Luu, A.N. Pham, H.T.T. Tran); Northumbria University, Newcastle upon Tyne, UK (L.G. Dover)

DOI: https://doi.org/10.3201/eid3005.231632

During a 2023 outbreak of *Mycoplasma pneumoniae*–associated community-acquired pneumonia among children in northern Vietnam, we analyzed *M. pneumoniae* isolated from nasopharyngeal samples. In almost half (6 of 13) of samples tested, we found known A2063G mutations (macrolide resistance) and a novel C2353T variant on the 23S rRNA gene.

Mycoplasma pneumoniae is a common etiologic agent of community-acquired pneumonia (CAP) among children. Although *M. pneumoniae* infection often causes a mild and self-limiting disease, pneumonia develops in ≈10%–20% of pediatric patients (*1*). First-line therapies for *M. pneumoniae* infection are based on macrolides, a group of antimicrobial drugs widely used in outpatient settings because of their high oral bioavailability. However, overuse and indiscriminate use of macrolides have contributed to the emergence of macrolide-resistant *M. pneumoniae* (MRMP). Point mutations in the V region of the *M. pneumoniae* 23S rRNA gene have been associated with macrolide resistance (*2*). In recent years, prevalence of MRMP has increased and is very high in Asia (13.6%–100%) (*2*–*4*). During spring/summer 2023, hundreds of children with CAP were admitted daily to each of the major hospitals in Hanoi, Vietnam. *M. pneumoniae* has emerged as the major pathogen detected in approximately one third of patients with CAP (5). We analyzed the mutations in the 23S rRNA gene of *M. pneumoniae* isolated from nasopharyngeal samples of pediatric CAP patients during the 2023 outbreak in Vinmec Times City Hospital, Hanoi.

During May 1–July 31, 2024, the real-time PCR Allplex Respiratory Panel 4 detected *M. pneumoniae* in 411 (26.1%) of 1,578 nasopharyngeal samples from children with suspected CAP. Among *M. pneumoniae*–positive samples with a cycle threshold <30, we randomly selected 13 samples from 13 patients for gene sequencing. We amplified the DNA sequence of the 748-bp region (nt 1963–2710) of the 23S rRNA gene containing all known MRMP mutations by using MRMP-F1 (5′-CGTCCCGCTTGAATGGTGTA-3′) and MRMP-R1 (5′-GGCGCTACAACTGGAGCATA-3′). We sequenced the amplicons according to the Sanger sequencing method by using a BigDye Terminator v3.1 Cycle Sequencing Kit and Applied Biosystems 3500 Dx Genetic Analyzer instrument (both Thermo Fisher Scientific, https://www.thermofisher.com). We assembled the generated sequence data and analyzed them for variations by comparing with the reference *M. pneumoniae* strain M129 23S ribosomal RNA gene (GenBank accession no. NR_077056.1), using BLAST (http://blast.ncbi.nlm.nih.gov). We used ClustalW to perform multiple alignments (*6*). Subsequently, we constructed the phylogenetic tree according to the maximum-likelihood method with bootstrap analysis (n = 500) by using MEGA11 software (https://www.megasoftware.net). The 2-dimensional secondary structure of the 23S rRNA gene was predicted by the R2DT tool (RNAcentral) according to an SA_LSU_3D template provided by RiboVision (*7*).

Of the 13 samples, 6 (46.2%) showed single-nucleotide variation from the type strain sequence in the V region of the 23S rRNA gene. A known A2063G mutation was detected in 4 samples, and a novel variant C2353T was found in 2 samples (Figure, panel A).

The known MRMP mutation A2063G is the most prevalent mutation reported to date compared with other infrequent mutations (e.g., A2063T/C, A2064G, A2067G, A1290G, and C2617A) (*2*,*8*). Mutations at site 2063 are also associated with a high level of macrolide resistance (*9*,*10*). The National Institutes of Health databases showed no recorded evidence for the sequences containing the C2353T variant observed in our study (Figure, panel B). We hypothesize that under selection pressure during CAP treatment with macrolides, C2353T mutants have emerged with macrolide resistance. Previous reports have shown that different mutations can lead to different levels of macrolide affinity as well as MIC elevation (*8*). Demonstration of MRMP by culture and MIC is not regularly done in clinical practice; thus, rapid detection of MRMP mutation may provide useful information for guiding antimicrobial drug therapy.

[1]These authors contributed equally to this article.

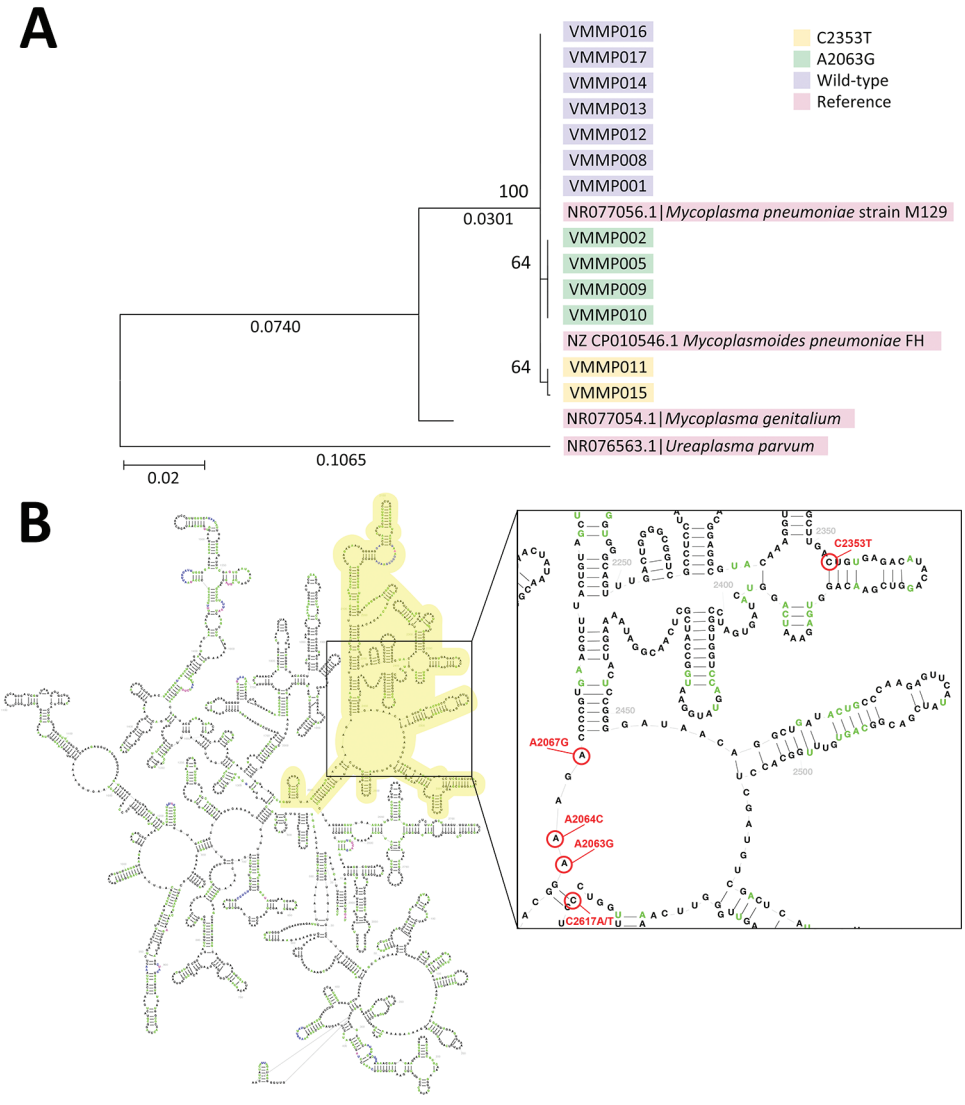

Figure. Phylogenetic tree and location of mutations for *Mycoplasma pneumoniae* strains identified in pediatric patients hospitalized with community-acquired pneumonia, Hanoi, Vietnam, spring/summer 2023. A) Maximum-likelihood phylogenetic analysis of the domain V region of the 23S rRNA gene. B) Predicted RNA secondary structure of 23S rRNA gene constructed with the description of known mutations (A2063G/C/T, A2064G, A2067G, C2617G) and novel variant (C2353T). Yellow highlights indicate the domain V region of 23S rRNA. Scale bar indicates base substitutions per site.

Clinical nonresponse to initial macrolide treatment was experienced by 3 (50%) of the 6 patients with the novel or known mutation and 2 (28.6%) of the 7 without (Table, https://wwwnc.cdc.gov/EID/article/30/5/23-1632-T1.htm; Appendix Table, https://wwwnc.cdc.gov/EID/article/30/5/23-1632-App1.pdf). Other respiratory bacteria were co-detected in approximately two thirds of patients in both groups, which might also affect clinical characteristics.

In summary, we detected the novel C2353T variant and known A2063G mutations in the 23S rRNA gene in nearly half of the pediatric patients with *M. pneumoniae*–associated CAP in Vinmec Times City Hospital during the 2023 outbreak in northern Vietnam. Our findings are consistent with those of other studies regarding the rising prevalence of MRMP in Southeast Asia. Our study findings may indicate circulation of different MRMP variants in Vietnam or emergence of new MRMP variants during the recent *M. pneumoniae*-associated CAP outbreak among children.

About the Author

Dr. Dinh-Dung Nguyen is a molecular genetics specialist at the Medical Genetics Department, Vinmec Hitech Center. His research interests are molecular biology, immunology, and gene-editing technology.

References

1. Krafft C, Christy C. *Mycoplasma* pneumonia in children and adolescents. Pediatr Rev. 2020;41:12–9. https://doi.org/10.1542/pir.2018-0016
2. Cao B, Qu JX, Yin YD, Eldere JV. Overview of antimicrobial options for *Mycoplasma pneumoniae* pneumonia: focus on macrolide resistance. Clin Respir J. 2017;11:419–29. https://doi.org/10.1111/crj.12379

3. Chen YC, Hsu WY, Chang TH. Macrolide-resistant *Mycoplasma pneumoniae* infections in pediatric community-acquired pneumonia. Emerg Infect Dis. 2020;26:1382–91. https://doi.org/10.3201/eid2607.200017
4. Yamazaki T, Kenri T. Epidemiology of *Mycoplasma pneumoniae* infections in Japan and therapeutic strategies for macrolide-resistant *M. pneumoniae.* Front Microbiol. 2016;7:693.https://doi.org/10.3389/fmicb.2016.00693
5. Hieu Vy NT. Children with increased *Mycoplasma* pneumonia, instructions for disease prevention [in Vietnamese] [cited 2023 Jun 26]. https://benhviennhitrung-guong.gov.vn/tre-mac-viem-phoi-do-mycoplasma-gia-tang-huong-dan-phong-benh.html
6. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22:4673–80.
7. Sweeney BA, Hoksza D, Nawrocki EP, Ribas CE, Madeira F, Cannone JJ, et al. R2DT is a framework for predicting and visualising RNA secondary structure using templates. Nat Commun. 2021;12:3494. https://doi.org/10.1038/s41467-021-23555-5
8. Kim JH, Kim JY, Yoo CH, Seo WH, Yoo Y, Song DJ, et al. Macrolide resistance and its impacts on *M. pneumoniae* pneumonia in children: comparison of two recent epidemics in Korea. Allergy Asthma Immunol Res. 2017;9:340–6. https://doi.org/10.4168/aair.2017.9.4.340
9. Lucier TS, Heitzman K, Liu SK, Hu PC. Transition mutations in the 23S rRNA of erythromycin-resistant isolates of *Mycoplasma pneumoniae.* Antimicrob Agents Chemother. 1995;39:2770–3. https://doi.org/10.1128/AAC.39.12.2770
10. Matsuoka M, Narita M, Okazaki N, Ohya H, Yamazaki T, Ouchi K, et al. Characterization and molecular analysis of macrolide-resistant *Mycoplasma pneumoniae* clinical isolates obtained in Japan. Antimicrob Agents Chemother. 2004;48:4624–30. https://doi.org/10.1128/AAC.48.12.4624-4630.2004

Address for correspondence: Huyen Thi Thanh Tran, Medical Genetics Department, Vinmec High Tech Center, 458 Minh Khai, Hai Ba Trung Hanoi 100000, Vietnam; email: v.huyenttt47@vinmec.com

Crimean-Congo Hemorrhagic Fever Virus in Ticks Collected from Cattle, Corsica, France, 2023

Paloma Kiwan, Shirley Masse, Geraldine Piorkowski, Nazli Ayhan, Morena Gasparine, Laurence Vial, Remi N. Charrel, Xavier de Lamballerie, Alessandra Falchi

Author affiliations: Unité des Virus Emergents, Aix Marseille Université, Università di Corsica, IRD140, INSERM 207 IRBA, Marseille, France (P. Kiwan, S. Masse, G. Piorkowski, N. Ayhan, M. Gasparine, R.N. Charrel, X. de Lamballerie, A. Falchi); Université de Corse–Institut National de Santé et de la Recherche Médicale, Corte, France (P. Kiwan, S. Masse, G. Piorkowski, N. Ayhan, M. Gasparine, R.N. Charrel, X. de Lamballerie, A. Falchi); Centre National de Référence des Arbovirus, Marseille, France (N. Ayhan, X. de Lamballerie); Université de Montpellier, Montpellier, France (L. Vial)

We report the detection of Crimean-Congo hemorrhagic fever virus (CCHFV) in Corsica, France. We identified CCHFV African genotype I in ticks collected from cattle at 2 different sites in southeastern and central-western Corsica, indicating an established CCHFV circulation. Healthcare professionals and at-risk groups should be alerted to CCHFV circulation in Corsica.

DOI: https://doi.org/10.3201/eid3005.231742

Crimean-Congo hemorrhagic fever (CCHF) is a tickborne disease caused by CCHF virus (CCHFV) (species *Orthonairovirus haemorrhagiae*, genus *Orthonairovirus,* family *Nairoviridae*, order *Bunyavirales*). Endemic in Africa, the Middle East, Asia, and Eastern Europe, CCHF has expanded to Western Europe (*1*). Repeated detection of CCHFV in Spain (*2*) raises questions about its circulation in neighboring countries, such as Portugal, Italy, and France.

In Corsica, a French Mediterranean island, a seroprevalence study of CCHFV conducted in livestock (cattle, goats, and sheep) during 2014–2016 showed an overall seroprevalence of 9.1%, and cattle harbored the highest rates (*3*). A subsequent surveillance study of 8,051 ticks collected from wild (wild boar, deer, and mouflon sheep) and domestic (cattle, horses, sheep) animals during 2016–2020 failed to detect CCHFV or nairovirus RNA (*4*).

Since 2022, we have continued CCHFV surveillance by collecting ticks from cattle at 2 slaughterhouses ≥2 times/month. Cattle originate from a broad