

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

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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'-CGTCCCCTTGAATGGTGTA-3') and MRMP-R1 (5'-GGCGCTACAACCTGGAGCATA-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.

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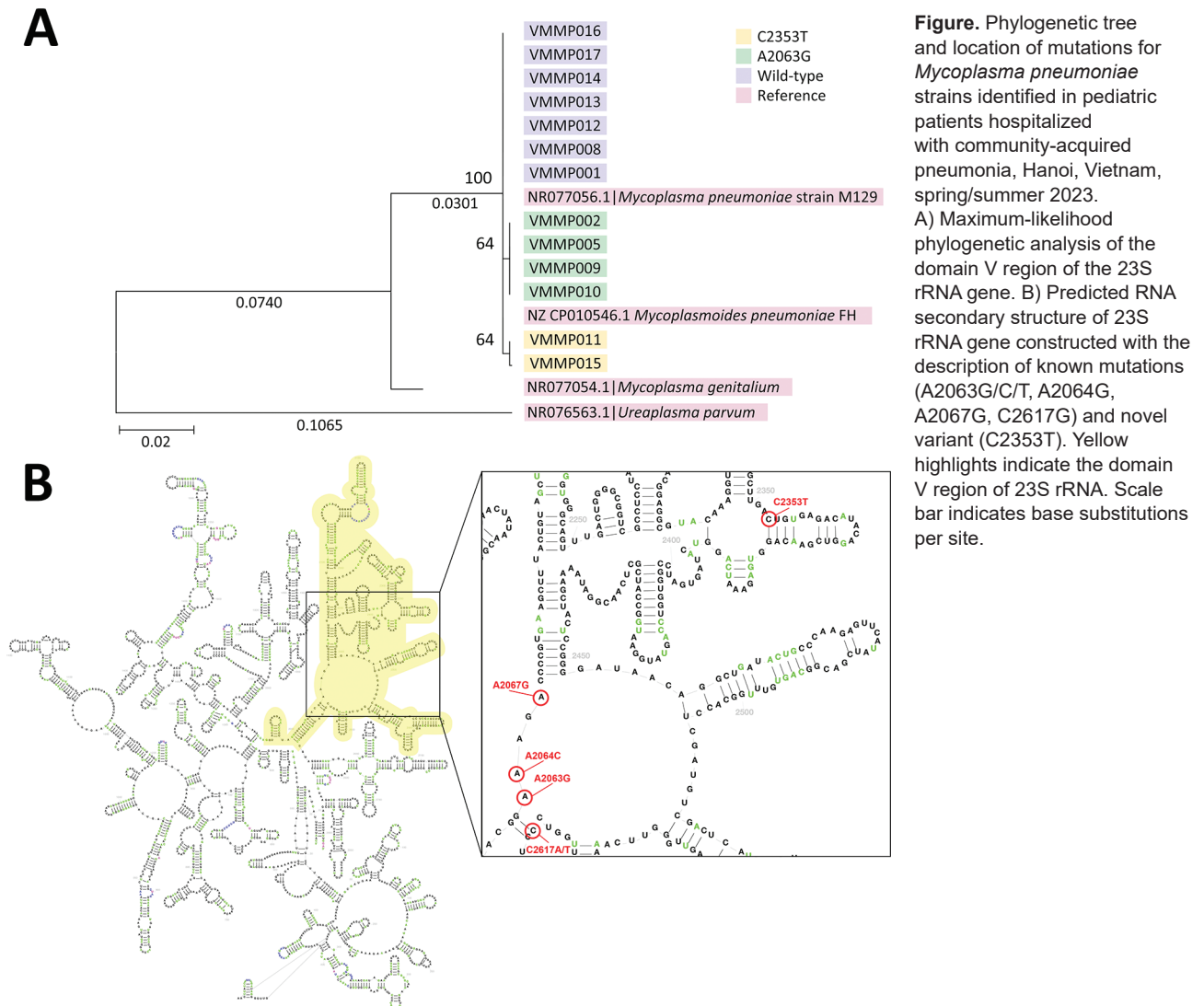


Figure. Phylogenetic tree and location of mutations for *Mycoplasma pneumoniae* strains identified in pediatric patients hospitalized with community-acquired pneumonia, Hanoi, Vietnam, spring/summer 2023. A) Maximum-likelihood phylogenetic analysis of the domain V region of the 23S rRNA gene. B) Predicted 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.

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

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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

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Appendix

Appendix Table. Characteristics of each of all patients

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13
Mutation	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No
Mutation type	A2063G	A2063G	A2063G	A2063G	C2353T	C2353T	Non-detected	Non-detected	Non-detected	Non-detected	Non-detected	Non-detected	Non-detected
Nasopharyngeal culture	Negative	Not done	Not done	Not done	Negative	Not done	Not done	Positive	Negative	Not done	Positive	Negative	Negative
- Positive Bacteria	NA	NA	NA	NA	NA	NA	NA	H. influenzae	NA	NA	S. pyogenes	NA	NA
PCR panel of 7 respiratory bacteria													
- Positive with <i>M. pneumoniae</i>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
- Positive with <i>H. influenzae</i>	No	No	Yes	No	Yes	No	No	Yes	No	No	No	No	Yes
- Positive with <i>S. pneumoniae</i>	No	No	No	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes
- Positive with other bacteria	No	No	No	No	No	No	No	No	No	No	No	No	No
Co_detection of bacteria by PCR panel	No	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes
Co_detection of bacteria by PCR or culture	No	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes
Mycoplasma IgM	Positive	NOT DONE	NOT DONE	Positive	NOT DONE	NOT DONE	NOT DONE	NOT DONE	Negative	NOT DONE	NOT DONE	NOT DONE	Positive
Flu rapid test	NOT DONE	NOT DONE	NOT DONE	NOT DONE	Negative	Negative	NOT DONE	NOT DONE	NOT DONE	NOT DONE	Negative	NOT DONE	NOT DONE
Gender	Female	Female	Male	Female	Male	Female	Male	Female	Female	Female	Male	Male	Male
Age (year)	4	6	4	2	13	6	1	1	4	7	7	4	3

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13
Hospitalized	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes
Date of hospital visit	2023-05-18	2023-06-28	2023-06-27	2023-05-19	2023-01-07	2023-06-28	2023-06-29	2023-02-07	2023-05-22	2023-07-01	2023-01-07	2023-06-26	2023-02-07
Day of illness at hospital visit and nasopharyngeal sample aspiration	5	3	4	5	3	4	5	4	5	7	4	5	5
Days with fever at hospital visit	5	3	4	5	3	4	5	4	5	2	4	5	5
Cough	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Respiratory distress	No	No	No	Yes	No	No	No	No	No	No	No	No	No
Wheezing	No	No	No	Yes	No	No	No	No	No	No	No	No	No
Chest pain	No	No	No	No	No	No	No	No	No	Yes	No	No	No
Pulmonary rales	Yes	No	No	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes
Lung lesion in chest X-ray	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Oxygen supplementation	No	No	No	Yes	No	No	No	No	No	No	No	No	No
Treatment before hospital visit	Yes	Yes	Yes	No	No	Yes	No	No	Yes	No	No	No	Yes
- Antibiotic use before hospital visit	Amoxicillin & Clavulanic acid	No	Clarithromycin	NA	NA	No	NA	NA	Azithromycin	NA	NA	NA	Amoxicillin & Clavulanic acid
- Duration of treatment before hospital visit	4	3	6	NA	NA	4	NA	NA	3	NA	NA	NA	4
Macrolide initiated at the beginning of hospital treatment	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
- Macrolide type	Azithromycin	Azithromycin	Clarithromycin	Azithromycin	Azithromycin	Azithromycin	Azithromycin	NA	Clarithromycin	Azithromycin	Clarithromycin	Azithromycin	Azithromycin
Other antibiotic use at the beginning of hospital treatment	Ceftriaxone	Amoxicillin & Clavulanic acid	Cefdinir	Amoxicillin & Clavulanic acid	Amoxicillin & Clavulanic acid	Amoxicillin & Clavulanic acid	NA	Amoxicillin & Clavulanic acid	Ceftriaxone	Amoxicillin & Clavulanic acid	Amoxicillin & Clavulanic acid	Amoxicillin & Clavulanic acid	Ceftriaxone
Switch to Clarithromycin	Yes	No	No	No	Yes	No	No	NA	NA	No	No	No	No
- Switch after how many day	6	NA	NA	NA	5	NA	NA	NA	NA	NA	NA	NA	NA
Macrolide was used after initial antibiotic (day)	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA
- Macrolide type	NA	NA	NA	NA	NA	NA	NA	Azithromycin	NA	NA	NA	NA	NA

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13
Clinical response to initial macrolide treatment (after >48h)	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes
- Evaluation after how many days of initial macrolide treatment?	3	3	6	3	3	5	2	3	2	6	3	3	3
Clinical non-response													
- Reason: persistent fever	No	No	No	Yes	No	No	No	Yes	Yes	No	No	No	No
- Reason: persistent or worse chest X-ray	Yes	No	No	No	Yes	No	No	No	No	No	No	No	No
- Reason: persistent respiratory distress	No	No	No	Yes	No	No	No	No	No	No	No	No	No
- Reason: new respiratory distress	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No
Alternative antibiotic use	Clarithromycin	No	No	Levofloxacin	Clarithromycin	No	No	Levofloxacin	Levofloxacin	No	NA	No	No
- Response to alternative antibiotic	Yes	NA	NA	Yes	Yes	NA	NA	Yes	Yes	NA	NA	NA	NA
Treatment results	Discharged with no complication	Recovered with outpatient treatment	Recovered with outpatient treatment	Discharged with no complication	Discharged with no complication	Recovered with outpatient treatment	Recovered with outpatient treatment	Discharged with no complication	Discharged with no complication	Recovered with outpatient treatment	Discharged with no complication	Discharged with no complication	Discharged with no complication