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

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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.

ycoplasma pneumoniae is a common etiologic Lagent 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. pneumoniaepositive 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 (5'-GGCGCTACAACTGGAGCATA-3'). MRMP-R1 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.

About the Author

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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.

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Crimean-Congo hemorrhagic fever (CCHF) is a tickborne disease caused by CCHF virus (CCH-FV) (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 Article DOI: https://doi.org/10.3201/eid3005.231632

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Appendix

Appendix Table. C	haracteristics of	each of all patier	nts										
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-	Non-	Non-detected	Non-	Non-detected	Non-	Non-
							detected	detected		detected		detected	detected
Nasopharyngeal	Negative	Not done	Not done	Not done	Negative	Not done	Not done	Positive	Negative	Not done	Positive	Negative	Negative
culture	-				-				-			-	-
- Positive	NA	NA	NA	NA	NA	NA	NA	H.	NA	NA	S. pyogenes	NA	NA
Bacteria								influenzae					
PCR panel of 7													
respiratory													
bacteria													
- Positive with	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
M. pneumoniae													
- Positive with	No	No	Yes	No	Yes	No	No	Yes	No	No	No	No	Yes
H. influenzae													
- Positive with	No	No	No	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes
S. pneumoniae													
- Positive with	No	No	No	No	No	No	No	No	No	No	No	No	No
other bacteria													
Co_detection of	No	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes
bacteria by PCR													
panel													
Co_detection of	No	No	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes
bacteria by PCR													
or culture													
Mycoplasma	Positive	NOT DONE	NOT DONE	Positive	NOT DONE	NOT DONE	NOT DONE	NOT DONE	Negative	NOT DONE	NOT DONE	NOT DONE	Positive
IgM													
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	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
visit													
Day of illness at	5	3	4	5	3	4	5	4	5	7	4	5	5
hospital visit													
and													
nasopharyngeal													
sample													
aspiration													
Days with fever	5	3	4	5	3	4	5	4	5	2	4	5	5
at hospital visit													
Cough	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Respiratory	No	No	No	Yes	No	No	No	No	No	No	No	No	No
distress													
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	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
chest X-ray													
Oxygen	No	No	No	Yes	No	No	No	No	No	No	No	No	No
supplementation													
Treatment	Yes	Yes	Yes	No	No	Yes	No	No	Yes	No	No	No	Yes
before hospital													
visit													
 Antibiotic use 	Amoxicillin &	No	Clarithromycin	NA	NA	No	NA	NA	Azithromicin	NA	NA	NA	Amoxicillin &
before hospital	Clavulanic												Clavulanic
visit	acid												acid
 Duration of 	4	3	6	NA	NA	4	NA	NA	3	NA	NA	NA	4
treatment before													
hospital visit													
Macrolide	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
initiated at the													
beginning of													
hospital													
treatment													
 Macrolide type 	Azithromycin	Azithromycin	Clarithromycin	Azithromycin	Azithromycin	Azithromycin	Azithromycin	NA	Clarithromycin	Azithromycin	Clarithromycin	Azithromycin	Azithromycin
Other antibiotic	Ceftriaxone	Amoxicillin &	Cefdinir	Amoxicillin &	Amoxicillin &	Amoxicillin &	NA	Amoxicillin &	Ceftriaxone	Amoxicillin &	Amoxicillin &	Amoxicillin &	Ceftriaxone
use at the		Clavulanic		Clavulanic	Clavulanic	Clavulanic		Clavulanic		Clavulanic	Clavulanic	Clavulanic	
beginning of		acid		acid	acid	acid		acid		acid	acid	acid	
hospital													
treatment													
Switch to	Yes	No	No	No	Yes	No	No	NA	NA	No	No	No	No
Clarithromycin													
- Switch after	6	NA	NA	NA	5	NA	NA	NA	NA	NA	NA	NA	NA
how many day													
Macrolide was	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA
used after initial													
antibiotic (day)													
 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	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes
response to													
initial macrolide													
treatment (after													
>48h)													
- Evaluation	3	3	6	3	3	5	2	3	2	6	3	3	3
after how many													
days of initial													
macrolide													
treatment?													
Clinical non-													
response													
- Reason:	No	No	No	Yes	No	No	No	Yes	Yes	No	No	No	No
persistent fever	Maria	NI.	N L	NL.	Mar	NL.	NL.	NL.	NI.	NL.	NL.	Ν.	Ν.,
- Reason:	Yes	NO	NO	NO	Yes	NO	NO	NO	NO	NO	NO	NO	NO
persistent or													
worse cnest X-													
Record	No	No	No	Vee	No	No	No	No	No	No	No	No	No
- Reason	NO	INO	NU	Tes	NU	NO	NO	NO	NO	NO	INO	NO	NO
persisterit													
distress													
- Reason' new	No	No	No	No	No	No	No	Vec	Vec	No	No	No	No
respiratory	NO	NO	NO	NO	NO	NO	NO	165	163	NO	NO	NO	NO
distress													
Alternative	Clarithromycin	No	No	l evofloxacin	Clarithromycin	No	No	Levofloxacin	l evofloxacin	No	NA	No	No
antibiotic use	e la			2010110/10/01				2010/10/10/10	2010/10/10/10				
- Response to	Yes	NA	NA	Yes	Yes	NA	NA	Yes	Yes	NA	NA	NA	NA
alternative													
antibiotic													
Treatment	Discharged	Recovered	Recovered	Discharged	Discharged	Recovered	Recovered	Discharged	Discharged	Recovered	Discharged	Discharged	Discharged
results	with no	with	with	with no	with no	with	with	with no	with no	with	with no	with no	with no
	complication	outpatient	outpatient	complication	complication	outpatient	outpatient	complication	complication	outpatient	complication	complication	complication
		treatment	treatment			treatment	treatment		•	treatment	•		