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Macrolide-Resistant *Bordetella pertussis*, Vietnam, 2016-2017

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Macrolide-resistant *Bordetella pertussis* emerged in Vietnam during 2016-2017. Direct analyses of swab samples from 10 patients with pertussis revealed a macrolide-resistant mutation, A2047G, in the 23S rRNA. We identified the MT104 genotype of macrolide-resistant *B. pertussis* (which is prevalent in mainland China) and its variants in these patients.

Pertussis (whooping cough) is a highly contagious disease caused by the gram-negative bacterium *Bordetella pertussis*. Vaccination is an effective method to prevent and control pertussis, but in many countries, pertussis incidence remains despite high vaccination coverage. Macrolides are commonly used to treat pertussis, but macrolide-resistant *B. pertussis* (MRBP) strains have been observed in mainland China and Iran (*1*–*4*). In China, MRBP is isolated with increasing frequency (57.5%–91.9%) and has been since the early 2010s (*4*,*5*). Most MRBP isolates from China have a homogeneous A2047G mutation in each of the 3 copies of the 23S rRNA gene, which is associated with macrolide resistance (*1*,*3*,*4*). In contrast, MRBP is rare in Iran; the A2047G mutation is not identified in the Iran MRBP isolate (*6*). China has several reports of MRBP, but our knowledge about these bacteria in other countries in Asia is limited.

To survey MRBP in Vietnam, which neighbors China, we performed a retrospective analysis of stored DNA samples from nasopharyngeal swabs collected during 2016–2018 from 53 patients with pertussis in northern Vietnam (median age 3 months [range 31 days–32 years]; 14 patients in 2016, 38 in 2017, and 1 in 2018) (Appendix Table, https://wwwnc.cdc.gov/EID/article/26/10/20-2035-App1.pdf). Nucleic acid amplification testing was used to diagnose

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B. pertussis in patients who were infected with this condition. We used the cycleave real-time PCR targeting the A2047 mutation in the *B. pertussis* 23S rRNA to examine the 53 DNA samples (Appendix). Of these DNA samples, 10 (19%) were positive for the A2047G mutation. PCR-based sequencing validated the presence of the mutation (*3*). Nine of these samples were from infants 32 days–4 months of age, and 1 was from a woman 29 years of age (Table). Geographically, 7 DNA samples were found in Hanoi and 3 in other provinces (Ha Nam and Thai Binh) (Appendix Figure 1). Five patients were treated with β-lactam antimicrobial drugs; the treatments for other patients and their epidemiologic links are unknown.

We used multilocus variable-number tandem-repeat analysis (MLVA) to determine the genotypes of the MRBP by direct genotyping (*7*). We classified the MLVA profiles into the following 3 genotypes: MT104 (n = 8) and new genotypes A and B (n = 1 each) (Table). Genotypes A and B were minor single-locus variants of MT104, differing in 1 of the 6 variable-number tandem-repeat (VNTR) loci. Phylogenetic analysis revealed that the MRBP belonging to genotypes A and B were closely related to MT104 (Appendix Figure 2). We also characterized *B. pertussis* virulence–associated allelic genes (*ptxP*, *ptxA*, *prn*, and *fim3*) by DNA sequence-based typing (*7*). Of the 10 MRBP DNA samples, 9 yielded a complete profile of virulence-associated allelic genes, 8 were *ptxP1/ptxA1/prn1/fim3A*, and 1 was *ptxP1/ptxA1/prn1/fim3B* (Table). The allelic profile *ptxP1/ptxA1/prn1* is common in MRBP strains prevalent in China (*8*). In addition, 9 of the MRBP DNA samples exhibited the C5330T mutation in *fhaB3*, which is frequently observed in MRBP in China (*9*) (Appendix Table).

Genotyping assays revealed that MRBP strains in Vietnam were closely related to an MRBP strain identified in China. The major MLVA types reported recently in China are MT55, MT104, and MT195 (*8*,*9*). These types are closely related; they have only 1 difference at 1 VNTR locus. All isolates of these genotypes contained the macrolide resistance A2047G mutation in the 23S rRNA. A clinical strain of MT104-MRBP was first identified in 2012 in Shannxi, China. Subsequently, this clinical strain of MT104-MRBP was found throughout the country (*9*).

In Vietnam, the *B. pertussis* population comprises 2 major strains, MT27 and MT104 (Appendix Figure 2). The MT27 strain is common in industrialized countries but not in China (*8*,*9*). In contrast, the MT104 strain is not common in industrialized countries but frequent in China. We define a clonal complex as genotypes differing in only 1 of the 6 VNTRs. We have 2 clonal complexes in the *B. pertussis* population in Vietnam, 1 containing MT104 and genotypes A and B and another containing MT18, MT27, and MT28. MRBP genotypes A and B differ from MT104 by a single repeat at 1 VNTR locus. MRBP genotypes A and B are grouped within the clonal complex of MRBP. This finding suggests that the MRBP-MT104 strain was imported from China to Vietnam before 2016 and subsequently mutated to genotypes A and B over time. Macrolides are the third most common antimicrobial drugs used in Vietnam (*10*), and they are commonly available at private pharmacies without prescriptions, suggesting that the uncontrolled use of macrolides might have selected MRBP in the country.

In conclusion, we reported the emergence of MRBP in Vietnam during 2016-2017. We detected MRBP strains that have the same or a similar phylogenetic lineage as 1 of the MRBP strains prevalent in China. Because MRBP is a serious threat to public health, global surveillance of MRBP is needed, especially in countries in Asia.

Table. Direct genotyping of *Bordetella pertussis* with the detected macrolide-resistant A2047G mutation in the 23S rRNA gene, Vietnam, 2016–2017*

Patient no.	Age/sex	Year/province	MLVA type	Repeat no. VNTRs†	Allele type of virulence-associated genes‡				C5330 in *fhaB*§
					ptxP	*ptxA*	*prn*	*fim3*	
1	2.5 mo/M	2016/Hanoi	MT104	8/6/0/7/6/10	1	1	1	A	NA
2	2 mo/F	2016/Ha Nam	New type A	8/6/0/6/6/10	1	1	1	A	C5330T
3	32 d/F	2016/Hanoi	New type B	9/6/0/7/6/10	1	1	1	A	C5330T
4	3 mo/F	2016/Hanoi	MT104	8/6/0/7/6/10	1	1	1	A	C5330T
5	2 mo/M	2016/Hanoi	MT104	8/6/0/7/6/10	1	1	1	A	C5330T
6	29 y/F	2016/Hanoi	MT104	8/6/0/7/6/10	1	1	1	B	C5330T
7	4 mo/F	2017/Thai Binh	MT104	8/6/0/7/6/10	NA	1	1	B	C5330T
8	52 d/F	2017/Ha Nam	MT104	8/6/0/7/6/10	1	1	1	A	C5330T
9	3 mo/M	2017/Hanoi	MT104	8/6/0/7/6/10	1	1	1	A	C5330T
10	3 mo/M	2017/Hanoi	MT104	8/6/0/7/6/10	1	1	1	A	C5330T

*MLVA, multilocus variable-number tandem-repeat analysis; NA, not analyzed; VNTR, variable-number tandem-repeat.
†The order is VNTR1/VNTR3a/VNTR3b/VNTR4/VNTR5/VNTR6.
‡*B. pertussis* virulence–associated allelic genes (*ptxP*, *ptxA*, *prn*, and *fim3*).
§*fhaB3* allele carries the single-nucleotide polymorphism mutation C5330T.

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COVID-19 in Patient with Sarcoidosis Receiving Long-Term Hydroxychloroquine Treatment, France, 2020

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Because of in vitro studies, hydroxychloroquine has been evaluated as a preexposure or postexposure prophylaxis for coronavirus disease (COVID-19) and as a possible COVID-19 curative treatment. We report a patient with sarcoidosis who was receiving long-term hydroxychloroquine treatment and contracted COVID-19, despite adequate plasma concentrations.

Because of in vitro studies suggesting potential activity on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (*1*,*2*), hydroxychloroquine has been one of the main candidate drugs evaluated for coronavirus disease (COVID-19), both as a curative treatment and as preexposure or postexposure prophylaxis. We report a case of COVID-19 in a patient receiving long-term hydroxychloroquine treatment despite plasma concentrations within the therapeutic range for autoimmune diseases, such as systemic lupus erythematosus.

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Macrolide-Resistant *Bordetella pertussis* in Vietnam, 2016–2017

Appendix

Cycleave Real-Time PCR Assay for Detection of the A2047G Mutation

A cycling probe technology assay was used (*1*,*2*). To discriminate between the mutant allele A2047G and wild-type allele (non-A2047G) of 23S rRNA in *B. pertussis*, two cycling probes were designed: 5′-Eclipse-GACGGgAAG-HEX-3′ for A2047G and 5′-Eclipse-AGACGGaAAG-FAM-3′ for non-A2047G. The upper- and lowercase letters in the sequences indicate DNA and RNA, respectively. The duplex Cycleave real-time PCR was performed using an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). PCR amplification was carried out in 20 μL reactions containing 10 μL of 2× CycleavePCR Reaction Mix (Takara Bio Inc, Japan), 0.4 μL of 50× ROX reference dye II (Takara Bio), 2 μL of DNA sample, 0.2 μM of each cycling probe, and 0.2 μM of each primer (Primer-F: 5′-GAATGGCGTAACGATG-3′ and Primer-R: 5′-TGCAAAGCTACAGTAAAGG-3′). The PCR conditions were the following: 20 s at 95°C, followed by 40 cycles of 95°C for 3 s, 60°C for 10 s, and 72°C for 25 s. The fluorescence signal of HEX was monitored on the VIC channel of the PCR system. In each assay, two PCR fragments (A2047- and A2047G-DNA fragments, 5× 10^3 DNA copies/μL each) were used as positive controls; sterile distilled water was used as a negative control. The detection limits of the PCR assay were »20 DNA copies/tube for both positive controls.

Construction of A2047- and A2047G-DNA Fragments

The positive controls (796 bp each) were constructed by PCR using *B. pertussis* Tohama DNA as a template (non-A2047G strain). The A2047-DNA fragment (wild-type allele) was amplified using the primers 23S rDNA-F (5′-GGTATACCCTGGTAGTGTGAAG-3′) and 23S rDNA-R (5′-CGACATCGAGGTGCCAAA-3′), and purified with the MinElute PCR Purification Kit (Qiagen). The A2047G-DNA fragment (mutant allele) was constructed by

overlap extension PCR method (*3*). Briefly, two DNA fragments were amplified by PCR using the primer sets, 23S rDNA-F and 23S A2047G-R (5′-AAGGTTCATGGGGTCTTCCCGTCTAGCCGCGGGTA-3′), and 23S A2047G-F (5′-TACCCGCGGCTAGACGGGAAGACCCCATGAACCTT-3′) and 23S rDNA-R, respectively. The DNA fragments were joined by overlap extension PCR using the primers 23S rDNA-F and 23S rDNA-R and purified with MinElute PCR Purification Kit. The substitution of A with G (at position 2047) in the primer sequences has been underlined.

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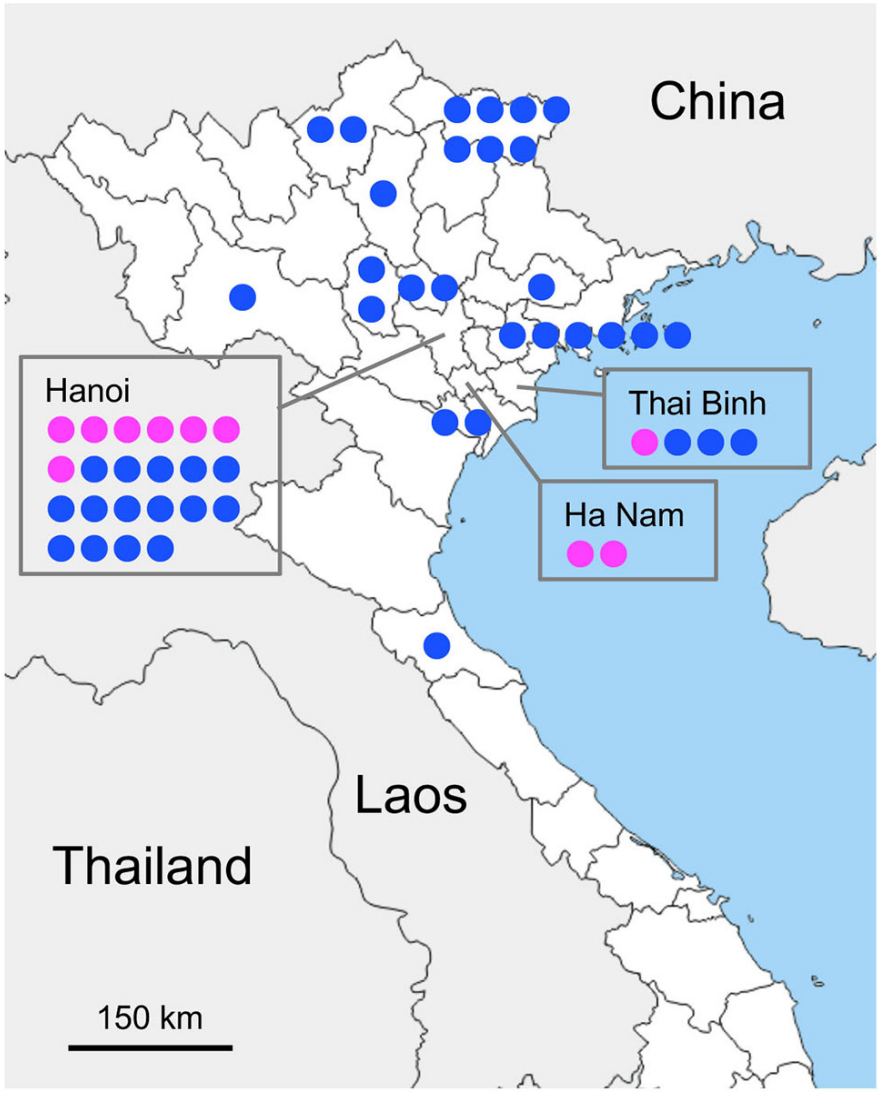
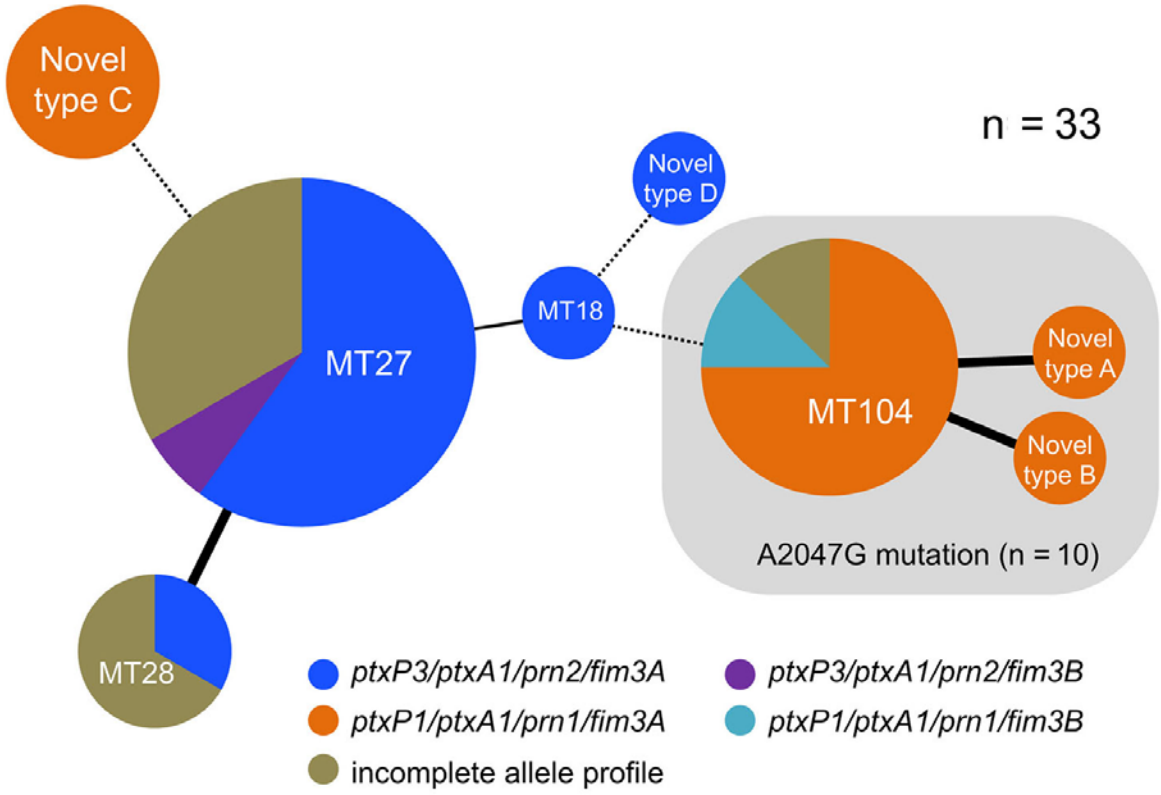
Appendix Table. Analysis of *Bordetella pertussis* in the DNA samples collected from 53 pertussis patients, Vietnam, 2016–2018*

Year of specimen collection	Province	Age/sex	Vaccine status	A2047G in 23S rRNA	MLVA type	Allele type of virulence-associated genes†				C5330 in *fhaB*‡
						ptxP	*ptxA*	*prn*	*fim3*	
2016	Hanoi	32 y/F	3 doses	Negative	MT18	3	1	2	A	C
2016	Hanoi	5 m/F	2 doses	Negative	MT27	3	1	2	A	C
2016	Hanoi	28 y/F	3 doses	Negative	MT27	3	1	NA	B	NA
2016	Cao Bang	26 y/F	Unknown	Negative	MT27	3	1	2	A	C
2016	Phu Tho	4 m/F	1 dose	Negative	NA	ND	ND	ND	ND	ND
2016	Phu Tho	3 m/F	1 dose	Negative	MT27	3	1	2	A	NA
2016	Hanoi	2.5 m/M	Unknown	Positive	MT104	1	1	1	A	NA
2016	Bac Giang	2 m/M	0	Negative	MT27	3	1	2	B	NA
2016	Ha Nam	2 m/F	0	Positive	Novel type A	1	1	1	A	C>T
2016	Hanoi	32 d/F	0	Positive	Novel type B	1	1	1	A	C>T
2016	Hanoi	3 m/F	1 dose	Positive	MT104	1	1	1	A	C>T
2016	Hanoi	2 m/F	0	Negative	NA	ND	ND	ND	ND	ND
2016	Hanoi	2 m/M	0	Positive	MT104	1	1	1	A	C>T
2016	Hanoi	29 y/F	3 doses	Positive	MT104	1	1	1	B	C>T
2017	Thai Binh	4 m/F	2 doses	Positive	MT104	NA	1	1	B	C>T
2017	Hanoi	2 m/F	0	Negative	NA	ND	ND	ND	ND	ND
2017	Cao Bang	11 m/M	Unknown	Negative	NA	ND	ND	ND	ND	ND
2017	Cao Bang	3 y/M	3 doses	Negative	Novel type C	1	1	1	A	C
2017	Cao Bang	4 y/F	3 doses	Negative	NA	ND	ND	ND	ND	ND
2017	Cao Bang	2 m/F	0	Negative	Novel type C	1	1	1	A	C
2017	Hanoi	2 y/M	3 doses	Negative	MT27	3	1	2	A	C
2017	Vinh Phuc	8 y/F	3 doses	Negative	MT27	3	1	2	A	C
2017	Vinh Phuc	2 m/M	0	Negative	MT27	3	1	2	A	C
2017	Hai Duong	4 m/F	2 doses	Negative	MT28	3	1	NA	B	C
2017	Thai Binh	4 m/M	1 dose	Negative	NA	ND	ND	ND	ND	ND
2017	Thai Binh	3 m/M	1 dose	Negative	MT27	3	1	2	A	C
2017	Hai Duong	45 d/M	0	Negative	MT28	3	1	2	A	NA
2017	Hanoi	3 m/F	1 dose	Negative	NA	ND	ND	ND	ND	ND
2017	Thai Binh	31 d/M	0	Negative	Novel type D	3	1	2	A	NA
2017	Hai Duong	52 d/M	0	Negative	NA	ND	ND	ND	ND	ND
2017	Cao Bang	6 y/M	3 doses	Negative	NA	ND	ND	ND	ND	ND
2017	Ha Giang	5 m/M	Unknown	Negative	NA	ND	ND	ND	ND	ND
2017	Ha Giang	18 m/F	3 doses	Negative	NA	ND	ND	ND	ND	ND
2017	Ha Tinh	2 m/M	Unknown	Negative	NA	ND	ND	ND	ND	ND
2017	Hanoi	20 m/F	3 doses	Negative	NA	ND	ND	ND	ND	ND
2017	Ninh Binh	4 m/F	1 dose	Negative	MT27	3	1	2	A	C
2017	Cao Bang	4.5 y/M	3 doses	Negative	MT27	3	1	NA	NA	NA
2017	Hai Duong	2 m/M	0	Negative	NA	ND	ND	ND	ND	ND
2017	Hai Duong	2 m/M	0	Negative	Novel type C	1	1	1	A	C
2017	Hai Duong	4 m/M	2 doses	Negative	MT27	3	1	2	A	C
2017	Hanoi	2 m/F	0	Negative	MT27	3	1	NA	B	C
2017	Hanoi	3 m/F	1 dose	Negative	NA	ND	ND	ND	ND	ND
2017	Ha Nam	52 d/F	0	Positive	MT104	1	1	1	A	C>T
2017	Tuyen Quang	45 d/F	Unknown	Negative	NA	ND	ND	ND	ND	ND
2017	Hanoi	3 m/M	1 dose	Positive	MT104	1	1	1	A	C>T
2017	Hanoi	23 y/F	3 doses	Negative	MT27	NA	1	NA	B	NA
2017	Hanoi	3 m/F	1 dose	Negative	MT28	NA	1	NA	NA	NA
2017	Hanoi	3 m/M	1 dose	Positive	MT104	1	1	1	A	C>T
2017	Son La	2.5 m/M	0	Negative	MT27	3	1	2	NA	C
2017	Hanoi	45 d/M	0	Negative	NA	ND	ND	ND	ND	ND
2017	Hanoi	3.5 m/M	1 dose	Negative	NA	ND	ND	ND	ND	ND
2017	Hanoi	3 m/F	1 dose	Negative	NA	ND	ND	ND	ND	ND
2018	Ninh Binh	28 d/M	0	Negative	NA	ND	ND	ND	ND	ND

*MLVA, multilocus variable-number tandem repeat analysis; NA, not analyzed; ND, not determined.
†The allelic genes were analyzed only in DNA samples that yielded a complete MLVA profile.
‡*fhaB3* allele carries the SNP mutation C5330T.



Appendix Figure 1. Geographic location of the 53 pertussis patients investigated in a study of macrolide-resistant *Bordetella pertussis*, Vietnam, 2016–2018. Red and blue circles indicate patients positive and negative for the macrolide-resistant A2047G mutation, respectively.



Appendix Figure 2. Minimum spanning tree revealing the genetic diversity of the *Bordetella pertussis* population, Vietnam, 2016–2017. Of the 53 DNA samples, 33 yielded a complete multilocus variable-number tandem repeat analysis profile by direct genotyping and were classified into 8 multilocus variable-number tandem repeat analysis types (MTs). The gray background represents macrolide-resistant *B. pertussis* carrying the A2047G mutation in the 23S rRNA (MT104, and novel genotypes A and B). The colors of the circles represent different allele profiles of the virulence-associated allelic genes (*ptxP/ptxA/prn/fim3*). Solid lines separate single-locus variants, dotted lines separate double-locus variants. Thick lines represent differences of one repeat at 1 variable-number tandem repeat.