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Short Communication

Molecular epidemiology of *Bordetella pertussis* in the Philippines in 2012–2014

Salvacion Rosario L. Galit [a], Nao Otsuka [b], Yuki Furuse [c], Daryl Joy V. Almonia [a], Lydia T. Sombrero [a], Rosario Z. Capeding [a], Socorro P. Lupisan [a], Mariko Saito [c,d], Hitoshi Oshitani [c], Yukihiro Hiramatsu [b], Keigo Shibayama [b], Kazunari Kamachi [b,*]
Tohoku-RITM Collaborating Research Team

[a] *Research Institute for Tropical Medicine (RITM), Metro Manila, Philippines*
[b] *Department of Bacteriology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan*
[c] *Department of Virology, Tohoku University Graduate School of Medicine, Miyagi, Japan*
[d] *Tohoku-RITM Collaborating Research Center on Emerging and Reemerging Infectious Diseases, Metro Manila, Philippines*

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SUMMARY

Objectives: The present study was designed to determine the genotypes of circulating *Bordetella pertussis* in the Philippines by direct molecular typing of clinical specimens.
Methods: Nasopharyngeal swabs (NPSs) were collected from 50 children hospitalized with pertussis in three hospitals during 2012–2014. Multilocus variable-number tandem repeat analysis (MLVA) was performed on the DNA extracts from NPSs. *B. pertussis* virulence-associated allelic genes (*ptxA*, *prn*, and *fim3*) and the pertussis toxin promoter, *ptxP*, were also investigated by DNA sequence-based typing.
Results: Twenty-six DNA extracts yielded a complete MLVA profile, which were sorted into 10 MLVA types. MLVA type 34 (MT34), which is rare in Australia, Europe, Japan, and the USA, was the predominant strain (50%). Seven MTs (MT29, MT32, MT33, and MT283–286, total 42%) were single-locus variants of MT34, while two (MT141 and MT287, total 8%) were double-locus variants of MT34. All MTs had the combination of virulence-associated allelic genes, *ptxP1–ptxA1–prn1–fim3A*.
Conclusions: The *B. pertussis* population in the Philippines comprises genetically related strains. These strains are markedly different from those found in patients from other countries where acellular pertussis vaccines are used. The differences in vaccine types between these other countries and the Philippines, where the whole-cell vaccine is still used, may select for distinct populations of *B. pertussis*.

1. Introduction

Bordetella pertussis, a highly communicable Gram-negative coccobacillus, is the etiological agent of whooping cough (pertussis), a major acute respiratory infection resulting in severe childhood illness and infant death. In the Philippines, pertussis is controlled by the administration of the whole-cell pertussis vaccine (WCV), given in three doses at ages 6, 10, and 14 months. The pentavalent DTwP-HepB-Hib vaccine (diphtheria, tetanus toxoids, whole-cell pertussis, hepatitis B, *Haemophilus influenzae* type b), Easyfive-TT (Panacea Biotec Ltd, India), is the most common vaccine currently used for immunization. Despite these controls, pertussis infections have occurred sporadically, primarily in unvaccinated children (<6 months of age). The molecular epidemiology of *B. pertussis* populations has been studied in several countries,[1–5] but no information of the specific strains present in the Philippines has been reported. Molecular typing of the organism can be performed on both bacterial isolates and clinical specimens.[6] The genotypes of the circulating strains of *B. pertussis* in the Philippines were therefore determined by direct molecular typing of clinical specimens.

2. Materials and methods

Between September 2012 and May 2014, 50 children hospitalized with severe respiratory distress (median age 2 months, range 0–53 months) were diagnosed with *B. pertussis* infections by PCR targeting IS*481*.[7] Patients were hospitalized at the Philippine

* Corresponding author. Tel.: +81-42-848-7101; fax: +81-42-561-7173.
E-mail address: kamachi@nih.go.jp (K. Kamachi).

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General Hospital (PGH; 26 patients), Ospital ng Palawan (ONP; 17 patients), and the Research Institute for Tropical Medicine (RITM; seven patients) (**Supplementary Material**, Figure S1). Nasopharyngeal swabs (NPSs) were obtained from the patients, and DNA was extracted from the NPSs using the QIAamp DNA Mini Kit (Qiagen).

Multilocus variable-number tandem repeat analysis (MLVA) typing was performed on DNA extracted from NPSs.[6] MLVA types (MTs) were assigned using the MLVA typing tool found at http://www.mlva.net. Novel MTs were assigned by Ing. H. van der Heide, National Institute for Public Health and the Environment, the Netherlands. To characterize the phylogenic relationships between the MTs, minimum spanning trees were generated using FPQuest software (Bio-Rad). DNA sequence-based typing for *B. pertussis* virulence-associated allelic genes (*ptxA*, *prn*, and *fim3*) and the pertussis toxin promoter, *ptxP*, was performed on DNA extracts that yielded a complete six-allele MLVA profile, with some modifications.[5] Briefly, PCR cycling conditions were 94 °C for 2 min, followed by 11 cycles of touchdown PCR (98 °C for 10 s, followed by annealing, initially at 65 °C for 30 s and decreasing 1 °C/cycle until 55 °C, and elongation at 68 °C for 45 s) and 25 cycles of standard PCR (98 °C for 10 s, 55 °C for 30 s, and 68 °C for 45 s). For *ptxP* and variable region 2 (R2) of *prn*, some DNA extracts that failed to yield DNA sequences were analyzed by nested PCR. The PCR primer sets used in this study are listed in the **Supplementary Material** (Table S1).

3. Results

DNA extracts from NPSs were collected from 50 patients and analyzed by MLVA typing. Twenty-six (52%) samples yielded a complete six-allele MLVA profile, and the remainder yielded either a partial profile (38%) or a negative MLVA result (10%) (Table 1). The complete MLVA profiles were obtained primarily from patients aged between 1 and <3 months; however, distributions of patient age among the three MLVA groups (complete profiles, partial profiles, no alleles) were not significantly different ($p = 0.87$, Fisher's exact test). The MLVA results therefore did not depend on patient age.

Among the 26 DNA extracts that yielded a complete MLVA profile, 10 distinct MTs were identified, of which five were novel: MT283, MT284, MT285, MT286, and MT287. Figure 1 shows a minimum spanning tree that revealed the genetic diversity of the *B. pertussis* population. MT34 was the most prevalent type ($n = 13$) and MT33 was the second most prevalent ($n = 4$). Eight MTs (MT29, MT32, MT141, MT283, and MT284–287) were minor subtypes, appearing only rarely ($n = 1$ or 2). All MTs had a combination of *ptxP1–ptxA1–prn1–fim3A* alleles, as demonstrated by DNA sequence-based typing. The MT distribution was not statistically different among the three hospitals, PGH, ONP, and RITM ($p = 0.23$, Fisher's exact test).

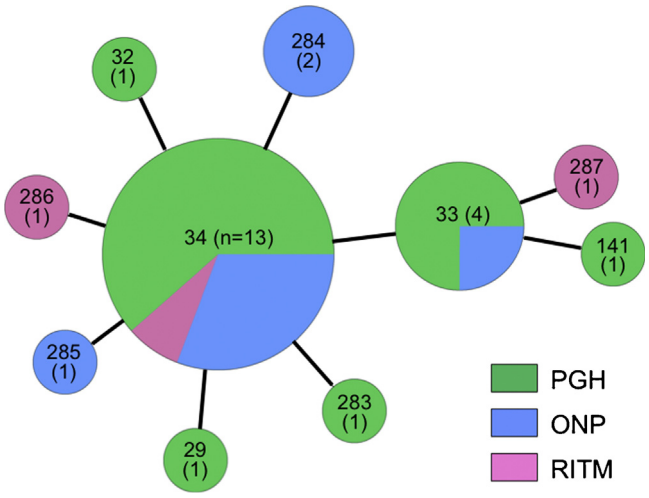

Figure 1. Minimum spanning tree revealing the genetic diversity of the *Bordetella pertussis* population in the Philippines during 2012–2014. MLVA types (MTs) were identified from DNA extracts of clinical specimens, collected from 26 patients. Each circle within a tree represents a unique MT, and the number denotes the MT. The sizes of circles are representative of the number of clinical specimens in each group, with the numbers indicated in parentheses. Lines connecting circles represent single-locus variants. All MTs carried *ptxP1*, *ptxA1*, *prn1*, and *fim3A* alleles. PGH, Philippine General Hospital; ONP, Ospital ng Palawan; RITM, Research Institute for Tropical Medicine.

4. Discussion

In this study, the *B. pertussis* MT34 strain was demonstrated to be the predominant (50%) subtype in the Philippines during 2012–2014. Seven MTs (MT29, MT32, MT33, and MT283–286, total 42%) were single-locus variants of MT34, and two others (MT141 and MT287, total 8%) were double-locus variants of MT34. All MT strains carried the same virulence-associated allelic genes, *ptxP1–ptxA1–prn1–fim3A*. These data suggest that the *B. pertussis* population in the Philippines comprises genetically related strains.

In Australia, Europe, and the USA, the *B. pertussis* MT27 strain (double-locus variant of MT34) was the predominant type during the past decade.[2–4,6,8,9] In Japan, both MT27 and MT186 were the predominant types during 2002–2012, with only one MT34 strain found out of 134 *B. pertussis* isolates tested.[5] The Japanese MT34 strain carried the same virulence-associated genes, *ptxP1–ptxA1–prn1–fim3A*, as the MT34 strain from the Philippines described in this study. MT34 strains have been identified very rarely throughout the world.[1–4,10] In Australia, the USA, Japan, and most

Table 1
Summary of MLVA results for 50 pertussis patients grouped by age

Patient age range	Number of clinical specimens	MLVA result[a]			MLVA type[b]
		Complete profile	Partial profile	No alleles generated	
0 to <1 month	2	1	1		MT286
1 to <2 months	21	11	8	2	MT29, MT32, MT34, MT141, MT284, MT285, MT287
2 to <3 months	14	8	5	1	MT33, MT34, MT284
3 to <12 months	8	4	3	1	MT33, MT34
1 to 4 years	4	2	2		MT34, MT283
Unknown	1[c]			1	
Total	50	26	19	5	

MLVA, multilocus variable-number tandem repeat analysis.
[a] Complete profile, six alleles; partial profile, from one to five alleles.
[b] MLVA types were determined using the complete profile.
[c] Patient age was <2 months.

European countries, acellular pertussis vaccines (ACVs) have been used, whereas a WCV is still used in the Philippines. Thus, the different vaccine types may select for different *B. pertussis* populations.

This study provides a baseline for future studies on the *B. pertussis* population in the Philippines.

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Conflict of interest: The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2015.04.001.

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