Genetic diagnosis and molecular epidemiology of Bordetella pertussis

Shu-Man Yao, Chuen-Sheue Chiang
Centers for Disease Control, Department of Health, Taipei, Taiwan

Summary:

Bordetella holmesii infection has never been reported in Taiwan. In current surveillance study, only one case of possible *B. holmesii* infection was found among 495 notified cases of pertussis (0.20%) in 2011 and 2012. This possible case was a 12-year-old boy who was notified in May 2011. According to this surveillance results, the prevalence of *B. holmesii* infection in Taiwan was extremely low among patients who had pertussis-like symptoms and were notified. Based on its high sensitivity, the IS*481*-based PCR was routinely used worldwide by many laboratories, however, it should be done with the awareness of its cross-reactivity. *B. holmesii* has been more and more frequently detected in biological samples from adolescents and adults of pertussis-like symptoms. Although differentiation might not be necessary for treatment, it is valuable in epidemiological settings to distinguish among *Bordetella* species. Improved specificity of detection would provide us an insight into the real burdens of *B. pertussis* and *B. holmesii* infection, and information regarding vaccine failure due to misdiagnosis and possible response strategy. In conclusion, surveillance of *B. holmesii* should be pursued, and correct identification of *Bordetella* species is important for active surveillance of *Bordetella* infections in the whole population, particularly in adolescents and adults.

I. Purpose:

In previous surveillance studies conducted in the USA and Canada, a low positive rate for *B. holmesii* infection (0.1–0.3%) was reported by culture or RT-PCR in patients with coughs (1, 2). In a recent study in France, however, *B. holmesii* DNA was detected in 20% of nasopharyngeal swabs (NPSs) collected from adolescent patients who had previously been diagnosed with *B. pertussis* infection (3). Furthermore, between 2010 and 2011, a pertussis outbreak caused by *B. pertussis* and *B. holmesii* infections occurred in Miyazaki Prefecture, Japan (4). These surveillance data indicated that *B. holmesii* infection has recently spread worldwide and that

accurate diagnosis is needed to distinguish between *B. holmesii* and *B. pertussis* infections. Therefore, the purpose of this surveillance study is to detect *B. holmesii* in NPSs received in our laboratory from all cases who had pertussis-like symptoms and were notified in 2011 and 2012.

II. Methods:

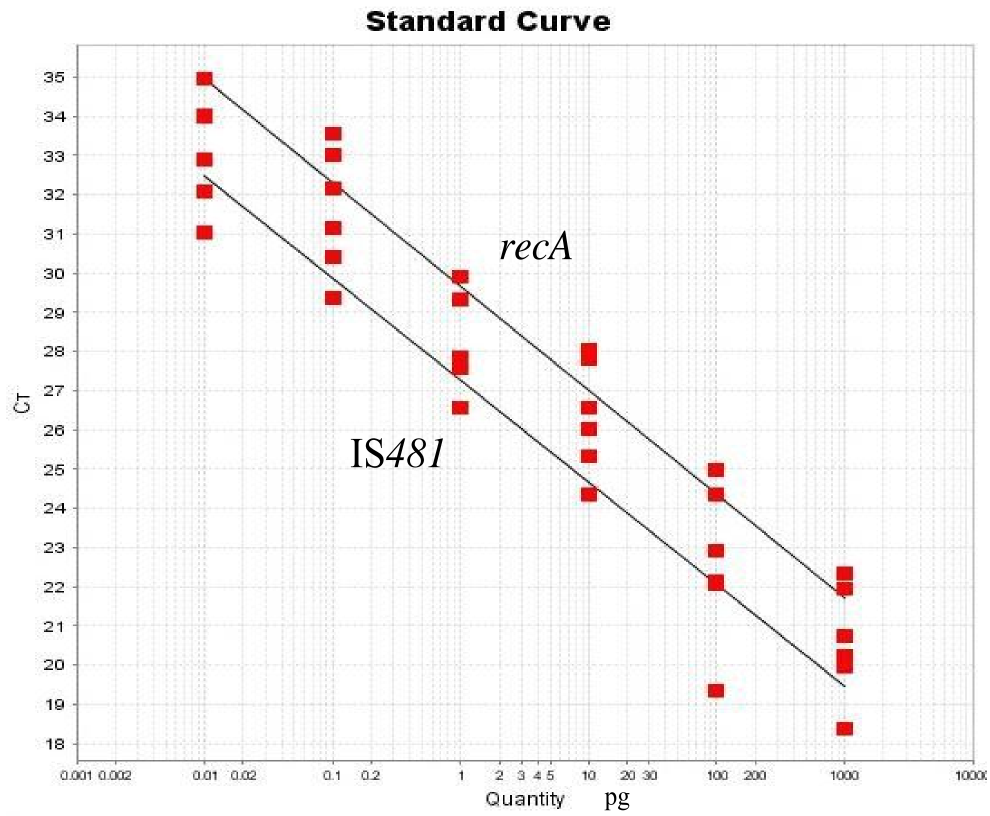
We applied *B. pertussis*-LAMP assay and a novel duplex real-time PCR assay to NPSs from 359 and 136 patients who had pertussis-like symptoms and were notified in 2011 and 2012, respectively. These patients were collected from Taiwan pertussis notified disease surveillance system. We obtained the NPSs using ESwab™ Nylon Flocked Swab and 1mL of modified Liquid Amies (Copan).

B. pertussis-LAMP assay (detection of *ptxP*): A 25µL reaction mixture containing 40 pmol (each) of BP-FIP and BP-BIP primers, 5 pmol (each) of BP-F3 and BP-B3 primers, 20 pmol (each) of BP-LF and BP-LB primers, 2X reaction mixture (12.5 µL), *Bst* DNA polymerase (1 µL), and template DNA (2 µL) was used. The mixture was incubated at 65°C for 40 min (for clinical specimens) and then heated at 80°C for 2 min to terminate the reaction. All oligonucleotides (high-performance liquid chromatography purification grade) for the LAMP primers were obtained from Invitrogen Taiwan Ltd. The LAMP amplification was confirmed with real-time monitoring of the increase of turbidity using LA-320C (Eiken Chemical Co., Ltd.) (5).

Novel duplex real-time PCR assay (detection of IS*481* and BH*recA*): The duplex PCR master mix consisted of 1X Premix master mix (Premix EX Taq, RR039A, Takara), 0.8 µM (each) BH*recA* forward and reverse primers, 0.4 µM BH*recA* probe, 1 µM (each) IS*481* forward and reverse primers, 0.25 µM IS*481* probe, 2 µL of template DNA, and enough sterile nuclease-free water to bring the total reaction volume to 20 µL. The samples were subjected to an initial amplification cycle of 95°C for 30s, followed by 40 cycles at 95°C for 5s and 60°C for 34s. Two microliters of *B. holmesii* ATCC51541 (500 pg/µL) with 6 series of 10-fold dilution was used as a positive PCR control; the negative control was 2 µL of sterile H_2O. Amplification, detection, and data analysis were performed with an Applied Biosystems 7500 real-time PCR system and the 7500 software v2.0 (1).

III. Results:

1) Detection Limit of the novel duplex real-time PCR assay



Detection limits are 0.01pg DNA for IS*481* and 0.1pg DNA for *recA* at threshold cycle (Ct) of 32±1.0.

2) Results of real-time PCR using IS*481*, BH*recA* and *ptxP* (LAMP) for NPSs in 2011-2012

Target	Target organism	2011		2012	
		No. of specimens	% of total specimens	No. of specimens	% of total specimens
IS*481** without BH*recA*	*B. pertussis*	60	16.7	30	22.1
IS*481* and BH*recA*	*B. holmesii*	1	0.3	0	0
ptxP (LAMP)	*B. pertussis*	47	13.1	25	18.4
Total specimens tesed		359		136	

B. holmesii DNA was detected only in one case notified in May 2011. This case was a 12-year-old boy. Positive rate of *B. pertussis* using IS*481*-based PCR was 16.7% and 22.1% in 2011 and 2012, respectively. Positive rate of *B. pertussis* using *ptxP*-based PCR was 13.1% and 18.4% in 2011 and 2012, respectively.

IV. Discussion:

Surveillance result of *B. holmesii* infection in Taiwan revealed that there was only one possible case, a 12-year-old boy, in May 2011. This result indicated that the prevalence of *B. holmesii* infection in Taiwan was very low among patients who had pertussis-like symptoms and were notified. Whether this case was a real case of *B. holmesii* infection was not certain. Although the duplex real-time PCR gave a positive result, sequencing of the DNA product was not successful because the *B. holmesii* DNA content in the specimen was too low.

From studies worldwide, most cases from whom *B. holmesii* was detected were adolescents and adults, especially significant occurrence in adolescents, but not in infants. However, the age distribution of our cases was 55.1% and 48.5% among infants less than 1 year old in 2011 and 2012, respectively, and 12.7% and 12.5% among children aged 10-19 years in 2011 and 2012, respectively. There might not be sufficient specimens from adolescents in our study, thus, leading to low prevalence. Nevertheless, *B. holmesii* was indeed present and associated with pertussis-like symptoms in patients, indicating that surveillance of *B. holmesii* infection is important.

Based on its high sensitivity, the IS*481*-based PCR was routinely used worldwide by many laboratories, however, it should be done with the awareness of its cross-reactivity. *B. holmesii* has been more and more frequently detected in biological samples from adolescents and adults of pertussis-like symptoms. Although differentiation might not be necessary for treatment, it is valuable in epidemiological settings to distinguish among *Bordetella* species. Improved specificity would advance our understanding of burdens from *B. pertussis* and *B. holmesii*, reduce concerns arising from apparent vaccine failures due to misdiagnosis, and might provide information on which vaccine-based outbreak response strategies can be based (6). In conclusion, surveillance of *B. holmesii* should be pursued and correct identification of *Bordetella* species is important for active surveillance of *Bordetella* infections in the whole population, particularly in adolescents and adults.

V. Reference list:

1. Guthrie JL, Robertson AV, Tang P, Jamieson F, Drews SJ. Novel duplex real-time PCR assay detects Bordetella holmesii in specimens from patients with Pertussis-like symptoms in Ontario, Canada. Journal of clinical microbiology. 2010 Apr;48(4):1435-7.
2. Yih WK, Silva EA, Ida J, Harrington N, Lett SM, George H. Bordetella holmesii-like organisms isolated from Massachusetts patients with pertussis-like symptoms. Emerging infectious diseases. 1999 May-Jun;5(3):441-3.
3. Njamkepo E, Bonacorsi S, Debruyne M, Gibaud SA, Guillot S, Guiso N. Significant finding of Bordetella holmesii DNA in nasopharyngeal samples from French patients with suspected pertussis. Journal of clinical microbiology. 2011 Dec;49(12):4347-8.
4. Kamiya H, Otsuka N, Ando Y, et al. Transmission of Bordetella holmesii during pertussis outbreak, Japan. Emerging infectious diseases. 2012 Jul;18(7):1166-9.
5. Kamachi K, Toyoizumi-Ajisaka H, Toda K, et al. Development and evaluation of a loop-mediated isothermal amplification method for rapid diagnosis of Bordetella pertussis infection. Journal of clinical microbiology. 2006 May;44(5):1899-902.
6. Rodgers L, Martin SW, Cohn A, et al. Epidemiologic and laboratory features of a large outbreak of pertussis-like illnesses associated with cocirculating Bordetella holmesii and Bordetella pertussis--Ohio, 2010-2011. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2013 Feb;56(3):322-31.

VI. Publication list for this work:

NIL