Pertussis Transmission in a Hospital Office that was Confirmed on the Basis of Loop-mediated Isothermal Amplification

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Objective: A hospital clerk developed whooping cough and its infectious condition throughout the work place was investigated to clarify the spread of pertussis infection using highly sensitive Loop-mediated isothermal amplification (LAMP).

Materials and Methods: LAMP for pertussis was performed against all employees in the personnel division of general hospital, then respiratory symptoms were noted in 12 (57%) of 21 clerical employees.

Results: LAMP for pertussis was positive in 7 (58.3%) of the 12 employees. The judgment was indeterminate in the first test in one employee, but it became negative later. An asymptomatic LAMP-negative employee subsequently developed cough and turned to positive in the second LAMP test 20 days after the first test. Cough was not noted even in positive cases in the third LAMP test 35 days after second test, or thereafter, and conversion to LAMP-negative took 76 days on average. No outbreak of whooping cough occurred in any of the other departments in the hospital throughout the course, and no transmission of infection occurred.

LAMP methods were useful for grasp of the situation of asymptomatic persons as well as symptomatic patients.

Conclusions: It is difficult to diagnose whooping cough based on cough symptoms alone. The LAMP test was useful for understanding the spread of pertussis infection. To prevent outbreak of whooping cough in adults, periodic administration of DTap vaccine is desired.

Key words: hospital office, pertussis, LAMP

Introduction

Pertussis has recently been attracting attention as a cause of prolonged cough in adults. Based on a serological survey of health care workers, employees are latently exposed to and infected with pertussis1). For diagnosis, in addition to conventional Bordetella pertussis culture and serological tests, loop-mediated isothermal amplification (LAMP) as a Bordetella pertussis gene test became covered by national health insurance in October 2016 in Japan and is now widely used for diagnosis in routine medical practice. In this study, a hospital clerk developed whooping cough and its spread in the work place was clarified using the LAMP method. LAMP methods were useful for grasp of the situation of asymptomatic persons as well as symptomatic patients.

Materials

Case 1 (index case) aged 24 years old female, who developed a cough on September 12, 20XX without certain sick contact. Her working place was administrative department in general hospital.
Clarithromycin (CAM) was prescribed from September 15, but the cough persisted. In Case 3, a cough developed from September 29, and whooping cough was diagnosed by the pertussis agglutinin titer test on October 14. By October 25, the number of clerical employees with prolonged cough in the same room increased to 8. Postnasal samples were collected from the clerical employees from the same room on October 26 and a screening test employing LAMP for pertussis was initiated.

Subjects: The subjects were 21 clerical employees (male: female=13:8) aged 23-63 years (mean: 44.3 years old, median: 44 years old).

Methods

Postnasal swabs were collected and DNA was extracted (QIAamp® DNA Blood Mini kit, QIAGEN K.K., Tokyo) and subjected to testing for pertussis infection using LAMP. For the LAMP test, the Loopamp Bordetella pertussis detection kit: (Eiken Chemical Co., Ltd., Tokyo) was used. Samples in which turbidity was noted but did not reach the threshold of positivity within the reaction time were considered as indeterminate. Antibiotic treatment was aimed to the persons with positive LAMP results or respiratory symptoms. For treatment, CAM 400 mg/day was administered twice daily or Azithromycin (AZM) 500 mg/day was administered once daily.

Informed consent for the examination of this study was obtained from each of the affected individuals.

This study was approved by the Ethical Committees of Koshigaya Municipal Hospital (Approval number 2109-7).

Results

First screening LAMP test for B. pertussis (n = 21) (figure 1)
Six (28%), 14 (67%), and 1 subject (5%) were LAMP-positive, -negative, and indeterminate, respectively. Cough was noted in 4 of the 6 positive subjects and one of them was accompanied by chest pain. One subject experienced pharyngeal discomfort and another subject had no cough. The antimicrobial agent administered was CAM in 4 and AZM in 2. Of the 14 LAMP-negative subjects, cough was noted in 4 and 2 experienced pharyngeal discomfort. Cough was not noted in 8 subjects. Antimicrobial agents were prescribed to 13 subjects with positive LAMP or respiratory symptoms and 1 indeterminate subject. CAM or AZM were administered to a total of 14 subjects.

Second screening LAMP test for B. pertussis (n = 10)
The second LAMP test was performed 20 days after the first test in the 6 LAMP-positive subjects, 1 indeterminate subject, and 3 subjects who were negative for LAMP but exhibited cough at the first test. Six of the 10 subjects were LAMP-positive.
and cough was only noted in one subject. Cough was noted in 1 of the 4 negative subjects. The indeterminate subject on the first test became LAMP-negative on the second test. A LAMP-negative subject with no cough on the first test developed cough and became LAMP-positive on the second test. The LAMP test-positive subject with cough and chest pain on the first LAMP test became negative.

Third screening LAMP test for *B. pertussis* (n = 6)

The third LAMP test was performed after 35 days in the 6 subjects who were positive on the second screening test. No cough was noted in these 6 subjects. Four subjects were positive for the third LAMP screening test and they received a second macrolide antibiotic administration.

Fourth screening LAMP test for *B. pertussis* (n = 4)

The fourth LAMP test was performed after 60 days in the 4 subjects who were positive on the third screening test. No cough was noted in any of these 4 subjects and only 1 subject was LAMP-positive. The presence or absence of cough as shown by the results of the pertussis LAMP test are presented in Figure 2. Cough was noted in 5 LAMP-positive and 6 LAMP-negative subjects in the first screening, but cough was noted in only one positive and one negative subjects in the second screening. Cough was not noted in any subject in the third or fourth screening.

Time to convert to LAMP-negative:

Seven subjects were LAMP-positive. Negative conversion was confirmed in 6 of them after 20–115 days (mean: 76 days, median: 85 days). Positivity persisted for more than 115 days in only one case (Case 2) and negative conversion could not be confirmed. For measures taken during this episode, symptomatic subjects wore a mask and continued work. Suspension of work was not applied as a rule. No outbreak of whooping cough occurred in any of the other departments in the hospital throughout the course, showing that the infection did not spread throughout the hospital.

Discussion

The incidence of whooping cough in adults has increased and became problematic because it serves as a source of infection in children. When antibodies acquired in infancy attenuate, whooping cough can develop in adulthood. Outbreaks of whooping cough have been reported in wards as well as among hospital clerical employees and in university laboratories\(^1\)\(^-\)\(^2\). Early diagnosis of atypical whooping cough in adult patients is difficult, unlike the diagnosis of cases in wards, being a major problem spreading whooping cough to patients. The active surveillance report of the

Figure 2 Symptomatic subjects with cough and asymptomatic subjects in each screening step are presented. The interval between the screenings is presented in braces. + : LAMP positive, - : LAMP negative
pertussis using the LAMP method is still unprecedented, and this is the first report.

The serum antibody titer was investigated in a previous report on whooping cough outbreak\(^2\), however, we surveyed the outbreak using LAMP. There are several types of Nucleic acid Amplification Test (NATs) available. The sensitivity of the LAMP method is comparable to that of the real-time PCR method, and the time to judgment of the test result is shorter than that of PCR. The analytical sensitivity of LAMP was 83% and was equal to that of the IS481 real-time PCR\(^4\).

Although clinical specimen of LAMP was incubated for 60 minutes, PCR results can be available in 2-24 hours\(^5\). Since the ptxp region of the _Bordetella pertussis_ gene is amplified, specificity is also higher than that of the PCR method, which tests the insertion sequence IS481.

Positivity of LAMP for pertussis was persistent in one subject in the present study, but infectivity was unclear and preferable to be investigated. While LAMP is useful in diagnosing the presence of _Bordetella pertussis_, it also detects dead bacteria. Thus, it is difficult to distinguish whether a positive case is an active infection. Long-term detection of _Bordetella pertussis_ has been reported using the NATs tests\(^6\). It was difficult to judge whether the detected _Bordetella pertussis_ was infectious.

Macrolide antibiotics were re-administered to the subjects who were LAMP-positive on the 3rd screening test. For treatment against _Bordetella pertussis_, generally, re-administration is not performed because the bacterium cannot be detected on culture test after administration of a proper antimicrobial agent for 5 days. However, relapse of whooping cough after proper macrolide antibiotic administration has been reported\(^7\), and persistent culture positivity due to failure of eradication of the bacteria has also been reported depending on the type of macrolide antibiotics\(^8\). Prevention of nosocomial infection spread by active preventive macrolide administration to _Bordetella pertussis_-exposed persons with no disease development has also been reported\(^9\). A significant booster effect was acquired by administration of 0.2 mL of DTap vaccine to young adults\(^10\) and additional administration of DTap vaccine to adults was included in the indication in 2017.

There are few opportunities to directly contact patients in hospital clerical departments, but pertussis infection may then be spread through health care workers, to which attention should be paid. The pertussis infection may spread before it is noted in the working place. It is difficult to diagnose whooping cough based on the cough symptom alone. Tdap vaccine is administered to prevent the outbreak of whooping cough in adults in western countries. In Japan, periodic administration of DTap vaccine instead of Tdap vaccine is desired.

**Conflict of interest**

The authors declare no conflicts of interest associated with this manuscript.

**Acknowledgements**

This study won the highest award in the 61st Annual conference of the Japanese Association for Infectious Diseases, Eastern regional conference.

We thank Hiromi Ikari and Ikumi Uragami for nasal sampling from affected individuals.

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