Original Article

Clinical Manifestations of Children with Microbiologically Confirmed Pertussis Infection and Antimicrobial Susceptibility of Isolated Strains in a Regional Hospital in Japan, 2008–2012

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SUMMARY: We conducted a retrospective study in 57 children (median age, 3.5 years; range, 1 month–14.5 years) with microbiologically confirmed pertussis infection over a recent 4-year period in a regional hospital in Japan. We obtained nasal swabs from all patients for *Bordetella pertussis* isolation as well as performed *B. pertussis* DNA detection using loop-mediated isothermal amplification (LAMP). Of the 57 cases, 34 (60%) were culture-positive and 57 (100%) were LAMP-positive. The frequency of each symptom was as follows: typical paroxysmal cough for over 14 days, 96% (55/57); paroxysms, 86% (49/57); posttussive vomiting, 33% (19/57); inspiratory whoop, 25% (14/57); and apnea, 12% (7/57). Hospitalization was required in 14 cases (25%), 93% (13/14) of which were aged <1 year. The proportion of patients previously immunized against diphtheria-tetanus-acellular pertussis vaccine (DTaP) was 19% (4/21) in children aged <1 year and 92% (11/12) in children aged ≥10 years. Minimum inhibitory concentrations for 6 antimicrobials (erythromycin, clarithromycin, azithromycin, minocycline, amoxicillin, and sulfamethoxazole/trimethoprim) were measured for 30 isolated strains, and all strains were susceptible to all aforementioned antimicrobials. Thus, an additional pertussis vaccination in older children is necessary, and the current macrolides-based treatment strategy is considered reasonable.

INTRODUCTION

Pertussis, an acute respiratory infection primarily affecting children, is caused by the bacterium *Bordetella pertussis*. Pertussis is sometimes serious, particularly in early infancy; therefore, rapid and accurate diagnosis is required for appropriate management. In Japan, the laboratory diagnosis of pertussis has been mainly performed by serology, with either single or paired serum samples; however, the accurate diagnosis is difficult because the results become complicated by the age and immunization status. The diagnosis of pertussis should be confirmed by microbiological methods that demonstrate *B. pertussis* infection more accurately, such as isolation of the bacterium and detection of its DNA.

Despite high diphtheria-tetanus-acellular pertussis (DTaP) vaccine coverage levels in children, pertussis outbreaks have occurred worldwide and their incidence has been increasing (1). It is well recognized that adolescents and adults are at risk of pertussis because of the waning of immunity provided by childhood vaccinations. These populations have been found to often act as reservoirs of infection (2). Therefore, clinical feature analysis and DTaP vaccine history of microbiologically confirmed pertussis patients will help in accurately understanding *B. pertussis* infection, vaccine efficacy, and

the ideal immunization strategy.

In this study, to assess the current situation of pertussis in children, we retrospectively examined the clinical manifestations and DTaP vaccine history of 57 children with microbiologically confirmed pertussis over a recent 4-year period in our hospital and measured the antimicrobial susceptibility of isolates.

PATIENTS AND METHODS

Subjects, sample collection, and diagnosis: Fifty-seven confirmed pertussis patients were retrospectively evaluated in the pediatric department of Konan Kosei Hospital (Aichi, Japan) between May 2008 and April 2012. The microbiological diagnosis of pertussis was confirmed by *B. pertussis* isolation and/or *B. pertussis* DNA detection using loop-mediated isothermal amplification (LAMP) with nasopharyngeal swabs. Moreover, we obtained serum samples for assessing bacterial agglutinin titers in acute and convalescent stages, if possible. We retrospectively analyzed the age, gender, family history, clinical manifestations, hospitalization, DTaP vaccine history, laboratory findings, and agglutinin titers of all patients.

Bacterial cultures and isolations were performed using charcoal agar (Oxoid Ltd, Hampshire, UK) with 10% defibrinated sheep blood and cephalixin. The culture plates were incubated at 37°C in a moist atmosphere and were observed for up to 1 week. We selected mercury-drop-like colonies and transferred them to blood agar and chocolate agar. If a colony grew in charcoal agar but did not grow in blood or chocolate

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agar, the colony was suspected of being *B. pertussis*. *B. pertussis* colonies were identified as small gram-negative rods that were oxidase-positive and *B. pertussis* antisera-positive (Denka Seiken Co., Ltd., Tokyo, Japan).

B. pertussis DNA was extracted from a nasopharyngeal specimen using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA), and LAMP reactions were conducted using the Loopamp DNA amplification kit (Eiken Chemical, Tokyo, Japan), as described previously (3). Primers for the LAMP assay were designed on the basis of PT promoter region of the *B. pertussis* strain Tohama (genome position, 159549–159755; GenBank accession number: BX640422). This assay demonstrated a high agreement level with conventional polymerase chain reaction (PCR) results (3).

Agglutinin titers against the *B. pertussis* strains Yamaguchi (epidemic strain) and Tohama (vaccine strain) were measured in serum samples. A serological test was considered positive if a single agglutinin titer was $\geq 40 \times$ in unvaccinated cases or if a ≥ 4 -fold increase was observed in the agglutinin titers in paired sera.

Antimicrobial susceptibility: In 30/34 strains identified by culture, minimum inhibitory concentrations (MICs) of 6 antimicrobials (erythromycin [EM], clarithromycin [CAM], azithromycin [AZM], minocycline [MINO], amoxicillin [AMPC], and sulfamethoxazole/trimethoprim [ST]) were measured using Etest® (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. Antimicrobial sensitivity was determined according to CLSI standards for *Haemophilus influenzae* (4).

Statistical analysis: Statistical analysis was performed using the Student's t-test and the chi square test. Significance levels were $p < 0.05$.

RESULTS

In total, 57 children were diagnosed with microbiologically confirmed pertussis (Table 1). We diagnosed 28 male children and 29 female children. The patients' median age was 3.5 years (range, 1 month–14.5 years). The median interval between symptom onset and sampling time was 10 days (range, 0–55 days). Of the 57 microbiologically confirmed pertussis infection cases,

34 (60%) were culture-positive, and 57 (100%) were LAMP-positive. No statistically significant differences were observed between the positive (34 cases) and negative cultures (23 cases) with regard to clinical manifestations such as the age, gender, duration of cough until testing, white blood cell count (lymphocyte count) at the acute phase, or number of DTaP vaccinations (data not shown). For serological tests, bacterial agglutinin titers were measured in 49 cases, and 26 of these comprised paired sera. A ≥ 4 -fold increase in these paired titers and a single titer of $\geq 40 \times$ in the unvaccinated cases were confirmed in 10 and 8 cases, respectively. The positive serological test rates were significantly higher in the cases of positive cultures (83%) than in those of negative cultures (17%; $p < 0.05$). A typical paroxysmal cough lasting for over 14 days was the most frequent symptom (96%), followed by paroxysms (86%), nocturnal cough (58%), posttussive vomiting (33%), and inspiratory whoop (25%). The average duration of cough was 28 days (0–63 days). In 2 cases, prolonged typical cough was not reported. In 1 of these cases, the initial symptom was apnea, and the other case consulted a doctor for mild cough in the catarrhal stage because a sibling was previously diagnosed with laboratory-confirmed pertussis. Hospitalization was required in 14 cases (25%), 93% (13/14) of which were aged < 1 year. Similar symptoms in another family member were observed in approximately 50% of cases: siblings in 26% (15/57), parents in 21% (12/57), and grandparents in 7% (4/57).

The annual age distribution among the pertussis cases over 4 years is presented in Fig. 1. An approximately 7-fold variation was observed in the total number of patients with pertussis between the May 2010–April 2011

Table 1. Diagnostic tests of 57 children with pertussis

Diagnostic test	No.
<i>B. pertussis</i> DNA detection (%)	57 (100%)
<i>B. pertussis</i> isolation (%)	34 (60%)
Serological confirmation (%)	18/49 (37%)
Pair ¹⁾	10/18
Single ²⁾	8/18

¹⁾: ≥ 4 -fold rise in the agglutination titers in paired sera.

²⁾: $\geq 40 \times$ in single agglutination titers in unvaccinated cases.

Table 2. Clinical manifestation and diphtheria-tetanus-acellular pertussis vaccination history of 57 children with pertussis

Clinical manifestation	Total (n = 57)	Non vaccination (n = 18)	≥ 1 dose vaccination (n = 33)	Unknown (n = 6)
≥ 14 days of cough (%)	55 (96%)	17 (94%)	33 (100%)	5 (83%)
Paroxysms (%)	49 (86%)	16 (89%)	29 (89%)	4 (67%)
Nocturnal cough (%)	33 (58%)	10 (56%)	20 (61%)	3 (50%)
Posttussive vomiting (%)	19 (33%)	10 (56%)	7 (21%)	2 (33%)
Whoop (%)	14 (25%)	8 (44%)	6 (18%)	0 (0%)
Fever $\geq 37.5^\circ\text{C}$ (%)	12 (21%)	6 (33%)	4 (12%)	2 (33%)
Apnea (%)	7 (12%)	7 (39%) ¹⁾	0 (0%)	0 (0%)
Pneumonia (%)	3 (5%)	2 (11%)	1 (3%)	0 (0%)
Hospitalization (%)	14 (25%)	11 (61%) ¹⁾	3 (9%)	0 (0%)

¹⁾: Significant differences ($p < 0.01$) detected between the clinical manifestation from non vaccination and ≥ 1 dose vaccination.

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Fig. 1. Total number and age distribution of patients with pertussis in each season.

Fig. 2. DTaP vaccination history according to age. DTaP, diphtheria-tetanus-acellular pertussis vaccines.

and May 2011-April 2012 seasons. Local epidemics of pertussis occurred during the May 2008-April 2009 and May 2011-April 2012 seasons. In the May 2011-April 2012 season, school-aged (7-14 years) children were most frequently affected, followed by early infants (<6 months). Seasonal epidemics were observed during spring and summer, although this did not occur every year.

A comparison of the clinical features of the 57 children with a DTaP vaccination history is presented in Table 2. The frequency of apnea and hospitalization was significantly higher in unvaccinated patients than in previously vaccinated patients ($p < 0.01$). No significant differences were observed for other clinical manifestations. Thirty-three patients (58%) with pertussis had a previous DTaP vaccination history (22: 4 doses; 7: 3 doses; 2: 2 doses; and 2: 1 dose), 18 patients

(32%) had no vaccination history, and 6 patients (10%) had no information about vaccination. The proportion of patients previously vaccinated with DTaP was 19% (4/21) in children aged <1 year and 92% (11/12) in children aged ≥ 10 years (Fig. 2).

MICs for 6 antimicrobials (EM, CAM, AZM, MINO, AMPC, and ST) could be measured for 30 isolated strains. All the strains were susceptible to these antimicrobials (Table 3).

DISCUSSION

At present, nucleic acid amplification methods, which have been reported to be rapid, sensitive, and specific, are available for the laboratory diagnosis of *B. pertussis* infections (3). LAMP is a nucleic acid amplification method that was developed in Japan as a simple, easy,

Table 3. Minimum inhibitory concentrations (MICs) of *Bordetella pertussis*

	MIC (μ g/ml)			Antimicrobial resistance (%)
	Range	MIC ₅₀	MIC ₉₀	
EM	0.016–0.032	0.023	0.023	0
CAM	0.016–0.023	0.023	0.023	0
AZM	0.016–0.032	0.023	0.032	0
MINO	0.016–0.032	0.023	0.032	0
AMPC	0.094–0.125	0.094	0.094	0
ST	0.016–0.047	0.032	0.047	0

EM, erythromycin; CAM, clarithromycin; AZM, azithromycin; MINO, minocycline; AMPC, amoxicillin; ST, sulfamethoxazole/trimethoprim.

and fast assay used to detect *B. pertussis* DNA (3,5). In our study, all 57 cases of microbiologically confirmed *B. pertussis* infections were LAMP-positive, while only 60% were culture-positive. This indicates that LAMP had a higher diagnostic sensitivity. In Japan, agglutinin titers against the *B. pertussis* strains Yamaguchi and Tohama have also been commonly measured for the serological diagnosis of pertussis. In our laboratory, of the 49 confirmed cases that were evaluated for agglutinin titers, 18 (37%) were serologically positive. However, several reports have indicated that measurement of bacterial agglutinin titers are less sensitive and specific than those of antibody to pertussis toxin (PT) IgG (6,7). Of the 57 microbiologically confirmed pertussis cases, PT IgG was measured in 4 cases. Of these, only 1 was serologically diagnosed with pertussis using a criteria of PT IgG of >100 EU/ml in acute-phase serum. The other case did not meet this criteria, and paired sera could not be acquired in the remaining 2 cases. Following the completion of this study period, serological diagnosis is being confirmed using PT IgG measurement at our hospital.

The World Health Organization (WHO) diagnostic criteria classify pertussis as a coughing illness lasting for at least 2 weeks with 1 of the following: paroxysms of coughing, inspiratory whoop, or posttussive vomiting (8). In our study, although most children with pertussis presented paroxysms of coughing, less than half of the children presented whooping and posttussive vomiting. In total, 55/57 cases (96%) met these criteria, and the remaining 2 cases that did not fulfill the criteria were aged <1 year. Wood et al. have reported that young infants may initially present with apnea alone (8). A previous study reported the following frequency of symptoms, which is similar to the results of our study: paroxysmal cough, 100%; prolonged cough over 14 days, 43%; inspiratory whoop, 39%; posttussive vomiting, 25%; and apnea, 7% (9). Clinical features, excluding apnea, were observed in unvaccinated and previously vaccinated patients. Furthermore, apnea was only observed in unvaccinated patients, presumably because apnea only occurs in infants.

Our study also showed that most older children with pertussis had completed a series of DTaP vaccines. This strongly suggests that additional booster vaccine doses are necessary. Although there no pertussis vaccines are currently licensed in Japan for use in children aged >7.5 years, revision of the vaccination program, including a booster dose for children aged 11–12 years, is

being considered at present. Meanwhile, infants (<12 months) accounted for 37% (21/57) of all pertussis cases and for 93% (13/14) of the hospitalized cases in our study. Moreover, 71% (10/14) of infants who are aged ≥ 3 months and should receive the DTaP vaccination did not receive it. A high DTaP vaccination coverage rate is maintained in Japanese infants; however, we suggest that a further rise in this rate is necessary (10).

Macrolides are commonly used as first-choice agents for treating pertussis. Till date, erythromycin resistance in *B. pertussis* has been reported in the U.S., with the first resistant isolate being detected in Arizona in 1994 (11). Therefore, monitoring for drug resistance is important not only to identify and investigate individual treatment failures but also to effectively target prevention and control programs by defining the frequency and clinical complications caused by resistant isolates. In our study, all isolated strains were susceptible to all 6 antimicrobials (EM, CAM, AZM, MINO, AMPC, and ST). Our results indicate that it is reasonable to consider the current treatment strategy using macrolides as the first choice.

In conclusion, our results indicate that an additional pertussis vaccination in older children is necessary and the current macrolides-based treatment strategy is effective.

Conflict of interest None to declare.

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