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Original Article

Effectiveness of rapid multiplex polymerase chain reaction for early diagnosis and treatment of pertussis

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Abstract *Background:* Pertussis, is an infectious respiratory disease caused by *Bordetella pertussis*. The incidence of pertussis has been increasing in South Korea to due to waning vaccine-induced immunity. Culture has a low sensitivity and a long turnaround time (TAT). Recently, a rapid multi-polymerase chain reaction (mPCR) test with a TAT of about 1 h was developed for the detection of respiratory pathogens (17 viruses and three bacteria), including *B. pertussis*. This study aimed to investigate the effectiveness of mPCR for early diagnosis and treatment of pertussis.
Methods: We performed a retrospective study of patients with pertussis diagnosed from May 2017 to June 2019 at a university hospital in South Korea. Nasopharyngeal swab specimens were tested using mPCR. Data were extracted from medical records.
Results: A total of 27 patients with a median age of 48.9 years (range: 3.3–82.2 years) were diagnosed with pertussis, of whom 9 (33.3%) were male. Eleven (40.7%) had fever, 12 (44.4%) had dyspnea, three (11.1%) had paroxysmal cough, and nine (33.3%) had inspiratory whooping. The median interval from symptom onset to diagnosis was 9.0 days (range: 1–31 days). Twenty-four patients (81.5%) were diagnosed within 2 weeks from symptom onset. All

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but one patient was prescribed macrolide antibiotics. Twenty-two patients (81.5%) required hospitalization, including three (11.1%) who required intensive care unit care for ventilation. *Conclusion:* Testing patients with respiratory symptoms using mPCR can improve early diagnosis of pertussis, ensure proper treatment, and may help with outbreak control.

Introduction

Pertussis is a highly contagious disease that causes a wide range of clinical symptoms from mild to life-threatening, resulting in the death of infants.[1] Pertussis spreads through respiratory droplets and can be transmitted by coughing, sneezing, or sharing breathing space.[2]

In South Korea, the government introduced the diphtheria, tetanus, and acellular pertussis (DTaP) vaccine in the National Immunization Program (NIP) in 1989. The reported incidence of pertussis gradually decreased from 1955 to the late 1990s, followed by a recent increase from the early 2000s.[3] Pertussis vaccination is administered to infants as three doses of DTaP at 2, 4, and 6 months of age. Pertussis booster vaccination is administered at 15–18 months and at 4–6 years with DTaP, and at 11–12 years with tetanus, diphtheria and pertussis (Tdap) vaccine. The Korean DTaP vaccination coverage among infants under 36 months of age was 96%–97% in 2017,[4] and the NIP budget is approximately 250 million dollars.[5]

Despite these efforts, pertussis outbreaks occur approximately every 3 years in South Korea and the number of outbreaks has increased recently.[6] Therefore, it is evident that pertussis is not adequately controlled. The reason for this includes non-immunization in adolescents and adults aged ≥15 years in a timely manner, and underdiagnosis due to atypical symptoms in adolescents and adults.[3,7] Thus, such patients are the major cause of transmission within their families.

The gold standard diagnosis for pertussis is culture. Nevertheless, polymerase chain reaction (PCR) is widely used because of the rapidity of diagnosis and the high sensitivity relative to culture. Multiplex PCR (mPCR) is particularly fast because it can detect different pathogens with one test. Therefore, mPCR is used for the diagnosis of many infectious diseases including respiratory infectious disease,[8–10] infectious gastroenteritis,[11] and infections of the central nervous system such as meningitis and encephalitis.[12] This study was conducted retrospectively to investigate the effectiveness of mPCR for the early diagnosis and treatment of pertussis.

Methods

Study population and samples

A retrospective study was conducted using the records of 27 patients diagnosed with pertussis from May 2017 to June 2019 at Yeungnam University Medical Center. Nasopharyngeal swabs were obtained from all patients and tested using mPCR. Data on age, sex, symptoms at the time of diagnosis, admission, hospitalization, isolation, vaccination history, past medical history, and accompanying diseases presenting symptoms, initiation of treatment, duration of hospitalization, and time to diagnosis were extracted from patient medical records.

Multiplex polymerase chain reaction

The mPCR was conducted with FilmArray respiratory panel (FA-RP; BioFire Diagnostics, Inc., Salt Lake City, UT, USA), using a BioFire FilmArray 2.0 analyzer, according to the manufacturer's instructions. The FA-RP utilizes a disposable hermetic pouch that provides the chemistry needed to isolate, amplify and detect nucleic acids from multiple respiratory pathogens in a single nasopharyngeal swab sample. The FA-RP is a fully automated nested mPCR test that simultaneously detects adenovirus, coronavirus (CoV)-229E, CoV-HKU1, CoV-NL63, hCoV-OC43, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A/H1N1, influenza A/H1-2009, influenza A/H3N2, influenza B, parainfluenza virus types 1–4, respiratory syncytial virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. In cases with coinfections, each of the infections by viruses or bacteria were recognized as positive.

Statistical analyses

Statistical analyses were performed using the IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Pearson's correlation test was used for analyzing the relationship between age and cough duration. P-values <0.05 were considered statistically significant.

Results

Demographics and clinical symptoms

Twenty-seven patients, with a median age of 48.9 years (range: 3.3–82.2 years) and a male to female ratio of 1:2, were diagnosed with pertussis using mPCR (Table 1). There were eight children and adolescents (0–19.9 years) and 17 adults (≥20 years). Heart disease was the most reported underlying medical history, with nine (33.3%) and eight (29.6%) patients reporting hypertension and chronic lung disease, respectively. Sputum expectoration (63.0%),

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Table 1 Patient characteristics (N = 27).

Demographic and clinical characteristics	N (%)
Male	9 (33.3)
Underlying medical history	
Diabetes	4 (14.8)
Hypertension	9 (33.3)
Chronic lung disease	8 (29.6)
Asthma	6 (22.2)
COPD	2 (7.4)
Interstitial lung disease	2 (7.4)
Heart disease	10 (37.0)
Presenting symptoms	
Fever	11 (40.7)
Chills	5 (18.5)
Cough	27 (100.0)
Sputum	17 (63.0)
Rhinorrhea	7 (25.9)
Dyspnea	12 (44.4)
Post-tussive vomiting	2 (7.4)
Paroxysmal cough	3 (11.1)
Inspiratory whooping	9 (33.3)
Laboratory test results	**Mean ± SD**
WBC (/μL)	14,193 ± 11,538
Lymph (%)	19.3 ± 10.0
Hb (g/dL)	12.8 ± 1.3
Platelet (/μL)	282,000 ± 73,000
AST (U/L)	33 ± 16
ALT (U/L)	22 ± 16
LDH (U/L)	617 ± 323
TB (mg/dL)	0.78 ± 0.41
DB (mg/dL)	0.21 ± 0.18
ESR (mm/hr)	36 ± 32
CRP (mg/dL)	6.68 ± 9.25
Procalcitonin (ng/dL)	1.841 ± 3.121
Lactate (mmol/L)	2.6 ± 4.2

ALT, alanine transferase; AST, aspartate transaminase; COPD, chronic obstructive pulmonary disease; DB, direct bilirubin; ESR, erythrocyte sediment rate; LDH, lactate dehydrogenase; WBC, white blood cell; TB, total bilirubin.

dyspnea (n = 12, 44.4%), and fever (n = 11, 40.7%) were the most common symptoms, besides cough.

The peak incidence of pertussis occurred in November with a total of seven cases. There were three cases in each of January, February, and August (Fig. 1 (a)). Based on the seasonal pattern, the incidence of pertussis was highest in the autumn (September to November), and lowest in the spring (March to May) (Fig. 1 (b)).

Laboratory and multiplex polymerase chain reaction results

The laboratory results are shown in Table 1. Of the 27 patients, 10 were found to have coinfections using mPCR. The most common coinfections were adenovirus and human rhinovirus/enterovirus (5, 18.5% each). There was one patient with coronavirus NL63 coinfection and one with *M. pneumoniae* coinfection. The median of turnaround time (TAT) of FA-RP was 94 min (range: 57–213 min).

Duration of cough

The median duration of cough was 7 days (range: 2–30 days). Seventeen (62.9%) and 24 (88.8%) patients had a coughing period of <8 days and ≤14 days, respectively. The median time from first symptom to diagnosis was 9.0 days (1–31 days). Twenty-four patients (81.5%) were diagnosed within 2 weeks (Table 2, Fig. 2). No significant differences were observed in cough duration according to age (Fig. 3; p = 0.711). The cough duration for patients diagnosed with pertussis was median 7 days (range: 2–30 days).

Treatment and clinical outcome

Patient treatment and outcomes are shown in Table 3. Twenty-five patients were treated with azithromycin and one was treated with clarithromycin. All patients were isolated, with 22 (81.5%) hospitalized, and five treated at the outpatient clinic. Three patients requiring ventilator care were treated at the intensive care unit. There were no patient deaths.

Discussion

Pertussis has an incubation period of 7–10 days.[13] The clinical course of pertussis progresses through the catarrhal, paroxysmal, and convalescent stage in sequence. Each stage lasts approximately 1–3 weeks. Infants and children, and adolescents and adults have similar progression. However, adults and adolescents have milder symptoms than infants and children. In the catarrhal stage, patients present symptoms of upper respiratory tract infections like low-grade fever, malaise, sore throat, nasal congestion, rhinorrhea, lacrimation, sneezing, and nocturnal cough paroxysms. Therefore, it is easy to overlook pertussis at the early stage. In the paroxysmal stage, patients show severe symptoms with intense and violent cough (five to ten coughs/paroxysms) that last several minutes and are associated with cyanosis, eye proptosis, tongue protrusion, salivation, thick oral mucus production, lacrimation, and engorgement of neck veins. Furthermore, the classical sign of pertussis—inspiratory whooping—manifests at this stage. Inspiratory whooping, paroxysmal cough, and post-tussive vomiting are the three classical signs of pertussis.[13] In the convalescent stage, patients' symptoms gradually decrease.

The World Health Organization clinical case definition is a coughing illness lasting at least 2 weeks with paroxysms of coughing, inspiratory whooping, or post-tussive vomiting. Diagnostic methods of pertussis include culture, PCR, serologic testing, and direct fluorescent antibody (DFA) staining. Serologic test needs to be calibrated to the reference standard for single time point assays after measuring immunoglobulin G (IgG) antibody, such as the World Health Organization International Standard.[14] After vaccination, pertussis could not be diagnosed due to vaccine-induced IgG. DFA staining cannot be used to diagnose due to its low sensitivity and specificity. The gold

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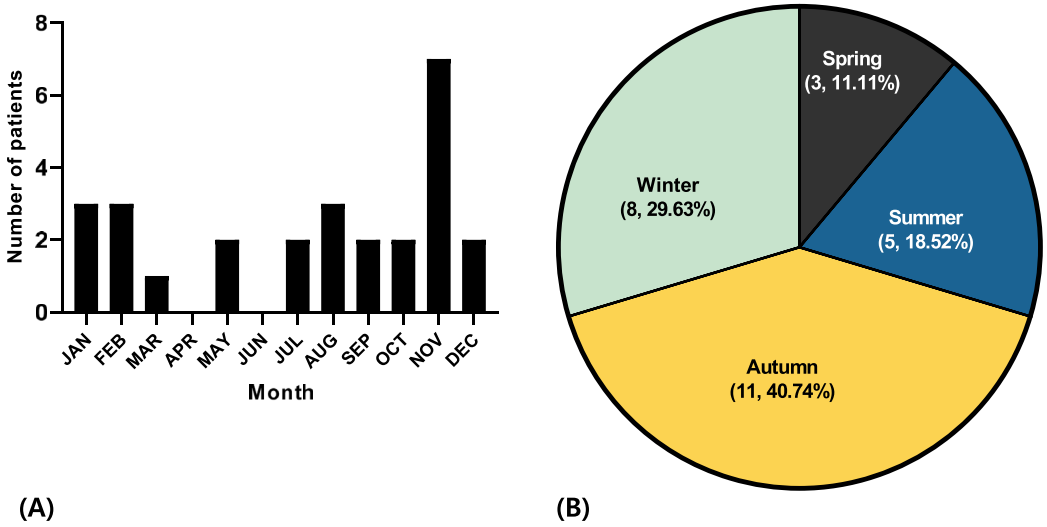

Figure 1. Monthly and seasonal trend of pertussis incidence. (A) Monthly trend of pertussis incidence (B) Seasonal trend of pertussis incidence. Spring (March to May), summer (June to August), autumn (September to November) and winter (December to February).

Table 2 Parameters related to the time to diagnose pertussis.

	n (%)	Median (range)
Turnaround time of the mPCR test (min)		94.0 (57.0–213.0)
Cough duration (days)		7 (2–30)
≤7	17 (63.0)	
8–13	4 (14.8)	
≥14	4 (14.8)	
Unknown	2 (7.4)	
Time from first symptom to diagnosis (days)		9.0 (1–31)
≤7	7 (25.9)	
8–13	15 (55.6)	
≥14	5 (18.5)	

mPCR: multiplex polymerase chain reaction.

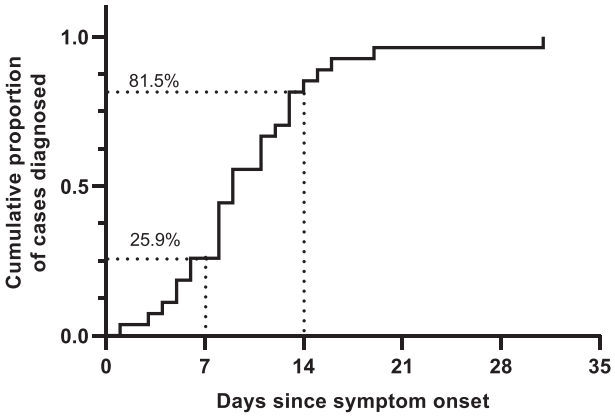

Figure 2. Time from symptom onset to diagnosis.

standard for diagnosis of pertussis is laboratory culture. However, the culture growth of *B. pertussis* can take up to 10 days and also requires antimicrobial susceptibility testing and molecular typing. Thus, PCR, which can be diagnosed in one day and has high specificity and sensitivity, can be used as a complement or can replace culture testing in diagnosing patients with clinical symptoms of pertussis.[4]

FA-RP, a recently developed mPCR test, can detect multiple pathogens at one test. A recent study found that this test significantly shortened the TAT compared to the conventional mPCR.[9] In this study, a median TAT was 94 min, and all patients were isolated and received specific antibiotic therapy after diagnosis of pertussis.

Yoon et al.,[6] reported that cough duration in patients with pertussis was an average 26.2 of (10–45) days for pediatric adolescents (0–19 years) during 2005–2017 in South Korea. Park et al.,[15] reported a median cough duration of 14.0 days (7–21 days for adolescents and adults) during 2011–2012. In our study, the median cough duration was 7 days which was shorter than that in previous studies. The shortened time from symptom onset to diagnosis may be due to the rapid mPCR test.

There was no significant difference in cough duration and age. The cough duration for patients diagnosed with pertussis was 7 days on median, 8.5 days on average, which was less than that in previous studies.[6,7,15] In this study, we retrospectively analyzed the clinical symptoms of patients diagnosed with pertussis. Only nine patients (33.3%) showed the classical signs of pertussis, which seemed to be the result of early diagnosis.

In South Korea, according to the National Vaccination Management Guidelines, all citizens received three basic doses of DTaP at 2, 4 and 6 months of age, three booster vaccinations at 15–18 months, with DTaP at 4–6 years of age; with Tdap at 11–12 years of age, followed by a dose of Td vaccine every 10 years.[16] In 2017, DTaP immunization rates for babies who were 12, 24, and 36 months were

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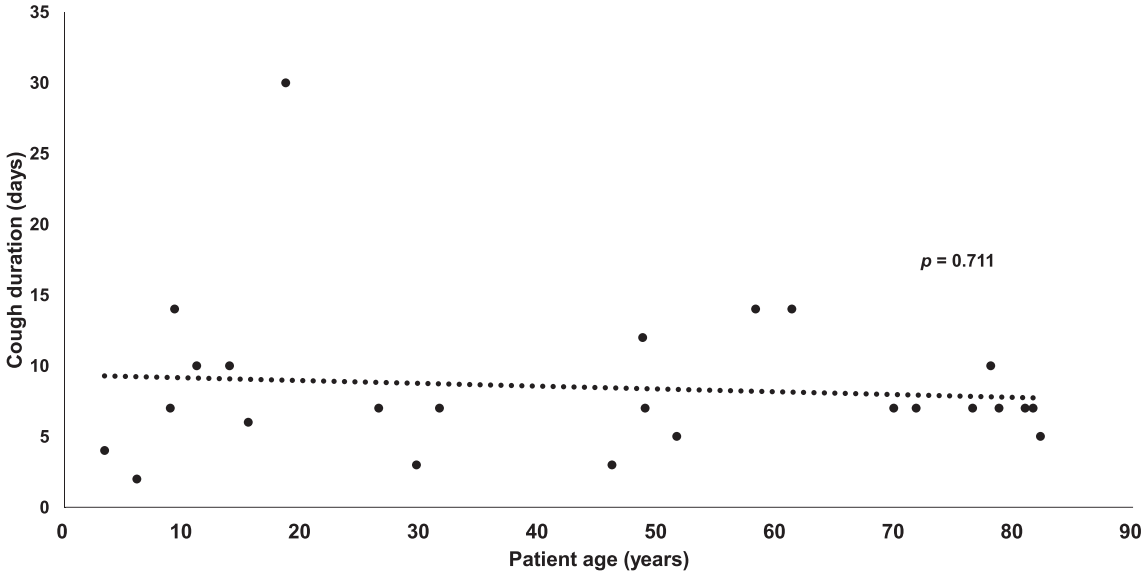

Figure 3. Correlation between the cough duration and age.

Table 3 Patient treatment and outcome.

Treatment	n (%)
Antibiotics	26 (96.2)
Azithromycin	25 (92.6)
Clarithromycin	1 (3.7)
Isolation	27 (100.0)
Admission	22 (81.5)
Intensive Care Unit	3 (11.1)
Ventilator care	3 (11.1)
Outcome	
Alive	27 (100.0)

reported to be 97.7%, 96.2%, and 96.6%, respectively.[5,17] Despite this high immunization rate, reports on the incidence of pertussis are increasing, and nonetheless, the Korean surveillance system for pertussis is likely to underestimate the burden of pertussis.[17,18]

In addition, most adolescents do not receive regular booster vaccines; it has been reported that the probability of pertussis as a cause of chronic cough has increased.[7] Furthermore, pertussis infection in adolescents and adults has been found to be responsible for household transmission of pertussis to susceptible infants.[19]

In the US, the reported pertussis incidence has increased since the 1980s, with peaks every 2–5 years. In 2013, there were 28,639 cases of reported pertussis in the US and 13 pertussis-related deaths.[20] In the US, a claims database analysis study showed that considerable underreporting of pertussis in people aged under 50 years exists, especially with increasing age. Thus, it is necessary to develop public health programs to reduce the pertussis burden.[21] In our study, adolescents and adults infected with pertussis accounted for 85.2% of the total burden. Therefore, if the mPCR test using FA-RP is applied to patients in this age group, the outbreak of pertussis may be more effectively controlled.

This study has the following limitations. This study was retrospectively analyzed the medical records of all patients diagnosed with pertussis for 2 years at a single institution. Although some patients were actual pertussis infection, there may have been some patients who were not diagnosed with pertussis because they were not properly screened. Because this is a retrospective study, specimens of those patients who were underdiagnosed were not tested again. This may lead to bias in the interpretation of the data collection. Culture was gold standard in the diagnosis of pertussis, it was not included in the routine clinical practice, so it could not be included in the study and analyzed.

Testing of patients with respiratory symptoms through mPCR containing pertussis can lead to early diagnosis. Early diagnosis and proper treatment may help in outbreak control of pertussis.

Ethical approval

This study was approved by the Institutional Review Board of the Yeungnam University Medical Center (IRB No: YUMC 2019-08-013).

Author's contribution

JML and JYA is the co-principal investigator for this study. JML, JHL, and YKK conceived the idea for this manuscript. SCO and SMP carried out the data analysis and wrote the first draft of the manuscript. SCO, SMP, JH, EYC, HJJ, YKK, JHL, JYA and JML interpreted the results and critically reviewed drafts of this manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

None.

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