



Identification of Etiological Agents of Pertussis-Like Syndrome in Children Younger Than 5 Years Old Hospitalized in Southwestern Iran

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Abstract

Background: Despite the global vaccination program, there are many new cases of pertussis in different societies annually.

Objectives: This study aimed to investigate the prevalence of some microorganisms associated with pertussis-like syndrome and compare the clinical presentations between *Bordetella pertussis* and pertussis-like syndrome in children.

Methods: Children younger than 5 years old suspected of pertussis-like syndrome were admitted to a hospital in Ahvaz, Iran, and examined from July 2018 to July 2019. Nasopharyngeal samples were evaluated using molecular methods. The studied microorganisms were the following: *Bordetella pertussis*, *B. parapertussis*, *Mycoplasma pneumoniae*, *Chlamydomydia pneumoniae*, adenovirus, respiratory syncytial virus, and parainfluenza virus type III.

Results: Forty-five children were enrolled. *Bordetella pertussis* was detected in 15 cases (33.3%), respiratory syncytial virus in 14 (31.1%), *C. pneumoniae* in 3 (6.7%), and parainfluenza virus type III in 3 (6.7%). The collected samples were negative in terms of *M. pneumoniae*, adenovirus, and *B. parapertussis*. In the case of paroxysmal cough, the clinical symptoms were significantly different between pertussis and pertussis-like groups.

Conclusions: The results indicated that children with pertussis-like syndrome are commonly infected with *B. pertussis* and respiratory syncytial virus, so more attention should be paid to this issue. The study also demonstrated the importance of molecular diagnosis methods, along with diagnosis based on clinical symptoms, in children suspected of pertussis-like syndrome.

Keywords: *Bordetella pertussis*, Respiratory Syncytial Virus, Pathogen, Children, Whooping Cough

1. Background

Pertussis is one of the main reasons for infectious disease-related deaths in children (1). *Bordetella* causes this respiratory disease. *Bordetella pertussis* is a small Gram-negative pathogen (2, 3). The common signs of pertussis are paroxysmal cough, wheezing, and post-cough vomiting. Such clinical signs are not only limited to pertussis infection, but other pathogens, including the influenza virus, parainfluenza virus type III (PIV-III), respiratory syncytial virus (RSV), adenovirus (ADV), *Mycoplasma pneumoniae*, *Chlamydomydia pneumoniae*, and *B. parapertussis* can also cause similar clinical signs, which are collectively referred to as the pertussis-like syndrome (4, 5). The pertussis-like syndrome might

happen at any age, but its prevalence is higher in children. It is challenging to differentiate the symptoms of *B. pertussis* from infections caused by viruses and some bacteria. Therefore, the pertussis diagnosis should not be solely based on the clinical signs, and the clinical diagnosis must be confirmed by laboratory tests (3, 5).

There is limited information on the etiology of pertussis-like syndrome worldwide (6). Furthermore, in Iran, culture and molecular methods are not routinely used in hospitals to confirm pertussis clinically, and they are only performed at the Pasteur Institute of Iran as a reference laboratory for pertussis. Therefore, accurate tracking of the prevalence of pertussis is difficult. In addition, it is important to know the pathogen causing the pertussis-like syndrome to reduce the rate of transmission

and accelerate the start of treatment. Besides, any decision to change national vaccination plans, including the replacement of new vaccines, should be made based on the results of local epidemiological studies in each region.

2. Objectives

This study aimed to evaluate the frequency of pertussis-like pathogens among children admitted to a children's hospital in southwestern Iran.

3. Methods

This cross-sectional study aimed to identify the prevalence of the etiologic causes of the pertussis-like syndrome in children under the age of 5 years suspected of pertussis and admitted to Aboozar Children's Hospital between July 2018 and July 2019. This hospital is the only children's referral hospital in the Khuzestan province and is located in the city of Ahvaz. The inclusion criteria were coughs lasting more than 14 days with one or more of the following symptoms: Whoop and paroxysmal cough, apneic paroxysm, post-tussive vomiting, or coughs for more than 21 days without the symptoms mentioned above. Demographic, clinical, and laboratory data were collected from questionnaires filled out by the patients' parents and the medical records.

3.1. Sample Collection

Two nasopharyngeal samples were taken from the patients using a Dacron swab, and 2 samples were taken from each patient. One of the swabs was then placed in the Regan-Lowe transport medium to detect bacterial agents. After that, the second swab was placed in the viral transport medium (VTM) to detect viral agents. All the specimens were transferred to the laboratory.

3.2. Molecular Diagnosis

3.2.1. Polymerase Chain Reaction for the Identification of Bacteria

According to the manufacturer's instructions, deoxyribonucleic acid (DNA) was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The DNA obtained was immediately assessed or stored at -80°C until use. Polymerase chain reaction (PCR) was performed with $5\ \mu\text{L}$ of the template DNA. The used primers and conditions for *B. pertussis* and *B. parapertussis* were described by Castillo et al. and Dragsted et al. (7, 8). The 191-bp fragment of the pertussis toxin S1 gene (PTxA) and a 145-bp fragment of the insertion sequence IS481 were amplified using the primers. Samples were positive

for *B. pertussis* when both the fragment of the pertussis toxin S1 gene (PTxA) and the insertion sequence IS481 were amplified. Primers BPPA amplify a 493 bp fragment of the insertion sequence IS1001, found in *B. parapertussis* (7-9). Moreover, the used primers and conditions for *M. pneumonia* and *C. pneumoniae* were described by Del Valle-Mendoza et al. (10).

3.2.2. Real-time PCR for the Detection of Adenovirus, Respiratory Syncytial Virus, and Parainfluenza Type III

Ribonucleic acid (RNA) extraction was performed using the High Pure RNA Isolation Kit (Roche Applied Science, Mannheim, Germany) based on the manufacturer's instructions. Next, RSV, PIV-III, and ADV were detected in the nasopharyngeal swab specimens using the real-time PCR described earlier (11). The primers and the probe for RSV and ADV were previously described by Liu et al. (12), and Heim et al. (13). For the case of PIV-III, the primers and conditions used for the real-time PCR were described by Coiras et al. (14).

3.3. Statistical Analysis

The data were analyzed in SPSS v. 22 (SPSS Inc., Chicago, IL, USA) and then expressed as appropriate numbers with percentages or medians and the interquartile range (IQR). Moreover, non-normally distributed variables and categorical data were analyzed using the chi-square test. A P-value < 0.05 was considered statistically significant.

4. Results

4.1. Demographic Characteristics

In this study, 45 eligible patients under the age of 5 years were examined. Of the 45 nasopharyngeal aspirates, 15 (33.3%) were *B. pertussis*-positive, referred to as the *B. pertussis* group; moreover, 30 (66.6%) children with other bacterial and viral pathogens or the absence of the studied microorganisms were classified into the pertussis-like group. Demographic data obtained from the study group are given in Table 1. The mean age of the children was 3.2 ± 2.5 months. Most children were in the age group of 1 to 8 months. The ratio of males to females was twice in the pertussis-like group; however, no significant differences regarding sex were observed between pertussis and pertussis-like groups ($P = 0.51$). All the patients were hospitalized for 3 - 7 days (mean: 3 days). In 17 (37.8%) patients, a history of coughs (lasting for more than 2 weeks) was observed in a family member 1 month prior to starting the study.

Table 1. Demographic and Clinical Presentations of the Pertussis-Like Syndrome^a

Parameters	Total Patients	Pertussis Group (n = 15)	Pertussis-Like Group (n = 30)	P-Value
Age (mo)	3.2 ± 2.5	2.837	3.155	0.152
Sex (M/F)	28/17	8/7	20/10	0.51
History of antibiotic use	28 (62.2)	8 (53.3)	20 (66.7)	0.517
Similar cases in the family	17 (37.8)	4 (26.7)	13 (43.3)	0.341
Length of hospital stay	5 (3 - 7)	4.8 (3 - 7)	5 (3 - 7)	0.082
Clinical symptoms				
Whoops	2 (4.4)	2 (13.3)	0	0.106
Paroxysmal cough	8 (17.8)	7 (46.6)	1 (3.3)	0.001
Vomiting	29 (64.4)	12 (80)	17 (56.6)	0.189
Apnea	15 (33.3)	4 (26.6)	11 (36.6)	0.738
Cyanosis	38 (84.4)	15 (100)	23 (76.6)	0.077
Fever	16 (35.6)	4 (26.6)	12 (40)	0.514
Tachypnea	15 (33.3)	2 (13.3)	13 (43.3)	0.053
Clinical signs				
Wheezing	19 (42.2)	2 (13.3)	17 (56.7)	0.005
Crackles	3 (6.7)	0	3 (10)	0.999
Rhonchi	2 (4.4)	1 (6.7)	1 (3.3)	0.846
Shaggy hearth	5 (11.1)	5 (33.3)	0	0.026
Parenchymal involvement	2 (4.4)	0	2 (6.7)	0.7
Laboratory values				
White blood cells median (interquartile range)	11558 (5000 - 25300)	11559 (5000 - 21000)	11558 (5000 - 25300)	0.782
Lymphocyte median (interquartile range)	64.5% (48 - 80)	64.7 (48 - 78)	64.5% (48 - 80)	0.845

^a Values are expressed as No. (%) unless otherwise indicated.

4.2. Comparison of the Clinical and Laboratory Characteristics Between Pertussis and Pertussis-Like Groups

Table 1 shows the distribution of signs and symptoms in the pediatric pertussis group and pertussis-like groups. Cyanosis (84.4%) and vomiting (64.4%) were the most common symptoms, and paroxysmal cough (17.8%) and whoop (4.4%) were the least common ones, respectively. Fever was present in only 16 cases (35.6%), and tachypnea was positive in 15 (33.3%) cases. There was no significant difference in terms of the clinical manifestations, including whoop, vomiting, apnea, cyanosis, fever, and tachypnea, between the two groups. Still, regarding paroxysmal cough, a significant difference was observed between pertussis and pertussis-like groups ($P = 0.001$). Lung hearing was normal in about half of the participants. Wheezing (42.2%) was the most common abnormal hearing problem, followed by crackling (6.7%) and rhonchi (4.4%), respectively. Among the symptoms mentioned above, there was a significant difference between the two *Bordetella* and *Bordetella*-like groups only

in wheezing ($P = 0.005$). Chest X-ray (CXR) findings also showed that most cases were normal.

Only 15.5% of these cases showed some abnormal findings, including shaggy hearth and parenchymal involvement. Among those who were positive for *B. pertussis*, 10 cases had a normal radiograph, and 5 cases (33.3%) had a shaggy heart. About the shaggy heart, a significant difference was observed between these two groups ($P = 0.026$). Moreover, 40% of all the patients had a white blood cell (WBC) count below 10,000/mL; 49% of them had 15,000 - 100,000 WBC/mL, and 11.1% had more than 150,000 WBC/mL. Among those with pertussis, only 3 children (20%) had more than 15,000 WC. No significant relationship was found between lymphocyte and leukocyte counts and the identification of *B. pertussis* as the causative agent of the disease ($P = 0.78$, $P = 0.84$).

4.3. Pathogens in the Children with the Pertussis-Like Syndrome

Of the 45 children with the pertussis-like syndrome, 35 (77.7%) children were positive for the pathogen. *Bordetella*

pertussis (33.3%), RSV (31.1%), *C. pneumonia* (6.7%), and PIV-III (6.7%) were the microbial pathogens found. Moreover, 30 children were negative for *B. pertussis*, and all the children were negative for *B. parapertussis*. Adenovirus and *M. pneumoniae* were found in none of the subjects. Additionally, no co-infection was observed among the patients (Figure 1).

5. Discussion

This study aimed to investigate the microorganisms involved in the pertussis-like syndrome and evaluate the clinical characteristics in children under 5 years old in southwestern Iran. Our findings showed that *B. pertussis* (33.3%) and RSV (31.1%) were the most common microorganisms, and *C. pneumoniae* (6.7%) and PIV-III (6.7%) were the least common ones involved in the pertussis-like syndrome, respectively. The prevalence rate of *B. pertussis* in children with the pertussis-like syndrome was estimated as 33.3%, which is higher than those reported in similar studies by Mahmoudi et al. in Iran (18%), Hajia et al. in Iran (9%) and Al Maani et al. in Oman (17%) (3, 15, 16). However, in the current study, the estimated frequency was lower than those obtained by Gu et al. (53%) and Jiang et al. (49%) in China, Saiki-Macedo et al. in Peru (41%), and Dumaidi and Al-Jawabreh in Palestine (49%) (5, 17-19).

The differences in the prevalence of *B. pertussis* in the suspected patients may be due to the different age ranges of the studied populations, the use of various diagnostic methods with different percentages of sensitivity and specificity, and differences in vaccination coverage in the studied regions. In addition, the transmission of pertussis from adults to infants may increase due to the hot climate in Ahvaz, the presence of more people in their houses, and, consequently, insufficient indoor air ventilation. In Iran, the pertussis vaccination program includes a total of 3 doses of the diphtheria and tetanus vaccine, combined with whole-cell pertussis vaccine, which is injected at 2, 4, and 6 months. In addition, booster doses are given at 18 months and 4-6 years old (20).

In the present study, 86% of the cases with confirmed pertussis-like syndrome (by PCR) were 0 to 4 months old and had not received the full vaccination dose. Due to the reduced efficacy of the pertussis vaccine with aging, adults play an important part in transmitting this disease to infants, especially those who have not received full vaccination (21). Therefore, some strategies should be used to support infants, including the vaccination of adolescents and adults with the acellular pertussis vaccine to prevent the home transmission of the disease, as well as the vaccination of pregnant women during the last

trimester of their pregnancy; the latter measure results in the transmission of maternal anti-pertussis antibodies to the fetus and, as a result, the newborns are protected against pertussis before being vaccinated (20, 22).

Since the whole-cell pertussis vaccine is not recommended for adults and there is no access to an acellular vaccine in Iran, it is necessary to start the vaccination program in the target groups with this type of vaccine and include it in the national vaccination program. In this study, RSV accounted for a high percentage of positive cases, as it was identified in 31% of cases. Similar results have been reported in Brazil (32%), China (31.4%), and Oman (32%) (16, 23, 24). The studies conducted by Mahmoudi et al. (20%) and Pourakbari et al. (17.2%) in Iran showed a lower rate. However, in these two articles, RSV was the most common viral pathogen (3, 25). In our study, PIV-III was detected in 6.7% of the children with the pertussis-like syndrome; this rate is lower than that of the parainfluenza virus obtained in Tao et al.'s (24) study in China (43%) and higher than that of Gu et al.'s study in China (3.2%) (5, 24).

Chlamydomphila pneumoniae was also identified in 6.7% of the children with the pertussis-like syndrome. A similar study in Peru identified 10.5% of this bacterium among children (10). In the present study, *M. pneumoniae* and adenovirus were detected in none of the subjects. Nevertheless, in similar studies, *Mycoplasma* was reported in 29.2% of the children with pertussis-like syndrome in China and 26% in Peru (17, 18); adenovirus was also identified in 49% of subjects in Peru, 3.2% in Oman, and 16% in Iran (3, 16, 18). As the etiology of pertussis, *B. parapertussis* is rare (26, 27). In our study, *B. parapertussis* was found in none of the participants.

However, paroxysmal cough and whoop are the important symptoms of pertussis and are used in the clinical definition of pertussis provided by the World Health Organization (WHO). In our study, only 13% of the patients with *B. pertussis* had whoop. Moreover, a significant difference was observed regarding paroxysmal cough between pertussis and pertussis-like groups, as about half of the patients with *B. pertussis* had this symptom. Regarding the other clinical symptoms, no significant difference was observed between the two study groups. This result is consistent with those of other studies in Iran and other parts of the world, showing a lack of specificity of clinical symptoms in pertussis diagnosis. This indicates the need for microbiological tests to confirm the diagnosis of suspected pertussis (3, 5, 22, 28, 29).

The present study observed no significant relationship between WBC, absolute lymphocyte counts, and *B. pertussis* positivity. However, some studies have shown that these

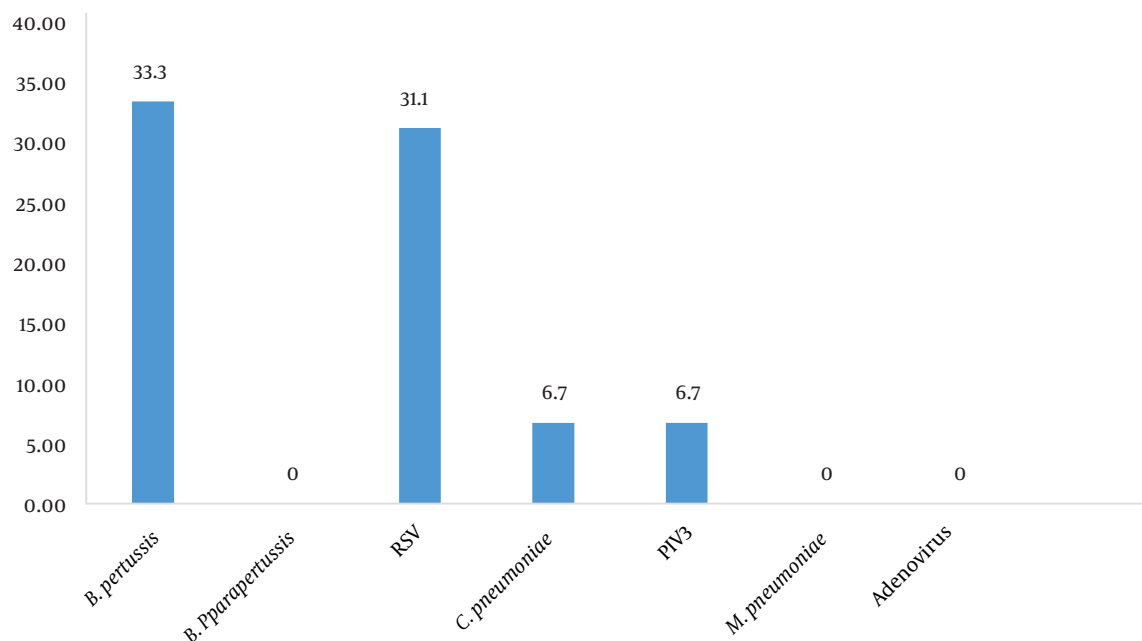


Figure 1. Overall detection of respiratory pathogens in 45 children with the pertussis-like syndrome (%)

two indexes can be used in laboratory separation of *B. pertussis* from other bacteria causing the pertussis-like syndrome (30). However, in 15 children with positive PCR test results for *B. pertussis*, the mean WBC count was 11,559. The mean lymphocyte percentage was 64.7%, which is higher than the average typical condition in pertussis (31). Accordingly, the lack of significance of these two parameters in the *B. pertussis* and pertussis-like groups can be explained by the fact that in this study, there was no healthy control group in which we assessed the percentages of WBC and lymphocytes. Therefore, a comparison of leukocytes and lymphocytes was made between the *B. pertussis* group and the pertussis-like group. The second group showed leukocytosis and lymphocytosis due to other bacteria and viruses, especially RSV. Chest X-ray changes were also found to be associated with *Bordetella* positivity. The classic *Bordetella* modification is a shaggy heart. Furthermore, diffuse infiltration and flattening of the diaphragm are known as the common causes of this bacterium. Still, its prevalence may not significantly differ from other causes of pertussis syndrome (30). In our study, there was a significant difference between the two study groups in terms of the shaggy heart.

A limitation of the present study was its small sample size. In the next stage, we should include more children suspected of having pertussis-like syndrome. In addition,

we did not examine all the pathogens involved in the pertussis-like syndrome, so we should detect more kinds of pathogens involved in the pertussis-like syndrome later.

5.1. Conclusions

The findings of this observational and prospective study showed that *B. pertussis* and RSV, with almost similar prevalence rates, are the causes of approximately 64% of the cases of pertussis-like syndrome in children under 5 years old hospitalized in Ahvaz. This finding should be considered in the clinic. Furthermore, clinically suspected cases should be confirmed using molecular methods.

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Footnotes

Authors' Contribution: Study concept and design: Ahmad Shamsizadeh; acquisition of data: Roya Nikfar and Elham Bavarsadiankhanh; analysis and interpretation of

data: Ahmad Shamsizadeh and Elham Bavarsadinkhah; drafting of the manuscript: Maniya Arshadi; critical revision of the manuscript for important intellectual content: Maniya Arshadi and Ahmad Shamsizadeh; statistical analysis: Maniya Arshadi and Elham Bavarsadinkhah; administrative, technical, and material support: Niloofer Neisi and Effat Abbasi-Montazeri; study supervision: Ahmad Shamsizadeh.

Conflict of Interests: We declare that one of our authors (Ahmad Shamsizadeh) is an editorial board member. The journal confirmed that the mentioned author with the conflict of interest (CoI) was completely excluded from all review processes. We also introduced this author with CoI during the submission as an opposed reviewer.

Data Reproducibility: All data are included in the manuscript. The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: The study design was approved by the Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Iran (IR.AJUMS.REC.1397.414).

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Informed Consent: The parents of all the included children provided written informed consent for participation.

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