MINISTRY OF EDUCATION AND TRAINING MINISTRY OF HEALTH
NATIONAL INSTITUTE OF MALARIOLOGY – PARASITOLOGY – ENTOMOLOGY

DO THI THUY NGA

CLINICAL, SUBCLINICAL CHARACTERISTICS, DETERMINATION OF MACROLIDE RESISTANCE GENE OF *BORDETELLA PERTUSSIS* AND OUTCOMES FOR TREATMENT IN PEDIATRIC WITH WHOOPING COUGH AT NATIONAL CHILDREN'S HOSPITAL

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- The National library
- The library of the National Insitute of Malariology - Parasitology - Entomology

LIST OF RESEARCH WORKS RELATED TO THE THESIS TO BE PUBLISHED

1. **Do Thi Thuy Nga**, Phung Thi Bich Thuy, Tran Minh Dien, Akihiko Saitoh, et al (2022). Association between Real-time Polymerase Chain Reaction Cycle Threshold Value and Clinical Severity in Neonates and Infants Infected with *Bordetella pertussis*. *Pediatric Infectious Disease Journal* 41:388–393.
2. **Do Thi Thuy Nga**, Nguyen Manh Cuong, Phung Thi Bich Thuy, Tran Minh Dien (2023). Several prognosis factors of severe pertussis in children treated at Vietnam national Children's hospital (2019-2020). *Vietnam Journal of Science Technology and Engineering 65(3): 75-79.*

INTRODUCTION

Pertussis, also known as whooping cough, is an acute, highly contagious respiratory tract infection caused by *Bordetella pertussis*. This disease predominantly affects infants and young children. Despite the availability of pertussis vaccines for nearly a century, the disease has not been completely eradicated. In many countries, including those with high vaccine coverage rates [2], pertussis tends to resurge. This resurgence presents significant challenges to public health [3] and poses considerable difficulties in clinical management [4], [5], [6]. Of particular concern is the development of pulmonary arterial hypertension, which remains a formidable issue in the critical care of pertussis patients. To date, there is no proven effective treatment approach for this condition [7], [8], [9]. Furthermore, the issue of pertussis antibiotic resistance, particularly to macrolide antibiotics, has garnered increasing attention [10], [11].

In Vietnam, pertussis has yet to be completely controlled, and particularly since 2015, there has been a rising trend in reported pertussis cases [16], [17], [18]. The adoption of new diagnostic standards and microbiological testing techniques for pertussis diagnosis has increasingly facilitated clinical research on pertussis [19], [20], [21], [22]. Severe complications of pertussis, such as severe pneumonia, severe pulmonary hypertension, respiratory failure, and circulatory failure, as well as the challenges in managing these complications, have garnered growing attention [23], [24]. The results of studies indicate a mortality rate of 1.5% to 2.8% due to pertussis [19], [20], [25], primarily affecting infants under three months of age [19], [20]. Some studies have explored severe prognostic factors [19], [26] and mortality in pertussis patients [23], [24]; however, these studies were descriptive with small sample sizes and analyzed only univariate correlations.

Moreover, in recent years, antibiotic resistance in pertussis, primarily macrolide resistance, has become a subject of evaluation. In Vietnam, a study with 15 culture-positive pertussis samples in the southern region reported no antibiotic-resistant pertussis bacteria [27], [28]. Nevertheless, the latest reports on pertussis antibiotic resistance in Northern provinces indicate a macrolide resistance rate of 19% (10/53) [29]. However, it's worth noting that both of these studies had small sample sizes.

Thus, despite high vaccine coverage for pertussis among children starting from 2 months of age, infants and young children continue to experience a high incidence of pertussis, often with severe manifestations and challenging

treatment. Consequently, we conducted this study with three primary objectives:

1. *To describe the clinical and subclinical characteristics of pertussis-infected pediatric patients at the National Children's Hospital from 2019 to 2020.*
2. *To determine the prevalence of Macrolide-resistant mutations and the genetic diversity of Bordetella pertussis bacteria.*
3. *To identify significant prognostic factors and treatment outcomes in pediatric pertussis cases.*

NOVEL CONTRIBUTIONS AND SCIENTIFIC SIGNIFICANCE, PRACTICAL IMPLICATIONS OF THE THESIS

This study contributes to the understanding that pertussis is frequently encountered in neonates, especially infants under four months of age, who have yet to receive vaccination or have incomplete vaccination coverage. The disease can lead to complications such as severe pulmonary hypertension, a life-threatening condition more common in younger children.

The research also plays a role in identifying prognostic factors for severe pertussis, aiding clinical practitioners in early detection and better prognosis for severe cases. This enables timely monitoring and treatment to improve survival rates. Furthermore, this study has determined the prevalence of Macrolide-resistant mutations and the genetic diversity of *Bordetella pertussis* bacteria, contributing to the molecular epidemiology of pertussis in Northern Vietnam.

THESIS STRUCTURE

The thesis consists of 130 pages and is structured as follows: Objectives (2 pages); Introduction (35 pages); 2 Subjects and methods (22 pages); Results (40 pages); Discussion (28 pages); Conclusion (2 pages); Recommendations (1 page). The thesis includes 46 figures, 30 tables and 5 appendices. It references 134 sources, with 42% of the references published within the last 5 years.

Chapter 1
LITERATURE REVIEW

1.1. General overview of pertussis

1.1.1. Epidemiology

Pertussis is a highly contagious respiratory disease in humans, primarily transmitted through respiratory droplets, with a high attack rate ranging from 70% to 100% [31]. Natural immunity or post-vaccination immunity does not provide lifelong protection. Post-vaccination immunity is effective for approximately 3 to 5 years.

1.1.2. Causative agent

Bordetella pertussis is the primary causative agent of whooping cough. It is a gram-negative, aerobic, non-sporulating bacterium that does not form endospores [35].

1.1.3. Physiopathological mechanism of pertussis

B. pertussis enters the respiratory epithelium of humans through respiratory droplets carrying the bacteria. They adhere to ciliated cells through binding factors, without deep tissue invasion or bloodstream entry. Upon attachment, they release toxic factors that cause necrosis of the respiratory epithelium, leading to acute respiratory tract inflammation and increased mucus secretion. Damage to the respiratory tract often results in superinfection with other bacteria.

1.2. Clinical, subclinical features and diagnosis of pertussis

1.2.1. Clinical features

Prolonged Cough Illness is divided into three stages: the initial stage, the paroxysmal stage and the convalescent stage, each lasting approximately 2 weeks:

- Initial stage (inflammatory stage): Lasts 3-14 days. Mild fever, often without fever [30]. Respiratory tract inflammatory symptoms include dry cough, sneezing, coryza, gradually progressing to paroxysmal cough.

- Paroxysmal stage (coughing stage): Lasts 1-2 weeks, characterized by typical bouts of whooping cough, consisting of 3 phases: cough, inspiratory whoop, and post-tussive emesis. After each coughing episode, children may appear fatigued and experience vomiting, sweating, rapid heart rate and rapid breathing. Auscultation of the lungs during coughing may reveal some wheezing.

- Convalescent Stage: Lasts 2-4 weeks. The frequency of coughing episodes decreases gradually, with shorter durations, reduced intensity of coughing, and less post-tussive vomiting, eventually ceasing altogether.

1.2.2. Subclinical features

Full blood count: Elevated leukocytes (15 - 100 G/L) due to increased lymphocytes.

Chest X-ray imaging: Shows infiltrates around the lung hilum or organized interstitial opacities (sometimes butterfly-shaped) with possible lung collapse.

Bacterial culture: High specificity but limited sensitivity (not exceeding 60%) [45].

Molecular biology: PCR testing yields higher positivity rates compared to bacterial culture.

Direct Fluorescent Antibody (DFA) testing has low sensitivity and specificity, necessitating bacterial culture, PCR or serology support.

Serologic testing employs enzyme immunoassay (EIA) to detect antibodies against B. pertussis components. This test has the highest sensitivity in older children and adults who have been vaccinated long ago (over three years) and have had a persistent cough for more than two weeks.

1.2.3. Diagnosis

According to the Global Pertussis Initiative (GPI) 2011, clinical diagnosis of pertussis is categorized into three age groups [52]:

- Infants 0 - < 4 months: Persistent cough, runny nose, with or without mild fever, accompanied by any following: inspiratory whoop, apnea, post-tussive vomiting, cyanosis, seizures or pneumonia.
- Children 4 months - < 10 years: Paroxysmal cough lasting ≥ 7 days, with or without mild fever, accompanied by any following: inspiratory whoop, apnea, post-tussive vomiting, seizures, conjunctival hemorrhage, or pneumonia.
- Children ≥ 10 years: Paroxysmal cough lasting ≥ 2 weeks, with or without mild fever, accompanied by any following: inspiratory whoop, apnea, sweating between coughing episodes, post-tussive vomiting, worsening symptoms at night.

Clinical diagnosis is made when a child meets one of the above criteria. The GPI 2011 also recommends using microbiological tests for pertussis diagnosis, including PCR, bacterial culture and serological testing [52].

1.2.4. Prognostic factors for severe pertussis

Vaccination: is a protective factor against severe pertussis [63], [64].

Age: Infants, especially those under three months of age, are a higher risk of developing severe and fatal pertussis [19], [64], [65].

Birth weight: Low birth-weight infants are a higher risk of severe disease than normal-weight infants.

Several other clinical factors contribute to the prognosis of severe pertussis, including prolonged hospitalization, fever, cyanosis, wheezing, and abnormal liver function [66].

Peripheral blood leukocytes: There is an association between elevated peripheral blood leukocyte counts, pulmonary hypertension, severe disease, and mortality in young children [19], [64], [65].

1.3. Macrolide resistance mutations and genetic polymorphism of *Bordetella pertussis*

Macrolides were the first-line antibiotics for treating pertussis infections. However, pertussis strains resistant to erythromycin have been reported since 1994 [68]. Since then, reports of macrolide-resistant pertussis have been increasing in numbers and proportions. Resistance to other antibiotics in pertussis is relatively limited; a report from Pakistan 2016 found that most pertussis bacteria were resistant to quinolones, and 71.4% were resistant to sulfamethoxazole [72].

There are three mechanisms of macrolide resistance in bacteria [11], [74], [75]: (1) Alteration of the macrolide binding site on the 50S ribosome. (2) Reduced cell membrane permeability or efflux pump activity (mef gene). (3) Drug inactivation. However, most studies have shown that B. pertussis resistance to macrolides is due to the A2047G mutation in the V region of the 23S rRNA gene (altering the binding site structure of macrolides with the 50S ribosome) [13], [15], [70], resulting in high-level resistance and cross-resistance to various macrolide antibiotics.

In Vietnam, there was no evidence of macrolide-resistant pertussis in the southern region in 2019 [27], [28]. In 2020, Kamachi and colleagues reported a macrolide resistance rate of 19% (10/53) in Vietnam [29].

Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) is a simple method that can be performed directly on clinical samples and isolated bacterial strains. Using this method, Kurniawan (2010) analyzed 316 pertussis strains isolated from 12 countries on 4 continents, identifying 66 MLVA types (MT) [85].

1.4. Pertussis treatment

1.4.1. Principles of treatment

- Isolation and early specific treatment when suspected of having pertussis symptoms.
- Monitoring, early detection, and management of complications
- Ensuring nutritional support to improve the patient's condition

1.4.2. Specific treatment

Indications when suspected or confirmed pertussis in children under 1 year old within 6 weeks and in children over 1 year old within 3 weeks from the onset of cough [87].

National Children's hospital and many countries use the following treatment protocols:

Preferred drugs: One of the following drugs may be used:

- Azithromycin: Children < 6 months: 10mg/kg/day for 5 days. Children > 6 months, adults: 10mg/kg (maximum 500 mg) on the first day; 5 mg/kg (maximum 250 mg) on days 2 – 5.
- Clarithromycin: Not recommended for children under 1 month old. Dose: 15 mg/kg/day (maximum 1 g/day), divided into 2 doses for 7 days
- Erythromycin: Not used for children under 1 month old. Dose: 40 - 50 mg/kg/day (maximum 2 g/day), divided into 4 doses for 14 days

Alternative drug: Trimethoprim-sulfammethoxazole (TMP-SMX): Contraindicated for children < 2 months. Dose: TMP 8 mg/kg/day (maximum TMP 320 mg/day), divided into 2 doses for 14 days [57].

1.4.3. Symptomatic treatment

Cough suppressants: Antihistamines, phenobarbital, and opioids have no proven benefit [92]. Corticosteroids are not recommended. β2-adrenergic stimulants, specific pertussis antibodies, have not shown apparent clinical effectiveness. Recent research on cough inhibitors or toxin-neutralizing agents in pertussis may help control symptoms and disease progression [94].

1.4.4. Treatment of complications

Treatment for respiratory failure and pulmonary hypertension should follow guidelines [57]. Other treatment measures, such as extracorporeal membrane oxygenation (ECMO), blood transfusion, hemodialysis, etc., are indicated in Appendix 1 [57], [95].

1.4.5. Care

Provide a quiet resting environment for the child, avoiding dust and cigarette smoke. Ensure the child eats regular meals. Monitor the child closely and provide oxygen and suction as needed.

1.4.6. Prevention

Prevent transmission and isolate suspected or confirmed pertussis cases. Post-exposure prophylaxis with macrolide antibiotics for all exposed individuals. Active prevention with vaccination is recommended [113].

Chapter 2
STUDY METHOD

2.1. Objective 1

2.1.1. Study population, location and duration

Study population: Children under 16 years of age are diagnosed with confirmed pertussis and receive treatment at the National Children's Hospital during the study period. All children with pertussis in this study are under 10 years of age.

Case selection criteria: Cases are diagnosed with confirmed pertussis following the guidelines of National Children's Hospital, applying the Global Pertussis Initiative 2011 as outlined in Figure 2.1 [52], [57].

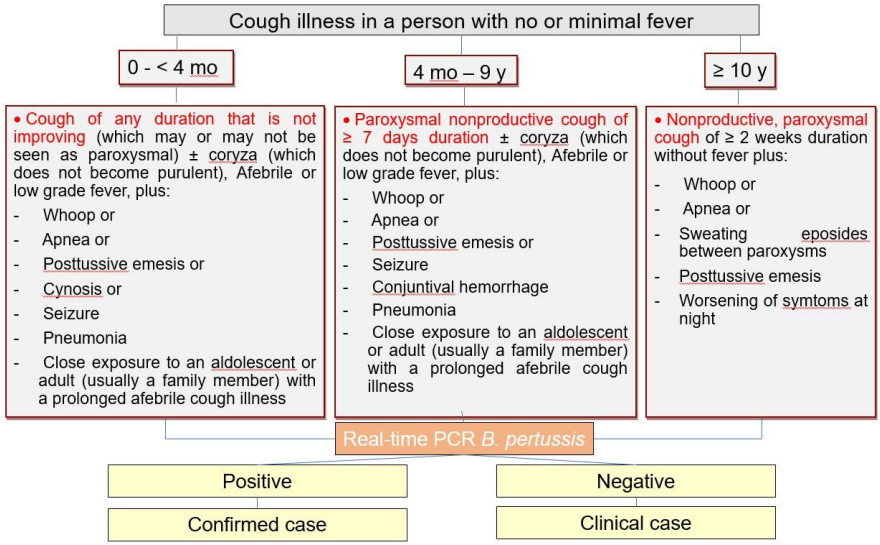


Figure 2.1: Diagnostic criteria for pertussis according to GPI 2011 [52]

Exclusion criteria: Caregivers who do not consent to participate in the study.

Study location: The study was conducted at the National Children's Hospital.

Study period: From January 2019 to December 2020.

2.1.2. Study methods

Study design: A prospective descriptive case series study.

Sample size: Sample size calculation for the descriptive study to determine a proportion [100]***:*** $\boldsymbol{n = Z^2_{1-\alpha/2}\frac{1-p}{p\varepsilon^2}}$

where: n: Minimum estimated sample size; Z: Confidence coefficient, with a 95% confidence level $Z_{1-\alpha/2}$ = 1,96; p: Estimated population proportion, p = 0,747 (the proportion of children with cyanosis in the pertussis group

according to Tran Minh Dien (2015)) [19]; ε: Allowable relative error, lấy ε = 0,06. With the selected values, the estimated sample size is 362. The actual sample size we collected was 382 patients.

Sampling method: Convenience sampling, including all patients who met the pertussis diagnostic criteria, as shown in Figure 2.1.

2.1.3. Study content

- General characteristics of the study group: Determine the distribution of characteristics such as age, gender, admission time, location, vaccination history, etc…
- Clinical features: determine the distribution of symptoms, complications of the disease, and the course of the disease.
- Paraclinical features: Laboratory test results, blood biochemistry, chest X-rays, echocardiography, etc…

2.2. Objective 2

i. 2.2.1. Study population

- Specimens of nasopharyngeal or tracheal aspirate with positive results for pertussis by Real-time PCR were collected to test the A2047G mutation and perform MLVA genotyping.
- Children whose specimens meet the research criteria as above

Exclusion criteria: Specimens with indeterminate results for the A2047G mutation test and MLVA genotyping.

Study location:

- The study was conducted at the National Children's Hospital.

+ Clinical departments that received and treated pertussis patients in the hospital.

+ The Molecular biology laboratory of infectious diseases: conducted Real-time PCR testing to identify *B. pertussis* bacteria.

- Bacterial laboratory department at the National Institute of Infectious Diseases, Japan: conducted testing to find the A2047G mutation and determine the MLVA genotyping on collected specimens.

Study period: Data for the study was collected in two phases:

- Retrospective phase: From 1/1/2017 to 31/12/2017, 75 specimen samples were collected.
- Prospective phase: From 1/1/2019 to 31/12/2020, 61 specimen samples were collected.

2.2.2. Study methods

Study design: Descriptive study with case series analysis

Sample size: Sample size calculation for the descriptive study to determine a proportion [100]: $\boldsymbol{n = Z^2_{1-\alpha/2}\frac{1-p}{p\varepsilon^2}}$

Where: n: Estimated minimum sample size; Z: Confidence coefficient,

with a 95% confidence level, $Z_{1-\alpha/2}$ = 1.96; p: Estimated population proportion, p = 0.491 (the proportion of A2047G mutation among *B. pertussis* strains according to Lin Xiao Juan 2021 [71]); ε: Allowable relative error, ε = 0.18. Thus, the estimated sample size is 123. The actual sample size collected was 136 specimens.

Sampling method: Randomly select specimens of nasopharyngeal or endotracheal secretions with positive B. pertussis real-time PCR results from 2017, 2019 and 2020.

2.2.3. Study content

- Determine the A2047G mutation associated with Macrolide resistance and calculate the proportion of bacteria carrying this mutation. Evaluate the severity of the disease, treatment measures, and treatment outcomes between the two groups: those with drug-resistant mutations and those without.

- Identify the MLVA genotypes of *B. pertussis* bacteria in the samples involved in the study, analyze differences and relationships between genotypes, and thereby assess the variation trends among bacterial strains.

2.3. Objective 3

2.3.1. Study population

All the pediatric patients participating in the study for objective 1.

Study location: The research was conducted at the National Children's Hospital.

Study period: From 1/1/2019 to 31/12/2020.

2.3.2. Study methods

Study design: Descriptive prospective study with case series analysis

Sample size: 382 pediatric patients who participated in objective 1 were included in the analysis for objective 3.

Sampling method: Convenience sampling, including all eligible cases in the study.

2.3.3. Study content

- Determine the rate of severe disease: Identify severe cases among hospitalized pertussis patients. Calculate the percentage of severe cases in the study sample.

- Identify prognostic factors for severe pertussis: Assess the relationship between various physiological, clinical, and laboratory factors and the severity of pertussis.

- Determine the rate of interventions and medication use in treating pertussis in children. Evaluate the treatment outcomes of pertussis in children.

2.4. Techniques used in the study

Real-time PCR for *B. pertussis* detection. Duplex Cycleave Real-Time PCR to identify the A2047G mutation. Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) for genotyping.

2.5. Data handling and analysis

- Data variables were collected following the research case report form (see Appendix 3)
- Data analysis was performed using SPSS 22.0 statistical software

2.6. Ethical considerations

This study obtained ethical approval from the Ethical Review Board for Biomedical Research at the National Children's Hospital, under the reference number VNCH-RICH-2019-60, dated October 31, 2019, National Institute of Malaria, Parasitology and Entomology.

Chapter 3

STUDY RESULT

During the study period, there were 382 pediatric patients diagnosed with pertussis and treated at the National Children's Hospital.

3.1. Clinical, subclinical characteristics of pediatric pertussis patients

3.1.1. General characteristics of the study group

Table 3.1: Some characteristics of the study group (n = 382)

Characteristics		Number	Percent %	p
Age (months)	Median	2,5		
	Min	0,6 (19 days)		
	Max	116 (9 years 8 months)		
	Mode	1,4		
	< 4 months	289	75,7	< 0,001
	≥ 4 months	93	24,3	
Sex	Male	211	55,2	0,046
	Female	171	44,8	
Exposure	Non-exposure	346	90,6	
	Suspected exposure	23	6,0	
	Exposure	13	3,4	
Vaccination	Not old enough to be vaccinated	163	42,7	
	No vaccinations	165	43,2	
	Vaccinated	54	14,1	

The median age of the study group was 2,5 months, with the youngest patient being 19 days old and the oldest being 116 months (9 years and 8 months). The group of children under 4 months old accounted for 75,7% (289/382), which was higher than the group aged 4 months and above, which accounted for 24,3% (93/382), $p < 0,01$.

The proportion of male children diagnosed with pertussis was higher than that of female children, with a male-to-female ratio of $55,2/44,8 \approx 1,23/1$. This difference was statistically significant with $p < 0,05$.

3.1.2. Clinical characteristics

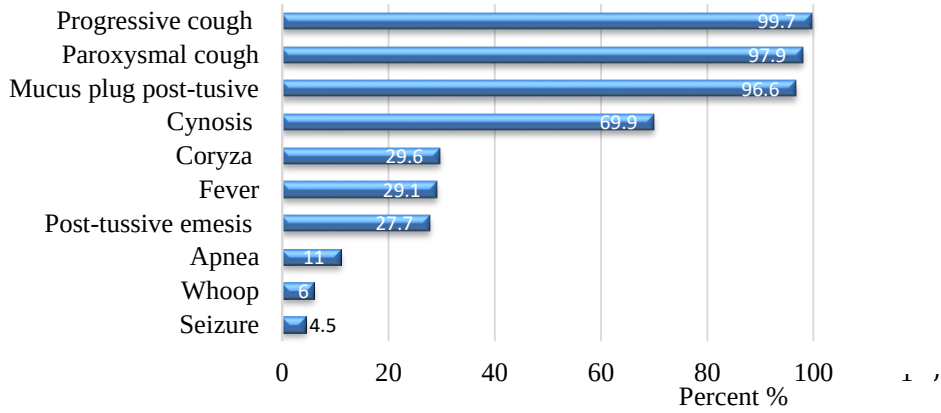


Figure 3.6: Common presenting symptoms (n = 382)

The most common symptoms of pertussis were progressive cough, paroxysmal cough, and expulsion of a mucus plug post-tusive in most cases. Severe coughing with cyanosis occurred in 69,9% of cases.

Table 3.3: Symptoms by age group (n = 382)

Symptom	**< 4 months (n_1 = 289)**		**≥ 4 months (n_2 = 93)**		**p**
	Number	%	Number	%	
Paroxysmal cough	284	98,3	90	96,8	0,38
Expulsion of a mucus plug post-tusive	281	97,2	88	94,6	0,3
Cyanosis	215	74,4	52	55,9	0,001
Coryza	71	24,6	42	45,2	**< 0,001**
Fever	70	24,2	41	44,1	**< 0,001**
Post-tusive emesis	78	27,0	28	30,1	0,5
Apnea	41	14,2	1	1,1	< 0,001
Whoop	13	4,5	10	10,8	**0,027**
Seizure	14	4,8	3	3,2	0,7

Cyanosis and apnea symptoms were more common in the group of infants aged < 4 months compared to the older group (≥ 4 months). Conversely, the older group often presented with fever, coryza and whoop. These differences were statistically significant with $p < 0,05$.

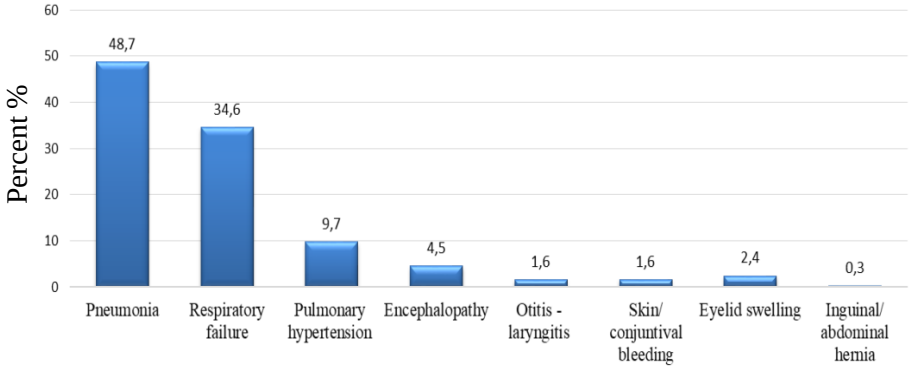


Figure 3.8: Some complications of pertussis (n = 382)

Table 3.4: Common complications by age group (n = 382)

Complications	< 4 months (n = 289)		≥ 4 months (n = 93)		p
	Number	%	Number	%	
Pneumonia	150	51,9	36	38,7	0,038
Respiratory failure	110	38,1	22	23,7	0,011
Pulmonary hypertension	32	11,1	5	5,4	0,1
Encephalopathy	15	5,2	2	2,2	0,2

The most common complications were pneumonia and respiratory failure, accounting for 48,7% and 34,6%, respectively. Severe complications such as pulmonary hypertension and encephalopathy were less common (9,7% and 4,5%, respectively). Pneumonia and respiratory failure were more prevalent in children under 4 months compared to those aged 4 months and older ($p < 0,05$).

3.1.3. Subclinical characteristics

Table 3.6: White blood cell (WBC) count and lymphocyte count in peripheral blood (n = 382)

Index	White blood cell		Lymphocyte	
	Number	Percent %	Number	Percent %
Mean ($\overline{x}$ ± SD) (G/l)	20,7 ± 13,9		13,2 ± 8,1	
≤ 10 (G/l)	55	14,4	167	43,7
>10 - 20 (G/l)	193	50,5	157	41,1
> 20 - 30 (G/l)	74	19,4	38	9,9
> 30 - 50 (G/l)	44	11,5	19	5,0
> 50 (G/l)	16	4,2	1	0,3

A total of 35,1% of children with pertussis had an elevated white blood cell count ≥ 20 G/l, 56,3% of children had an elevated lymphocyte count above 10 G/l, with the majority showing lymphocyte counts between 10 - 20 G/l (41,1%).

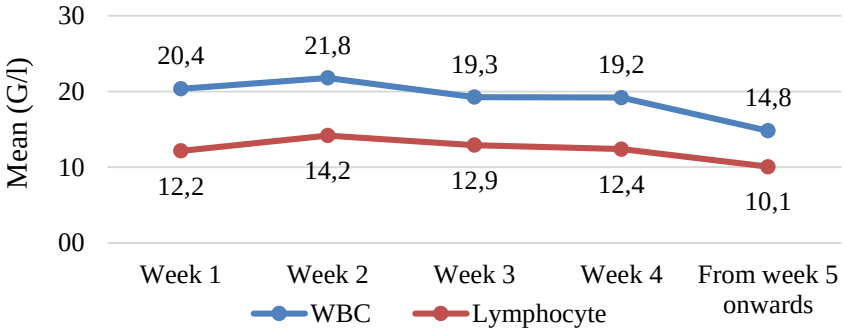


Figure 3.9: Mean values of total white blood cell count and lymphocyte count in peripheral blood over time (n = 382)

The total white blood cell count and lymphocyte count increased gradually from the end of the first week, reaching their highest levels in the second week (corresponding to 21,8 and 14,2 G/l respectively). From the third week onwards, both the total white blood cell count and lymphocyte count decreased gradually.

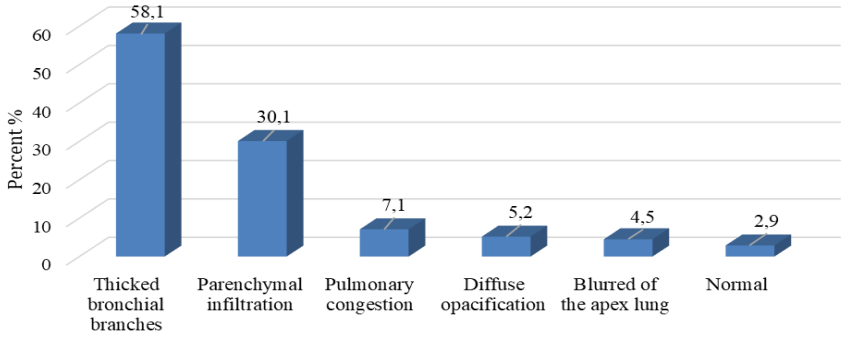


Figure 3.11: Images of lung damage on chest X-ray (n = 382)

The predominant lung damage seen on chest X-rays in children with pertussis mainly consists of marked thickening of the bronchial branches in both lung regions, accounting for 58,1% (222/382), while parenchymal infiltration and pulmonary congestion were less common.

Table 3.11: Superinfections with other microorganisms (n = 382)

Superinfections	**Microorganisms**	**Number (%)**	**Total (%)**
Superinfected	Virus	63 (70,0)	**90 (23,6)**
	Bacteria	36 (40,0)	
	Fungi	1 (1,1)	
	Parasites	1 (1,1)	
Non-superinfected			**292 (76,4)**
Total			**382 (100)**

Of the children with pertussis, 23,6% (90/382) had superinfections with other microorganisms, including viral superinfections in 70,0% (63/90) and bacterial superinfections in 40,0% (36/90). Notably, 17,8% (16/90) of the children had superinfections with at least two microorganisms.

3.2. The proportion of *Bordetella pertussis* bacteria having Macrolide-resistant A2047G mutation and bacterial polymorphism

Analysis of 136 samples with positive Real-time PCR pertussis obtained the following results:

3.2.1. The proportion of B. pertussis having the Macrolide-resistant A2047G mutation

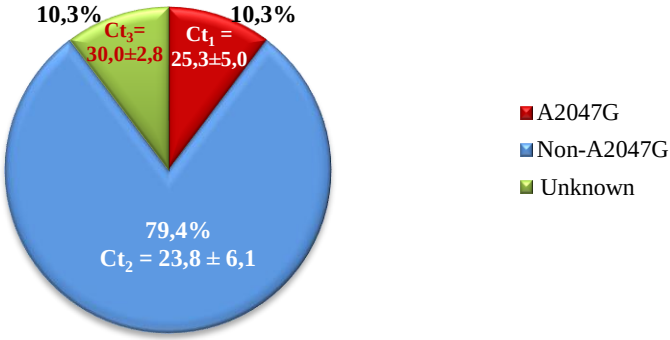


Figure 3.13: Proportion of B. pertussis having A2047G mutation

Of the 136 samples sent for genetic analysis of Macrolide resistance, 14 samples (10,3%) could not be determined, while results were obtained for 122 samples. Among them, 14 samples were identified to have the A2047G Macrolide-resistant mutation, accounting for a proportion of 10,3% (14/136). Thus, among the samples with determined results, the percentage of samples with Macrolide-resistant bacteria is 11,5% (14/122).

3.2.2. Genetic polymorphism of Bordetella pertussis bacteria

3.2.2.1. Distribution of Bordetella pertussis bacterial strains

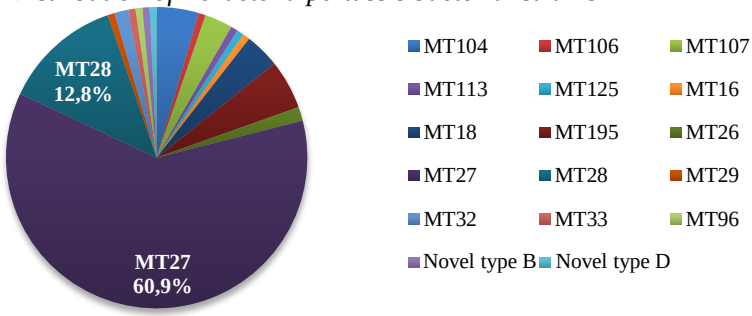
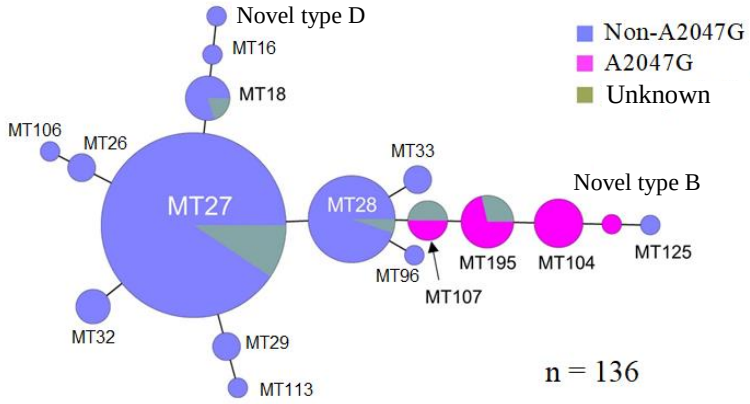


Figure 3.15: Proportion of MLVA types of B. pertussis strains (n = 133)

Out of the 136 samples sent for MLVA analysis, we identified 17 MLVA genotypes in 133 B. pertussis samples (97.8%), while the remaining 3 samples could not be determined for MLVA genotypes (MT). The MT27 genotype accounted for the majority at 60,9% (81/133) among the pertussis strains found in Vietnam, followed by MT28 at 12,8% (17/133).

3.2.2.2. Distribution of MLVA genotypes and A2047G mutation



Hình 3.16: Phân bố các kiểu gen MLVA của vi khuẩn B. pertussis

(Distribution tree model with the area of circles corresponding to the frequency of genotypes, connecting lines indicating genetic relationships)

There are 4 MLVA genotypes: MT195, MT104, MT107 and a novel type B with the A2047G Macrolide-resistant mutation.

3.2.2.3. Percentage of B. pertussis with Macrolide-resistant mutations over time

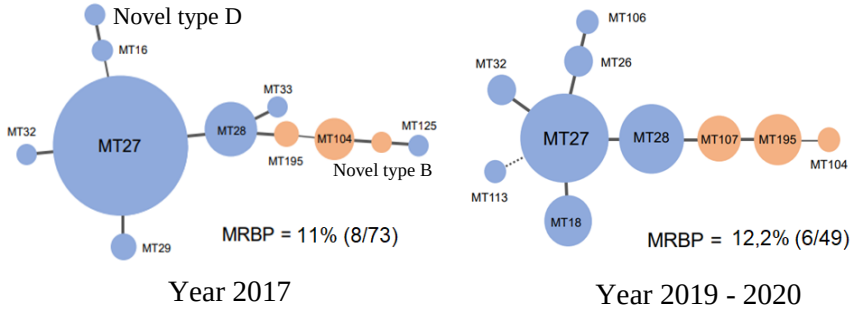
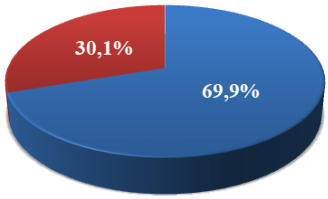


Figure 3.17: Percentage distribution of B. pertussis MLVA genotypes over time

The percentage of bacteria carrying Macrolide-resistant mutations slightly increased from 11% in 2017 to 12,2% năm 2019 - 2020.

3.3. Some prognostic factors of severe pertussis and outcomes

3.3.1. Percentage of severe cases



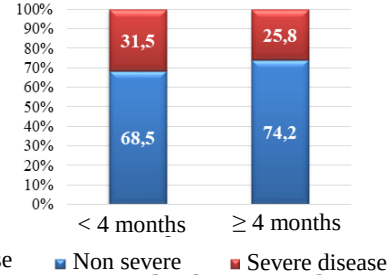


Figure 3.18: Severe disease rate

Figure 3.19: Severe disease rate by age group

The percentage of severe pertussis cases was 30,1% (115/382), with 31,5% (91/289) in the group under four months and 25,8% (24/93) in the group aged four months and older.

3.3.2. Some predictive factors of severe pertussis

3.3.2.1. Some physiological characteristics of children

Table 3.17: The relationship between some epidemiological characteristics and severe disease

Features	**Severe disease** (n_1=115)		**Non-severe disease** (n_2 = 267)		p	OR: 95% CI
	Number	%	Number	%		
Premature	13	11,3	13	4,9	0,026	2,5: 1,1-5,6
Malnutrition	8	7,0	3	1,1	0,004	6,6: 1,7-25,3
Base diseases	16	13,9	15	5,6	0,008	2,7: 1,3-5,7
No vaccination	108	93,9	220	82,4	0,005	3,3: 1,4-7,5

Some characteristics of premature infants, malnutrition, underlying medical conditions and lack of pertussis vaccination were found to be related to the severity of the disease ($p < 0,05$).

3.3.2.2. Clinical symptoms and complications

Table 3.18: Some symptoms and complications associated with severe disease

Symptoms, complications	Severe disease (n_1=115)		Non-severe disease (n_2 = 267)		p	OR: 95% CI
	Number	%	Number	%		
Fever	50	43,5	61	22,8	< 0,01	2,6: 1,6 – 4,1
Whoop	7	6,1	16	6,0	0,9	-
Cyanosis	110	95,7	157	58,8	< 0,01	15,4: 6,1-39,0
Apnea	29	27,8	13	4,9	< 0,01	6,6: 3,3 – 13,2
Seizure	15	13,0	2	0,7	< 0,01	19,9: 4,5 – 88,5
Pneumonia	107	93,0	79	29,6	< 0,01	31,8: 14,8 – 68,4
Pulmonary hypertension	30	26,1	7	2,6	< 0,01	13,1: 5,6 – 30,9

Fever, cyanosis, apnea, seizures and complications such as pneumonia and pulmonary artery hypertension were associated with an increased risk of severe pertussis $p < 0,05$.

3.3.2.3. Subclinical signs

Table 3.20: Blood counts and some related indices concerning severity

Index	Severe disease (n_1=115)		Non-severe disease (n_2 = 267)		p
	$\bar{x}$	SD	$\bar{x}$	SD	
WBC (G/l)	28,6	19,1	17,1	8,9	< 0,001
Lymphocyte (G/l)	16,3	9,9	11,9	6,9	< 0,001
Neutrophils (G/l)	8,4	7,7	3,4	2,7	< 0,001
Plateletes (G/l)	507,9	181,0	457,4	153,2	0,006
CRP (mg/l)	13,4	30,3	2,5	9,1	< 0,001
Cycle threshold (Ct)	23,8	6,2	26,1	5,9	0,001

The mean of total white blood cells, lymphocytes, neutrophils, platelets, and CRP levels in the severe disease group were higher than the respective averages of these parameters in the non-severe disease group ($p < 0,05$).

3.3.2.4. Superinfection with other microorganisms

Table 3.23: Superinfection status related to the severity of the disease

	Superinfection	Non-superinfection	p	OR: 95% CI
Severe disease (n_1=115)	50 (43,5%)	65 (56,5%)	< 0,001	4,4: 2,7-7,2
Non-severe disease (n_2=267)	40 (15,0%)	227 (85,0%)		

The proportion of children with whooping cough who had superinfections in the severe disease group was 43,5% (50/115), which was higher than the proportion in the non-severe disease group, which was 15% (40/267), $p < 0,01$.

3.3.2.5. Determining prognostic factors for the disease through multivariable logistic regression analysis

Table 3.24: Multivariable regression analysis of prognostic factors for severity

Associated factors	**B**	**p**	**OR: 95% CI**
Age (months)	0,003	0,88	-
Onset time ≤ 5 days	0,742	**0,024**	2,10: 1,10 - 4,00
Fever	0,91	**0,017**	2,40: 1,17 - 4,93
Cyanosis	2,61	< **0,001**	13,54: 4,22 - 43,49
Pneumonia	2,67	< **0,001**	14,49: 6,27 - 33,47
Pulmonary hypertension	1,62	**0,015**	5,07: 1,37 - 18,78
Leukocytes/(10)	0,37	**0,009**	1,45: 1,10 - 1,91
Ct/(-5)	0,28	**0,048**	1,32: 1,01 - 1,76
Superinfection	1,19	**0,001**	3,29: 1,58 - 6,83

Multivariable regression analysis identified several prognostic factors for severe disease with OR and 95% CI: Onset of illness ≤ 5 days increased the risk of severe disease by 2,10 times (95% CI: 1,10 – 4,00 times). Fever: [2,40: 1,17 – 4,93]; Cyanosis: [13,54: 4,22 – 43,49]; Pneumonia: [14,49: 6,27 – 33,47]; Increased pulmonary artery pressure: [5,07: 1,37 – 18,78]; White blood cell count increased by 10 G/l: [1,45: 1,10 – 1,91]; Ct value decreased by 5 cycles: [1,32: 1,01 – 1,76]; Super-infection: [3,29: 1,58 – 6,83].

3.3.3. Antibiotic use in the hospital

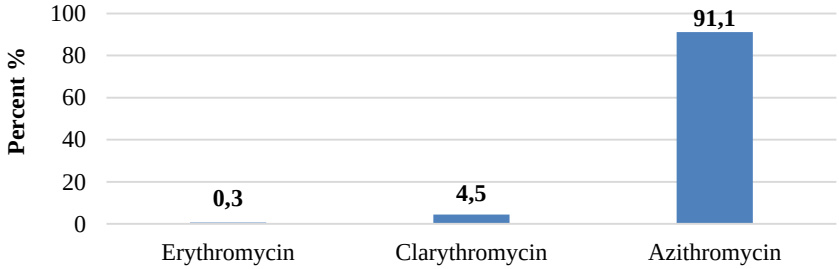


Figure 3.24: Percentage of Macrolide antibiotic use (n = 382)

A total of 95.5% (365/382) of children with pertussis were prescribed Macrolide antibiotics, with 91.1% (348/382) receiving azithromycin.

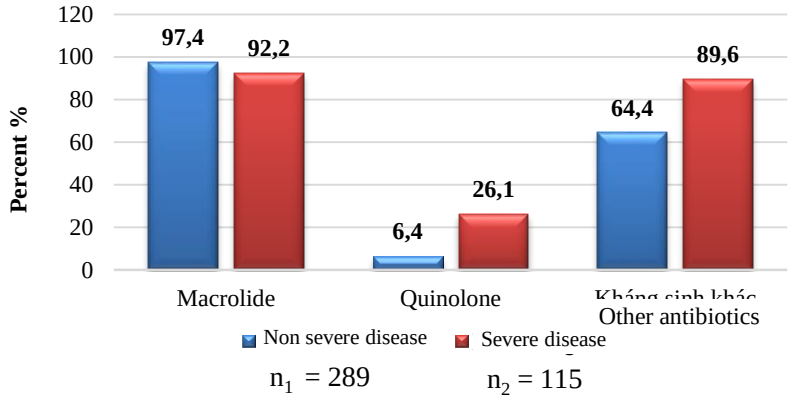


Figure 3.26: Antibiotic use by severity of illness

Children with severe disease had a lower rate of Macrolide antibiotic use (92,2%). In comparison, the use of Quinolone antibiotics (26,1%) and other antibiotics (89,6%) was higher compared to the non-severe disease group (97,4%; 6,4% and 64,4%, respectively), These differences were statistically significant, p < 0,05.

3.3.4. Treatment result

3.3.4.2. Treatment time

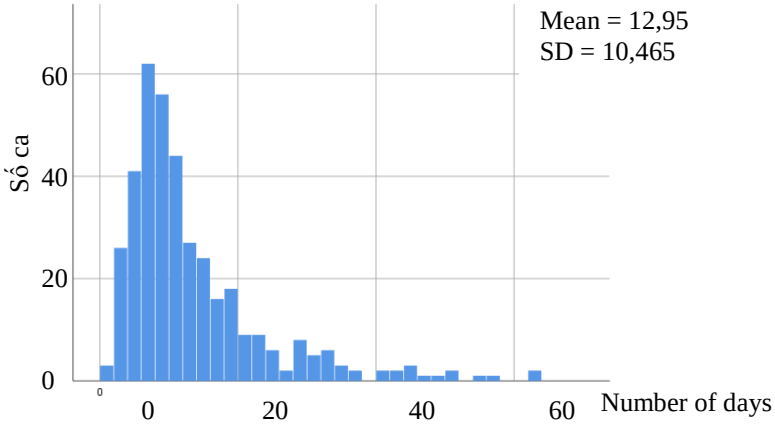


Figure 3.29: Length of hospital stay (n = 382)

The average length of hospital stay was $\bar{x} \pm SD = 13,0 \pm 10,47$ (days), with a median of 10 days (ranging from a minimum of 1 day to a maximum of 62 days), and only 8,6% (33/382) of children were hospitalized for less than one week.

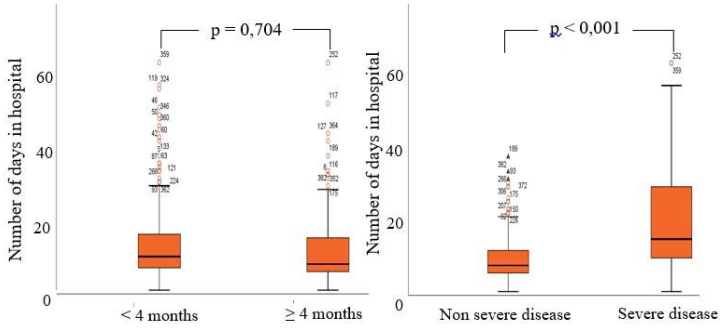
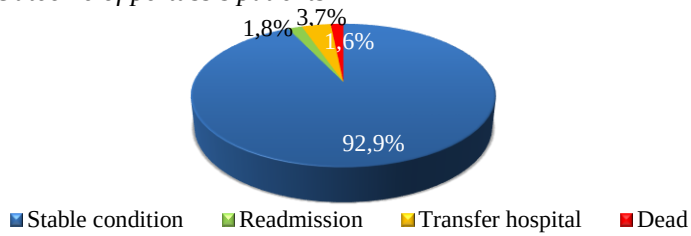


Figure 3.30: Length of Hospital Stay by age group and disease severity (n = 382)

The length of hospital stay did not vary significantly among age groups (p > 0,05). However, the median length of hospital stay for the severe disease group was 15 days, nearly double that for the non-severe disease group, which was 8 days (p < 0,01).

3.3.4.3. Outcome of pertussis patients



Hình 3.31: Tình trạng ra viện của bệnh nhân ho gà (n = 382)

92,9% (355/382) trẻ mắc ho gà được điều trị khỏi và ra viện ổn định, một số ít (1,8%) trẻ phải vào viện lại. Có 1,6% (6/382) trẻ ho gà tử vong.

Chapter 4
DISCUSSION

4.1. Clinical and subclinical characteristics of pertussis patients treated at the National Children's Hospital in 2019 – 2020

4.1.1. General characteristics of the study group

The age distribution of the study group ranged from 0 to < 10 years, with a median age of 2,5 months. Infants under 4 months of age accounted for 75,7% (289/382) of the group. This age group is considered an immunological gap in the population, as infants under 2 months are not yet eligible for vaccination, and those starting their primary vaccinations at 2 months are not yet entirely immune. Additionally, maternal immunity to pertussis in this age group is typically low and insufficient to provide protection, especially in the early stages of life [108]. Male infants had a higher incidence of pertussis compared

to female infants, with a male-to-female ratio of approximately 1,23:1. This difference may be attributed to the predominantly young age of pertussis cases in this population, where males tend to outnumber females [117].

4.1.2. Đặc điểm lâm sàng bệnh ho gà

The most common clinical symptoms observed in pertussis patients included gradually increasing cough (99,7%), paroxysmal coughing fits (97,9%), and post-coughing sticky white sputum (96,6%). These symptoms were prevalent in nearly all pertussis cases. Cynosis was noted in 69,9% of cases. Other symptoms, such as runny nose, fever, and post-cough vomiting, were present in 29,6%, 29,1%, and 27,7% of cases, respectively. Additionally, less frequently encountered symptoms included apnea (11,0%), whoop (6,0%), and seizures (4,5%). The most common complications were pneumonia and respiratory failure, occurring in 48,7% and 34,6% of cases. Complications such as pulmonary hypertension and brain damage were less common, accounting for 9,7% and 4,5%, respectively.

Children aged ≥ 4 months were more likely to exhibit symptoms such as coryza, fever, and whoop (45,2%, 44,1%, 10,8%) compared to the group of children aged < 4 months (24,6%, 24,2%, 4,5%). Conversely, younger infants were more likely to experience pallor and apnea (74,4%, 14,2% vs. 55,9%, 1,1%). Additionally, infants under 4 months had a higher incidence of complications like pneumonia and respiratory failure (51,9%, 38,1% vs. 38,7%, 23,7%) ($p < 0,05$). Complications such as pulmonary hypertension and brain damage were more common in infants under 4 months, although these differences were not statistically significant ($p > 0,05$).

4.1.3. Subclinical characteristics

Children diagnosed with pertussis exhibited an average white blood cell count of 20,7 ± 13,9 G/l and an average lymphocyte count of 13,2 ± 8,1 G/l. Among them, 35,1% of children had a peripheral blood total white cell count exceeding 20 G/l, with only 4,2% exceeding 50 G/l. Furthermore, 56,3% of pertussis patients had an increased lymphocyte count of more than 10 G/l, with lymphocytes primarily increasing within the range of 10 - 20 G/l (41,1%). The total white blood cell and lymphocyte counts showed an increasing trend starting from the end of the first week, reaching the highest values in the second week, and then gradually decreasing from the third week onwards.

The most common lung lesions observed in chest X-ray images were darkened bronchial branches in both lung regions (accounting for 58,1%). This image corresponds to the known pathophysiological mechanism of pertussis, which involves inflammatory and necrotic damage to the respiratory tract mucosa caused by pertussis toxin. Literature also describes chest X-ray images showing lesions near the heart on both sides [30].

In this study, 23,6% (90/382) of children had super-infections with other microorganisms, with a majority being viral super-infections (70,0%), followed by bacterial super-infections (40%).

4.2. Proportion of *Bordetella pertussis* strains carrying Macrolide resistance mutations and genetic polymorphism

4.2.1. Proportion of Macrolide-Resistant Bordetella pertussis

Our study identified 14 DNA samples carrying the A2047G mutation in the 23S rRNA gene associated with Macrolide resistance out of 122 DNA samples from *Bordetella pertussis*, accounting for 11,5%. Despite the current recommendations favoring Macrolide antibiotics as the first-line treatment for pertussis [30], reports of *B. pertussis* resistance to erythromycin have been documented worldwide since 1994 in multiple countries [12], [68], [80]. The highest rates were reported in China [11], with the isolates all carrying the A2047G mutation in the V region of the 23S rRNA gene, resulting in high-level Macrolide resistance with MIC > 256 µg/ml [69]. In Vietnam, a report from southern Vietnam in 2020 stated that there were no macrolide-resistant pertussis cases in 2015-2017 [27]. However, in late 2020, Kamachi and colleagues reported the first instance of *B. pertussis* strains carrying the A2047G Macrolide resistance mutation in Vietnam, with a rate of 19% (10/53) [29]. Our results align with Kamachi's findings, indicating that Macrolide-resistant pertussis strains with the A2047G mutation have emerged in Vietnam. Therefore, using the estimated parameter p = 49,1% for in sample size calculations determining the proportion of macrolide-resistant *B. pertussis* strains differs from the observed p (11,5%). Further studies with larger sample sizes conducted on a broader scale are needed to provide a more accurate assessment of this proportion in Vietnam.

4.2.2. Genetic polymorphism of Bordetella pertussis

In this study, we employed the Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) method to identify 17 MLVA genotypes of *Bordetella pertussis* in Vietnam, namely MT16, MT18, MT26, MT27, MT28, MT29, MT32, MT33, MT96, MT104, MT106, MT107, MT113, MT125, MT195, and a new type, D. Among these, the two most common MLVA genotypes were MT27 (59,6%) and MT28 (12,5%). *Bordetella pertussis* strains carrying the A2047G Macrolide resistance mutation (MRBP) exhibited MLVA genotypes MT195, MT104, MT107 and novel type B.

Kamachi and colleagues reported a pertussis study in Vietnam for the period 2016-2017, identifying 8 MLVA genotypes of *B. pertussis*, including 3 MLVA genotypes with the A2047G mutation, namely MT104, a novel type A, and a novel type B [29]. Thus, the MLVA genotypes of *B. pertussis* strains in Vietnam during the 2017-2020 period showed more diversity compared to the 2016-2017 period. Notably, the trend showed an increase in the number of MRBP MLVA genotypes from one MLVA genotype, MT104, in the 2016-2017 period [29] to two MLVA genotypes, MT104 and MT195, in 2017, and then to three MLVA genotypes, MT104, MT195, and MT107, in 2019-2020 (with MT104 exhibiting a decreasing trend, while MT195 and MT107 showed increasing trends). In the 2017-2020 period, we also found one case of a new MLVA genotype, similar to Kamachi's research. This new genotype, type B,

was closely related to the MT104 genotype, differing by only one repeat segment in one of the six VNTR loci. According to the authors, it is hypothesized that the MRBP MT104 strain may have been introduced from China to Vietnam before 2016 and subsequently mutated into novel type B genotype [29].

4.3. Some prognostic factors for severe pertussis and outcomes

4.3.1. Proportion of severe cases

In this study, the proportion of children with severe pertussis was 30.1%, with 31,5% in children under 4 months and 25,8% in the group of children aged 4 months and older.

4.3.2. Some prognostic factors for severity

The results of the univariate analysis indicated that preterm birth (< 37 weeks gestation), malnutrition, underlying medical conditions, lack of pertussis vaccination, shorter disease onset time, and several symptoms such as fever, cynosis, apnea, seizures, and complications like pneumonia and pulmonary arterial hypertension were associated with severe pertussis. Laboratory parameters, including total white blood cell count, lymphocyte count, neutrophil count, and platelet count were higher in the severe pertussis group compared to the non-severe group, suggesting an association between these parameters and disease severity.

The average Ct value in the severe pertussis group was lower than in the non-severe group, indicating a correlation between low Ct values and disease severity. This could be explained by the hypothesis that a low Ct value is synonymous with a high bacterial load (in one unit of the specimen) and is equivalent to an increased concentration of bacterial toxins (in one unit of serum or body tissue), thereby increasing the risk of severe complications and disease severity. Furthermore, superinfection with other microorganisms also increased the risk of severe pertussis.

Multivariate logistic regression analysis revealed several prognostic factors for severe pertussis, including a shorter disease onset time, the presence of symptoms such as fever and cynosis, the presence of pneumonia, pulmonary arterial hypertension, elevated white blood cell count, low Ct value, and superinfection with other microorganisms.

4.3.3. Treatment modalities

The fundamental principle in treating pertussis is early specific treatment (with antibiotics) when the disease is suspected. Macrolide antibiotics are the first-line choice, with azithromycin being the preferred antibiotic due to its widespread use in all age groups [30]. In this study, we observed that most (95,5%) of children with pertussis were prescribed Macrolide antibiotics, with 91,1% (348/382) receiving azithromycin. However, in severe cases and cases with superinfections, the use of other antibiotics and Quinolone antibiotics increased.

4.3.4. Treatment outcomes in pertussis patients

The average length of hospitalization in this study was 13,0 ± 10,47 days, with a median of 10 days. The median length of hospitalization for children

under 4 months was 10 days (IQR 1-62 days), while for children aged 4 months and older, it was 8 days (IQR 1-62 days). The median length of hospitalization in the severe pertussis group was 15 days, nearly double that of the non-severe group at 8 days ($p < 0,05$).

Most (92,9%) of children with pertussis were treated and discharged in stable condition, with 1,6% (6/382) of pertussis cases resulting in death. This highlights that despite widespread vaccination efforts worldwide over the past 40-50 years, pertussis remains a life-threatening illness, especially for young children.

4.4. Limitations of the study

This study is descriptive research with case series analysis and lacks a control group, so some variables may not have accounted for confounding factors. Additionally, although the sample size for the study was calculated, the assumed prevalence used for sample size estimation may not perfectly align with the actual prevalence in the study population in Vietnam, making the sample size potentially inadequate. Furthermore, the sampling method was conducted solely at the National Children's Hospital, which may limit the generalizability of the study results to the overall pertussis situation in Vietnamese children.

CONCLUSION

1. Clinical and subclinical characteristics of pertussis in children

Most children admitted with pertussis were infants under 4 months old, accounting for 75,7%, and a significant proportion were too young to receive vaccination (42,7%). The male-to-female ratio was 1,23:1. Most pertussis cases presented with the classic triad of symptoms: progressive cough (99,7%), paroxysmal coughin (97,9%), and posttusive emesis (96,6%). Approximately 69,9% of affected children exhibited cyanosis. Common complications included pneumonia (48,7%) and respiratory failure (34,6%). Pulmonary hypertension, a severe and potentially life-threatening complication, was observed in 9,7% of cases. Children aged 4 months and older were more likely to experience fever, coryza and whoop. In comparison, those under 4 months were more prone to cyanosis, apnea, and complications such as pneumonia and respiratory failure.

Peripheral blood total white blood cell count averaged 20,7 ± 13,9 G/L, and lymphocyte count averaged 13,2 ± 8,1 G/L. Elevated lymphocyte count (>10 G/L) was present in 56,3% of children, while only 35,1% had total white blood cell counts exceeding 20 G/L. The counts of both total white blood cells and lymphocytes increased during the first week of illness, peaked during the second week, and gradually decreased from the third week onwards. The most common radiological finding on chest X-rays was a prominent perihilar bronchovascular pattern (58,1%). Superinfections were observed in 23,6% of cases, with viral superinfections being predominant 70%).

2. The rate of Macrolide resistance mutation and genetic diversity of *Bordetella pertussis* bacteria

The rate of *Bordetella pertussis* bacteria harboring the A2047G mutation in the 23S rRNA gene, which causes Macrolide resistance, was 11,5% (14 out of 122).

We identified 17 MLVA genotypes within the studied population, with MLVA genotypes carrying the A2047G mutation being MT104, MT195, MT107, and a new type designated as type B. These genotypes exhibited genetic similarities with prevalent A2047G-mutated strains in China. Over time, there appears to be an increasing diversity in Macrolide-resistant MLVA genotypes.

3. Prognostic factors for disease severity and treatment outcomes of pertussis in children

Severe pertussis was observed in 30,1% of cases, with infants under 4 months of age having a severity rate of 31,5%. Several prognostic factors for severe pertussis included a shorter onset of illness, fever, cyanosis, pneumonia complications, pulmonary artery hypertension, increased white blood cell counts, lower Ct values, and superinfection with other microorganisms.

A total of 95,5% of children with pertussis received specific treatment with Macrolide antibiotics, primarily azithromycin (91,1%). However, due to the emergence of Macrolide-resistant pertussis, more severe cases with superinfections required using alternative antibiotics such as Quinolones (14,1%) and third-generation cephalosporins (61,0%)...

Most pertussis-infected children (92,9%) successfully recovered from the disease, with an average hospital stay of 12,95 ± 10,47 days. Children with severe pertussis had significantly more prolonged hospitalizations, averaging 15 days compared to 8 days for non-severe cases. Pertussis remains a dangerous disease that can lead to fatalities in young children, with a mortality rate of 1,6%.

RECOMMENDATIONS

It is essential to diagnose and promptly manage severe pertussis cases, particularly those with pulmonary artery hypertension, to control mortality risk in children with pertussis.

Continuous monitoring and in-depth research with larger sample sizes should be conducted at major pediatric centers in Vietnam to provide more accurate assessments of pertussis antibiotic resistance. This will help recommend suitable antibiotics for pertussis treatment in Vietnam.

Given the significant number of pertussis cases in infants under 2 months of age who are not yet eligible for vaccination, it is advisable to administer Tdap (Tetanus, Diphtheria, and Pertussis) vaccines to pregnant women after 20 weeks of gestation. This can help reduce the risk of pertussis in young infants who are not yet eligible for vaccination. Additionally, adherence to the vaccination schedule for infants is crucial to ensure they acquire sufficient immunity against pertussis early in life.