MINISTRY OF EDUCATION AND TRAINING MINISTRY OF HEALTH
CENTRAL INSTITUTE OF MALARIOLOGY, PARASITOLOGY AND ENTOMOLOGY

STUDY OF SEVERAL CLILICAL, PARACLINICAL CHARACTERISTICS, ANTIBIOTIC RESISTANCE AND TREATMENT EFFICIENCY OF PNEUMOCOCCAL PNEUMONIA FOR CHILDREN BELOW 5 YEARS OLD IN NGHE AN OBSTETRICS AND PEDIATRICS HOSPITAL (2019 – 2022)

Major: Infectious diseases and tropical disease
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LIST OF ABBREVIATIONS

Abbreviation	English
ADN	Deoxyribonucleic acid
CAP	Community Acquired Pneumonia
CDC	Centers for Disease Control and Prevention
CRP	C-reactive protein
ENSP	Erythromycin non-susceptible pneumococci
erm(B)	Erythromycin ribosome methylase
IPD	Invasive pneumococcal disease
IVIG	Intravenous Immunoglobulin Therapy
KS	Antibiotic
MDR	Multi-Drug-Resistant
MIC	Minimum inhibitory concentration
mef(A)	Macrolide efflux gene
NIPs	National immunization programs
PBPs	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PNSP	Penicillin non-susceptible pneumococci
RSV	Respiratory syncytial virus
SGOT	Serum Glutamic-Oxaloacetic Transamine
SGPT	Serum Glutamate Pyruvate Transaminase
SIADH	Syndrome of inappropriate *antidiuretic hormone*
UNICEF	United Nations International Children's Emergency Fund
VPCĐ	Community pneumonia
VP	Pneumonia
VPN	Severe pneumonia
WHO	World Health Organization
VPPC	Pneumococcal pneumonia
VNVC	Vietnam Vaccine Joint Stock Company

INTRODUCTION

Streptococcus pneumonia or Pneumococcus is a Gram (+) bacterium which is sphere-shaped, aerobic and causes alpha hemolysis, and also is the important pathogen causing community pneumonia, sinusitis, otitis media, meningitis, sepsis for children below 5 years old with high dealth rate [1], [3]. The pneumococcal diseases are the public health problems paid much attention of the whole world [2]. According to Wahl and et al. (2018), annually in the world, about 317,300 children below 5 years old are dead by pneumococcus, mainly in countries with low income [3]. This bacterium also causes the invasive infections for elderly people and people with weak immune system [1].

Pneumococcal pneumonia causes about 12.4 million cases of pneumonia, with 318,000 cases of death for children below 5 years old [4]. In Vietnam, annual about 3 million children below 1 year old catch this disease and about 4000 children are dead by pneumonia. Our country lie in the list of 15 countries occupying 75% cases of pneumonia over the world, the ratio of dead people by pneumonia lies in the top of respiratory diseases (70%) and occupying 30 – 35% dead cases for children generally [5].

The ratio of macrolide resistant pneumococcus changes according to geographic regions, fluctuating from 10% to above 90% [8]. The studies show that the clinical, paraclinical characteristics of pneumococcal pneumonia and drug resistant condition changes depending on time and location. In addition, the studies also show that the ratio of antibiotic resistant cases belonging to group of macrolide in pneumococcus is rather high (above 75%) [14], [15] and appearance of several genes as *erm*(A), *erm*(B), *mef*(A) and *msr*(D) related to drug resistance [16], [17], [18]. Until now, there is not any work studying deeply these problems in Nghe An, therefore this study is implemented for targets, as follows:

1. Description of clinical, paraclinical characteristics of pneumococcal pneumonia for children from 2 months old to below 5 years old treated in Nghe An Obstetrics and Pediatrics Hospital (2019 – 2022).

2. Identification of antibiotic resistant ratio and several genes related to drug resistance of Streptococcus pneumonia.

3. Evaluation of treatment results and several factors affecting the pneumococcal pneumonia treatment results for children from 2 months old to below 5 years old treated in Nghe An Obstetrics and Pediatrics Hospital (2019 – 2022).

NEW CONTRIBUTIONS AND SCIENTIFIC MEANINGS, PRACTICAL MEANINGS OF THESIS

This is the first study researching deeply, methodically with scientific researching methods of description with analysis and comparison before and after pneumococcal pneumonia treating for children below 5 years old in Nghe An, its samples are enough big with deep analyses of variables describing the clinical, paraclinical characteristics and results of intervention and treatment results by specific

antibiotic. On the other hand, this is the first time when Nghe An has a study on factors related to hospital stay and treatment results.

This is the first time when the PCR molecular biology technologies and genome sequencing method are applied for identifying the drug resistant gene of streptococcus pneumonia, and on this basis, it proposes the petitions of selecting the most suitable and most effective antibiotics.

STRUCTURE OF THESIS

The thesis has 123 pages, including: 2 pages of Introduction, 30 pages of Overview; 26 pages of Research objects and methods; 30 pages of research results; 30 pages of Discussion; 2 pages of conclusion; 1 page of petition. The thesis has 22 figures, 35 datasheets and 2 appendixes. There 183 references, in which > 50% references are done for 5 recent years.

Chapter 1: OVERVIEW

1.1. Several general characteristics of pneumonia and pneumococcal pneumonia

1.1.1. Concept of pneumonia

Pneumonia is one of main reasons of death for children below 5 years old, occupying 19% reasons. In developing countries, the indicator of new pneumonia cases in this age is 0.29 onset/ child/ year. Children suffered from pneumonia, who have any life threatening risk and shall be hospitalized, occupy 7 – 13%. The main risk factors leading to pneumonia for children include children not breast-fed totally, malnutrition, air pollution, low new birth weight and not vaccinated measles sufficiently [19]. Depending on places where patients are suffered from pneumonia pathogens, the authors classify them into two kinds: community pneumonia and hospital acquired pneumonia [22]/. The community pneumonia is the pneumonia that a child is suffered from in the community before being hospitalized [22], [23].

1.1.2. Reasons

Reasons of pneumonia for children maybe bacteria, virus or other microorganisms. Bacteria: According to WHO, the most popular reason of pneumonia is *S. pneumoniae*, *Haemophilus influenzae* (HI). The reasons of pneumonia caused by bacteria may change according to age.

1.1.3. Pathogenesis and favorable factors in pneumococcal pneumonia

- Normally, pneumococcus can reside without symptoms in nose and throat. When catching nasopharyngeal mucosa inflammation, the density of pneumococcal increases, the epithelial cells expose the receptors which create good conditions for pneumococcus to stick on and destroy the entireness of epithelia of respiratory track. Several virulent factors help pneumococcus to stick, intrude and avoid the immune system [4], decreasing the preventing ability of immune system in the host and actuating the intrusion of pneumococcus [32]. When the host cannot destroy pneumococcus after invading the respiratory tract, bacteria will multiply, breaking the balance of microorganism system in the respiratory tract, pneumococcus continue enter the parenchyma of lung and causing pneumonia [33].

- Risk factors: Children below 2 years old, especially infants; low birth weight infants < 2500 gram; suffered from many diseases: measles, diarrhea, malnutrition, birth defects, immunodeficiency...; sudden season change, high humidity; Children are not vaccinated sufficiently [35], [36].

1.1.4. Several epidemiological characteristics of pneumococcal pneumonia

In our country, until now, there is no statistical data on rate of children suffered from pneumonia in the whole country. However, most studies of authors conducted in different time and places conclude that pneumococcus is the leading reason of pneumonia for children below 5 years old. Dinh Thi Yen and et al. (2015) studies about lobar pneumonia for children in Hai Phong Pediatrics Hospital, pneumococcus occupies 50% [44]. According to Hoang Ngoc Anh and et al. (2017), the study is conducted in Respiration Department, Hai Phong Pediatrics Hospital, pneumococcus occupies 60% [5]. According to Dang Duc Anh, in the acute respiratory infection among children below 5 years old, the ratio of isolation for bacteria: 41.9%, virus: 9.1%, in which the pneumonia cases caused by bacteria *S. Pneumoniae* occupy 38.5% [47]. Quach Ngoc Ngan and et al. (2014) studies children from 2 months old to 5 years old in Can Tho Pediatrics Hospital, showing that the ratio of pneumonia cases caused by *S. pneumoniae* (47,1%), nextly *S. aureus* (20,6%); *M. catarrhalis* (14,7%), *H. influenzae* (8,8%), *K. pneumoniae* (5,9%) and *P. aeruginosa* (2,9%) [48].

Previously, when antibiotic was not used, the ratio of death/ cases suffered from pneumonia in hospitals were 20 – 40%, this ratio decreased to 5-10% after antibiotic was used broadly. But for malnourished children, premature infants or children suffered from inborn diseases as cardiovascular diseases, the ratio was about 30%, even above 50% [49].

1.1.5. Clinical, paraclinical characteristics of pneumococcal pneumonia

Patients suffered from pneumonia frequently have the combination signs of many symptoms, including: respiratory symptoms, specially cough (75%), shortness of breath (65%), expectoration (30%), and chest pain (30%) with symptoms of whole body, such as: fever, shivery, muscle pain and consciousness disorder. Consciousness disorder usually happens in elderly patients and patients with several pneumonia [31]. For newborn children, the signs maybe only poor suckling, unpleasantness, nervousness, fussiness. The older children may complain about chest pain, stomachache (lower lobe pneumonia), neck pain or neck stiffness [54].

Pneumococcal pneumonia has enough signs of pneumonia caused by bacteria as high fever, excessive coughing and respiratory tract infection [54]. The older children have cough with sputum or bloody sputum. Through clinical examination, they are discovered rapid breathing, wheezes and rales in lung, more seriously chest indrawing, and symptoms of pleural effusion [13], [54], [55].

Chest X-ray is considered as an important standard, objective evidence to diagnose pneumonia [55], in addition, X-ray is also a good mean to diagnose the complications in lung as collapsed lung, pleural effusion, hydro-pneumothorax or

lung abscess [56], [57]. By testing, they discover that the number of peripheral blood leucocytes and concentration of CRP frequently increase highly in pneumococcal pneumonia [19], [54], [55]. The number of leucocytes is > 15,000/ mm^3, neutrophils predominate, concentration of CRP increases and the speed of blood sedimentation increases, all of them are the foundation to suggest the reason of bacteria [54].

1.2. Physiological characteristics, immunity and pathogenicity of pneumococcus

Streptococcus pneumoniae (*S. pneumoniae*, S. pneumococcus, *S. pneumococci*) is Gram positive coccus belonging to *Streptococcus* genus, aranging into pair or chain [60]. *S. Pneumoniae* bacterium is called as *Streptococcus pneumonia* or Pneumococcus [61].

1.3. Diagnosis of Streptococcus pneumonia infection

The clinical symptoms or biochemical tests, haematological tests, infection indicators and lung X-ray help diagnose pneumonia but not identify the sources of Streptococcus pneumonia. On the contrary, the biochemical tests and molecular biological tests permit to identify *Streptococcus pneumonia* in specimens of patients. Depending on type of disease, the specimen may be: throat fluid, fluid from sinus, nasopharynx fluid, sputum, blood, pus, cerebrospinal fluid and etc. [60], [61].

- Direct diagnosis: the specimen is dyed Gram to observe the morphological characteristics and color catching property of bacteria [60], [61]. Isolated culture: any suspicious specimen is cultured in blood agar with gentamycin (5µg/ml) at 37ºC in the condition 5-10% CO_2 [60], [61]. If any suspicious colony grows, this colony will be dyed Gram, tested catalase, optochin, tested bile solubility…. to identify *Streptococcus pneumonia* [61].

- Gene amplification technique PCR: PCR is valuable with specimen of pleural fluid, cerebrospinal fluid and blood [61]. PCR technique is able to diagnose Streptococcus pneumonia infection even qhen the patient has been used antibiotic.

1.4. Treatment of pneumococcal pneumonia

The principle of pneumococcal pneumonia treatment for children: Treating respiratory failure; Treating infection; Treating other symptoms and disorders; Treating complications, if any.

- ***Treatment of respiratory failure:*** is applied for cases in emergency room, supervising closely pulse, breath, SaO_2, PaO_2, $PaCO_3$. The patient is placed in the position of shoulder placed highly, head turned to one side, clothes and diapers loosened to breathe easily. Clearing airway, breathe oxygen or supported respiration when appointed. Only get IV fluid when having high fever. In case of apnea, the patient must be placed endotracheal intubation, handbagged, and supported respiration [25].

- ***Treatment of body temperature alterations:*** Getting fever down, measuring temperature at armpit, if a child has fever above 38,5ºC, let the child take Paracetamol 10-15mg/kg/time, putting drug in the anus for 6 hours per time. Taking the child lie in airy room, washing body by warm water. Preventing temperature from getting down:

for babies, they can put temperature down until the temperature of armpit < 36ºC. Children are treated by warmed up, lying in airtight room and let children eat sufficiently to avoid hypoglycemia [19].

- Treatment of infection

Guiding father/ mother to care child at home: how to let child take medicines, how to nourish, how to clean nose, supervising and discovering heavy cases to re-exam promptly [81].

+ *Treating by antibiotic [81]:* Let children below 5 years old take one of following antibiotics: Amoxicilin 90mg/kg/24 hours in 2-3 times or Amoxicilin - clavulanic 90mg/kg/24 hours in 2-3 times, for 5 days. If any child is allergic to Beta-lactam or suspected to have pneumonia caused by atypical bacteria, let him take azithromycin, clarithromycin or erythromycin.

+ Inpatient treatment: The time of antibiotic using is at least 5 days. During the treatment process, the development of disease should be supervised. After 48 hours, the disease will progress favorably if the full status is better, the child eats and drinks better, reduces fever, breathes slower, reduces exhausted breathing, and increases oxygen saturation. The tests of leucocyte, CRP or PCT improve. On the contrary, if the disease becomes more serious or does not improve after 72 hours, the patient must take X-ray of lung or lung abscess or making antibiogram to discover any antibiotic resistant case [86], [87]. When the antibiogram has any result, using antibiotic according to antibiogram [81], [84].

1.5. Drug resistance, several genes related to drug resistance in *S. pneumoniae*

The data over the world shows that the antibiotic resistance condition in *S. pneumonia* is increasing and becomes an important estimation factor because it relates directly to persistent happening or death in patients [11], [82].

The resistance to different antibiotic kinds in *S. pneumonia* happens according to very different mechanisms [62]. Macrolide resistance in *Coccus* in general and *S. pneumonia* in particular is frequently caused by 2 mechanisms and has roles of many related genes. In details, any changes of main position on gene 23S ribosome activate to generate enzyme (encoded by genes belong to family erm, such as: erm(A), erm(B), erm(C), erm(E) lieing on plasmid of bacteria) prevent the process of sticking drug on bacteria, leading drugs ineffective [8], [9]. The antibiotics belonging to Quinolone group has the main mechanism and effects of inhibiting two important enzymes of bacteria, they are DNA gyrase (DNA topoisomerase II) and DNA topoisomerase IV. When two enzymes are inhibited, DNA of bacteria is fragmented and fractured [88].

Chapter 2: RESEARCH METHOD

2.1. Description of clinical, para-clinical characteristics of pneumococcal pneumonia for children below 5 years old in Nghe An Obstetrics and Pediatrics

Hospital

2.1.1. Research object, place and time

Research object: Pediatric patients with age from 2 months old to below 60 months old, are treated in Nghe An Obstetrics and Pediatrics Hospital and diagnosed to have pneumococcal pneumonia and pneumococcal pneumonia at serious level.

Standards for diagnosing whether a pediatric patient has pneumonia or not: the pediatric patients are diagnosed pneumonia according to standards of Ministry of Health [19]. The children have cough, fever and associated with at least one of symptoms: Fast breath (under age): Children < 2 months old, breathing frequency ≥ 60 cycles/ minute; Children 2 - < 12 months old, breathing frequency ≥ 50 cycles/ minute; Children 1 – 5 years old, breathing frequency ≥ 40 cycles/ minute; Chest retraction (the lower part of chest retracts, inhaling); Examining lung and discovering abnormal signs: reducing ventilation and having abnormal sounds.

Diagnosis of pneumococcal pneumonia [19], [112]: Culturing quantitatively the specimen of nasopharynx fluid or sputum (+) with *Streptococcus pneumonia*, density VK ≥ 10^6/ml.

Diagnosis of pneumococcal pneumonia case at serious level [19]

Any pediatric patients are diagnosed to have pneumococcal pneumonia and associated with at least one of signs, as follows: symptoms of respiratory failure: Grunting; chest retraction and heavy chest; Cyanosis or index SpO_2< 90%; The full status happens seriously: quit breastfeeding or unable to drink; Perception disorder: groggy or coma; Appearing seizure symptoms; Children below 2 months old;

***Standard of elimination*:** Cases are suffered from pneumonia in hospital.

Research place: The pneumonia patients are collected information in several departments in Nghe An Obstetrics and Pediatrics Hospital, including: Respiratory Department, Pediatric Intensive Care and Toxic Management Department and Voluntary Treatment Department;

- ***Research time:*** From January 2019 to December 2022.

2.1.2. Research contents

- Researching clinical characteristics: Identifying the frequency of symptoms in pediatric patients suffered from pneumococcal pneumonia, the differences of symptoms between groups according to characteristics of object...

- Identifying the appearance frequency of changes in tests of peripheral blood cell analysis (changes of leukocyte, platelet, hemoglobin), changes of CRP value; X-ray of heart and lung, pleura ultrasound scanning.... Identifying the frequency of serotypes of *S. pneumonia;*

2.1.3. Research method

- ***Study design:*** The study describes a series of cases done in Nghe An Obstetrics and Pediatrics Hospital from January 2019 to December 2022.

- ***Researched sample size:*** 193 children with age from 2 months old to below

60 months old identified to have pneumococcal pneumonia with be described their clinical and para-clinical characteristics; The number of pediatric patients is calculated by calculating the ratio of children suffered from pneumococcal pneumonia among children suffered from pneumonia. In details, the number of children suffered from pneumonia should be surveyed as: $\boldsymbol{n = Z^2_{1-\alpha/2}\frac{p(1-p)}{d^2}}$; *In which:* n: Number of children with pneumonia;$Z^2_{1-\alpha/2}$: value based on statistical meaning level, with d = 5%; p: Ratio of patients with pneumococcal pneumonia, in this study, we use the value p = 0,471 (p = 47,1%), based on ratio of patients with pneumococcal pneumonia in the study of Quach Ngoc Ngan (2013) for children from 2 months old to 5 years old in Can Tho Pediatrics Hospital [48]; d: approved absolute error, taking d = 0.05. With selected values, the calculated sample size is 383. Among 383 children suffered from pneumonia, 193 children have pneumococcal pneumonia.

2.1.4. Techniques used in the study

- Clinical examination technique;
- Clinical information collection technique about signs and symptoms.
- Signs: Hard to breathe
- Para-clinical data collecting technique: Blood formula; Number of leucocytes; C-reactive protein (CRP); Typing serum of *S. pneumonia*; X-ray of heart and lung; Ultrasound scan of lung and pleura.

2.1.5. Research index

Ratio of children vaccinated *S. pneumonia*; Ratio of clinical symptoms before hospitalized; Ratio of leukocyte increase, CRP increase, blood donation; Ratio of children using antibiotic before hospitalized; Rate of patients who are recovered, getting better, do not recover or dead.

2.1.6. Date analyzing and processing methods

The information of patient, clinical signs, values of para-clinical tests are encoded and processed, analyzed by software SPSS 20.0. Using the audit T-tets in the comparative analyses ($p < 0.05$) when identified as having statistical meaning.

2.2. Identification of antibiotic resistance ratio and genes related to drug resistance of *Streptococcus pneumonia*

2.2.1. Research object, place and time

- ***Streptococcus pneumonia*** isolate in children with age from 2 to 60 months old. *Streptococcus pneumoniae* ATCC® 49619 is the reference species.

- **Research place:** Department of Microorganism, Nghe An Obstetrics and Pediatrics Hospital. Identifying serum type and genes *erm*(B) and *mef*(A) in ADN Technology Institute and genetic analysis (GENLAB).

- **Research time:** From January 2019 to December 2022.

2.2.2. Research contents

Identifying the sensitive degree of *Streptococcus pneumoniae* to several antibiotics of *Streptococcus pneumoniae*, including: β-lactam group; Quinolon group,

Macrolide group; Cyclin group. Identifying the appearance frequency of genes *erm*(B) and *mef*(A) related to Macroline's antibiotic resistance of *Streptococcus pneumoniae*, including: Frequency of bearing gene *erm*(B); Frequency of bearing gene *mef*(A);

2.2.4. Research method

- **Study design:** The study is experimented in laboratory.

- **Sample size**: Sample sizes for identifying the antibiotic resistance ratio include: 193 *Streptococcus pneumoniae* species which are separated from 193 diferent children suffered from pneumococcal pneumonia after identified by identification system VITEK® 2 Compact (bioMérieux, North Carolina 27712, USA); The sample sizes for identifying the frequency of bearing genes *erm*(B) and *mef*(A) relate to macrolide group's drug resistance: including 126 macrolide resistant *Streptococcus pneumoniae* species taken from 193 cpecies seperated from children suffered from pneumococcal pneumonia.

2.2.5. Techniques used in study

The *S. pneumoniae* seperating technique; *S. pneumoniae* identifying technique; Gene sequencing technique; Technique for identifying the sensitiveness of *S. pneumoniae* to antibiotic.

2.2.6. Research index

Frequency of *S. pneumoniae* sensitive, resistant to a kind of antibiotic; Frequency of bearing gene gen erm(B). Frequency of bearing gene mef(A). Frequency of bearing both genes erm(B) and mef(A). Frequency of bearing at least one of two genes erm(B) and mef(A).

2.2.7. *Research data processing*

The research data is inputed and analyzed by statistical software IBM SPSS version 20.0 (Armonk, NY, US). The sequences collected will be compared with reference order on gene bank using BLAST tool in website http://ncbi.nlm.nih.gov of National biotechnology Information Center of US.

2.3. Result evaluation and several factors related to pneumococcal pneumonia treatment results

2.3.1.Research object, place and time

- Pediatric patients from 2 months old to below 60 months old are identified to have pneumococcal pneumonia as stated in Target 1.

- The study is done in Nghe An Obstetrics and Pediatrics Hospital.

- The research time is from January 2019 to December 2022.

2.3.2. Materials and equipments used

Medical records researched; Certificate of agreeing with participating into research; research's information card; Antibiotic kinds: Amoxillin, xít Clavulanic, Cefazidime, Cefuroxim, Ceftriaxon, Ceftizoxim, Azithromycin, Vancomycin…and other drugs for supporting treatment.

2.3.3. Research content

The number of antibiotics used in the treatment procedure; The other drugs/ treatment methods used; Treatment time; Rate of patients who are recovered, getting better, have sequelae or dead when discharged from hospital….; Identifying several factors related to treatment results.

2.3.4. Research method

- Study design*:* Non-control intervention study

- Research sample size:193 pediatric patients are diagnosed to have pneumococcal pneumonia as stated in Target 1.

2.3.5. Techniques used in the study

Intravenous injection technique; Nebulization technique according to guidelines of Ministry of Health according to Decision No. 5344/QĐ-BYT dated 28/11/2017 issuing the pediatric technical procedure [119].

2.3.6. Research index

Rate of patients who are recovered, get better, transferred to other hospital or dead; Average treatment time; Ratio of patients treated in long time (≥ 14 days).

2.3.7. Date processing and analyzing methods

Happenings of patient, treatment results are encoded and processed, analyzed by software SPSS 20.0, operating system windows. Using the audit ($\chi 2$), value p-valua

2.4. Ethics in researching

The research is approved by Medical Ethics Council of National Institute of Malariology – Parasitology and Entomology in March 2018 in Decision No. 225/ QĐ-VSR.

Chapter 3: RESEARCH RESULTS

3.1. Several clinical and para-clinical characteristics of pneumococcal pneumonia

3.1.1. Ratio of children with pneumococcal pneumonia among total number of children with pneumonia

With 193 pediatric patients with enough standards for diagnosing pneumococcal pneumonia.

Table 3.1. Several characteristics of research object (n =19)

Characteristics	Number	Ratio %
Age group		
2 months - ≤ 24 months	145	75,13
25 months - < 60 months	48	24,87
Sex		
Male	130	67,36
Female	63	32,64

Ethnic group		
Kinh	191	98,96
Others	2	1,04

Ratio of children with pneumococcal pneumonia who have age ≤ 24 months (75.13%) are male (67.36%), and have ethnic group of Kinh (98.96%).

- Several physical symptoms

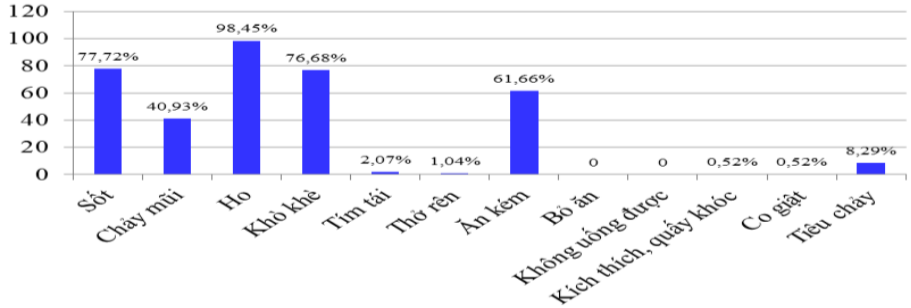

Figure 3.1. Clinical symptoms before hospitalized

Cough occupies the highest ratio (98.45%), nextly fever (77.72%), zheeze (76.68%), poor eating (61.66%), runny nose (40.93%).

- Signs

Table 3.1. Fever levels discovered when hospitalized (n =193)

Temperature	Number	Ratio %
No fever (< 37,5ºC)	40	20,73
Mild fever (37,5 - 38,4°C)	103	53,37
Moderate fever (38,5°C - 39,4°C)	46	23,83
High fever (≥39,5°C)	4	2,07

Children with pneumococcal pneumonia frequently have signs of fever from mild level (53.37%) and moderate level (23.83%), 2.07% high fever and 20.73% no fever.

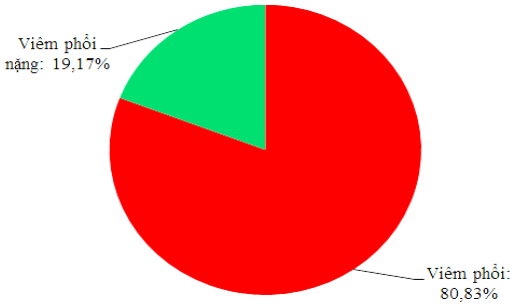

Figure 3.2. Classification of pneumococcal pneumonia levels in children (n =193)

80,83% children with pneumonia at mild and moderate levels and 19.17% children with pneumonia at serious level.

3.1.3. Several clinical characteristics

- X-ray results

Table 3.2. Damages on Lung X-ray (n = 193)

Image on X-ray	Number	Ratio %
Lung bronchitis	173	89,64
Lobar pneumonia	7	3,63
Unclear image of damage	13	6,74

Thanks to results of Lung X-ray, 89.64% results show that the image of lung bronchitis, the images of lobar pneumonia occupy 3.63% and 6.74% pediatric patients do not discover or have not discovered any image of lung damage on X-ray film.

- Results of molecular examination and identification of gene type of Streptococcus pneumoniae

Selected 126/193 species separated from patients who have not been vaccinated pneumococcal disease to examinate molecular. Results: All of 126 species have PCR results positive to gene *cpsA.*

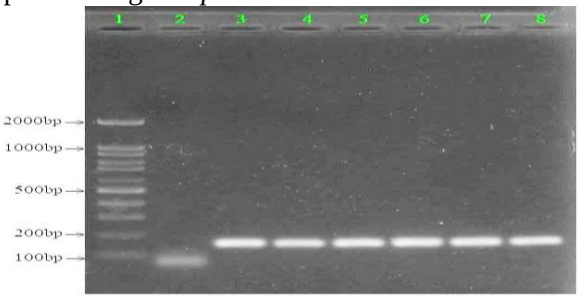

Figure 3.5. Results of PCR product electrophoresis for amplifying gene cpsA by specific bait pair cpsA-F and cpsA-R

Well 1: scale DNA standard 100 bp; Well 2: negative control; Wells 3-7 (species Sp8107, Sp8279, Sp8281, Sp8294 and Sp8298): Well 8: positive control.

Sequencies of apart of gene 16S of 22 representative species are compared with gene bank and have the result suitable to *Streptococcus pneumoniae* (Ratio of similarity > 98%). These sequences have been registered and issued codes on NCBI gene bank of National Biotechnology Information Center of US with codes from MW672550 to MW672562 and from MZ007491 to MZ007499.

Analysis of genealogical relation shows that the *Streptococcus pneumoniae* species in this study have close relation to *Streptococcus pneumoniae* species in China, Netherlands (Figure 3.6).

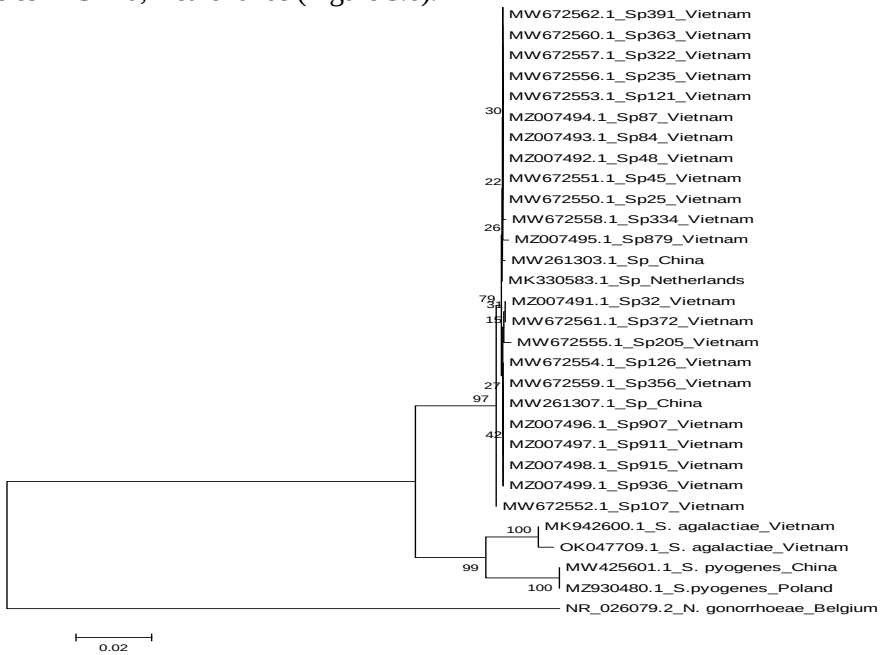

Figure 3.6. Phylogenetic tree for identifying relation on species between Streptococcus pneumoniae types *(Based on sequence of 16S gene fragment built by MEG6.06 program, using Neighborjoining method NJ with certainty factor bootstrap of 1,000 repeating times)*

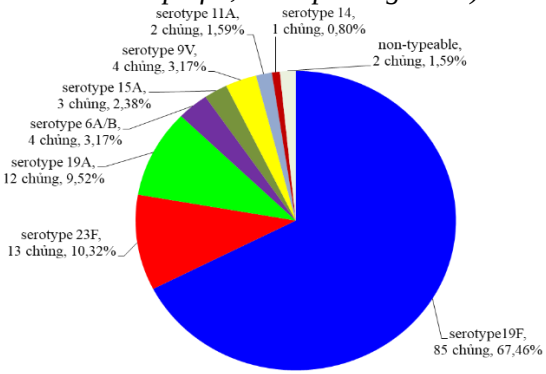

Figure 3.7. Distribution of Streptococcus pneumoniae's gene kinds

Among 126 *S. pneumonia* species (separated from 126 patients who have not vaccinated) that have been identified serum type, 124 (98.41%) species have results and each patient is only infected by 1 serum type. 2 species are not

identified serum type, occupying 1.59%. The most popular serum type is 19F (85 species; 67.46%), nextly type 23F (12 species; 10.32%), 19A (12 species; 9.52%), 6A/B (4 species; 3.17%), 15A (4 species; 2.38%), 9V (4 species; 3.17%), 11 A (2 species; 1.59%) and 14 (1 species, 0.80%).

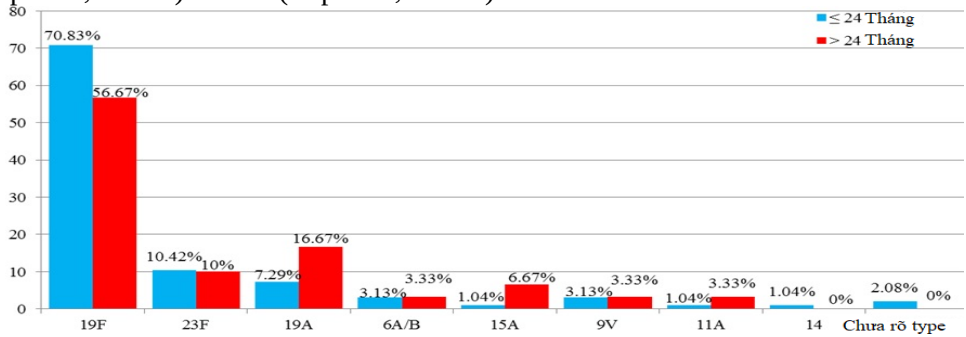

Figure 3.9. Distribution of serum type under age group (n = 126)

3.2. Antibiotic resistance ratio identification result and several genes related to drug resistance of *Streptococcus pneumoniae*

Table 3.3. Antibiotic resistance situation of Streptococcus pneumoniae (n = 193)

The antibiotic resistance rate observed in 193 *Streptococcus pneumoniae*

Name of antibiotic	Sensitiveness degree (number, %)		
	Sensitive	Mediate	Resistant
Benzylpenicillin (PEN)	97 (50,26)	87 (45,08)	9 (4,66)
Cefotaxim (CXM)	42 (21,76)	62 (32,13)	89 (46,11)
Ceftriaxone (CEF)	47 (24,36)	73 (37,82)	73 (37,82)
Levofloxacin (LEV)	190 (98,45)	0 (0)	3 (1,55)
Moxifloxacin (MXF)	192 (99,48)	1 (0,52)	0 (0)
Erythromycin (ERY)	2 (1,04)	0 (0)	191 (98,96)
Azithromycin (AZM)	1 (0,52)	0 (0)	192 (99,48)
Clarithromycin (CLA)	1 (0,52)	0 (0)	192 (99,48)
Clindamycin (CLI)	10 (5,18)	1 (0,52)	182 (94,30)
Linezolid (LIN)	193 (100)	0 (0)	0 (0)
Vancomycin (VAN)	193 (100)	0 (0)	0 (0)
Tetracycline (TET)	11 (5,70)	0 (0)	182 (94,30)
Chloramphenicol (CLP)	187 (96,89)	0 (0)	6 (3,11)
Rifampicin (RIF)	193 (100)	0 (0)	0 (0)
Trimethoprim/sulfamethoxazole (SXT)	9 (4,66)	1 (0,52)	183 (94,82)

species for CLA, AZM, ERY, SXT, CLI, TET, CXM, CEF, PEN, CLP, and LEV

are 99,48%(192), 99,48%(192), 98,96%(191), 94,82%(183), 94,30%(182), 94,30%(182), 46,11%(89), 37,82%(73), 4,66%(9), 3,11%(6) and 1,55(3) successively. There are 100% *Streptococcus pneumoniae* species sensitive to MXF, LIN, VAN and RIF.

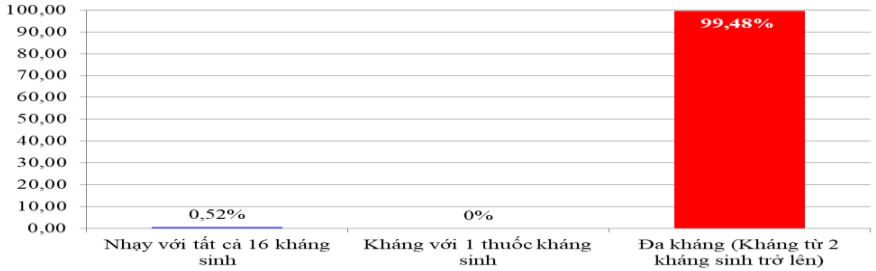

Figure 3.10. Multi-drug resistance frequency of Streptococcus pneumoniae causing pneumonia in children below 5 years old in Nghe An (n = 193)

The multi-drug resistance frequency of *Streptococcus pneumoniae* occupies 99.48%.

3.2.2. Results of Streptococcus pneumoniae's antibiotic resistance situation analysis

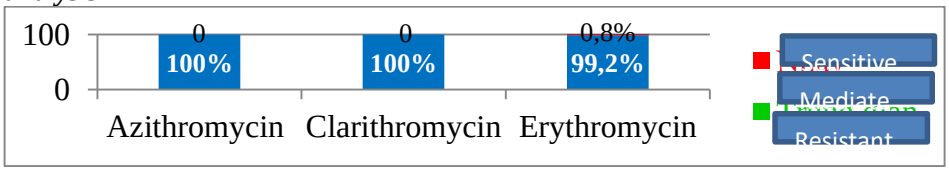

Figure 3.12. Results of identifying macrolide group's drug resistance situation of 12 Streptococcus pneumoniae species

100% *Streptococcus pneumoniae* species in this study are resistant to at least one of 3 antibiotics Azithromycin, Clarithromycin and Erythromycin. For antibiotic Erythromycin has 99.2% species (125 species) appearing resistance.

3.2.3. Results of identifying frequency of bearing genes erm(B) and mef(A) related to macrolide resistance

Table 3.4. Frequency of Macrolide group's antibiotic resistant Streptococcus pneumoniae species bearing genes erm(B) and mef(A) (n = 126)

Gene bearing type	Number	Ratio %
Bearing *erm*(B)	116	92,1
Bearing *mef*(A)	73	57,9
Bearing simultaneously 2 genes *erm*(B) and *mef*(A)	69	54,8
Bearing gene *erm*(B), not bearing gene *mef*(A)	47	37,3
Bearing gene *mef*(A), not bearing gene *erm*(B)	4	3,2
Not bearing 2 genes *erm*(B) and *mef*(A)	6	4,8

Frequencies of macrolide resistant *Streptococcus pneumoniae* species bearing genes erm(B) and mef(A) are successively 92.1% and 57.9%. The frequency of bearing simultaneously 2 genes is 54.8%.

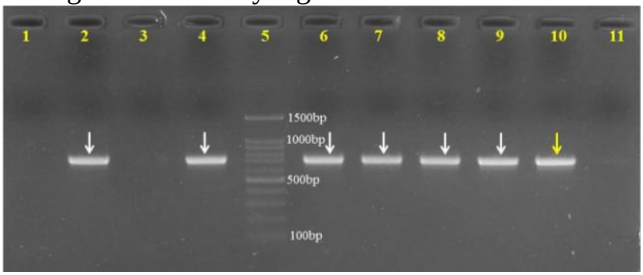

Figure 3.13. Result of PCR running for discovering gene ermB in S. pneumoniae

Wells 1 and 3: species do not bear gene erm(B); wells 2, 4, 6-9: species bear gene erm(B); well 5: scale ADN standard 100 – 1500bp; well 10: positive control; well 11: negative control.

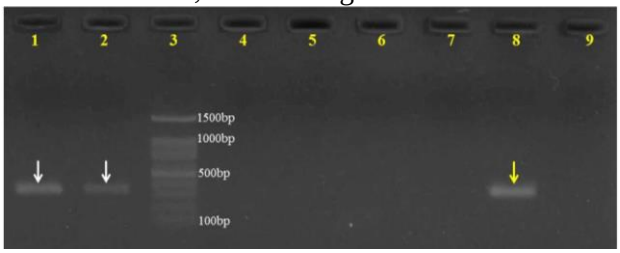

Figure 3.14. Result of PCR running for discovering gene mef(A) in S. pneumoniae

Wells 1 and 2: species do not bear gene *mef*(A); well 3: scale ADN standard 100 – 1500bp; wells 4-7: *Streptococcus pneumoniae* species do not bear gene *mef*(A); well 8: positive control; well 9: negative control.

3.3. Treatment results and several factors related to pneumococcal pneumonia treatment results

3.3.1. Pneumococcal pneumonia treatment results

Table 3.5. Hospital stay of children with pneumococcal pneumonia (n =193)

Time (day)	Pneumonia (n_1, %)	Serious pneumonia (n_2, %)	Common (number, %)
Number of average treatment days	8,22 ± 3,87	9,46 ± 4,98	8,46 ± 4,12
Shortest - Longest	1-22	2-25	1 - 25
≤ 6 days	52 (33,33)	12 (32,43)	64 (33,16)
7 - 13 days	84 (53,85)	19 (51,35)	103 (53,37)
14 - 20 days	19 (12,18)	4 (10,81)	23 (11,92)
≥ 21 days	1 (0,64)	2 (5,41)	3 (1,55)

The average treatment day number of pediatric patients with pneum ococcal pneumonia is 8,46 ± 4,12. For patients with serious pneumonia, the average number of treatment days is longer than normal pneumonia (9,46 ± 4,98 days in comparison with 8,22 ± 3,87 days). Most of patients must be treated within above 7 days (> 67%). The longest hospital stay is 25 days.

3.3.2. Several factors affecting treatment results

Table 3.6. Impacts of age on treatment results (n =193)

Treatment results	Age of children with pneumonia (number, %)		*p*
	≤ 24 months old (n_1 = 145)	25 - 60 months old (n_2= 48)	
Treatment time ± SD	8,80 ± 4,37	7,42 ± 3,03	0,0437
Status when discharged from hospital			
Recovered	104 (71,72)	29 (60,42)	0,1434
Better	41 (28,28)	19 (39,58)	

The average hospital stay of children ≤ 24 months old suffered from pneumococcal pneumonia is higher than children above 24 months old (8,80 ± 4,37 days in comparison with 7,42 ± 3,03 days), the difference between 2 groups have the statistical meaning (p < 0,05). The ratio of recovered children in group of children ≤ 24 months old suffered from pneumococcal pneumonia is higher than group of children above 24 months old but this difference has no statistical meaning (71,72% compared with 60,42%, p > 0,05).

Table 3.7. Impacts of antibiotics using before hospitalized on treatment result (n = 193)

Treatment result	Using antibiotics before hospitalized (number, %)		p
	Yes (n_1 = 134)	No (n_2 = 59)	

Average treatment time	8,40 ± 4,12	8,59 ± 4,16	0,7689
Status when discharged from hospital			
Recovered	86 (64,18)	47 (79,66)	**0,0327**
Better	48 (35,82)	12 (20,34)	

The ratio of patients recovered from disease in group of patients without antibiotics is higher with statistical meaning than group of patients with antibiotics before hospitalized (79,66% compared with 64,18%, p < 0,05).

Table 3.8. Impacts of history of respiratory diseases on treatment result (n = 193)

Treatment result	**History of respiratory tract infection**		***p***
	Yes (n_1 = 52)	No (n_2 = 141)	
Average treatment time	8,83 ± 4,97	8,32 ± 3,77	0,4469
Status when discharged from hospital			
Recovered	41 (78,85)	92 (65,25)	0,0709
Better	11 (21,15)	49 (34,75)	

Group of children with pneumococcal pneumonia who have been suffered from respiratory tract infection have the longer treatment time than group of children not suffered from respiratory tract infection 8,83 ± 4,97 days in comparison with 8,32 ± 3,77 days, however this difference has no statistical meaning (p > 0,05).

Table 3.9. Multivariate analysis results of factors affecting treatment results of pneumococcal pneumonia in children (n = 193)

Analysis factor	**OR (95%CI)**	***p***
History of using antibiotics before hospitalized	2,27 (1,07-4,80)	0,032
Pneumonia degree	3,51 (1,65-7,46)	0,001

The multivariate analysis results that both factors of using antibiotics before hospitalized and pneumonia degree have impacts on treatment results of patients with pneumococcal pneumonia. In details, children who do not use antibiotics before hospitalized have the recovering ratio (95%CI: 1,07-4,80) 2,27 times as high as children using antibiotics; Children with normal pneumonia has the recovering ratio (95%CI: 1,65-7,46) 3,51 times as high as children with serious pneumonia.

Chapter 4: DISCUSSION

4.1. Several clinical and paraclinical characteristics of pneumococcal pneumonia

Age is an important risk factor of pneumococcal pneumonia. According to estimation, the rate of patients with pneumococcal pneumonia in children < 2 years old and adult > 65 years old are 50 times as high as other ages [37]. The research results show that among 193 children participating into study, children from 2 to 24

months old occupy 75.13%, the left 24.87% is children from 25 to 60 months old. The distribution of children's age group in this study is rather similar to study of Nguyen Dang Quyet and et al. (2021) implemented for children with pneumococcal pneumonia in National Pediatrics Hospital.

In our study, 96,37% children suffered from pneumonia live in rural areas/ mountainous areas, the left 3.63% is children coming from urban area. This result is different from study of Nguyen Dang Quyet (2022). According to this author, 74% children suffered from pneumococcal pneumonia coming from urban areas, 26% children coming from rural areas, the ratio of urban/ rural is 2,8:1 [13]. In the multicenter study done in 3 countries, including: Argentina, Brazil and Republic of Dominica by Cardoso and et al. (2014), the ratio of children with pneumococcal pneumonia coming from urban areas occupy 90% [122]. This difference maybe caused by National Pediatrics Hospital locating in Hanoi, thus most of patients hospitalized have the family record in Hanoi.

4.1.2. Several clinical characteristics of pneumococcal pneumonia

Pneumomia patients usually have signs of fever, respiratory tract infection before several days as cough, runny noise [19]. The patients have fever, fast breathing, and increase of subsidiary respiratory muscles' activities, intercostals muscle contraction, chest indrawing, serious cases may have cyanosis, apnoea especially infants. Medical examination can recognize moist rales, triple reduction syndrome, sodification…The clinical signs also depend on different pneumonia periods, depending on age of patient and species causing pneumonia [29], [135]. In addition, there is a difference on clinical symptoms according to serious degrees of patients [21].

In this study, cough and fever are 2 most popular symptoms in children with pneumococcal pneumonia. In details, 98,45% children have coughing symptom and 77,72% children have the fever symptom before hospitalized. Actually, the ratio of children with fever syndrome maybe higher because many children have mild fever but their children do not use thermometer and consequently do not discover disease. Cough and fever are two most important physical symptoms and are the obligatory standard in standard of diagnosing pneumonia caused by bacteria in children [19], [112].

Fever and cough are 2 obligatory standards in pneumonia diagnosing standard [19], [112]. In this study, among children hospitalized and diagnosed pneumonia, 40 cases have no fever (occupying 20.73%), 153 left pediatric patients (79.27%) have fever. Most of pediatric patients have mild fever from $37,5^0$C - $38,4^0$C, occupying 53,38%. The ratio of children with moderate fever and high fever occupy 25,9%. In comparison with other study in country, the ratio of children suffered from pneumococcal pneumonia having fever syndrome is similar to study of Nguyen Thi Ha and et al. (2020) conducted in National Pediatrics Hospital (75%), but higher than study of Vu Thi Tam and et al. (2021) on children with

pneumonia in Hoa Binh (ratio of children with fever is 52,3%) [124].

In this study, we identify that 78,24% children with pneumococcal pneumonia have wheeze; 64,77% children have fast breathing and 3,11% children have cyanosis. The domestic studies also show that the wheezing syndrome is recognized rather frequently. In the study of Trinh Thi Ngoc (2020) for children below 5 years suffered from pneumonia in Thanh Hoa, the wheezing syndrome has in 70% children with pneumonia [46]. In the other study of Vu Thi Tam and et al. (2021) in Hoa Binh, the wheezing syndrome appearance in > 79% children with pneumonia [124]. The syndrome of fast breathing in this study is lower than study of Nguyen Thi Ha and et al. (2020) conducted in National Pediatrics Hospital (64,77% compared with 91,2%) [123].

4.1.3. Several paraclinical characteristics of pneumococcal pneumonia

In pneumonia caused by bacteria, leucocytes and CRP usually increase highly, while these values are normal in pneumonia caused by virus and atypical pneumonia [19]. For children with pneumonia, after at leas 12 hours when having fever, the number of leucocytes usually increases highly, especially neutrophils. They also recognize that in pneumonia caused by bacteria, CRP infection marker also frequently increase highly after this time [50]. Therefore, when there is not any bacterium culturing test, the formulas of leukocyte and CRP are the important suggestions to distinguish between pneumonia caused by bacteria and pneumonia caused by virus. In this study, we take the threshold CRP = 6 to classify according to guidelines of Ministry of Health. The findings show that 45,08% children with pneumococcal pneumonia have leukocyte increase and 56,48% children increase CRP values. The findings of this study are rather similar to findings of study done by Trinh Thi Ngoc (2020) for children with pneumonia in Thanh Hoa Pediatrics Hospital, in which the ratio of leukocyte and CRP increase are successively 45,5% and 51,8% [46]. The rate of pediatric patients with increases of leukocyte and CRP in our study are lower than studies done in National Pediatrics Hospital.

Lung X-ray is objective evidence and a standard of pneumonia diagnosis but for 2-3 first days of disease onset, X-ray images maybe normal [19]. In this study, bronchopneumonia is the popular damage and occupying 89,64%. In left cases, they observe the blurred lung lobe (3,63%) and unclear damage images (6,74%). In comparison with domestic study, X-ray images in our study are rather similar. The study of Nguyen Dang Quyet (2022) shows that the X-ray images of bronchopneumonia occupy 80%, images of lobar pneumonia occupy 20%, images of pleural effusion occupy 4.85% [13]. In the study of Quach Ngoc Ngan and et al. (2014) in Can Tho, the images of bronchopneumonia occupy 99%, images of lobar pneumonia occupy 1% [48].

The invasive infections caused by *Streptococcus pneumonia* are the main reasons of death in children below 5 years old, especially children below 2 years old, although the prevention activities have been implemented in many countries

[114], [146]. According to previous studies, the serum types of *Streptococcus pneumonia* play an important role in causing invasive infections and changes according to geographic areas [10], [131], [147]. Therefore, regular screening for identifying the distribution of serum types of *Streptococcus pneumonia* is necessary [11]. According to WHO, the nation-wide vaccination is the best way to prevent from diseases caused by *Streptococcus pneumonia* [65]. In our study, in Nghe An, 8 serum types 6A/B, 9V, 11A, 14, 15A, 19F, 19A, and 23F have been discovered in children below 5 years old who have not vaccinated. In these types, 3 serum types 19F, 19A and 23 occupy above 90% *S. Pneumoniae* species separated, while the serum types 6A/B, 9V, 11A, 14, and 15A occupy lower ratio, fluctuating from 0.08 to 3.17%.

4.2. Identification of antibiotic resistance ratio, several genes related to drug resistance

4.2.1. Situation of antibiotic resistance of Streptococcus pneumonia

The ratio of antibiotic resistant patients observed in 193 *Streptococcus pneumonia* species for antibiotics CLA, AZM, ERY, SXT, CLI, TET, CXM, CEF, PEN, CLP, and LEV are respectively 99,48% (192), 99,48% (192), 98,96% (191), 94,82% (183), 94,30% (182), 94,30% (182), 46,11% (89), 37,82% (73), 4,66% (9), 3,11% (6) and 1,55 (3). The findings also show that 100% *Streptococcus pneumonia* species are sensitive to MXF, LIN, VAN and RIF. The findings of this study show that the drug resistance characteristics of *S. Pneumoniae* should be paid more attention to. The ratio of *S. Pneumoniae* species resistant to AZM, CLA, CLI, ERY, SXT, and TET antibiotics is higher than 94%. In our study, all *S. Pneumoniae* species are sensitive to RIF, VAN, MXF and LIN. The rate of *S. Pneumoniae* species resistant to LEV and CLP are rather low (1,55% and 96,89%). These findings show that RIF, CLP, VAN, MXF, LIN and LEV antibiotics are the suitable selection to treat according to experiences of *S. Pneumoniae*'s diseases; especially, the cases of *S. Pneumoniae* species resistant to β-lactam, Macrolides, Lincosamide, Tetracyclines, and Cotrimoxazole antibiotics in Vietnam.

For 3 antibiotics of Macrolide group (Azithromycin, Clarithromycin, Erythromycin), the analysis results show that 100% *S. Pneumoniae* species in this study are resistant to at least a kind of Macrolide group's antibiotics and 99,5% species (192 species) are resistant to many Macrolide group's antibiotics of *S. Pneumoniae* at the same time in Nghe An, which is very popular and should be paid much attention to. The situation of Macrolide group's drug resistance is rather troubling as mentioned in previous studies [5], but this is important guideline to help clinician select suitable antibiotics for patients suspected to have *S. Pneumoniae* infection even when there is no antibiogram's result.

4.2.2. Genes related to Macrolide group's drug resistance of Streptococcus pneumonia

The genes *erm*(B), *mef*(A) participating into 2 different macrolide drug

resistance mechanisms of *S. pneumonia* have been identified the appearance frequency. The analyzing results show that the frequency of macrolide resistant *S. Pneumoniae* species bearing genes *erm*(B) and *mef*(A) are successively 92,1% and 57,9%. All species bearing genes *erm*(B) và *mef*(A) are resistant to at least one of antibiotics: azithromycin, clarithromycin, erythromycin. The frequency of macrolide resistant *S. Pneumoniae* species bearing gene *erm*(B) in this study is higher than almost previous studies over the world. The different studies in the world show that the frequency of macrolide resistant *S. Pneumoniae* species bearing gene *erm*(B) fluctuates from 5 to 90.2% [8].

In Vietnam, according to study of Tong Thi Ha (2017) done in 3 provinces/ cities, including Hanoi, Hai Duong and Khanh Hoa, the frequencies of species bearing genes *erm*(B) and *mef*(A) are 86,4% and 39,3% [16]. Meanwhile, the study of Le Van Duyet and et al. (2017) on 26 *S. Pneumoniae* species do not recognize any species bearing gene *erm*(B) and only species bearing gene *mef*(A) [17]. In comparision with our study, the rate of species bearing gene *erm*(B) and *mef*(A) in 2 studies are lower. According to Hoban and et al. (2021), data on genes relate to macrolide drug resistance and drug resistant data are an important foundation to select the suitable antibiotics in clinical practice of *S. Pneumoniae* disease treatment [102].

4.3. Treatment results and several factors related to pneumococcal pneumonia treatment results

4.3.1. Treatment results

In this study, the hospital stay of children fluctuates from 1 – 25 days, on average 8,46 ± 4,12 days. Most children with pneumococcal pneumonia should be treated within 14 days (86,53%). The average treatment time in this study is higher than studies of Trinh Thi Ngoc (2020) [46], Hoang Tien Loi and et al. (2022) [165] done on pediatric patients with pneumococcal pneumonia in Thanh Hoa Pediatrics Hospital (8,46 ± 4,12 days compared with 6,58 ± 3,03 days and 7,91 ± 3,54 days), the study of Chau Long and et al. (2021) done on children with pneumonia in Can Tho Pediatrics Hospital (8,46 ± 4,12 ngày so với 7,53 ± 2,74 days) [137]. The average treatment time of children with pneumococcal pneumonia in our study is higher than this time in several studies over the world, such as: study of Chen and et al. (2023) in Thanh Do, China (7 days) [168], study of Wieteska and et al. (2022) in Lublin, Finland (7,8 days) [169].

The evaluation of treatment results in hospital stay shows that not case has complication and dead, the ratio of children recovered from disease reaches 68,91% and 31,19% children have better progress. The ratio of pediatric patients recovered in this study is lower than studies of Hoang Ngoc Anh and et al. (2017) [5] for children in Hai Phong (97,2% patients recovered from disease; 2,8% patients must be transferred to upper route, no case is dead); Nguyen Dang Quyet and et al. (2021) done on children treated in National Pediatrics Hospital (the ratio of patients

recovered totally is 83,4%; 14,2% patients are better; 2,4% patients have the complication of pleural thickening and no pediatric patient is dead) [14].

4.3.2 Several factors affecting the pneumococcal pneumonia treatment results

The average treatment time of children ≤ 24 months old suffered from pneumococcal pneumonia is longer than children from 25 to 60 months old (8,80 ± 4,37 days compared with 7,42 ± 3,03; $p < 0,05$). The research results of Gajewska and et al. (2016) for children with pneumonia caused by bacteria in Finland show that the treatment time in hospital fluctuates from 7,4 to 9,7 days. The treatment time of older children is much shorter than group of children ≤ 2 years old [175]. In study of Wieteska and et al. (2022) in Finland shows that the average treatment time of children with pneumococcal pneumonia is 7,8 days [169]. The analyzing results of this study show although the average treatment times and rate of patients recovered from disease in 2 groups of children who have been vaccinated and not vaccinated *S. Pneumoniae* are different with no statistical meaning, but they can be recognized, the average treatment time of children who have not been vaccinated *S. Pneumoniae* is higher than children who have been vaccinated 8,60 ± 4,35 days compared with 7,87 ± 3,00 days). Vaccination is proved to reduce the death ratio in hospital, hospital stay and treatment costs in old patients who are suffered from diseases caused by invasive *S. Pneumoniae* and must be hospitalized [178], [179]. In details, according to Naito and et al. (2020), the ratio of dead patients and average treatment time in old people who have been vaccinated to prevent invasive *S. Pneumoniae* are 3,9% and 6,4 ± 35,8 days, these data for patients who have not been vaccinated are 8,2% and 40,9 ± 59,9 days [178].

In this study, the average treatment time in group of children with pneumococcal pneumonia who have used antibiotics and fever relievers before treating is shorter than group of children who do not use antibiotics before hospitalized 8,40 ± 4,12 days compared with 8,59 ± 4,16 and 7,62 ± 3,68 compared with 8,81 ± 4,26) but this difference has not statistical meaning ($p < 0,05$). The study of Nguyen Dang Quyet (2022) shows that the ratio of children who have the treatment time in hospital < 14 days in group of children using antibiotics before hospitalized is lower than children who do not use antibiotics before hospitalized but this difference has no statistical meaning (78,52% compared with 86,67%, $p < 0,05$) [13]. Thanks to analyses stated above, we can recognize that treatment by antibiotic before hospitalized does not decrease the treatment time in children who are suffered from pneumococcal pneumonia and must be hospitalized [181].

The univariate and multivariate analyses show that the ratio of patients recovered from serious pneumonia is lower (45,95% compared with 74,36%), this difference has the statistical meaning ($p < 0,05$). The study of Nguyen Dang Quyet (2021) in National Pediatrics Hospital also shows that the treatment time of children with serious pneumonia is longer than pneumonia [14]. The other study of Chau Long and et al. (2021) in Can ho Pediatrics Hospital shows that the average treatment time

is 10,64 ± 3,26 days in children with serious pneumonia in comparison with 7,16 ± 2,68 days in children with pneumonia. The ratio of pediatric patients recovered and better in pneumonia is 87,86% compared with 11,31% in serious pneumonia, this difference has the statistical meaning [137]. In Taiwan, the study of Hsieh and et al. (2004) has similar results. So, the treatment time in hospital for serious pneumococcal pneumonia is 25,2 ± 12,0 days compared with 12,6 ± 6,8 days in pneumonia [182].

CONCLUSION

1. Clinical, paraclinical characteristics of pneumococcal pneumonia in children below 5 years old

The study of 193 children from 2 months old to 60 months old suffered from pneumococcal pneumonia treated in Nghe An Obstetrics and Pediatrics Hospital show that:

- The ratio of children with pneumococcal pneumonia who have been hospitalized by parents for 3 first disease onset days is 61.14%; The most popular clinical symptoms are cough, fever and moist rales with respective rate of 98,45%; 77,72% and 93,26%. The ratio of children with serious pneumonia is 19,17%.

- The ratio of children with leucocyte increase is 45,59% and CRP increase is 56,48%. The damage images of bronchopneumonia on X-ray film is 89,64%. 8 different serums of *S. Pneumoniae* have been identified as present in Nghe An. In which, 19F, 23F and 19A are the most popular. Serum type 19A with high infection ratio is characteristics that should be paid much attention to.

2. Identification of antibiotic resistance ratio and several genes related to drug resistance of *S. pneumonia*

- The ratio of *S. Pneumoniae* species sensitive to Levofloxacin 98,45%, Moxifloxacin 99,48%, Chlorampenicol 96,89%, Rifamicin 100%.

- The rate of *S. Pneumoniae* species resistant to clarithromycin, azithromycin, erythromycin, trimethoprim/sulfamethoxazole, tetracyclin, clindamycin are respectively 99,48%, 99,48%, 98,96%, 94,86%, 94,3% , 94,3%. The situation of macrolid group's antibiotic resistant *S. Pneumoniae* species is high (> 98%). There are below 5% *S. Pneumoniae* species resistant to linezolid, levofloxacin, vancomycin, rifampicin, chloramphenicol and moxifloxacin.

- Frequency of *S. Pneumoniae* species bearing genes *erm*(B) and *mef*(A) related to macrolide group's antibiotic resistance are 92,1% and 57,9% respectively. In which, the frequency of *S. Pneumoniae* species bearing at least one of two genes is 95.2%; the frequency of *S. Pneumoniae* species bearing simultaneously 2 genes is 54,8%.

3. Treatment results and several factors related to pneumococcal pneumonia treatment results

- The average number of treatment days is 8,46 ± 4,12. The ratio of patients recovered totally is 68,91%, getting better is 31,09% and no case is dead.

- Children ≤ 24 months old have longer treatment time than children from 25 to 60 months old. The treatment time in hospital of children with serious pneumococcal pneumonia is longer than children with pneumococcal pneumonia.

- 2 factors of using antibiotics before hospitalized and serious pneumococcal pneumonia have impacts on pneumococcal pneumonia treatment results in children.

PETITION

- Do not select antibiotics as Clarithromycin, Azithromycin, Erythromycin, Trimethoprim/sulfamethoxazole, Tetracyclin and Clindamycin as the initial treatment drugs for acute pneumonia children. When identifying cause of pneumonia as *S. Pneumoniae*, they should make the antibiogram and treat based on the results of antibiogram.

- The study should be expanded to clarify the molecular mechanism of drug resistance on genes related to macrolide resistance and other antibiotic groups.

- For children with serious pneumococcal pneumonia, they should be appointed to use antibiotics which rarely happen the drug resistance to treat (linezolid, levofloxacin, vancomycin, rifampicin và moxifloxacin).

LIST OF THESIS-RELATED PUBLICATIONS OF THE AUTHOR

1. Bui Anh Son, Tang Xuan Hai, Tran Van Cuong, Duong Dinh Chinh, Thi-Hong-Hanh Le, Nguyen Manh Dung, Vu Nhat Dinh, Do Ngoc Anh(2022), Serotype distribution and antibiotic resistance of *Streptococcus pneumoniae* isolates collected from unvaccinated children with pneumonia at a province in central Vietnam, *Iranian Journal of Microbiology*, 14(5):653-661.
2. Bui Anh Son, Duong Dinh Chinh, Thi-Hong-Hanh Le, Nguyen Manh Dung, Vu Nhat Dinh, Do Ngoc Anh Do Ngoc Anh (2023), Identification of *erm(B)* and *mef(A)* genes among macrolide-resistant *Streptococcus pneumonia* species collected from children below 5 years old in Nghe An (2019 – 2021), Vietnam Journal of Science and Technology, 65(2): 9-13.