MINISTRY OF EDUCATION AND TRAINING MINISTRY OF HELTH

CENTRAL INSTITUTE OF MALARIOLOGY, PARASITOLOGY AND ENTOMOLOGY

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LE THI HOA

CLINICAL AND SUB-CLINICAL CHARACTERISTICS OF RESPIRATORY SYNCYTIAL VIRUS PNEUMONIA WITH BACTERIAL CO-INFECTION IN PEDIATRIC PATIENTS AND THE SUPPORTIVE EFFECT OF PROBIOTICS AT THE VIETNAM NATIONAL CHILDREN'S HOSPITAL (2022-2024)

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Supervisors:

1. Assoc. Prof. Dr. Le Thi Hong Hanh
2. Assoc. Prof. Phung Thi Bich Thuy

Reviewer 1:

Reviewer 2:

Reviewer 3:

This thesis is defended before the institute-level thesis examination council met in Central Institute of Malariology, Parasitology and Entomology

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LIST OF THESIS-RELATED PUBLICATIONS OF THE AUTHOR

1. Le Thi Hoa, Le Thi Hong Anh, Phung Thi Bich Thuy et al (2025), "Clinical, sub-clinical characteristics of children with pneumonia caused by respiratory syncytial virus bacterial co-infections", *Vietnam Journal of Community Medicine*, Vol. 66(1), 290-295.
2. Le Thi Hoa, Le Thi Hong Anh, Phung Thi Bich Thuy et al (2025), "Clinical, sub-clinical characteristics and antibiotic resistance of co-infecting bacteria in children with respiratory syncytial virus (RSV) pneumonia", *Vietnam Journal of Community Medicine*, Vol. 66(1), 116-121.
3. Le Thi Hoa, Le Thi Hong Anh, Phung Thi Bich Thuy et al (2024), "Bacterial co-infection in pediatric patients with pneumonia caused by respiratory syncytial virus", *Vietnam Medical Journal,* Vol. 545(3), 49-53.

INTRODUCTION

RSV pneumonia accounts for a high proportion of pneumonia cases in children. The bacterial co-infection rate is relatively high, ranging from 26,3% to 43,6%. Children with co-infections tend to have more severe disease and require longer treatment durations. Treatment follows the standard pneumonia protocol, including antibiotics and symptomatic treatment. Probiotics have emerged as a promising therapy in supporting the treatment of respiratory tract infections in children. At the Vietnam National Children's Hospital, several studies have investigated the effects of probiotics on upper respiratory tract infections caused by RSV and influenza; however, no studies have been conducted on children with pneumonia. Therefore, we conducted this study with three primary objectives:

1. *Characterize the clinical, sub-clinical of RSV pneumonia with bacterial co-infection in children aged 1 to 24 months at the Vietnam National Children's Hospital from 2022 to 2024.*
2. *Evaluate the clinical efficacy and safety of a nasal-spraying probiotic containing Bacillus subtilis and Bacillus clausii in these patients.*
3. *Measure changes in viral load, bacterial load, and cytokine levels in nasopharyngeal samples before and after 3 days of probiotic nasal spray treatment.*

This study is of critical importance for four main reasons:

- The bacterial co-infection rate in children with RSV pneumonia is quite high (57.2%), yet no large-scale studies have been conducted on this issue in Vietnam.
- Identifying the most common bacterial co-infections in children with RSV pneumonia can help clinicians make more informed decisions when selecting appropriate antibiotic therapy, potentially

reducing treatment duration.

- Evaluating probiotic therapy therapy as an appropriate supportive treatment method while there is no specific drug for the virus and antibiotic resistance is increasing.
- The study findings have the potential for rapid clinical application, reducing hospital stay duration and healthcare costs.

New Contributions and scientific meanings of the thesis

- The study identifies *Haemophilus influenzae* as the most common bacterial co-infection in children with RSV pneumonia (53,4% detected by Real time RT-PCR, 55,7% by culture), whereas previous studies have emphasized the greater role of *Streptococcus pneumoniae*.
- The study shows that *H. influenzae* has a beta-lactamase production rate of 72,1%, demonstrating high resistance to ampicillin while remaining highly susceptible to Cefotaxime and Ceftriaxone.
- The safety and efficacy of a probiotic nasal spray containing *B. subtilis* and *B. clausii* were evaluated in reducing clinical symptoms and objective markers, including viral and bacterial loads and cytokine levels, before and after 3 days of treatment. These findings support the use of probiotics as a potential therapy for RSV pneumonia with bacterial co-infection

Structure of thesis

The thesis covers 129 pages, including: INTRODUCTION (2 pages); OVERVIEW (33 pages); RESEARCH OBJECTS AND METHODS (26 pages); RESULTS (33 pages); DISCUSSION (32 pages); CONCLUSION (2 pages). The thesis has 32 tables, 24 figures. There are 168 references.

Chaper 1
OVERVIEW

1.1. RSV pneumonia with bacterial co-infection in children

1.1.1. Definitions

Pneumonia is a term commonly used to describe inflammation of the lung parenchyma, most often caused by bacteria and viruses, leading to the alveoli being filled with fluid or pus.

Community-acquired pneumonia (CAP) refers to pneumonia caused by pathogens acquired outside the hospital setting.

Co-infection is defined as the isolation of at least one microbial pathogen from blood or nasopharyngeal samples collected within 48 hours of hospital admission.

1.1.2. Characteristics of Respiratory Syncytial Virus (RSV)

RSV belongs to the Paramyxoviridae family and the Pneumovirinae subfamily. It is an enveloped, single-stranded RNA virus, spherical in shape, with a size ranging from 65 to 300 nm. The viral envelope plays a crucial role in viral egress through budding. Two specific glycoproteins, G and F, are present on the viral envelope. Glycoprotein G facilitates viral attachment to host cells. Glycoprotein F mediates viral membrane fusion with the host cell membrane, enabling viral entry. It also promotes the fusion of RSV-infected cells into multinucleated giant cells (syncytia).

1.1.3. Diagnostic tests for Respiratory Syncytial Virus (RSV)

- Rapid antigen detection tests aim to detect viral antigens and provide results within 30 minutes.

- Real-time RT-PCR has high sensitivity and specificity, offering fast and accurate results.

- Viral culture from respiratory secretions is rarely performed in clinical practice.

1.1.4. Mechanism of bacterial co-infection in RSV pneumonia

When the virus enters host cells, alterations in mucin secretion, modulation of adhesion molecule regulation on the epithelial surface, and increased extracellular iron levels promote biofilm formation and bacterial invasion.

RSV can damage ciliated epithelial cells, leading to cilia dysfunction and impaired mucociliary clearance, which reduces the elimination of mucus and trapped pathogens. Virus-induced cell death disrupts epithelial integrity, exposing new receptors that facilitate bacterial adhesion and invasion

1.1.5. Clinical and sub-clinical of community-acquired pneumonia due to RSV with bacterial co-infection

1.1.5.1. Epidemiological factors: living in areas with RSV outbreaks, close contact with other infected children or adults.

1.1.5.2. Clinical symptoms: RSV pneumonia with bacterial co-infection typically presents in two stages:

Prodromal stage

- Incubation period: Approximately 4 days.
- Upper respiratory tract symptoms: Cough, rhinorrhea, fever, chills, irritability, nausea, vomiting, poor feeding, abdominal distension, diarrhea.
- Pulmonary symptoms may not yet be evident.

Full-blown stage

- Subjective symptoms: fever, cough, fatigue, irritability, headache, chills, vomiting, abdominal distension, diarrhea, abdominal pain.
- Pulmonary examination reveals abnormal lung sounds, including dry rales, rhonchi, and moist rales.

1.1.5.3. Sub-clinical symptoms

- Viral pneumonia: White blood cell (WBC) and neutrophil counts are typically normal, with no significant increase. C-reactive protein (CRP) levels are usually below 6 mg/L, and procalcitonin (PCT) levels are typically below 0.1 ng/L.

- Mixed viral-bacterial pneumonia: CRP and PCT levels are elevated

- Chest X-ray findings**:** Diffuse nodular opacities, scattered infiltrates in both lung fields, consolidation, interstitial involvement, air trapping, and patchy opacities due to atelectasis or bilateral infiltration.

- Pathogen detection for viral and bacterial co-infection:

+ Rapid diagnostic tests for influenza A/B, parainfluenza…

+ Molecular techniques (PCR, single-plex and multiplex RT-PCR) with high sensitivity and specificity.

+ Bacterial culture from respiratory specimens: nasopharyngeal aspirate, tracheal aspirate, bronchoalveolar lavage fluid; as well as blood and sputum cultures.

1.1.6. The role of cytokines in Respiratory Syncytial Virus (RSV) Pneumonia

Cytokines are low-molecular-weight soluble protein molecules produced by various cells in response to antigens, acting as signaling mediators to regulate inflammatory and immune responses. In individuals infected with RSV, elevated IL-6 levels have been associated with more severe disease. A 2020 meta-analysis by two independent researchers involving 921 children (207 non-RSV-infected and 714 RSV-infected) demonstrated that IL-8 levels in nasopharyngeal secretions were significantly higher in RSV-infected children, with variations depending on the testing method. Although some clinical studies have suggested that tumor necrosis factor-alpha (TNF-α) plays a major role in RSV-induced respiratory inflammation (e.g., bronchospasm and persistent wheezing), experimental evidence remains inconclusive or conflicting. A 2024 systematic review by Divya Sinha et al. highlighted the role of secretory IgA (sIgA) in defending against respiratory viruses (including SARS-CoV-2, RSV, and influenza) and emphasized the future potential of mucosal immunity-based vaccine research.

1.2. The role of probiotics in respiratory infections

1.2.1. Overview of probiotics

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host".

Among probiotics, *Lactobacillus* and *Bifidobacterium* species are the most predominant. Other genera include *Lactococcus*, *Bacillus*, *Streptococcus*, and *Saccharomyces*.

The genera *Bacillus* and *Lactobacillus* belong to the phylum Firmicutes. *Bacillus* comprises a diverse range of species, most of which are harmless. These bacteria are Gram-positive, rod-shaped, and can be either aerobic or facultative anaerobic. Notably, they form nearly spherical endospores, enabling them to remain dormant for extended periods

Common *Bacillus* species include: *B. clausii, B. polyfermenticus, B. subtilis, B. coagulans.*

1.2.2. Antiviral mechanisms of probiotics

Antiviral mechanisms of probiotics: The potential antiviral mechanisms of probiotics include: (1) viral adsorption and (2) inhibiting viral entry into host cells (1) cản trở sự hấp phụ và (2) sự xâm nhập tế bào của virus; (3) producing metabolites and bioactive compounds with direct antiviral effects; (4) modulating cellular immune responses to establish antiviral protection.

1.2.3. Research on the role of B. clausii and B. subtilis in RSV respiratory infections

Early clinical trials have demonstrated that probiotics containing *B. clausii* can shorten the duration of respiratory tract infections and reduce antibiotic-associated side effects during treatment. *Bacillus* species produce lipopeptides such as fengycin, which exhibit antimicrobial activity against *Staphylococcus aureus*. Specifically, *Bacillus* fengycins inhibit *S. aureus* by preventing its cell replication.

At the Vietnam National Children's Hospital, a clinical trial evaluating a nasal-spraying probiotic in children with RSV and influenza-related upper respiratory infections showed promising results. The probiotic group experienced a shorter treatment duration and a one-day reduction in clinical symptoms compared to the control group. Additionally, the probiotic group exhibited a greater reduction in viral load and cytokine levels than the control group, further supporting the therapeutic potential of probiotic nasal sprays.

Chapter 2
RESEARCH OBJECTS AND METHODS

Objective 1

2.1. Subjects, study period, and location (Objective 1)

2.1.1. Study subjects

Inclusion criteria: Children aged 1 to 24 months diagnosed with RSV pneumonia who had not been hospitalized or were admitted within the first 48 hours.

Exclusion criteria: Children with underlying conditions (e.g., congenital heart disease, airway malformations); preterm infants; co-infection with other viruses (e.g., influenza, adenovirus).

Diagnostic criteria for RSV pneumonia in children:

Pneumonia is diagnosed based on the WHO-2013 criteria, which include cough or difficulty breathing along with at least one of the following: age-specific fast breathing, chest depression, presence of fine crackles, moist rales, pleural rub, or reduced lung ventilation upon auscultation. Chest X-ray test include irregularly sized opacities with unclear margins in one or both lungs. Laboratory confirmation requires a positive RSV rapid antigen test and/or real-time PCR for RSV in nasopharyngeal samples.

Diagnostic criteria for RSV pneumonia with bacterial co-infection in children: Children diagnosed with RSV pneumonia with a positive real-time PCR and/or bacterial culture from nasopharyngeal samples identifying at least one bacterial species.

2.1.2. Study period and location

- Study period: From August 2022 to November 2023.
- Location: Center for Pulmonology and Respiratory Care, Vietnam National Children's Hospital.

2.2. Research Methods (Objective 1)

2.2.1. Study design: prospective descriptive study

2.2.2. Sample size and sampling method

- Sample size determined using the prevalence estimation formula.
- Minimum required sample size: $n \geq 254$ pediatric patients.

Objectives 2 and 3

2.3. Subjects, study period, and location (Objectives 2, 3)

2.3.1. Study subjects

- Children aged 1 to 24 months diagnosed with RSV pneumonia and bacterial co-infection by real-time PCR/ RT-PCR in nasopharyngeal samples.
- The nasal-spraying probiotic in the study is LiveSpo® Navax (Registration number: 190001347/PCBA-HN), developed by ANABIO R&D Co., Ltd

2.3.2. Study period and location: Same as Objective 1

2.4 Research methods (Objective 2, 3)

2.4.1. Study design

A randomized, double-blind, controlled clinical trial.

Comparison between two groups:

- Control group: Received 0.9% physiological saline (0.9% NaCl).
- Probiotic group: Received probiotic spore nasal spray (LiveSpo® Navax).

2.4.2. Sample size and sampling method

The sample size was calculated based on the hypothesis that probiotic spores improve symptom resolution by more than 25%: the proportion of patients becoming symptom-free between days 3 and 6 was 90% in the probiotic spore group, compared to 65% in the control group. The required sample size was calculated as $n \geq 43$.

We conducted an intervention on 101 pediatric patients, who were divided into two groups: 50 patients in the control group and 51 patients in the probiotic group.

Study procedure

- Random selection of 101 patients diagnosed with RSV pneumonia and bacterial co-infection (confirmed by real-time PCR/RT-PCR for seven bacterial species). Group allocation was performed using the lottery method.
- Standard treatment adherence and administration of the assigned nasal spray according to protocol
- Clinical symptom assessment from hospital admission to discharge. Comparisons were made before treatment, after 3 days, and after 5 days in both groups.
- Safety evaluation of the probiotic nasal spray.
- RSV viral load, co-infecting bacterial load, and the concentrations of IL-6, IL-8, TNF-α, and IgA in nasopharyngeal secretions were measured on day 0 and after 3 days, and the results were compared between the two groups.

2.5. Data collection and statistical analysis methods

Data were collected and analyzed using SPSS 23.0 and GraphPad Prism v8.4.3 software.

2.6. Bias, confounders, and control measures

- Random errors and systematic errors
- Methods to minimize bias: randomization and blinding.
- Identical packaging, labeling, and coding to maintain blinding.
- Emergency unblinding protocol established.

2.7. Ethical issues

- The study was approved under Decision No. 1241/BVNTƯ-HĐĐĐ by the Ethics Committee of the Vietnam National Children's Hospital
- Pediatric patients were ensured full rights to medical examination and comprehensive evaluation, with confidentiality of personal information strictly maintained

Chapter 3
RESULTS

The study was conducted on 283 pediatric patients with RSV pneumonia aged 1–24 months, yielding the following results

1.1. Clinical and sub-clinical characteristics of RSV pneumonia with bacterial co-infection

1.1.1. Clinical and sub-clinical characteristics of RSV pneumonia

Table 3.1. Distribution of age, gender, and geographic location

Characteristics		***n***	**Percentage (%)**
Age	<6 months	178	62,9
	6-11 months	63	22,3
	12-24 months	42	14,8
Gender (*n* = 283)	Male	181	64,0
	Female	102	36,0
Location (*n* = 283)	Rural	182	64,3
	Urban	101	35,7

Comment:

- The most commonly affected age group in the study was infants under 6 months (62.9%). The male-to-female ratio was 1.8:1, with a higher prevalence in rural areas than in urban settings.

- Respiratory tract inflammation symptoms (cough, runny nose, wheezing) were present in nearly all patients. Anorexia was a major reason for hospitalization, observed in 83,7% of cases. Fever occurred in 61,8% of cases, while diarrhea, a notable gastrointestinal symptom, was present in 18% of patients. Symptoms typically appeared 3–4 days before hospital admission.
- Children with saturation of peripheral oxygen ($SpO_2 \leq 94\%$) was observed in 76,3% of patients. Classic pneumonia symptoms, including chest depression and fast breathing (age-adjusted), were noted in 67,8% and 41,7% of cases, respectively. Among pulmonary auscultation findings, the combination of dry rales and moist rales was the most common (51,2%), followed by isolated moist rales (25,4%).
- Most patients had normal white blood cell counts, neutrophil counts, and CRP levels (81,3%, 80,9%, and 55,0%, respectively). Among children aged ≥ 6 months, anemia was prevalent in 74,0% of cases.
- Chest X-rays test showed patchy or nodular opacities in both lung fields (75,6%), while other findings (e.g., perihilar opacities, interstitial infiltrates, air trapping, right upper lobe consolidation, and bronchial wall thickening) were less frequent.

3.1.2. Bacterial co-infection in pediatric RSV pneumonia

3.1.2.1. Pathogens identified through different methods

- The detection rate of bacteria in nasopharyngeal samples was 47,3% using real-time PCR for seven bacterial species and 36,9% using bacterial culture.

- *Real-time PCR: H. influenzae* was the most frequently detected bacterium (53,4%), followed by *S. pneumoniae* (24%). Co-infection with *S. pneumoniae* and *H. influenzae* was found in 15,5% of cases, *M. pneumoniae* in 3,1%, and dual infection with *S. pneumoniae* and *M. pneumoniae* in 1,6%.

- *Bacterial culture*: *H. influenzae* was the most prevalent isolate (55,7%), followed by *S. pneumoniae* (14,4%), *Moraxella catarrhalis* (11,5%), and *S. aureus* (10,6%).

- *Antibiotic resistance profile of H. influenzae*: Beta-lactamase production was detected in 72,1% of *H. influenzae* isolates, contributing to high resistance rates (>90%) against Ampicillin, Cefuroxime, and Cefaclor. Reduced susceptibility was observed for Ampicillin/Sulbactam (32,1%). In contrast, Cefotaxime retained high sensitivity (98,2%), and no resistance was detected for Ceftriaxone, Imipenem, or Ciprofloxacin.

3.1.2.2. Bacterial co-infection rate

A total of 162 out of 283 children (57.2%) had bacterial co-infections.

3.1.3. Clinical and sub-clinical characteristics of RSV pneumonia with bacterial co-infection

3.1.3.1. Age and gender distribution according to bacterial co-infection

Table 3.2. Age and gender distribution according to bacterial co-infection

		Co-infected group (n_1 = 162)		**Non-co-infected group (n_2 = 121)**		***p***
		n	**%**	***n***	**%**	
Age	<6 months	95	58,6	83	68,6	$p < 0,05$
	6-11 months	45	27,8	18	14,9	
	12-24 months	22	13,6	20	16,5	
Gender	Male	95	58,6	86	71,1	$p < 0,05$
	Female	67	41,4	35	28,9	

Comment: The proportion of children aged 6-11 months, the proportion of female children in the co-infected group was 27,8%; 41,4% higher than in the non-co-infected group 14,9% ($p < 0,05$)

3.1.3.2. Clinical symptoms according to co-infection

Table 3.3. Clinical symptoms of the two groups

Symptoms	Co-infected group (n_1=162)		Non-co-infected group (n_2=121)		*p*
	n	%	*n*	%	
Fever	**121**	**74,7**	**54**	**44,6**	**< 0,01**
Wheezing	161	99,4	119	98,3	> 0,05
Runny nose	158	97,5	119	98,3	> 0,05
Anorexia	**128**	**79,0**	**109**	**90,8**	**< 0,05**
Chest depression	113	69,8	79	65,3	> 0,05
Respiratory failure	108	66,7	79	65,3	> 0,05
Moist rales	30	18,5	42	34,7	**<0,05**
Dry rales + moist rales	99	61,1	46	38,0	**< 0,05**
Rhonchi, dry rales	33	20,4	33	27,3	>0,05

Comment: Fever was observed in 74,7% of the co-infected group, significantly higher than in the non-co-infected group ($p < 0,01$). In contrast, poor feeding was more common in the non-co-infected group ($p < 0,05$). Moist rales and dry rales findings also showed a statistically significant difference between the two groups ($p < 0,05$). Children with bacterial co-infection had a 3,66 times higher risk of fever compared to those without co-infection (OR = 3,66, 95% CI: 2,21–6,06).

3.1.3.3. Sub-clinical symptoms according to co-infection

Table 3.4. White blood cell count, neutrophil count, hemoglobin platelet count, CRP of the two study groups

Test results	Co-infected group (n_1=162)	Non-co-infected group (n_2=121)	*p*
White blood cell count (G/l) (median)	12,08 (4,99-26,96)	9,12 (2,62-26,94)	< 0,01
Neutrophil count (%) (median)	41,0 (5,2-89,4)	27,2 (5,3-85,5)	< 0,01
Mean Hb (G/l)	111,28 ± 10,21	111,08 ± 12,89	> 0,05
Platelet count (G/l) (median)	407 (177-857)	135,7 (153-751)	> 0,05
CRP (mg/dl) (median)	10,01 (0,17-177,78)	1,61 (0,04-117,63)	< 0,01

Comment: The co-infected group had significantly higher white blood cell counts, neutrophil percentages, and CRP levels compared to the non-co-infected group ($p < 0,01$), while hemoglobin and platelet counts were similar between the two groups ($p > 0,05$).

- The risk of elevated white blood cell count, neutrophil count, and CRP levels was 2,37 folds, 2,58 folds, and 4,3 folds higher, respectively, in the co-infected group compared to the non-co-infected group (OR = 2,37, 95% CI: 1,17–4,80; OR = 2,58, 95% CI: 1,15–6,69; OR = 4,3, 95% CI: 2,6–7,2, $p < 0,0001$).
- Interstitial infiltrates and hyperinflation were significantly more common in the co-infected group, ($p < 0,05$).

3.2. Clinical efficacy and safety of probiotic containing *B. subtilis* and *B. clausii* in children with RSV pneumonia and bacterial co-

infection

3.2.1. Effect of probiotics on clinical symptoms of RSV pneumonia with bacterial co-infection.

Table 3.5. Comparison of the number of days of clinical symptom relief in the two study groups

Symptoms	Time to symptom relief (days) Median (min-max)		*p*
	Control group $n_1 = 50$	**Probiotics group $n_2 = 51$**	
Runny nose	5 (3-10)	4 (1-8)	0,0113
Fever	2 (1-6)	1 (1-3)	0,0002
Chest depression	4 (1-8)	3 (1-8)	0,0283
Dry rales	*4 (1-10)*	*4 (1-8)*	*0,0883*
Moist rales	5 (3-10)	4 (2-8)	0,0241
Wheezy	5 (3-10)	4 (1-8)	0,0202
Cough	6 (3-10)	5 (1-9)	0,0385
Vomiting	*5 (3-9)*	*4 (2-9)*	*0,0800*
Diarrhea	5 (3-9)	4 (1-9)	0,0200
Times required for oxygen therapy (day)	4 (1-9)	2 (1-8)	0,0063
Hospital duration (day)	7 (4-12)	6 (3-14)	0,0487

Comment: The probiotics group had a 1-day shorter recovery time for most clinical symptoms compared to the control group ($p < 0,05$)

3.2.2. Safety of the nasal-spraying probiotics containing B. subtilis and B. clausii

- Across nine spray applications, changes in respiratory rate (Δ

breaths/min) and heart rate (Δ beats/min) remained within safe limits, with mean fluctuations of ±2 breaths/min (range: -5 to +5) and ± 2 beats/min (range: -15 to +12).

- Saturation of peripheral oxygen (Δ SpO2%) showed a slight increasing trend post-spray (mean: +1%, range: -4% to +9%) in both groups.
- No choking, nasal congestion, mucosal irritation, local infections, or allergic reactions (either cutaneous or systemic) were observed.

3.3. Evaluation of RSV load, bacterial co-infection, and cytokine levels in nasopharyngeal samples before and after 3 days of treatment

3.3.1. Changes in RSV load and bacterial co-infection

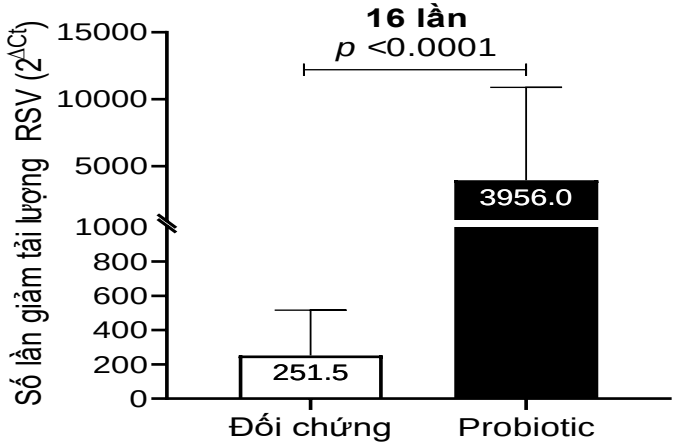

Figure 3.1. Reduction in RSV load ($2^{\Delta Ct}$) after 3 days compared to day 0 in the Control group and the Probiotics group

- After 3 days, the nasal-spraying probiotic effectively reduced RSV load 16 folds more than physiological saline ($p < 0{,}0001$).
- The Probiotics group showed a significant reduction in total bacterial co-infection (*H. influenzae*, *S. pneumoniae*, *M. pneumoniae*, *B. pertussis*) by 4096-fold, which was 16 folds higher

than the Control group (256-fold reduction) after 3 days of treatment ($p < 0.0001$).

3.3.2. Changes in cytokine levels in nasopharyngeal samples

Table 3.6. Changes in IL-6, IL-8, TNF-α, and IgA levels in nasopharyngeal samples of both groups after 3 days compared to day 0.

Cytokines and IgA levels (median)	**Control group $n_1 = 50$**				**Probiotics group $n_2 = 51$**			
	Day 0	**After 3 days**	**Fold change (+/-)**	***p*1**	**Day 0**	**After 3 days**	**Fold change (+/-)**	***p*2**
IL-6 (pg/mL)	54,25	15,61	-3,5 folds	0,1491	63,73	13,84	-4,6 folds	**<0,0001**
IL-8 (pg/mL)	547,07	480,29	-1,1-fold	0,6109	655,1	452,6	-1,5 folds	**0,0094**
TNF-α (pg/mL)	48,77	2,4	-20,3 folds	**< 0,0001**	47,94	0,08	-599,3 folds	**<0,0001**
IgA (µg/mL)	6,3	7,17	+ 1,1 -fold	0,3052	6,65	11,12	+1,7 folds	**0,038**

After 3 days of treatment:

- IL-6 and IL-8 levels significantly decreased in the probiotics group by 4,6-fold and 1,5-fold, respectively ($p < 0,0001$), whereas in the control group, the reduction was only 3,5-fold and 1,1-fold, respectively ($p > 0,05$).
- TNF-α levels significantly decreased in both groups ($p < 0,00001$), with the control group showing a 20,3-fold reduction (from 48,77 to 2,4 pg/mL), while the probiotics group achieved complete normalization to baseline levels (from 47,94 to 0 pg/mL).
- IgA levels showed a mild increase in both groups after 3 days. However, the probiotics group exhibited a 1,7-fold increase ($p < 0,05$), whereas the control group showed only a slight, non-significant increase (1,3-fold, $p > 0,05$).

Chapter 4
DISCUSSION

4.1. Clinical and sub-clinical characteristics of Respiratory Syncytial Virus (RSV) pneumonia with bacterial co-infection

4.1.1. Clinical and sub-clinical characteristics of RSV pneumonia

Our study indicates that younger children have a higher incidence of RSV pneumonia, with the highest proportion observed in infants under six months of age. The disease was more prevalent in male children and those living in rural areas, which aligns with previous studies on RSV pneumonia. Most patients exhibited respiratory tract inflammation and wheezing. Given that this study was conducted at a tertiary referral hospital, a high proportion of patients presented with severe disease and respiratory distress (76,3%), along with classical pneumonia symptoms such as fever, moits rales, and dry rales, which were higher than in prior studies.

The majority of children had normal white blood cell and neutrophil counts, while C-reactive protein (CRP) levels were elevated in 44,2% of cases. Chest X-ray tests were consistent with pneumonia, with consolidation and nodular opacities being the most common radiological features.

4.1.2. Bacterial co-infection rates, clinical, and sub-clinical characteristics of RSV pneumonia with bacterial co-infection

The bacterial co-infection rate varies across studies, depending on the methods used to detect bacterial and viral pathogens. In our study, 57,2% of RSV pneumonia cases had bacterial co-infection, which is comparable to the findings of Jung Jiwon et al. and Tran Quang Khai et al., who reported high co-infection rates in children with severe pneumonia (67,5%) using real-time PCR, while bacterial culture yielded a 67,1% positivity rate.

Compared to data from two years ago at the Center for Pulmonology and Respiratory Care, Vietnam National Children's Hospital, Truong Thi Viet Nga et al. observed a 10% increase in antibiotic resistance rates in our study. Additionally, β-lactamase-producing bacterial strains accounted for 72,1%, which is higher than previous reports

4.1.2. Clinical characteristics of RSV pneumonia with bacterial co-infection

4.1.2.1. Age and gender distribution in relation to bacterial co-infection

The 6–11-month-old age group had a higher proportion of bacterial co-infection (27,8%) compared to the non-co-infected group (14,9%). This can be attributed to passive immunity from maternal antibodies during the first six months, which declines thereafter, increasing susceptibility to infections. Additionally, older infants are more exposed to environmental factors as they begin crawling and walking, increasing their risk of bacterial infections.

Regarding gender distribution, 41,4% of co-infected children were female, compared to 28,9% in the non-co-infected group ($p < 0,05$). Although male children generally have a higher overall incidence of RSV pneumonia, as demonstrated in multiple studies, the reason for this higher co-infection rate among females remains unclear.

4.1.2.2. Clinical symptoms according to bacterial co-infection

In the RSV pneumonia with bacterial co-infection group, 74,7% of patients presented with fever, significantly higher than the 44,6% in the non-co-infected group ($p < 0,01$). However, when comparing other clinical symptoms such as tachypnea, respiratory distress, hypoxemia, and hypotension, Hsiao-Chi Lin et al. found no significant difference ($p > 0,05$) between the co-infected and non-co-infected groups. This suggests that most RSV pneumonia cases requiring hospitalization already have moderate to severe disease, which may obscure differences in these symptoms.

4.1.2.3. Sub-clinical characteristics of RSV pneumonia with bacterial co-infection

Elevated WBC and neutrophil counts are suggestive of bacterial co-infection. Helmia Farida et al. (2023) reported that clinical signs (fever, rainy season in Indonesia) and CRP levels ≥5,7 mg/dL could be indicative of bacterial or viral-bacterial co-infection, with a moderate predictive value (sensitivity: 62,28%, specificity: 65,52%).

Compared to Hsiao-Chi Lin's (2022) study on 620 children with RSV pneumonia, which found that 33,8% of co-infected cases had pulmonary consolidation versus 23,9% in the non-co-infected group, our study did not show a significant difference in radiological findings. This discrepancy may be due to our smaller sample size, which may not have been sufficient to detect a significant difference.

4.2. Clinical efficacy and safety evaluation of *B. subtilis* and *B. clausii* probiotic nasal spray in patients with Respiratory Syncytial Virus (RSV) pneumonia and bacterial co-infection

A study by Tran Minh Dien et al. (2022) in children with RSV-induced bronchiolitis demonstrated that nasal-spraying containing *Bacillus* spores shortened the treatment duration by one day compared to the control group and improved runny nose symptoms by the fourth day of treatment. The effectiveness of fever reduction and chest depression in Probiotics group 1 day earlier than the Control group in our study is similar to some studies by Tran Minh Dien (2022) and Tran Thanh Tu (2023) when using nasal-spraying *Bacillus* spores in children infected with RSV and influenza virus. Notably, the earlier resolution of moist rales in the probiotic group - 1 day sooner than in the control group - is a strong indicator of improved pneumonia progression.

We observed a significant reduction in oxygen therapy duration by two days and a one-day shorter total hospitalization period in the probiotic group compared to the control group ($p < 0,05$). This finding is clinically relevant as it contributes to reducing hospital stay and treatment costs for pediatric pneumonia.

No abnormal changes in respiratory rate, heart rate, or blood oxygen saturation (SpO_2) were recorded following *Bacillus* spore nasal spray administration. During the first 3 days, the recorded changes (Δ) in heart rate, respiratory rate, and SpO_2 before and after each administration of *Bacillus* spores or 0,9% physiological saline remained within safe limits. Additionally, no cases of aspiration, nasal obstruction, or mucosal irritation were reported in either group. Furthermore, no local infections, skin reactions, or systemic allergic responses were observed following probiotic spray administration.

4.3. Evaluation of Respiratory Syncytial Virus (RSV) load, bacterial co-infection, and cytokine levels in nasopharyngeal samples before and after 3 days of nasal-spraying probiotic

The reduction in RSV load in pneumonia patients using probiotic was 1,6 to 3,3 folds lower than that reported in previous studies by Tran Minh Dien (2022) and Tran Thanh Tu (2023). This discrepancy may be attributed to the severity of illness in our study cohort, which included a higher proportion of pneumonia patients requiring oxygen therapy, potentially limiting the nasal spray's efficacy.

Beyond its antiviral properties, the probiotic nasal spray demonstrated significant inhibitory effects on co-infecting bacteria. The bacterial loads of *S. pneumoniae* and *H. influenzae* were reduced by 2807- and 8801-fold, respectively, which was 10 to 11 folds more effective than in the control group ($p < 0.05$).

After 3 days of treatment, both groups exhibited a decreasing trend in inflammatory cytokine levels. However, the probiotic group demonstrated significantly greater reductions in IL-6 and IL-8 levels 4,6- and 1,5-fold, respectively, from baseline ($p < 0,05$) compared to 3,5- and 1,1-fold reductions in the control group ($p > 0,05$). TNF-α levels decreased similarly in both groups ($p < 0,05$). Additionally, nasal-spraying probiotic significantly enhanced IgA production, increasing levels by 1,7-fold after 3 days of treatment ($p < 0,05$).

CONCLUSION

1. Clinical and sub-clinical characteristics of RSV pneumonia with bacterial co-infection in children

1.1. Clinical and sub-clinical characteristics of RSV pneumonia

- Pediatric patients (aged 1–24 months) with RSV pneumonia presented with 100% cough, 98,9% wheezing, 97,9% runny nose, 61,8% fever, and 67,8% chest depression. Pulmonary auscultation revealed 51,2% with both dry rales and moist rales, 25,4% with moist rales only, and 23,3% with dry rales and rhonchi. Saturation of peripheral oxygen (SpO2) ≤94% was noted in 76,3% of cases.
- Test results indicated 17,0% leukocytosis, 11,7% elevated neutrophil count, and 44.2% increased CRP. Chest X-rays showed 75,6% with diffuse opacities, 6,7% with pericardiac infiltrates, 6.0% with interstitial lesions and air trapping, 3,9% with upper lobe consolidation, and 7,8% with bronchial wall thickening.

1.2. Bacterial co-infection rate, clinical and sub-clinical characteristics of RSV pneumonia with bacterial co-infection

- 57,2% of RSV pneumonia cases had bacterial co-infections, with *H. influenzae* being the most common (53,4% detected by real-time PCR, 55,7% by culture).
- *H. influenzae* beta-lactamase production was 72,1%, with over 90% resistance to Ampicillin, Cefuroxime, and Cefaclor, while remaining highly susceptible to Cefotaxime and Ceftriaxone.
- The bacterial co-infection group had significantly higher rates of fever, wheezing, and crackles compared to the non-co-infected group ($p < 0,05$). Children with bacterial co-infection were 3,66 times more likely to have a high fever (95% CI: 2,21–6,06).
- The bacterial co-infection group showed higher leukocyte counts and neutrophil ratios ($p < 0,01$) than the non-co-infected group, while hemoglobin levels and platelet counts were similar ($p > 0,05$).
- CRP levels were 4,3 times higher in co-infected children than in non-co-infected cases (95% CI: 2,6–7,2).

- Chest X-ray tests of interstitial lesions, and air trapping were significantly more common in the bacterial co-infection group ($p < 0,05$).

2. Clinical efficacy and safety of *B. subtilis* và *B. clausii* probiotic in children RSV pneumonia with bacterial co-infection

- Nasal-spraying robiotic reduced the duration to relief of most clinical symptoms by 1 day compared to the physiological saline ($p < 0,05$) and improved 10–30% of typical symptoms of RSV pneumonia with bacterial co-infection.
- The probiotic group had a median of 2 days of oxygen therapy compared to 4 days in the physiological saline group ($p < 0,05$) and a median hospital stay of 6 days compared to 7 days in the physiological saline ($p < 0,05$).
- No local or systemic adverse events were recorded during probiotic nasal spray administration. Heart rate, respiratory rate, and SpO_2 remained within safe ranges before and after administration.

3. Reduction in RSV load, bacterial co-infection, and cytokine levels in nasopharyngeal samples after 3 days of probiotic nasal spray treatment

- Nasal-spraying probiotic reduced RSV loads 16-fold compared to saline spray after 3 days ($p < 0,0001$).
- The total bacterial co-infection load (*H. influenzae, S. pneumoniae, M. pneumoniae, B. pertussis*) decreased 4096-fold in the probiotic group, 16 folds higher than the control group (256-fold reduction) ($p < 0,0001$).
- IL-6 and IL-8 levels decreased 4,6- and 1,5-fold, respectively, in the probiotic group ($p < 0,05$), whereas the control group showed reductions of 3,5- and 1,1-fold ($p > 0,05$). TNF-α levels declined similarly in both groups ($p < 0,05$).
- IgA levels in the probiotic group increased 1,7-fold after 3 days ($p < 0,05$), compared to a 1.1-fold increase in the control group ($p > 0,05$).

RECOMMENDATIONS

1. Applying multiplex Realtime-PCR to diagnose more types of viruses and bacteria for faster results

2. Since *H. influenzae* and *S. pneumoniae* are the most common bacterial co-infectors in RSV pneumonia, antibiotic selection should cover these pathogens. Third-generation cephalosporins should be considered for RSV pneumonia cases suspected of bacterial co-infection.

3. Nasal-spraying probiotics should be used as supportive therapy in RSV pneumonia with bacterial co-infection to help alleviate symptoms and support patient recovery.