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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

THE CLINICAL AND DIAGNOSTIC SIGNIFICANCE OF THE METABOLIC ACTIVITY OF THE OROPHARYNGEAL AND INTESTINAL MICROBIOTA AND ITS ANTIBIOTIC SUSCEPTIBILITY IN CHILDREN IN THERAPY DEPARTMENTS

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Field of science: Medical Sciences

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GENERAL CHARACTERISTICS OF THE DISSERTATION

Relevance of the Research. Pneumonia in children is an infectious disease of considerable clinical significance. Its management is provided in outpatient or inpatient settings, depending on the clinical condition, severity of the disease, the child's age, and associated risk factors. Microbial colonization is detected in 12–20% of children hospitalized with pneumonia. Nosocomial pneumonia accounts for approximately 15–53% of all hospital-acquired infections[1].

Pneumonia is a severe infectious disease caused by bacteria, viruses, or other microorganisms and characterized by damage to the pulmonary alveoli. The mortality rate associated with pneumonia ranges from 15.5% to 38.2%[2].

At present, antibiotic therapy is widely used in the management of severe infections requiring intensive care. Treatment includes antibacterial agents with the broadest possible spectrum of activity. The causative agent of the infectious process and its susceptibility to antibiotics are determined through microbiological investigations. When the infectious agent cannot be identified, a high-dose course of broad-spectrum antibiotics, generally lasting 10–14 days, should be administered[3].

Ventilator-Associated Pneumonia (VAP) usually develops approximately 48 hours after endotracheal intubation and the initiation of mechanical ventilation (MV). Studies report that the incidence of VAP varies widely, ranging from 9% to 60%. In intubated patients, the risk of ventilator-associated pneumonia (VAP) increases by approximately 1–3% for each day of mechanical ventilation[4].

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Experts from the European Respiratory Society state that the younger the child, the broader and more diverse the range of potential bacterial pathogens responsible for pneumonia. Pathogens such as *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pyogenes*, methicillin-resistant *Staphylococcus epidermidis*, and *Escherichia coli* remain highly relevant. These microorganisms can cause severe ventilator-associated pneumonia, including destructive forms of pneumonia[5].

In children, the morphological and functional immaturity of organs and systems, prolonged hospitalization, and invasive procedures may facilitate the colonization of the mucous membranes of the upper respiratory tract and gastrointestinal tract by opportunistic microorganisms in the hospital environment, potentially leading to the subsequent development of an infectious process. As a result, clinical deterioration may occur in newborns older than 72 hours of life, while the intensive therapeutic interventions administered may contribute to the development of infection[6]. According to the World Health Organization (WHO), a healthcare-associated infection (HAI) refers to any infection associated with the provision of healthcare, regardless of the type of healthcare setting or service provided[7].

Numerous authors have demonstrated that prolonged mechanical ventilation predisposes patients to the development of nosocomial pneumonia. Some studies have reported that the uncontrolled use of antibiotics and the duration of hospitalization are stronger prognostic factors. This further confirms the multifactorial nature of hospital-acquired pneumonia[8].

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Currently, tracheal intubation and mechanical ventilation in intensive care and resuscitation units constitute major risk factors for the development of hospital-acquired pneumonia[9].

Several risk factors are associated with an increased likelihood of developing ventilator-associated pneumonia (VAP), including age, sex, prolonged mechanical ventilation, and prior antibiotic administration[10].

In addition to these risk factors, the state of the microbiome also plays an important role in the development of infectious processes. The presence of the physiological microbiome (i.e., the colonization of the skin surface and mucous membranes by microorganisms) is essential for human growth and development, as well as for the proper establishment of metabolic and immune homeostasis[11].

Adherent microbial colonies on the intestinal wall form a microbe–tissue complex comprising bacterial microcolonies, the metabolites they synthesize, mucin, the glycocalyx, epithelial cells, and stromal cells of the mucous membrane. Within this complex, there is continuous exchange of genetic material, signaling and regulatory molecules, as well as metabolites[12]. One of the most important low-molecular-weight metabolites is short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate. These substances influence the adhesion of pathogenic and opportunistic microorganisms, local immune responses, and the proliferation and differentiation of colonocytes. They also participate in the regulation

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of ion exchange, microcirculation, and mucus secretion, provide energy for epithelial cells, and reflect various physiological processes occurring in the colon[13].

Considering the above, it is necessary to investigate the metabolic activity, clinical and diagnostic significance, and antibiotic susceptibility of the oropharyngeal and intestinal microbiota in children receiving treatment in intensive care units.

Object and subject of the research. The study included 350 children and adolescents who developed nosocomial respiratory tract and gastrointestinal infections at least 48 hours after hospital admission respiratory tract infections were observed in 200 children, while gastrointestinal tract infections were identified in 150 children. The subject of the study was the microbiological status of nosocomial respiratory tract and gastrointestinal infections in children receiving intensive care, the structural and metabolic parameters of their microbiocenosis, and the analysis of the resistance profiles of the isolated microorganisms.

Aim of the Study: To evaluate the composition, colonization characteristics, and metabolic activity of the oropharyngeal and gastrointestinal microbiota in children of different ages admitted to intensive care units, as well as their clinical and diagnostic significance and the antibiotic susceptibility of the isolated microorganisms.

Objectives of the Study:

1. To determine and analyse the age-specific prevalence of concurrent hospital-acquired respiratory tract and gastrointestinal infections in children;
2. To determine the structure and prevalence of comorbidities in children of different age groups with concurrent respiratory tract and gastrointestinal infections in the intensive care unit, and assessment of their role in the infectious process;
3. To determine the frequency and structure of colonization by various microorganisms in infectious foci samples across the study groups.

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4. To study the colonization levels of opportunistic and nosocomial microorganisms, including *P. aeruginosa, A. baumannii, K. pneumoniae,* and *S. maltophilia*, in oropharyngeal and rectal swab samples obtained from children with respiratory and gastrointestinal infectious complications in hospital settings.

5. To assess the metabolic activity of the oropharyngeal and intestinal microbiota by studying changes in the levels of short-chain fatty acids, urease/proteolytic activity, and metabolic markers such as lactate;

6. To determine the antibiotic susceptibility of microorganisms isolated from respiratory infection foci to various antibiotics;

7. To determine early diagnostic and prognostic criteria for infectious complications in children receiving intensive care based on indicators of microbiota metabolic activity, colonization levels, and antibiotic resistance.

Research Methods: Microbiological examinations were performed in the children included in the study, including bacteriological analysis of oropharyngeal and rectal swabs, assessment of the colonization level and metabolic activity of microorganisms, and determination of their susceptibility to antibiotics.

Main Provisions of the Dissertation Submitted for Defense:

1. Emergency and urgent intubation, prolonged mechanical ventilation via an endotracheal tube, repeated intubations, and contaminated ventilator equipment increase the risk of ventilator-associated pneumonia (VAP).

2. It has provided valuable information on the existence of associations between the intestinal microbiota and certain diseases, while a number of both exogenous and patient-related endogenous factors may contribute to the development of dysbiosis acquired in the intensive care unit.

3. Continuous surveillance of the various rotavirus strains currently circulating will make a significant contribution to evaluating the effectiveness of these vaccines and facilitating the development of new vaccines.

4. Microorganisms colonizing the upper respiratory tract may originate from the oropharynx, paranasal sinuses, nose, and dental plaque. In the intensive care unit, potential exogenous sources inclu-

de a contaminated environment, contaminated medical equipment, enteral feeding, aerosols, and neighboring patients in the ward.

Scientific Novelty of the Study:

-The epidemiological characteristics, age-specific incidence, and microbiological associations of concomitant nosocomial infectious complications involving the respiratory and gastrointestinal systems were comprehensively studied in children and adolescents receiving treatment in the intensive care unit.

- The structural characteristics of concomitant pathologies observed in the intensive care unit and their impact on the severity of the infectious process in the development of concomitant respiratory and gastrointestinal infectious complications were determined.

- The metabolic activity of the oropharyngeal and intestinal microbiota in children admitted to the intensive care unit was comprehensively investigated, and the patterns of changes in metabolic markers, including short-chain fatty acid levels, urease and proteolytic activity, and lactate levels, were determined.

- An association was established between the metabolic activity of the microbiota and the severity of nosocomial infectious processes, the level of colonization, and antibiotic resistance indicators.

- The effect of the duration of mechanical ventilation on the time of onset and duration of hospital-acquired respiratory and gastrointestinal infections in children belonging to different age groups was evaluated.

- The susceptibility and resistance profiles of microorganisms isolated from hospital-acquired infectious foci to various antibiotics were determined, and the characteristics of antibiotic resistance among nosocomial strains in intensive care settings were identified.

- Based on the microbiological, metabolic, and antibiotic susceptibility findings obtained, early diagnostic and prognostic criteria for nosocomial infectious complications in children admitted to the intensive care unit were developed.

Practical significance of the study:

- It was determined that the risk of developing respiratory and gastrointestinal infectious complications in the intensive care unit is particularly high among children in the younger age groups. The-

refore, strengthening infection control measures and implementing intensive microbiological monitoring in these patients is necessary.

- During hospital-acquired infectious processes, an increase in the metabolic and colonization activity of microorganisms such as *Candida albicans, Clostridium spp., Corynebacterium spp., Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus spp., Pseudomonas aeruginosa*, and *Staphylococcus aureus,* in addition to the primary etiological pathogens, was identified. Taking these characteristics into account enables diagnostic errors to be minimized when interpreting laboratory results and facilitates the more accurate detection of concomitant nosocomial infections.

- A concomitant increase in the colonization and metabolic activity of *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia* was identified in both the oropharyngeal and intestinal environments of children with respiratory and gastrointestinal infectious complications. This finding indicates that nosocomial infectious processes may have a systemic nature and provides a basis for a new clinical approach to assessing the potential for multisystem dissemination of infection.

- A comprehensive assessment of the metabolic activity of the microbiota, colonization levels, and antibiotic susceptibility indicators was of practical importance for the early diagnosis of infectious complications in intensive care units, identification of high-risk groups, and selection of appropriate antimicrobial therapy.

- It was determined that the inclusion of probiotic preparations in conventional treatment regimens contributes to reducing the duration of respiratory and gastrointestinal infections and accelerating clinical improvement.

Approbation of the Study:

The results of the study were presented and discussed at the following conferences:

- Наука и инновации-современные концепции (Москва 2022), Актуальные вопросы образования и науки (Россия, Тамбов 2022);

- International Apitherapy and Nature Congress İANCO23 (Naxçıvan 2023);

- The Impact of Climate Change on Health (Nakhchivan, 2024); Collection of Abstracts of the Medical Festival Dedicated to the 102nd Anniversary of National Leader Heydar Aliyev and the 95th Anniversary of Azerbaijan Medical University (Baku, 2025);

-Сборник статей поматериалам XCVI международнойнаучно-практической конференции “Современная медицина:новые подходы и актуальные исследования (Москва 2025), LXXXIII Международной научно-практической конференции «Естественные науки и медицина: теория и практика» (Россия г. Новосибирск 2025);

- International Conference Dedicated to the 100th Anniversary of the Department of Medical Microbiology and Immunology of Azerbaijan Medical University (Baku, 2026).

The preliminary discussion of the dissertation was conducted at an extended meeting of the Departments of Clinical Subjects and Military Medicine, Basic Medical Sciences, and Biology of Nakhchivan State University (March 11, 2025; Protocol No. 3), as well as at a meeting of the Discussion Council conducting scientific seminars in the specialty 2414.01—Microbiology at Azerbaijan Medical University (June 10, 2026; Protocol No. 2).

Application of the Research Findings in Practice

The findings of the study are being implemented in the examination and treatment of patients in the Resuscitation and Intensive Care Unit of the Aziz Aliyev Central Children's Hospital of the Nakhchivan Autonomous Republic, a public legal entity, as well as in the research plan and educational process of the Department of Basic Medical Sciences at Nakhchivan State University.

Organization Where the Dissertation Was Conducted

The dissertation was conducted at the Department of Basic Medical Sciences of Nakhchivan State University.

Published Scientific Works

According to the research findings, 14 scientific works have been published, including 6 scientific articles and 8 conference abstracts.

Structure and Volume of the Dissertation. The dissertation was written in Azerbaijani in A4 format, using 14-point Times New Roman font and 1.5 line spacing. Dissertation contains 161 pages (223.056 characters in total) including Introduction (18.704 characters), Chapter

I,Literature review (64.330characters), Chapter II, Materials and Methods of the Study (17.720 characters), Chapter III, comprising a comparative analysis of sociodemographic and clinical-microbiological parameters across the study groups (79.470 characters), Chapter IV, Development of Clinical and Microbiological Criteria for the Early Diagnosis and Prediction of Infectious Complications (20.972 characters), Discussion (17.951 characters), Conclusions (2.678 characters), practical recommendations (1.231 characters).Dissertation has been illustrated with 15 graphs and 28 tables. The reference list comprises 141 sources, including 8 local sources,21 Russian-language sources, and 112 studies conducted by foreign scholars.

MATERIALS AND METHODS OF THE RESEARCH

The study was conducted between 2018 and 2022 in the Department of Resuscitation and Intensive Care of the Central Children's Hospital of the Nakhchivan Autonomous Republic named after Aziz Aliyev. The study included 350 children aged 0–13 years who developed nosocomial infections at least 48 hours after hospital admission. Of these, 200 had hospital-acquired respiratory tract infections, while 150 had hospital-acquired gastrointestinal tract infections. The patients were divided into five age groups: under 2 years, 2–4 years, 5–6 years, 7–10 years, and 11–13 years.

The study included children receiving treatment in the Department of Resuscitation and Intensive Care whose nosocomial infections had been confirmed by clinical and laboratory examinations and whose medical records were complete. Children with active infections prior to hospital admission, severe congenital immunodeficiency, incomplete medical records, or without parental consent were excluded from the study.

All patients underwent clinical examinations, complete blood and urinalysis, biochemical and microbiological investigations, bacteriological analysis of oropharyngeal and rectal swabs, assessment of the colonization levels and metabolic activity of microorganisms, and antimicrobial susceptibility testing. Instrumental and radiological examinations were performed when clinically indicated.

A total of 53 patients were selected to evaluate the clinical efficacy of Enterogermina, a probiotic containing Bacillus clausii (Opella Healthcare Italy S.r.l., Italy). Among the 25 children with nosocomial respiratory tract infections, 14 received standard treatment, while 11 received a combination of standard treatment and the probiotic. The 28 children with gastrointestinal tract infections were divided into two equal subgroups: 14 patients received standard treatment, and the remaining 14 received standard treatment supplemented with the probiotic.

Statistical analysis was performed using Statistica 7.0 software. Quantitative variables were presented as the mean (M), standard error of the mean (m), minimum and maximum values, percentages, and 95% confidence intervals. Intergroup comparisons were performed using Student's t-test, the Mann–Whitney U test, and Pearson's chi-square (χ^2) test. ROC analysis was conducted to determine the sensitivity, specificity, and optimal cut-off point of diagnostic indicators. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS OF THE STUDY AND THEIR DISCUSSION

The children included in the study were divided into age groups (Table 1).

Table 1.

The age intervals in the study groups were as follows:

Age	Respiratory infection (n=200)		Gastrointestinal infection (n=150)	
	Number	%	Number	%
0-2	125	62,5	87	58,0
2-4	26	13,0	22	14,7
5-6	19	9,5	16	10,7
7-10	21	10,5	20	13,3
11-13	9	4,5	5	3,3
Total	200	100,0	150	100,0

In children with hospital-acquired respiratory tract infections, ENT pathologies accounted for 20.0%, chronic conditions for 9.0%, allergic conditions for 4.5%, malabsorption syndrome for 7.5%,

dysbiosis for 3.0%, and functional gastrointestinal disorders for 7.5%. In children with hospital-acquired gastrointestinal tract infections, chronic ENT pathologies accounted for 10.0%, chronic conditions affecting other systems for 6.7%, allergic conditions for 9.3%, malabsorption syndrome for 22.7%, dysbiosis for 13.3%, and functional gastrointestinal disorders for 23.3% ($p = 0.0110$).

According to the results of virological examinations, the following viruses were identified in patients with respiratory tract infections: rhinoviruses in 67.5%, rotaviruses in 20.5%, and influenza viruses in 12.0%. Among children with gastrointestinal tract infections, rotaviruses were detected in 78.7%, enteroviruses in 15.3%, and influenza viruses in 6.0% ($\chi^2 = 199.03$; $p < 0.0001$).

The species composition and frequency of occurrence of bacterial and fungal microorganisms isolated from the study groups were analysed (Table 2).

Table 2

Species Composition and Frequency of Occurrence of Microorganisms Detected in Patients with Nosocomial Respiratory and Gastrointestinal System Infections

Microorganisms	Respiratory infection (n=200)		Gastrointestinal infection (n=150)	
	n	%	n	%
C.albicans	5	2,5	5	3,33
Clostridium spp.	2	1	-	-
Corynebacterium spp.	3	1,5	-	-
E.faecalis	7	3,5	10	6,67
E.coli	29	14,5	21	14,00
K.pneumoniae	47	23,5	32	21,33
Micrococcus spp.	2	1	-	-
Ps.aeruginosa	83	41,5	65	43,33
S.aureus	17	8,5	11	7,33
S.epidermidis	5	2,5	6	4,00

In the group of patients with respiratory tract infections, the following microorganisms were identified: *C. albicans* (2.5%), *Clostridium spp*. (1.0%), *Corynebacterium spp.* (1.5%), *E. faecalis* (3.5%), *E. coli*

(14.5%), *K. pneumoniae* (23.5%), *Micrococcus spp*. (1.0%), *Ps. aeruginosa* (41.5%), *S. aureus* (8.5%), and *S. epidermidis* (2.5%).

In the group of patients with gastrointestinal tract infections, the following microorganisms were identified: *C. albicans* (3.33%), *E. faecalis* (6.67%), *E. coli* (14.00%), *K. pneumoniae* (21.33%*), Ps. aeruginosa* (43.33%), S. aureus (7.33%), and *S. epidermidis* (4.00%). *Clostridium spp., Corynebacterium spp.,* and *Micrococcus spp*. were not detected in this group.

Among patients with hospital-acquired respiratory tract infections, *Pseudomonas aeruginosa* was detected in 47.5% of cases, *Acinetobacter baumannii* in 46.0%, *Klebsiella pneumoniae* in 36.5%, and *Stenotrophomonas maltophilia* in 28.0%.

Among children with hospital-acquired gastrointestinal tract infections, *Pseudomonas aeruginosa* was identified in 62.67% of cases, *Acinetobacter baumannii* in 50.67%, *Klebsiella pneumoniae* in 31.33%, and *Stenotrophomonas maltophilia* in 7.33%.

Co-detection of multiple microorganisms was observed in 20 patients with hospital-acquired respiratory tract infections. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were detected together in 25.0% of cases, while *Klebsiella pneumoniae* and *Stenotrophomonas maltophilia* were co-detected in 15.0%.

The following combinations were each detected in 10.0% of cases: *A. baumannii* and *K. pneumoniae, A. baumannii* and *S. maltophilia,* and *P. aeruginosa and K. pneumoniae.* The combination of *P. aeruginosa* and *S. maltophilia* was observed in 5.0% of cases.

The simultaneous detection of three microorganisms was observed in the following combinations: *A. baumannii, P. aeruginosa,* and *S. maltophilia* in 5.0% of cases; P. *aeruginosa, S. maltophilia*, and *K. pneumoniae* in 10.0%; and A. baumannii, S. maltophilia, and *K. pneumoniae* in 5.0%. Four microorganisms—*A. baumannii, P. aeruginosa, S. maltophilia,* and *K. pneumoniae*—were detected simultaneously in 5.0% of cases.

Associative combinations of microorganisms were identified in 20 patients with hospital-acquired gastrointestinal tract infections. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were detected together in 30.0% of cases, while *Acinetobacter baumannii* and

P.aeruginosa, and *A. baumannii* and *K. pneumoniae,* were each identified in 20.0% of cases.

The simultaneous detection of three microorganisms was recorded in the following combinations*: A. baumannii, P. aeruginosa,* and *K. pneumoniae* in 10.0% of cases; *A. baumannii, Stenotrophomonas maltophilia,* and *K. pneumoniae* in 10.0%; and *P. aeruginosa, S. maltophilia*, and *K. pneumoniae* in 10.0%.

The timing of onset and duration of hospital-acquired infectious complications among patients in the Department of Resuscitation and Intensive Care were analysed.

Among the 20 patients with hospital-acquired respiratory tract infections, the duration of mechanical ventilation was up to 5 days in 4 patients (20.0%), 5–10 days in 11 patients (55.0%), and more than 10 days in 5 patients (25.0%). Among the 15 patients with gastrointestinal tract infections, mechanical ventilation lasted up to 5 days in 3 patients (20.0%), 5–10 days in 9 patients (60.0%), and more than 10 days in 3 patients (20.0%).

Patients with respiratory tract and gastrointestinal tract infections were subdivided into age-based subgroups to analyse the number of days from hospital admission to infection onset and the duration of infection.

Among patients with respiratory tract infections, the time from hospital admission to the onset of the infectious process ranged from 2.11 ± 0.098 to 2.33 ± 0.220 days across the age subgroups. The lowest value was recorded in patients aged 5–6 years (2.11 ± 0.098 days), while the highest was observed in children aged 2–4 years (2.33 ± 0.220 days). The duration of the infectious process was 3.11 ± 0.063 days in the 11–13-year age group, 4.33 ± 0.185 days in the 7–10-year age group, 5.16 ± 0.206 days in the 5–6-year age group, 5.22 ± 0.472 days in the 2–4-year age group, and 5.27 ± 0.200 days in children under 2 years of age. Thus, a decrease in the duration of the infectious process was observed with increasing age.

Among patients with gastrointestinal tract infections, the time to onset of the infectious process ranged from 2.05 ± 0.064 to 2.60 ± 0.289 days. The highest value was recorded in the 2–4-year age group (2.60 ± 0.289 days), whereas the lowest was observed in the 11–13-year age

group (2.05 ± 0.064 days). The duration of infection was 5.75 ± 0.273 days in children under 2 years of age, 6.70 ± 0.382 days in the 2–4-year age group, 5.25 ± 0.514 days in the 5–6-year age group, 4.80 ± 0.231 days in the 7–10-year age group, and 3.43 ± 0.113 days in the 11–13-year age group. A similar decrease in the duration of the infectious process with increasing age was observed in this group.

The detection of nonspecific symptoms and their duration were determined according to age in the study groups.

The frequency of clinical symptoms in children and adolescents with hospital-acquired respiratory tract infections was analysed across the age subgroups. The study included 125 children under 2 years of age, 26 children aged 2–4 years, 19 children aged 5–6 years, 21 children aged 7–10 years, and 9 patients aged 11–13 years.

A loss of more than 10% of body weight was observed in 48 children under 2 years of age (38.4%), 8 children in the 2–4-year age group (30.8%), 7 children in the 5–6-year age group (36.8%), 8 children in the 7–10-year age group (38.1%), and 3 patients in the 11–13-year age group (33.3%). The highest frequency of this finding was recorded in the under 2 years of age (38.4%), whereas the lowest was observed in the 2–4-year age group (30.8%).

An increased heart rate was one of the most common symptoms across all age groups. This sign was identified in 77 children under 2 years of age (61.6%), 18 children aged 2–4 years (69.2%), 12 children aged 5–6 years (63.2%), 13 children aged 7–10 years (61.9%), and 6 patients aged 11–13 years (66.7%). The highest frequency was observed in the 2–4-year age group (69.2%), while the lowest was recorded in children under 2 years of age (61.6%).

A body temperature exceeding 39°C was predominant across all age subgroups. This symptom was recorded in 116 children under 2 years of age (92.8%), 23 children aged 2–4 years (88.5%), 17 children aged 5–6 years (89.5%), 19 children aged 7–10 years (90.5%), and 8 patients aged 11–13 years (88.9%). Thus, high fever was most frequently observed in children under 2 years of age (92.8%).

A body temperature ranging from 37.5°C to 39°C was observed less frequently. This finding was identified in 9 children under 2 years of age (7.2%), 3 children aged 2–4 years (11.5%), 2

children aged 5–6 years (10.5%), 2 children aged 7–10 years (9.5%), and 1 patient aged 11–13 years (11.1%).

The frequency of clinical symptoms in children and adolescents with hospital-acquired gastrointestinal tract infections was analysed across the age subgroups. The study included 87 children under 2 years of age, 22 children aged 2–4 years, 16 children aged 5–6 years, 20 children aged 7–10 years, and 5 patients aged 11–13 years.

A loss of more than 10% of body weight was observed in 28 children under 2 years of age (32.2%), 6 children in the 2–4-year age group (27.3%), 5 children in the 5–6-year age group (31.3%), 6 children in the 7–10-year age group (30.0%), and 2 patients in the 11–13-year age group (40.0%). The highest frequency of this symptom was recorded in the 11–13-year age group (40.0%), while the lowest was observed in the 2–4-year age group (27.3%).

An increased heart rate was common across all age groups. This sign was identified in 59 children under 2 years of age (67.8%), 16 children aged 2–4 years (72.7%), 11 children aged 5–6 years (68.7%), 14 children aged 7–10 years (70.0%), and 3 patients aged 11–13 years (60.0%). The highest frequency was observed in the 2–4-year age group (72.7%), whereas the lowest was recorded in the 11–13-year age group (60.0%).

A body temperature exceeding 39°C was one of the most frequently observed symptoms in the study groups. This finding was recorded in 79 children under 2 years of age (90.8%), 16 children aged 2–4 years (72.7%), 15 children aged 5–6 years (93.8%), 18 children aged 7–10 years (90.0%), and 5 patients aged 11–13 years (100%). Thus, the highest frequency was observed in the 11–13-year age group. However, as this subgroup included only five patients, the results should be interpreted with caution.

A body temperature ranging from 37.5°C to 39°C was recorded in 8 children under 2 years of age (9.2%), 6 children aged 2–4 years (27.3%), 1 child aged 5–6 years (6.2%), and 2 children aged 7–10 years (10.0%). No cases within this temperature range were recorded in the 11–13-year age group. The highest frequency of this temperature range was observed in the 2–4-year age group (27.3%).

When body temperature exceeded 39°C, the duration was 5.69 days in patients with gastrointestinal tract infections and 4.65 days in those with respiratory tract infections. A similar difference was observed in cases where body temperature ranged from 37.5°C to 39°C, with corresponding durations of 5.18 and 4.52 days, respectively.

Microbiological examinations were conducted to assess the susceptibility of pathogens to antibacterial agents in cases of hospital-acquired respiratory tract infections.

Among the microorganisms examined, *Staphylococcus epidermidis, Staphylococcus aureus,* and *Klebsiella pneumoniae* were predominant. Among *Staphylococcus epidermidis* strains, susceptibility to ampicillin was 50.0%, ciprofloxacin 40.0%, oxacillin 25.0%, vancomycin and erythromycin 15.0%, amoxicillin, rifampicin, and lomefloxacin 10.0%, and gentamicin and neomycin 5.0%. No susceptibility to imipenem was recorded.

Among *Staphylococcus aureus* strains, the highest susceptibility was observed to ciprofloxacin (75.0%) and lomefloxacin (55.0%). Susceptibility to oxacillin and erythromycin was 45.0%, to ampicillin 40.0%, rifampicin 30.0%, vancomycin 25.0%, gentamicin 20.0%, amoxicillin 15.0%, neomycin 10.0%, and imipenem 5.0%.

Among *Klebsiella pneumoniae* strains, susceptibility to rifampicin was 90.0%, gentamicin 85.0%, ciprofloxacin 80.0%, lomefloxacin 65.0%, and neomycin 50.0%.

Overall, the findings indicate that susceptibility to antibacterial agents varies depending on the microorganism species.

To evaluate the effects of different treatment regimens on the duration of respiratory symptoms, outcomes were compared between patients receiving standard treatment alone and those receiving standard treatment supplemented with a probiotic. The analysis included 25 patients with respiratory tract infections and 28 patients with gastrointestinal tract infections.

In patients with respiratory tract infections, the addition of a probiotic to standard treatment was associated with a reduction in the duration of severe cough from 4.71 ± 0.322 to 3.82 ± 0.296 days, respiratory insufficiency symptoms from 2.86 ± 0.231 to 2.73 ± 0.304 days, sore throat from 3.36 ± 0.199 to 3.09 ± 0.285 days, and

profuse sputum production from 5.43 ± 0.228 to 5.09 ± 0.211 days. However, the duration of nasal discharge increased from 2.36 ± 0.225 days with standard treatment to 2.55 ± 0.207 days with probiotic supplementation.

In patients with gastrointestinal tract infections, probiotic supplementation was associated with a reduction in the duration of severe nasal discharge from 3.86 ± 0.206 to 3.43 ± 0.173 days, severe cough from 5.36 ± 0.372 to 4.57 ± 0.228 days, and respiratory insufficiency symptoms from 1.57 ± 0.173 to 1.43 ± 0.137 days. The duration of sore throat decreased from 4.14 ± 0.206 to 3.43 ± 0.202 days, while the duration of profuse sputum production decreased from 7.29 ± 0.244 to 5.43 ± 0.251 days.

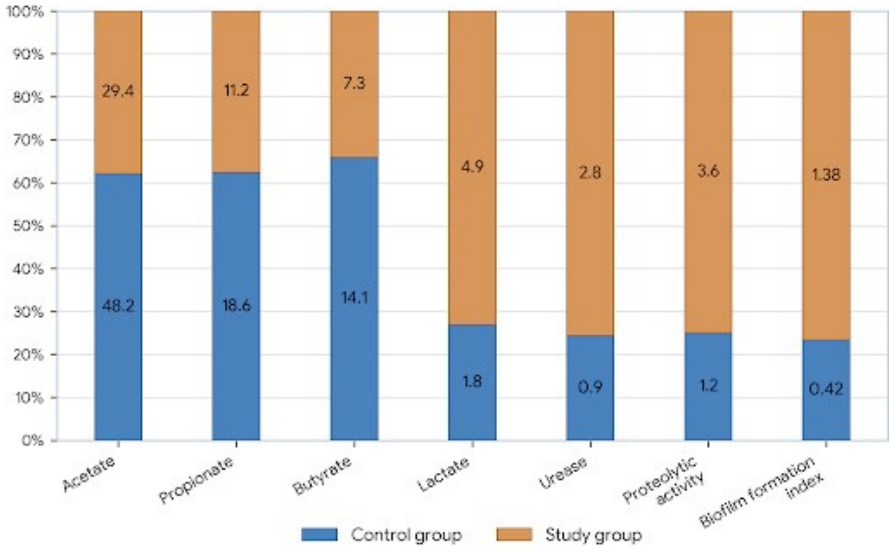
Thus, the addition of a probiotic to standard treatment was associated with a reduction in the duration of several respiratory symptoms in both groups.

In patients with respiratory tract infections, the addition of a probiotic to standard treatment reduced the duration of diarrhoea from 3.79 ± 0.318 to 3.36 ± 0.244 days, the duration of toxicosis from 1.29 ± 0.125 to 1.18 ± 0.122 days, and the duration of abdominal pain from 4.86 ± 0.275 to 4.64 ± 0.152 days.

In patients with gastrointestinal tract infections, probiotic supplementation was associated with a reduction in the duration of vomiting from 1.57 ± 0.137 to 1.36 ± 0.169 days, diarrhoea from 4.36 ± 0.225 to 3.71 ± 0.163 days, and toxicosis from 1.43 ± 0.137 to 1.21 ± 0.114 days. The duration of nausea decreased from 4.14 ± 0.206 to 3.43 ± 0.202 days, while the duration of abdominal pain decreased from 7.29 ± 0.244 to 5.43 ± 0.251 days.

Thus, the addition of a probiotic to standard treatment was associated with a reduction in the duration of gastrointestinal symptoms, particularly nausea and abdominal pain, in patients with gastrointestinal tract infections.

The metabolic activity of the oropharyngeal and intestinal microbiota was assessed. For this purpose, the levels of short-chain fatty acids (acetate, propionate, and butyrate), lactate concentration, urease and proteolytic activity, and biofilm-forming potential were evaluated.



Graph 1. Graphical Representation of the Metabolic Activity Indicators of the Oropharyngeal and Intestinal Microbiota

A comparative analysis of the metabolic activity of the oropharyngeal and intestinal microbiota revealed marked differences between the control and study groups. Compared with the control group, the study group exhibited lower levels of short-chain fatty acids, alongside increased lactate concentrations, urease and proteolytic activity, and biofilm formation index.

Specifically, the acetate level was 48.2 ± 3.1 mmol/L in the control group, compared with 29.4 ± 2.8 mmol/L in the study group. Propionate levels were 18.6 ± 1.4 and 11.2 ± 1.1 mmol/L, respectively, while butyrate levels were 14.1 ± 1.2 and 7.3 ± 0.8 mmol/L. Thus, acetate levels decreased by approximately 39%, propionate by 40%, and butyrate by 48% in the study group.

In contrast, the lactate concentration increased from 1.8 ± 0.2 mmol/L in the control group to 4.9 ± 0.4 mmol/L in the study group. Urease activity increased from 0.9 ± 0.1 units/mL to 2.8 ± 0.3 units/mL, while proteolytic activity rose from 1.2 ± 0.2 units/mL to 3.6 ± 0.4 units/mL. The biofilm formation index also increased from

0.42 ± 0.03 in the control group to 1.38 ± 0.09 in the study group, representing an approximately 3.3-fold increase.

Thus, the study group demonstrated a reduction in acetate, propionate, and butyrate levels, which are indicators of beneficial microbial metabolic activity, accompanied by increased lactate concentrations, urease and proteolytic activity, and biofilm formation index.

Development of a Programme for the Metabolic-Prognostic Assessment of Nosocomial Infectious Complications

Based on the results of the clinical, microbiological, and metabolic investigations, an original programme entitled "Metabolic-Prognostic Assessment of Nosocomial Infectious Complications" was developed for the early diagnosis and prediction of nosocomial infectious complications in children receiving treatment in the Department of Resuscitation and Intensive Care. The primary objectives of the programme were the early identification of children at high risk, the prevention of complications, and the optimisation of individualised antimicrobial therapy.

A scoring system was established in which each parameter was assigned a specific score according to its prognostic significance for the development of nosocomial infectious complications (Table 3).

Table 3
Risk Scale of the "Metabolic-Prognostic Assessment of Nosocomial Infectious Complications" Program

Indicator	Point
ASV >10 days	3
High biofilm formation index	3
Carbapenem resistance	3
High colonization level	2
Lactate>4 mmol/L	2
Increased ureasea ctivity	2
Increased proteolytic activity	2
Decreased butyrate level	1
Recurrent hospitalization	1
Long-term antibiotic therapy	1

Risk interpretation:

- 0–3 points- low risk
- 4–7 points - moderate risk
- ≥8 points - high risk

ROC analysis demonstrated that the program had high diagnostic efficacy.

Table 4

Diagnostic Performance Indicators of the "Metabolic-Prognostic Assessment of Nosocomial Infectious Complications" Program

Indicator	Result
Sensitivity	88,4%
Specificity	81,7%
AUC	0,89
Positive predictive value	84,2%
Negative predictive value (NPV)	86,9%
Overall diagnostic accuracy	85,6%

Based on the results obtained, it was established that the biofilm formation index, colonisation level, and carbapenem resistance were the strongest prognostic components of the programme. In particular, high metabolic activity and elevated biofilm indices in *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains were associated with a severe clinical course and prolonged hospitalisation.

Furthermore, a decrease in short-chain fatty acids and an increase in lactate levels reflected destabilisation of the intestinal microbiota and increased the risk of developing a systemic inflammatory response.

Algorithm of the Programme for the Metabolic-Prognostic Assessment of Nosocomial Infectious Complications

Stage I: Initial Clinical Assessment

The following risk factors are identified:

- Duration of mechanical ventilation;
- Length of hospitalisation;
- Number of invasive procedures;
- Previous antibiotic therapy.

Stage II: Microbiological Monitoring

Oropharyngeal and rectal swabs are collected to determine:

- Colonisation level;
- Identification of nosocomial flora;
- Antibiotic resistance.

Stage III: Assessment of Metabolic Activity

The following parameters are assessed:

- Lactate level;
- Urease activity;
- Proteolytic activity;
- Biofilm index;
- Acetate, propionate, and butyrate levels.

Stage IV: Calculation of the Risk Score

All parameters are scored in accordance with the scale developed for the Programme for the Metabolic-Prognostic Assessment of Nosocomial Infectious Complications.

Stage V: Risk Stratification

- 0–3 points: Low risk.
- 4–7 points: Moderate risk.
- ≥8 points: High risk.

In patients at high risk, microbiological monitoring is intensified, and probiotic/prebiotic intervention is recommended. Metabolic activity and colonisation are monitored closely to facilitate the development of an early intensive antimicrobial strategy.

Thus, the developed Programme for the Metabolic-Prognostic Assessment of Nosocomial Infectious Complications demonstrated high effectiveness in the early detection and prediction of nosocomial infectious complications in children of different age groups admitted to the intensive care unit.

CONCLUSION

1. In the group of patients who developed infectious complications of the respiratory system after hospital admission, children aged up to 2 years accounted for 125 patients, representing 62.5% of the total cohort and constituting the highest proportion [8, 9].

2. In the group of patients who developed respiratory infections (n = 200), active rotavirus infection was detected in 41 cases, accounting for 20.5 ± 2.85% of the patients in this group.

3. Among children and adolescents who developed infectious complications of the respiratory system under hospital conditions, 8.5% of cases were associated with the activity of *S. aureus*, while complications of the gastrointestinal system were associated with *S. aureus* activity in 7.33% of cases. For *K. pneumoniae*, the corresponding rates were 36.5% in the first group and 31.33% in the second group [8, 9].

4. In the study group of children with hospital-acquired secondary infection of the gastrointestinal system, simultaneous colonization by *A. baumannii*, *S. maltophilia*, and *K. pneumoniae* was detected in two rectal swab samples, accounting for 10.0 ± 6.71% of the total selected cohort in the group[8].

5. During the assessment of the metabolic activity of the oropharyngeal and intestinal microbiota, a statistically significant decrease in short-chain fatty acid levels was identified in children with nosocomial infectious complications. Specifically, acetate levels decreased from 48.2 ± 3.1 mmol/L to 29.4 ± 2.8 mmol/L, propionate levels from 18.6 ± 1.4 mmol/L to 11.2 ± 1.1 mmol/L, and butyrate levels from 14.1 ± 1.2 mmol/L to 7.3 ± 0.8 mmol/L ($p < 0.001$). At the same time, an increase in lactate levels from 1.8 ± 0.2 mmol/L to 4.9 ± 0.4 mmol/L, urease activity from 0.9 ± 0.1 to 2.8 ± 0.3 units/mL, and proteolytic activity from 1.2 ± 0.2 to 3.6 ± 0.4 units/mL indicated metabolic destabilization of the microbiota ($p < 0.001$).

6. When assessing the antibiotic susceptibility of microorganisms isolated from respiratory infection foci, *Klebsiella pneumoniae* demonstrated high levels of resistance. Susceptibility to rifampicin among *Klebsiella pneumoniae* strains was 90.0%, while susceptibility to gentamicin was 85.0%. The low susceptibility of *Staphylococcus aureus* strains to oxacillin suggested the presence of methicillin-resistant forms[7, 8].

7. A comprehensive analysis of microbiota metabolic activity, colonization levels, and antibiotic resistance indicators identified the main risk factors for nosocomial infectious complications in children receiving intensive care. High colonization levels (OR =

6.1), a high biofilm index (OR = 5.2), a duration of mechanical ventilation of >10 days (OR = 4.8), carbapenem resistance (OR = 4.4), increased urease activity (OR = 3.9), and decreased levels of short-chain fatty acids (OR = 3.2) were statistically significantly associated with the development of infectious complications. The developed metabolic-prognostic programme demonstrated high diagnostic efficacy, with a sensitivity of 88.4%, specificity of 81.7%, and an AUC of 0.89.

PRACTICAL RECOMMENDATIONS

1. In children at risk of nosocomial infectious complications, the colonization levels of microorganisms such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia* should be assessed regularly.

2. Children with particularly high colonization levels, elevated biofilm formation indices, and carbapenem resistance should be classified as a high-risk group, and their antimicrobial treatment should be administered under more rigorous microbiological monitoring.

3. The assessment of the metabolic activity of the oropharyngeal and intestinal microbiota—including lactate levels, urease and proteolytic activity, short-chain fatty acids, and the biofilm index—can be used as an additional practical marker for the early diagnosis and prediction of nosocomial infections.

4. The “Metabolic-Prognostic Assessment Program for Nosocomial Infectious Complications” can be applied as a practical tool for identifying risk groups, planning early preventive measures, and selecting appropriate treatment strategies in children receiving intensive care.

5. For the prevention of infectious complications, it is advisable to implement a comprehensive approach involving the rational use of antibiotics, minimization of the duration of invasive interventions, colonization monitoring, and microbiota-targeted corrective measures.

The main content of the dissertation has found its reflection in the works published by the author:

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CONVENTIONAL SIGNS

VAP – Ventilator-associated pneumonia
ICU – Intensive Care Unit
MV – Mechanical ventilation
MV – Mechanical ventilation of the lungs



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