ORIGINAL ARTICLE

CONTENTS

Cytomorphological characteristics of the nasopharyngeal mucosa in children with acute respiratory infections

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ABSTRACT

Aim: This study aims to investigate the relationships between cytomorphological markers of pathological processes and the development of acute respiratory infections in children.

Materials and Methods: A microbiological study was conducted to identify pathogens based on their morphological, cultural, and biochemical properties using nasopharyngeal swabs. Pure culture isolation and subsequent colonization were performed on standard nutrient media. Cytological studies were carried out using electron microscopy.

Results: A total of 114 strains of conditionally pathogenic microorganisms were identified, including 33 strains (29.0%) of Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71.0%) of Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli). Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%) were the predominant pathogens. The study identified key metabolic markers, including sucrose (n=69), maltase (n=87), and lactoperoxidase (n=89), with lactoperoxidase showing the highest levels. Aminoacids and alcohols were analyzed to assess their role in the inflammatory response. Electron microscopy confirmed bacterial localization within epithelial cells and extracellular areas, with morphological signs of epithelial cell destruction, including nuclear degradation and increased vascularization.

Conclusions: The findings highlight the significant role of nasopharyngeal microbiota in respiratory infections and their correlation with inflammation markers. The high prevalence of Gram-negative bacteria, particularly *Escherichia coli and Staphylococcus aureus*, underscores the need for targeted prevention and treatment strategies. Understanding the cytomorphological and biochemical changes in the nasopharyngeal mucosa contributes to a better comprehension of pathogen-host interactions in respiratory infections.

KEY WORDS: acute respiratory infections, cytomorphological study, children

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INTRODUCTION

The colonization of the nasopharyngeal zone is the first stage in the development of respiratory pathology. The next stage of primary colonization involves the transmission of infection within the environment. Nasopharyngeal carriage of microorganisms can play a key role in the development and spread of respiratory infections, and the so-called «healthy» carriage, under the influence of various pathological factors, may transform into an infectious process [1, 2].

During acute respiratory viral infections, there is active proliferation of microorganisms. Under the influence of infectious agents and other immunosuppressive factors, the bacterial process progresses [3].

AIM

To investigate the relationships between cytomorphological markers of the pathological process and the development of acute respiratory pathology.

MATERIALS AND METHODS

The microbiological study included the isolation of pathogens of the pathological process, their identification based on morphological, cultural, and biochemical properties through the analysis of nasopharyngeal swabs. The algorithm for isolating pure cultures and their subsequent colonization was carried out using standard nutrient media (meat-peptone agar, blood agar, meat-peptone broth, Endo medium, Sabouraud medium), as well as specialized chromogenic differential diagnostic media (Biomerieux). Cytological studies were conducted at the interfaculty laboratory of experimental research methods (electron microscopy) at Ivan Franko National University of Lviv.

RESULTS

In the analysis of the nasopharyngeal microflora of patients with acute upper respiratory tract infections, 114 strains of opportunistic microorganisms were isolated.

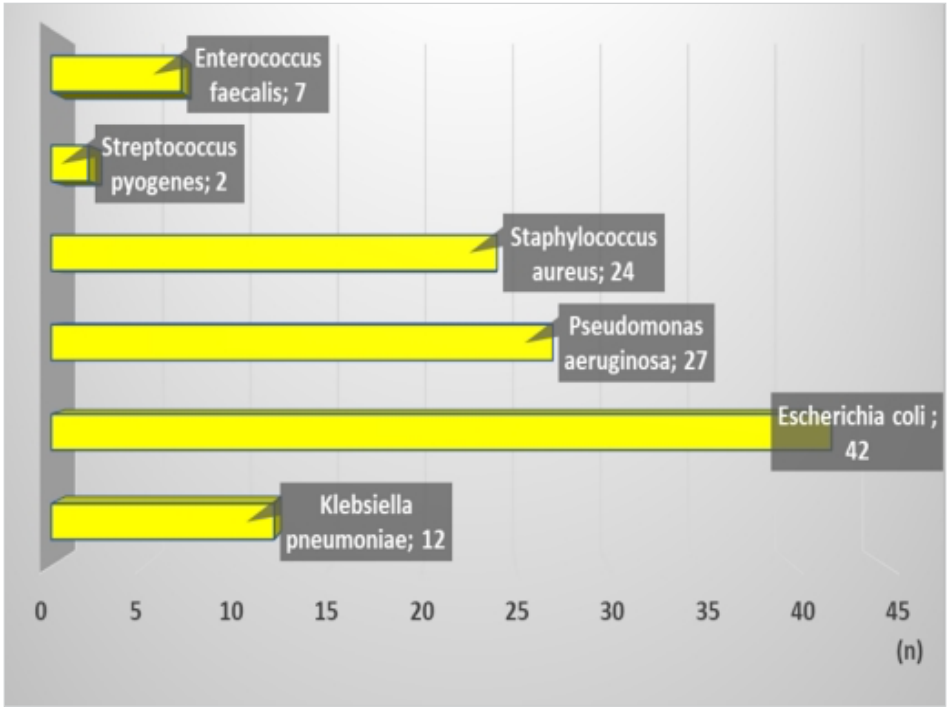

Fig. 1. Characteristics of identified pathogens in children (absolute values).

Table 1. Correlation relationships between the structural components of the microbiome and inflammatory process markers

Parameters		Correlation coefficient (r)	Statistical significance (p)
Escherichia coli	Free T_4	0,19	0,05
	TNF-α	0,20	0,04
	Cu	-0,21	0,03
Staphylococcus aureus	Lactoperoxidase	-0,20	0,04
	Acetone	-0,21	0,03
Pseudomonas aeruginosa	Free T_3	0,20	0,04
	Free T_4	-0,28	0,003
	TNF-α	-0,20	0,04
Streptococcus pyogenes	IFN-γ	0,32	0,001
Klebsiella pneumoniae	Free T_4	0,20	0,04
Enterococcus faecalis	Cortisole	0,26	0,007

Among them, 33 strains (29.0%) were Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*), while 81 strains (71.0%) were Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*) (Fig. 1).

According to the obtained research data, there is a predominance of Gram-negative bacterial strains—81 (71.0%), specifically *Klebsiella pneumoniae* (11%), *Pseudomonas aeruginosa* (24%), and *Escherichia coli* (37.0%). Gram-positive bacteria accounted for 33 strains (29.0%), including *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), and *Enterococcus faecalis* (6.0%). Among the groups, the leading pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%). The composition of the nasopharyngeal microflora in healthy children depends on various factors, including age category, hormonal status, and pathological conditions [4].

The biomaterial study design included the identification of enzyme groups such as sucrase (*n*=69), maltase (*n*=87), and lactoperoxidase (*n*=89). The highest level in our research was observed for lactoperoxidase, which is a heme-containing glycoprotein. Its function involves utilizing H_2O_2 for the synthesis of hypothiocyanite (OSCN), which has the ability to inhibit bacterial replication, fungi, viruses, and parasites, as well as neutralize intestinal pathogens in infants. The enzyme maltase (α-glucosidase) breaks down the disaccharide maltose into glucose, while sucrase also hydrolyzes sucrose and maltose [5].

The obtained glucose levels in the analysis of children (*n*=84) indicate the functionality of the degradation system and monosaccharide formation. The biomaterial

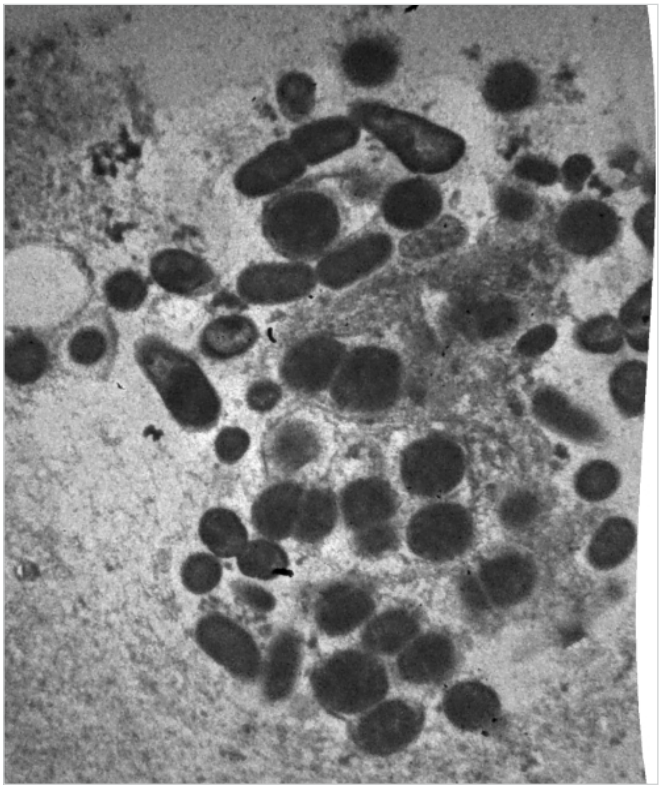

Fig. 2. Electron Micrograph (Magnification x10,000).

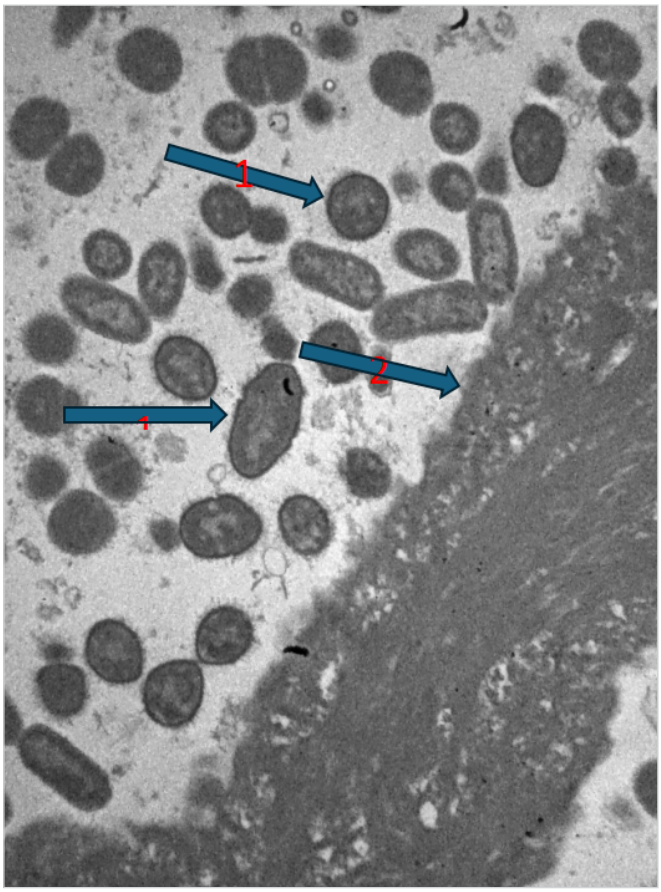

Fig. 3. Electron Micrograph (Magnification x10,000).

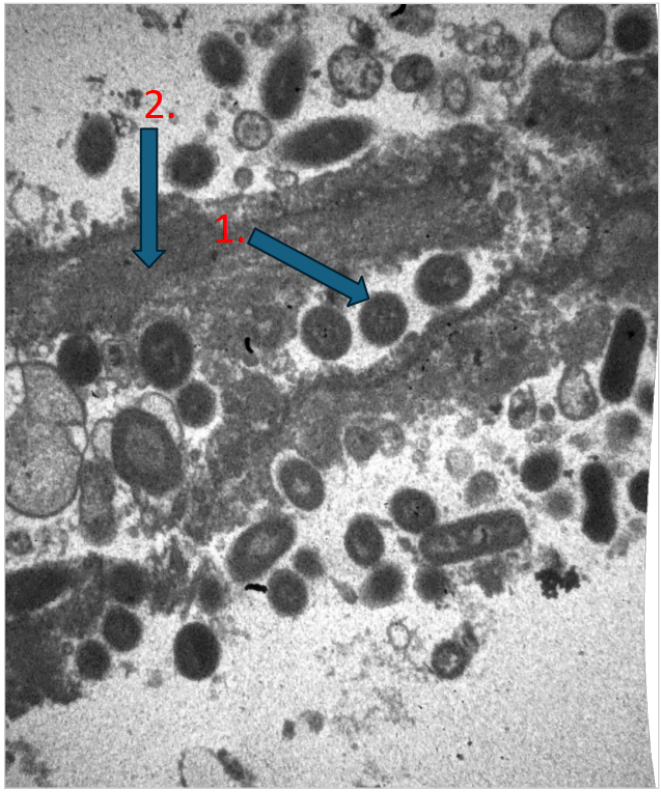

Fig. 4. Electron Micrograph (Magnification x10,000).
1. Electron-dense bacteria in the peri- and perinuclear space of the cell
2. Partial nucleus destruction

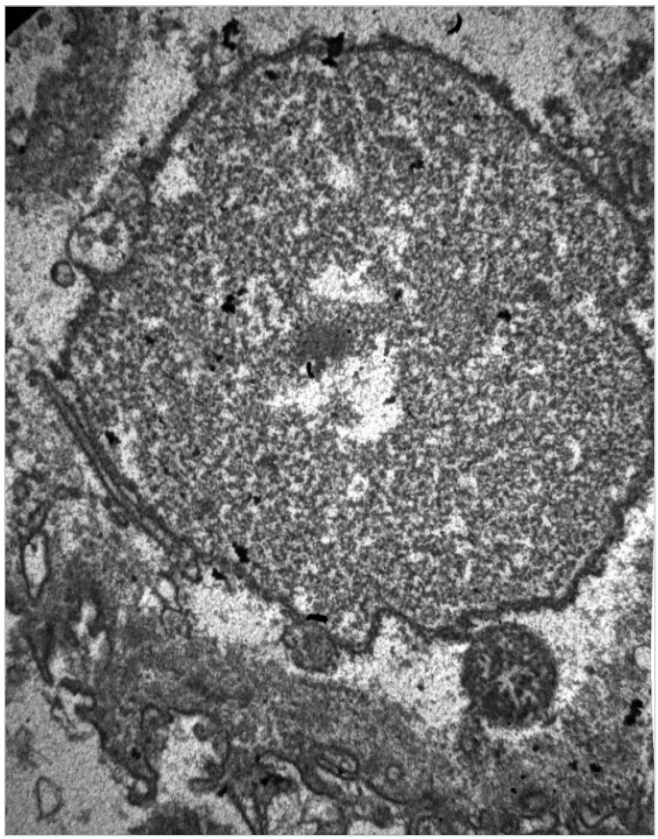

Fig. 5. Electron Micrograph (Magnification x10,000).
Macrophage in the process of phagocytosis of electron-dense bacteria.

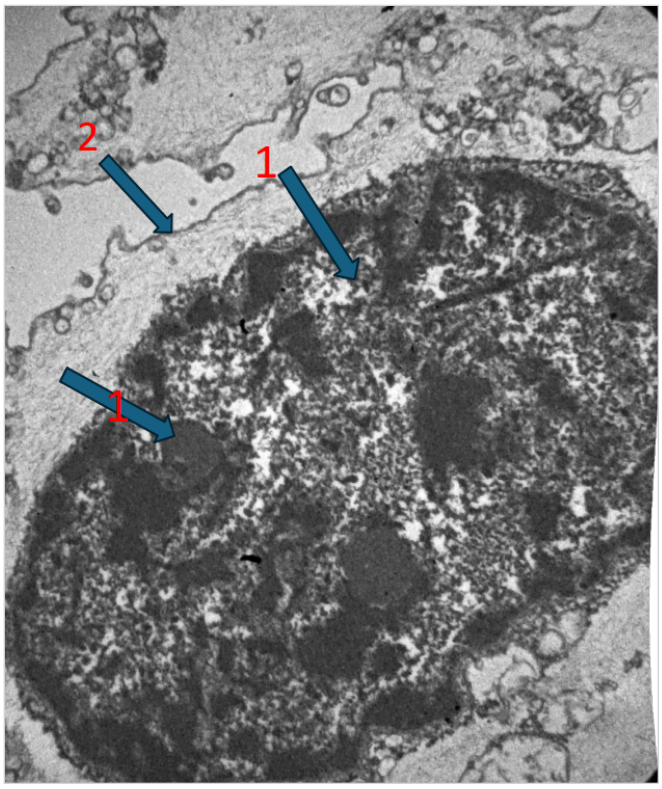

Fig. 6. Electron Micrograph (Magnification x10,000).
1. Destruction of the epithelial cell nucleus with the formation of a significant number of nucleoli and cellular debris.
2. Peripheral thinning of the basal cell membrane

also revealed the presence of amino acids: lysine (*n*=96), ornithine (*n*=62), and arginine (*n*=30). The presence of alcohols—sorbitol (a hexahydric alcohol, *n*=102), mannitol (a hexahydric alcohol, *n*=84), and xylitol (a pentahydric alcohol, *n*=86) - suggests the detoxification capabilities of the child's body. Additionally, intoxication-related factors were detected: acetone (*n*=105), which indicates excessive bacterial replication in the oral cavity. The identification of indole in 72 cases suggests its influence on the regulation of various aspects of bacterial physiology and virulence levels. Tryptophan, a derivative of indole and a precursor of the neurotransmitter serotonin, may cause vomiting and angiospasm in patients [3].

The presence of relationships between inflammation markers and microflora will be considered (Table 1).

According to Table 1, we will consider the correlation relationships between the levels of detected microorganisms and indicators of the inflammatory response in the child's body. The following significant correlations were identified. The colonization level of *Escherichia coli* increases with the positive influence of free T4 and TNF-α and decreases with the negative impact of Cu levels. *Staphylococcus aureus* has negative correlations with lactoperoxidase enzyme levels ($r = -0.20$, $p = 0.04$) and acetone ($r = -0.21$, $p = 0.03$). According to the correlation data, the replication of *Pseudomonas aeruginosa* is supported by an increase in free T3 levels, while growth inhibition occurs due to the negative impact of T4 and TNF-α levels. The level of *Streptococcus pyogenes* increases with elevated IFN-γ levels, *Klebsiella pneumoniae* is positively influenced by free T4 levels, and the growth and development of *Enterococcus faecalis* are supported by high cortisol levels.

Given the high prevalence of acute respiratory diseases in childhood, there is a need for scientific research to identify pathogenetic factors for treatment and prevention of this pathological process. The respiratory microbiome plays a key role in diseases. Microflora imbalances and bacterial carriage contribute to the clinical presentation of acute respiratory illnesses. Disturbances in balance and the presence of pathogenic microorganisms trigger an inflammatory response in the respiratory mucosa. Changes in enzymatic, detoxification, and characteristic features of the microbial landscape reflect the inflammatory response of the child's body. Positive correlations are observed between T4 levels, hormones, and TNF-α in the presence of *Escherichia coli*. *Staphylococcus aureus* disrupts enzymatic properties in the oral cavity, particularly lactoperoxidase activity, and contributes to signs of intoxication in the child's body.

Respiratory pathology is the most common issue in clinical pediatrics, and its relevance in childhood is linked to its prevalence, the potential for severe progression and complications, and the need for continued treatment. Addressing the etiopathogenetic aspects is crucial for understanding the disease and its prevention [5, 6].

The obtained data showed a predominance of Gram-negative bacterial strains—81 (71.0%), specifically *Klebsiella pneumoniae* (11%), *Pseudomonas aeruginosa* (24%), and *Escherichia coli* (37.0%). Gram-positive bacteria accounted for 33 strains (29.0%), including *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), and *Enterococcus faecalis* (6.0%). The predominant pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%). The most representative electron microscopy images (Fig. 2, Fig.3, Fig.4, Fig.5, Fig.6) are provided based on scrapings from the nasopharyngeal mucosa.Кінець форми

Scraping of the palatine tonsil from a patient with acute respiratory infection (ARI). The cytoplasm of the epithelial cell is filled with clusters of electron-dense bacteria, forming multiple colonies.

Patient T., 12 years old. Electron-dense bacteria (1) are located in close proximity to the cell membrane (2). An

increase in blood flow in the cytoplasmic membrane of the cell is observed.

Based on the electron micrographs obtained from patient T., 12 years old, diagnosed with acute respiratory infection (ARI), and based on material from a scraping of the mucosal area of the oropharynx, primarily the palatine tonsil, the following changes were observed: the cytoplasm of the epithelial cell was filled with electron-dense bacteria, resembling grape-like clusters, and their multiple colonies. Additionally, loci of electron-dense bacteria were observed in close proximity to the cell membrane, in both the peri- and perinuclear space of the cell. An increase in blood flow in the cytoplasmic membrane, peripheral thinning of the basal cell membrane, and destruction of the epithelial cell nucleus with the formation of a significant number of nucleoli and cellular debris were noted. A representative finding was the electron microscopy documentation of the macrophage in the process of phagocytosis of electron-dense bacteria (Fig. 4).

Apoptosis of the host cell is an intrinsic immune defense mechanism in response to the invasion of infectious agents. However, bacterial pathogens utilize various strategies to manipulate host cell death and survival pathways to enhance their replication and persistence. Bacteria employ different mechanisms to evade host immunity and cause diseases in humans. The susceptibility of the organism to bacterial infection depends on the effectiveness of the immune system, overall health status, and genetic factors [7,8].

Scientific sources describe several mechanisms of infectious invasion, particularly streptococcal invasion into epithelial cells. Two mechanisms of focal adhesion and penetration through the cellular membrane have been demonstrated—the zipper-like mechanism and the membrane-ruffling mechanism. Many bacteria can also induce their own internalization into non-professional phagocytes. In this case, two main entry mechanisms are involved: the zipper and the trigger mechanism. Both rely on the activation of signaling cascades that lead to the reorganization of the actin cytoskeleton at the level of the host plasma membrane. In the "zipper" mechanism, the interaction of bacterial surface proteins with host proteins triggers cytoskeletal and membrane remodeling, leading to bacterial internalization. In the "trigger" mechanism, bacterial effectors injected into the host cell cytoplasm initiate extensive cytoskeletal rearrangements and membrane ruffling, allowing bacterial engulfment and internalization [9].

Invasive bacteria actively induce their own uptake via phagocytosis in normally non-phagocytic cells, subsequently either creating a protected niche where they survive and proliferate or spreading from cell to cell through actin-based motility. Bacterial adhesion to host surfaces is a crucial aspect of host colonization, as it prevents mechanical clearance of pathogens and provides a selective advantage to bacteria of endogenous flora. Accordingly, bacteria have developed a vast arsenal of molecular strategies that enable them to target and adhere to host cells.

DISCUSSION

Local immunity of the respiratory tract mucous membranes remains insufficiently studied [10]. At the same time, the variability in the course of respiratory infections - from mild or asymptomatic forms in the upper respiratory tract to autoimmune disorders and severe purulent infections - is determined both by direct viral or bacterial aggression and by the immune response of the body to infection [11].

Among the most important nonspecific factors of local defense are the phagocytic function of granulocytes and alveolar macrophages, mucociliary clearance of the airway mucosa, as well as a number of antibacterial and antiviral components present in the respiratory tract secretions, including lysozyme, lactoferrin, complement, interferon, and surfactant [12, 13].

During evolution, a specific lymphoid tissue - Mucosa-associated lymphoid tissue (MALT) - formed in the mucous membranes, where innate and adaptive defense reactions develop in response to pathogenic aggression [14, 15]. At all stages of immune system formation, the first barrier preventing the development of infectious processes is the local MALT defense of the respiratory tract [16]. Under normal conditions, the mechanisms of local protection have sufficient potential to prevent acute respiratory viral infections (ARVI) even in the early stages of immune system development. Viruses entering the upper respiratory tract with inhaled air encounter several local immunity factors, such as viscous secretions, ciliary epithelial movement, the antiseptic properties of lysozyme and lactoferrin, the competitive effect of natural microflora, the enzymatic activity of secretions, and the specific action of IgA, among others [17, 18].

Thus, the mucous membranes of the nasal and oropharynx serve as the primary entry points for respiratory infections. Aggressive environmental conditions, the presence of chronic infection foci, and disturbances in the microbial balance of the saprophytic microflora impair the colonization resistance of the respiratory tract MALT in children. Therefore, the activation of the body's own defense mechanisms is not only a means of treatment but also a strategy for preventing upper respiratory tract infections (rhinitis, sinusitis, nasopharyngitis, tonsillitis, pharyngitis, tonsillopharyngitis) [19].

CONCLUSIONS

A total of 114 strains of opportunistic microorganisms were isolated, including 33 strains (29.0%) of Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71.0%) of Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*). The predominant pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%).

The biomaterial analysis included the identification of enzyme groups such as sucrase (n=69), maltase (n=87), and lactoperoxidase (n=89). The highest level in our studies was found for lactoperoxidase. Sucrase also hydrolyzes sucrose and maltose. The detected glucose level in the children's analysis (n=84) indicates the functionality of the degradation system and monosaccharide formation.

The presence of alcohols - sorbitol (a hexahydric alcohol, n=102), mannitol (a hexahydric alcohol, n=84), and xylitol (a pentahydric alcohol, n=86) - suggests the detoxification capabilities of the child's body. Additionally, intoxication-related factors were identified: acetone (n=105), which indicates excessive bacterial replication in the oral cavity. The identification of indole in 72 cases suggests its involvement in regulating various aspects of bacterial physiology and virulence levels. Tryptophan, a derivative of indole and a precursor of the neurotransmitter serotonin, may cause vomiting and angiospasm in patients.

Most Frequently Identified Microorganisms the next: *Escherichia coli* showed significant positive correlations with Free T4 (r=0.19, p=0.05) and TNF-α (r=0.20, p=0.04), while exhibiting a negative correlation with Cu levels. *Staphylococcus aureus* demonstrated negative correlations with lactoperoxidase enzyme levels (r=-0.20, p=0.04) and acetone (r=-0.21, p=0.03).

Bacterial invasion involves effector molecules that support pathogenic adhesion to the cells of the child's body and intracellular changes induced by the pathogen [7].

The data obtained showed a predominance of Gram-negative bacterial strains—81 (71.0%), specifically *Klebsiella pneumoniae* (11%), *Pseudomonas aeruginosa* (24%), and *Escherichia coli* (37.0%). Gram-positive bacteria accounted for 33 strains (29.0%), including *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), and *Enterococcus faecalis* (6.0%). The predominant pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%).

Electron Microscopy Findings. Electron microscopy results revealed the localization of coccal flora both inside epithelial cells and in the extracellular area. The main pathological manifestations in the epithelial cells of the nasopharyngeal zone included nuclear destruction with the formation of a significant number of nucleoli and cellular debris in the cytoplasmic membrane, increased blood flow in the cytoplasmic membrane, and peripheral thinning of the basal cell membrane.

Morphological analysis of the studied images revealed all stages of apoptosis, from initiation to degradation.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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