DISTRIBUTION OF THE CAUSATIVE AGENTS OF RESPIRATORY TRACT INFECTIONS IN CHILDREN

Mishyna M.[1], *Gonchar M.*[1], *Logvinova O.*[1], *Isaieva H.*[1], *Basiuk M.*[2]

Kharkiv National Medical University1
Kharkiv Regional Children's Clinical Hospital, Ukraine2

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Abstract

The study aimed to investigate the prevalence of microorganisms depending on the site of isolation and disease. The study involved 48 children aged 1 to 17 years. The patients were diagnosed with acute bronchitis (54.17%), community-acquired pneumonia (CAP) (33.33%), bronchial asthma (12.50%). 173 strains of microorganisms were isolated. 106 strains (61.3%) of Gram-positive microorganisms, 49 strains (28.3%) of Gram-negative microorganisms, 18 strains (10.4%) of fungi were detected. The study implied assessment of 100 samples from the nose (nasal swabs), pharynx (throat swabs) and sputum. Gram-positive microorganisms were isolated in 83 cases, Gram-negative microorganisms in 36 cases, fungi in 18 cases. The assessment showed that *Staphylococcus aureus* was most often isolated from patients with acute bronchitis; Gram-negative microorganisms were most often detected from throat swabs, comparing with microorganisms detected from nose swabs and sputum.

Key words: *microorganisms, biofilms, respiratory diseases, children.*

Introduction

Lower respiratory tract infections are still one of the leading causes of mortality in young children worldwide, accounting for 1.4 to 1.8 million deaths annually [1]. It has been hypothesized that the composition of the microbiota in the respiratory tract might be altered during respiratory diseases [2]. There is a lack of knowledge regarding the role of respiratory microbiota in susceptibility to pneumonia [3]. By the age of 2 years, almost 95% of children are colonized in the nasopharynx, in the form of a bacterial biofilm, by one of the greater than 90 serotypes of *Streptococcus pneumoniae*. Biofilm formation is a fundamental step in pathogenesis, as biofilms promote bacterial persistence, competence, immune evasion, and resistance to antibiotics, all while serving as reservoirs for local and invasive disease [4–7]. Another common upper respiratory tract opportunistic pathogen is *Staphylococcus aureus*. This microorganism colonizes the anterior nares and nasopharynx of 30 to 80% of individuals, often in biofilms, which serve as a reservoir for local and invasive disease [8–11]. Pneumonia caused by *Klebsiella pneumoniae* is characterized by an exacerbated inflammatory response, associated with excessive neutrophil and macrophage infiltration, high production of pro-inflammatory cytokines and severe lung injury [12, 13]. The opportunistic pathogen *Pseudomonas aeruginosa* is known to be an important human pathogen that produces several virulence factors. Its Quorum sensing (QS) systems are probably the best characterized among Gram-negative bacteria [14]. QS systems and biofilm formation are extemelly important components in the development of acute and chronic infections, particularly for *Pseudomonas aeruginosa* [15, 16]. Nontypeable *Haemophilus influenzae* (NTHI) biofilm formation has been associated with respiratory tract infections such as chronic rhinosinusitis and exacerbations of both chronic obstructive pulmonary disease (COPD) and cystic fibrosis [17]. Bacteria residing within

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Corresponding Author:
Hanna Isaieva, MD, PhD student of D.P. Grynyov Department of Microbiology, Virology and Immunology, Kharkiv National Medical University, Kharkiv, Ukraine. E-mail: anna1989isaeva@ukr.net

biofilms are up to 1,000 times more resistant to antibiotics and innate immune effectors than their planktonic counterparts [18], which contributes to the chronic and recurrent nature of these biofilm-associated diseases. Consequently, there is a great need for novel ways to manage biofilm-associated NTHI infections [19].

2. Purposes, subjects and methods:

2.1. The purpose of the work was to investigate the prevalence of microorganisms depending on the site of isolation and disease.

2.2. Subjects and Methods

The study involved 48 children aged 1 to 17 years with respiratory diseases: community-acquired pneumonia (CAP), acute bronchitis, bronchial asthma, which were treated at Kharkiv Regional Children's Clinical Hospital in the intensive care unit, pulmonary department. There were 29 boys (60.42±7.06%) and 19 girls (39.58±7.06%). (*Fig. 1*).

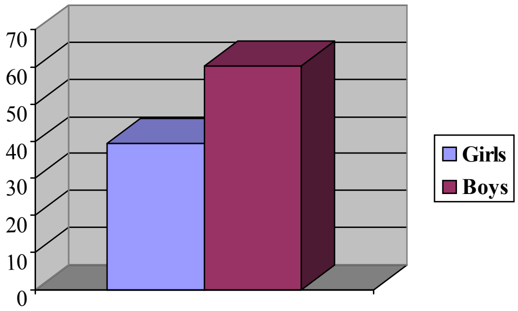

Fig. 1. Distribution of boys and girls in the research (%)

Isolation and identification of microorganisms from the nose (nasal swabs), pharynx (throat swabs), and sputum was performed according to the Order No. 535 of 22 April 1985 "On unification of microbiological methods of the research used in clinical-diagnostic laboratories in hospitals". Sputum production was induced by inhalation of 5.0% hypertonic saline solution, and the sputum sample was obtained by aspirating the nasopharynx through the nostrils or by expectoration if the child was old enough to produce an adequate sputum sample [20, 21].

The data were first entered in the Microsoft Excel and were analysed by SPSS V.19. Differences between places of isolation were assessed by means of χ^2 analysis. Statistical significance was set at 0.05.

Conflict of interests. There is no conflict of interests.

3. Results and discussion

Acute bronchitis developed in 26 cases (54.17±7.19%), CAP in 16 cases (33.33±6.8%), bronchial asthma in 6 cases (12.50±4.77%) (*Fig. 2*).

We investigated 100 samples from the nose (nasal swabs), pharynx (throat swabs) and sputum. Gram-positive microorganisms were isolated in 83 cases, Gram-negative microorganisms in 36 cases, fungi in 18 cases. *Table 1* shows that Gram-negative microorganisms in most cases were isolated from the pharynx as compared with the nose and sputum.

Overall, 173 strains of microorganisms were cultured. We detected 106 strains (61.27±3.70%) of Gram-positive microorganisms, 49 strains (28.32±3.43%) of Gram-negative microorganisms, 18 strains (10.40±2.32%) of fungi (*Fig. 3*).

100 strains were isolated from the pharynx. There were 52 strains (52%) of Gram-positive

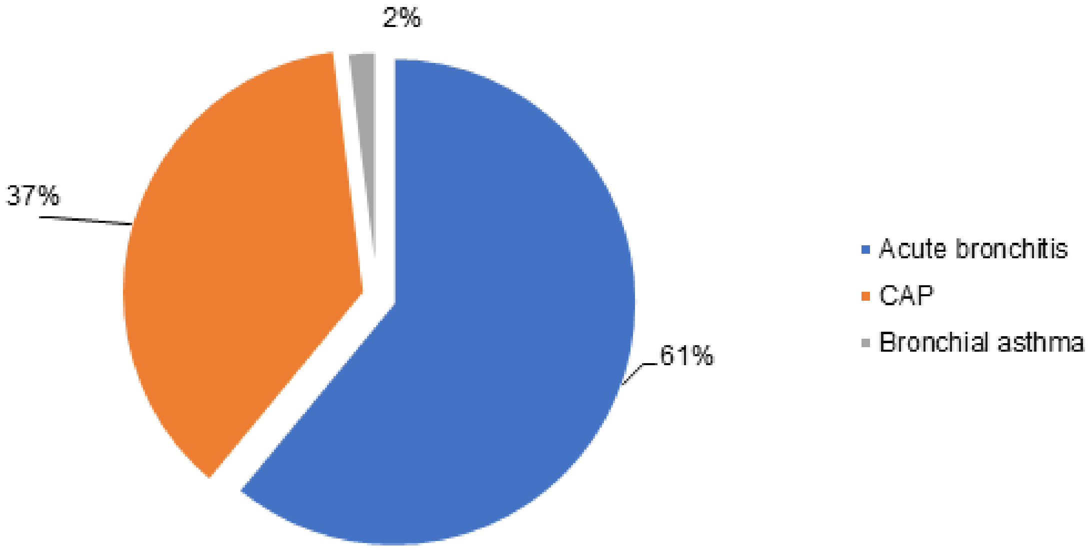

Fig. 2. Distribution of acute bronchitis, pneumonia and bronchial asthma in the research (%)

Table 1

Frequency of Gram-negative organisms distribution depending on the site of detection

	Pharynx	Nose	Sputum	*p*
Gram-negative microorganisms	24 (66.67%)	8 (22.22%)	4 (11.11%)	0.002

Note: differences between the samples with Gram-negative microorganisms from the pharynx, nose, sputum are statistically significant ($p<0.05$).

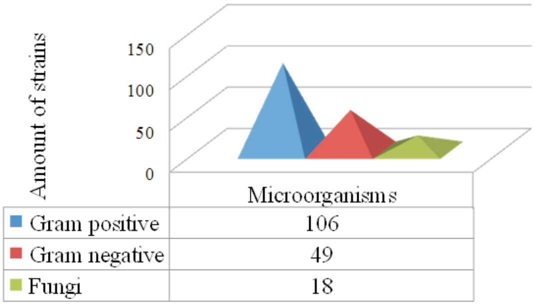

Fig. 3. Groups of microorganisms isolated from the patients

microorganisms: *Streptococcus pneumoniae, viridans streptococci, group A β-hemolytic streptococci, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* (*Table 2*).

Gram-negative microorganisms detected 34 strains (34%): *Klebsiella pneumoniae, Neisseria spp., Haemophilus influenzae, Pseudomonas aeruginosa* and other *Nonfermenting Gram-negative bacilli (NFGNB)* (*Table 3*). 14 strains of fungi were detected (14%): *Candida spp.* and other fungi.

Table 2

The number and percentage of Gram-positive microorganisms isolated from the pharynx (throat swabs)

Serial number	Name of organism	Number of organism (n=52)	Percentage of total organisms
1	*viridans streptococci*	17	32.69±6.50 %
2	*Staphylococcus aureus*	15	28.85±6.28 %
3	*group A β-hemolytic streptococci*	10	19.23±5.47 %
4	*Streptococcus pneumoniae*	7	13.464.73 %
5	*Staphylococcus epidermidis*	2	3.85±2.67 %
6	*Enterococcus faecalis*	1	1.92±1.90 %

We found that *group A β-hemolytic streptococci, Streptococcus pneumoniae, Klebsiella pneumoniae, Neisseria spp., Candida spp.*, were isolated from throat swabs more often as compared to nasal swabs and sputum (*Table 4*).

Staphylococcus epidermidis was most often isolated from nasal swabs – 22 cases (88%), as compared to throat swabs – 2 cases (8%) and sputum – 1 case (4%) (*Fig 4*).

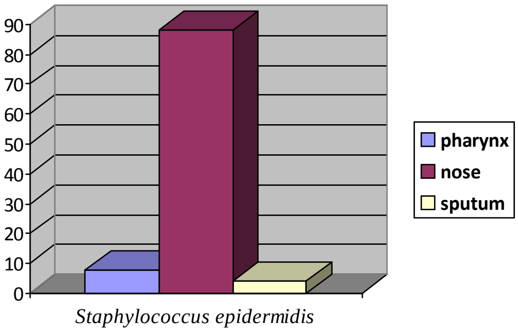

Fig. 4. The distribution of Staphylococcus epidermidis depending on the place of isolation (%)
Note: Amount of probability type 1 error, $p=0.000006<0.05$

Staphylococcus aureus predominated in patients with acute bronchitis – 23 cases (63.89%), comparing with CAP – 6 cases (16.67%) and bronchial asthma – 7 cases (19.44%) (*Fig. 5*).

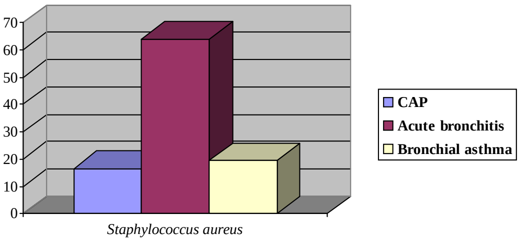

Fig. 5. The distribution of Staphylococcus aureus depending on the diagnosis (%)
Note: Amount of probability type 1 error, $p=0.025<0.05$

Discussion:

We showed that *Staphylococcus aureus* most often was isolated from patients with acute

Table 3

The number and percentage of Gram-negative organisms isolated from the pharynx (throat swabs)

Serial number	Name of organism	Number of organism (n=34)	Percentage of total organisms
1	*Klebsiella pneumoniae*	12	35.29±8.20%
2	*Neisseria spp.*	12	35.29±8.20%
3	NFGNB	3	8.83±4.86 %
4	*Pseudomonas aeruginosa*	5	14.71±6.07%
5	*Haemophilus influenzae*	1	2.94±2.90 %
6	*Escherichia coli*	1	2.94±2.90 %

Table 4

The frequency of microorganisms distribution depending on the site of detection

	Pharynx	Nose	Sputum	*p*
Streptococcus pneumoniae n=12	7 (58. 34 %)	1 (8. 33%)	4 (33. 33%)	0.0004
viridans streptococci n=20	17 (85. 0 %)	0 (0%)	3 (15.0%)	0.0004
group A β-hemolytic streptococci n=12	10 (83.34%)	1 (8.33%)	1 (8.33%)	0.015
Candida spp. n=16	12 (75.0%)	2 (12.50%)	2 (12.5%)	0.013
Klebsiella pneumoniae n=18	12 (66.66%)	3 (16.67%)	3 (16.67%)	0.017
Neisseria spp. n=13	12 (92.31%)	0 (0%)	1 (7.69%)	0.001

Note: differences between specimens from pharynx, nose, sputum are statistically significant ($p<0.05$).

bronchitis. This microorganism has a lot of virulence factors, but most important in the developing respiratory diseases are toxins (α-toxin, β-toxin, Panton-Valentine leukocidin) and biofilms formation. Theophilus K.Adiku et al. from Ghana [22], who investigated etiology of acute lower respiratory infections among children, noted that *Staphylococcus aureus* was a prevalent bacterial organism isolated from the throat (8%) and from the nasopharynx (16%). On the one hand *Staphylococcus aureus*, which was the most common bacterial organism in the nasopharyngeal aspirate samples, is known to occur as a normal flora in the nasopharynx of most healthy children. On the other hand, their data shows that *Staphylococcus aureus* may be an important cause of septicemia among children in Ghana [22]. In our research we could not prove the exact microorganism prevailing in patients with CAP, but we can suppose that without adequate treatment, bronchitis could transform to pneumonia. El Seify M.Y. et al., from Cairo, Egypt [23] showed that *Staphylococcus aureus* (*n* = 12; 13.3%) was the most common typical bacterial cause of pneumonia followed by *Streptococcus pneumonia* (*n* = 7; 7.8%) and *Klebsiella pneumoniae* (*n* = 7; 7.8%). In their study, *Staphylococcus aureus* was found to be the most common typical respiratory pathogen causing CAP (13.3%) [23]. Bhuyan G.S. et al. from Dhaka, Bangladesh [24], detected microorganisms from nasal swabs in children with acute respiratory infections and demonstrated that the most commonly isolated bacteria were *Streptococcus pneumoniae (39%), Klebsiella pneumoniae (22%), and Haemophilus influenzae (6%)*. Our investigation showed that Gram-negative microorganisms in most cases detected from throat swabs as compared to microorganisms detected from nosal swabs and sputum. It may be the purpose for further investigation of virulence factors of *Klebsiella pneumoniae* (capsule, endotoxin, siderophores, biofilm formation), *Pseudomonas aeruginosa* (pigments, siderophores, elastases, biofilms formation), *Haemophilus influenzae* (capsule, biofilm formation) and their mechanisms of resistance to the action of antibiotics. We found that *Streptococcus pneumoniae* was isolated from the pharynx more often compared with nose and sputum. It is a common commensal that quiescently colonizes the upper respiratory tract, forming biofilms adhering to the epithelium of the nasopharynx, lungs, rather than planctonic cells. [25–30]. Capsule and pneumolysin are the main virulent factors that protect *Streptococcus pneumoniae* against phagocytosis. Honkinen M. [31], and Gentile A. [32] showed that

Streptococcus pneumoniae and *Haemophilus influenzae* predominated in frequency in children with CAP [31, 32].

Conclusions

1. The present study demonstrates that group *A β-hemolytic streptococci, Streptococcus pneumoniae, Klebsiella pneumoniae, Neisseria spp., Candida spp.*, were isolated from the pharynx more frequently as compared to the nose and sputum.

2. The study showed that Gram-negative microorganisms were most often detected in the pharynx, than in the nose and sputum.

3. Our findings suggest that *Staphylococcus aureus* most often was isolated from patients with acute bronchitis.

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