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УДК 616.2-022.6-06:616.21-008.87

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HIERARCHIC STRUCTURE OF NASOPHARYNX MICROBIOTIC COMMUNITY IN PATIENTS WITH ACUTE RESPIRATORY INFECTION

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The results of the study of microbiota of nasal mucosa of patients with acute respiratory infections and acute upper respiratory tract pathology are presented. It is revealed that the microbe variety of the nose and nasopharynx forms associations of staphylococci, streptococci, Neisseria, Moraxella, hemophilic bacteria, sometimes Enterobacteriacea and Pseudomonadaceae, mostly of streptococci and staphylococci. Populations of organisms differ in their levels of colonization. The highest values were characteristic for streptococcal community – 7,88 lg CFU/ml.

Key words*: acute respiratory infections, nasopharynx, microbiocenosis.*

At the current stage of medicine development microflora of the human body is seen as extracorporeal organ with many functions, the most important of which is, undoubtedly, immune. The rising interest of practitioners to comprehensive and thorough studies of body microbiocenosis is associated with, on the one hand, their participation in providing numerous physiological processes in the body, and, on the other hand, – their potential role in the development of various complications of the basis disease, including purulent-septic processes [1 5]. Upper respiratory tract anatomically and physiologically is adapted for deposition of microorganisms from the inhaled air, so often they serve as «gateway» for infection and potential source of endogenous infections [7]. Many diseases are associated with the constant violations of nasopharyngeal flora, because this biotope is an unique ecological system that has developed evolutionarily and contains a wide variety of microorganisms, fungi, protozoa, viruses [1,3]. Most diseases of the respiratory system result from the formation of dysbiosis in this microbiocenosis influenced by various factors, including viral upper respiratory tract diseases such as influenza, acute respiratory infections (ARI) and acute upper respiratory tract conditions [1,2]. Nowadys these diseases remain uncontrolled infections that cause significant damage to the health of the population. Another important characteristic of respiratory diseases is that they can cause epidemics in the process of encompassing the general public of all ages, often causing severe diseases with the significant proportion of complicated forms. Often the cause of mortality are bacterial complications (sinusitis, meningitis, pneumonia, etc. that occur in the background or as a result of acute viral disease, resulting in the formation of dysbiosis nasal mucosa) [6,7].

The aim of study was to evaluate population composition and colonization level of the nasopharynx microbiota in patients with acute respiratory infections for further exploration the possibility of predicting the risk of complications.

Materials and methods

The nasopharynx microbiocenosis material of 52 patients with acute respiratory infections and their complications (acute sinusitis) was investigated by recognized microbiological method [9, 6]. Nasopharyngeal smears were taken with a sterile cotton swab, followed close by suspending in 1 ml of sterile saline solution. Later tenfold dilutions were inoculated on elective and selective nutrient media, which were then incubated later at optimum temperature for 24-48 hours. After the incubation was completed, the number of colonies that grew on the medium was counted. The level of colonization of the test material was expressed by the logarithm of the number of colonies forming units of bacteria in 1 ml of clinical sample (lg CFU/ml), the total population-level was expressed by the geometric mean of all values. Microbes were identified according to the Bergey's classification [8]. In some cases the identification was made with semi-automatic microbiological analyzer «Vitek-2» [10].

Results and discussions

There were such groups of exanimated patients. More than 2/3 of them (71, 0 %) suffered from acute respiratory infections, among which 63,0 % persons had moderate progress, 29.0 % of patients had acute sinusitis, 6,0 % – with a pronounced hyperthermia syndrome, indicating the severity of the course; in 2,0 % of cases occurred complicated forms.

The population structure of the of nasal mucosa microbe variety in examined patients was represented by 98 strains of facultative anaerobic, aerobic and anaerobic bacteria, which formed associations.

All identified microorganisms were belonged to bacterial flora. They were divided into 3 types and 5 classes. All selected microorganisms were divided into such types: *Firmicutes* owned 72,4 % of strains, *Actinobacteria* – 18,4 %, *Proteobacteria* – 9,2 %. *Firmicutes* type was represented by two classes of bacteria: *Bacilli* (97,2 % of strains) and *Clostridia* (rest ones). Type *Actinobacteria* formed the class of the same name, and in type *Proteobacteria* there were two classes *Betaproteobacteria* and *Gammaproteobacteria* (33,3 % and 66,7 % of strains accordingly)

The most studied patients' biotopes were colonized by Gram-positive and Gram-negative cocci. *Staphylococcus spp.* were presented on the mucosa of about two thirds of patients (76,9 %), *Streptococcus spp.* – in 40,4 % (Table 1).

Table 1

The frequency of certain bacteria carrying in nasopharynx in patients with Acute respiratory infections

Microorganism	Frequency of carrying n=52	
	absolute	%
Staphylococcus spp.	40	76,9
Streptococcus spp.	21	40,4
Enterococcus spp.	5	9,6
Micrococcus spp.	8	15,4
Corynebacterium spp.	10	19,2
Neisseria spp.	3	5,8
Moraxella spp.	3	5,8
Pseudomonas spp.	1	1,9
Enterobacter spp.	1	1,9
Granulicatella spp.	3	5,8
Haemophylus spp.	1	1,9
Clostridium spp.	2	3,8

Micrococcus spp. were presented in 15,4 %, *Corynebacterium spp.* – in 19,2 % of patients. Some persons were carriers of *Moraxella spp., Neisseria spp., Granilicatella* (5, 8 % of cases), *Haemophilus influenza*, *Enterobacteriaceae*, *Pseudomonadaceae* (1,9 % of patients). *Clostridia spp.* were isolated from two patients (3,8 %) (Table 1).

Most of bacterial genera formed appropriate communities. The structure of some of them is shown in Table 2.

Table 2

Composition of certain bacteria communities, that colonize the nasal mucosa

Microorganism	Community composition		Level of bacterial colonization
	absolute	%	lg CFU/ml
streptococci			
Streptococcus spp.	21	80,8	7,88
Enterococcus spp.	5	19,2	4,25
staphylococci			
Staphylococcus spp.	40	100,0	5,10
Staph. aureus	10	25,0	6,94
Staph. epidermidis	17	42,5	4,72
Staph. haemolyticus	5	12,5	5,26
Staph. hominis	3	7,5	4,26
Staph. intermedius	1	2,5	6,72
Staph. lentus	1	2,5	6,83
Staph. simulans	2	5,0	3,99
Staph. warneri	1	2,5	3,72
micrococci			
Kokuria kristinae	3	37,5	4,11
Rothia mucilaginosa	4	50,0	5,96
Micrococcus lylae	1	12,5	4,56
corynebacteria			
Corynebacterium spp.	10	100,0	5,09
neisseria and moraxella			
Moraxella cataralis	3	50,0	5,75
Neisseria spp.	3	50,0	4,43
others			
Granulicatella spp.	3	42,8	6,22
C. difficile	2	28,6	4,57
Ps. fluorescens	1	14,3	6,83
E. coli	1	14,3	8,68

Among the Staphylococcus communities 8 species of cocci were identified. Coagulase-positive *S. aureus* and *S. intermedius* formed almost a third of the respective group (27,5 %), the rest staphylococci belonged to coagulase- negative cocci, including *S. epidermedius*, which was dominated (42,5 %). It should be noted that population-level of coagulase-positive staphylococci was 1-2 orders of magnitude higher comparing with coagulase-negative ones (Table 2).

In streptococcal community there were three groups of streptococci: alpha-hemolytic, beta-hemolytic, and gamma-hemolytic variants.

Gamma-hemolytic streptococci were dominated in this community (61,9 % of its composition). Beta-hemolytic streptococci were almost 3 times less – 23,8 %. Alpha-hemolytic variants accounted for only 14.3 %.

It should be mentioned that the highest level of colonization was characteristic for beta-hemolytic ones and it reached 8,24 lg CFU/ml, while in alpha- and gamma-hemolytic forms it was smaller (accordingly 7,15 and 7,86 lg CFU/ml). The species diversity represented *S. mitis*, *S. pyogenes*, *S. oralis*, *S. salivarius*, *S. pneumoniae*, with dominance of *S. mitis.*

Miicrococcal community was represented by three genera of cocci: Micrococcus, Kokuria and Rothia. The density of colonization of these bacteria was significantly lower, comparing with staphylococci and streptococci, reaching 4.8 lg CFU/ml according to geometric mean. The highest colonization level was observed in *Rothia spp.* – 5,96 lg CFU/ml and lowest (4,11 lg CFU/ml) – in *Kokuria spp.* (Table 2).

Thus, it can be noted that the high level of microbial colonization of the mucosa was exposed to strains of *E. coli* and *Pseudomonas spp.* – 7,70 lg CFU/ml (according to geometric mean). The grades of colonization by *Staphylococcus spp.* (5,10 lg CFU/ml), *Corynebacterium spp.* (5,09 lg CFU/ml), *Moraxella spp.*, (5,75 lg CFU/ml), *Haemophilus spp.* (4,68 lg CFU/ml, *Enterococcus spp.* (4,25 lg CFU/ml) were lower. The lowest density of colonization was in *Neisseria spp.* (4,43 lg CFU/ml and *Kokuria kristinae* – 4,11 lg CFU/ml (Table 2).

Some differences in the structure of nasopharynx microbiotic community as well as colonization levels of its bacterial populations in patient with different clinical picture of diseases were observed.

In particular, patients, who had the phenomenon of acute sinusitis in materials were dominating staphylococci such as *S. aureus* (37,5 % of cases), other staphylococci (31,25 %) and various types of streptococci (25 %). Besides, in materials from these patients the survey found *Neisseria spp.* (12,5 %) and *Rothia mucilaginosa* (12,5 %) and in 6,25 % of cases *Granilicatella elegans, C. difficile, Corynebacterium spp., Moraxella cataralis, Ps. fluorescens.*

In patients with a diagnosis of acute respiratory infection, among bacteria isolated from nasopharynx mucosa population of staphylococci (80,6 % of cases) and streptococci were dominated (80,6 % and 47,2 % of cases respectively). *S. mitis population* was found in 22,2 % of patients, *S. pyogenes* – in 11,1 % ones. and Staphylococcal community in such patients was represented coagulase-negative staphylococci – *S. epidermidis* and *S. haemolyticus* – 36,1 % and 11,1 % of patients respectively. Population of coagulase-positive *S. aureus* was revealed in 11,1 % of persons. In some cases nasoparynx mucosa of these patients was colonized such populations *Corynebacterium spp.* (25,0 %), *Enterococcus spp.* (13,9 %), *Kokuria kristinae* (8,3 %), *Moraxella cataralis* (5,6 %), *Granilicatella adiacens* (5.6 %). In rare cases populations of *Neisseria spp., Micrococcus lylae*, and *H.influenzae, E. coli*, and *C. difficile* were identified.

The results basically confirm the published data on the structure of microbiota of the nasal and nasopharynx mucosa in humans [1,2,5]. Simultaneously, they evidencing significant differences in the microbial biotope composition during various diseases.

Making the analysis of nasal and nasopharynx mucosa microbiocenosis in patients with acute respiratory viral infection, it should be noted that together with are a number of ubiquitous bacteria, that represent resident and transient populations (*Staphylococcus spp.*, *Streptococcus spp.*, *Neisseria spp.*, *Moraxella spp.*, *Hemophilus spp.* etc. of corresponding microbiotic community. But sometimes bacteria that met rarely, under certain conditions, especially in a weakened body by major disease, could complicate its course. For example, *Rothia mucilaginosa* may be liable to cause infectious granulomatous dermatitis and bacteremia, *Granilicatella elegans* – bacteremia, peritonitis as complication of pain pathological process [3, 4].

Conclusion

1. Microbiota of nasal mucosa in patients with acute respiratory infections and acute upper respiratory tract pathology is represented by associations of populations of staphylococci, streptococci, neisseria, moraxella, hemophilic bacteria, Enterobacteriacea, Pseudomonadaceae et al., mostly of streptococci spp. and staphylococci spp.
2. The highest rates of colonization levels are reached by streptococci – 7,88 lg CFU/ml. However, *E. coli*, which is found in single cases, also had high levels of nasopharynx mucosa colonization – 8,68 lg CFU/ml.
3. There are some quantitative differences in the composition nasal mucosa microbiocenosis in patients who have different comorbidities and different levels of cause the main process, make it possible to analyze and predict the probable etiology of bacterial complications and therefore, in time to prevent them.

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ІЄРАРХІЧНА СТРУКТУРА МІКРОБІОТИ НОСОГЛОТКИ У ХВОРИХ НА ГОСТРУ РЕСПІРАТОРНУ ІНФЕКЦІЮ

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РЕЗЮМЕ. Досліджено мікробіоту носоглотки у хворих з гострою респіраторною інфекцією. Показано, що до складу мікробіоценозу носоглотки входять аеробні, факультативно анаеробні та анаеробні мікроорганізми. Серед них виявляються популяції стафілококів, стрептококів, нейсерій, мораксел, мікрококів, коринебактерій, гемофільних бактерій, деколи представники родин Enterobacteriacea та Pseudomonadaceae з переважання угруповань стрептококів і стафілококів. Показано, що Staphylococcus spp. представлено на слизовій носоглотки 76,9 % хворих, а Streptococcus spp. – 40,4 %. Найвищі колонізаційні рівні має угруповання стрептококів – 7,88 lg КУО/мл. Майже на 2 порядки поступається їм щільність колонізації слизової стафілококами – 5,10 lg КУО/мл. Разом з убіквітарними представниками мікробіоценозу при патології можуть з'являтися такі алохтонні мікроорганізми як Rothia, spp., Granulicatella spp. та інші.

Ключові слова: *гострі респіраторні інфекції, носоглотка, мікробіоценоз.*

Отримано 20.11.2014 р.