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Research Article

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Nasal Microflora in Patients with Flu and Acute Upper Respiratory Viral Infections, Which was Isolated in Ternopil Clinics in 2017

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Introduction

During annual influenza epidemics the incidence is about 10% of the population, and during pandemics that number increases by 4-6 times. In fact all influenza epidemies accompanied increase mortality. Worldwide annual deaths from influenza and acute upper respiratory tract infections (URTI) is over 4,5 million people (for comparison, the death rate from tuberculosis – 3,1 million people, malaria – 2,2 million people hepatitis – 1,1 million. people). In Ukraine in 2014 about 6 million citizens with symptoms of influenza and URTI appealed for medical help [1]. Pulmonary complications of influenza are most common include secondary bacterial infection [2].The human upper respiratory tract is the reservoir of a diverse community of commensals and potential pathogens, including Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Staphylococcus aureus, which occasionally turn into pathogens causing infectious diseases [3-5]. Bacterial complications such as otitis media and acute sinusitis and others are possible [6].

Materials and Methods

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

The specimen taken from nasal cavity of 52 patients with acute respiratory infections and flu were collected by sterile swabs taking care to avoid a contact with nares. Samples were investigated by microbiological technique [7, 8]. Tested material was suspended in 1 ml of sterile saline solution, tenfold dilution were made. Later tenfold dilutions were inoculated on elective and selective nutrient media, which were then incubated at optimum temperature for 24-48 hours under the proper conditions. After the completed incubation, the numbers of colonies that grew on the media were calculated. To normalize the microbial data, the numbers were transformed logarithmically. Colonies forming units of bacteria in 1 ml of clinical sample (lg CFU/ml) was used. Microbes were identified according to the Bergey's classification. In some cases microbiological analyzer «Vitek-2 Compact-15" was used [9].

Results

The population structure of the nasal mucosa microbe variety in examined patients was represented by 98 strains of facultative anaerobic, aerobic and anaerobic bacteria, which formed associations. The most patients' biotopes were colonized by cocci. Staphylococcus spp. was presented on the mucosa of about two thirds of patients (76.9 %), Streptococcus spp. – in 40.4 % [Table 1].

Micrococcus spp. and Corynebacterium spp. colonized nasopharyngeal mucosa of 15.4 and 19, 2 % of patients accordingly. Some persons (5, 8 %) were carriers of Moraxella spp., Neisseria spp., and Granilicatella spp. Enterobacteria were isolated from 3.8 % of patients; Haemophilus spp., Pseudomonadaceae – from 1, 9 % of patients [table 1].

Table 1: Characteristic of microorganisms – habitants of the nasopharyngeal biotope of patients with acute URTI and influenza

Microorganism	Frequency of carrying		Level of bacterial colonization, lg CFU/ml
	absolute	%	
Staphylococcus spp.	40	76.9	5.31
Streptococcus spp.	21	40.4	7.88
Enterococcus spp.	5	9.6	4.25
Micrococcus spp.	8	15.4	4.88
Granulicatella spp.	3	5.8	6.22
Corynebacterium spp.	10	19.2	5.09
Neisseria spp.	3	5.8	5.75
Moraxella spp.	3	5.8	4.43
E. coli	1	1.9	8.68
Enterobacter spp.	1	1.9	4.25
Pseudomonas spp.	1	1.9	6.83
Haemophylus spp.	1	1.9	4.68
Clostridium spp.	2	3.8	4.57

Eight populations of cocci were identified in Staphylococcaceae family. Coagulase-positive S. aureus and S. intermedius colonized mucosa of 19.2 % and 1, 9 % of patients accordingly. Their population level reached 6.94-6.72lg CFU/ml. S. epidermidis dominated among coagulase-negative Staphylococci. These strains were isolated from 32.7 % of patients and its colonization level was 4, 72lg CFU/ml. Other coagulase-negative staphylococci colonized nasal mucosa rarely but mostly in clinically significant growth: S. warneri – in 3.72lg CFU/ml, S. simulans – in 3.99lg CFU/ml, S. hominis – in 4.26lg CFU/ml, S. haemolyticus – in 5.26lg CFU/ml, S. lentus – in 6.83 lg CFU/ml. It should be noted that population levels of coagulase-positive staphylococci were 1-2 orders of magnitude higher comparing with coagulase-negative ones: (6,83+0,16) contrary to (4,79+1,14) lg CFU/ml).

Gamma-hemolytic streptococci were dominated between streptococci (61.9 %). Beta-hemolytic streptococci were almost 2.6 (23.8%) and alpha-hemolytic variants – 4.3 (14.3 %) times less. Beta-hemolytic streptococci were demonstrated the highest level of colonization (8.24lg CFU/ml). While in alpha- and gamma-hemolytic forms it was smaller (accordingly 7.15 and 7.86lg CFU/ml). The population diversity represented by S. mitis, S. pyogenes, S. oralis, S. salivarius, S. pneumoniae, with dominance of S. mitis.

Micrococcaceae family was represented by Micrococcus, Kocuria, Stomatococcus (Rothia) genera. Strains of Rothia mucilaginosa were isolated in 50 % of all micrococci, Kocuria kristinae – in 37.5 % Micrococcus lylae – in 12.5 %. The colonization density of these bacteria was significantly lower, in comparing with staphylococci and streptococci, reaching 4.88lg CFU/ml. The highest colonization level among micrococci was observed in Rothia spp. (5.96lg CFU/ml) and lowest one – in Kocuria spp. (4.11lg CFU/ml).

It can be noted that gram-negative rods colonized nasopharyngeal mucosa with high level too, but frequencies of their carrying were low: from 1.9 % till 5.8 % of all cases. The highest degree of microbial colonization was exposed to strains of E. coli (8.68lg CFU/ml). The colonization level of Pseudomonas strain reached 6.83lg CFU/ml; Haemophilus spp. – 4.68lg CFU/ml; Moraxella spp. – 4.43lg CFU/ml.

Discussion

The results basically confirm the published data on the structure of microbiota of the nasal and nasopharynx mucosa in humans [10, 11]. Populations of various staphylococci (76.9 % of cases) and streptococci were dominated (40.4 % of cases) on nasal mucosa of patients with acute URTI. Nasopharyngeal mucosa of these patients was colonized by such populations as Corynebacterium spp. (25.0 %), Enterococcus spp. (13.9 %), S. aureus (11 %), Kocuria kristinae (8.3 %), Moraxella cataralis (5, 6 %), Granilicatella adiacens (5.6 %). In rare populations of Neisseria spp., M. lylae, H. influenza e, E. coli, and C. difficile were revealed.

Some differences in the colonization levels of bacterial populations as well as structure of nasopharyngeal microbial community in patient with viral URTI and secondary bacterial infections were observed. In particular, in specimen of patients with acute sinusitis staphylococci such as S. aureus (37.5 % of cases), other staphylococci (31.25 %) and various types of streptococci (25 %) were dominating. Besides, in specimen from these patients Neisseria spp. (12.5 %) and Rothia mucilaginosa (12.5 %) and in 6.25 % of cases Granilicatella elegans, C. difficile, Corynebacterium spp., Moraxella cataralis, P. fluorescens were found.

According to given results the microbiota of nasal mucosa in patients with acute respiratory infections is represented by associations of populations of staphylococci, streptococci, neisseria, moraxella, hemophilic bacteria, Enterobacteriacea, Pseudomonadaceae et al., mostly of streptococci spp. and staphylococci spp. The highest rates of colonization levels are reached by streptococci – 7.88lg CFU/ml. However, E. coli, which is found in single cases, also had high levels of nasal mucosa colonization – 8.68lg CFU/ml. 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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