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ANTIMICROBIAL SUSCEPTIBILITY OF S. PNEUMONIAE, ISOLATED FROM ADULTS

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Abstract

Objective: to study of antimicrobial susceptibility of *S.pneumoniae* isolated from nasopharyngeal swabs of adult patients with acute respiratory infections.

Material and methods: a bacteriological study was carried out on 240 samples of nasopharyngeal mucus taken from patients with a diagnosis of "Acute respiratory infection" aged 18 years and older who were inpatient treatment at the City Clinical Infectious Diseases Hospital No. 1 in Tashkent (CCIDH No. 1). Determination of sensitivity to antimicrobial drugs was carried out by the disk-diffusion method and the E-test method. The data were interpreted according to the recommendations of the European Committee of antimicrobial susceptibility test (EUCAST).

Results and discussion: from all examined 240/100% of patients with acute respiratory infections of the upper and lower respiratory tract, *S. pneumoniae* was isolated from nasopharyngeal mucus in 56 / 23.3% of patients. The most significant factors in maintaining the carriage of *S. pneumoniae* were such factors as chronic diseases of the ENT organs, unsatisfactory living conditions, and bad habits. The obtained isolates of *S. pneumoniae* in 73.3% of cases retained sensitivity to penicillin, in 26.7% of the strains were resistant. Also, resistant strains were detected in 25.8% of isolates to azithromycin and in 27.3% to erythromycin.

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Conclusions: In patients with ARI, from whose nasopharyngeal mucus *S. pneumoniae* was isolated, 66.1% of cases had chronic diseases of the ENT organs (chronic tonsillitis, chronic sinusitis / sinusitis). The isolated pneumococcal strains showed resistance to penicillins and macrolides. In risk groups for chronic respiratory diseases, vaccination with pneumococcal conjugate vaccine (PCV-13) is recommended.

Key words: pneumococci, nasopharyngeal carriage, antibiotic sensitivity.

抽象的

目的：研究成人急性呼吸道感染患者鼻咽拭子分离的肺炎链球菌的药敏性。

材料与方法：对塔什干市第一临床传染病医院住院治疗的18岁及以上确诊为“急性呼吸道感染”患者的240份鼻咽黏液样本进行细菌学研究（CCIDH 第 1 号）。对抗菌药物敏感性的测定通过圆盘扩散法和E-test法进行。根据欧洲抗菌药物敏感性试验委员会(EUCAST)的建议解释数据结果和讨论：从所有检查的 240/100% 的上呼吸道和下呼吸道急性呼吸道感染患者中，56 / 23.3% 的患者从鼻咽粘液中分离出肺炎链球菌。维持肺炎链球菌携带的最重要因素是耳鼻喉器官慢性疾病、生活条件不理想、不良生活习惯等因素。获得的肺炎链球菌分离株在 73.3% 的病例中对青霉素保持敏感，26.7% 的菌株具有耐药性。此外，在 25.8% 的阿奇霉素分离株和 27.3% 的红霉素分离株中检测到耐药菌株。

结论：在鼻咽黏液分离出肺炎链球菌的 ARI 患者中，66.1% 的病例患有耳鼻喉器官的慢性疾病（慢性扁桃体炎、慢性鼻窦炎/鼻窦炎）。分离的肺炎球菌菌株显示出对青霉素和大环内酯类的耐药性。在慢性呼吸道疾病的危险人群中，建议接种肺炎球菌结合疫苗(PCV-13)。

关键词：肺炎球菌，鼻咽部携带，抗生素敏感性。

Introduction

According to the literature, *S. pneumoniae* is responsible for 25-30% of the 500,000 annual cases of bacterial pneumonia in the United States [2], with a mortality rate of 5-7%. *S. pneumoniae* is also the most common microorganism excreted in patients with chronic obstructive pulmonary diseases, represented by pneumonia or exacerbations of chronic bronchitis, is the cause of community-acquired and nosocomial pneumonia in 50% of hospitalizations [1, 2, 3]. 20% of all cases of pneumococcal pneumonia with a mortality rate of up to 50% in the elderly

and an inpatient care cost of approximately US \$ 2 billion annually [2, 4, 5, 15].

It is noted that the incidence of invasive pneumococcal diseases (IPD) is high in the age group <2 years and ≥60 years, in those who often suffer from acute respiratory viral infections, in the autumn-winter period [2, 3, 4]. The burden of pneumococcal disease is especially high among children in the first years of life and the elderly [6, 7, 8, 9]. The prevention of IPD in adults depends on ongoing surveillance, widespread implementation of vaccination programs, access to health care, promotion of the harmful effects

of smoking, and adherence to standard guidelines for diagnosis and treatment of diseases. In Uzbekistan, vaccination against pneumococcal infection of children according to the scheme of 2 months - 3 months - 12 months has been included in the National Schedule of Vaccinations since 2015 [1].

To date, the position and recommendations of the WHO on vaccination against pneumococcal infection is that vaccination is the only way to significantly affect the morbidity and mortality from pneumococcal infection, as well as the growth and spread of microbial resistance to antimicrobial drugs.

So, there are no data on the frequency of nasopharyngeal carriage of *S. pneumoniae* in the adult population and the sensitivity of the isolated strains to antimicrobial drugs.

The aim of the study: to study of the antimicrobial susceptibility of *S.pneumoniae* isolated from nasopharyngeal swabs of adult patients with acute respiratory infections.

Material and research methods.

From January 2017 to December 2020, a bacteriological study was carried out on 240 samples of nasopharyngeal mucus taken from patients with a diagnosis of "Acute respiratory infection" aged 18 years and older who were inpatient treatment at the City Clinical Infectious Diseases Hospital No. 1 in Tashkent (CCIDH No. 1). Also, the analysis of anamnestic, clinical, epidemiological and laboratory data of patients included in the study was carried out, in accordance with the current standards. Bacteriological examination of nasopharyngeal

mucus with the release of *Streptococcus pneumoniae* and setting a test for sensitivity to antibiotics was carried out on the basis of the bacteriological laboratory of the clinic, as well as in the laboratory of the Center for the Study of Antibiotic Resistance of the Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases of the Ministry of Health of the Republic of Uzbekistan.

The age distribution of the examined patients for nasopharyngeal carriage of *S. pneumoniae* is shown in Table 1.

Table 1

Distribution of examined patients with ARI by age (n = 240)

S.pneumoniae from n/a mucus	18-29 years old		30-39 years old		40-49 years old		50 years and more		Total	
	N	%	N	%	N	%	N	%	N	%
Isolated	1	5	5	2	3	1	2	8	2	1
	2	3.	7	3,	4	4.	1	.	4	0
	8	3	7	7	3	3	7	0	0	0
Not isolated	3	1	4	1.	1	4.	7	2	5	2
	4	4.	2	6	1	6	.	.	6	3.
	9	3	5	2	2	9,	1	5	1	7
	4	9.	3	2.	3	7	4	.	8	6.
	1	1	1	1	1	1	8	4	4	7

Of all examined 240/100% of patients with acute respiratory infections of the upper and lower

respiratory tract, in 56 / 23.3% of patients *S. pneumoniae* was isolated from nasopharyngeal swab. With the distribution by age, the percentage of nasopharyngeal carriage among patients aged 18-29 years was 14.2%, at the age of 30-39 years - 1.6%, in the age group 40-49 years 4.6% and for the share of 50 years and 2.9% of cases were older (Table 1). Of 56 patients from whom *S.pneumoniae* was isolated , 31 (55.3%) patients were diagnosed with ARI of the lower respiratory tract. Community-acquired pneumonia, moderate form ", in 8 (14.3%) patients" ARI of the upper respiratory tract, purulent rhinosinusitis ", in 11 (19.6%) patients purulent nasopharyngitis, in 7 (12.5%) patients - acute tracheobronchitis.

The distribution of patients according to the severity of the course showed that in 92.8% of patients the disease proceeded in a moderate form, and a severe form was observed in 7.15% of patients.

The total number of bed-days spent by patients in the hospital was 8.3 ± 1.4 days.

Isolation of *S. pneumoniae* from nasopharyngeal mucus taken from patients with ARI was carried out by cultural method.

Cultural method (isolation, identification of *S. pneumoniae* cultures). Bacteriological inoculation of nasopharyngeal swabs was carried out on plates with chocolate and blood agar (HiMedia, India), previously taken from the refrigerator and heated in a thermostat at 37°C for at least 30 minutes. [7]. In the bacteriological laboratory, the inoculations were incubated at 37°C for 24-48 hours in an atmosphere containing

5% CO_2 . In the presence of growth on solid nutrient media, a visual assessment of the grown colonies was performed, a Gram smear was prepared, oxidase and catalase were determined, and, depending on the result obtained, further identification of the pathogen and determination of antibiotic sensitivity were carried out. Identification of pneumococci was performed according to the following tests: detection of diplococci in the material, surrounded by a capsule; growth on media containing blood with the formation of alpha hemolysis; characteristic morphology of the culture smear according to Gram; positive test with optochin; sensitivity to bile acids (deoxycholate test or bile solubility test). To control the quality of identification of pneumococcus used strain *S. pneumoniae* ATCC 49619 [5].

Antibiotic susceptibility test. Antimicrobial susceptibility test was performed using the disk diffusion method and the E-test method. Quality control of the sensitivity determination was performed using control strains, *S. pneumoniae* ATCC 49619. The data were interpreted according to the recommendations of the European Committee of antimicrobial susceptibility test (EUCAST) [5].

The data obtained during the study was subjected to statistical processing on a personal computer using the Microsoft Office Excel-2019 software package, including the use of built-in statistical processing functions.

Results and discussion.

The nasopharyngeal carriage of *S. pneumonia*, as a process that constantly circulates the pathogen

among the population, is also supported by factors that determine the lifestyle of people and their health, the outcome and course of which depends on the intensity and timeliness of the inclusion, first of all, of the functional adaptive systems of the macroorganism associated with specific and non-specific protection factors [9, 13].

When analyzing the data of 56 patients with acute respiratory infections of the upper and lower respiratory tract, *S. pneumoniae* was isolated from their nasopharyngeal mucus, the indicators of the seasonality of the registered cases of the disease coincided with the season of the epidemic rise of acute respiratory viral diseases.

It is known that in most cases, respiratory diseases develop as a bacterial complication in acute respiratory viral diseases, which explains the increase in cases of community-acquired pneumonia and exacerbation of chronic obstructive pulmonary diseases during the seasonal rise in acute respiratory viral infections [2, 15].

The available literature data showed that there is a connection between the development of pneumococcal diseases (pneumonia, meningitis) and acute respiratory viral diseases. Thus, an analysis of the long-term epidemiological situation of community-acquired pneumonia revealed an unfavorable trend towards an increase in pneumonia, which coincided with seasonal increases in the incidence of influenza in Russia. It was also noted that after the decline in the incidence of influenza, there were

consistently high rates of morbidity and mortality from pneumonia [2, 3].

In our study, a significant number of patients with ARI were recorded in the winter (January, February, December) and early spring (March, April) months of the year.

Thus, according to the data obtained, in patients with chronic diseases of the respiratory tract, nasopharyngeal carriage of *S. pneumoniae* is characteristic and during the period of the seasonal rise of acute respiratory viral infections, the risk of developing invasive pneumococcal diseases in this group of persons increases, which in turn requires taking measures to specific prevention of bacterial complications.

Also, an assessment of the social status of patients with ARI with nasopharyngeal carriage of *S. pneumoniae* was carried out. It was revealed that 39 (70%) patients were cigarette smokers, 24 (42.8%) patients worked at construction sites, 16 (28.6%) patients lived in unsatisfactory living conditions, 12 (21.4%) patients lived in hostels. Thus, the results of the study showed that the most significant factors in maintaining the carriage of *S. pneumoniae* were such factors as chronic diseases of the ENT organs (chronic tonsillitis, chronic sinusitis / sinusitis). At the same time, smoking, cramped and unsatisfactory living conditions certainly affect the somatic health of a person, and also determine an unfavorable epidemiological environment. Passive smoking is of great importance as one of the most pathogenic risk factors, which reduces the effectiveness of protective immune mechanisms both at the local and systemic levels.

In addition, the presence of chronic foci of infection from the ENT organs, aggravated allergic background lead to an imbalance in the relationship between the micro - and the macroorganism, create favorable conditions for the colonization of pneumococcus, the activation of its pathogenic properties.

The results of the analysis confirm the opinion of the researchers that persons with chronic respiratory diseases, JIOP diseases and aggravated allergic history are threatened by the incidence of various forms of pneumococcal infection, since, first of all, these persons may be at increased risk for the formation of pneumococcal carriage.

Resistance to antibacterial drugs is one of the factors that form the epidemiological significance of pneumococcal infections due to the risk of an epidemic variant of *S. pneumoniae*. Since in most cases the choice of antibiotic therapy in the treatment of pneumococcal diseases is made empirically, it is necessary to know the sensitivity of these pathogens to antibacterial drugs. For this purpose, 56 nasopharyngeal strains of *S. pneumoniae* isolated from patients with various forms of ARI were tested.

Thus, the results of the test for sensitivity to antimicrobial allocated the 56 strains of *S.pneumoniae* shown , that in 73.3% of cases were sensitive to penicillin, in 26.7% of the strains showed resistance to penicillin, the isolates with intermediate sensitivity is not identified were. To determine the sensitivity to antibiotics of the fluoroquinolone group, the disc

diffusion method with norfloxacin was used as a screening test. At the same time, 65.1% of *S. pneumoniae* strains were found to be sensitive to norfloxacin, and resistance was found in 34.9% of isolates. For isolates insensitive to norfloxacin, the sensitivity to each fluoroquinolone drug was determined individually. A sensitivity of 100% was observed to antibiotics of the fluoroquinolone group - moxifloxacin and levofloxacin, while isolates with intermediate sensitivity were not detected. To drugs of the macrolide group - azithromycin and erythromycin in 54.8% and 43.2%, respectively, susceptible strains were determined, while resistant strains were determined in 25.8% of isolates to azithromycin and in 27.3% to erythromycin. In addition, 19.4% of isolates had intermediate sensitivity to azithromycin and 29.5% of isolates to erythromycin.

To date, more and more data appear on the identification of penicillin-resistant strains of *S. pneumoniae* in some countries of the world. So, for example, according to literary sources, in the USA, Spain, France and some Asian countries, resistance to penicillin reaches 50%, but in countries such as Finland, Sweden, Germany is registered in less than 5% of cases. The main risk factors for the formation of resistance are clearly the excessive use of antimicrobial drugs, recent hospitalizations, HIV infection, chronic pulmonary diseases, and frequent colds of the respiratory tract. Also, the authors noted that the formation of resistance to penicillin is followed by resistance to macrolides, which arises through

the transfer of non- β -lactam-resistant determinants by transposons. Thus, in Canada, resistance to macrolides increased from 3.7% in 1995 to 19.0% in 2005 ($P = 0.003$). It should be noted that, according to literature data, resistance to macrolides ranges from less than 3% to more than 70% in different countries [7, 14].

Thus, it should be noted that an epidemiologically important problem is the emergence of *S. pneumoniae* isolates resistant to penicillins and macrolides. In this regard, as recommendations for the use of antibacterial drugs, it is necessary to point out the need for the rational use of the above drugs in the treatment of non-invasive and invasive pneumococcal diseases, given the spread of *S. pneumoniae* strains that are insensitive to the drugs of these groups. At the same time, the ongoing development of new vaccines is encouraging, but it is very important to focus on preventive measures such as adequate medical treatment and education of the population (especially those with high risk factors), studying epidemiology and strict adherence to standard recommendations for antibiotic therapy.

Conclusions

1. In patients with ARI, from whose nasopharyngeal mucus *S.pneumoniae* was isolated in 66.1% of cases, they had chronic diseases of the ENT organs (chronic tonsillitis, chronic sinusitis / sinusitis).
2. Epidemiologically important is the appearance of *S. pneumoniae* isolates resistant to penicillins (26.7%) and macrolides (25.8% of isolates to azithromycin and 27.3% to erythromycin).

3. Considering the isolation of empirical antibiotic resistant strains of *S.pneumoniae* from older people, in order to create protective immunity, it is recommended to consider the issue of vaccination of adults suffering from chronic respiratory diseases, chronic diseases of ENT organs, pneumococcal conjugate vaccine (PCV-13).

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