Nasopharyngial carriage, serotypic landscape and sensitivity to S. Pneumoniae antibiotics in children before and after the implantation of vaccination in Uzbekistan

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Abstract. This article presents the results of a study of the nasopharyngeal carriage of Streptococcus pneumoniae in 76 organized children before vaccination and 77 unorganized vaccinated children in Tashkent. It was found that the frequency of nasopharyngeal carriage of pneumococcus is higher in children of closed children's groups. The disappearance of the most common pneumococcal serotypes after vaccination indicates the effectiveness of vaccination.

Keywords: Pneumococcus; vaccination; children

Introduction. Bearing of S. pneumoniae, according to epidemiological studies, varies in the range of 20–65% [3,4,6]. Under certain conditions, these nasopharyngeal colonizing pathogens can cause acute infections of the middle ear, sinuses and lungs. In some cases, these same bacteria can cause the development of severe systemic bacterial infections in children [1]. Colonization of the nasopharynx by opportunistic flora is considered as a special risk factor for the development of infectious diseases in children with primary immunodeficiencies and chronic respiratory diseases [9]. S. pneumoniae serves as the main etiological agent for severe invasive infections, such as bacteremia and meningitis, as well as the most common bacterial causative agent of community-acquired pneumonia, acute otitis media, and sinusitis in children around the world [7, 8]. A special risk group is young children [3]. Since S. pneumoniae carriage is the main prerequisite for the spread of pneumococcal diseases, dynamic monitoring of both the frequency of carriage and the spectrum of circulating serotypes is necessary. No less important is the monitoring of antimicrobial resistance of S. pneumoniae. Empirical antibiotic therapy should be based on regional microflora sensitivity data. It should also be borne in mind that since the beginning of the 2000s. international programs for monitoring antimicrobial resistance reveal significant age-related differences with the prevalence of pneumococci insensitive to certain classes of antibiotics in children [4].

The emergence of two new pneumococcal conjugate vaccines (PCV10 and PCV13) was aimed at reducing the nasopharyngeal carriage of vaccine strains of pneumococcus. It led to a decrease (by 90%) in the frequency of invasive pneumococcal infections caused by vaccine serotypes.

The aim of this study was to study the microbial spectrum in nasopharyngeal carriage in healthy children of preschool institutions and unorganized children under 5 years of age with analysis of serotype diversity and antimicrobial resistance of S. Pneumoniae before and after vaccination is included in the vaccination calendar.

Material and methods.

To study nasopharyngial carriage of S. pneumoniae, a bacteriological study of nasopharyngeal mucus was performed in 76 healthy children. Of these, 42 children are pupils of kindergartens aged 3 to 6 years who have not received vaccination against pneumococcal infection, the remaining 34 are unorganized children aged 1.5 to 3 years. 3 years after the introduction of vaccination, 77 healthy children were examined, who were vaccinated with three PCV 13 vaccines Prevenar according to the scheme.

Patients documented age, vaccination status, and antibiotic intake during the last month before sampling. Exclusion criteria were: the presence of acute infectious diseases at the time of the study; the presence of exacerbation of chronic diseases at the time of the study; the use of antimicrobial drugs at the time of the study and the refusal of the parents to study.

Nasopharyngeal swabs were collected using a kit consisting of a probe and a container with a transport medium. Swabs from the nasopharyngeal mucosa in children were taken with a dry sterile nasopharyngeal swab on a plastic applicator. The probe was inserted with a slight movement along the outer wall of the nose to a depth of 2-3 cm to the lower conch, slightly lowering downward, introduced into the lower nasal passage under the lower nasal concha, made a rotational movement and removed along the outer wall of the nose. The total depth of insertion of the probe should have been approximately half the distance from the nostril to the ear opening (3-4 cm for children). After taking the material, the end of the probe with a swab was lowered into a sterile disposable tube with a transport medium to the place of breakage, while the flexible part of the probe is folded in a spiral, then, covering the tube with a lid on top, the probe handle was lowered to achieve complete breaking off of the upper part of the probe. The tube should be sealed.

After taking a smear from the nasopharynx, the material was delivered to a bacteriological laboratory for several hours (4-12 hours), placed in an enrichment medium and plated. The time from taking the material to entering the laboratory did not exceed 12 hours. The study of nasopharyngeal mucus was carried out by staining according to Gram and bacteriological method.

Bacteriological seeding of materials was carried out on plates with chocolate and blood agar (HiMedia, India), preheating in an incubator at 37 ° C for at least 30 minutes. In a bacteriological laboratory, crops were incubated at 37 ° C for 24-48 hours in an atmosphere containing 5% CO2. In the presence of growth on solid nutrient media, the grown colonies were visually evaluated, a Gram smear was prepared, oxidase and catalase were determined and, depending on the result, further identification of the pathogen and determination of sensitivity to antibiotics were performed. The identification of pneumococcus was carried out on the basis of morphological and cultural properties, as well as using the test with optokhin. Pneumococcal isolates were stored at - 80 ° C.

In the case of positive seeding, the sensitivity of pneumococcus to antibiotics was determined by diffusion of the disk into agar. The widespread and uncontrolled use of antibacterial drugs by patients before seeking medical help leads to an increase in the antibiotic resistance of clinical isolates of S. pneumoniae. To conduct adequate antipneumococcal therapy, it is necessary to determine the sensitivity profile of the selected clinical isolate to antimicrobial agents. Sensitivity was determined to amoxicillin / clavulanate, cefazolin, azithromycin, cefepime, ceftriaxone, cefuroxime, metronidazole, chloramphenicol. In the interpretation used definitions are resistant, insensitive, sensitive, highly sensitive.

Nasopharyngeal swabs were collected with the informed consent of the parents of the children, during the routine medical examination of children, as well as children who came to the clinic for routine vaccination.

Results.

Distribution by gender and age is shown in the table1.

Table 1: Age distribution of children examined for nasopharyngeal carriage *S.pneumoniae* (n=76)

Children's age	Boys	%	Girls	%	Total	%
Unorganized, 1.5-3 years	15	44,1	19	55,9	34	44,7
Organized 3-6 years	18	42,8	24	57,1	42	55,3
Total	34	44,7	42	55,3	76	100,0

All children included in the study had no signs of respiratory infection and did not receive antibacterial drugs for 2 weeks before the study. The study was conducted before the inclusion of pneumococcal vaccination in the national calendar of vaccinations.

A total of 76 nasopharyngeal samples were collected. When studying the microbial spectrum during nasopharyngeal carriage, 41% (31/76) cases showed an increase in normal microflora (Table 2).

Table 2: Microbial spectrum in nasopharyngeal carriage in organized and unorganized children before vaccination

	Microflora	Unorganized children (n-34)		Kids attending kindergarten (n-42)		Total	
		abs	%	abs	%	abs	%
1	*S.pneumoniae*	6	17,6	13	31*	19	25
2	*H influenzae*	3	8,8	9	21,4*	12	16
3	*M catarrhalis*	6	17,6	8	19,0	14	18,4
4	*S.aureus*	6	17,6	4	9,5*	10	13,1
5	*S.viridans*	1	2,9	7	16,7*	8	10,5
5	*Other*	12	35,3	1	2,4*	13	17,1
7	Total	34	100	42	100	76	100

Note: * - validity of data between organized and unorganized children (P<0,05)

The normal flora was mainly represented by staphylococci, green streptococci, and various types of enterobacteria. The growth of opportunistic flora was observed in 59.9% (45/76) of the children included in the study. In 25% (19/76) of the examined children, simultaneous growth of 2 or 3 types of bacteria was recorded in nasopharyngeal samples. The most common associations were the associations of S. Pneumoniae with H. influenzae (n = 12), S. Pneumoniae with M. cataralis (n = 16), combinations of H. influenzae and M. cataralis (n = 6), as well as S .pneumoniae, H. influenzae and M cataralis (n = 4). In 3 cases, S.aureus participated in the combinations.

The proportion of S. pneumoniae in nasopharyngeal carriage was 25% and was higher than the proportion of H influenzae 16%; and M cataralis 18%.

The high frequency of nasopharyngeal carriage of conditionally pathogenic microflora in children from organized children's groups is explained by a large number of contacts with peers. The growth of potentially non-pathogenic flora (we designated it as normoflora) was

observed in one third of children from kindergartens (33%). The frequency of microbial associations of significant respiratory pathogens was 57%.

In general, the frequency of nasopharyngeal carriage of S. pneumoniae was 25% and was higher than the carriage of H influenzae (16%) and M cataralis (18%). Our data are consistent with the results of other studies conducted in different years, which show that the incidence of pneumococcus carriage is a significant proportion compared with other pathogens. So, according to the literature, the prevalence of nasopharyngeal colonization of pneumococcus in children under 5 years of age is estimated in the range of 23-56% [30-32].
In addition, we studied the nasopharyngeal carriage of S. pneumoniae in patients with acute respiratory bacterial infections hospitalized in Tashkent hospitals in 2015-2016. A clinical and laboratory examination of 96 children with lower respiratory tract lesions who were hospitalized in the clinic of the Tashkent Pediatric Medical Institute and City Children's Clinical Hospital No. 1 was carried out.

As is known, to confirm the etiology of a respiratory disease, a microbiological examination of sputum taken from the lower respiratory tract is necessary. We took a swab from the nasopharynx and suggested a particular etiology of the disease. Obtaining sputum for a cultural study in children in most cases is extremely difficult, and if it can be obtained, a positive test result cannot be completely interpreted as one or another etiology of a respiratory disease. We took into account that the positive inoculation of the biomaterial obtained from the nasopharynx on the flora is not an unequivocal confirmation of the etiology of pneumonia or other respiratory bacterial infection and characterizes, first of all, the carriage of opportunistic bacteria.

In the table below it can be seen the structure of bacterial pathogens in respiratory diseases in children (table. 3).

The examined were isolated 11 strains of S. Pneumoniae (11.4%). Based on the results of the study, it can be said that pneumococcus is the predominant pathogen in respiratory diseases of the lower respiratory tract in children. The study showed that S. Pneumoniae is the main pathogen in the structure of nasopharyngeal carriage in children under the age of 5 years who were hospitalized for acute respiratory bacterial infection.

Table 3: Structure of bacterial pathogens of respiratory diseases in inpatient children

Pathogens	Number of surveyed children	
	Abs.	%
Str.pneumonia	11	11,4
Str.pneumon.+Enterobact. Aerog	4	4,1
Klibsiella pneumon.	5	5,2
Klibsiella pneumon.+ Candida	5	5,2
St. Aureus	24	25
St. Aureus+ Candida	6	6,2
St. Aureus+ Str. Haemolitics	4	4,1

Candida	5	5,2
Candida+ Enterobact	6	6,2
Str. Haemolitics	5	5,2
Str. Viridans	6	6,2
Str. Pyogenes	2	2
Not found.	13	13,5

Cultural blood tests give a positive result in 13-26.5% of patients with complicated pneumonia, but less than 5% of patients with not severe and uncomplicated forms of community-acquired pneumonia. This is primarily due to the fact that most cases of pneumonia in children are not bacteremic.

Of course, the appointment of antibiotic therapy prior to the collection of biomaterial for sowing will also affect the results of culture blood tests. The use of molecular biological methods (polymerase chain reaction method, PCR) can increase the sensitivity of identification of a bacterial pathogen, in particular S. Pneumoniae.

We determined the sensitivity of the isolated pneumococcal strains to antibiotics used in hospitals in Tashkent (Table 4).

Table 4

The results of determining the sensitivity of S. pneumoniae strains to antibiotics (%)

Antibiotic	Highly sensitive	Sensitive	Low sensitivity / Resistant
Azithromycin	87,5	-	12,5
Amoxicillin / clavulanate	-	62,5	37,5
Cefazolin	100	-	-
Cefepim	100	-	-
Cefuroxime	100	-	-
Metronidazole	-	-	100

The results of the studies showed that the antibiotic sensitivity to amoxacillin / clavulanate has a slight tendency to decrease - of the obtained samples, 37.5% had weak or no sensitivity to this antibiotic. 87.5% of patients had low sensitivity to azithromycin, as well as a lack of sensitivity to metronidazole.

It should be noted that pneumococcus remains highly sensitive to injectable cephalosporins, but cephalosporins are not recommended for widespread use in community-acquired pneumonia in children. To preserve the antibiotic sensitivity of pathogens, it is advisable to limit their use in community-acquired pneumonia.

Thus, pneumococcus remains the most common causative agent of bacterial respiratory infections in children. An important way of epidemiological control of pneumococcal infections is to study the spectrum of pathogens circulating in this territory. Our study revealed a tendency to increase antibiotic resistance of pneumococcus to macrolides and metronidazole. This result coincides with the global trend of the rapidly growing prevalence of resistant pneumococci, when resistance to macrolides has grown from almost 5% to 25%.

Thus, our data once again demonstrate the need for continuous monitoring of the prevalence of antibiotic-resistant strains of pneumococci and the study of their sensitivity in order to improve empirical antibiotic therapy. The study provides important information on the prevalence of pneumococcus in clinical specimens for respiratory infections in children.

Since S. Pneumoniae carriage is the main prerequisite for the spread of pneumococcal diseases, dynamic monitoring of the spectrum of circulating serotypes is necessary. To assess the effectiveness of vaccine prophylaxis, it is necessary to take into account the correspondence of their composition to the serotypes circulating in each specific region. In the development of the epidemic process of pneumococcal infection, a constantly changing serotypic landscape of invasive pneumococci plays a role, which can lead to an increase in the incidence of serotypes that are not part of the vaccine (the "substitution effect"), and, in turn, poses a new task for the scientific world to expand the number serotypes included in the vaccine. American studies suggest that the colonized airways of children are a reservoir in which the pathogen evolves, leading to a change in its resistance and invasive properties. No less important is the monitoring of antimicrobial resistance of S. pneumoniae.

As you know, the pneumococcal vaccine was included in the vaccination schedule in October 2015. In order to evaluate the serotype landscape of S. pneumonia, after vaccination was introduced, we studied the microbial spectrum of nasopharyngeal carriage of 77 healthy unorganized children under 5 years of age.

All children included in the study were vaccinated against pneumococcal infection according to the 2 + 1 scheme: two doses with an interval of at least 4 weeks between administrations. The study was conducted 1.5-2 years after the child received 3 doses of pneumococcal vaccine (table. 4).

Table 4: Prevenar vaccination schedule for children

Vaccination onset age	Dose	Number of doses	Scheme
2 to 6 months	0.5 ml	2 + 1 revaccination	2 + 1: two doses with an interval of at least 4 weeks between administrations. The first dose is administered from 2 months of life, the second - from 3 months of life. Revaccination is carried out once in 12 months

Exclusion criteria were: the presence of acute infectious diseases at the time of the study; the presence of exacerbation of chronic diseases at the time of the study; the use of antimicrobial drugs within 2 weeks before the study and the parents refuse the study.

The study of the microbial spectrum in healthy children showed that out of 77 children, S. pneumoniae was isolated in 12 children, which amounted to 15.6%. We identified only the most significant respiratory pathogens. The frequency of isolation of other pathogens was significantly lower (Table 5).

Table 5: The structure of the isolated strains in healthy children in nasopharyngeal swabs

Microflora	Number of examined children	
	Abs.	%
S.*pneumoniae*	12	15,6
Str. Viridans H influenzae	3	3,9
Str. Pyogenes M catarrhalis	2	2,6
S.*aureus*	9	11,7
Other	34	44
Not detected	17	22
Total	77	100

In the study of nasopharyngeal carriage, 12 strains of S. pneumoniae were isolated. Primers for serotyping 6A / B / C / D, 9A / V, 23F, 19F, 18A / B / C / F, 15A / F serotypes were used. In 5 of 12 isolated samples, only serotype 6A was detected, the remaining serotypes were not detected. The isolated 6A pneumococcal serotype in the examined children is part of PCV-13. Serotypes of pneumococcus isolated from the respiratory tract of patients with acute community-acquired pneumonia prior to vaccination in the republic were represented by serotypes 1, 3, 5, 6A, 14, 19, while according to foreign studies, serotypes 19 were the most common serotypes isolated from patients with pneumonia , 23, 1, 5, 6B and 3. When they compared the serotypes that make up the 13-valent vaccine (PCV-13) with the S. pneumoniae serotypes isolated from patients, overlap with vaccine serotypes was observed in 78.3% of cases, and overlapping serotypes included in The remaining 10-valent pneumococcal vaccine (PCV-10) in 62.7% of cases.

Our data on the disappearance of the most common pneumococcal serotypes after vaccination indicates a good vaccination efficiency.

Data on the current pneumococcal serotypes circulating in Uzbekistan are important for assessing the efficacy of PCV after its introduction into the domestic immunization calendar, since it allows us to assess the overlap of serotypes with existing vaccines. In countries where PCV7 vaccination was introduced, vaccine strains of pneumococcus have virtually disappeared [2,5,10,11]. Given the fact that invasive pneumococcal infections are necessarily preceded by colonization of non-sterile loci with the pathogen, our data allow us to predict a decrease in the incidence of respiratory infections in Uzbekistan.

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