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Role of *Streptococcus pneumoniae* in the structure of bacterial infections in the children hospitalized to inpatient hospitals in Moscow in 2011-2012

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Data on the spread of Streptococcus pneumoniae serotypes in the RF are extremely limited. 3 pneumococcal conjugate vaccines are approved in Russia; however, neither has yet been employed in the framework of the national population immunization program. At the same time, it is the data on the serotype range of pneumococcal infections that may be considered the prognostic efficacy criterion for the national vaccination programs. The objective of this research is identification of the circulating S. pneumoniae serotypes and spread of pneumococcal etiology infections in the structure of bacterial infections in the infants hospitalized to 5 inpatient hospital of Moscow in 2011-2012. The trial involved 864 patients in tote. Vast majority of patients (86%) had acute purulent otitis media and sinusitis. Community-acquired pneumonia was diagnosed in 9% of patients, sepsis and bacteremia – in 3.6%; purulent meningitis – 1.2% of patients. It has been revealed that S. pneumoniae is the primary pathogen in the structure of nasopharyngeal carriage in the children under 5 years of age hospitalized with acute bacterial infections, and the primary bacterial causative agent of acute otitis media at this age. Nasopharyngeal pneumococcal carriage analysis revealed the prevalent serotypes – 19F, 14, 23F, 3, 6A and B; they were present in ¾ of all cases; 19F was the most frequent (>20%). Diversity of the S. pneumoniae serotypes detected in middle ear liquid was less significant – 17 serotypes (in comparison with 24 serotypes in nasopharynx). The 5 prevalent serotypes were 19F, 3, 14, 23F, 6B and 19A (>75% in tote). Detection rate of serotypes 3 and 19A in middle ear liquid significantly exceeded the detection rate of these serotypes in case of nasopharyngeal carriage. The study of invasive infections revealed serotypes 14, 23F, 3 and 15C. These data may be used as a benchmark for future monitoring and evaluation of effect of PCV vaccines on epidemiology of serotypes and antibiotic resistance of S. pneumoniae in Russia after introduction of PCV into the national vaccinal prevention calendar.
Keywords: *pneumococcal infections, children, Streptococcus pneumoniae serotypes, antibacterial therapy, vaccinal prevention, conjugate polysaccharidic vaccine.*

Streptococcus pneumoniae (*S. pneumoniae*, pneumococcus) is the most widespread causative agent of respiratory bacterial infections, meningites and bacteremia in children [1, 2]. The World

Health Organization (WHO) considers pneumococcal infection the main cause of morbidity and mortality in all regions of the world [3]. The burden of pneumococcal diseases is especially heavy among younger children, the elderly and people with chronic diseases [4]. *S. pneumoniae* is one of the microbes that usually colonize nasopharynx – the main reservoir of infection and its further spread – in infants [5]. 90 serotypes of pneumococcus different in the structure of capsule polysaccharides, which determine the microbe's virulence and immunogenicity, are known. Not all serotypes are equally pathogenic; most pneumococcal infections are connected with a limited number of serotypes [6]. Severity of pneumococcal diseases is different as well: in most cases, *S. pneumoniae* causes mucosal infections, such as acute otitis media and acute sinusitis. At the same time, pneumococcus is the most widespread reason of severe invasive infections. According to the WHO, 1.6 mn people die of pneumococcal infection every year; 700,000-1,000,000 of them are children under 5 years of age. This statistics mainly reflects situation in the countries where vaccination against pneumococcal infection has not been adopted yet. The annual rate of invasive pneumococcal infections in such regions varies from 10 to 100 cases per 100,000 people. Thus, pneumococcus is the main cause of acute pneumonias in children under 2 years of age. Pneumococcal pneumonia, meningitis and bacteremia are considered to be severe and, often, life-threatening diseases, which pose a serious problem for public healthcare [7-9].

At the same time, the exact spread of pneumococcal infections, especially of pneumococcal bacteremia, in Russia is unknown. Etiological confirmation of bacterial infections is rare for daily pediatric practice; doctors focus on clinical symptoms; this makes prescription of etiotropic therapy more difficult. The epidemiologic surveillance system for pneumococcal infections is underdeveloped. Extremely rare blood and cerebrospinal fluid tests together with the standard practice of treating children with suspected bacteremia and meningitis with antibacterial drugs rule out the possibility of measuring the real rate of invasive pneumococcal diseases. Moreover, there are only several laboratories in Russia, where it is possible to isolate, identify and serotype *S. pneumoniae* sufficiently accurately. Thus, the statistical data on the incidence rate of invasive pneumococcal infections in Russia are mainly based on expert judgments.

The data on the spread os pneumococcal serotypes in the RF are extremely limited; the international literature has featured only several reports from Russia for the last decade [10-12]. 3 pneumococcal conjugate vaccines are licensed in Russia (7-, 10- and 13-valent), although neither of them has been implemented into the national public immunization program. At the same time, it is the data on the serotype range of pneumococcal infections that may be considered a prognostic criterion of efficacy of national vaccination programs. The recent publications indicate a considerable reduction in the incidence rate of invasive pneumococcal infections in many countries of the world after introduction of cohort vaccinal prevention of pneumococcal diseases [13-18].

Program "Identification of the role of pneumococcal infection in the structure of bacterial infections in the children hospitalized to inpatient departments of Moscow medical institutions in 2011-2012 for therapy optimization and disease prevention purposes" was substantiated by high priority of scientific research in this sphere of pediatrics. The Program was aimed at identification of the circulating *S. pneumoniae* serotypes and spread of infections of pneumococcal etiology in the structure of bacterial infections in the infants hospitalized to inpatient departments.

Study design and methods

Doctors of the following Moscow medical institutions took part in the study: FSBI "Scientific Center of Children's Health", SBHI "Morozov children's municipal hospital #1, MHD", SBHI "Speranskiy children's municipal clinical hospital #9, MHD", SBHI "Filatov children's municipal clinical hospital #13, MHD", SPHI "Children's isolation hospital #6, MHD".

The patients hospitalized to inpatient departments of these medical institutions were involved in the study on the basis of inclusion criteria:

- <24 hours after admission;
- established diagnosis of an acute bacterial infection: sepsis, meningitis, bacteremia, community-acquired pneumonia, acute purulent otitis media, acute purulent sinusitis.

In compliance with the current legislation of the Russian Federation, we obtained informed and signed consent to the diagnostic and medical procedures at hospitalization to an inpatient department of legal representatives of all the patients involved in the study.

The study inclusion criteria included:

- previous antibacterial therapy: >2 antibiotic doses;
- diagnosed urinary tract infection;
- diagnosed acute intestinal infection.

In order to obtain reliable data on the spread of pneumococcal infection and *S. pneumoniae* serotypes, we sampled biomaterials from sterile (blood, cerebrospinal fluid, middle ear fluid) and non-sterile (middle ear discharge, nasopharyngeal smear) loci in the children involved in the study with clinical symptoms of bacterial infections. The sampling was conducted using eSWAB Collection Ki (Copan Diagnostics, Italy) consisting of a probe and a transport medium container.

The obtained samples were daily taken to the microbiological laboratory of the Federal State Budgetary Institution "Scientific Center of Children's Health" by a special express delivery service observing temperature conditions and other transportation regulations.

In the microbiological laboratory of the FSBI "Scientific Center of Children's Health", the obtained biomaterial samples were studied in compliance with the standard protocol. The revealed *S. pneumoniae* cultures were typed using a serological method and polymerase chain reaction (PCR). Thus, in the course of realization of the Program, we identified the *S. pneumoniae* serotypes revealed in the hospitalized patients with respiratory bacterial infections, meningites, bacteremia, fever without nidus of infection, acute purulent otites mediae and sinusites.

We used consumables and equipment manufactured by BioMerieux (France) for microbiological analysis. Biomaterials were inoculated on the nutrient agar with 3% donor concentrated red human blood cells and 3% horse serum and on a chocolate agar with 10 mcg/ml of nicotinamide adenine dinucleotide (NAD). NAD was added to the medium previously cooled down to 50-60^{o}C. The revealed *Haemophilus influenzae* cultures were identified on the basis of research of morphology, cultural properties and need in growth factors X and V in the API NH system. The inoculations were incubated in a thermostat with increased CO_2 content (5%) at the temperature of 37^{o}C for 24-48 hours. Pneumococcus was identified on the basis of morphological and cultural properties and also using an optochin test and latex-agglutination reaction (Slidex Pneumo-Kit). Beta-hemolytic streptococci were identified on the basis of bacitracin sensitivity and using a latex agglutination method with Slidex Strept Kit reagent. The revealed microbiological cultures were identified using analyzer Vitek2 and MALDI mass spectrometer (Bio-typer, Bruker, Germany). We applied disk technique on the Mueller-Hinton agar with 5% human blood in order to identify sensitivity of causative agents to antibiotics.

S. pneumoniae serotypes were identified using specific pooled and grouped serums (Statens Serum Institut, Denmark) in agglutination and/or Neufeld capsular swelling reactions.

STUDY RESULTS

The study involved 864 patients in tote. The SCCH laboratory admitted 1,716 biological material samples throughout the project realization period (2 samples per patient at the average; tb. 1). Nasopharyngeal smears (50%) and middle ear fluid (MEF; 43%) constituted the bulk of the samples. We conducted 106 (6%) blood inoculations and analyzed 10 cerebrospinal fluid samples (1%).

S. pneumoniae was revealed in 20% (352/1,716) of samples. The vast majority of strains were revealed in nasopharynx (208/352) and middle ear fluid (140/352): 59 and 40%, accordingly. We

identified 4 *S. pneumoniae* strains in sterile loci (blood and cerebrospinal fluid) by means of a cultural method.

Description of patients

The vast majority (86%) of patients involved in the study had acute purulent otitis media and sinusitis (pic. 1). Community-acquired pneumonia was diagnosed in 9% of patients, sepsis and bacteremia – in 3.6%, purulent meningitis – in 1.2% of patients. 484 (56%) out of 864 patients involved in the study were boys. Concurrent atopic diseases were observed in 44 (5%) children. 155 (18%) patients attended children's preschool institutions. 104 (12%) children had been prescribed antibiotics within the latter 3 months at least once. At the same time, only 7 (0.8%) and 2 (0.2%) children underwent vaccination against Haemophilus influenza and pneumococcal infection (one was vaccinated with a polysaccharidic vaccine Pneumo 23, another – with a septivalent conjugate vaccine Prevenar 7), respectively.
Real practice in the RF has been characterized by high rate of prescription of antibacterial drugs before sampling the patients' biomaterials, i.e. without etiological diagnostics. In our trial, 240 (28%) of the involved patients had received 1 or 2 antibacterial drug's doses before biomaterial sampling. It ought to be mentioned that the share of samples with the platable *S. pneumoniae* reduced almost twice in the setting of intake of antibiotics.

Results of microbiological trials

In the course of study, we revealed 19 invasive strains of different microbes in blood and cerebrospinal fluid by means of a cultural method, including 4 *S. pneumoniae* strains (tb. 2). In 2 more cases (1 blood sample and 1 cerebrospinal fluid sample), pneumococcus was revealed by means of PCR-diagnostics (LytA+), when the cultural method yielded negative result. Thus, we observed 6 cases of invasive pneumococcal infections in the hospitalized children involved in the study. In many patients (7 out of 19), positive hemoculture ought to be considered possible contamination at blood sterility sampling.
Pneumococcus was revealed in nasopharyngeal smears of 208 (24% of all nasopharyngeal smear samples) patients (see tb. 1). The rate of *S. pneumoniae* detection in middle ear fluid was 19% of all the obtained MEF samples.
Prevalence (detection rate – 47%) of pneumococcus was observed in the structure of the revealed microbes at inoculations of nasopharyngeal samples (pic. 2).
According to the cultural MEF trial, pneumococcus was prevalent in the structure of causative agents of acute otitis media – 53% of all the detected pathogens (pic. 3).

Results of *S. pneumoniae* serotyping

181 isolates were typed out of 208 nasopharyngeal *S. pneumoniae* strains (tb. 3). We identified 24 different serotypes in tote. The most frequently observed (n>10) were serotypes 19F, 14, 23F, 3, 6A and 6B – 76.2% of the spread in tote.
140 *S. pneumoniae* strains were revealed in MEF (19% of all the MEF samples), 128 of them were typed (tb. 4). 17 different *S. pneumoniae* serotypes were present in MEF. The dominant (n>10) were 5 serotypes, including 19F, 3, 14, 23F, 6B and 19A – 76.6% of the spread in tote.
Structure of the pneumococcal serotypes revealed in nasopharyngeal smears and middle ear fluid does not significantly differ in children of different age (pic. 4, 5). High rate of serotype 19F carriage in children of 0-1 years of age and serotype 3 occurrence rate increase in patients over 3 years of age were observed.

DISCUSSION OF STUDY RESULTS

Diseases of mucous tunics, primarily otites and sinusites, are prevalent in the structure of the acute bacterial infections causing most hospitalizations of infants to inpatient departments. In most cases these respiratory diseases do not pose life threat; however, their prevalence reconfirms that it is the most frequent cause of prescription of antibiotics to children. Acute otitis media is considered the second most frequent childhood disease (after respiratory viral infections) all over the world [19]. At the same time, such a high share of otites mediae as a cause of hospitalization of children may, without any doubt, be discussed from the point of view of reasonability of admitting such patients to a twenty-four-hour inpatient department. Thereupon, our study vividly demonstrates real pediatric practice of Moscow children's hospitals: almost all children addressing a reception ward are hospitalized; treatment of mucosal infections, which do not usually require infusion therapy, is prolonged (up to 7-10 days) and conducted at inpatient hospital; tympanocentesis is a routine procedure in patients with otitis media (almost all patients with this diagnosis undergo this procedure). This is significantly different from the practice generally accepted in most developed countries and requires urgent revision. This conclusion concerns the sphere of public healthcare organization; however, it is evident at analysis of the obtained hospitalization structure.

Real practice of prescribing antibiotic therapy to children with suspected acute bacterial infection on the preclinical stage ought to be mentioned as well. Before biomaterial sampling, 240 children had received an antibiotic, 197 (82%) out of them – on the reception ward or inpatient department stage, before diagnosis establishment and inclusion in the study. The antibacterial drug of choice was the third-generation cephalosporins (primarily ceftriaxone) in 50% of cases and the first-generation cephalosporins (cefazolin) in 33% of cases. In outpatient practice in the setting of empirical antibiotic therapy, the preference is given to such drugs as Sumamed and Suprax. The drug of choice at otites, sinusites and pneumonias in children – amoxicillin – was empirically selected by doctors on the preclinical stage only in 8% of cases. Thus, antibacterial drug's selection in case of suspected acute bacterial infection in children does not usually comply with recommendations of the WHO and international guidelines in real practice of Moscow medical institutions. Moreover, parenteral administration of antibacterial drugs is prevalent, although most otites mediae and pneumonias in children may be cured by peroral amoxicillin intake [20]. Wide application and irrational selection of antibiotics promote growth of *S. pneumoniae* resistance to antimicrobial drugs [21-23].

The conducted study showed that S. pneumoniae is the primary pathogen in the structure of nasopharyngeal carriage in the children under 5 years of age hospitalized with acute bacterial infection, and the primary bacterial causative agent of acute otitis media at this age. The data on serotypical diversity of pneumococcus detected in all those cases determine prognosis of vaccinal prevention with the pneumococcal conjugate vaccines (PCV) available in Russia.

The study of invasive infections revealed serotypes 14, 23F, 3 and 15C. The spread of the serotypes responsible for invasive pneumococcal infections is different from the spread of non-invasive serotypes. The most frequently revealed serotypes at these infections in Europe are 14, 6B, 1, 19F and 23F [9, 13]. Serotypes 1, 14, 3 and 5 are the most virulent and, as both Russian and foreign researchers mention, cause severe pneumonias with pleurisy and destruction more often than other serotypes [9, 24, 25]. Serotypes 1 and 5 are called epidemic serotypes as they often cause eruptions of pneumococcal infections in the developing countries.

According to the results of our study and analysis of nasopharyngeal pneumococcus carriage, the prevalent serotypes were 19F, 14, 23F, 3, 6A and 6BB: they occurred in ¾ of all cases; the most frequent serotype was 19F (>20%). Diversity of the revealed *S. pneumoniae* serotypes in middle ear fluid was less significant (only 17 serotypes against 24 in nasopharynx). The dominant serotypes among them were 19F, 3, 14, 23F, 6B and 19A (>75% in tote). The detection rate of serotypes 3 and 19A in middle ear fluid was significantly higher than in case of nasopharyngeal carriage.

Experience of cohort PCV vaccinal prevent in many countries of the world shows significant alteration of serotype range of *S. pneumoniae*. Thus, after the PCV7 vaccination had been

introduced, the pneumococcal serotypes included in the vaccine virtually disappeared [26, 27]. The studies conducted by the SCCH in 2010 and results of this project showed that the range of *S. pneumoniae* serotypes had remained stable and suffered only slight changes in comparison with 1990. According to the Russian studies, structure of the pneumococcal serotypes revealed in respiratory tract of the patients with acute community-acquired pneumonia is 80% the same as in 1990; the only difference is the rate of certain strains in certain periods [25, 28]. We may expect alteration of the range of widespread pneumococcal serotypes when cohort vaccination is introduced in the RF.

Real efficacy of the applied pneumococcal vaccines may be evaluated given correspondence of their composition to the *S. pneumoniae* serotypes circulating in a certain region. In case there are no epidemiologic data on the pneumococcal serotypes circulating in the country, the data on their efficacy in other countries and regions ought not to be unconditionally extrapolated.

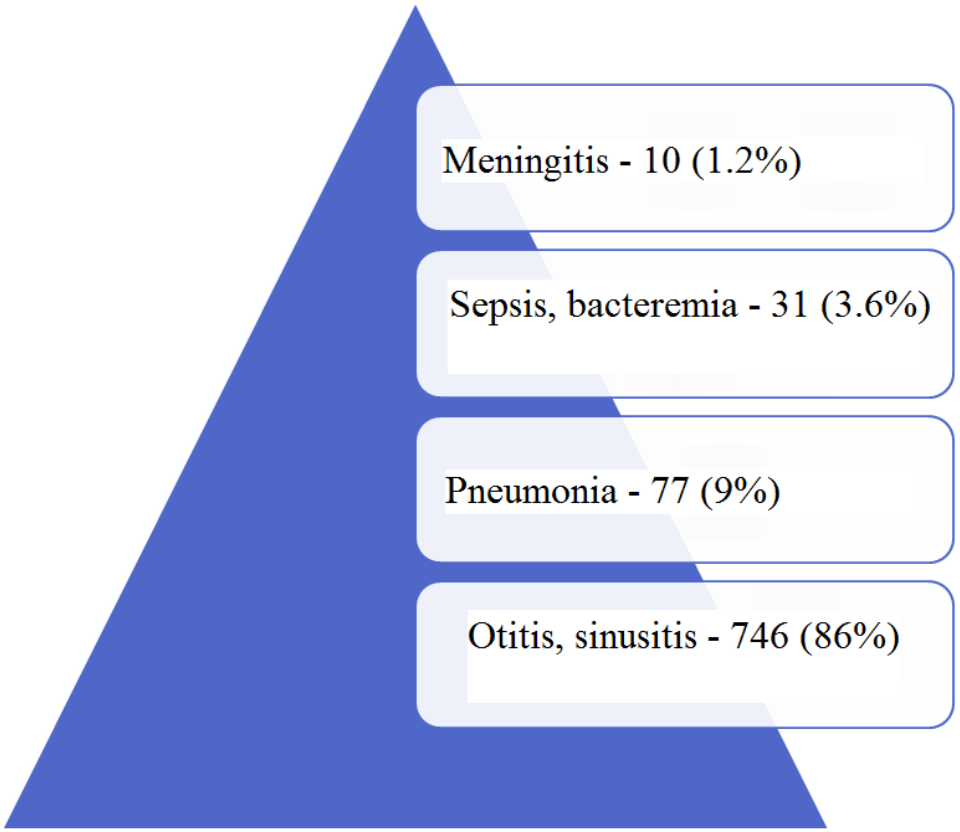
According to the results of our study conducted in Moscow, comparison of the *S. pneumoniae* serotypes constituting 7-, 10- and 13-valent vaccines with the serotypes detected in the carriers revealed 60.3% correspondence of the serotype range for PCV7, 64.2% correspondence for PCV10 and 94.5% correspondence for PCV13. Analysis of structure of the serotypes revealed in middle ear fluid in patients with otitis media revealed the 65.8% serotype range correspondence for PCV7, 67.4% correspondence for PCV10 and 93.8% correspondence for PCV13. These are the data obtained in Moscow.

A similar in object large-scale study conducted in 8 Federal Districts of the RF revealed a significantly lower correspondence of pneumococcal serotypes in case a patient carried serotypes constituting the vaccine: 80.4, 80.7 and 84.6% for 7-, 10- and 13-valent vaccines, respectively [29]. It ought to be mentioned that the study identified not pneumococcal serotypes, but only serogroups. In that trial correspondence of the serotypes obtained from middle ear fluid to the vaccinal serotypes was very low – 56.5, 56.5 and 66.7%, respectively, for 7-, 10- and 13-valent vaccines. However, this structure was based on the study of only 39 strains. The expected vaccinal efficacy for the serotypes of invasive pneumococcal infections widespread in the RF may reach 71.4, 92.8 and 100% for 7-, 10- and 13-valent vaccines, respectively [29]. The study of children with acute otites and pneumonias conducted in Saint Petersburg measured the expected efficacy of PCV7 at 75%, of PCV13 – at 95.1% [30]; this is comparable to our data.

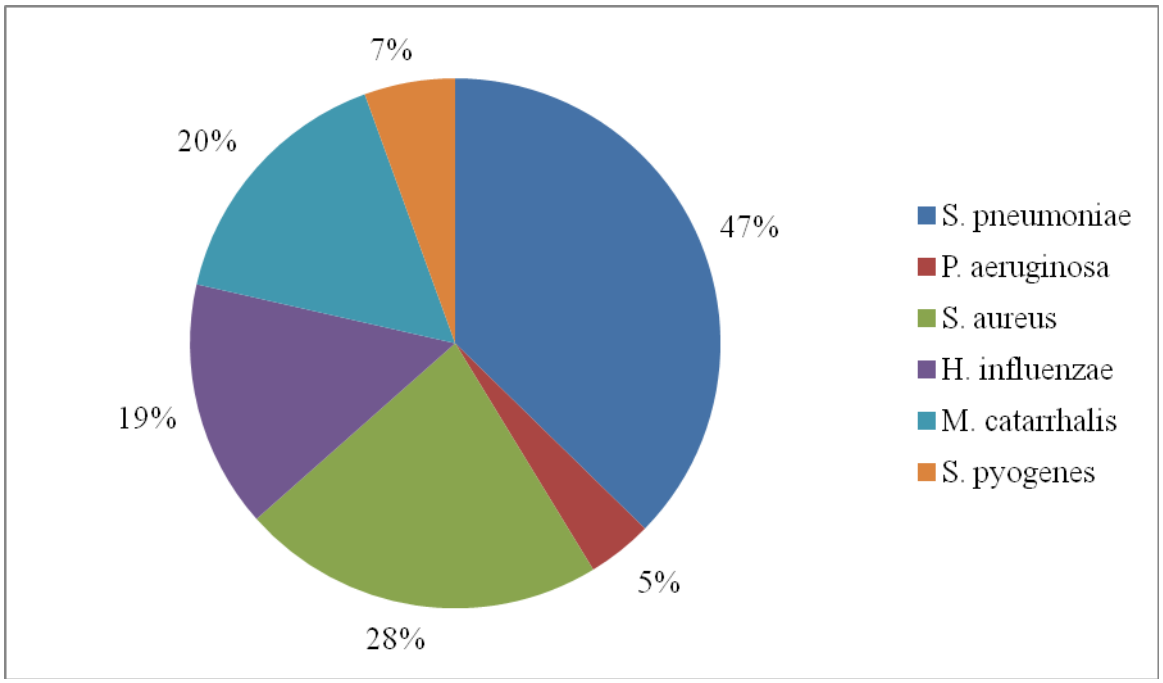
In conclusion, it ought to be mentioned that this study has been one of the most large-sale studies conducted in Russia; it presents extensive information on the spread of serotypes of non-invasive pneumococci in Russia. These data may be used as a starting point for future monitoring and evaluation of influence of PCV vaccines on epidemiology of serotypes and antibiotic resistance of *S. pneumoniae* in Russia (after PCV are introduced in the national vaccinal prevention calendar).

Credits: the authors express gratitude to doctors of the inpatient hospitals participating in the projects. This study was conducted under the aegis of a Pfizer grant.

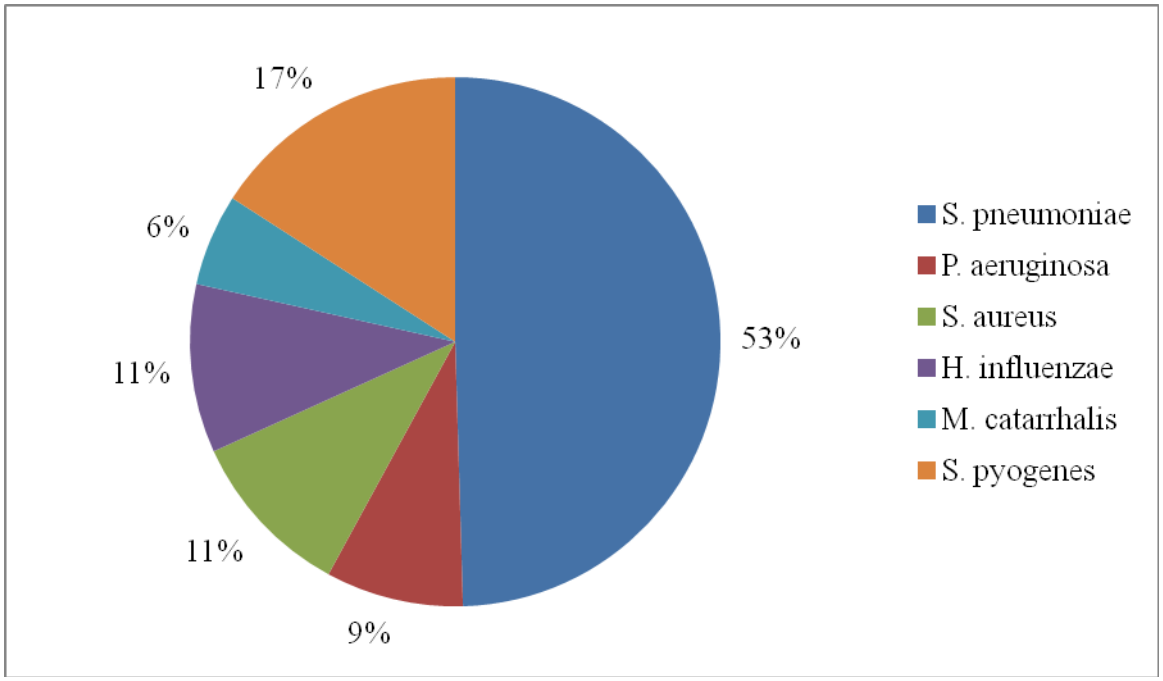
Pic. 1. Nosological structure of the patients involved in the study



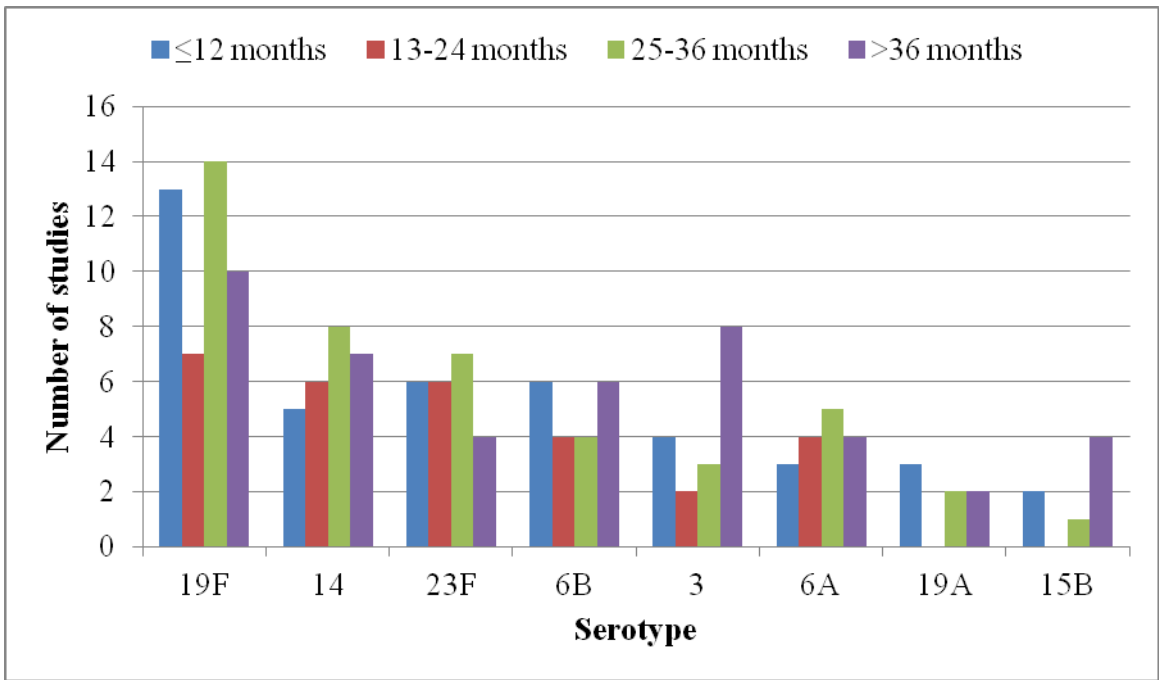
Pic. 2. Pathogenic nasopharyngeal flora in children with acute bacterial infections



Pic. 3. Range of the bacterial causative agents of acute otitis media revealed in middle ear fluid



Pic. 4. Age structure of the S. pneumoniae serotypes revealed in nasopharyngeal smears



Pic. 5. Age structure of the S. pneumoniae serotypes revealed in middle ear fluid

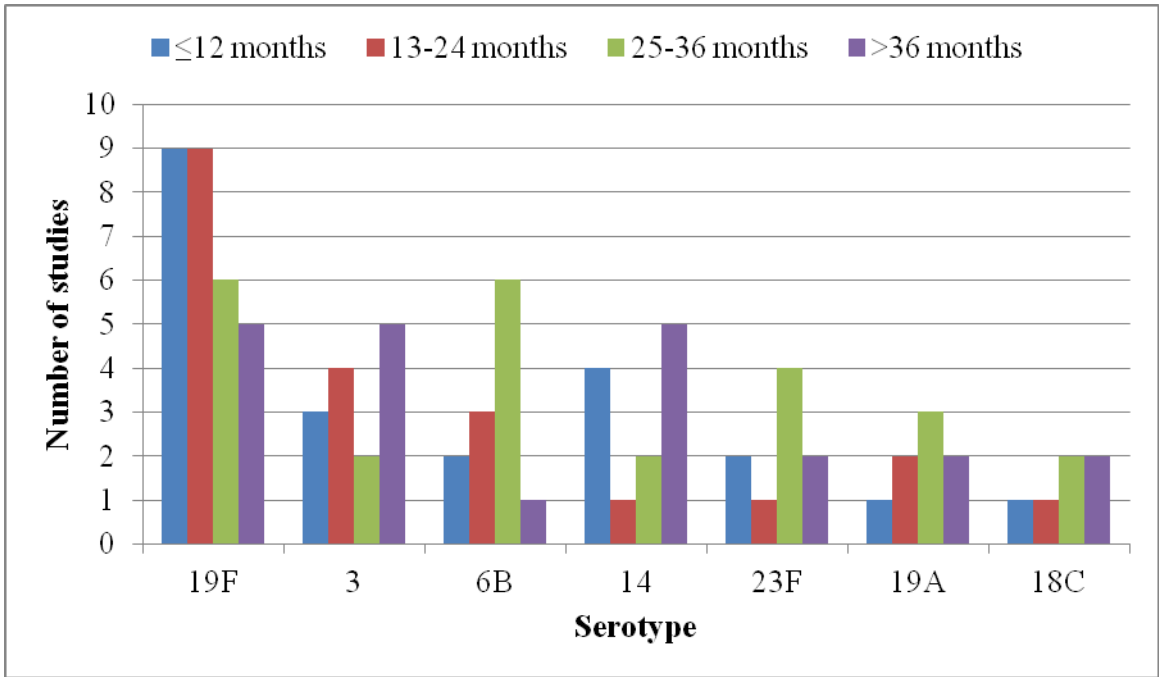


Table 1. General description of study results

Biomaterial samples	**Total samples** (n, %)	***S. pneumoniae*** (n, %)
Nasopharyngeal smear	857 (50)	208 (59)
Middle ear fluid	742 (43)	140 (40)
Blood	106 (6)	3 (0.8)
Cerebrospinal fluid	10 (1)	1 (0.2)
Total	**1,716 (100)**	**352 (100)**

Table 2. The invasive S. pneumoniae serotypes revealed by means of a cultural method

Parameters	**Material** (n, %)		**Total**
	Blood	**Cerebrospinal fluid**	
Total	**106 (100)**	**10 (100)**	**116 (100)**
Negative culture	94 (89)	3 (30)	97 (84)
Positive culture	12 (11)	7 (70)	19 (16)
Real pathogens:			
S. pneumoniae	3 (serotypes 14, 23F and 3)	1 (serotype 15C)	4
H. influenzae	1	5	6
N. meningitides	1	-	1
Possible contamination:			
S. epidermidis	2	-	2
E. faecalis	1	-	1
Micrococcus spp.	1	-	1
S. hominis	1	-	1
S. viridans	1	-	1
S. anginosus	-	1	1
S. capitis	1	-	1

Table 3. Nasopharyngeal *S. pneumoniae* serotypes

Serotype	**n**	**%**	**Accumulated, %**

19F	41	22.7	22.7
14	25	13.8	36.5
23F	23	12.7	49.2
3	17	9.4	58.6
6A	16	8.8	67.4
6B	16	8.8	76.2
15B	7	3.9	80.1
19A	6	3.3	83.4
7F	5	2.8	86.2
11A	4	2.2	88.4
35F	4	2.2	90.6
10A	2	1.1	91.7
9N	2	1.1	92.8
4	2	1.1	93.9
1	2	1.1	95.0
9V	1	0.6	95.6
15C	1	0.6	96.1
23A	1	0.6	96.7
18C	1	0.6	97.2
13	1	0.6	97.8
42	1	0.6	98.3
35C	1	0.6	98.9
28F	1	0.6	99.4
39	1	0.6	100.0
Total	**181**	**100.0**	**-**
Not typed	14 (7%)		
Non-typeable	4 (2%)		
Total	**199**		

Table 4. *S. pneumoniae* serotypes in middle ear fluid

Serotype	**n**	**%**	**Accumulated, %**
19F	34	26.6	26.6
3	18	14.1	40.6
14	13	10.2	50.8
23F	12	9.4	60.2
6B	12	9.4	69.5
19A	9	7.0	76.6
18C	7	5.5	82.0
6A	6	4.7	86.7
9V	4	3.1	89.8
15C	3	2.3	92.2
7F	2	1.6	93.8
15B	2	1.6	95.3
4	2	1.6	96.9
18	1	0.8	97.7
8	1	0.8	98.4
13	1	0.8	99.2
39	1	0.8	100.0
Total	**128**	**100.0**	**-**

Not typed	5 (3.6%)	-	-
Non-typeable	4 (2.9%)	-	-
Total	**137**	**-**	**-**

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