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Characteristics of *Streptococcus pneumoniae* carriage in the pediatric population

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Abstract

Objective: to investigate the regional peculiarities of *Streptococcus pneumoniae* carriage in the pediatric population and characterize the dominant serotypes of the pathogen.

Materials and methods. The clinical study group consisted of 509 healthy children attending preschool institutions. Examination of nasopharyngeal samples for the detection of *S. pneumonae* was carried out by classical bacteriological and molecular biological methods. The serotype was determined by real-time PCR. Genome-wide sequencing of the serogroups 15 and 11 isolates and bioinformatic analysis were performed.

Results. The *S. pneumoniae* bacterial carriers in the group of healthy children was detected in 207 children (40.7%), while the frequency of detection of *S. pneumoniae* in urban children living in Kazan was significantly higher than in children living in rural area and amounted to 53.4 and 31.1%, respectively ($p < 0.05$). Among children vaccinated with the 13-valent pneumococcal conjugate vaccine (PCV-13), *S. pneumoniae* carriers were not detected in 57.5% of cases. There were no significant differences in the degree of nasopharyngeal contamination depending on the vaccination status. Analysis of the serotype composition indicates the predominance of vaccine serotypes (57.7%), while the share of serotypes included in the PCV-13 vaccine accounts for only 24.7%, the share of non-vaccine serotypes was 32.1%, untyped — 10.2%. In unvaccinated children, vaccine serotypes that are part of the PCV-13 and 23-valent polysaccharide pneumococcal vaccine prevailed (PPSV-23): 6ABCD (21%), 11 AD (15%), 14 (13%). In vaccinated children, serotypes not included in the active vaccines dominated: 15AF (17.4%), 23A (19.2%), as well as 11AD (19.6%) (11A is included in PPSV-23). The 27 Kz isolate (serotype 15C) belonged to one of the most common sequence types ST1025. The 105_Kz isolate (serotype 11D) belonged to another common sequence type ST 62.

Conclusion. In order to improve epidemiological surveillance of pneumococcal infection, it is necessary to introduce the monitoring of circulating clonal complexes of dominant *S. pneumoniae* serogroups and analyze the genetic determinants of antibiotic resistance and virulence depending on the sequence type.

Keywords: *Streptococcus pneumoniae, bacterial carrier, serotypes, sequence types*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the Kazan Scientific Research Institute of Epidemiology and Microbiology (protocol No. 1, March 12, 2020).

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Характеристика бактерионосительства *Streptococcus pneumoniae* в детской популяции

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Аннотация

Цель: изучение региональных особенностей бактерионосительства *Streptococcus pneumoniae* в детской популяции и характеристика доминирующих серотипов возбудителя.

Материалы и методы. Обследованы 509 здоровых детей, посещающих детские дошкольные учреждения. Исследование мазков из носоглотки на обнаружение *S. pneumoniae* проводили классическим бактериологическим и молекулярно-биологическим методами. Определение серотипа осуществляли методом полимеразной цепной реакции в реальном времени. Проведено полногеномное секвенирование изолятов серогрупп 15 и 11.

Результаты. Бактерионосительство *S. pneumoniae* в группе здоровых детей выявлено у 207 (40,7%) детей, при этом частота обнаружения *S. pneumoniae* у городских детей, проживающих в Казани, была достоверно выше, чем у сельских детей, и составила 53,4 и 31,1% соответственно ($p < 0,05$). Среди детей, вакцинированных 13-валентной пневмококковой конъюгированной вакциной (ПКВ-13), в 57,5% случаев носительства *S. pneumoniae* не наблюдалось. Достоверных различий по степени обсеменённости носоглотки в зависимости от вакцинального статуса не установлено. Анализ серотипового состава указывает на преобладание вакцинных серотипов (57,7%), при этом на долю серотипов, входящих в состав вакцины ПКВ-13, приходится всего 24,7%; доля невакцинных серотипов составила 32,1%; нетипируемых — 10,2%. У невакцинированных детей преобладали вакцинные серотипы, входящие в состав ПКВ-13 и 23-валентной полисахаридной пневмококковой вакцины (ППСВ-23): серогруппа 6ABCD (вакцинными являются серотипы 6A и 6B; 21%), 11AD (15%), 14 (13%). У вакцинированных детей доминировали серотипы, не входящие в состав действующих вакцин: 15AF (17,4%), 23A (19,2%), а также 11AD (19,6%; 11A входит в ППСВ-23). Изолят 27_Kz (серотип 15C) относился к одному из наиболее распространённых сиквенс-типов ST1025. Изолят 105_Kz (серотип 11D) относился к другому распространённому сиквенс-типу ST62.

Выводы. В целях совершенствования эпидемиологического надзора необходимо внедрение мониторинга за циркулирующими клональными комплексами доминирующих серогрупп пневмококков и проведение анализа генетических детерминант антибиотикорезистентности и вирулентности в зависимости от сиквенс-типа.

Ключевые слова: *Streptococcus pneumoniae, бактерионосительство, серотипы, сиквенс-типы*

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Introduction

Streptococcus pneumoniae is one of the most common bacterial respiratory pathogens. Infections caused by this microorganism continue to be a pressing issue, with young children and the elderly belonging to the high-risk group [1]. According to the structure of capsular polysaccharide, 100 serotypes of *S. pneumoniae* have been identified to date [2]. The wide application of molecular genetic methods allows further differentiation of pneumococci by clones and sequences-types. The spectrum of dominant serotypes may depend on the age of the subjects and the geographic region, although similar distribution patterns have been observed in different countries. According to multicenter studies, approximately 20 serotypes account for more than 80% of invasive pneumococcal infection (IPI) cases in all age groups, with 13 of the most common serotypes responsible for 70–75% of pediatric IPI cases [3].

Pneumococcal vaccines include the most relevant serotypes that are associated with IPIs. Since the introduction of specific prophylaxis against pneumococcal infection in national childhood immunization programs, an increase in immune coverage has been observed not only among the child population but also among adults. As part of a research project to assess the shift and spread of pneumococcal pathogen serotypes, data from 44 surveillance sites (Europe, North America, Africa, Latin America, Asia and Oceania) were analyzed to examine the direct and indirect effects of routine use of childhood vaccination programs with 10- and 13-valent pneumococcal conjugate vaccines (PCV-10 and PCV-13) on the incidence of IPIs. Routine childhood immunization with PCV was observed to result in an 85% reduction in IPIs among children and adults of all ages 6 years after the vaccine introduction [3].

Despite the undoubted achievements of vaccine prophylaxis, which can be attributed to the reduction of IPIs in all age groups of vaccinated and unvaccinated individuals, there is evidence of an increase in the frequency of nasopharyngeal colonization with non-vaccine and untypable serotypes, including encapsulated strains of pneumococci. Numerous studies of PCV-13 efficacy in different regions have shown that the use of this vaccine can not only reduce the incidence of pneumococcal infection, but also cause changes in the serotype composition of circulating *S. pneumoniae* strains [4, 5].

One of the priority areas of pneumococcal infection control is conducting scientific research to study the direct and indirect effect of vaccination on the results of morbidity and bacterial carriers after the introduction of PCV to the pediatric population in different territories.

The objective of our study was to investigate regional peculiarities of *S. pneumoniae* carriage in the pediatric population and characterize the dominant serotypes of the pathogen.

Materials and methods

As part of the regional monitoring in the Republic of Tatarstan in 2020–2022, 509 children aged 3 years to 5 years 11 months and 29 days were examined in accordance with the inclusion criteria (acceptable age, parents or legal representatives signing a voluntary informed consent form). The studies were approved by the Local Ethical Committee of the Kazan Research Institute of Epidemiology and Microbiology (protocol No. 1 of 12.03.2020). The group of healthy children (n = 509) included those attending preschool institutions in Kazan (n = 204) and Vysokaya Gora (n = 305), in the absence of acute respiratory disease symptoms. Vaccine status of children was studied according to their developmental checklist.

Nasopharyngeal swabs served as the main material for the study. ESwab (Copan) fluid collection and transportation system was used for collection and transportation of biomaterial. *S. pneumonae* was determined by bacteriological and molecular-biological methods. Culturing was performed on Colombian CNA agar with 5% defibrinated sheep blood (Sredoff). Petri dishes were incubated for 18–24 h at 37ºC in an atmosphere containing 5% CO_2. *S. pneumoniae* was identified by morphological (Gram-positive diplococci), cultural (S-form colonies with alpha-hemolysis) and biochemical properties (catalase test, sensitivity to optochin and bile salts) in accordance with the methodological guidelines[1].

DNA was extracted from pure *S. pneumoniae* cultures using the AmpliSens DNA-Sorb-B Nucleic Acid Extraction Kit (InterLabService). Typing of the obtained samples by polymerase chain reaction (PCR) was carried out in two stages. The first stage was detection of marker genes of *S. pneumoniae* — *lytA* and *cpsA*; the second stage — serotype determination by real-time PCR using fluorescently labeled oligonucleotides and primers according to CDC recommendations[2]: 6A/B/C/D, 9A/V, 23F, 19F, 18A/B/C/F, 15A/F, 19A, 3, 12F/A/B/44/46, 7A/F, 4, 5 11A/D, 16F, 9L/N, 14, 1, 2, 22A/F, 23 A, 33A/33F/37. *S. pneumoniae* isolates that were not categorized into the studied groups were labeled as untypable.

Whole genome sequencing (WGS) was performed for 2 *S. pneumoniae* isolates, whose serotypes were determined by PCR as 15AF and 11AD. The genome analysis of the first isolate showed that it belonged to serotype 15C (*Streptococcus pneumoniae*

[1] Methodological guidelines MG 4.2. 0114-16 “Laboratory diagnosis of community-acquired pneumonia of pneumococcal etiology” (approved by the Federal Service for Surveillance on Consumer Rights Protection and Human Welfare, Chief State Sanitary Doctor of the Russian Federation).

[2] Table 1: List of oligonucleotide primers used in 41 conventional multiplex* PCR assays for pneumococcal serotype deduction of 70 serotypes. URL: http://www.cdc.gov/streplab/downloads/pcr-oligonucleotide-primers.pdf

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105_Kz CP125291), while the second isolate belonged to serotype 11D (*Streptococcus pneumoniae* 105_Kz CP125291). The obtained data were uploaded to GenBank (BioProject PRJNA971376 (NZ_CP126249.1) and PRJNA1009429). The choice of serotypes for sequencing is explained by the significant spread of pneumococci serogroups 15 and 11 against the background of vaccination, with only serotypes 11A, 15A and 15C included in the new PCV-20 (Pfizer) [6], serotypes 11A and 15B — in the composition of PV23.

For WGS, DNA was isolated from pure *S. pneumoniae* cultures, using the QIAamp DNA Mini Kit (Qiagen). WGS was performed on DNBSEQ-G50 (MGI) and GridION (Oxford Nanopore Technologies) platforms. Libraries for WGS were prepared using the MGIEasy Fast FS DNA Library Prep Set (MGI), Native Barcoding Expansion and Ligation Sequencing Kit (Oxford Nanopore Technologies), respectively. The median length of the library fragments was 430 bp (identified using the QIAxcel Advanced System capillary gel electrophoresis system). Sequencing to obtain paired-end reads was performed on the DNBSEQ-G50 platform (MGI) using the DNBSEQ-G50RS kit (FCL PE150/FCS PE150).

The quality of the obtained nucleotide sequences was assessed using the FastQC v. 0.11.8 program (Babraham Bioinformatics). Quality read filtering and removal of PCR adapters and primers used in library preparation were performed using the Cutadapt v. 1.15 program. For *de novo* genome assembly, the SPAdes v. 3.15.4 algorithm was used [7], for hybrid assembly – Unicycler v. 0.4.7 [8]. The final quality assessment was performed using the Quast v. 5.0.2 program [9]. MLST (Multilocus sequence typing) was performed using the MLST v. 2.0 program[3] [10]. Genomes were annotated using the RAST (Rapid Annotations using Subsystems Technology) server [11]. The CARD database was used to identify genes and mutations associated with antibiotic resistance [12]. The search for prophage sequences in the genomes of the studied isolates was performed using the Phaster online service [13], the search for pathogenicity islands in the genomes was performed using the IslandViewer v. 4 online service [14].

The results obtained during the study were processed using the Statistica for Windows v. 6.0 program system. The level of $p < 0.05$ was considered as the criterion of statistical reliability of the obtained data.

Results

S. pneumoniae bacterial carriers in the group of healthy children were detected in 207 (40.7%) children, and the frequency of *S. pneumoniae* detection in children living in Kazan was significantly higher than in children living in rural areas – 53.4 and 31.1%, respectively ($p < 0.05$). When studying the vaccination status according to the development checklists, it was found that out of 509 healthy children, 315 were vaccinated with PCV-13 (**Table 1**). Of the 207 *S. pneumoniae* carriers, 134 children were vaccinated, with 43 children receiving the full course of vaccination, 47 receiving 2 doses, and 44 receiving only 1 dose.

According to the Immunoprophylaxis Republican Center, vaccination of the child population against pneumococcal infection has been conducted in the Republic of Tatarstan according to the national calendar of preventive vaccinations since 2014. The PCV-13 pneumococcal vaccine is used for vaccination in accordance with the schedule, which includes vaccination at 2, 4, 5 months and subsequent revaccination at 15 months. As of 01.01.2021, 82% of children aged 0–7 years were vaccinated with 1-3 doses of the vaccine, with the share of those who had undergone the full course of vaccination and revaccination amounting to 60.4%.

No *S. pneumoniae* bacterial carriage was observed among vaccinated children in the majority of cases (57.5%). Only 20.7% of children who underwent the full course of vaccination were found to be *S. pneumoniae* bacterial carriers ($p < 0.01$). Thus, in most cases the bacterial carriers were unvaccinated or children who had not undergone the full course of vaccination.

When studying the degree of nasopharyngeal *S. pneumoniae* contamination, it was found that low contamination (10^1–10^2 CFU/tampon) was observed in 62 (30%) cases, moderate contamination (10^3–10^4 CFU/tampon) in 113 (54.6%), high contamination (10^5–10^6 CFU/tampon) in 32 (15.4%). At the same time, in the groups of bacterial carrier children, both vaccinated and unvaccinated, a moderate degree of contamination prevailed, no significant differences in the degree of contamination depending on PCV-13 vaccination were found (**Table 2**).

The serotype composition of pneumococcal strains isolated from healthy children was studied and analyzed depending on the vaccine status of the bacterial carrier (**Table 3**).

The total number of identified serotypes of *S. pneumoniae* isolated from nasopharyngeal swabs exceeded the number of cultures isolated, indicating mixed colonization by several serotypes (from 2 to 4), with mixed colonization more frequently observed in vaccinated children in most cases. This phenomenon has also been observed by other researchers [15].

The results of the serotype composition analysis indicate low coverage of serotypes circulating among child carriers by pneumococcal vaccines used in Russia, and we were unable to differentiate individual serotypes within some serogroups during the study. The results obtained can be assessed as tentative and requiring further deciphering. The share of serotypes included in the PCV-13 vaccine was 24.7%, and the share of vaccine serotypes of the 23-valent pneumococcal polysac-

[3] Center for Genomic Epidemiology. URL: http://www.cbs.dtu.dk/services/MLST

Table 1. The frequency of *S. pneumoniae* bacterial carriage in vaccinated healthy children, *n* (%)

Vaccination	Number of vaccinated children	Number of children who are not *S. pneumoniae* carriers	Number of the *S. pneumoniae* carrier children
V1	108	64 (59,3%)	44 (40,7%)*
V2	115	68 (59,2%)	47 (40,8%)*
V3	92	49 (53,3%)	43 (46,7%)
Total	315	181 (57,4%)	134 (42,5%)*

Note. *$p < 0.01$ compared to the non-carrier group.

Table 2. The degree of *S. pneumoniae* contamination of the nasopharynx depending on vaccination by PCV-13, *n* (%)

The degree of contamination	Number of *S. pneumoniae* carrier children	The number of cases of *S. pneumoniae* carriage in vaccinated children	Number of cases of *S. pneumoniae* carriage in unvaccinated children	*p*
Low	62 (30%)	35 (26,2%)	27 (43,5%)	0,152
Medium	113 (54,6%)	78 (58,2%)	35 (47,1)	< 0,01
High	32 (15,4%)	21 (15,6%)	11 (34,4%)	0,013
Total	207	134 (64,7%)	73 (35,3%)	< 0,01

charide vaccine (PPSV-23), which is not used for vaccination of children, was 33%. The share of non-vaccine serotypes amounted to 32.1%, and untypable serotypes — 10.2%. Analysis of serotype composition depending on the vaccination status of the children showed significant differences in the frequency of serotypes. In unvaccinated children, serotypes belonging to vaccine serotypes prevailed — 6ABCD (21%), 11AD (15%), 14 (13%), although we were unable to differentiate vaccine and non-vaccine serotypes in several serogroups. In vaccinated children, predominant serotypes were 15AF (17.4%), 23A (19.2%), i.e. serotypes not included in current vaccines, and 11AD (19.6%), part of the serogroup, a representative of which (11A) is included in PPSV-23, which is very rarely used to prevent pneumococcal infection in children.

Of the isolates associated with the most common serogroups 15 and 11 among pediatric carriers that are not included in the PCV-13 vaccine, one representative isolate each was selected for sequencing (**Table 4**). The 27_Kz isolate (serotype 15C) belonged to sequence type ST1025. The 27_Kz genome isolate contained one intact prophage (Streptococcus phage SpSL1, NC_027396(23), 39.7 bp) and residues of 3 prophages. The 27_Kz genome contained components of phosphotransferase systems of different specificity (galactose-specific, mannitol-specific, beta-glucosidase-specific, cellobiose-specific); sortase genes; IgA1-protease gene; LanM lanthionine-containing bacteriocin synthesis genes; asparagine synthesis genes; *piaABCD* locus (encoding iron ion transporters); ABC transporter genes of different specificity (amino acids, polyamines, metal ions). Variants of the type I restriction-modification system (types of S-subunit specificity) of the 27_Kz isolate were associated with pathogenicity (based on the annotation in IslandViewer v. 4). The presence of antibiotic resistance determinants of different classes of antibiotics was predicted using RAST-online. A variant of the *folA* dihydrofolate reductase gene associated with trimethoprim resistance was identified in the 27_Kz genome (Table 4).

The 105_Kz isolate (serotype 11D) belonged to ST62. The 105_Kz isolate genome contained residues of 4 prophages and a mobile genetic element Tn5252 containing a lantibiotic synthesis locus. The 105_KZ isolate genome also contained components of phosphotransferase systems of different specificity, soraptase genes, IgA1-protease gene, *piaABCD* locus, ABC-transporter genes of different specificity (amino acids, polyamines, metal ions). The 105_Kz isolate was characterized by the presence of cytosine-DNA methyltransferase gene (most genetic lines of *S. pneumoniae* contain adenine-DNA methyltransferases). The presence of a cytosine-specific methyltransferase that is not widely distributed among *S. pneumoniae* may explain the absence of intact prophages in the genome of this strain, and may also be related to the genetic stabilization and spread of ST62. Furthermore, the 105_Kz isolate has ATP synthase V (not F type) and may have features of energy metabolism.

Thus, based on vaccination in Russia, there is a spread of serogroups 15 and 11 in child bacterial carriers of *S. pneumoniae*, representatives of which may be associated with genetic lines that have potentially increased virulence or other adaptive changes that ensure stabilization and successful spread of these clones.

Discussion

The results of our study show that PCV-13 vaccination does not completely exclude the phenomenon of bacterial carriage among preschool children, but at the same time, children who have undergone a full course of vaccination and revaccination have significantly lower rates of bacterial carriage compared to the group

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Table 3. Serotype composition of *S. pneumoniae* isolated from healthy bacterial carrier children depending on the vaccination status, *n* (%)

Vaccine	Identified serotypes	All vaccinated and unvaccinated	Unvaccinated with PCV-13	Vaccinated with PCV-13	*p*
PCV-13 vaccine serotypes	4	0	0	0	–
	6ABCD (only 6A and 6B are included in PCV-13)*	48	21 (43,75%)	27 (56,25%)	0,223
	9AV (only 9V is included in PCV-13)*	0	0	0	–
	14	20	13 (65%)	7 (35%)	0,061
	18ABCF (only 18C is included in PCV-13)*	4	0	4	–
	19F	1	0	1	–
	23F	0	0	0	–
	1	1	0	1	–
	5	0	0	0	–
	7AF (only 7F is included in PCV-13)*	0	0	0	–
	3	6	1 (16,67%)	5 (83,33%)	0,021
	19A	0	0	0	–
PPSV-23 Vaccine serotypes	2	1	1	0	–
	8	0	0	0	–
	9LN (only 9N is included in PPSV-23)*	28	11 (39,29%)	17 (60,71%)	0,112
	10A	0	0	0	–
	11AD (only 11A is included in PPSV-23)*	59	15 (25,42%)	44 (74,58%)	0,214
	12F	3	0	3	–
	15BC (only 15B is included in PPSV-23)*	0	0	0	–
	17F	0	0	0	–
	22F	11	4 (36,36%)	7 (63,64%)	0,211
	33F	5	0	5	–
	12AF (only 12F is included in PPSV-23)*	0	0	0	–
Non-vaccine serotypes	15AF	49	10 (20,41%)	39 (79,59%)	0,213
	16F	1	0	1	–
	23A	54	11 (20,37%)	43 (79,63%)	0,213
Untyped serotypes	–	33	13 (39,39%)	20 (60,61%)	0,087
Total		324	100 (30,86%)	224 (69,14%)	0,216

Note. *Serotypes of some serogroups were not differentiated by real-time PCR.

of children who have not been vaccinated or undergone its full course. We have not revealed the influence of vaccination on the degree of pneumococcal colonization of the nasopharynx in healthy bacterial carriers. Possibly, the degree of colonization is influenced by other factors related to immunological features of the host, virulence of the pathogen or environmental factors, which undoubtedly requires further research.

In this study, a correlation between the frequency of bacterial carriage and the living environment of the children was established: in urban children the frequency of nasopharyngeal colonization with *S. pneumoniae* was significantly higher than in children living in rural areas, which can be explained by more frequent contact in the urban environment. These data should be taken into account when planning regional monitoring

Table 4. Characteristics of genomes of serotypes 15C and 11D isolates obtained from nasopharyngeal swabs of children with identified *S. pneumoniae* carriage

Sample	Genbank accession number	Year of isolation	Serotype	Sequence type	Patient's age, years	PEN	ERY	TET	CHL	TXT
27_Kz	NZ_CP126249.1	2020	15C	1025	3	S	S	S	S	R
105_Kz	PRJNA1009429	2020	11D	62	4	S	S	S	S	S

Note. PEN — penicillin; ERY — erythromycin; TET — tetracycline; CHL — chloramphenicol; TXT — co-trimoxazole; R — presence, S — absence of resistance determinants.
Source: Prediction of antimicrobial resistance in PATRIC and RAST. URL: https://www.bv-brc.org/job

studies of *S. pneumoniae* circulation among different groups of the pediatric population. Monitoring studies conducted in different countries indicate the existence of common patterns in the distribution of *S. pneumoniae* serotype composition after the introduction of routine immunization. For example, in Portugal, PCV-13 has been available since 2010, after a decade of using PCV-7. S. Felix et al. evaluated changes in the serotype distribution and antimicrobial susceptibility of pneumococcal pneumococci carried by children living in two regions of Portugal (one urban and one rural) over 3 epidemiologic periods: before the introduction of PCV-13 (2009–2010), the early PCV-13 period (2011–2012), and the late PCV-13 period (2015–2016) [16]. Nasopharyngeal samples (n = 4232) obtained from children aged 0–6 years attending day care centers were studied. PCV-13 immunization rates were very high in both regions (> 75%). Pneumococcal carriage remained consistently high: 62.1, 62.4, and 61.6% during the study periods, respectively (p = 0.909) in the urban region and 59.8, 62.8, 59.5% (p = 0.543) in the rural region. Meanwhile, the carriage of serotypes comprising PCV-13 decreased in both urban (16.4, 7.3, and 1.6%; $p < 0.001$) and rural areas (13.2, 7.8, and 1.9%; $p < 0.001$). This decrease was mainly due to serotype 19A (14.1, 4.4 and 1.3% in the urban region and 11.1, 3.6 and 0.8% in the rural region; $p < 0.001$), while serotypes 11D, 15A/B/C, 16F, 21, 22F, 23A/B, 24F, 35F and untyped variants were most prevalent in the late stages of PCV-13 immunization [16].

Monitoring of serotype composition of circulating pneumococci allowed in our study to identify some trends in the structure of dominant serotypes depending on the vaccine status of children. Thus, we found that, despite the predominance of vaccine serotypes, the proportion of *S. pneumoniae* variants included in PCV-13 tends to decrease and to be replaced by serotypes not included in its composition. We observed serotype replacement processes in both groups, but with different intensity. The groups of vaccinated children showed greater genetic diversity with predominance of non-vaccine serotypes (15AF — 17.4%; 23A — 19.2%), while in the group of unvaccinated children their share was lower (15AF — 10%; 23A — 11%).

The replacement of vaccine serotypes with non-vaccine serotypes was reported by researchers soon after the introduction of mass immunization with PCV in various countries. Following the introduction of routine immunization with PCV-7 in the USA in 2003–2005, profound changes in the distribution of serotypes colonizing the nasopharynx of children were reported, with some of the non-vaccine serotypes becoming more prevalent and more aggressive due to antibiotic resistance [17, 18].

Since the introduction of PCV-13, researchers from countries that have included this vaccine in their national immunization programs have reported an increase in the number of infections with *S. pneumoniae* serogroup 15, which is not covered by this vaccine [19–21]. Pneumococci of this serogroup have caused outbreaks and deaths in children [22, 23].

In China, continuous monitoring at the Beijing Children's Hospital, partially reflecting the prevalence of *S. pneumoniae* in Chinese children during the study period, has shown the share of serogroup 15 pneumococci in the pediatric population to be as high as 6.12%. After the introduction of PCV-13 in China in May 2017, the isolation rates of *S. pneumoniae* serogroup 15 in 2018 and 2019 were 7.41% and 10.53%, respectively, showing an upward trend [24]. Chinese researchers found that *S. pneumoniae* serogroup 15 strains exhibited good sensitivity to common antibiotics, but the most common clonal complex (CC) CC3397 was 100% resistant to penicillin, suggesting the influence of antibiotics in altering the predominant CCs [24]. Many studies have previously reported clonal shift phenomena in other serotypes, for example, CC271 replaced CC983 among serotype 19F strains [25], ST81 replaced ST342 among pneumococcal isolates of serotype 23F [26], CC320 replaced CC230 in 19A strains [27], CC876 replaced CC875 in serotype 14 strains [28]. These examples of clonal shift phenomena in one serotype may be caused by the selection effect of antibiotics, according to which sequence types expressing high antibiotic resistance replace sequence types with lower resistance. Long-term epidemiologic studies of antibiotic resistance of *S. pneumoniae* strains among different serotypes, including serogroup 15, are necessary to confirm this theory.

Over the period of our observations since 2016, we also note an increasing trend in the prevalence of serogroup 15 serotypes: the proportion of 15AF sero-

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types in 2016–2019 and 2020–2021 increased from 2.4% to 7.0%, with 17.4% in the group of vaccinated children in 2021; for the part of serogroup 15BC, there is also an increasing trend from 2.4% in 2016 to 3.9% in 2019 [29]. Serogroup 15B, 15A and 15C serogroup 15 are known to be among the most common serotypes of *S. pneumoniae* associated with invasive pneumococcal disease after the introduction of PCV-13, furthermore, 15B contributes significantly to the development of acute otitis media. The capsular polysaccharides of serotypes 15A, 15B, and 15C are closely related, with 15A having a linear structure of repeating units, and 15B and 15C having a branched structure of repeating units of carbohydrate residues [30].

According to the results of the Russian multicenter PeGAS studies 2015–2018 on invasive strains of *S. pneumoniae*, the dominant serotypes belonging to serogroups 3 (21%), 19F and 6ABE (11% each), 15B (6.5%) were identified. Sequence types were determined in all 46 strains studied and 6 previously undescribed sequence types were identified: ST15247–ST15252, while MLST did not reveal a predominant sequencing type or identify a CC, with the exception of serotype 3 strains [31]. According to the results of another multicenter study from 2016, the genetic lines CC505 (serotype 3), CC236/CC271/CC320 (19F), CC1025 (15BC), CC143 (different serotypes), CC311 (23F), which are often associated with invasive diseases, are predominantly widespread in Russia. For CC1025, which includes serotype 15BC, an increasing trend in abundance has also been noted. The genetic lines CC505, CC1025 and CC311 are associated with sensitivity to most classes of antibiotics. The genomes of representatives of the widespread genetic lines carry a variety of virulence determinants [32].

Conclusion

The studies indicate a high frequency of nasopharyngeal colonization in preschoolers, especially among urban children. At the same time, the serotype landscape is influenced by the vaccination status of the children: reliable differences in the frequency of occurrence of different serotypes in vaccinated and unvaccinated children have been established. At the present stage, monitoring only the serotype (serogroup) composition of circulating *S. pneumoniae* strains is insufficient. In order to improve epidemiologic surveillance of pneumococcal infection, it is necessary to introduce the monitoring of circulating *S. pneumoniae* CC and analysis of genetic determinants of antibiotic resistance depending on ST, using WGS and bioinformatics analysis.

СПИСОК ИСТОЧНИКОВ | REFERENCES

1. Государственный доклад «О состоянии санитарно-эпидемиологического благополучия населения в Российской Федерации в 2022 году». М.; 2023. State Report «About the state of sanitary and epidemiological welfare of the population in the Russian Federation in 2022». Moscow; 2023.
2. Ganaie F., Saad J. S., McGee L., et al. A new pneumococcal capsule type, 10D, is the 100th serotype and has a large cps fragment from an oral streptococcus. *mBio.* 2020;11(3):e00937-20. DOI: https://doi.org/10.1128/mbio.00937-20
3. WHO. Pneumococcal conjugate vaccines in infants and children under 5 years of age. *Wkly Epidemiol. Rec*. 2019;94(8):85–104.
4. Ubukata K., Takata M., Morozumi M., et al. Effects of pneumococcal conjugate vaccine on genotypic penicillin resistance and serotype changes, Japan, 2010–2017. *Emerg. Infect. Dis*. 2018;24(11):2010–20. DOI: https://doi.org/10.3201/eid2411.180326
5. Kim S.H., Chung D.R., Song J.H., et al. Changes in serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates from adult patients in Asia: emergence of drug-resistant non-vaccine serotypes. *Vaccine.* 2020;38(38):6065–73. DOI: https://doi.org/10.1016/j.vaccine.2019.09.065
6. Hurley D., Griffin C., Young M., et al. Safety, tolerability, and immunogenicity of a 20-valent pneumococcal conjugate vaccine (PCV20) in adults 60 to 64 years of age. *J. Clin. Infect. Dis*. 2021;73(7):e1489–97. DOI: https://doi.org/10.1093/cid/ciaa1045
7. Prjibelski A., Antipov D., Meleshko D., et al. Using SPAdes de novo assembler. *Curr. Protoc. Bioinformatics*. 2020;70(1):e102. DOI: https://doi.org/10.1002/cpbi.102
8. Wick R.R., Judd L.M., Gorrie C.L., Holt K.E. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput. Biol.* 2017;13(6):e1005595. DOI: https://doi.org/10.1371/journal.pcbi.1005595
9. Gurevich A., Saveliev V., Vyahhi N., Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013;29(8):1072–5. DOI: https://doi.org/10.1093/bioinformatics/btt086
10. Larsen M.V., Cosentino S., Rasmussen S., et al. Multilocus sequence typing of total genome sequenced bacteria. *J. Clin. Microbiol.* 2012;50(4):1355–61. DOI: https://doi.org/10.1128/JCM.06094-11
11. Olson R.D., Assaf R., Brettin T., et al. Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): a resource combining PATRIC, IRD and ViPR. *Nucleic. Acids. Res*. 2023;51(D1):D678–89. DOI: https://doi.org/10.1093/nar/gkac1003
12. Alcock B.P., Huynh W., Chalil R., et al. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the comprehensive antibiotic resistance database. *Nucleic. Acids. Res*. 2023;51(D1):D690–9. DOI: https://doi.org/10.1093/nar/gkac920.
13. Arndt D., Grant J.R., Marcu A., et al. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic. Acids. Res*. 2016;44(W1):W16–21. DOI: https://doi.org/10.1093/nar/gkw387
14. Bertelli C., Laird M.R., Williams K.P., et al. IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. *Nucleic. Acids. Res*. 2017;45(W1):W30–5. DOI: https://doi.org/10.1093/nar/gkx343
15. Sidorenko S., Rennert W., Lobzin Y., et al. Multicenter study of serotype distribution of *Streptococcus pneumoniae* nasopharyngeal isolates from healthy children in the Russian Federation after introduction of PCV13 into the National Vaccination Calendar. *Microbiol. Infect. Dis.* 2020;96(1):114914. DOI: https://doi.org/10.1016/j.diagmicrobio.2019.114914
16. Felix S., Handem S., Nunes S., et al. Impact of private use of the 13-valent pneumococcal conjugate vaccine (PCV13) on pneumococcal carriage among Portuguese children living in urban and rural regions. *Vaccine* 2021;39(32):4524–33. DOI: https://doi.org/10.1016/j.vaccine.2021.06.035
17. Hicks L.A., Harrison L.H., Flannery B., et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. *J. Infect. Dis.* 2007;196(9):1346–54. DOI: https://doi.org/10.1086/521626
18. Pai R., Moore M.R., Pilishvili T., et al. Postvaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *J. Infect. Dis.* 2005;192(11):1988–95. DOI: https://doi.org/10.1086/498043
19. van der Linden M., Perniciaro S., Imöhl M. Increase of serotypes 15A and 23B in IPD in Germany in the PCV13 vaccination era. *BMC Infect. Dis*. 2015;15:207. DOI: https://doi.org/10.1186/s12879-015-0941-9
20. Sheppard C., Fry N. K., Mushtaq S., et al. Rise of multidrug-resistant non-vaccine serotype 15A *Streptococcus pneumoniae* in the United Kingdom, 2001 to 2014. *Euro. Surveill.* 2016;21(50):30423. DOI: https://doi.org/10.2807/1560-7917.ES.2016.21.50.30423
21. Nakano S., Fujisawa T., Ito Y., et al. Spread of meropenem-resistant *Streptococcus pneumoniae* serotype 15A-ST63 clone in Japan, 2012–2014. *Emerg. Infect. Dis.* 2018;24(2):275–83. DOI: https://doi.org/10.3201/eid2402.171268
22. Fleming-Dutra K., Mbaeyi C., Link-Gelles R., et al. *Streptococcus pneumoniae* serotype 15A in psychiatric unit, Rhode Island, USA, 2010–2011. *Emerg. Infect. Dis.* 2012;18(11):1889–93. DOI: https://doi.org/10.3201/eid1811.120454
23. Arushothy R., Ramasamy H., Hashim R., et al. Multidrug-resistant *Streptococcus pneumoniae* causing invasive pneumococcal disease isolated from a paediatric patient. *Int. J. Infect. Dis.* 2020;90:219–22. DOI: https://doi.org/10.1016/j.ijid.2019.10.037
24. Shi W., Du Q., Yuan L., et al. Antibiotic resistance and molecular biological characteristics of non-13-valent-pneumococcal conjugate vaccine serogroup 15 *Streptococcus pneumoniae* isolated from children in China. *Front. Microbiol.* 2022;12:778985. DOI: https://doi.org/10.3389/fmicb.2021.778985
25. Li Q.H., Yao K.H., Yu S.J., et al. Spread of multidrug-resistant clonal complex 271 of serotype 19F *Streptococcus pneumoniae* in Beijing, China: characterization of serotype 19F. *Epidemiol. Infect.* 2013;141(12):2492–6. DOI: https://doi.org/10.1017/S0950268813000514
26. Ma X., Yao K.H., Yu S.J., et al. Genotype replacement within serotype 23F *Streptococcus pneumoniae* in Beijing, China: characterization of serotype 23F. *Epidemiol. Infect.* 2013;141(8):1690–6. DOI: https://doi.org/10.1017/S0950268812002269
27. Маянский Н.А., Алябьева Н.М., Пономаренко О.А. и др. Динамика распространенности серотипов и антибиотикорезистентности носоглоточных пневмококков, выделенных у детей в 2010–2016 гг.: результаты ретроспективного когортного исследования. *Вопросы современной педиатрии*. 2017;16(5):413–23. Mayanskiy N.A., Alyab'eva N.M., Ponomarenko O.A., et al. Serotypes and antimicrobial susceptibility of nasopharyngeal pneumococci isolated from children in 2010–2016: a retrospective cohort study. *Current Pediatrics.* 2017;16(5):413–23. DOI: https://doi.org/10.15690/VSP.V16I5.1806 EDN: https://elibrary.ru/ztiykx
28. He M., Yao K., Shi W., et al. Dynamics of serotype 14 *Streptococcus pneumoniae* population causing acute respiratory infections among children in China (1997–2012). *BMC Infect. Dis.* 2015;15:266. DOI: https://doi.org/10.1186/s12879-015-1008-7
29. Исаева Г.Ш., Баязитова Л.Т., Зарипова А.З. и др. Региональные особенности серотипового состава *Streptococcus pneumoniae*, выделенных от детей-бактерионосителей дошкольного возраста в Республике Татарстан. *Эпиде-*

миология и вакцинопрофилактика. 2023;22(3):26–35. Isaeva G.Sh., Bayazitova L.T., Zaripova A.Z., et al. Regional features of the serotype composition of *Streptococcus pneumoniae* isolated from bacterial carriers of preschool age in the Republic of Tatarstan. *Epidemiology and Vaccinal Prevention*. 2023;22(3):26–35.
DOI: https://doi.org/10.31631/2073-3046-2023-22-3-26-35
EDN: https://elibrary.ru/avelpt

30. Hao L., Kuttel M.M., Ravenscroft N., et al. *Streptococcus pneumoniae* serotype 15B polysaccharide conjugate elicits a cross-functional immune response against serotype 15C but not 15A. *Vaccine.* 2022;40(33):4872–80.
DOI: https://doi.org/10.1016/j.vaccine.2022.06.041
31. Миронов К.О., Корчагин В.И., Михайлова Ю.В. и др. Характеристика штаммов *Streptococcus pneumoniae*, выделенных от больных инвазивными пневмококковыми инфекциями, с использованием высокопроизводительного секвенирования. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2020;97(2):113–8. Mironov K.O., Korchagin V.I., Mikhaylova Yu.V., et al. Characterization of *Streptococcus pneumoniae* strains causing invasive infections using whole-genome sequencing. *Journal of Microbiology, Epidemiology and Immunobiology.* 2020;97(2):113–8.
DOI: https://doi.org/10.36233/0372-9311-2020-97-2-113-118
EDN: https://elibrary.ru/lnxmqy
32. Цветкова И.А., Никитина Е.В., Александрова Е.В. и др. Характеристика распространенных генетических линий *Streptococcus pneumoniae*, циркулировавших в различных регионах России с 2003 г. по 2022 г. *Проблемы медицинской микологии*. 2023;25(2):195. Tsvetkova I.A., Nikitina E.V., Aleksandrova E.V., et al. Wide-spread *Streptococcus pneumoniae* genetic lines in different regions of Russia, 2003–2022. *Problems in Medical Mycology*. 2023;25(2):195.
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