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Serotypes and Antimicrobial Susceptibility of Nasopharyngeal Pneumococci Isolated from Children in 2010–2016: A Retrospective Cohort Study

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Background. *Pneumococci (Streptococcus pneumoniae) represent major pathogens that cause acute infections in children.* ***Objective***. *Our aim was to analyze dynamics of the distribution of nasopharyngeal pneumococcal serotypes and their antimicrobial susceptibility in children.* ***Methods***. *A retrospective cohort study was conducted. We examined nasopharyngeal pneumococci isolated from children getting care at the National Medical Research Center of Children's Health (Moscow) in 2010–2016. Serotyping was performed using specific antisera and/or by molecular typing employing PCR. Susceptibility to oxacillin (OXA), erythromycin (ERY), clindamycin (CLI), trimethoprim/sulfamethoxazol, chloramphenicol and tetracycline was tested by the disk diffusion method. In 2013–2016, penicillin (PEN), amoxicillin (AMX), ERY and CLI minimal inhibitory concentrations (MIC) were measured.* ***Results***. *A total of 1,111 pneumococcal isolates were examined; the sample was obtained from children with a median age of 4 years (P25–P75, 2.4–6.5 years). We identified 48 pneumococcal serotypes; six leading serotypes were serotypes 3, 6A, 6B, 14, 19F and 23F aggregating a proportion of 63.2% in the overall distribution. From 2010 to 2016, the distribution of serotypes has not changed. Wherein, 13-valent pneumococcal conjugate vaccine covered 74% of serotypes in children under 5 years. The five leading serotypes (6A, 6B, 14, 19F, 23F and serotype 19A) had the highest resistance rate. Within 2010–2016, the proportion of OXA- and ERY-resistant pneumococci grew from 21.3% to 35.9% and from 24.5% to 36.9%, respectively. The majority (81.3%) of ERY-resistant isolates possessed an MLSB-phenotype, i.e. were resistant to macrolides, lincosamides, and streptogramin B. In 2013–2016, the rate of PEN- and AMX-resistant pneumococci was 34.6% and 3.5%, respectively.* ***Conclusion***. *Within the seven year study period, no major shifts in the nasopharyngeal pneumococcal serotype distribution were observed. The pneumococci remained highly susceptible to AMX, but activity of macrolides was significantly reduced. Considering the leading mechanism of macrolide resistance, the use of any macrolides or lincosamides for empiric treatment of pneumococcal infections in children is questionable.*
Key words*: children, pneumococcus, serotype, isolates, antibiotics, resistance, dynamics.*
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RESULTS

Table 1. Minimum inhibitory concentrations of penicillin, amoxicillin, erythromycin, and clindamycin in nasopharyngeal pneumococci isolated in 2013–2016

AMA	Proportion (%) of isolates with corresponding MIC (mg/L)[a]													MIC breakpoints (mg/L)[b] and proportion (%) of isolates in the relevant category						MIC_{50}	MIC_{90}
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256	Susceptible		Moderately resistant		Resistant			
														MIC	%	MIC	%	MIC	%	mg/L	
PEN (*n* =633)	65.4	6.5	4.4	6.3	11.1	4.1	1.3	0.6	0.3	0	-	-	-	≤0.06	65.4	0.12−2	32.4	>2	2.2	≤0.06	1
AMX (*n* =631)	68.8	6.5	2.4	4.8	9.0	5.1	2.9	0.6	0	0	-	-	-	≤2	96.5	4	2.9	>4	0.6	≤0.06	1
ERY (*n* =633)	-	-	64.8	0	0.5	0.9	1.4	2.2	1.9	1.1	0.2	0	27	≤0.25	64.8	0.5	0	>0.5	35.2	≤0.25	≥256
CLI (*n* =631)	-	-	-	78.1	0.3	0.3	0	0	0.2	0	0	0	21.1	≤0.5	78.1	None	-	>0.5	21.9	≤0.5	≥256

Note. [a] — range of the studied MICs (mg/L): PEN and AMX — from ≤ 0.06 to ≥ 32; ERY — from ≤ 0.25 to ≥ 256; CLI — from ≤ 0.5 to ≥ 256.
[b] — MIC breakpoints for PEN, ERY and CLI are specified according to EUCAST criteria [20], for AMX — according to CLSI criteria [21]. AMA — antimicrobial agent, PEN — penicillin, AMX — amoxicillin, ERY — erythromycin, CLI — clindamycin, MIC — minimum inhibitory concentration.

Table 2. Serotype distribution of nasopharyngeal pneumococcal isolates (*n* =1,111) obtained in 2010–2016

Serotype	Abs. (%)	Cumulated %
19F	209 (18.8)	18.8
23F	127 (11.4)	30.2
6B	125 (11.3)	41.5
14	109 (9.8)	51.3
6A	69 (6.2)	57.5
3	63 (5.7)	63.2
15B/C	53 (4.8)	68.0
11A	42 (3.8)	71.7
Non-typeable	**25 (2.3)**	**74.0**
23A	23 (2.1)	76.1
19A	20 (1.8)	77.9
18C	20 (1.8)	79.7
35C	18 (1.6)	81.3
9V	17 (1.5)	82.8
33F	17 (1.5)	84.3
7F	16 (1.4)	85.8
9N	15 (1.4)	87.1
35F	14 (1.3)	88.4
10A	14 (1.3)	89.6
6C	14 (1.3)	90.9
37	10 (0.9)	91.8
6D	10 (0.9)	92.7

22F	9 (0.8)	93.5
34	8 (0.7)	94.2
15A	8 (0.7)	95.0
8	7 (0.6)	95.6
17F	6 (0.5)	96.1
16F	6 (0.5)	96.7
Other	**37 (3.3)**	**100**

Note. Serotypes are arranged in descending order of their frequency. 27 serotypes with a frequency of ≥5 isolates and non-typeable isolates are shown. The remaining serotypes were included in the category «Other» (total 21 serotype, in descending order of frequency): 35B — 4; 23B — 3; 31 — 3; serotypes 1, 4, 12F, 13, 20, 21, 28A, 38, 39 — 2 each; serotypes 7C, 9A, 10B, 11D, 15F, 24A, 24F, 28F, 42 — 1 each.

Table 3. Antibiotic resistance of nasopharyngeal pneumococci isolated in 2010–2016

Serotype	**The number (%) of resistant isolates in the corresponding serotype distribution**						
	OXA	ERY	CLI[a]	SXT	CHL	TET	MDR[b]
6A	18/67 (26.9)	21/67 (31.3)	6/66 (9.1)	44/67 (65.7)	2/22 (9.1)	4/17 (23.5)	7/67 (10.4)
6B	36/125 (28.8)	67/125 (53.6)	55/124 (44.4)	71/125 (56.8)	4/77 (5.2)	21/33 (63.5)	52/125 (41.6)
14	74/109 (67.9)	59/109 (54.1)	25/109 (22.9)	81/109 (74.3)	3/109 (2.8)	16/46 (34.8)	54/109 (49.5)
19A	13/20 (65)	8/20 (40)	5/20 (25)	12/20 (60)	0/19 (0)	5/10 (50)	7/20 (35)
19F	113/207 (54.6)	112/207 (54.1)	100/207 (48.3)	138/205 (67.3)	11/138 (8.0)	50/74 (67.6)	98/207 (47.3)
23F	45/126 (35.7)	20/126 (15.9)	8/125 (6.4)	64/126 (50.8)	5/78 (6.4)	7/50 (14)	15/126 (11,9)
Other	37/440 (8.4)	41/440 (9.3)	27/438 (6.2)	111/439 (25.3)	4/226 (1.8)	21/129 (16.3)	24/440 (5.5)[c]
All serotypes	336/1,094 (30.7)	328/1,094 (29.9)	227/1,089 (20.8)	521/1,091 (47.8)	29/669 (4.3)	124/359 (34.5)	257/1,094 (23.5)

Note. OXA — oxacillin, ERY — erythromycin, CLI — clindamycin, SXT — sulfamethoxazole / trimethoprim, CHL — chloramphenicol, TET — tetracycline, MDR — multi-drug resistant.
[a] — the results of CLI susceptibility determination without evaluation of inducible CLI-resistance in ERY-resistant isolates in the presence of macrolide (see «Methods») are presented. [b] — MDR was determined by susceptibility to 4 antibiotics **—** PEN, ERY, CLI, and SXT. [c] — other MDR-serotypes (the number of insusceptible isolates / total isolates, in the order of serotypes nomenclature): 6C (1/13), 9N (1/15), 9V (5/17), 11A (3/42), 13 (1/2), 15A (1/8), 20 (1/2), 23A (3/23), 24F (1/1), 31 (1/3), 35C (2/18), non-typeable (4/22).

Table 4. Dynamics of antibiotic resistance of nasopharyngeal pneumococcal isolates in 2010–2016

Antibiotic	**Testing period, years[a]**					***P*[b]**
	2010/2011	**2012**	**2013**	**2014/2015**	**2016**	
OXA	40/189 (21.3)	69/274 (25.4)	63/197 (32)	58/142 (40.8)	106/295 (35.9)	0.001
ERY	46/189 (24.5)	58/274 (21.2)	55/197 (27.9)	60/142 (42.3)	109/295 (36.9)	0.001
CLI[c]	37/186 (19.9)	47/272 (17.3)	43/192 (21.8)	31/142 (21.8)	69/295 (23.4)	0.482
SXT	110/186(59.1)	135/274 (49.3)	103/197 (52.3)	64/142 (45.1)	111/295 (37.6)	0.001
CHL	6/41 (14.6)	3/40 (7.5)	1/151 (0.7)	5/142 (3.5)	14/295 (4.7)	0.002
TET	15/25 (60.0)	17/28 (60.7)	7/14 (53.8)	NT	85/293 (29.0)	0.001

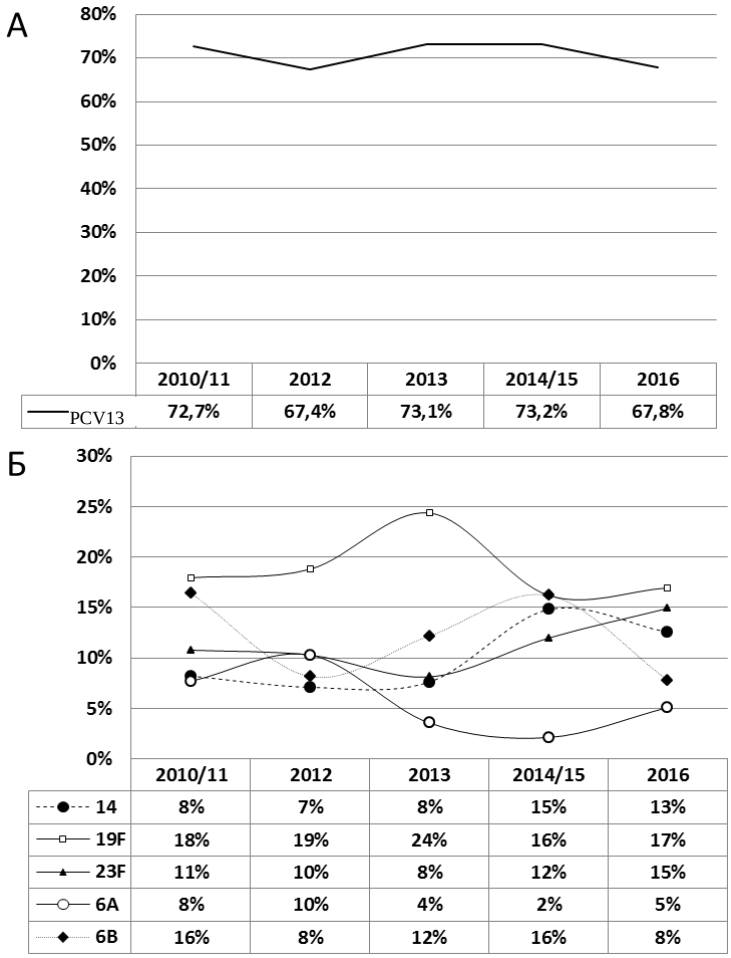
Note. OXA — oxacillin, ERY — erythromycin, CLI — clindamycin, SXT — sulfamethoxazole / trimethoprim, CHL — chloramphenicol, TET — tetracycline.
[a] — the number of resistant / tested isolates and the proportion (%) of resistant isolates are given. [b] — *p*-value is calculated for df = 4 (for TET, df = 3); a statistically significant result indicates a change in the incidence of resistant isolates during 2010-2016. [c] — the results of CLI susceptibility determination without evaluation of inducible CLI-resistance in ERY-resistant isolates in the presence of macrolide (see «Methods»). NT — not tested.

Table 5. Polymorphic variants of the *ermB* and *mef* genes in erythromycin-resistant pneumococci and their susceptibility to clindamycin

Phenotype	**Genotype, abs. (%)**			
	***ermB*+/*mef*-**	***ermB*+/*mef*+**	***ermB*-/*mef*+**	***ermB*-/*mef*-**
Total ERY-resistant, *n* =224	85 (37.9)	92 (41.1)	40 (17.9)	7 (3.1)
• $cMLS_B$-phenotype (CLI-resistance), *n* =142	57 (40.0)	81 (57.0)	0	4 (3.0)
• $iMLS_B$-phenotype (CLI-resistance), *n* =40	28 (70)	10 (25)	1 (2.5)	1 (2.5)
• M-phenotype (CLI-resistance), *n* =42	0	1 (2.0)	39 (93.0)	2 (5.0)

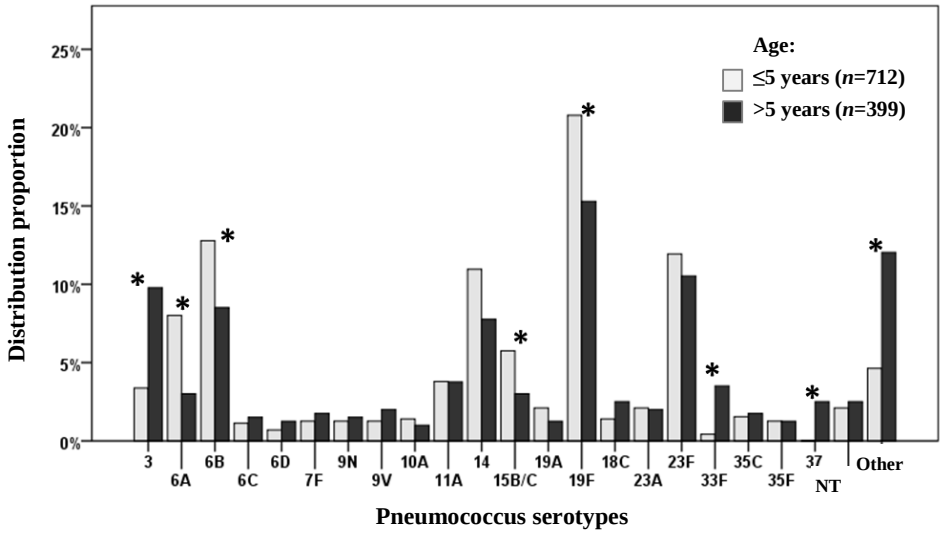
Note. ERY — erythromycin, CLI — clindamycin. $cMLS_B$-phenotype — constitutive resistance to all macrolides, lincosamides, streptogramin B; $iMLS_B$-phenotype — inducible resistance to all macrolides, lincosamides, streptogramin B (the presence of inducible resistance was recorded when the growth suppression zone was flatted around the disk with CLI adjacent to ERY disk); M-phenotype — resistance to 14- and 15-membered macrolides.

Fig. 1. Dynamics of the prevalence (%) of specific serotypes of nasopharyngeal pneumococcal isolates in 2010–2016.



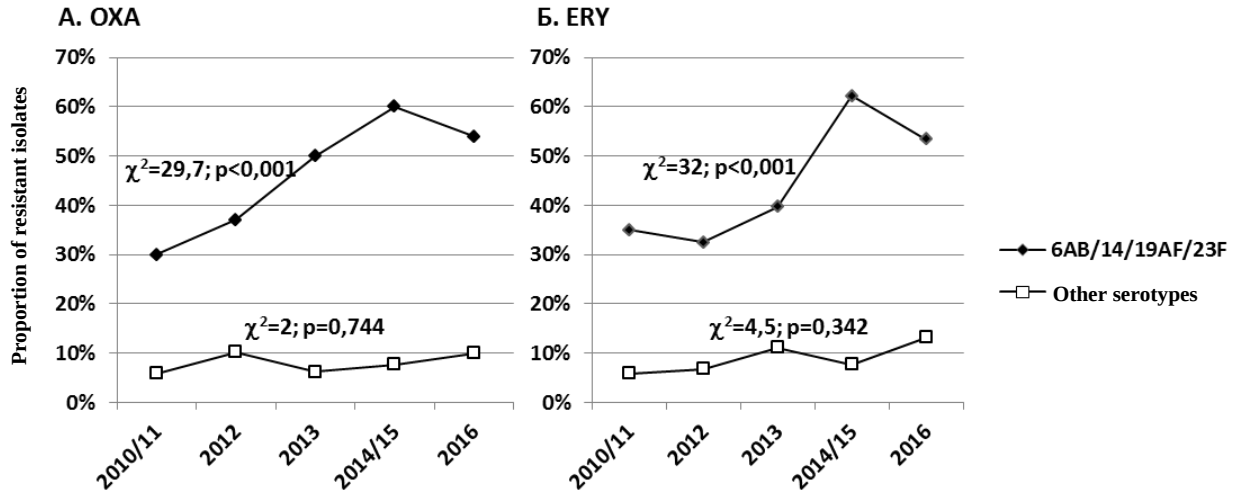
Note. PCV13 — 13-valent pneumococcal conjugate vaccine.
A — the total proportion of serotypes that make up PCV13 [1, 3, 4, 5 (not found), 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F]; B — proportion of the 5 most common serotypes.
The figure indicates the proportion of serotypes in the corresponding period of time. Number of isolates by period: 2010/11 —195; 2012 —282; 2013 —197; 2014/15 —142; 2016 —295.

Fig. 2. Serotype distribution of nasopharyngeal pneumococcal isolates in children of different age groups



Note. Serotypes are arranged in the order of their nomenclature. There are 21 serotypes represented by ≥10 isolates each. * — significant differences in proportions between two age groups: 6B — p =0.031; 19F — p =0.024; 3, 33F, 37, Other — p <0.001.
NT — non-typeable serotypes.

Fig. 3. Dynamics of the resistance of nasopharyngeal pneumococcal isolates to oxacillin (A) and erythromycin (B), depending on the serotype



Note. χ^2 and p criteria values for the change in frequency of resistant isolates during 2010–2016 are indicated above the curves. OXA — oxacillin, ERY — erythromycin.

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CONFLICT OF INTERESTS

Nikolay A. Mayanskiy — receiving research grants and fees for being a speaker from pharmaceutical companies Pfizer and GlaxoSmithKline.

Tatiana V. Kulichenko — receiving research grants from pharmaceutical companies Pfizer, Abbott and fees for being a speaker from Merck, Pfizer, Pierre Fabre.

The other contributors confirmed the absence of a reportable conflict of interests.

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