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Changing serotype distribution and resistance patterns among pediatric nasopharyngeal pneumococci collected in Moscow, 2010–2017

Nikolai Mayanskiy *, Tatiana Kulichenko, Natalia Alyabieva, Ekaterina Brzhozovskaya, Olga Ponomarenko, Tatiana Savinova, Anna Lazareva

National Medical Research Center for Children's Health, 119991, Lomonosovskiy prosp., 2, Moscow, Russia

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ABSTRACT

Serotype distribution and antimicrobial resistance were analyzed in 632 nasopharyngeal pneumococcal isolates collected at a single pediatric center in 2010–2017 before and following the introduction of the 13-valent pneumococcal conjugated vaccine (PCV13) in Russia in 2014. The mean prevalence of PCV13 serotypes was 77.7% in 2010–2015 with a significant decline to 58.5% in 2017, which was accompanied by an elevation in serotype 15B/C prevalence (15.1% in 2017), 66% and 26% of 15B/C-pneumococci related to ST1025 and ST1262, respectively. The rate of oxacillin, erythromycin, and clindamycin resistance has increased by 15–20 percentage points from 2010 to 2016, approaching a 40–45% prevalence in 2016. The resistance rates significantly increased over time only in a group of PCV13 serotypes. The growing resistance among serotype 14 pneumococci was associated with expansion of a multidrug-resistant clone of ST143. These results emphasize the need for close monitoring of the constantly changing pneumococcal population.

1. Introduction

The pneumococcus (*Streptococcus pneumoniae*) remains among the most important causes of infectious morbidity and mortality, especially in children and elderly (Ceyhan et al., 2016; O'Brien et al., 2009). Prophylaxis of invasive pneumococcal disease (IPD) using polysaccharide conjugate vaccines (PCVs) that contain from 7 to 13 pneumococcal capsule polysaccharide serovariants has dramatically decreased the rate of severe pneumococcal infections (Balsells et al., 2017; Richter et al., 2014; van der Linden et al., 2016). The reduction of the vaccine type-associated IPD rate coincided with expansion of several nonvaccine, previously rare serotypes among IPD isolates as well as in carriage. The impact of PCV on pneumococcal serotype epidemiology is well documented, although vaccine-independent changes in serotype prevalence over time have been reported (Black, 2010; Choi et al., 2008; Dagan et al., 2009).

Changing prevalence of a given serotype may be accompanied by alterations in the genetic composition of that particular serotype, as it has been demonstrated for serotype 19A and, more recently, for serotype 35B (Hulten et al., 2013; Olarte et al., 2017; Richter et al., 2013; van der Linden et al., 2013). Worryingly, clonal evolution of these serotypes resulted in the expansion of multiple drug–resistant (MDR) lineages of sequence types (ST) 320 and 156, respectively (Olarte et al., 2017; van der Linden et al., 2013). Growing resistance of pneumococci against commonly used antimicrobials poses a major health care concern.

Hence, the constantly changing pneumococcal population experiencing various selective challenges requires close monitoring. In Russia, investigation of pneumococcal serotype epidemiology and resistance patterns has gained a new impulse on the threshold of PCV13 introduction into the National immunization program in 2014, which secured a universal 2 + 1-dose vaccine schedule in children at 2, 4.5, and 15 months of age with full reimbursement. A number of studies from several regions describing serotype distribution and resistance of pneumococci in the pre-PCV period in Russia have been published (Kalinogorskaya et al., 2015; Mayanskiy et al., 2014; Tatochenko et al., 2014). In the present work, we analyzed the dynamics of serotype diversity and resistance profile among nasopharyngeal pneumococcal isolates collected from children below 5 years of age at a single center in Moscow in the period of 2010–2017 with the emphasis on temporal changes in genotype prevalences among pneumococci of serotype 14 and 15B/C.

2. Materials and methods

2.1. Pneumococcal isolate collection

This retrospective cohort study included all pneumococcal isolates that were recovered from nasopharyngeal swabs of children ≤5 years of age in the microbiology laboratory of the National Medical Research

* Corresponding author. Tel.: +7-4991340218.
E-mail address: mayansky@nczd.ru (N. Mayanskiy).

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Center for Children's Health (Moscow) in 2010–2017. Biological material was collected during the routine local diagnostic procedure from children with symptoms of an acute respiratory infection visiting the outpatient department of this hospital using an eSWAB kit (Copan, Italy). Signed informed consent was obtained from the parents or legal representatives of enrolled children before sampling. PCV vaccination status of the participants was not available.

The specimens were plated on blood agar medium with 5% sheep blood and 2% horse serum and incubated at 37 °C with 5% CO_2 for 24–48 h. *S. pneumoniae* was identified by optochin test and latex agglutination with the Slidex pneumo-kit (bioMérieux, France). Serotyping was performed by pool antisera for latex agglutination and type/factor antisera in the Quellung reaction using Staten Serum Institut products (SSI, Copenhagen, Denmark). Isolates that agglutinated none of the pool sera (A to I and P to T) were considered nontypeable. To ensure the pneumococcal identity, such isolates were further PCR-tested for the *ply* and *psaA* genes, which are considered as reasonably accurate pneumococcal markers (Verhelst et al., 2003).

2.2. Antimicrobial susceptibility testing

Antibiotic susceptibility testing for oxacillin (OXA), erythromycin (ERY), clindamycin (CLI), trimethoprim/sulfamethoxazole (SXT), chloramphenicol (CHL), and tetracycline (TET) was done using the disk diffusion method with disks from Bio-Rad (USA). Starting from 2013, in OXA- and/or ERY-resistant isolates, we determined minimum inhibitory concentrations (MICs) for penicillin (PEN), amoxicillin (AMX), and ERY by E-test strips (Oxoid, UK). The PEN and ERY MIC category interpretations were based on updated standards of European Committee on Antimicrobial Susceptibility Testing (EUCAST) (The European Committee on Antimicrobial Susceptibility testing (EUCAST), 2018); AMX MICs were assessed according to Clinical and Laboratory Standards Institute (CLSI) breakpoints (CLSI, 2016) because EUCAST does not offer MIC interpretations for this antimicrobial. Intermediate and resistant isolates were collectively grouped as nonsusceptible (NS). MDR was defined as nonsusceptibility to ≥3 antimicrobial classes (Magiorakos et al., 2012).

2.3. Genetic analysis

PCR was used to detect the *erm(B)* and *mef* (without differentiation between *mefA* and *mefE*) determinants in ERY-resistant pneumococci, as described previously (Mayanskiy et al., 2014). Multilocus sequence typing (MLST) of pneumococcal isolates of serotypes 14 and 15B/C was performed according to the *S. pneumoniae* MLST protocol, as described elsewhere (https://pubmlst.org/spneumoniae, n.d.; Mayanskiy et al., 2017).

2.4. Statistics

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Contingency table analysis for comparing serotype distribution and resistance rate changes over time was done by Chi-square test. Proportions were compared by means of the *Z*-criterion. The tests were considered statistically significant at $P < 0.05$.

3. Results

3.1. The overall collection

Our analyses included 632 nasopharyngeal pneumococcal isolates obtained from children with a median age of 3.0 years (interquartile range, 1.7–4.0 years) in 2010–2017. The isolate collection was divided in 6 time periods for dynamic analysis (Table 1). In 2015, fewer pneumococcal isolates was obtained due to a reduction of patient flow, which was related to restructuring of the outpatient care at our hospital. The age difference between periods was not statistically significant (Kruskal–Wallis test; $H = 6.5$; $P = 0.263$).

In the overall collection, 32 serotypes were discovered (Table 1). Nine (1.4%) isolates were nontypeable; all these isolates tested positive for *ply* and *psaA* carriage, supporting their initial pneumococcal identification. In 2010–2015, the prevalence of PCV13 serotypes was relatively stable, ranging from 83.1% to 74.6% (mean, 77.7%) ($\chi^2 = 3$; $P = 0.7$). In 2016, we observed a small reduction of the PCV13 serotype rate to 70%, but in 2017, the proportion of PCV13 serotypes significantly declined to 58.5% ($z = -4.0$, $P < 0.001$ vs 2010–2015 period).

3.2. Serotype 15B/C characterization

The rise of non-PCV13 serotypes was predominantly associated with serotype 15B/C (Table 1). At the beginning of the study in 2010–2014, the prevalence of this serotype was relatively stable (mean, 4.1%; range, 3.5% to 4.5%). Later, however, serotype 15B/C isolates demonstrated a volatile prevalence comprising 12.7%, 5.8%, and 15.1% of the overall distribution in 2015, 2016, and 2017, respectively (Table 1). Overall, the increase of serotype 15B/C proportion over time was statistically significant ($\chi^2 = 18$; $P = 0.002$). Forty-three of 45 serotype 15B/C isolates were typed using factor antisera; of these pneumococci, 31 (72%) and 12 (28%) had serotype 15B and 15C, respectively. The MLST analysis of 35 15B/C-pneumococci revealed that 66% (23/35) and 26% (9/35) of isolates related to sequence types 1025 and 1262, respectively, with similar prevalences over 2010–2015 and 2016–2017.

Serotype 15B/C isolates were almost universally susceptible to all tested antimicrobials, except SXT (60% were SXT-resistant); 2 isolates had increased MICs for PEN (0.5 and 2 mg/L), another isolate had *mef*-associated resistance to ERY (ST199).

3.3. Antimicrobial resistance

Using the disk diffusion method over the whole study period, we demonstrated that the overall resistance rate varied from 3.1% for CHL to 52% for SXT with an MDR phenotype prevalence of 29% (Table 2; "Overall" column). All antimicrobials, except TET, were examined during at least 4 study periods; thus, a dynamic analysis was possible (Table 2; "All" rows). The resistance rates for OXA, ERY, CLI, and SXT showed statistically significant fluctuations during the study period; variations in the rate of CHL resistance were marginal. From 2010 to 2016, the rate of the OXA-, ERY-, and CLI-resistant pneumococci has grown by 15–20 percentage points (pp) approaching a 40–45% prevalence in 2016. In 2017, however, these prevalences dropped by approximately 10 pp compared to 2016 (Table 2; "All" rows). The SXT resistance rate declined from 64% in 2010/11 to 41.5% in 2017.

The majority of resistant pneumococci, including isolates with an MDR phenotype, belonged to a narrow spectrum of serotypes represented by 5 most prevalent serotypes, 6A, 6B, 14, 19F, and 23F, and serotype 19A (Table 2, "6AB/14/19AF/23F" rows). In fact, the resistance rates for OXA, ERY, and CLI have significantly increased over time only in this group of serotypes, whereas non-6AB/14/19AF/23F pneumococci demonstrated no statistically significant temporal trends in resistance to any antimicrobial tested. Since 2015, the resistance rates of the 6AB/14/19AF/23F serotype group have not been below 50% for OXA, ERY, and CLI; SXT resistance has been high, although declining over time. The proportion of the CHL-resistant pneumococci was stably small over time in all serotypes. In non-6AB/14/19AF/23F serotypes, the resistance rate was low for all tested antimicrobials, except SXT (30.7%), and did not differ significantly between time periods. The proportion of MDR-pneumococci in the 6AB/14/19AF/23F group was 40.9%, whereas among the remaining isolates, only 6.4% had an MDR phenotype (Table 2).

Starting from 2013, we introduced an E-test–based MIC examination for PEN, AMX, and ERY in isolates that were resistant to OXA or/and ERY as measured by the disk diffusion method (see Appendix A). The rates of

Table 1
Serotype distribution of *Streptococcus pneumoniae*, Moscow, 2010–2017.

Serotype	Period, no. of isolates (proportion of isolates in the period)						
	2010/11	2012	2013/14	2015	2016	2017	Overall
PCV13							
19F	17 (19.1%)	25 (18.0%)	31 (27.0%)	10 (15.9%)	27 (22.5%)	18 (17.0%)	128 (20.3%)
6B	20 (22.5%)	14 (10.1%)	20 (17.4%)	13 (20.6%)	7 (5.8%)	10 (9.4%)	84 (13.3%)
23F	9 (10.1%)	17 (12.2%)	16 (13.9%)	5 (7.9%)	21 (17.5%)	8 (7.5%)	76 (12.0%)
14	5 (5.6%)	13 (9.4%)	8 (7.0%)	11 (17.5%)	16 (13.3%)	8 (7.5%)	61 (9.7%)
6A	11 (12.4%)	21 (15.1%)	3 (2.6%)	1 (1.6%)	4 (3.3%)	7 (6.6%)	47 (7.4%)
3	2 (2.2%)	6 (4.3%)	5 (4.3%)	3 (4.8%)	1 (0.8%)	2 (1.9%)	19 (3.0%)
19A	3 (3.4%)	3 (2.2%)	2 (1.7%)	2 (3.2%)	5 (4.2%)	3 (2.8%)	18 (2.8%)
9 V	2 (2.2%)	1 (0.7%)	2 (1.7%)	2 (3.2%)	1 (0.8%)	2 (1.9%)	10 (1.6%)
18C	4 (4.5%)	3 (2.2%)	0	0	0	3 (2.8%)	10 (1.6%)
7F	1 (1.1%)	2 (1.4%)	2 (1.7%)	0	2 (1.7%)	1 (0.9%)	8 (1.3%)
Subtotal	74 (83.1%)	105 (75.5%)	89 (77.4%)	47 (74.6%)	84 (70%)	62 (58.5%)	461 (72.9%)
Non-PCV13							
15B/C	4 (4.5%)	6 (4.3%)	4 (3.5%)	8 (12.7%)	7 (5.8%)	16 (15.1%)	45 (7.1%)
11A	1 (1.1%)	6 (4.3%)	5 (4.3%)	1 (1.6%)	7 (5.8%)	5 (4.7%)	25 (4%)
35F	0	2 (1.4%)	2 (1.7%)	4 (6.3%)	0	5 (4.7%)	13 (2.1%)
23A	1 (1.1%)	4 (2.9%)	3 (2.6%)	0	1 (0.8%)	3 (2.8%)	12 (1.9%)
10A	1 (1.1%)	3 (2.2%)	2 (1.7%)	1 (1.6%)	2 (1.7%)	0	9 (1.4%)
6C	2 (2.2%)	0	1 (0.9%)	1 (1.6%)	1 (0.8%)	4 (3.8%)	9 (1.4%)
9N	2 (2.2%)	2 (1.4%)	1 (0.9%)	0	3 (2.5%)	0	8 (1.3%)
35C	1 (1.1%)	1 (0.7%)	2 (1.7%)	0	3 (2.5%)	0	7 (1.1%)
15A	0	2 (1.4%)	1 (0.9%)	0	0	2 (1.9%)	5 (0.8%)
Other (n < 5) [a]	3 (3.4%)	6 (4.3%)	5 (4.3%)	1 (1.6%)	8 (6.7%)	6 (5.7%)	29 (4.6%)
Nontypeable	0	2 (1.4%)	0	0	4 (3.3%)	3 (2.8%)	9 (1.4%)
Subtotal	15 (16.9%)	34 (24.5%)	26 (22.6%)	16 (25.4%)	36 (30%)	44 (41.5%)	171 (27.1%)
Total	89 (100%)	139 (100%)	115 (100%)	63 (100%)	120 (100%)	106 (100%)	632 (100%)

[a] Other: serotypes 6D, 16F, 34 (4 each); 8, 22F, 35B (3 each); 33F (2); 15F, 17F, 28F, 37, 39, 42 (1 each).

PEN-, AMX-, or ERY-resistant isolates did not differ significantly between time periods in the course of 2013–2017 (data not shown); thus, we combined the data from these periods. Among 149 OXA-resistant pneumococci, 5 (3.4%) isolates were susceptible to PEN having an MIC ≤0.06 mg/L. Thus, 144 (35.7%) isolates were PEN-nonsusceptible (MIC >0.06 mg/L), including 11 (2.7%) resistant isolates (MIC >2 mg/L). The same 11 isolates were nonsusceptible to AMX; the remaining 391 (97.3%) pneumococci had an AMX MIC corresponding to the susceptible category (≤2 mg/mL). PEN-resistant isolates had serotypes 19A (2), 19F (6), 23A (1), and 23F (2). All 3 AMX-resistant pneumococci (MIC >4 mg/L) had serotype 19F.

As shown in Appendix A, the vast majority of ERY-resistant bacteria (77.9%; 123/158) showed high ERY MICs (≥256 mg/L). Examination of the macrolide resistance genotype revealed that 130 (81.8%) ERY-resistant pneumococci carried *ermB* (alone or in a combination with *mef*). Among 144 PEN-NS isolates, 115 (79.9%) were resistant to ERY; thus, the overall prevalence of PEN-NS/ERY-R pneumococci was 28.5% (115/403).

3.4. Clonal evolution of serotype 14

In 2015–2016, we noticed an elevation of serotype 14 prevalence approaching a mean rate of 14.8% (27/183) (see Table 1). Moreover, this

Table 2
Antimicrobial resistance of *Streptococcus pneumoniae*, Moscow, 2010–2017.

Antimicrobial [a]	Serotype (*n*)	Period, no. of resistant isolates (proportion of resistant isolates in the period)							*p* value [b]
		2010/11	2012	2013/14	2015	2016	2017	Overall	
OXA	6AB/14/19AF/23F (413)	18 (28%)	32 (35%)	39 (49%)	24 (57%)	44 (55%)	29 (54%)	186 (45%)	***0.001***
	Other (218)	0	5 (11%)	3 (9%)	2 (10%)	5 (13%)	4 (8%)	19 (8.7%)	0.627
	All (631)	18 (20%)	37 (26.8%)	42 (36.5%)	26 (41%)	49 (40.8%)	33 (31.1%)	205 (32.4%)	***0.009***
ERY	6AB/14/19AF/23F (413)	23 (35%)	32 (35%)	34 (43%)	28 (67%)	47 (59%)	33 (61%)	197 (47.7%)	***<0.001***
	Other (218)	1 (4%)	3 (7%)	4 (11%)	2 (10%)	6 (15%)	5 (10%)	21 (9.6%)	0.717
	All (631)	24 (27%)	35 (25.4%)	38 (33%)	30 (48%)	53 (44.2%)	38 (35.8%)	218 (34.5%)	***0.004***
CLI	6AB/14/19AF/23F (409)	21 (33%)	27 (30%)	29 (36%)	23 (55%)	43 (54%)	27 (50%)	170 (41.6%)	***0.004***
	Other (218)	1 (4%)	3 (7%)	2 (6%)	1 (5%)	4 (10%)	3 (6%)	14 (6.4%)	0.939
	All (627)	22 (25%)	30 (22.1%)	31 (27%)	24 (38%)	47 (39.2%)	30 (28.3%)	184 (29.3%)	***0.028***
SXT	6AB/14/19AF/23F (411)	46 (73%)	68 (74%)	50 (63%)	26 (62%)	45 (56%)	25 (46%)	260 (63.3%)	***0.008***
	Other (218)	10 (42%)	14 (30%)	9 (26%)	7 (33%)	8 (20%)	19 (37%)	67 (30.7%)	0.358
	All (629)	56 (64%)	82 (59.4%)	59 (51.3%)	33 (52%)	53 (44.2%)	44 (41.5%)	327 (52%)	***0.006***
TET	6AB/14/19AF/23F (133)	NT	NT	NT	NT	36 (46%)	26 (48%)	62 (46.6%)	NA
	Other (92)	NT	NT	NT	NT	6 (15%)	8 (15%)	14 (15.2%)	NA
	All (225)	NT	NT	NT	NT	42 (35.3%)	34 (32.1%)	76 (33.8%)	NA
CHL	6AB/14/19AF/23F (252)	NT	NT	2 (3%)	2 (5%)	6 (7%)	1 (2%)	11 (4.4%)	0.417
	Other (141)	NT	NT	0	0	1 (3%)	0	1 (0.7%)	0.397
	All (393)	NT	NT	2 (1.9%)	2 (3%)	7 (5.8%)	1 (0.9%)	12 (3.1%)	0.159
MDR	6AB/14/19AF/23F (413)	22 (34%)	30 (33%)	31 (39%)	20 (48%)	41 (51%)	25 (46%)	169 (40.9%)	0.106
	Other (218)	1 (4%)	2 (4%)	3 (9%)	1 (5%)	3 (8%)	4 (8%)	14 (6.4%)	0.949
	All (631)	23 (26%)	32 (23.2%)	34 (29.6%)	21 (33%)	44 (36.7%)	29 (27.4%)	183 (29%)	0.225

Note. NT = not tested; NA = not analyzed.
[a] OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; CHL, chloramphenicol; MDR, multiple drug resistance.
[b] P value of the χ^2 test for trend; statistically significant changes in proportions of resistant isolates during the study period are in bold typeface.

increase coincided with a growing resistance of serotype 14 pneumococci to several antimicrobials, including PEN, ERY, and CLI. The rate of MDR isolates increased from 35% (6/17) in 2010–2012 to 89% (24/27) in 2015–2016. To elucidate underlying reasons for the observed phenomena, we analyzed genetic lineages of available serotype 14 pneumococci ($n = 60$) by means of MLST.

In total, 28 different STs were detected (14 were recovered for the first time), of which 18 STs (64%) related to 3 clonal complexes (CCs) including CC15 (6 STs), CC143 (8 STs), and CC156 (4 STs); the data on the clonal and ST diversity of serotype 14 pneumococci are given in Table 3 and Appendix B in more detail. Although ST143 is a DLV of ST156 (at *gdh* and *recP* loci) (Magiorakos et al., 2012), for purposes of the present study, we considered ST143 and its SLVs as a separate clonal group that differed from ST156 by prevalence and antimicrobial susceptibility (see below).

CC15, CC143, and CC156 collectively covered the majority of serotype 14 pneumococci (90%, 54/60), with CC143 being the most prevalent clone (47%, 28/60). ST143 was the single most abundant sequence type ($n = 20$). The prevalence of clones as well as individual STs was significantly different between the study periods ($\chi^2 = 14$; $P = 0.004$ and $\chi^2 = 35$; $P = 0.029$ for CCs and STs, respectively). In 2010–2014, the rate of CC15 was 36% (9/25), whereas CC143 comprised a 20% proportion (5/25) (Table 3). In contrast, in 2015–2017, CC143 became the leading lineage with a proportion of 66% (23/35; $z = 3.5$; $P < 0.001$); this was accompanied by reductions in prevalence of CC15 ($z = -1.38$; $P = 0.167$), CC156 ($z = -1.29$; $P = 0.198$), and the group of other genotypes ($z = -2.18$, $P = 0.029$).

Serotype 14 pneumococcal clones demonstrated different antimicrobial resistance profiles (Table 4). Remarkably, all the 28 CC143 isolates (ST143 and 7 SLVs) possessed an MDR phenotype demonstrating nonsusceptibility to PEN, ERY, and CLI; a high proportion of these isolates were resistant to SXT (93%) and TET (71%). Among CC15 and CC156 pneumococci, the MDR rate was lower (56% and 40%, respectively), although all 10 CC156-related isolates were nonsusceptible to PEN. In contrast to a 100% ERY-resistance rate in CC143, CC15 and CC156 contained a smaller proportion of ERY-resistant isolates (63% and 40%, respectively).

Table 3
Clonal diversity of serotype 14 pneumococci, by study period.

CC	ST	*n* (% of all isolates)	*n* (% of isolates in the period)		Comment [a]
			2010–2014	2015–2017	
143		28 (47%)	5 (20%)	23 (66%) [b]	
	143		4	16	DLV Spain^{9V}-3 (ST156)
	SLV of 143		1	7	
15		16 (27%)	9 (36%)	7 (20%)	England^{14}-9 (ST9)
	15		6	2	
	SLV/DLV of 15		3	5	
156		10 (17%)	6 (24%)	4 (11%)	Spain^{9V}-3 (ST156)
	156		3	1	
	SLV of 156		3	3	
Other		6 (10%)	5 (20%)	1 (3%)	
	124		3	0	Netherlands^{14}-35 (ST124)
	Other		2	1	
Overall		60 (100%)	25 (100%)	35 (100%)	

Note. SLV and DLV = single- and double-locus variant, respectively.
[a] Indicated are the Pneumococcal Molecular Epidemiology Network (PMEN) clones (Pneumococcal Molecular Epidemiology Network (PMEN), n.d.) and their identifier STs (in brackets).
[b] Significant differences between the proportions of CC143 in 2010–2014 and 2015–2017 ($\chi^2 = 13.6$; $P = 0.004$).

4. Discussion

Hereby, we described the serotype spectrum and antimicrobial resistance among pediatric nasopharyngeal pneumococci isolated at a single center in Moscow over an 8-year period (2010–2017) that included pre-PCV and post-PCV periods in Russia. PCV13 vaccination has been introduced in the Russian National immunization program in 2014. Data on the vaccination status of children in our cohort were not available; however, official figures estimated the PCV vaccination coverage in Moscow as being 20.9%, 52.3%, and 52% for children born in 2015, 2016, and 2017, respectively (The official site of the Ministry of Health of the Russian Federation, n.d.). During 2010–2015, the rate of PCV13 serotypes remained above 75% with a marginal fluctuation year by year. However, in 2017, we observed a significant reduction in PCV13 serotype prevalence to 58.5% that was associated with a growing rate of serotype 15B/C. This increase was associated with growing prevalence of the preexisting genotypes of ST1025 and ST1262, which have been related predominantly to serotype 15B/C pneumococci in other countries (https://pubmlst.org/spneumoniae, n.d.; Ho et al., 2015). These clones were universally susceptible to β-lactams and macrolides with a 40% resistant rate to SXT.

A serotype 15B/C increase was documented in the post-PCV period in several countries. In the USA before the introduction of PCV7 in 1999–2000, the prevalence of serotype 15B/C among noninvasive isolates from children ≤5 years of age was 1.7% but climbed up to 10.4% in the post-PCV13 period (years 2010–2011) (Richter et al., 2013). An increase in the nasopharyngeal carriage of 15B/C pneumococci after PCV implementation was observed in the UK and Hong Kong (Devine et al., 2017; Ho et al., 2015). In Germany, the rate of serotype 15B/C among pediatric IPD isolates has increased from 1.4% in 1997–2006 to 10.9% in 2013–2014 (van der Linden et al., 2015). A recent meta-analysis of serotype distribution among pneumococci causing IPD in children in the post-PCV era (included 20 studies from 24 countries) indicated that, currently, serotype 15B/C is among the predominant non-PCV13 serotypes, ranging from 4% in Latin America to 9.6% in Europe (Balsells et al., 2017). Thus, serotype 15B/C pneumococci not only have become common colonizers but also represent important causes of IPD in the post-PCV period.

Antimicrobial resistance in our collection was high. The overall nonsusceptibility rate to PEN, ERY, CLI, and TET was close to, or above, 30%. The presence of *ermB* was detected in 80% of ERY-resistant pneumococci conferring an MLS_B phenotype, i.e., nonsusceptibility to all macrolides, lincosamids, and streptogramin B (Schroeder and Stephens, 2016). Although the rate of bacteria resistant to PEN and AMX was low (2.7% and 0.7%, respectively), an appreciable proportion of isolates demonstrated elevated MICs against these antimicrobials within a range of 1–2 mg/L; MIC_{90} for PEN and AMX reached 1 mg/L. Reportedly, in the decade of 1999–2009 in Russia, MIC_{90} for PEN and AMX has varied between 0.06–0.125 mg/L and 0.06–0.25 mg/L, respectively (Kozlov et al., 2010).

Our current results were in close agreement with a recently published report that included pneumococci from 3 regions in Russia, including Moscow, Saint Petersburg, and Smolensk (Torumkuney et al., 2018). The rate of nonsusceptibility to PEN was 33%; resistance to macrolides including ERY, azithromycin, and clarithromycin was demonstrated in 31.2% of isolates. The majority of examined isolates were susceptible to AMX with a resistance rate of 5% (Torumkuney et al., 2018). However, MIC_{90} was 1 mg/L and 2 mg/L for AMX and PEN, respectively. In general, our data coincided with pneumococcal resistance estimates obtained in European countries in 2009–2012 (Tomic and Dowzicky, 2014). That study reported the rates of nonsusceptibility to PEN, ERY, CLI, and AMX as 28.9%. 28.5%, 19.5%, and <5%, respectively. Taken together, these results suggest qualitative changes of β-lactam resistance mechanisms over the last decade in Russia.

Antimicrobial resistance rates were serotype-dependent. The resistance was predominantly attributable to a limited number of

Table 4
Clonal group-related antimicrobial susceptibility and macrolide resistance genotype in serotype 14 pneumococci.

Antimicrobial [a]	Category	CC143 ($n = 28$)	CC15 ($n = 16$)	CC156 ($n = 10$)	Other ($n = 6$)	Total ($n = 60$)
PEN	NS [b]	28 (100%)	8 (50%)	10 (100%)	3 (50%)	49 (82%)
	MIC_{50}; MIC_{90}	1; 1	≤0.06; 1	1; 2	≤0.06; 1	0.5; 1
AMX	NS	1 (4%) [c]	0	1 (10%) [d]	0	2 (3%)
	MIC_{50}; MIC_{90}	1; 2	≤0.06; 1	1; 2	≤0.06; 1	0.5; 2
ERY	R	28 (100%)	10 (63%)	4 (40%)	1 (17%)	43 (72%)
	MIC_{50}; MIC_{90} (mg/L)	≥256; ≥256	≥256; ≥256	≤0.25; ≥256	≤0.25; ≤0.25	≥256; ≥256
	ERY-R genotype	*ermB* 22/28 (79%); *ermB* + *mef* 5/28 (18%); no *ermB*, no *mef* 1/28 (3%)	*ermB* 9/10 (90%); *mef* 1/10 (10%)	*ermB* 2/4; *ermB* + *mef* 2/4	*ermB* 1/1	*ermB* 34/43 (79%); *ermB* + *mef* 7/43 (17%); *mef* 1/43 (2%); no *ermB*, no *mef* 1/43 (2%)
CLI	R	28 (100%)	9 (56%)	4 (40%)	1 (17%)	42 (70%)
SXT	R	26 (93%)	13 (81%)	7 (70%)	3 (50%)	49 (82%)
TET [f]	R	12 (71%)	1 (17%)	1 (14%)	NT	14 (47%)
CHL	R	0	2 (13%)	0	0	2 (3%)
MDR	Yes	28 (100%)	9 (56%)	4 (40%)	1 (17%)	42 (70%)

Note. NS = nonsusceptible; R = resistant; NT = not tested.
[a] OXA, oxacillin; ERY, erythromycin; CLI, clindamycin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; CHL, chloramphenicol; MDR, multiple drug resistance.
[b] MIC range, 0.12–2 mg/L.
[c] MIC = 4 mg/L.
[d] MIC = 8 mg/L.
[f] TET susceptibility was determined in 30 isolates.

pneumococcal serogroups/serotypes including 6, 14, 19, and 23F that covered more than 90% of resistant isolates. Furthermore, during the study period, growing resistance to OXA, ERY, and CLI was observed specifically within this group of pneumococci. The rise of resistance rates within a pneumococcal serotype could be related to expansion of preexisting, more resistant clones of the same serotype, as we observed for serotype 14. Indeed, using MLST, we demonstrated a pronounced shift in genotypes among serotype 14 isolates over the study period. In the last years, a single MDR clone, CC143, has replaced has increased at the expense of other clonal groups that were abundant before. ST143 is a DLV of ST156, the founder of the international clone Spain^{9V}-3, which is characterized by nonsusceptibility to β-lactams and susceptibility to macrolides (Pneumococcal Molecular Epidemiology Network (PMEN), n.d.). Having a similar level of β-lactam nonsusceptibility as ST156, all ST143-pneumococci displayed resistance to ERY with high MICs ≥256 mg/L. This might suggest that one of competitive advantages of CC143 promoting the proliferation of this genotype could be resistance to macrolides. In fact, examination of the clonal composition of nasopharyngeal pneumococci performed in communities receiving mass azithromycin distributions for trachoma has demonstrated that the rate of macrolide-resistant clones present before mass azithromycin usage significantly increased after treatment (Keenan et al., 2015). This finding indicates that the selection pressure of antibiotics may stimulate expansion of existing resistant strains. Moreover, pharmacodynamics characteristics of azithromycin may facilitate selection of macrolide resistant pneumococcal clones that possess an MDR phenotype (Barkai et al., 2005; Dagan et al., 2008). Current misuse of antimicrobials, especially in pediatrics, which is documented in Russia, corroborates this speculation. A study carried out in Russia in 2011–2013 indicated that up to 40% of antimicrobial prescriptions in children with acute respiratory infections in outpatient settings were unjustified. Notably, macrolides (azithromycin) were among the most prescribed antibiotics with a prescription proportion of 22% (Rachina et al., 2016).

The present study has several limitations. Although large, our pneumococcal collection contained only noninvasive isolates, thus complicating the extrapolation of the results to PCV13 impact on the IPD serotype distribution. Unfortunately, there are no reliable published data on the IPD incidence and its dynamics over time for Russia yet. The same is true for disease-causing serotype distribution. In addition, the data on carriage rates were not available in this study. According to our unpublished experience, the pneumococcal carriage rate varies from 30% to 50% in children below 5 years of age. PCV vaccination expels vaccine-type pneumococci from carriage but hardly has an impact on overall pneumococcal carriage (Nicholls et al., 2016). Another limitation was the lack of PCV13 vaccination data from enrolled children, which precluded direct evaluation of the vaccine effect on pneumococcal carriage serotypes.

5. Conclusion

In conclusion, to the best of our knowledge, this is the first report comparing pneumococcal serotype distribution before and after the introduction of PCV in Russia. The reduction in prevalence of PCV13 serotypes among nasopharyngeal pneumococci likely demonstrates an early impact of PCV13 in restricting vaccine type pneumococcal colonization. Moreover, in 2017, we observed reductions of the resistant rates against several antimicrobials that could be attributed to decreasing prevalences among resistant vaccine type pneumococci and concurrent increment of replacing susceptible clones such as those of serotype 15B/C. In fact, expulsion of resistant vaccine serotypes from circulation by the usage of PCV has been shown to reduce pneumococcal resistance in the post-PCV period in several countries (Janoir et al., 2016; Richter et al., 2014). Thus, implementation of programs for rational antimicrobial use would be helpful for preventing spread of resistant nonvaccine serotype clones. Apparently, the bacterial population is dynamically changing under various clinical and natural challenges requiring a continued monitoring.

Conflict of interest

NM and TK have received a research grant through their institution from Pfizer as well as honoraria for speaking at symposia and/or financial support for attending symposia from Pfizer, GSK, Sandoz.

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