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Nasopharyngeal pneumococcal carriage in healthy Turkish children after 13-valent conjugated pneumococcal vaccine implementation in the national immunization program

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ABSTRACT

Background: In Turkey, pneumococcal conjugated vaccine (PCV) was introduced to the national immunization program as PCV7 in 2008, and was replaced with PCV13 in 2011. The aims of this study were to investigate the effects of PCV13 on nasopharyngeal pneumococcal carriage (NPC) by determining the serotype distribution, and to identify risk factors for carriage, in healthy Turkish children.
Methods: This prospective study was conducted on 500 healthy children aged 0–13 years between April and November 2014. Nasopharyngeal swab samples were taken, and molecular method for capsular serotyping was performed by multiplex PCR.
Results: Of 500 children, 43.4% were unvaccinated with a PCV (7- or 13-valent), 56.6% were vaccinated and The NPC rate was found to be 9.8%. Of 49 positive *Streptococcus pneumoniae* isolates, 26 (53%) were PCV13 vaccine strains (VSs), and 17 (34.7%) were non-VS. Six isolates (12.2%) were not typeable by the method applied. The most common serotypes detected were serotype 3 (18.3%), serotype 19F (14.2%), serotype 6A/B (8.1%), serotype 11A (8.1%), and serotype 15B (8.1%). The total coverage rate of the PCV13 serotypes was 60.4%.
Conclusion: A significant decrease in carriage rate was detected within three years after the introduction of PCV13 in Turkey. However, the nasopharyngeal carriage of PCV13 strains was found to be interestingly high.

Abbreviations: ACIP, advisory committee on immunization practices; DNA, deoxyribonucleic acid; EDTA, ethylene diamine tetraacetic acid; IPD, invasive pneumococcal disease; mPCR, multiplex polymerase chain reaction; NIP, national immunization program; NMMRL, national molecular microbiology reference laboratory; NPC, nasopharyngeal pneumococcal carriage; PCR, polymerase chain reaction; PCV, pneumococcal conjugated vaccines; PPV, polysaccharide pneumococcal vaccine; RT-PCR, real-time polymerase chain reaction; Rxn, reaction; SPSS, statistical package for the social sciences; UK, United Kingdom; UV, ultraviolet; VS, vaccine strains; WHO, World Health Organization.

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Introduction

Streptococcus pneumoniae is an important bacterial pathogen that causes invasive pneumococcal disease (IPD) which may result in high mortality and morbidity. According to the World Health Organization (WHO) reports, nearly 500,000 children under 5 years of age are infected by *S. pneumoniae* annually, especially in developing countries [1]. IPD is estimated to cause up to one million deaths per year in resource-poor or undeveloped countries [2]. The source of pneumococcal infections is mainly asymptomatic carriers, and the main reservoir in carriage is human nasopharynx [3,4]. The rate of nasopharyngeal pneumococcal carriage (NPC) provides information about the development of pneumococcal diseases and the person-to-person transmission of the bacteria [4]. NPC rate varies depending on geographical region and population, but is highest in children and within the first 24 months of life [4,5].

Although there are more than 90 defined serotypes of *S. pneumoniae*, about 15 serotypes (1–9, 12, 14, 18, 19, 23 and 25) are frequently responsible for IPD [2]. The distinction of pneumococcal serotypes can be made by determining the chemical structure of capsular polysaccharide using serological tests and/or some molecular techniques [2,5]. Based on these mechanisms, serotype-specific polysaccharide and conjugated pneumococcal vaccines were developed [6]. The polysaccharide pneumococcal vaccine (PPV), the first developed pneumococcal vaccine form, was 23-valent and covered a large majority of serotypes effective in IPH for that period. But, the poor immunogenicity of PPV23 in infants led to development of the conjugated vaccines for pneumococci [5,6]. Currently, in addition to the PPV23, 7-, 10- and 13-valent pneumococcal conjugated vaccines (PCVs) are in use. New and broader PCV formulations are also expected to be licensed in the near future [7].

With the widespread use of PCVs worldwide, the frequency of IPD and also the proportion of vaccine-type carriers are expected to be significantly reduced, and the serotype profiles of circulating pneumococci will eventually change in the coming years. The findings of recently published studies from all over the world support this expectation [2,7]. The impact of the PCVs on carriage is important to identify circulating strains and to know the possible serotypes that are involved in IPD. In addition, the herd immunity effect of PCVs on unvaccinated children and adults could not be ignored [2,4]. Because the impact of PCVs tends to vary across countries and settings [2,4,8,9], each country should conduct and monitor its own pneumococcal surveillance regularly. In Turkey, PCV7 (covering serotypes 4, 6B, 9V, 14,18C, 19F and 23F) was introduced to the National Immunization Program (NIP) in April 2008, and was switched to PCV13 (covering the six additional serotypes 1, 3, 5, 6A, 7F and 19A) in November 2011. Despite the studies of different centers conducted to determine pneumococcal carriage status and related risk factors, post vaccine era studies about NPC status and distribution of circulating serotypes are insufficient in Turkey [10–12]. In few studies conducted on children after the introduction of PCV7 to the NIP, the impact of the vaccine in Turkey were documented, but data is lacking for the situation after PCV13 introduction [10–13]. The aims of this study were (I) to investigate the effects of PCV13 on nasopharyngeal carriage rates of *S. pneumoniae* by determining the pneumococcal serotype distribution, and (II) identify risk factors for carriage; in healthy Turkish children, by comparing with pre and post vaccine era studies, on both general and basis of age groups.

Materials and methods

Study setting, target population and data collection

This prospective study was conducted with 500 children between April and November 2014 at two government tertiary level hospitals (Ankara Hematology Oncology Children's Training and Research Hospital, and Gazi University Medical Faculty Hospital) located in Ankara, central of Turkey. Ankara is the capital city, and also is the second most populated settlement center with more than five million inhabitants. The two centers serve similar patient groups being tertiary level referral centers and centrally located.

Healthy children aged 0–13 years visiting general pediatric outpatient clinics for non-disease reasons (such as height and weight follow-up, vaccination, or development evaluation) were enrolled in the study. Children having a history of infection and antibiotic usage within the last two weeks, and the children with a chronic disease were excluded. Data on demographic characteristics, health status, vaccination history (via individual vaccination card), and risk factors was collected by using a questionnaire form with face-to-face interviews. The risk factors questioned included; day-care or school attendance for participant and his/her sibling(s), number of sibling(s), number of household members, passive smoking history, breast feeding history, recent illness such as upper/lower respiratory tract infection history during last year, antimicrobial agent usage in the previous months (15–29 days, 1–3 months, 4–6 months and 7–12 months), history of hospitalization, and presence of health personnel at home. According to current National Childhood Vaccination Schedule PCV13 is administered at the end of 2nd, 3rd and 6th months with a booster dose at 12th month in Turkey. This study included 0–13 years old children either vaccinated with PCV7 or with a shift from PCV7 to PCV13 after November 2011, or not vaccinated with any PCV. The vaccination status of the children was determined as unvaccinated, incomplete vaccinated and fully vaccinated according to the Advisory Committee on Immunization Practices (ACIP) criteria [14,15]. The following groups were formed in the comparison of vaccine status; fully vaccinated ($\geq$3 doses) with PCV7, mixed vaccination with PCV7 and PCV13 (3 doses of PCV7 + 1 dose of PCV13, 2 doses of PCV7 + 2 doses of PCV13, or 1 dose of PCV7 + 3 doses of PCV13), incomplete or fully vaccinated ($\geq$3 doses) with PCV13. In order to make comparisons more consistent, participants were allocated in three age groups, according to age groups considered in previous studies [10–13]: group I (0–23 months), group II (24–59 months), and group III ($\geq$60 months). The effects of PCV13 implementation on age groups were also investigated for this study.

Sample collection and identification of S. pneumoniae *isolates*

Specimens were taken as nasopharyngeal swab samples from 500 participants using a sterile dacron swab with flexible twisted wire body (Copan Italia™ Swap Amies Agar Gels, Italy) by trained pediatricians according to the WHO guidelines [16]. The procedure of sample collection was performed as follows: after the child's head was fixed and tilted slightly backward, swap was moved parallel to the base of the NP passage until reaching the nasopharynx without resistance; once the swap was in the right location, the swap was rotated 180° or waited for 5 s for saturation; when sufficient and appropriate sample was obtained, swap was removed and the procedure was terminated. After collection, the specimens were delivered to the laboratory with appropriate transport conditions (at 2–8 °C, in the transport media [Copan Italia™ Amies

Agar Gels, Italy] to prevent drying) within two hours at the most. Samples were inoculated onto 5% defibrinated sheep blood agar plate (BD™ Columbia Agar, Heidelberg/Germany) and incubated at 37 °C with 5–10 % CO^2 for 18–24 h. *S. pneumoniae* isolates were identified using standard laboratory procedures including colony morphology and gram staining, after that identification was confirmed via optochin susceptibility with 5 μg disc and bile solubility testing [16]. The isolates were stored at −80 °C in 16% glycerol medium then delivered to National Molecular Microbiology Reference Laboratory (NMMRL) for identification of their serotypes. All procedures including sample collection, swap transport, culture and identification of isolates were conducted in accordance with WHO recommendations [16].

Molecular identification and serotyping

The DNAs of *S. pneumoniae* positive bacterial isolates were obtained by using the boiling method. Briefly, the samples received in 16% glycerol medium were transferred into microcentrifuge tubes and centrifuged for removal of the medium. After discarding supernatants, the bacterial cell pellets were suspended in 1 ml of 0.85% NaCl. This cell washing step was repeated once more and then each pellet was suspended in 100 μl TE (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) buffer. Sample tubes were boiled at 100 °C for 10 min, and then the cell lysates containing bacterial DNAs were stored at −20 °C until use. All PCR reagents were purchased from Qiagen Inc. (Hilden, Germany). The sequences of primer pairs specific for *S. pneumoniae* genes used in this study were from previous publications [17–19]. All primers, synthesized in Alpha DNA (Quebec, Canada) were suspended in PCR grade water (10 pmol/ml) and then stored at −20 °C until use. The presence of *S. pneumoniae* DNA was searched as described in a study using a very sensitive real-time PCR (RT-PCR) method based on amplification of autolysin-encoding gene, *lytA*, [20]. The RT-PCR positive samples were then subjected to a multiplex PCR (mPCR) based assay which was previously optimized in the NMMRL (Ankara) for detection of the invasive and commonly circulating pneumococci serotypes. This assay allows detection of 28 serotypes by running only three different mPCR reactions (Rxn I: PCV13 serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F [21], Rxn II: serotypes 8, 9L, 9N, 10A, 15A, 16F, 17F, 23A and 33F, and Rxn III: serotypes 11A, 15B, 22F, 31, 35B and 35F). Assay reactions are carried out in a stepwise manner so that the Rxn I is performed for all DNA samples, then the Rxn II for non-PCV13 samples, and finally the Rxn III for only the negative samples of the previous steps. Before running the assay, the master mixtures of Rxns I, II and III were individually prepared in bulks depending on the number of the samples to be tested (20 μl/sample) by mixing the relevant primer pairs in the range of 0.75–6.0 pmol consantrations with 2X QuantiTect Multiplex RT-PCR NoRox Master Mixbuffer as described in the manufacturer's manual. As an internal control, an additional primer pair amplifying the *cpsA* gene (coding for capsular polysaccharide biosynthesis) common in all pneumococci was also added (0.75 pmol) into all three master mixes. For each isolate, 5 μl of cell lysate was added into a 0.2 ml PCR tube containg 20 μl of the desired master mix, and PCR cycling was performed in Corbett model thermocycler (CorbettLife Science, Australia). All three mPCRs were conducted under the same cycling conditions: following the initial denaturation at 95 °C for 10 min, 30 cycles at 95 °C for 50 s, 55 °C for 30 s, 65 °C for 2.5 min, and 1 cycle of 65 °C for 10 min. The PCR products of 10–15 μl were loaded into the wells of 3% NuSieve agarose gel (Gamma micropor, Prona, EU) containing ethidium bromide. After electrophorefsis at 150 V for 3–5 h, the gels were visualized under the UV. The serotypes of isolates were determined by comparing the immigration of sample DNA bands with the serotype specific DNA marker bands. The coverage rates were calculated as the rate of strains targeted by each vaccine in all of detected isolates [22].

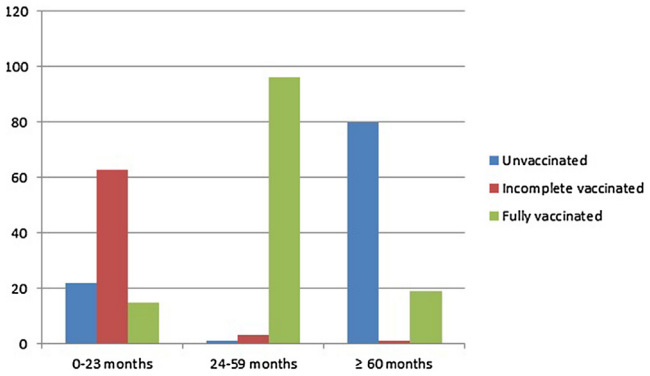

Fig. 1. The vaccination status of the children according to the age groups (%).

Statistical analysis

The initial sample size calculation was performed with a 0.05 significance level, 95% confidence interval and 80% power using a carriage rate of 20%. Based on this calculation, the minimum number of participants to be enrolled for the study was calculated as 383. A weighted sample size was calculated for each center and a total of 500 children were enrolled in the study. Statistical analysis was performed using SPSS v25.0 (IBM Corp., Armonk, New York, USA) statistical package. Descriptive statistics are presented as frequencies, percents, arithmetical mean ± standard deviation, and median (minimum, maximum). Pearson Chi-square, Chi-square for trend and Fisher's Exact tests were used for statistical analysis. A p value of <0.05 was considered statistically significant.

Results

Study population and vaccination status

Of the 500 participants, 278 (55.6%) were male. The mean age of the children was 55.97 ± 50.23 (0–156) months and median age was 46.5 months. Numbers of children according to groups were 195 (39%) at ages 0–23 months, 95 (19%) at ages 24–59 months, and 210 (42%) at ages ≥60 months. The mean number of household members was 4.34 ± 1.26 (2–12) with a median of 4, the mean number of children in the family was 2.1 ± 1.01 (1–9), and the mean number of children attending school / college was 1.49 ± 0.7 (0–12).

Of the children, 43.4% (n = 217) were unvaccinated with a PCV (7 or 13-valent), and 56.6% (n = 283) were vaccinated. The vaccinated children was scattered as follows; 43% (n = 215) were fully vaccinated and 13.6% (n = 68) were incomplete vaccinated. The distribution of the fully (≥3 doses) vaccinated participants was; 12.4% (n = 62) fully vaccinated with PCV7, 2.2% (n = 11) 3 doses PCV7 and 1 dose PCV13, 1.8% (n = 9) 2 doses PCV7 + 2 doses PCV13 or 1 dose PCV7 + 3 doses PCV13, and 26.6% (n = 133) fully vaccinated with PCV13. The vaccination status of the children according to groups independent of PCV type is shown in Fig. 1.

NPC and risk factors for carriage

The overall carriage rate was 9.8% (49/500). The NPC rates were 11.8% (n = 23/195), 8.4% (n = 8/95), and 8.6% (n = 18/210) at age groups 0–23 months, 24–59 months, and ≥60 months, respectively. Although NPC rate appears to be higher in the 0–23 months age group, there was no significant difference between the groups (p = 0.487). Distribution of carriers in the three predefined age

Table 1
Distribution of carriers in the age groups according to the demographic data and risk factors.

Demographic data/risk factors	0–23 months (Group I)		24–59 months (Group II)		≥ 60 months (Group III)	
	Carriers *n* (%)	*p* Value	Carriers *n* (%)	*p* Value	Carriers *n* (%)	p Value
Gender						
Female	10 (5.1)	0.865	4 (4.2)	0.709	9 (4.3)	0.703
Male	13 (6.7)		4 (4.2)		9 (4.3)	
Day-care or school attendance						
Yes	2 (1)	0.107	3 (3.2)	0.15	16 (7.6)	0.56
No	21 (10.8		5 (5.3)		2 (1)	
History of breast						
Not breast-fed	–		–		–	
Still breast-feeding	16 (8.3)	0.336	–	–	–	–
Breast-fed before	7 (3.6)		8 (8.4)		8 (8.4)	
Numbers of household members						
≤3	6 (3.1)	0.167	–	–	1 (0.5)	0.706
≥4	17 (8.7)		8 (8.4)		17 (8.1)	
Number of children at home						
1	7 (3.6)		1 (1.1)		2 (1)	
2	8 (4.1)	0.108	4 (4.2)	0.528	11 (5.3)	0.821
≥3	8 (4.1)		3 (3.2)		5 (2.4)	
Monthly income level						
Low <500 $	6 (3.1)		4 (4.2)		3 (1.4)	
Middle 500–1000 $	16 (8.2)	0.442	2 (2.1)	0.107	15 (7.2)	0.125
High ≥1000 $	1 (0.5)		2 (2.1)		–	
Presence of a health provider at home						
Yes	1 (0.5)	0.574	–	–	–	–
No	22 (11.3)		8 (8.4)		18 (8.6)	
Passive smoking						
Yes	11 (5.7)	0.497	5 (5.3)	0.322	11 (5.3)	0.354
No	12 (6.2)		3 (3.2)		7 (3.3)	
Respiratory infection						
Last 15–29 days	16 (6.1)	0.298	1 (1.1)	0.195	8 (3.8)	0.201
Last 1–3 months	18 (6.1)	0.465	5 (5.3)	0.163	8 (3.8)	0.638
Last 4–6 months	12 (4.6)	0.844	1 (1.1)	0.119	8 (3.8)	0.291
Last 7–12 months	8 (4.2)	0.657	–	–	8 (3.8)	0.04*
Antibiotic consumption						
Last 15–29 days	6 (3.7)	0.368	1 (1.1)	0.465	4 (1.9)	0.486
Last 1–3 months	14 (7.3)	0.465	3 (3.2)	0.436	7 (3.3)	0.201
Last 4–6 months	9 (4.1)	0.841	2 (2.1)	0.592	6 (2.9)	0.238
Last 7–12 months	8 (3.8)	0.663	–	–	7 (3.3)	0.059
Hospitalization						
Last 15–29 days	7 (5.2)	0.025*	1 (1.1)	0.324	4 (1.9)	0.431
Last 1–3 months	6 (4.7)	0.139	1 (1.1)	0.471	2 (1)	0.344
Last 4–6 months	4 (3.2)	0.280	1 (1.1)	0.362	3 (1.4)	0.057
Last 7–12 months	5 (3.8)	0.170	–	–	3 (1.4)	0.057

* Statistically significant.

groups according to the demographic data and risk factors are shown in Table 1. In addition to the risk factors in the Table 1, which have a significant relationship with NPC, the presence of a sibling attending day-care/school was found to increase the NPC at 0–23 months age group (p = 0.029).

The overall carriage rate in children who have received the full schedule (≥3 doses) of PCV (7-valent, 13-valent, or PCV7 followed by PCV13) was 9.7%. Incomplete vaccination with 13-valent vaccine was found to increase NPC (%21.2), at 0–23 months age group (p = 0.001). However, incomplete vaccination was not associated with the increased risk of carriage in the other age groups. The carriage rate decreased as the number of doses increased, independent of PCV type for the total study group (p = 0.017) but, this relationship was not determined within separate age groups.

Among the carriers, 75.5% (37/49) had received at least one dose of either PCV7 or PCV13. Vaccination status of the carriers is shown in Fig. 2. In children vaccinated with PCV7 followed by PCV13 (2 doses PCV7 + 2 doses PCV13 [n = 6], 1 dose PCV7 + 3 doses PCV13 [n = 3]), no carriage was detected. Of the non-carriers, 55.2% (n = 249) were vaccinated with at least one dose of a PCV, and 31.4% (n = 142) were fully vaccinated (with PCV7, PCV13, or PCV7 followed by PCV13).

Serotype prevalence and distribution

A total of 49 isolates positive for *S. pneumonia* were obtained from the nasopharyngeal swap specimens of 500 children by standard culture method, and these isolates were subjected to molecular method for serotyping. Sixteen different strains were identified in the typeable isolates. Type of the six isolates (12.2%) was not identified by the method applied. The most common serotypes detected were serotype 3 (n = 9, 18.3%), serotype 19 F (n = 7, 14.2%), serotype 6A/B (n = 4, 8.1%), serotype 11A (n = 4, 8.1%), and serotype 15B (n = 4, 8.1%).

Of the isolates, 26 (53.0%) were in PCV13 vaccine strains (VSs), and 17 (34.7%) strains were non-VS. Distribution of VSs was as follows; 9 were serotype 3 (18.4%), 7 were serotype 19 F (14.3%), 4 were serotype 6A / B (8.2%), 2 were serotype 23F (4.1%), 2 were serotype 18C (4.1%), 1 was serotype 19A (2%), and 1 was serotype 9V (2%). Distribution of non-VSs was; serotype 11A 8.2%, serotype 15B 8.2%, serotype 23A 6.1%, serotype 9L / N 2%, serotype 15A 2%, serotype 16F 2%, serotype 31 %2, serotype 35B 2%, and serotype 35F 2%. Serotype coverage rate was 60.4% for PCV13, and 37.2% for PCV7.

In 0–23 months age group, VS carriage ratio was 56.5% (13/23), the non-VS carriage 26% (6/23), and the non-typable 17.5% (4/23);

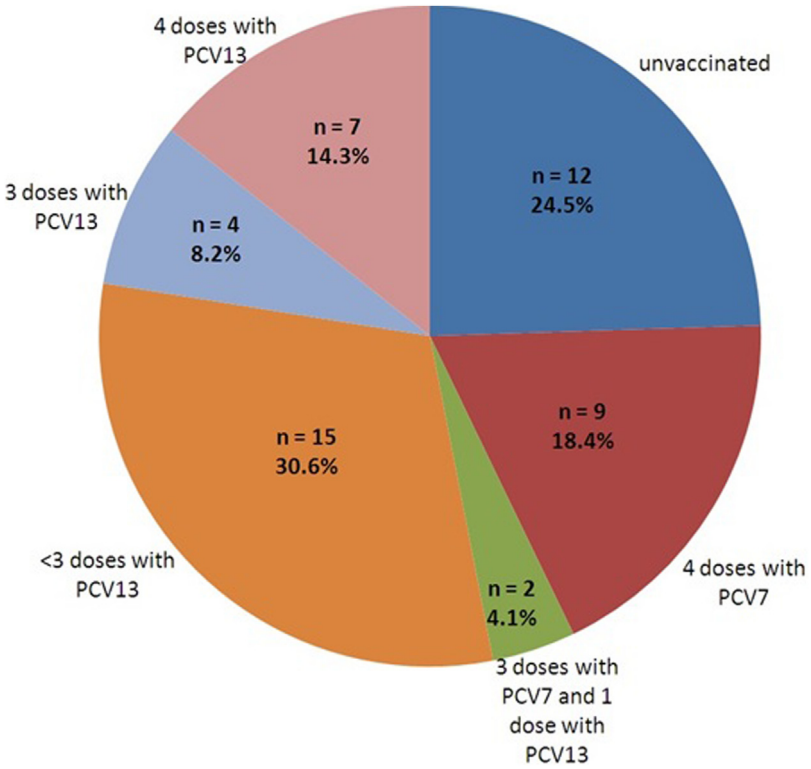

Fig. 2. Vaccination status of the carriers.

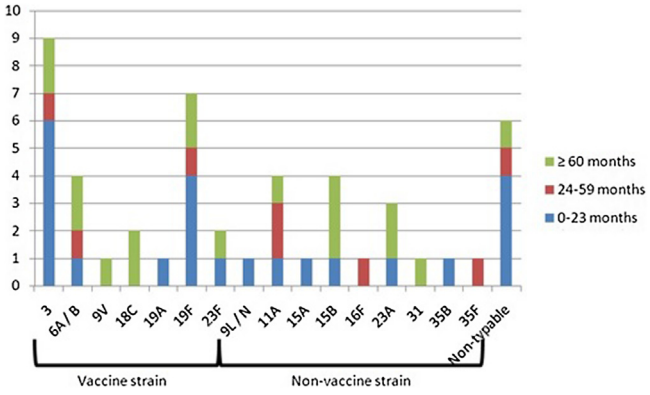

Fig. 3. The number of isolated strains with respect to age groups.

in 24–59 months age group, VS carriage ratio was 37.5% (3/8), non-VS carriage was 50% (4/8) and non-typable 12.5% (1/8); in ≥60 months age group, VS carriage ratio was 55.5% (10/18), non-VS carriage 39% (7/18) and the non-typable 5.5% (1/18). There was no significant difference between the groups in terms of VS carriage ratio in this distribution. The serotypes most frequently detected by the age groups were; in 0–23 months age group serotype 3 and non-typable; in 24–59 months age group serotype 3, 19F, 6A/B, 11A, 35F, 16F and non-typable; in ≥60 months age group serotype 3, 15B and 19F (Fig. 3). There was no significance in the overall distribution of serotypes with respects to gender (p = 0.49), age groups (p = 0.446), and vaccination status (p = 0.842). No association was found between the risk factors and general distribution of serotypes.

In 0–23 months age group, serotype 3 (n = 4), 19 F (n = 2) and non-typable (n = 3) were detected most frequently in incomplete vaccinated participants, and serotype 3 (n = 2) and 19 F (n = 2) were most frequently detected in fully vaccinated children. In 24–59 months age group, only serotype 11A (n = 1) was detected in incomplete vaccinated children, whereas serotype 3, 19F, 6A / B, 11A, 16F, 35F, and non-typable (n = 1, in all) were detected in fully vaccinated cases. In ≥60 months age group, serotype 3 (n = 2) and 19F (n = 2) were the most common in incomplete vaccinated cases, and serotype 15B (n = 3) was detected most frequently in fully vaccinated children. When examined independently from age groups; the most common serotypes in unvaccinated children were 3 and 19F; in incomplete vaccinated children, the most common serotypes were 3, 19F, 11A and non-typable; and in fully vaccinated children, the most common serotypes were 3, 19F, 6A / B, 15B and non-typable.

Among all participants, the risk of VS carriage increased in ≥60 months and female gender. Apart from these, there was no significant relationship between grouped (VS/non-VS) serotypes and the other risk factors. Overall VS carriage rate in children received the full schedule (≥3 doses) of PCV was found to be 4.6%. And the VS carriage rate in carriers received the full schedule (≥3 doses) of PCV was 47.6%.

Discussion

In the pre-PCV7 studies conducted during the 10-year period before 2008, the NPC rates ranged from 4.2 to 37.5% in healthy Turkish children [23–28]. The absence of a common age group and differences in study techniques make the results of these studies difficult to compare and interpret. Since 2008, when the PCV7 has been introduced to our NIP, there are far fewer studies about NPC in Turkey, than the studies conducted in pre-vaccine era. Ozdemir et al. [10], the executives of the first NPC study after PCV7 (three years after the introduction of vaccine to the NIP), found the carriage rate as 21.9%, and highest in 0–24 month age group. This study was conducted on 1101 healthy Turkish children between ages 1 month and 18 years in a single center. The study design and the age groups were similar to ours. In contrast to our study, serotyping

was performed by conventional method and antibiotic susceptibility of strains was also investigated. The mean age was 45.7 ± 49.6 months, and the male/female ratio of children was 1.18. Of 500 participants included in the study, 61.7% received PCV7 and 38.3% were non-vaccinated. In the study performed five years after the introduction of PCV7 and two years after the introduction of PCV13 to the NIP, Soysal et al. [13] found the carriage rate as 6.4% in 2165 healthy children aged 0–18 year, and higher in 0–24 month age group. The design and age groups of this study were also consistent with our study, and serotyping was performed by molecular method as in ours. Antibiotic susceptibility was additionally evaluated by Soysal et al. The mean age was found as 76 ± 56 months, and 53% of the participants were female. Among the children, 60% were not vaccinated with any PCV; 10% of children received at least 1, 2 or 3 doses of either PCV7 or PCV13, and 30% of the children received the full schedule of either PCV7 or PCV13. Our study results reveal that the NPC rate decreased significantly (2-fold) in three years after the introduction of PCV13 to NIP of Turkey, compared to post-PCV7 study of Özdemir et al. Although Özdemir and his colleagues claimed in their report that PCV7 does not affect the carriage rates, when evaluated together with the results of the study conducted by Soysal et al. and our study, it can be said that the NPC rate tends to decrease in healthy children in Turkey after PCV period. However, unlike the two aforementioned studies, it is an interesting result of our study that a significant difference was not detected at carriage rates between the age groups. It is unlikely to explain this decline with the rates of vaccinated children in studies. Because, it is obvious that there is a decrease in the vaccination rates of the children received at least one dose of a PCV in that studies (Özdemir et al. 61.7%, Soysal et al. 40%, our study 56.6%). This data may be explained by the increase in the mean age of vaccinated children and the herd immunity, as a result of the late PCV period. This effect is particularly evident in the 25–60 months age groups, according to the results of the mentioned studies (rates of unvaccinated children in 25–60 months age groups; Özdemir et al. 35.7%, Soysal et al. 11.3%, our study 1%). In addition, the effect of wider serotype coverage by the introduction of PCV13 may also be mentioned both in our study and the survey by Soysal et al. The achieved carriage rate from our report is also evident as one of the lowest rates when compared to reported *S. pneumoniae* carriage rates by both European countries and other world scales after PCV applications [29–36]. It has also been reported in several studies that the NPC has not changed due to the increased rate of non-vaccine pneumococci types in children vaccinated with a PCV [37–40]. In our study, it was found that the NPC rate significantly decreased as the number of doses increased independent of a PCV type. However, the carriage rate did not change in children who received the full schedule of PCV (9.8% vs 9.7%). Arvas et al. [12] from Turkey found the NPC rate as 14%, interestingly higher than our study, in all vaccinated healthy children aged 0–6 years old in 2014. Although it seems lower in the study of Soysal et al., the proportion of children who received at least one dose of a PCV in the carriers group was found to be higher than that of the non-carriers, in both studies. The decrease in this rate in the study of Soysal et al. may be attributed to the herd immunity effect of the vaccine, but the unchanging result is that incompleted vaccine schedule does not interfere with the change in carriage rates. When the vaccination status of carriers is evaluated in general, the basic result of our study is that vaccination does not provide protection against carriage, even if reducing the carriage rate in our society.

As mentioned above, a risk factor associated with any age group was not determined in this study. Hospitalisation history, recent upper/lower respiratory tract infections, and the presence of a sibling attending day-care/school were found as group-based and time related risk factors for carriage rate. With these results, a dominant risk factor for carriage was not found. Soysal et al. reported that a risk factor for carriage was not identified in their study, similarly. Differently, Özdemir et al. stated the risk factors for NPC as respiratory infection within the last month, attendance at daycare, at least one sibling attending at day-care, absence of a health care provider at home, and low income. Arvas et al. found that the only risk factor for NPC was with a respiratory infection in the last 3 months. Several risk factors associated with NPC, such as age, recent and frequent respiratory tract infections, antibiotic usage, passive smoking, attendance at daycare centers, living in a crowded family, having siblings, lower socioeconomic status, have been mentioned in the literature [41–45].

The most remarkable result of this study is that our pediatric population continues to carry primarily VS serotypes despite a marked decline in NPC. Post-vaccine era studies from around the world have reported a decrease in VS serotypes and a 'serotype replacement' to non-VS serotypes [29,33–36,46]. The serotype shift to non-VS strains has been frequently mentioned in studies conducted from various countries, especially after PCV7 implementations [37,38,47,48]. Similarly, a significant decline in VS serotypes was reported in post-vaccine studies by countries applied PCV10 [49–51]. The most commonly carried serotypes reported from all over the world after PCV7 administrations were 19A, 6A, 15B/C, 35B, and 11A [46–48]. Especially, the problem of increase in serotype 19A was remarkable [52–54]. The ineffectiveness of PCV7 to some serotypes that in its content of has also been discussed. As an example, the findings of previous studies determined that PCV7 was less effective in reducing the carriage of serotype 19F [39,55]. Özdemir et al. reported that serotype 6B, serotype 19F and serotype 23F were the most common isolated VS serotypes; and the most common non-VS serotypes were 15A/B/C/F, 23A, 10A/B, 35F/A/B/C, 6C with a remarkable increase in 6A [10]. In the same study, the absence of serotype 19A detected in any child was interpreted as a surprising result by the authors, compared with the results of the studies from all over the world after PCV7 implementations. Serotype 19A does not appear to be a problematic serotype as a result of our study, but it is difficult to interpret it as the effect of PCV13 considering report of Özdemir et al. It should also be added that the study of Özdemir et al. supports low efficacy of PCV7 against serotype 19F. In a study conducted just before introduction of PCV7 to the NIP of Turkey by Ercan et al., children who received a fully schema of PCV7 were compared with the control group, and most frequently isolated VS serotype was found as 23F in the vaccinated group, and 19F in the non-vaccinated group [11]. As a striking result of that study, vaccine ineffectiveness against certain vaccine serotypes in both vaccinated and unvaccinated groups can be mentioned. With the implementation of PCV13 including serotype 19A and 6A, in which the increase was observed after PCV7, serotype shift with increase in different serotypes started to be detected, all over the world [34,35,39,40,56]. This increase after PCV13 was observed especially at serotype 15, although not as obvious as at serotype 19A [57–59]. Serotypes 35B, 23A / B, 11A and 6C are also reported as other serotypes with increasing trend [56,60,61]. With the application of PCV13, it can be said that some serotypes which are problematic in the PCV7 period are stay back and new serotypes are starting to cause problems. Such that, Desai et al. claimed in their study from USA that the effect of PCV13 on NPC decline mainly depends on the reduction of serotype 19A [56]. As in PCV7, the ineffectiveness of PCV13 against certain vaccine serotypes, primarily serotype 19F, has been addressed with various studies and meta-analyzes [62–67].

The most common isolates detected in the study by Soysal et al. were serotype 6A/B/C, 19F, 23F, 9V/A, 22A/F, 12F, and 35B [13]. The interesting result of this study is that the four most common serotypes detected were in PCV7 (naturally in PCV13) while the only one serotype was in PCV13 (non-PCV7). The authors have already interpreted this result as continuing to host VT serotypes

by pediatric population of our country. The three most common serotypes detected in our study were serotype 3, 19F, and 6A/B, which are the serotypes within the scope of PCV13, and additional two common non-PCV13 serotypes 11A and 15B. It is an interesting result that serotype 3 has not detected as a problematic serotype in previous post-vaccine era studies in our country, and moreover it is the most frequently carried serotype in our study. When evaluated on groups basis, it is striking that serotype 3 is carried frequently in incomplete vaccinated children in 0–23 months age group and ≥60 months age group, and was found to have a relatively low incidence in fully vaccinated children in all groups. It is possible to interpret this finding as that herd immunity poses a problem for serotype 3, largely independent of individual vaccine status. In fact, in many studies conducted from around the world, PCV13's ineffectiveness on serotype 3 has been expressed, and some authors claim that elimination of serotype 3 may not be achieved despite PCV13 and may pose a risk for IPD [32,67,68]. As reported in post-PCV13 studies in the literature, serotype 19F, which is frequently problematic, supports findings as the second serotype carried most frequently in our study. When evaluated specifically for our country, based on the serotype specific results of both our and Soysal et al. study, PCV13 does not appear to reduce serotype 19F carriage (Soysal et al. 13.5%, our study 14.2%) more than PCV7 (Özdemir et al. 15.2%). Serotype 19A, which has an increased frequency with 'serotype replacement' in post PCV7 period around the world, was shown by Özdemir et al. that it is not a trouble for our country. Both ours and Soysal et al. studies showed that serotype 19A is still not a problem for us. When non-VS strains were examined, an increase in serotype 15 was observed in accordance with the literature in results of our study (Özdemir et al. for serotype 15A/F, 10.8%; Soysal et al., for serotype 15A/F, 5%; our study for serotype 15A/B, 10.2%).

Serotype coverage rates for PCV7 ranged from 30% to 51% in the pre-vaccine studies conducted in Turkey [23,25,27]. This rate was found as 60.4% for PCV13, and 37.2% for PCV7 in our study results. When these ratios are considered, the study of our group had the lowest PCV7 coverage rate among the post-vaccine era studies of our country (PCV7 coverage rates for Özdemir et al. 46.2%, and for Soysal et al. 51.4%). This low PCV7 coverage can be explained by that serotype 3, a PCV13 strain, is the prominent strain of the study, and with the proportion of non-VS strains. In other words, it can be interpreted as the late period effect of PCV7. However, it is confusing that the coverage rate of PCV7 is higher in Soysal et al. When coverage rate for PCV13 is evaluated, the results of the two studies can be assessable as consistent (PCV13 coverage rates for Soysal et al. 56%, and for our study 60.4%). Briefly, PCV13 strains were the main problem and remarkable in both studies. In fact, the reduction of serotype coverage in carriers by the effect of PCVs is an expected result and examples are available. In a study from UK, an emphasis has been made to the declining serotype coverage of new pneumococcal conjugate vaccines about the pneumococcal carriage [37]. In another study from the UK, serotype coverage of PCV7 and PCV13 was reported to be very low, although the overall prevalence of pneumococcal carriage remained stable [69]. And, in a recent study by Abu Seir et al., a significant decrease in serotype coverage by the effects of PCV7, PCV10 and PCV13 was reported in detail [70].

This study has some limitations. It covers only a limited geographic location in Turkey, which is a country with fairly large surface area. Consequently, the carriage rates and serotype distributions which vary according to geographical settlement differences can not be ignored. Also, it does not have any pre-PCV control group. For this reason, the results of pre- and post-vaccine era studies from different centers and different regions were used in the comparisons. Additionally, our study was conducted three years after PCV13 introduction to the NIP of Turkey, and six years after PCV7. Because of this, the late period effect of PCV7 can not be ruled out. In addition, some children enrolled to the study are vaccinated with both PCV7 and PCV13. Therefore, it is difficult to interpret the effect of each vaccine, separately. Finally, antibiotic susceptibility test could not applied to isolates identified. That is, the effect of PCV13 on the carriage of resistant strains was not detected.

Monitoring epidemiological tendency of a vaccine-preventable disease is critical to evaluating the effectiveness of the vaccination programs and obtainning disease precursor data for each country. Because the shortage of clinical surveillance data about NPC compared with IPD in our country, this study is very valuable due to providing important information about the post vaccine era. Considering the damage caused by pneumococcal diseases in developing countries such as Turkey, the results obtained with this study will shed light on other developing countries that have implemented PCVs in their NIPs, as well.

Conclusions

Compared with nasopharyngeal carriage studies in Turkey especially conducted in the post-vaccine era, a significant decrease in carriage rates was detected in three years after the introduction of PCV13. But, a difference between the age groups for carriage was not found. The carriage rate in children who have received the full schedule was not different from overall carriage rate. The NPC is particularly high in PCV13 strains regardless of vaccination status. Serotype 3 is the most frequently carried strain with serotype 19F. This data is meaningful to reflect the efficacy of PCV13 on the vaccine strain carriage in our country. Since pneumococcal surveillance data varies from country to country, monitoring the national pneumococcal surveillance is important for each country.

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Conflict of interest

The authors declare they have no competing interests.

Ethical approval

Research ethics board approval was obtained for the study at Ankara Hematology Oncology Children's Training and Researh Hospital Ethics Committee (Approval number: 03.2014/052), and all investigational procedures conform to the Declaration of Helsinki guiding principles. Written informed consents before participation were provided from legal caregivers of all participants.

Author contributions

Conception and design of the research plan: HT,SKY, FNA, SK. Data collection: SKY, BG, TBD, AKU, MK. Performing the experiments: DG, FA, SS, MÇ. Data analysis: FNA, SKY, HT. Drafting of manuscript: SKY, AOP, HT. All authors performed critical review of the manuscript. All authorsread and approved the final manuscript.

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