Risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy Turkish children after the addition of heptavalent pneumococcal conjugate vaccine (PCV7) to the national vaccine schedule

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The purpose of this study was to investigate the effects of pneumococcal conjugate vaccine (PCV7) on nasopharyngeal (NP) carriage rates of *Streptococcus pneumoniae* in healthy Turkish children. The study was conducted on 1101 healthy Turkish children between 1 month and 18 years of age. The median and mean ages of the children were 25 months (1 month-18 years) and 45.7±49.6 months, respectively. *S. pneumoniae* was isolated in 241/1101 (21.9%) children included in the study. According to multivariate analysis, being <5 years of age, presence of a child attending a daycare center, recovery from respiratory infection within the last month, low income level of the family, and presence of more children in the family were found to be the risk factors for the NP pneumococcal carriage. The carriage rate of NP pneumococci in healthy children was not influenced by PCV7 in Turkey.

Key words: child, pneumococcal carriage, pneumococcal conjugate vaccine, Streptococcus pneumoniae.

Streptococcus pneumoniae is the most common bacterial cause of community-acquired pneumonia, occult bacteremia, acute sinusitis, and acute otitis media. It is the most common cause of childhood bacterial meningitis in developing countries where routine heptavalent pneumococcal conjugate vaccine (PCV7) has not been administered. In the United States (US) in 2000, *S. pneumoniae* caused an estimated 17,000 cases of invasive disease, including 700 cases of meningitis, 13,000 of bacteremia and 200 deaths, among children aged <5 years[1]. On the other hand, in developing countries, pneumonia accounts for ~30% of deaths among children aged <5 years, and it is believed that many of these deaths are caused by pneumococci[2]. Asymptomatic nasopharyngeal (NP) carriage of *S. pneumoniae* is widely prevalent among young children in both developed and developing countries, which is important since it is related both to development of disease and to spread of the pathogen to other individuals[3]. NP colonization of pneumococci starts during early infancy. Carriage rates vary between 11% and 93% according to the geographic region and population[1]. Pneumococcal conjugate vaccines were developed to prevent invasive pneumococcal diseases, and after the introduction of the PCV7, a substantial decrease in the incidence of invasive pneumococcal diseases has been reported in the target population aged <5 years. PCV7 also showed a herd effect among non-vaccinated populations by reducing NP colonization and transmission of vaccine-type pneumococci from vaccinated children[4]. In some countries, decreases in the

pneumococcal carriage rates were seen after the implementation of PCV7, but in most of the studies conducted after community-wide vaccination with PCV7, NP carriage of *S. pneumoniae* did not change because of the increase in the non-vaccine types of pneumococci in children. The PCV7 was introduced in Turkey in September 2005, and it was added to the Turkish national vaccine schedule in November 2008 for children born in May 2008. The purpose of this study was to investigate the effects of PCV7 on NP carriage rates of *S. pneumoniae* in healthy Turkish children. We also studied the possible changes in known related risk factors for NP colonization of pneumococci in the era of community-wide PCV7 use for the first time.

Material and Methods

The study was conducted on 1101 healthy Turkish children between 1 month and 18 years of age, visiting a well-child outpatient clinic and a general pediatric outpatient clinic in Ankara University Faculty of Medicine, Ankara, Turkey. The children with active respiratory infections and/or with antibiotic therapy within the last two weeks and children with chronic diseases were excluded from the study. The vaccination status of the children was determined as non-vaccinated, fully vaccinated (3 or 4 doses for <24 months and 1 dose for ≥24 months) and partially vaccinated (1 or 2 doses for <24 months) according to the Advisory Committee on Immunization Practices (ACIP) criteria[5,6]. Nasopharyngeal (NP) specimens were collected with calcium alginate-tipped swabs on a flexible aluminum wire swab by trained pediatricians between April 2011 and June 2011. Swab specimens were placed in transport medium and processed in the microbiological laboratory at Ankara University Medical School within 3 hours. Swabs were inoculated in agar plates supplemented with 5% defibrinated sheep's blood and incubated overnight at 37°C in 5-10% CO_2 atmosphere. *S. pneumoniae* was identified using standard laboratory procedures, including morphology following Gram's stain, susceptibility to 5 μg optochin disk and bile solubility test. The isolates were confirmed at Refik Saydam National Public Health Agency, Molecular Microbiology Research and Application Laboratory.

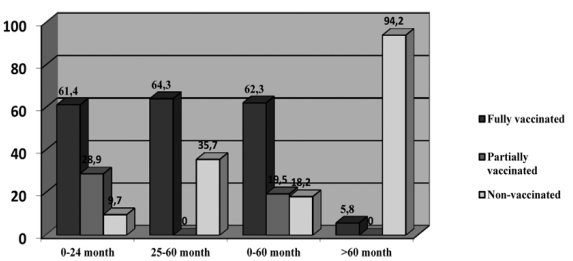

Fig. 1. Vaccination status of the children.

Questionnaires were administered to the parents by face-to-face interview to obtain information about the demographic features and risk factors for pneumococcal carriage, including vaccine history, history of respiratory infection within the last 1 year and 1 month, history of breastfeeding, attendance at daycare centers and the presence of siblings attending daycare centers, number of members in the household, presence of a smoker in the house, antibiotic consumption, hospitalization within 1 year, 3 months and 1 month prior to enrollment, operation history within the last 1 year and 6 months, presence of a health care provider at home, and income level of the parents.

Informed consent was obtained from the parents of the children, and the study was approved by the ethical committee of Ankara University Faculty of Medicine. The study was supported by Ankara University Scientific Research Projects Office (Project no: 10B3330033).

All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS) software. Chi-square test was used for univariate analysis, with a value of $p<0.05$ considered to be statistically significant. Logistic regression analysis was used to identify the potential risk factors for NP colonization with *S. pneumoniae*, and a p value of <0.1 was considered to be statistically significant.

Results

Of the study population, 504/1101 (45.8%) of the children were female and 597/1101 (54.2%) were male. The median and mean ages of the children were 25 months (1 month-18 years) and 45.7±49.6 months, respectively. According to the age distribution, about half of the children (49.6%) were ≤2 years old and nearly three-fourths (73.5%) were ≤5 years old. Of the children included in the study, 679 (61.7%) received PCV7 and the remaining 422 children (38.3%) were in the non-vaccinated group. The vaccination status of the children

Table I. Demographic Features of the Children

Features	%
Respiratory infection within last year	78.8
Respiratory infection within last month	46.7
Antibiotic consumption within last year	56.9
Antibiotic consumption within last 3 months	34
Antibiotic consumption within last month	19.3
Hospitalization within last year	7.4
Hospitalization within last 3 months	2.5
Hospitalization within last month	1.1
Operation history within last year	2.5
Operation history within last 6 months	1.9
Attendance at daycare centers	6
Attendance at daycare centers (participant and siblings)	11.8
History of breastfeeding	9.4
Exposure to smoke at home	33
Presence of a health care provider at home	13.7
Monthly income level of the parents (TL*)	
<500	2.9
500-1000	34.6
1001-2000	42.2
2001-5000	18.6
>5000	1.7

*1TL=0.43€

Table II. The Relationship between NP Pneumococcal Carriage and Age of the Children

Age groups	*S. pneumoniae* (-) %	*S. pneumoniae* (+) %	p value
0-24 months	72.5	27.5	
25-60 months	79.8	20.2	<0.001
>60 months	87	13	
0-24 months	72.5	27.5	
>24 months	83.6	16.4	<0.001
0-60 months	74.9	25.1	
>60 months	87	13	<0.001

with PCV7 according to the age groups and demographic features are shown in Figure 1 and Table I, respectively.

S. pneumoniae was isolated in 241/1101 (21.9%) children included in the study. Increase in the percentage of pneumococcal carriage was significantly correlated with a decrease in age. The NP colonization rates of pneumococci were 27.5%, 20.2% and 13% in children aged between 0-24 months, 25-60 months and >60 months, respectively (p<0.001) (Table II).

Univariate analysis of risk factors potentially associated with NP carriage of *S. pneumoniae* indicated as significant were: attendance at daycare center (participant or siblings), respiratory infection within the last month, increased mean number of persons and children in the family, increased mean number of respiratory infections within the last year, decrease in the monthly income level of the parents, and lack of health care in the family in children aged 0-24 months (Table III). However, there were no relationship between gender; attendance at daycare center; hospitalization

Table III. The Risk Factors of NP Pneumococcal Carriage in Children Aged 0-24 Months

Risk factors	*S. pneumoniae* (-) %	*S. pneumoniae* (+) %	p value
Attendance at DC (participant or siblings) (+)	60	40	
Attendance at DC (participant or siblings) (-)	73.8	26.2	<0.05
Respiratory infection within last month (+)	63.5	36.5	
Respiratory infection within last month (-)	78.1	21.9	<0.001
Mean number of persons in family	3.86±1.12	4.2±1.3	<0.001
Mean number of children in family	1.57±0.71	1.85±0.74	<0.001
Mean number of respiratory infections within last year	1.48±1.72	1.91±2.04	<0.05
Health care provider at home (+)	83.5	16.5	
Health care provider at home (-)	70	30	0.006
Monthly income level of the parents (TL)			
<500	43.8	56.2	
500-1000	73.7	26.3	
1001-2000	68.7	31.3	<0.05
2001-5000	80.3	19.7	
>5000	72.7	27.3	

DC: Daycare center.

- duration of hospitalization - number of hospitalizations within the last year, last 3 months and last month; admission to the intensive care unit; operation history within the last 6 months and last year; antibiotic consumption within the last year, last 3 months and last month; exposure to smoke at home; vaccination status with PCV7; and the number of PCV7 dosages in these children. On the other hand, attendance at a daycare center was the only risk factor in children aged 25-60 months and >60 months (Tables IV, V).

The pneumococcal carriage rate was lower in children vaccinated with PCV7 than in non-vaccinated children aged ≤2 years, but it was not statistically significant (Table VI). Lastly, according to multivariate analysis, being <5 years of age, presence of a child attending a daycare center, recovery from a respiratory infection within the last month, low income level of the family, and presence of more children in the family were found to be the risk factors for the NP pneumococcal carriage in healthy Turkish children (Table VII). There was no relationship between the other risk factors and NP pneumococcal carriage.

Discussion

S. pneumoniae is a bacterium that frequently colonizes the human nasopharynx. Apart from

Table IV. The Risk Factors of NP Pneumococcal Carriage in Children Aged 25-60 Months
DC: Daycare center.

Risk factors	*S. pneumoniae* (-) %	*S. pneumoniae* (+) %	p value
Attendance at DC (+)	62.5	37.5	
Attendance at DC (-)	72.6	27.4	0.009
Attendance at DC (participant or siblings) (+)	65.8	34.2	
Attendance at DC (participant or siblings) (-)	82.2	17.8	<0.05

Table V. The Risk Factors of NP Pneumococcal Carriage in Children Aged >60 Months

Risk factors	*S. pneumoniae* (-) %	*S. pneumoniae* (+) %	p value
Attendance at DC (+)	75	25	
Attendance at DC (-)	88.5	11.5	<0.05

DC: Daycare center.

Table VI. Relationship between NP Pneumococcal Carriage and Vaccination with PCV7

Age groups	Vaccination status with PCV7	*S. pneumoniae* (+) %	*S. pneumoniae* (-) %	p value
0-24 months	Non-vaccinated	32.1	67.9	
	Partially vaccinated	25.3	74.7	>0.05
	Fully vaccinated	27.8	72.2	
25-60 months	Non-vaccinated	19.1	80.9	
	Partially vaccinated	0	0	>0.05
	Fully vaccinated	20.7	79.3	
>60 months	Non-vaccinated	12.7	87.3	
	Partially vaccinated	0	0	>0.05
	Fully vaccinated	17.6	82.4	

Table VII. Risk Factors for Pneumococcal Carriage by Logistic Regression Analysis

Risk factors	Odd ratio	CI (95%)	*p
Age 0-24 months	3.68	2.42-5.59	<0.001
Age 25-60 months	2.05	1.28-3.29	0.003
A child attending daycare center in family	1.93	1.26-2.95	0.002
Respiratory infection within last month	1.43	1.05-1.94	0.021
Increase in the number of children in family by 1	1.38	1.13-1.68	<0.001
Family income <200 TL	3.96	0.88-17.83	0.072
Family income 200-400 TL	1.62	0.43-6.09	0.471
Family income 400-800 TL	2.25	0.6-8.37	0.226
Family income 800-2000 TL	1.11	0.28-4.27	0.877

CI: Confidence interval.
*p<0.1 is significant.

disease outcomes such as sinusitis, otitis media, and community-acquired pneumonia, which result from direct spread from the nasopharynx, the pneumococcus can invade the bloodstream and cause septicemia, meningitis, and invasive pneumonia[7]. In other words, all pneumococcal disease is preceded by NP colonization, and asymptomatic carriers are the main source of pneumococcal disease. These asymptomatic carriers infect other individuals by the respiratory tract. NP colonization of pneumococci starts during early infancy. Carriage rates vary according to the geographic region and population[1,8]. In African and Asian countries, the NP pneumococcal carriage rates are higher than in developed countries: nearly 90% in Gambia for children <14 years and in the highlands of Papua New Guinea for children <5 years, 48% in Indonesia for children <2 years, and 62% in Pakistan among children aged <5 years. In industrialized countries, the rates are 53% for children aged <5 years in Israel, 3.5%–8.6% for Italian children aged <8 years, 43% for 2-year-old children in Finland, and 26%–38% for children aged <7 years in the eastern United States (and 50% for children aged <5 years in Alaska)[2].

Widespread use of the conjugate vaccine has enormously reduced the incidence of pneumococcal disease in all age groups. In children, this effect is a direct result of vaccination; in nonvaccinated children and adults, it represents what is called the "herd effect," in which the protection of the entire population

Table VIII. The NP Pneumococcal Carriage Rates in Other Countries in the Pre- and Post-Vaccine Era

Country	Age (month)	Pre-vaccine era			Post-vaccine era			Reference no
		Year	No of children	Carriage rate (%)	Year	No of children	Carriage rate (%)	
France*	3-39	1999	298	54	2008	343	45.2	10
Greece*	13-76	2005	769	48.1	2009	820	40.7	11
The Netherlands	11	2005	319	67	2009	329	47	12
The Netherlands	24	2005	321	66	2009	330	49	12
Hong Kong*	24-72	1999	1978	19.4	2009	2211	15.7	13
Singapore*	17-78	1997	395	25.8	2007	418	14.1	14
US	3-59	2000	450	38	2002	450	35	15
US	3-59	2000	450	38	2004	450	41	16
US	3-84	2000	678	27	2006	972	30	17
Norway*	45**	2006	611	77.7	2008	602	80.2	19
Hungary*	36-72	2003	95	35.8	2010	121	31.4	20
UK	0-48	2006	324	32.1	2008	328	31.1	21
Taiwan	2-60	2005	1506	12.9	2008	764	13.9	22
Brazil*	4-72	2001	717	64.9	2006	571	68.7	23
Turkey***	1-216				2011	1101	21.9	

*Children attending day care centers, **Median age, ***This study

results from reduced NP carriage of infective strains in the vaccinated population[8]. As stated earlier, control of NP carriage of pneumococci is the key to managing pneumococcal disease and person-to-person spread of *S. pneumoniae*. The nonconjugate pneumococcal vaccines do not have a significant effect on carriage of *S. pneumoniae* in children and adults. In contrast, the conjugate vaccines do have a significant effect on carriage. Despite variations in the nature of the conjugate vaccines, populations, and the ages at which the vaccines were administered, a significant reduction in carriage of the serotypes included in the vaccine clearly was observed in all studies. However, in most of the studies, a "replacement" phenomenon occurred: an increase in the carriage of *S. pneumoniae* serotypes not included in the vaccine was observed in conjunction with a decrease in the carriage of serotypes included in the vaccine. Although this replacement phenomenon is remarkable, its clinical significance is not clear[9]. In this study, we evaluated the effect of PCV7 on the NP carriage of *S. pneumoniae* in healthy Turkish children aged between 1 month and 18 years three years after the addition of PCV7 to the Turkish national vaccine schedule. Many comparative studies were carried out about NP pneumococcal carriage before and after PCV7 vaccination in certain periods in the US and in European, South American and Far Eastern countries. However, in Turkey and in other developing countries, there is only information about the prevaccination period because of the lack of PCV7 inclusion in national immunization programs.

Significant declines were obtained in the pneumococcal carriage rates in children attending daycare centers after introduction of PCV7 in France (age group 3-39 months) and Greece (age group 13-76 months) as 54% to 45.2% and 48.1% to 40.7%, respectively[10,11]. Furthermore, these declines were seen in Dutch children aged 11 months (67% to 47%) and 24 months (66% to 49%)[12]. In Asian countries, Hong Kong and Singapore, where vaccination with PCV7 is done, the NP pneumococcal carriage in children aged 2-6 years was also significantly decreased (19.4% vs. 15.7% and 25.8% vs. 14.1%, respectively) (Table VIII)[13, 14].

In the US, the pneumococcal carriage was stable during 2000, 2001 and 2002, as PCV7 was being introduced into the routine immunization schedule. The carriage rate decreased from 38% to 35% in children aged 3-59 months, but the result was not statistically significant[15]. Park et al.[16] and Huang et al.[17] in the US and Kellner et al.[18] in Canada also found no statistically significant effect of PCV7 on the NP carriage of *S. pneumoniae* in healthy children aged <7 years. In European countries, the frequency of carriage was as high as nearly 80% in Norway (in children attending daycare centers with a median age of 45 months), but it was about 30% in the United Kingdom (UK) (in children aged <4 years) and Hungary (in children attending daycare centers aged 3-6 years), and these rates were not significantly influenced by PCV7[19-21]. The NP pneumococcal carriage rate did not change in Taiwan despite the widespread use of PCV7 for four years (Table VIII)[22].

In studies from Brazil, Sa-Leao et al.[23] and Rodrigues and colleagues[24] determined that NP pneumococcal carriage frequency was quite high in children attending daycare centers and increased from 64.9% to 68.7% five years after introduction of PCV7 (Table VIII).

In our country, various studies have been conducted about NP pneumococcal carriage before the introduction of PCV7 into our national vaccine schedule. The NP carriage rates ranged between 8.5% and 37.2%, and all of the children included in those studies were aged under 13 years[3,25-33]. Three of these studies were conducted in Ankara, and the NP carriage rates were found as 23.9% (year: 1997, age range: 0-11 years), 30% (year: 1997, age range: 0-12 years) and 22.5% (year: 2004, age range: 0-2 years)[25,26,29]. The second of these Ankara studies was conducted in our clinic[26]. In our study, we found that the carriage rate was 21.9% in children aged 1 month - 18 years, and nearly half of the children were under 5 years old. The carriage rates were 27.5% and 25.1% for children aged 0-24 months and 0-60 months, respectively. As shown in these previous studies, the NP carriage of *S. pneumoniae* was not significantly influenced by PCV7. Because, as with many other microorganisms, *S. pneumoniae* finds its ecologic niche in colonizing the nasopharynx. In most of the NP carriage studies, no remarkable decrease was seen for NP pneumococcal carriage because of the increase in the carriage of *S. pneumoniae* serotypes not included in the vaccine[15-17,23]. Thus, in our study, the absence of the expected decrease in NP pneumococcal carriage may be associated with a possible increase in carriage of the non-vaccine serotypes of pneumococci.

Several risk factors, such as age <5 years, attendance at daycare centers, frequent occurrence of viral respiratory tract infections, living in a crowded household, lower socioeconomic status, use of antibiotics, and exposure to tobacco smoke, which favor the NP carriage of *S. pneumoniae*, have been implicated in the literature[3,17,18,24]. In this study, we found that being <5 years old, presence of a child attending daycare centers, recovery from respiratory infection within the last month, low income level of the family, and presence of more children in the family were the risk factors for the NP pneumococcal carriage in healthy Turkish children. Eventually, PCV7 did not change these risk factors, as observed in the results of other studies conducted after the widespread use of PCV7 in Turkey and other countries[3,15-18,22,24,25,28-30,34-37].

In conclusion, this study is the first research about NP carriage of *S. pneumonia* in healthy children aged 1 month - 18 years after the community-wide PCV7 vaccination in Turkey. The carriage rate of NP pneumococci in healthy children was not influenced by community-wide PCV7 vaccination, because the carriage rate was similar to the rates reported in previous studies in our country, as in most of the other countries. The risk factors for NP pneumococcal colonization remained unchanged despite the community-wide PCV7 vaccination.

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