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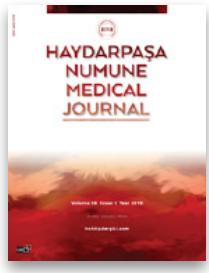

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ORIGINAL ARTICLE

Nasopharyngeal Carriage of *Streptococcus Pneumoniae* and Related Risk Factors in Children Attending Day Care Centers

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Abstract

Introduction: Streptococcus pneumoniae (pneumococcus) is a leading cause of severe illness among pneumoniae (pneumococcus children worldwide. In this study, we aimed to evaluate nasopharyngeal carriage of Streptococcus) in healthy children, related risk factors and pneumococcus serotypes isolate in nasopharyngeal swabs.

Methods: This study was performed in 310 healthy children aged six months to six years who were attending day care centers. Family size, number of siblings, vaccination status and duration of attendance at day care centers were evaluated. Nasopharyngeal specimens were collected, and serotypes of the isolates were identified.

Results: Family size, number of siblings, and vaccination status were not risk factors affecting the nasopharyngeal carriage of pneumococcus. However, we found significantly higher rates of nasopharyngeal pneumococcus carriage in children attending day care centers for less than six months compared to children attending for longer than six months.

Discussion and Conclusion: We demonstrated that the duration of attendance at day care centers played a significant role in the development of nasopharyngeal pneumococcus carriage.

Keywords: Child; nasopharyngeal carriage; streptococcus pneumonia.

Streptococcus pneumoniae (pneumococcus) is a leading cause of severe illness among children worldwide. Pneumococcus carriage plays a significant role in the development of respiratory system infections and the spread of penicillin-resistant serotypes[1]. In this study, we aimed to evaluate the nasopharyngeal carriage of *Streptococcus pneumoniae* in healthy children, related risk factors and pneumococcus serotypes isolated in nasopharyngeal swabs.

Colonization with *Streptococcus pneumoniae* increases to a peak incidence of 55% at three years of age. Increasing incidence rates occur at 10 years of age, with a constant 8% prevalence[2]. Other studies have reported that although the nasopharyngeal carriage incidence is 13% in children younger than six months, it increases to 43% in children older than 19 months[3].

The major risk factors of nasopharyngeal colonization are age, family size, number of siblings, income level, tobacco smoke exposure, vaccination status and attendance at day care centers or school[4].

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The isolation incidence in nasopharyngeal swabs increases to 40-60% in children[5]. Initial nasopharyngeal colonization appears between four days and 18 months of age. Serotypes 6, 14, 19, and 23 are the most commonly isolated in nasopharyngeal swabs of children younger than three years old[6].

Materials and Methods

This study included 310 healthy children aged between six months and six years who attended a day care center for at least one month and had no respiratory infection or antibiotic usage during the period of data collection. This study was performed after being approved by the Local Ethics Committee of our hospital. Questionnaires, which included interviews regarding demographic features, tobacco smoke exposure, number of siblings at home, family size, duration of attendance at a day care center, and vaccination status, were administered to children for whom maternal approval was obtained. Nasopharyngeal swabs were collected from 310 children attending a day care center. The specimens were inoculated onto a 5% defibrinated sheep blood agar plate and incubated overnight at 37 ºC in a 5% CO_2 chamber.

Positive cultures that grew alpha-hemolytic colonies were gram stained and isolate serotypes were identified with the Quelling reaction using antiserum.

Statistical analyses were performed using SPSS software, version 19.0 (SPSS, Chicago, IL, USA). The serotype proportion was analyzed using the chi-squared test.

Results

This study was performed from January 2012 to April 2012. This study included healthy children aged six months to six years who attended day care centers and had no respiratory infection or antibiotic usage during the analyses.

In total, 92 (30%) of the children were immunized with the 7-valent pneumococcal conjugate vaccine (PCV7), and 215 (70%) of the children were not vaccinated (Table 1). Twenty-one children (7%) had received all of the scheduled vaccine doses, while 278 (93%) of the children had only incomplete vaccination (Table 1).

The nasopharyngeal pneumococcal carriage was detected in 29 children (9.4%), and the remaining 281 (90.6%) subjects did not carry any pneumococcal serotypes (Table 1).

This study included 147 female (47.4%) and 163 (52.6%) male children with a mean age of 38.94±15.71 months (Table 2). The duration of attendance at day care centers was between 1 and 54 months, with a mean value of

Table 1. *Streptococcus pneumoniae* and distributions related to vaccination

	Min-Max	Mean value
MIC value (mcg/ml)	0.16±16.0	2.45±3.92
	n	**%**
Pneumococcus vaccine		
Present	92	30
Absent	215	70
Vaccination status		
Complete	21	7
Incomplete	278	93
Streptococcus pneumoniae carriage		
Present	29	9.4
Absent	281	90.6
Isolated Pneumococcus serotypes		
Serotype 1	5	17.2
Serotype 4	5	17.2
Serotype 6	2	6.9
Serotype 11	8	27.6
Serotype 14	7	24.1
Serotype 23	2	6.9

Table 2. Demographic features of *Streptococcus pneumoniae* carriage

	***Streptococcus pneumoniae* carriage**		p
	Present Mean value	**Absent Mean value**	
Age (months)	32.5±12.72	39.60±15.85	0.021*
	n (%)	**n (%)**	
Gender			
Female	18 (62.1)	129 (45.9)	0.097**
Male	11 (37.9)	152 (54.1)	
Duration of breastfeeding			
≥6 months	24 (82.8)	235 (83.6)	0.904**
<6 months	5 (17.2)	46 (16.4)	
Family size			
<5	25 (86.2)	217 (77.2)	0.266**
≥5	4 (13.8)	64 (22.8)	
Amount of siblings			
<3	25 (86.2)	232 (82.9)	0.646**
≥3	4 (13.8)	48 (17.1)	
Duration of attendance at day-care centers			
≤6 months	29 (100)	61 (21.7)	0.001**
>6 months	0 (0)	220 (73.8)	

*Student's t-test; **the chi-square test.

12.71±9.46 months. The number of family members residing at home ranged from three to eight, with a mean of 3.95±0.89 (median 4) (Table 2).

The number of siblings ranged from one to four, with a mean of 1.82±0.74 (median 2) (Table 2).

The duration of breastfeeding ranged from 0 to 36 months, with a mean value of 13.35±7.30 months (Table 2).

Pneumococcus serotype 1 was isolated in five of the children (17.2%), serotype 6 was isolated in 2 (6.9%), serotype 11 was isolated in 8 (27.6%), serotype 14 was isolated in 7 (24.1%), and serotype 23 was isolated in 2 (6.9%) (Table 1), the mean age of children (39.60±15.85 months) who had no nasopharyngeal pneumococcus carriage was significantly higher than the mean age of non-carriers (32.5±12.72 months) (p=0.021) (Table 2).

There was no significant difference between the genders according to the nasopharyngeal pneumococcus carriage status, but the carriage was typically higher in girls than in boys (Table 2).

There was no significant difference in family sizes, the number of siblings or duration of breastfeeding according to the nasopharyngeal pneumococcal carriage (p>0.05) (Table 2).

Our data showed a statistically significant difference between the duration of day care attendance according to the nasopharyngeal pneumococcus carriage (p<0.01). The rate of nasopharyngeal pneumococcus carriage was significantly higher in children who had attended day care for less than six months compared to children who had attended for longer than six months (p=0.001) (Table 2).

No significant difference in the nasopharyngeal pneumococcal carriage was identified between completely vaccinated, unvaccinated or incompletely vaccinated children (Table 3).

Table 3. Evaluation of vaccination status according to *Streptococcus pneumoniae* carriage

	***Streptococcus pneumoniae* Carriage**		p*
	Present n (%)	**Absent n (%)**	
Pneumococcus vaccine			
Absent	20 (69.0)	195 (70.1)	0.895
Present	9 (31.0)	83 (29.9)	
Vaccination status			
Complete	2 (6.9)	19 (7.0)	0.978
Incomplete	27 (93.1)	251 (93.0)	

*The chi-square test.

Discussion

Streptococcus pneumoniae is one of the leading causes of mortality in children <5 years old[7]. *Streptococcus pneumoniae* accounts for approximately 30-50% of pneumonia-related deaths[8,9]. There are over 91 serotypes of pneumococcus, and worldwide, 6-11 serotypes are responsible for ≥70% of invasive disease (IPD) in children <5 years old[10]. The distribution of pneumococcal capsular serotypes in nasopharyngeal carriage isolates and IPD changes with age, geography and socioeconomic status[11,12]. Pneumococcus is also part of the normal microbial flora in the nasopharynx, its main reservoir. Although harmless and asymptomatic by itself, the carriage is a precondition for invasive and non-invasive diseases, including acute otitis media, bacteremia and pneumonia[13,14].

Before immunization with the conjugate vaccine, many cases of invasive disease among children younger than five years of age were caused by pneumococcus. In some cases, the nasopharyngeal pneumococcal carriage may progress to respiratory or even systemic pneumococcal disease in the host and also serves as a reservoir for the horizontal spread of pneumococcus within the community. The routine use of PCV7 resulted in a decreased incidence of invasive pneumococcal disease in children[15,16]. However, there are increasing data suggesting that universal infant immunization with PCV7 changed the serotype patterns of invasive disease, nasopharyngeal colonization and antibiotic resistance status. Some studies have supported the hypothesis that vaccination decreases the carriage rate; other studies have reported that the carriage of vaccine serotypes has almost disappeared and that they were replaced by non-vaccine serotypes[17].

Kim et al. compared pneumococcal nasopharyngeal carriage between vaccinated and non-vaccinated children (PCV7). Nasopharyngeal pneumococcal swabs were obtained from 400 children aged 18-59 months, and the serotypes and antibiotic susceptibilities were analyzed. The pneumococcal carriage rate was lower in the vaccinated group (36/200, 18%) compared to the control group (63/200, 31.5%). The resistance rates for penicillin and erythromycin were also lower in the vaccine group. PCV7 reduced carriage of the vaccine serotypes, which resulted in the replacement of the pneumococcal by antibiotic-susceptible vaccine-related types or no vaccine-type strains[18]. In our study, pneumococcal carriage did not differ according to vaccination status. There was no significant difference between fully vaccinated and unvaccinated subjects according to nasopharyngeal pneumococcal carriage.

Hashida et al. investigated the turnover of nasopharyngeal pneumococcal carriage in healthy children in day care centers. A total of 363 nasopharyngeal mucus samples were collected from children aged 0 to 6 years who attended day care centers. They obtained 157 *S. pneumoniae* isolates and analyzed them with antibiotic susceptibility testing. The overall carriage rate was 35.7% (56/157). Their results indicated the spread of *S. pneumoniae*, particularly strains with antibiotic resistance genes, and the horizontal transmission of this pathogen in healthy children attending day care centers in Japan[19]. Our study reported a nasopharyngeal carriage rate of 9.4% among 350 healthy children. However, a higher proportion of children aged six months to six years (93%) were incompletely vaccinated. We demonstrated that a complete vaccination status did not change the rate of nasopharyngeal pneumococcal carriage. Serotypes 14 and 11 were the most commonly observed isolates in the nasopharyngeal swabs of our subjects.

Cho et al. investigated the serotype distribution and antimicrobial resistance of pneumococcal carriage isolates from children after optional immunization with PCV7 in Korea. A total of 1243 nasopharyngeal aspirates of children were analyzed, and the serotypes and antibiotic susceptibility were determined. The results were comparable to previous studies performed in the pre-PCV7 period. In their study, the most common serotypes were 6A (15.3%), 19A (14.7%), 19F (10.2%), 35B (7.3%) and 6D (5.6%). The proportion of vaccine serotypes decreased from 61.9% to 23.8% ($p<0.001$). The proportion of all PCV7 serotypes, except 19F, decreased as antimicrobial-resistant serotypes 6A and 19A increased[20]. In our study, the serotype distribution could not be evaluated because a high proportion of our subjects (278 children [93%]) were incompletely vaccinated. However, although the subjects had a low rate of vaccination, we found a low proportion of nasopharyngeal pneumococcal carriage (29 children [9.4%]). We, therefore, concluded that nasopharyngeal carriage of *S. pneumoniae* did not change according to vaccination status. Our data showed a serotype distribution with higher rates of serotypes 14 and 11. However, our study indicates that the nasopharyngeal carriage of pneumococcus is significantly higher in children who have attended day care centers for a shorter duration (<6 months) compared to those who have attended for longer than six months.

Cohen et al. studied the nasopharyngeal carriage of pneumococcus in children with acute otitis media and healthy children in two age groups (6-24 months versus 25-60 months). They collected nasopharyngeal swabs from children 6-60 months with acute otitis media and healthy controls to analyze the carriage of pneumococcus and other pathogens. Of the 1557 enrolled children, 1258 were six to 24 months of age, and 299 were 25 to 60 months. More than 85% were PCV7 vaccinated, and the children 25-60 months of age were rarely PCV13 vaccinated (14.1%) compared to the younger children. For children 6-24 months, *S. pneumoniae* carriage was higher in the acute otitis media group compared to the healthy group. By contrast, the difference was not significant for older children. In the healthy group, older children more often carried *S. pneumoniae* than younger children. A multivariate analysis of the healthy group showed that siblings and attendance at a day care center increased the carriage rate of *S. pneumoniae*. These nasopharyngeal carriage data in children six to 60 months old showed that pneumococcal carriage was high in patients less than two years of age, particularly in the healthy group[21]. Our data showed a pneumococcal carriage rate of 9.4%, which is lower than that reported by Cohen. They reported high pneumococcal carriage rates in healthy children younger than two years. However, our data are supported by their study because they also reported an increased carriage of *S. pneumoniae* in the healthy group related to attendance at day care centers.

Millar et al. investigated the effects of vaccination on the nasopharyngeal carriage of pneumococcus. They found exactly the same ratios in vaccinated and unvaccinated groups, 63.9% and 60.5%, respectively[22]. In our study, the nasopharyngeal pneumococcal carriage rates did not differ significantly between vaccinated (2.9%) and unvaccinated (6.5%) groups.

Conclusion

We analyzed the risk factors affecting the nasopharyngeal carriage of pneumococcus and found a nasopharyngeal pneumococcal carriage rate of 9.4%. The duration of breastfeeding, family size, the number of siblings, and vaccination status did not increase the nasopharyngeal carriage of the organism.

The rates of nasopharyngeal pneumococcal carriage differed significantly according to the duration of attendance at day care centers among healthy children, regardless of vaccination status.

We believe that larger multi-center studies, including more subjects from different socioeconomic statuses and regions will further elucidate the relationships between the risk factors and nasopharyngeal carriage of pneumococcus in healthy children.

Ethics Committee Approval: The Ethics Committee of Haydarpaşa Numune Training and Research Hospital provided the ethics committee approval for this study (2010/13-29.11.2010).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: N.A.D.,Ç.N.,Ö.C.; Design: T.G., Ç.N., Ö.C.; Data Collection or Processing: N.A.D.; Analysis or Interpretation: N.A., Z.E.Ö., T.G.; Literature Search: N.A., Z.E.Ö., T.G.; Writing: N.A., Z.E.Ö.

Conflict of Interest: None declared.

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