Original Article

Prevalence and Risk Factors of Nasopharyngeal Carriage of *Streptococcus pneumoniae* in Healthy Children in Japan

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SUMMARY: The findings of this study revealed a 22% prevalence of *Streptococcus pneumoniae* among healthy children in Japan; the prevalence tended to increase with age. We identified attendance at day-care facilities and the presence of older siblings as the major risk factors associated with pneumococcal carriage. A significant decrease in pneumococcal colonization was observed among children during the summer; however, a seasonal variation was independent of various factors. Our findings suggest that prior immunization with heptavalent pneumococcal conjugate vaccine within the current recommended schedule is required for children exposed to these risk factors.

INTRODUCTION

Streptococcus pneumoniae is a clinically important pathogen that causes invasive pneumococcal diseases (IPDs) such as sepsis, meningitis, and pneumonia in children. *S. pneumoniae* is part of the commensal flora of the nasopharynx (1), but only a few reports have been published in Japan on the prevalence of nasopharyngeal carriage of *S. pneumoniae* in children (2,3). The risk factors cited for nasopharyngeal colonization with pneumococci in children in the United States and Europe are ethnicity, attendance at day-care centers, the presence of older siblings, recent antibiotic use, and respiratory tract infections (1,4–7). In Japan, a report based on routine public medical checkups (2), suggests that the presence of older siblings and day-care center attendance are the major risk factors for pneumococcal carriage in healthy children. To the best of our knowledge, no reports on seasonal variations in pneumococcal colonization among healthy children have yet been published in Japan. The aims of this study were to estimate the prevalence of nasopharyngeal carriage of *S. pneumoniae* in healthy children in Japan, and to examine the potential risk factors for the infection.

MATERIALS AND METHODS

Study population: During the study period (July 2010 through March 2012), 229 healthy children were enrolled. Three institutions in Saitama Prefecture and Tokyo were involved. Parents were informed about this study when they brought their children for their first routine heptavalent pneumococcal conjugate vaccine (PCV7) immunizations. Written informed consent was obtained from the parents who agreed to let their children participate in the study. All the participants were between 2 months and 6 years of age; none had any prior history of immunization with a pneumococcal vaccine, and none had any obvious infection. The Ethical Review Board of Toho University approved this study.

Specimen collection and testing: Pediatricians took nasopharyngeal swabs (Amies charcoal transport medium) of the children during their first routine PCV7 immunizations. The swabs were plated onto a medium containing 5% sheep blood. The plates were incubated overnight at 35°C in 5% CO₂. Pneumococci were isolated and identified on the basis of colony morphology and susceptibility to optochin. Colonies that were difficult to identify with the optochin susceptibility test were identified by means of *lytA* gene polymerase chain reaction (8).

Potential risk factors: Information on exposure to potential risk factors was collected from the parents or caregivers of each participating child, examination of medical records, and/or via a questionnaire. The following factors were examined: age (<1, 1–2, 3–6 years), sex, day-care attendance, presence of older/younger siblings, vaccination history (acellular pertussis vaccine

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combined with diphtheria toxoid and tetanus toxoid, *Haemophilus influenzae* type b vaccine, or influenza vaccine), treatment with antibiotics during the previous 3 months, and seasons when specimens were collected (winter, January to March; spring, April to June; summer, July to September; autumn, October to December).

Statistical analysis: The association of the prevalence of pneumococcal carriage with potential risk factors of the streptococcal infection was analyzed first by univariate analysis, and then by multivariate analysis using logistic regression models. The estimates of the association were expressed as odds ratios (OR) and 95% confidence intervals (CI). The STATA software, version 11 (STATA Corp., College Station, Tex., USA) was used for all data analyses.

RESULTS

The demographic characteristics of the study participants are shown in Table 1; further, 97 (42%) participants were less than 1 year of age, majority (55%) of the participants constituted boys, 53 (23%) participants were attending day-care centers, and 105 (46%) had older siblings.

Table 1. Characteristics of the 229 study children

Characteristic	No. (%) of children
Age (yr)	
< 1	97 (42)
1–2	75 (33)
3–6	57 (25)
Sex	
Boy	125 (55)
Girl	104 (45)
Day-care attendance	
Yes	53 (23)
No	176 (77)
Presence of older siblings	
Yes	105 (46)
No	121 (53)
Not known	3 (1)
Presence of younger siblings	
Yes	37 (16)
No	179 (78)
Not known	13 (6)
Other vaccination history ¹⁾	
Yes	195 (85)
No	32 (14)
Not known	2 (1)
Treatment with antibiotics during the previous 3 months	
Yes	82 (36)
No	141 (62)
Not known	6 (2)
Seasonality	
Winter (January–March)	75 (33)
Spring (April–June)	30 (13)
Summer (July–September)	75 (33)
Autumn (October–December)	49 (21)

¹⁾: acellular pertussis vaccine combined with diphtheria toxoid and tetanus toxoid, *Haemophilus influenzae* type b vaccine, and influenza vaccine.

S. pneumoniae was isolated from 51 of the 229 children (22%). The association between pneumococcal colonization and the potential risk factors are shown in Table 2. The prevalence of carriage appeared to increase with age (19%, 23%, and 28% in the <1 year old, 1–2 years old, and 3–6 years old age groups, respectively), although without statistical significance. Crude analysis showed statistical significance with respect to day-care attendance, the presence of older siblings, treatment with antibiotics during the previous 3 months, and specimen collection during summer. However, multivariate analysis after including these factors and age, showed statistically significant associations only with respect to day-care attendance ($P = 0.049$), the presence of older siblings ($P = 0.006$), and specimen collection during summer ($P = 0.028$). The adjusted OR (95% CI) for day-care attendance and the presence of older siblings were 3.11 (1.00–9.65) and 2.64 (1.32–5.27), respectively. The prevalence of pneumococcal carriage was lowest in summer (12%); the prevalence in winter was 27%, in spring, 37%; and in autumn, 22%. The adjusted OR (95% CI) was 0.36 (0.14–0.90) for summer compared to winter, even after controlling other factors such as day-care attendance.

DISCUSSION

None of the participants had received prior PCV7 immunization. All were healthy at the time of specimen collection and were thus suitable subjects for the evaluation of *S. pneumoniae* colonization. A 22% prevalence of pneumococcal carriage was noted among the study participants.

Previous reports demonstrated a 38–87% prevalence of *S. pneumoniae* colonization in children in Japan (9,10), which is higher than the rate we found. This discrepancy may be explained by the fact that these previous studies specifically targeted children attending day-care centers, i.e., children exposed to one of the risk factors for pneumococcal colonization. In our study, the prevalence of pneumococcal colonization in children attending day-care centers was 34%, closer to the previously reported data (9,10). In Japan, few studies have analyzed groups that have not been exposed to risk factors for pneumococcal carriage. Previous studies have reported a 14% pneumococcal prevalence among children aged 0–14 years on routine immunization visits (11), 46% among infants undergoing routine public medical checkups at age 18 months (3), and 17–48% among infants undergoing routine medical checkups at 3 or 4 months to 18 months (2). In the present study, the pneumococcal prevalence showed a tendency to increase with age: 19% at <1 year, 23% at 1–2 years, and 28% at 3–6 years. In a study carried out in the Netherlands, Bogaert et al. reported that nasopharyngeal colonization of *S. pneumoniae* in children varied with age, with a peak prevalence of pneumococcal colonization of 55% aged 3 years. Thereafter, there was a steady decline of *S. pneumoniae* colonization until a stable prevalence of 8% was attained in children over the age of 10 years (1).

In this study, day-care center attendance was found to be significantly associated with increased pneumococcal carriage; the prevalence of pneumococcal colonization

Table 2. Univariate and multivariate analysis of potential risk factors associated with *Streptococcus pneumoniae* colonization

Variable	No./total no. (%)	Crude OR (95% CI)	P	Adjusted OR (95% CI)	P
Age (yr)					
<1	18/97 (18.6)	1		1	
1–2	17/75 (22.7)	1.29 (0.61–2.71)	0.507	0.89 (0.38–2.09)	0.783
3–6	16/57 (28.1)	1.71 (0.79–3.71)	0.172	0.74 (0.22–2.54)	0.633
Sex					
Girl	22/104 (21.2)	1			
Boy	29/125 (23.2)	1.12 (0.60–2.11)	0.711	—	—
Day-care attendance					
No	33/176 (18.8)	1		1	
Yes	18/53 (34.0)	2.23 (1.13–4.41)	0.021	3.11 (1.00–9.65)	0.049
Presence of older siblings					
No	18/121 (14.9)	1		1	
Yes	33/105 (31.4)	2.70 (1.37–5.01)	0.004	2.64 (1.32–5.27)	0.006
Presence of younger siblings					
No	41/179 (14.9)	1			
Yes	7/37 (32.0)	0.79 (0.32–1.92)	0.596	—	—
Other vaccination history					
No	6/32 (18.8)	1			
Yes	45/195 (23.1)	1.30 (0.50–3.36)	0.588	—	—
Treatment with antibiotics during the previous 3 months					
No	26/141 (18.4)	1		1	
Yes	25/82 (30.5)	1.94 (1.03–3.66)	0.041	1.54 (0.73–3.25)	0.253
Seasonality					
Winter (January–March)	20/75 (26.7)	1		1	
Spring (April–June)	11/30 (36.7)	1.59 (0.65–3.92)	0.312	1.56 (0.60–4.02)	0.358
Summer (July–September)	9/75 (12.0)	0.38 (0.16–0.89)	0.026	0.36 (0.14–0.90)	0.028
Autumn (October–December)	11/49 (22.4)	0.80 (0.34–1.85)	0.596	0.82 (0.33–2.02)	0.664

in children attending day-care centers was higher than in those who did not attend (34% versus 19%). In other countries, children attending day-care centers have been shown to be at higher risk of pneumococcal infection (1), with an OR (95% CI) of day-care attendance with pneumococcal colonization in healthy children of 2.27 (1.89–2.75) reported (7). In Japan, a study of healthy children undergoing routine public medical checkups revealed an OR (95% CI) for pneumococcal colonization among those attending day-care of 1.7 (1.3–2.3)–3.5 (2.4–5.1) (2). Takeuchi et al. found that almost all children were colonized with *S. pneumoniae* within a year of starting kindergarten (12). In crowded settings like day-care centers, there appear to be frequent opportunities for contact with pneumococcal reservoirs, and nasopharyngeal colonization of *S. pneumoniae* increases because of horizontal spread among those present at such places.

The present study also revealed a significantly higher prevalence of pneumococcal colonization in children with older siblings than in those without (31% versus 15%). In Alaska, Moore et al. found that a high OR (95% CI) of pneumococcal colonization in healthy children with siblings (1.55 [1.12–2.16]–2.27 [1.83–2.81]) (6). In contrast, a study of healthy children in Japan attending routine public medical checkups demonstrated an OR (95% CI) of 3.5 (2.6–4.7)–3.9 (2.8–5.3) in children with older siblings (2). A likely explanation for this is that the increased range of social activities undertaken by the older siblings' increases the opportunities for nasopharyngeal colonization with *S. pneumoniae* and transmission to their younger siblings (13,14).

This study demonstrated a seasonal variation in pneumococcal colonization, with a significant decrease among participants in summer. Gray et al. also found that the acquisition rate was seasonal, with a marked peak during winter peak and drop during summer (15). Dowell et al. reported that seasonal patterns of IPD prevalence correlated with changes in temperature and daylight hours (16). However, Hashida et al. reported that nasopharyngeal carriage of *S. pneumoniae* in infants at day-care centers in Japan did not differ between summer and winter (9). Meanwhile, Matsubara et al. reported a bimodal peak (spring and autumn) in childhood IPDs. They also associated the spring peak with children entering day-care centers (the fiscal and academic years start in April in Japan) (17). Our multivariate analysis showed that pneumococcal colonization decreased significantly in summer, independent of day-care attendance. It has been suggested that respiratory viruses, especially rhinovirus and respiratory syncytial virus (RSV), are associated with IPDs in children that do not attend day-care centers (18). Hasegawa et al. have reported that RSV is detected mainly during late summer to winter in cases of pediatric community-acquired pneumonia in Japan (19). The increasing prevalence of pneumococcal colonization we identified in autumn may have been associated with the prevalence of such respiratory infections. However, it should be noted that our study participants were healthy when pneumococcal colonization was surveyed. Whether seasonal variation in pneumococcal colonization in healthy children (as opposed to those with IPDs) is independent of day-care attendance, and whether it is associated with

respiratory infection, and/or changes in temperature and daylight hours, needs to be studied further.

The main limitation of our study is its lack of statistical power because of the relatively small sample size.

In summary, we found a 22% prevalence of *S. pneumoniae* among healthy children in Japan, which tended to increase with age. We identified day-care attendance and the presence of older siblings as the major risk factors associated with pneumococcal carriage. A significant decrease in pneumococcal colonization was observed in summer. In addition, seasonal variation was independent of various factors. This is the first report of seasonal variation in pneumococcal colonization among healthy children in Japan. Our findings suggest that prior immunization with PCV7 within the current recommended schedule is required for children exposed to these risk factors.

Conflict of interest None to declare.

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