

# Prevalence of Streptococcus Pneumoniae in Conjunctival Flora and Association with Carriage in Nasopharynx Among Healthy Vietnamese Children

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## Introduction

*Streptococcus pneumoniae* is one of the top 3 most commonly isolated micro-organisms from the conjunctival flora. *S. pneumoniae* colonization in the nasopharynx is a prerequisite for pneumococcal diseases.<sup>1-3</sup>

Although the most prevalent serotypes of nasopharyngeal *S. pneumoniae* carriage were described in literatures, but we did not find any literatures that reported serotypes of conjunctival *S. pneumoniae* carriage. Also, association between *S. pneumoniae* carriage in conjunctiva and that in nasopharynx have not been described before.

Our aim in the study is to survey the prevalence of *S. pneumoniae* in conjunctival flora in children below 24 months before introduction of pneumococcal conjugate vaccine (PCV), investigate whether *S. pneumoniae* in conjunctival flora is associated with that in nasopharynx, and determine the serotype distribution of *S. pneumoniae* in children's conjunctiva and compare it with ones detected in the nasopharynx.

## Methods and Materials

Study was conducted at six communes in Nha Trang city, Vietnam. Sixty children each from two age groups; younger group (<12month) and older group (12-23month) in each commune were randomly selected.

Demographic, socioeconomic and clinical informations were collected. Child's conjunctival, nasopharyngeal and mother's nasopharyngeal swabs were obtained by a doctor and sent to the Pasteur Institute Nha Trang to perform DNA extraction and realtime PCR for detecting the autolysin-encoding gene (*lytA*) of *S. pneumoniae* for all samples, and microbiological culture for *lytA* positive. We extracted DNA from the colony growth using QIAcube HT (Qiagen, Hilden, Germany). The extracted DNA were sent to Murdoch Children's Research Institute, Australia, for serotyping by microarray analysis.

### Statistical analysis

Prevalence of conjunctival *S. pneumoniae* was calculated. Crude odds ratio of having conjunctival *S. pneumoniae* was analyzed in each characteristic using logistic regression.

*S. pneumoniae* serotype distribution in conjunctival and nasopharyngeal carriage were shown graphically and conformity was evaluated. Statistical analyses were conducted using STATA version 14.0 (Stata Corp, USA).

## Results

Among the 698 children enrolled in this study, 54.2% were boys and the median age at examination was 11.7 months. Sixty-two children (8.9%) had *S. pneumoniae* in the conjunctiva.

### Factors associated with *S. pneumoniae* in conjunctiva

Respiratory hospitalization history, eye symptom in the last two weeks, day-care attendance and *S. pneumoniae* carriage in child's and in mother's nasopharynx were more likely to be associated with *S. pneumoniae* positive conjunctiva by univariate analysis (Table 1).

After adjusting potential confounders, we determined *S. pneumoniae* in child's nasopharynx were positively associated with *S. pneumoniae* in the child's conjunctiva. Low birthweight and day-care attendance independently increased *S. pneumoniae* carriage in conjunctiva (Table 1).

### *S. pneumoniae* serotype in conjunctiva and nasopharynx

Serotype was determined in 87% conjunctival samples positive for *S. pneumoniae*. Among the isolates, NT (59%), 6A (12%), 6B (8%), and 14 (8%) were frequently detected in conjunctiva of children. (Fig 1).

30% of children had *S. pneumoniae* in the nasopharynx. 6A (28.9%) followed by NT (22.2%), 19F (16.7%), 6B (12.1%), and 23F (7.5%) was the most frequently detected in nasopharynx of children (Fig 1).

Serotype was determined in 17 of 18 mother's nasopharyngeal samples positive for *S. pneumoniae* (94%). Eighteen strains were isolated from them. NT was the most commonly found among them (39%) (Fig 1).

87% of children with serotype-determined conjunctival pneumococcal carriage had at least one serotype matched with *S. pneumoniae* isolates in the child's nasopharynx (Table 2).

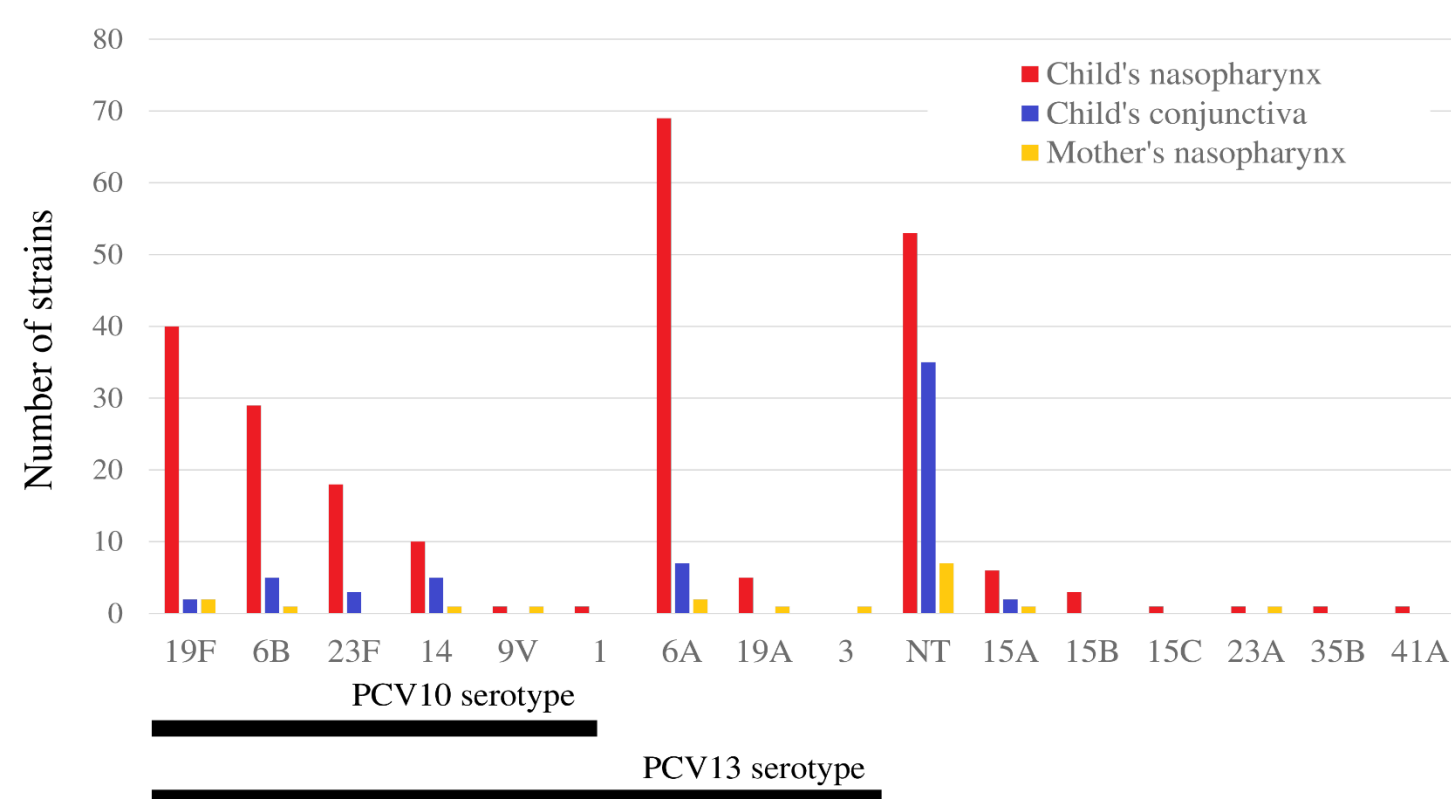


Figure (1) Distribution of *S. pneumoniae* serotypes.

## Discussion

\*The higher prevalence of *S. pneumoniae* in this study may be attributed to many factors in which age group is an important one. Tao H *et al.*'s study found the prevalence of 4.2% in children ≤6 years and 1.6% among those 7-65 years old.<sup>1</sup>

\*The differences may be explained by age-related changes in general immune responsiveness, tear composition and dynamics, patterns of exposure to bacteria, past antibiotic utilisation, and the flora of adjacent areas such as skin and upper respiratory tract.<sup>4</sup>

\**S. pneumoniae* is a causative agent of many documented conjunctivitis outbreaks in which NT has been identified as the etiological agent.<sup>3,5</sup>

\**S. pneumoniae* can be transmitted from nasopharynx to conjunctiva through the nasolacrimal duct by retrograde passage of fluid from nose to conjunctiva during nasal congestion and because of short duct during infancy and early childhood.

## Conclusions

\*This study is the first study to demonstrate the significant association between *S. pneumoniae* in the conjunctiva and that in the nasopharynx statistically, and also showed the conformity in serotypes among healthy children.

\*Prevalence of *S. pneumoniae* in the conjunctiva among children aged less than 24 months was 8.9% in this study.

\*In our study, the lower rate of conjunctival carriage than nasopharyngeal carriage and conformity of serotypes in them might indicate that colonization of *S. pneumoniae* starts in the nasopharynx and spreads to the conjunctiva.

\*To our knowledge, this is the first study to discuss risk factors related to conjunctival *S. pneumoniae* carriage per se.

Characteristics		S. pneumoniae Positive conjunctiva N (%) (n=62)	S. pneumoniae Negative conjunctiva N (%) (n=636)	crude odds ratio of having S. pneumoniae in conjunctiva (95%CI)	adjusted odds ratio* of having Streptococcus pneumoniae in conjunctiva (95%CI)	No	Conjunctiva	Child's nasopharynx	Mother's nasopharynx	Matched serotype <sup>§</sup>
<b>Demographics</b>										
Sex	Boy	28 (7.4)	350 (92.6)	0.67 (0.40-1.14)	0.61 (0.27-1.39)	1	14	14		Yes
	Girl	34 (10.6)	286 (89.4)	reference	reference	2	14	14		Yes
Age months	<12	24 (6.8)	328 (93.2)	reference	reference	3	14	.		No
	12-23	38 (11.0)	308 (89.0)	1.69 (0.99-2.88)	0.84 (0.65-1.09)	4	14 NT	14 19F		Yes
Birth weight (gram)	≥2500	58 (8.5)	622 (91.5)	reference	reference	5	15A	15A		Yes
	<2500	4 (22.2)	14 (77.8)	3.06 (0.98-9.61)	10.67 (3.95-28.85)	6	15A	15A		Yes
<b>Hospitalization for respiratory disease</b>										
	Yes	11 (20.8)	42 (79.3)	3.05 (1.48-6.28)		7	19F	19F		Yes
	No	51 (7.9)	594 (92.1)	reference		8	19F	19F		Yes
<b>Child's symptom in last two weeks</b>										
Cough	Yes	37 (11.9)	274 (88.1)	1.96 (1.15-3.33)		9	23F	23F		Yes
	No	25 (6.5)	362 (93.5)	reference		10	6A	6A		Yes
Running nose	Yes	41 (12.2)	295 (87.8)	2.26 (1.30-3.91)		11	6A	6A		Yes
	No	21 (5.8)	341 (94.2)	reference		12	6A	6A		Yes
Eye symptom	Yes	10 (30.3)	23 (69.7)	5.13 (2.32-11.34)		13	6A	6A		Yes
	No	52 (7.8)	613 (92.2)	reference		14	6A	6A		Yes
Day-care attendance	Yes	31 (19.4)	129 (80.6)	3.93 (2.30-6.70)	2.24 (1.44-3.50)	15	6A	6A 19F		Yes
	No	31 (5.8)	507 (94.2)	reference	reference	16	A6A NT	6A NT 19F		Yes
<b>S. pneumoniae carriage (PCR and culture)</b>										
Child's nasopharynx	Positive	58 (27.4)	156 (72.6)	45.19 (16.15-126.51)	47.55 (23.30-97.06)	17	6B	6B		Yes
	Negative	4 (0.8)	480 (99.2)	reference	reference	18	6B	6B		Yes
Mother's nasopharynx	Positive	5 (27.8)	13 (72.2)	4.20 (1.45-12.21)	1.97 (0.50-7.72)	19	6B	6B 19F 23F		Yes
	Negative	57 (8.4)	623 (91.6)	reference	reference	20	6B	14		No
						21	6B 23F	6B		Yes
						22	NT	19F NT		Yes
						23	NT	19F NT		Yes
						24	NT	19F NT		Yes
						25	NT	6A NT	NT	Yes
						26	NT	6A NT		Yes
						27	NT	6A NT		Yes
						28	NT	6A NT		Yes
						29	NT	6A NT 19F	NT	Yes
						30	NT	6B NT		Yes
						31	NT	NT	NT	Yes
						32	NT	NT	NT	Yes
						33	NT	NT		Yes
						34	NT	NT		Yes
						35	NT	NT		Yes
						36	NT	NT		Yes
						37	NT	NT		Yes
						38	NT	NT		Yes
						39	NT	NT		Yes
						40	NT	NT		Yes
						41	NT	NT		Yes
						42	NT	NT		Yes
						43	NT	NT		Yes
						44	NT	NT		Yes
						45	NT	NT		Yes
						46	NT	NT 19F		Yes
						47	NT	NT 6A		Yes
						48	NT	.	19A	No
						49	NT	.		No
						50	NT	19F		No
						51	NT	19F		No
						52	NT	6B		No
						53	NT 14	14		Yes
						54	NT 23F	23F		Yes

Table (1) Effect of each characteristic on having *S. pneumoniae* in conjunctiva, estimated using logistic regression model.

Table (2) *S. pneumoniae* serotypes detected in the conjunctiva and the nasopharynx.

## References

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