Prevalence and serotype distribution of nasopharyngeal carriage of Streptococcus pneumoniae among healthy children under 5 years old in Hainan Province, China

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Abstract

Background The thirteen-valent pneumococcal conjugate vaccine (PCV13) is not included in the national immunization program and administered voluntarily with informed consent in China. In preparation for assessing the impact of pilot introduction in Hainan Province, we conducted a carriage study among children under 5 years old from four regions in Hainan Province.

Methods In 2022, nasopharyngeal (NP) swabs collected from healthy children were tested for pneumococcus using conventional culture. Pneumococcal isolates were serotyped using the Quellung reaction. Risk factors associated with pneumococcal colonization were assessed using univariate analysis and multivariable logistic regression adjusting for age, daycare attendance and other factors.

Result Pneumococcus was isolated in 710 (30.4%) of the 2333 children enrolled. Of 737 pneumococci, 29 serotypes were identified; 60.9% were PCV13 serotypes; the most common were 6B (20.4%), 19F (13.04%), 6A (11.9%) and 23F (6.1%); and the most common non-vaccine serotypes were 23A (12.9%), 34 (6.08%) and nontypeable (NT) pneumococci (5.6%). Children vaccinated with PCV13 had lower carriage (17.7% vs 32.5%; \( P=0.0001 \)) and fewer were PCV13-type (41.9% vs 62.7%; \( P=0.0017 \)) compared to unimmunized children. After adjustment, NP carriage was higher among children attending daycare and those with siblings; living in urban areas, mothers who had completed senior high/technical secondary school and completion of 3-4 doses of PCV13 were associated with a lower carriage rate.

Conclusions We established the baseline of pneumococcal carriage, serotype distribution and PCV13 immunization rates among healthy children under 5 years old in Hainan Province, prior to the introduction of PCV13 into national immunization. The high proportion of PCV13 serotype suggests that PCV13 introduction will likely have substantial impact on pneumococcal carriage in Hainan Province.

Background

Streptococcus pneumonia (Spn) is the leading cause of pneumonia, meningitis, and other serious infections in children, which was associated with the most deaths in children under than 5 years\[1, 2\]. Globally, the implementation of pneumococcal conjugate vaccines (PCVs) has significantly reduced invasive pneumococcal disease (IPD) and pneumonia caused by vaccine serotypes (VTs) pneumococci \[3\]. The World Health Organization recommended the inclusion of PCVs in childhood immunization programs worldwide\[4\].

Nasopharyngeal (NP) carriage of pneumococci is usually asymptomatic, and is a prerequisite for the occurrence of pneumococcal mucosal infection, invasive infection and transmission. Young children are more commonly colonized with \( \text{Spn} \) than older children or adults and are a major source of pneumococcal transmission to persons of all ages. Studies have shown that the implementation of PCVs into national immunization programs has significantly reduced NP carriage of vaccine serotypes \[5, 6\]. Consequently, young children who receive the PCVs indirectly protect vaccinated and unvaccinated individuals (including adults) by reducing the prevalence of carriage of vaccine serotypes \[7, 8\]. Thus, the
changes in pneumococcal carriage rates and serotypes distribution before and after pneumococcal vaccination are a key biological link to evaluate the vaccine efficacy.

China has not established a national or population-wide disease surveillance system for pneumococcal disease, which was unable to carry out surveillance of IPD as exemplified by the Centers for Disease Control in the USA and European countries [9–10]. Therefore, assessing the pneumococcal carriage among children provides a simple and practical approach to evaluate the potential disease burden and vaccine effect in China. Furthermore, PCV13 has been licenced for optional use since 2016 and has not been included in national immunization programs[11]. Moreover, there is a lack of baseline data on NP carriage nationwide and across all age groups. Therefore, it is essential to conduct carriage studies among the multi-age groups of the vulnerable population before the wide use of PCV13 around China, as this can provide crucial data for vaccine evaluation.

This study aimed to analyse the prevalence and serotype distribution of NP carriage among children under 5 years of age from 4 different regions of Hainan Province. These analyses are expected to provide valuable epidemiological baseline data of pneumococcal carriage in Hainan Province, before the introduction of PCVs into national immunization schedules

**Methods**

**Setting**

Hainan Province is located in the southernmost part of the Chinese mainland, where the annual average temperature is 22~27°C and the rainfall is abundant. By the end of 2020, there are 10.12 million residents, of which the non-registered population was less than 6%. The per capita GDP (55 131) and per capita disposable income of households (27 904) are lower than the national average[12, 13]. PCV13 has not been included in the immunization program in Hainan Province or in other provinces. Completing a four-dose series requires an out-of-pocket expenditure of 1,900–2,800 Chinese Yuan (CNY). In the less economically developed regions, the IPD burden is higher due to the natural environment and relatively weaker healthcare conditions[14], whereas PCV13 coverage is lower which may be related to high cost[15]. Unbalanced regional development and disparities in health care between different areas can also be found in Hainan Province [13].

**Study design**

This investigation was a cross-sectional study conducted from March to June 2022. Using data from other carriage studies conducted in China[16], we determined that enrolling 2250 children in 5 age groups (<12months, 12–23 months, 24–35 months, 36–47 months and 48-59months, 450 subjects per age group) should enable detection of 25% carriage of pneumococci. According to the latest census data[13], the ratio of urban to rural population in Hainan is approximately 6 to 4. Therefore, we planned to enroll 60% of the sample from urban areas and 40% from rural areas. A total of 4 regions (Haikou, Wanning,
Baisha and Qiongzhong) are sampled from 18 regions stratified by urban-rural population proportions and income levels (Supplementary Table 1).

In China, point of vaccinations (POV) carries out regular health management and establishes vaccination files for children aged 0–6 years in community health service centers. POV staff can use these files to recruit respondents. Children visiting POV are likely to be healthy. In this study, we recruited children in 33 POVs (13 from Haikou, 8 from Wanning, 6 from Qiongzhong and 6 from Baisha, respectively), accounting for 16.9% of the total number of local POVs.

**Study population**

Study participants were healthy children aged younger than 59 months who lived in the 4 different regions of Hainan Province. Children were excluded if they had upper or lower respiratory illness or a documented a febrile episode within the last 24 hours (axillary temperature of ≥ 37.3 ℃), if they had used antibiotics within the previous 10 days, or if they had congenital malformation or injury of the nasopharynx that would prevent the taking of an NP swab. The parents or guardians of the participants were interviewed about demographics, epidemiological factors, vaccination history related to pneumococcus, and risk factors that have been associated with pneumococcal carriage.

**NP sample collection**

We followed the previously published recipe and method for the preparation of STGG (skim milk-tryptone-glucose-glycerin) medium[17]. STGG vials were brought to room temperature and vortexed immediately at high speed for 10-20s before being used. An NP specimen was obtained at the clinics by otolaryngologists from each participant using flexible mini tip size nylon swab (Copan Diagnostics, Inc., Italy). The swab was inserted to the bottom of the STGG medium, and the handle of the swab was cut off using sterile scissors, leaving the tip immersed in 1 mL STGG medium, and the cap was secured. The vial was vortexed for 10–20 s to disperse the organisms from the swab. These NP swabs within STGG medium were placed on dry ice within 30 minutes and transferred to the local microbiology laboratory within 6 hours and stored in a -80℃ refrigerator. After collection, all NP-STGG specimens were transported to the Beijing Centers for Disease Prevention and Control (CDC) on dry ice, and kept at -80℃ until they were processed.

**Laboratory methods**

NP-STGG specimens were thawed at 37℃ and vortexed for approximately 10–20 s. For each NP specimen, 400 µL of STGG media was transferred to 6.0 mL enrichment broth (5 ml of Todd Hewitt broth containing 0.5% yeast extract to which 1 ml rabbit serum was added) and the broth was incubated in 5% carbon dioxide at 37℃ for 6 hours. Enriched culture (10 µL) was plated on 5% sheep blood agar with 10 µg polymyxin B and nalidixic acid, and incubated in 5% carbon dioxide at 37℃ for 18–20 hours. Two or more (for each different morphology type) typical pneumococcal colonies were selected and subcultured on 5% sheep blood agar. Pneumococci were identified by optochin susceptibility and bile solubility tests.
Pneumococcal isolates were serotyped by performing the Quellung reaction with antisera from the Staten Serum Institute (Copenhagen, Denmark).

**Statistical methods**

ANOVA was used to calculate and compare mean age among different regions. The Chi-square test was used for inter-group comparisons of categorical variables such as NP carriage and gender proportions. To evaluate potential risk factors for NP carriage, first univariate analysis using Chi square was performed to identify potential factors associated with carriage. Variables that were significant (P < 0.05) in the univariate analysis were further tested by multiple logistic regression using the forward procedure to assess contribution to carriage. P < 0.05 was considered statistically significant. Analyses were performed using SPSS 19.0 and WPS Office.

**Result**

**Population and pneumococcal colonization**

A total of 2333 children were enrolled from 4 different regions in the study: 764 from Haikou, 645 from Wanning, 450 from Baisha and 474 from Qiongzhong (Table 1). Of those, 443 ~ 508 children were in each age group. Pneumococcus was isolated from 710 (30.4%) children: 213 from Haikou, 221 from Wanning, 145 from Baisha and 131 from Qiongzhong, and the carriage rates were 27.9%, 34.3%, 32.2% and 27.6%, respectively.

**Serotype Distribution and Vaccine Coverage**

Twenty-seven children had 2 serotypes isolated from a single specimen, for a total of 737 pneumococcal isolates. Among those pneumococcal isolates, 29 serotypes were identified and the coverage rate of PCV13 was 60.9% (449/737) (Fig. 1). The most common vaccine serotypes were 6B (n = 150, [20.4%]), 19F (n = 99, [13.04%]), 6A (n = 88, [11.9%]) and 23F (n = 45, [6.1%]). Four vaccine serotypes (1,4, 5, 7F and 8) were not isolated from any children, and vaccine serotypes 9V was only isolated from 1 child. The most common non-vaccine serotypes were 23A (n = 95 [12.9%]), 34 (n = 44 [6.08%]) and nontypeable (NT) pneumococci (n = 41 [5.6%]).

**PCV13 Usage and pneumococcal colonization**

For all the participants, 327 (14.0%) children (PCV13-group) completed 1–4 doses of PCV13 and 2006 (86.0%) children (non-PCV13 group) did not received PCV13 or received 1 dose of 23-valent pneumococcal polysaccharide vaccine (PPV23). There was a statistically significant difference in carriage between the two groups for both carriage rate and vaccine coverage. We compared the prevalence of serotype colonization between the two groups. Pneumococcus was isolated in 58 children (17.7%) and 652 children (32.5%) in the PCV13 and non-PCV13 groups, respectively (17.7% vs. 32.5%, P = 0.0001). A total of 61 and 676 pneumococcal isolates were identified, and the coverage rates of PCV13 were 41.9% (26/62) and 62.7% (423/675), respectively, in the PCV13 and non-PCV13 groups (41.9% vs.
62.7%, \( P = 0.0017 \). The serotype distribution was similar in the two groups. The most common vaccine serotypes were 6B, 19F and 6A, and the most common non-vaccine serotypes were 23A, 34 and NT pneumococcus.

As a comparison, we also looked at carriage patterns in the 4 different regions. The proportions of PCV-13 vaccinated children were 30.2% (231/767), 6.5% (42/645), 6.4% (29/450), and 4.6% (22/474) in Haikou, Wanning, Baisha and Qiongzhong, respectively. Among all pneumococcal isolate serotypes, the coverage rates of PCV13 were different and the proportions were 48.9%, 75.8%, 60.7% and 56.6%, respectively. Haikou, with the highest vaccination rate, had significantly lower PCV13 serotype coverage than Wanning and Qiongzhong (Fig. 2). The serotype distribution patterns were also similar in the 4 regions (Supplementary Table 2). It is worth noting that 23A was the most common serotype among all isolates identified in Haikou.

**Risk factors associated with pneumococcal colonization**

Seven factors including age, daycare attendance, presence of siblings, residential area, educational degree of parent, per capita monthly disposable income and PCV13 vaccination history were associated with NP carriage (\( P < 0.05 \) for all) (Table 2). Factors that remained associated with NP carriage after forward elimination included the following: age, daycare attendance, presence of siblings, residential area, educational degree of parent, per capita monthly disposable income and PCV13 vaccination history (Table 3). After adjustment for these factors, daycare attendance, presence of siblings, residential area and mothers who completed senior high/technical secondary school were statistically significant. For the PCV13 vaccination history, only completion of 3-4 doses PCV13 was associated with a lower likelihood of NP carriage.

**Discussion**

To our knowledge, this cross-sectional study is the largest NP carriage investigation in healthy children in Hainan Province. We present the results of a population-based survey on NP carriage prior to adopting PCVs in China’s national immunization schedules. The baseline data will enable the estimation of the vaccine impact of PCV13 implementation. The overall NP carriage rate was 30.4% in our study. Previous surveillance studies of NP carriage, prior to the introduction of PCV13, have revealed a higher rate of NP carriage in children under 5 years of age in some Asian countries. In Thailand, Indonesia and India, the overall carriage rates were 35.9%, 49.5% and 54.5%, respectively[18–20]. A meta-analysis performed from studies conducted in southeast Asia showed that the pooled prevalence of NP carriage in healthy children under 5 years of age was 36.0% (95% CI: 34.2%–37.8%)[21]. The overall NP carriage in our study was higher than that reported from other regions in China. Earlier studies of NP carriage in Beijing, Shanghai and Chongqing indicated that the overall carriage rates were 22%, 16.6% and 16.6%, respectively[22–24]. In 2017, prior to the introduction of PCV13 in China, a meta-analysis of data from young children found that the pooled prevalence of NP carriage was 21.4% (95% CI: 18.3–24.4%)[24]. Several factors could account for the discrepancy observed in our study, including geography, socioeconomic status, sample
collection and differences in laboratory procedures used for \textit{Spn} identification. For example, higher NP carriage rates were observed in less-developed countries\cite{25,26}. In the sample collection and processing procedures, we ensured that the NP swabs were placed within STGG medium on dry ice as quickly as possible (within 30 minutes). This allowed for storage and transport of NP specimens at temperatures below \(-70^\circ\text{C}\), which proved to be optimal conditions without loss of colony-forming units (CFU)\cite{17}. In addition, molecular biology methods were used for detection, thereby enhancing sensitivity and detection rates compared to culture-based procedures alone. In our laboratory procedures, we extracted DNA from enriched cultures and detected the targeting \textit{lytA} gene using real-time PCR method, and this result was very useful for the detection of pneumococci in culture procedures, especially for the samples with low organism concentrations.

The top 5 serotypes in our study (6B, 19F, 23A, 6A, and 23F) accounted for 64.7\% of all carriage strains. In line with other carriage studies and systematic reviews conducted in China\cite{22,23,27,28}, vaccine serotypes 6B, 19F, 6A and 23F were highly prevalent; other vaccine serotypes 1, 4, 5, 7F, 8 and 9 were relatively rare in our study. Notably, the serotype distribution was relatively consistent with the serotypes frequently associated with IPD in China\cite{29}. Of significant concern was the high prevalence of certain non-vaccine serotypes, which could potentially reduce the benefits of vaccination. The top 3 non-vaccine serotypes (23A, 34 and NT) accounted for 24.4\% of all carriage strains. Serotype 23A was prevalent in our study, particularly in Haikou, where it was the dominant serotype. In contrast, it was rarely observed in other carriage studies conducted in different regions of China and other countries. Considering the relatively high PCV13 immunization rate in Haikou, the potential risk of serotype replacement by non-vaccine serotypes requires vigilance. Throughout Asia and Australia\cite{18,19,30,31}, NT pneumococci were the most commonly isolated organisms, with some NT pneumococci being co-colonizing isolates. Our findings also revealed that some NT pneumococci co-colonized with other serotypes. Although NT pneumococci are infrequent causes of invasive disease in young children, they are associated with a variety of mucosal diseases and may serve as an essential reservoir for antimicrobial resistance genes\cite{32}.

The NP carriage rate and serotype distribution vary by geography and are altered by the implementation of PCVs and socioeconomic status. We recruited healthy children from 4 different regions in Hainan Province. Haikou, the provincial capital with a higher economic level than other regions, had the majority of children vaccinated with PCV13. Consequently, the lowest PCV13 serotype coverage rates were also observed in Haikou. Although the overall carriage rates remained stable or relatively moderately declined after the introduction of PCVs in several studies\cite{33–35}, lower carriage rates were found in children vaccinated with PCV13 in our study. This could be attributed to higher economic levels and better living conditions, which reduce the likelihood of pneumococcal carriage.

Several expected epidemiologic factors showed associations with pneumococcal carriage. Consistent with previous findings\cite{25,34,36}, factors such as age, daycare attendance, presence of siblings, residing in a rural area and having a lower socioeconomic status were significantly associated with higher rates of pneumococcal colonization. Previous studies have reported that breastfeeding was associated with lower
rates of pneumococcal colonization[37,38]. Considering that the majority of Chinese children are weaned between 1 and 2 years old, and that breastfeeding has minimal impact on pneumococcal colonization in children over 2 years old, only children under 2 years old were included in the bivariate analysis. However, in our study, breastfeeding showed no relationship to colonization. Antibiotic therapy, previously associated with reduced odds of pneumococcal colonization[34], showed no such association in our study. After adjustment for multiple factors, only 5 factors remained statistically significant. Consistent with several previous studies[34,36], having siblings and daycare attendance were identified as risk factors for carriage. This is likely due to the transmission of pneumococci between children within the same family and kindergarten through close contact. Studies conducted in the UK suggested that reduced-dose schedules have been shown to be immunogenic and have little impact on IPD or pneumococcal community-acquired pneumonia (CAP) cases[39,40]. However, 2 primary doses of the PCVs received in the first year of life have a weak effect on colonization. In line with this study, we found that completion of 3-4 doses of PCV13 was associated with a lower likelihood of NP carriage[41]. Several factors could explain this observation. First, echoing previous findings, high IgG concentrations are required to reduce and prevent NP carriage[42]. After the infant series (3 doses of PCV13) and the toddler dose (4 doses of PCV13), children obtained high IgG concentrations, contributing to the clearance of NP-carriage and prevention of new colonization. In addition, since PCV13 was not widely used in China, non-vaccine serotypes replacement was less obvious than that in other countries with high PCV13 vaccination coverage[43].

Our study has several limitations. First, our investigation was a single-center study confined to Hainan Province; thus, the findings might not represent the overall NP carriage trends in China. Conducting a multicenter investigation of NP carriage and serotype distribution is essential to provide important epidemiological baseline data for assessing the impact of PCVs on NP carriage, especially prior to the introduction of PCV13 into national immunization. Second, carriage patterns also varied with seasonality. Our investigation took place over a 1-month period in each region, and some vaccine serotypes were not found or were uncommon in our study; these serotypes are common causes of IPD. Therefore, the NP carriage patterns may not accurately predict the distribution of specific serotypes causing severe disease in the local area.

**Conclusions**

In conclusion, this study provides the baseline of pneumococcal carriage, serotype distribution and PCV13 immunization rates among healthy children under 5 years of age in Hainan Province, prior to the introduction of PCV13 into national immunization. The high proportion of PCV13 serotype suggests that PCV13 introduction will likely have a substantial impact on pneumococcal carriage in Hainan Province. Due to the diversity of pneumococcal serotype distribution, it is essential to perform future longitudinal and multicenter surveillance of NP carriage to monitor the effectiveness of current PCVs.
Abbreviations

PCV13, 13-valent pneumococcal conjugate vaccine
NP, Nasopharyngeal
NT, Nontypeable

*S. pneumoniae*

IPD, Invasive pneumococcal disease
VTs, Vaccine serotypes
NVTs, Non-vaccine serotype

CNY, Chinese Yuan
POV, Point of vaccinations
STGG, Skim milk-tryptone-glucose-glycerin
IQR, Interquartile range
OR, odds ratio
aOR, Adjusted odds ratio;
CI, Confidence interval
CFU, Colony-forming units
CAP, Community-acquired pneumonia

Declarations

Authors’ contributions

Conceptualization and design: JW, ML QZ and JW; supervision, coordinating and sample collection: LQ, BH, RQ, ZH, JXL, TBW, XS; sample testing: JW, SB, WZ, JNZ and ZSS; Data analysis: JW, AZ and JL; draft manuscript preparation: all authors; final manuscript preparation: JW and ML

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Ethics approval and consent to participate

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no conflicts of interest.

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5. Davis SM, Deloria-Knoll M, Kassa HT, O'Brien KL. Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: Review of evidence on


Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

Figure 1
Serotype distribution of pneumococci isolated from Hainan Province children under 5 years of age (N = 737). Abbreviations: NT, nontypeable serotype; PCV13, 13-valent pneumococcal conjugate vaccine

Figure 2

The coverage rate of PCV13 vaccine and non-vaccine serotypes in 4 regions.

Abbreviations: VTs, vaccine serotypes; NVTs, non-vaccine serotypes; PCV13, 13-valent pneumococcal conjugate vaccine.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xls
- Table2.xlsx
• Table3.xls
• Supplementary.doc