## Letter to the Editor

# A Cross-sectional Survey Assessing Carriage of *Streptococcus pneumoniae* in a Healthy Population in Xinjiang Uygur Autonomous Region of China*

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**The carriage rate and serotype distribution of *Streptococcus pneumoniae* (*S. pneumoniae*) in a healthy population in China remains unclear. In this study, we collected the oropharyngeal swabs from 513 individuals in Xinjiang, China. Real-time PCR targeting the *lytA* gene and 12 serotypes were assessed to identify *S. pneumoniae* carriage. The total carriage rate of *S. pneumoniae* was 70.4% (361/513). The most prevalent serotypes were 19B/F, 18B/C, 5, and 6A/B. The highest carriage rate of *S. pneumoniae* was noted in children aged 6-10 years (88.6%), which merits further attention. The co-colonization rate of two or more *S. pneumoniae* serotypes was 79.8% (264/331). This study aimed to investigate the baseline pneumococcal carriage rate among healthy individuals in China to improve our understanding of the epidemiology of *S. pneumoniae*.**

Key words: Cross-sectional survey; *Streptococcus pneumoniae* carriage; Real-time PCR; Xinjiang; China

*Streptococcus Pneumoniae* (*S. pneumoniae*) infections can result in serious invasive pneumococcal disease (IPD)[1]. IPD usually results in a high case fatality rate of 5%-50% worldwide. In China, about 30,000 children die of pneumococcal disease each year. Patients with pneumococcal disease and healthy carriers are all sources of infection.

Studying carriage and serotype distribution of *S. pneumoniae* will improve our understanding of the epidemiology of *S. pneumoniae* as well as support the introduction and measurement of the impact of widespread pneumococcal vaccination. Cross-sectional surveys are usually performed to investigate pneumococcal carriage by culturing and agglutination reactions. Real-time PCR methodology could be an optimal choice, and the pneumococcal *lytA* gene was considered a good target gene for *S. pneumoniae* species testing by real-time PCR.

The use of pneumococcal vaccines (PVC) has resulted in profound declines in IPD, pneumonia, and otitis media[2,3]. In China, PCVs have not been adopted into the national immunization schedules; therefore, data on pneumococcal carriage are limited. Population-based studies are warranted. Understanding of pre-vaccine carriage rates of *S. pneumoniae* will provide a baseline estimate, which permits evaluation of the effect of upcoming PCV mass immunization and facilitates monitoring of serotype replacement in Xinjiang, China.

*Ethics Statement Exemption* The study consent and questionnaire forms were all submitted and approved by the Xinjiang Autonomous Region center for disease control and prevention ethical review committee. Each participant was informed in writing before they were sampled and provided a signed consent form. For children, the consent form was signed by their guardian.

*Study Sites and Population* Aksu City and Yining City were chosen as the sentinel sites for this study. Aksu City is located in the south of Xinjiang and adjoins with the desert of Taklamakan. Yining City is located in the northwest of Xinjiang and on the northern side of the Ili River in the Dzungarian Basin.

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The ethnic composition, quality of healthcare, and living conditions are different in the two cities. Healthy individuals and those without acute respiratory infections before the enrollment date were included in the study. The study population were recruited from kindergartens, vaccination clinics, and schools. Based on a 5% maximum permissible error, we estimated a 50% carriage rate[4] at a 5% significance level.

*Laboratory Testing* Each participant was swabbed at the oropharynx using fiber swabs (Classiq Swabs, COPAN Italy S.p.A.). Initially, real-time PCR targeting *lytA* gene was performed to identify the *S. pneumoniae* species. Twelve serotypes (4, 5, 6A/B, 7A/F, 9V/A, 10A/B, 14, 15, 18B/C, 19A, 19B/F, and 23F) were detected in the *lytA*-positive samples (CT value was < 38). Primers and probes for real-time PCR are listed in Supplementary Table S1[5] (available in www.besjournal.com). A negative control (ultrapure water) and a positive control (DNA extracted from cultures positive for *S. pneumoniae* serotype) were included in every run. PCR reaction conditions were as follows: 1 cycle of 95 °C for 30 s, 50 cycles of 95 °C for 5 s, and 60 °C for 30 s. The result was considered positive when the CT value was < 38.

*Statistical Analysis* Data were analyzed using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). The values (%) for categorical variables such as region, age, ethnicity, gender, and other pneumococcal carriage rates, and constituent ratios were assessed using chi-square test to test for statistical significance of compared variables. *P*-values < 0.05 were considered statistically significant. The significance level was adjusted for multiple testing of age groups using the Bonferroni correction; therefore, the *P*-value for statistical significance was $P = 0.003$ (0.05/15).

*Carriage Rate of S. pneumonia* A total of 513 healthy individuals (female, 294, 57.3%; male, 219, 42.7%) were enrolled in this study, including 250 from Aksu City and 263 from Yining City. Participants' age ranged from 2 months to 53 years. All participants were not vaccinated with pneumococcal vaccine.

The total carriage rate of *S. pneumoniae* was 70.4% (361/513). Results of the chi-square test showed that there were statistically significant differences between region, ethnicity, and occupation ($P < 0.001$). The carriage rate of *S. pneumoniae* in Aksu City (81.2%, 203/250) was higher than that in Yining (60.1%, 158/263) ($P < 0.001$). The carriage rate of *S. pneumoniae* was highest among kindergarten children (80.4%) and lowest among medical workers (33.3%). Significant differences were observed among scattered children (66.3%), kindergarten children (80.4%), and students (75.8%) when compared with medical workers (33.3%) ($P < 0.001$, Table 1).

*Serotype Distributions of S. pneumoniae* In 361 *lytA*-positive individuals, 331 specimens were serotyped using real-time PCR, which targeted 12 serotype-specific primers and probes. The other 30 specimens were all negative for the 12 serotypes. Serotypes 19B/F, 18B/C, 5, and 6A/B were the major serotypes of *S. pneumoniae*, accounting for 51.3%, 30.4%, 24.6%, and 15.0%, respectively (Figure 1).

The carriage rates of serotypes 19B/F, 5, 6A/B, 19A, 18B/C, 4, and 7A/F were significantly different between the two cities. The carriage rates of 19B/F, 5, 6A/B, and 19A were higher in Aksu than in Yining; carriage rates of serotypes 18B/C, 4, and 7A/F were higher in Yining than in Aksu. However, there was no statistically significant difference between serotypes 14, 23F, 15, 9V/A, and 10A/B.

The carriage rate for each age group was higher in Aksu City than that observed in Yining City. In addition, it was significantly different between age groups ($P < 0.001$), increasing from 67.1% (57/85) in a group of individuals aged < 3 years, peaking at 88.6% (70/79) in those aged 6-10 years, and progressively decreasing to 51.1% (48/94) in those aged ≥ 21 years. In 12 serotypes, most of the high carriage rates were evident among individuals aged 3-5 years, 6-10 years, and 11-15 years.

*Co-colonization with Multiple Serotypes* A total of 67 (20.24%) samples had only one serotype in the 331 samples that could be serotyped. Co-colonization with two to six serotypes was identified in 264 specimens (79.8%). The proportion of samples with two to six serotypes decreased successively, where 20.5% ($n = 105$) had two co-colonizations, 18.3% ($n = 94$) had three co-colonizations, 9.4% ($n = 48$) had four co-colonizations, 2.9% ($n = 15$) had five co-colonizations, and 0.4% had six co-colonizations ($n = 2$).

A total of 107 multiple serotype combinations were identified. Two and three multiple serotypes were common ($n = 199$). Major serotypes in co-carried specimens were 19B/F, 18B/C, 5, and 6A/B, accounting for 71.0%, 46.7%, 40.2%, and 28.0%, respectively. The most common combinations for two serotypes were 19B/F + 18B/C (9.7%, $n = 32$), 19B/F + 5 (3.9%, $n = 13$), and 19B/F + 6A/B (3.6%, $n = 12$). The major