

Preplanned Studies

Impact of PCV13 Vaccination on Pharyngeal Detection of *Streptococcus pneumoniae* Among Children — Suqian City, Jiangsu Province, China, February 2023–February 2025

Lisha Ma^{1,&}; Mingwei Wei^{2,3,&}; Tao Wu³; Ran Tao³; Jinbo Gou⁴; Yue Liu¹; Manrong Zhang¹;
Mengjuan Yuan²; Lunbiao Cui^{3,#}; Jingxin Li^{1,3,#}

Summary

What is already known on this topic?

Pneumococcal conjugate vaccines (PCVs), including the 13-valent PCV (PCV13), effectively reduce the nasopharyngeal carriage of vaccine-type *Streptococcus pneumoniae* (*S. pneumoniae*) and prevent invasive pneumococcal disease in children. However, an increasing prevalence of non-vaccine serotypes and serotype replacement has been documented globally, with notable variations in the predominant replacement serotypes across different geographic settings.

What is added by this report?

This study demonstrated that PCV13 vaccination significantly reduced the pharyngeal detection of *S. pneumoniae* among children with acute respiratory infections, with three to four doses decreasing the detection rate by 30.2%. This reduction encompassed both vaccine and non-vaccine serotypes. Additionally, non-PCV13 serotypes, particularly 10A and 15A/15F, were predominant in positive samples, underscoring the current dominance of non-vaccine serotypes in the study population.

What are the implications for public health practice?

The PCV13 program successfully reduced pneumococcal detection rates, particularly among children who complete the 3–4 dose series. Continued surveillance of circulating *S. pneumoniae* serotypes is essential to monitor serotype replacement patterns and develop future vaccination strategies.

Methods: This study conducted a 2-year prospective cohort study (February 2023–February 2025) in Suqian, Jiangsu Province, enrolling 2-month-old infants and monitoring for acute respiratory infections (ARIs). Pharyngeal swabs collected during ARIs were analyzed using targeted next-generation sequencing (tNGS) to identify respiratory pathogens, and *Streptococcus pneumoniae* (*S. pneumoniae*)-positive samples were serotyped using multiplex PCR. Risk ratios (*RRs*) for *S. pneumoniae* detection were estimated using Poisson regression, with sensitivity analysis performed using inverse probability of treatment weighting (IPTW).

Results: Among 579 children, 1,527 swabs were collected, yielding an overall *S. pneumoniae* detection incidence of 35.2%. Vaccinated children receiving 3–4 PCV13 doses demonstrated significantly lower detection rates than unvaccinated children [23.1% *vs.* 40.2%; adjusted *RR*=0.70; 95% confidence interval (*CI*): 0.50, 0.98; *P*=0.036]. IPTW analysis confirmed these findings. Non-vaccine serotypes predominated, particularly the 10A and 15A/15F strains.

Conclusion: PCV13 vaccination was associated with reduced *S. pneumoniae* detection among children with ARIs, despite dominance of non-vaccine serotypes. Our findings emphasize the importance of ongoing surveillance for *S. pneumoniae* and highlight the need to expand the serotype coverage of pneumococcal vaccines.

ABSTRACT

Introduction: The 13-valent pneumococcal conjugate vaccine (PCV13) has reduced vaccine-type carriage rates, though regarding serotype replacement remain.

Streptococcus pneumoniae (*S. pneumoniae*) represents a major bacterial pathogen responsible for significant infections and mortality among children under five years of age. Nasopharyngeal colonization serves not only as the primary reservoir for horizontal transmission within communities but also as an essential prerequisite for invasive disease development

(1). Young children serve as the principal reservoir, with point prevalence estimates of nasopharyngeal colonization ranging from 27% to 85% (2). The introduction of pneumococcal conjugate vaccines (PCVs) has substantially reduced the incidence of vaccine-type carriage and pneumococcal disease among young children. However, the selective pressure exerted by vaccination has fundamentally altered the microbial landscape, resulting in an increased prevalence of non-vaccine serotypes, with a growing proportion of infections now attributed to replacement serotypes, such as 8, 10A, 12F, 15A, and 24F (3). In recent years, global interest has intensified regarding the protective effects of PCVs and the associated changes in pneumococcal serotype distribution across diverse populations. Therefore, based on a prospective acute respiratory infection (ARI) surveillance cohort in eastern China, the present study aimed to evaluate the impact of PCV13 vaccination on the risk of *S. pneumoniae* detection and characterize the current distribution of predominant pneumococcal serotypes among young children.

This study was based on a prospective cohort of healthy infants established in February 2023 in Suqian, Jiangsu Province, Eastern China, as previously reported (4). Briefly, infants aged 2 months at recruitment were enrolled and actively monitored for ARI episodes over 24 months. Demographic data and baseline pharyngeal swabs were collected at enrollment. During the surveillance, the researchers contacted caregivers biweekly via telephone to monitor their health status. When a child exhibited ARI symptoms such as cough, nasal congestion, tachypnea, or throat redness and swelling, researchers conducted home visits within 48 hours to collect pharyngeal swabs. Trained healthcare personnel collected pharyngeal swabs. With the child's head tilted slightly backward, a disposable flocked swab was inserted into the posterior pharyngeal wall and tonsillar areas, rotated 3–5 times, and immediately placed in a tube containing the viral transport medium. All specimens were transported on dry ice and stored at –80 °C prior to laboratory testing.

Pharyngeal swab specimens were analyzed using the Respiratory Pathogen Detection Kit (KS608-100HXD96, KingCreate, Guangzhou, China) for targeted next-generation sequencing (tNGS), which can identify 107 respiratory pathogens, including *S. pneumoniae*, *Mycoplasma pneumoniae*, and influenza A and B viruses. Detailed tNGS procedures are provided in the [Supplementary Material](https://weekly.chinacdc.cn/) (available at <https://weekly.chinacdc.cn/>). Serotyping was

performed using multiplex PCR for specimens that tested positive for *S. pneumoniae*. DNA extraction and analysis of the amplified products from earlier surveys have been described previously (4). PCV13 vaccination status during the follow-up period was obtained from the National Immunization Information System.

All statistical analyses were conducted using the R Software (version 4.4.1; The R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics, including medians, interquartile ranges (IQRs), and percentages, were calculated to summarize the baseline characteristics of the study population. Categorical variables were compared using the Chi-square test, and continuous variables were compared using the Mann-Whitney U test. We compared the overall incidence of *S. pneumoniae* detection in PCV13-vaccinated and unvaccinated children. Risk ratios (RRs) were estimated using Poisson regression with robust standard errors by PCV13 doses, and adjusted RRs were obtained after controlling for sex, disease history, mode of delivery, maternal age at delivery, maternal education level, maternal monthly income, number of household members, presence of children under 18 years of age in the household, monthly household income, and baseline *S. pneumoniae* carriage. We employed inverse probability of treatment weighting (IPTW) using propensity scores to reconstruct comparable cohorts of PCV13-vaccinated and unvaccinated individuals for sensitivity analysis to examine the impact of PCV13 on the detection of *S. pneumoniae*. The reduction in pneumococcal occurrence attributable to vaccination was calculated as $(1 - \text{adjusted } RR) \times 100\%$. For samples with failed serotyping, multiple imputations were applied using chained equations. Missing serotype values were imputed using classification and regression tree methods to generate 20 imputed datasets. The results from all the imputed datasets were subsequently combined using Rubin's rules. A two-sided *P* value of <0.05 was considered statistically significant.

Of the 796 children enrolled in the longitudinal ARI surveillance cohort study, 579 (72.7%) provided at least one valid pharyngeal swab sample during an ARI episode and were included in the present analysis. The baseline demographic characteristics of the 579 participants did not differ significantly from those of the overall cohort ([Supplementary Table S1](https://weekly.chinacdc.cn/), available at <https://weekly.chinacdc.cn/>). Among the included children, 310 (53.5%) were boys, 21 (3.6%) carried *S. pneumoniae* at enrollment, and 54 (9.3%) received one dose of PCV13 before enrollment ([Table 1](#)).

TABLE 1. Baseline demographic characteristics of 579 participants analyzed in this study.

Characteristics	Participants included in the analysis
Infant sex, <i>n</i> (%)	
Female	269 (46.5)
Male	310 (53.5)
Infant age, median (IQR), days	71.0 (66.0, 78.0)
Infant height, median (IQR), cm	60.0 (59.0, 62.0)
Infant weight, median (IQR), kg	6.3 (5.8, 6.8)
Infant birth length, median (IQR), cm	50.0 (50.0, 50.0)
Infant birth weight, median (IQR), kg	3.4 (3.2, 3.7)
Infant disease history*, <i>n</i> (%)	
Yes	18 (3.1)
No	561 (96.9)
Maternal mode of delivery, <i>n</i> (%)	
Full-term vaginal delivery	291 (50.3)
Cesarean delivery	288 (49.7)
Maternal feeding pattern, <i>n</i> (%)	
Breastfeeding	523 (90.3)
Not breastfeeding after birth	47 (8.1)
Mixed feeding	9 (1.6)
Maternal age, <i>n</i> (%), years	
<35	503 (86.9)
≥35	76 (13.1)
Maternal education, <i>n</i> (%)	
Middle school or below	162 (28.0)
High school or vocational school	100 (17.3)
Junior college	152 (26.3)
Bachelor's degree or above	165 (28.5)
Maternal monthly income, <i>n</i> (%), CNY	
<1,000	245 (42.3)
1,000–6,999	270 (46.6)
≥7,000	64 (11.1)
No. of household members, <i>n</i> (%)	
≤4	421 (72.7)
>4	158 (27.3)
With household members <18 years of age, <i>n</i> (%)	
Yes	329 (56.8)
No	250 (43.2)
Monthly household income, <i>n</i> (%), CNY	
<10,000	175 (30.2)
≥10,000	404 (69.8)
Baseline <i>S. pneumoniae</i> carriage	
Yes	21 (3.6)
No	553 (95.5)
Missing	5 (0.9)
Received first dose of PCV13 before enrollment, <i>n</i> (%)	
Yes	54 (9.3)
No	525 (90.7)

Abbreviation: PCV13=13-valent pneumococcal conjugate vaccine; CNY=Chinese Yuan; COVID-19=coronavirus disease 2019.

* Infant disease history includes pneumonia, jaundice, COVID-19, and lactose intolerance.

During the study period, 2,996 ARI episodes were recorded, yielding an incidence rate of 196.6 episodes per 100 person-years (95% *CI*: 189.5, 203.6). A total of 1,527 pharyngeal swab samples were collected from 579 participants. Among them, 204 tested positive for *S. pneumoniae* at least once, with an overall detection rate of 35.2% (95% *CI*: 31.3%, 39.1%). Overall, 181 (31.3%) children received 1–4 doses of PCV13, whereas 398 (68.7%) remained unvaccinated. Notably, PCV13 vaccination was not associated with a reduction in the overall ARI incidence (Table 2).

For each of the 204 children with *S. pneumoniae*, one positive sample was randomly selected for serotyping analysis. Of these, 137 (67.2%) were successfully serotyped. Among the 137 successfully serotyped samples, 105 (76.6%) contained a single serotype, 26 (19.0%) contained two serotypes, and 6 (4.4%) contained three serotypes. The most frequently detected non-vaccine serotypes were 10A (30.3%) and 15A/15F (22.3%), with 23F (3.4%) being the most common (Figure 1). For samples for which serotyping failed, we additionally applied multiple imputations that showed a consistent serotype distribution. The detection rates of most non-vaccine serotypes were higher in unvaccinated children than in vaccinated children. Statistically significant differences were observed for serotypes 10A ($P=0.041$) and 15A/15F ($P=0.003$) based on the Pearson's Chi-square test, whereas no significant differences were detected for the other serotypes (Figure 2).

Among children in the overall pneumococcal group, those who received 1–2 doses of PCV13 demonstrated an incidence of *S. pneumoniae* detection of 28.9% (95% *CI*: 15.4%, 45.9%), compared to 40.2% (95% *CI*: 35.3%, 45.2%) in unvaccinated children, with an adjusted *RR* of 0.80 (95% *CI*: 0.49, 1.32; $P=0.392$) (Table 3). In contrast, children who received 3–4 doses exhibited a 30.2% reduction in *S. pneumoniae* detection (adjusted *RR*=0.70; 95% *CI*: 0.50, 0.98; $P=0.036$). A significant 39.0% reduction in non-vaccine-type *S. pneumoniae* detection was also observed in this group (adjusted *RR*=0.61; 95% *CI*: 0.38, 0.97; $P=0.037$) (Table 3). Owing to the limited sample size,

no significant effect of PCV13 was observed on vaccine-type *S. pneumoniae* detection, although point estimates suggested some differences. Sensitivity analyses using IPTW yielded consistent results, demonstrating a 61.9% reduction in non-vaccine-type *S. pneumoniae* detection (*RR*=0.38; 95% *CI*: 0.23, 0.64; $P<0.001$) (Table 3).

DISCUSSION

This study evaluated the impact of PCV13 vaccination on the detection of *S. pneumoniae* among children with ARI through a comprehensive 24-month active surveillance program. During the follow-up period, the overall incidence of *S. pneumoniae* detection was 35.2%. Children who received 3–4 doses of PCV13 demonstrated a significantly reduced risk of *S. pneumoniae* detection compared with their unvaccinated counterparts, with particularly pronounced reductions observed for non-vaccine serotypes 10A and 15A/15F.

The detection rate of *S. pneumoniae* observed in our cohort was 35.2%, exceeding that reported in previous studies of healthy children under 5 years of age (30.4%) (5) and the pooled prevalence estimate from a recent meta-analysis of Chinese children (21.4%) (6). This elevated detection rate can be attributed to our prospective cohort design, which enabled the comprehensive identification of ARI episodes and the implementation of tNGS, a highly sensitive molecular technique that achieves superior detection rates compared with traditional culture-based methods.

Our findings demonstrated that PCV13 vaccination significantly reduced the risk of pneumococcal detection during childhood, particularly among children who completed the 3–4 dose immunization series, reflecting the direct protective effect of the vaccine on vaccinated individuals. However, no significant effect was observed on the overall incidence of ARI. Unlike previous studies that primarily focused on vaccine serotypes, our results suggest that PCV13 vaccination may also be associated with a reduced detection of non-vaccine serotypes. Following the

TABLE 2. Incidence of acute respiratory infection episodes per 100 person-years by PCV13 vaccination status.

PCV13 vaccination status	Person years	No. of events	Rate (95% <i>CI</i>)	Incidence rate ratio (95% <i>CI</i>)	<i>P</i>
Unvaccinated	819	1,676	204.6 (194.8, 214.4)	Reference	–
1–2 doses	78	150	191.9 (161.2, 222.6)	0.94 (0.79, 1.10)	0.448
3–4 doses	294	591	201.0 (184.8, 217.2)	0.98 (0.89, 1.08)	0.695

Note: The incidence rate ratio and 95% *CI* were estimated using Poisson regression models with log (person-years) as the offset. Abbreviation: PCV13=13-valent pneumococcal conjugate vaccine; *CI*=confidence interval.

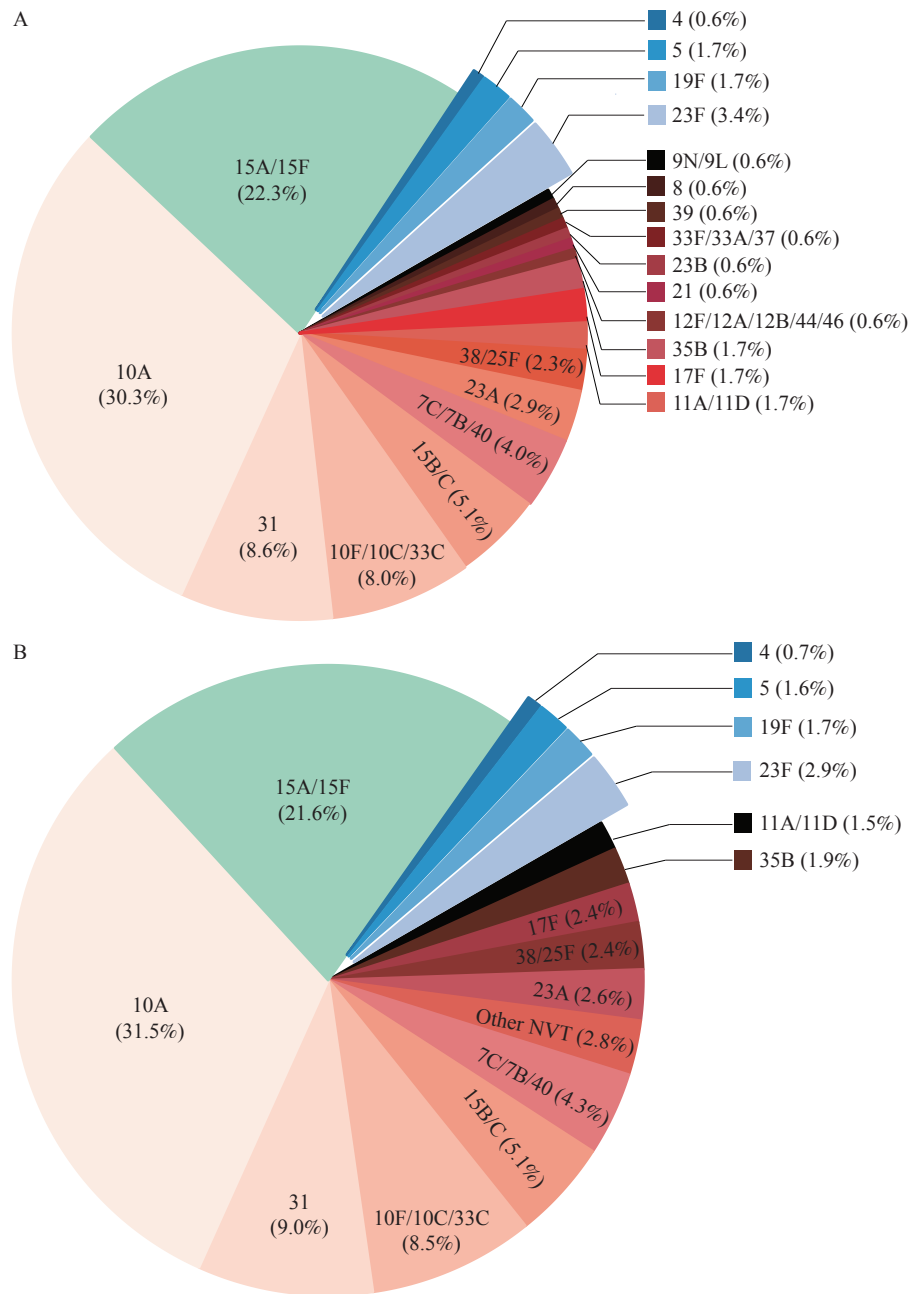


FIGURE 1. Distribution of pneumococcal serotypes during ARI episodes among children. (A) Serotype distribution of *Streptococcus pneumoniae* detected during ARI episodes; (B) Serotype distribution of *Streptococcus pneumoniae* after multiple imputation.

Note: In panels A and B, the vaccine serotypes are highlighted in blue. Other NVT included 12F/12A/12B/44/46, 21, 23B, 33F/33A/37, 39, 8, and 9N/9L.

Abbreviation: NVT=non-vaccine serotypes; ARI=acute respiratory infection.

introduction of PCVs, vaccine-type serotypes are markedly suppressed, whereas non-vaccine serotypes may not colonize efficiently, and therefore may not fully occupy the niche vacated by vaccine types, leaving ecological space for competing colonizers (7). In a randomized controlled trial on PCV7, a negative association in co-colonization between *S. pneumoniae*

(both vaccine-type and non-vaccine serotypes) and *Staphylococcus aureus* was observed (8).

Our results also revealed that non-vaccine serotypes of *S. pneumoniae* were predominant over vaccine serotypes, with serotypes 10A and 15A/15F being the most prevalent, whereas vaccine serotypes accounted for only 7.4%. These findings are consistent with

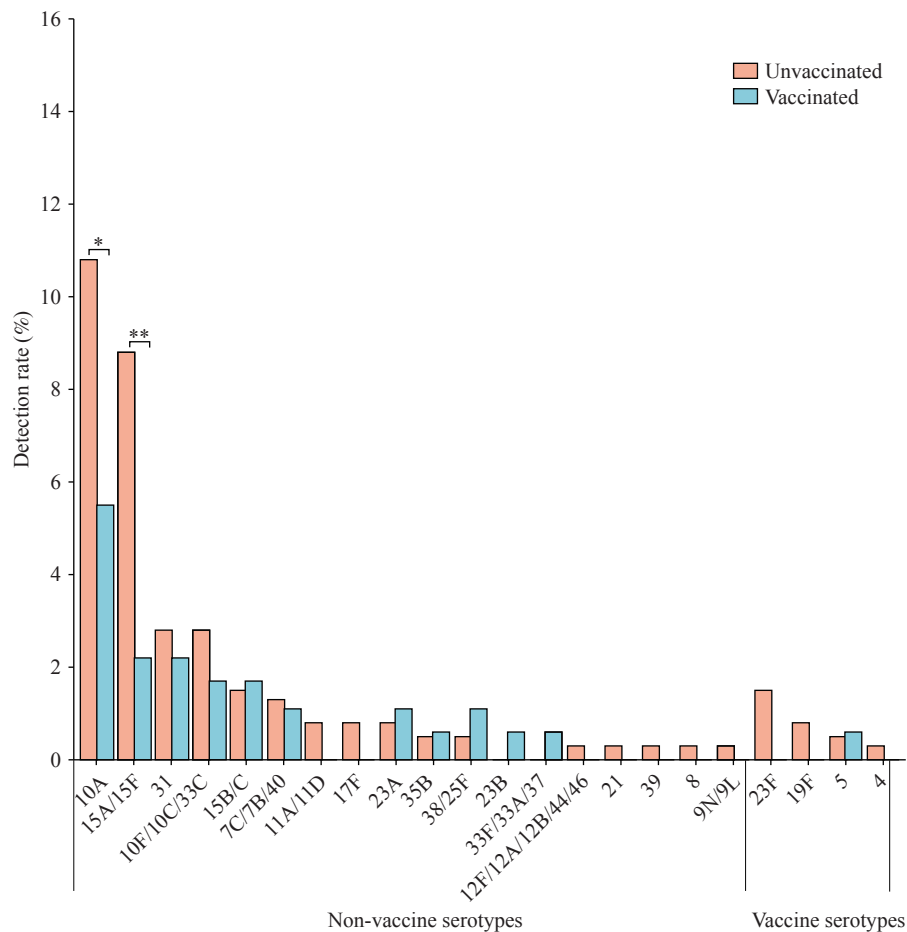


FIGURE 2. Detection rates of vaccine-type and non-vaccine serotypes during ARI episodes. Abbreviation: ARI=acute respiratory infection.

previously reported patterns. In Ulaanbaatar, Mongolia, a 44% reduction in vaccine-type carriage was observed following PCV13 vaccination, with serotypes 15A and 10A being the dominant serotypes (9). In Hong Kong, serogroup 15 has been reported to be the most frequently detected (10). Furthermore, a meta-analysis conducted between 2017 and 2024 identified 15A as the major non-vaccine serotype in Chinese children (11). Notably, the study reported 19F as the most prevalent serotype, which differed from our findings. This discrepancy may reflect differences in specimen types; the analysis included sterile site specimens and bronchoalveolar lavage fluid, which better represent serotypes causing clinical disease, whereas our study focused on ARI cases with mild symptoms, likely reflecting serotypes circulating within the community.

PCV immunization reduces the individual carriage of vaccine-type *S. pneumoniae*, thereby diminishing the transmission of these serotypes within the community (12). Although serotype replacement occurs frequently,

these emerging serotypes generally exhibit a lower invasive potential, resulting in a net benefit from vaccination with important public health implications. Long-term monitoring remains essential to determine whether vaccine-driven reductions in invasive pneumococcal persist and whether the invasive potential of non-vaccine serotypes remains stable or evolves as vaccine coverage expands.

The study had some limitations. First, although we evaluated the overall impact of PCV13 on pneumococcal detection, the small number of vaccine-type cases limited comprehensive subgroup analyses. Second, a substantial proportion of samples (32.8%) could not be serotyped, likely because of methodological factors. Multiplex PCR requires adequate template DNA, which makes low-load samples identified by tNGS more prone to serotyping failure. Moreover, some untypeable samples may have resulted from the limited serotype coverage of the PCR primers. Finally, our study was conducted exclusively in Eastern China, and given the regional variations in

TABLE 3. Cumulative incidence and risk ratios of pneumococcal detection during ARI episodes by PCV13 vaccination status in the original and IPTW cohorts.

Pneumococcal type	PCV13 vaccination status	n/N (%)	Cumulative incidence (%; 95% CI)	Unadjusted RR (95% CI)	P (unadjusted)	Adjusted RR (95% CI)*	P (adjusted)
Original cohort							
All pneumococci	Unvaccinated	160/398 (40.2)	40.2 (35.3, 45.2)	Reference	–	Reference	–
	1–2 doses	11/38 (28.9)	28.9 (15.4, 45.9)	0.74 (0.44, 1.23)	0.260	0.80 (0.49, 1.32)	0.392
	3–4 doses	33/143 (23.1)	23.1 (16.4, 30.9)	0.57 (0.41, 0.78)	0.001	0.70 (0.50, 0.98)	0.036
Vaccine serotypes	Unvaccinated	12/398 (3.0)	3.0 (1.6, 5.2)	Reference	–	Reference	–
	≥1 dose	1/181 (0.6)	0.6 (0.0, 3.0)	0.18 (0.02, 1.36)	0.096	0.29 (0.04, 2.39)	0.251
Non-vaccine serotypes	Unvaccinated	99/398 (24.9)	24.9 (20.7, 29.4)	Reference	–	Reference	–
	1–2 doses	7/38 (18.4)	18.4 (7.7, 34.3)	0.75 (0.38, 1.48)	0.408	0.78 (0.41, 1.49)	0.454
	3–4 doses	18/143 (12.6)	12.6 (7.6, 19.2)	0.50 (0.31, 0.79)	0.003	0.61 (0.38, 0.97)	0.037
IPTW cohort†							
All pneumococci	Unvaccinated	222/578 (38.4)	38.4 (33.5, 43.3)	Reference	–	–	–
	1–2 doses	152/530 (28.7)	28.7 (13.3, 44.0)	0.75 (0.43, 1.30)	0.299	–	–
	3–4 doses	124/573 (21.6)	21.6 (13.9, 29.2)	0.56 (0.39, 0.82)	0.003	–	–
Non-vaccine serotypes	Unvaccinated	138/578 (23.9)	23.9 (19.7, 28.2)	Reference	–	–	–
	1–2 doses	82/530 (15.5)	15.5 (4.4, 26.6)	0.65 (0.31, 1.35)	0.247	–	–
	3–4 doses	52/573 (9.3)	9.1 (4.8, 13.5)	0.38 (0.23, 0.64)	<0.001	–	–

Note: Data are presented as n/N (%) unless otherwise specified.

Abbreviation: ARI=acute respiratory infection; PCV13=13-valent pneumococcal conjugate vaccine; RR=risk ratio; CI=confidence interval; IPTW=inverse probability of treatment weighting.

* Adjusted for sex, disease history, mode of delivery, maternal age at delivery, maternal education level, maternal monthly income, number of household members, presence of children under 18 years old in the household, monthly household income, and baseline *S. pneumoniae* carriage.

As there were no cases in the 1–2 dose group and only one case in the 3–4 dose group, the two groups were combined for analysis.

† Weighted analysis was performed using IPTW estimated from multinomial logistic regression. The weighted results for vaccine serotypes were not estimated because of the small number of events. For the IPTW cohort, n/N values represent the sum of the IPTW weights and not the actual sample size.

epidemiological patterns and transmission dynamics, our findings may not be fully generalizable to ARI populations in other geographic regions.

In conclusion, PCV13 vaccination reduced pneumococcal detection in children with ARI, highlighting the importance of complete early immunization. As serotype shifts occur, continuous surveillance is essential for guiding strategic adjustments and evidence-based control policies.

Conflicts of interest: No conflicts of interest.

Ethical statement: The Scientific Review Committee of Jiangsu Provincial Center for Disease Control and Prevention approved the study protocol (approval number: JSJK2022-B014-02).

Funding: Supported by Science Fund for Distinguished Young Scholars of Jiangsu Province (grant number: BK20220064).

doi: [10.46234/ccdcw2025.222](https://doi.org/10.46234/ccdcw2025.222)

Corresponding authors: Lunbiao Cui, lbcui@jscdc.cn; Jingxin Li, Lijingxin@jscdc.cn.

¹ School of Public Health, Southeast University, Nanjing City, Jiangsu Province, China; ² School of Public Health, National Vaccine Innovation Platform, Nanjing Medical University, Nanjing City, Jiangsu Province, China; ³ Jiangsu Provincial Medical Innovation Center, National Health Commission Key Laboratory of Enteric Pathogenic Microbiology, Jiangsu Provincial Center for Disease Control and Prevention (Jiangsu Provincial Academy of Preventive Medicine), Nanjing City, Jiangsu Province, China; ⁴ CanSino Biologics Inc., Tianjin, China.

& Joint first authors.

Copyright © 2025 by Chinese Center for Disease Control and Prevention. All content is distributed under a Creative Commons Attribution Non Commercial License 4.0 (CC BY-NC).

Submitted: August 04, 2025

Accepted: September 25, 2025

Issued: October 17, 2025

REFERENCES

- Weiser JN, Ferreira DM, Paton JC. *Streptococcus pneumoniae*: transmission, colonization and invasion. *Nat Rev Microbiol* 2018;16(6):355 – 67. <https://doi.org/10.1038/s41579-018-0001-8>.
- Danigrat WOD, Amalia H, Ayu IM, Satzke C, Safari D. Carriage of

- Streptococcus pneumoniae* in children under five years of age prior to pneumococcal vaccine introduction in Southeast Asia: a systematic review and meta-analysis (2001-2019). *J Microbiol Immunol Infect* 2022;55(1):6 – 17. <https://doi.org/10.1016/j.jmii.2021.08.002>.
3. China National Clinical Research Center for Respiratory Diseases, National Center for Children's Health, Group of Respiratory Chinese Pediatric Society, Chinese Medical Association, Chinese Medical Doctor Association Committee on Respiratory Pediatrics, China Medicine Education Association Committee on Pediatrics, Chinese Research Hospital Association Committee on Pediatrics, et al. Chinese experts' consensus statement on diagnosis, treatment, and prevention of pneumococcal diseases in children. *Chin J Appl Clin Pediatr* 2020;35(7):485 – 505. <https://doi.org/10.3760/cma.j.cn101070-20200306-00329>.
 4. Li YQ, Wei MW, Cui LB, Huo X, Ma LS, Tao R, et al. Respiratory pathogens in infants and young children with acute respiratory illness: a prospective cohort study, China, 2023-2024. *Front Public Health* 2025;13:1548190. <https://doi.org/10.3389/fpubh.2025.1548190>.
 5. Wang J, Qiu L, Bai S, Zhao W, Zhang A, Li J, et al. Prevalence and serotype distribution of nasopharyngeal carriage of *Streptococcus pneumoniae* among healthy children under 5 years of age in Hainan Province, China. *Infect Dis Poverty* 2024;13(1):7. <https://doi.org/10.1186/s40249-024-01175-7>.
 6. Wang L, Fu JJ, Liang ZX, Chen JC. Prevalence and serotype distribution of nasopharyngeal carriage of *Streptococcus pneumoniae* in China: a meta-analysis. *BMC Infect Dis* 2017;17(1):765. <https://doi.org/10.1186/s12879-017-2816-8>.
 7. Mika M, Maurer J, Korten I, Allemann A, Aebi S, Brugger SD, et al. Influence of the pneumococcal conjugate vaccines on the temporal variation of pneumococcal carriage and the nasal microbiota in healthy infants: a longitudinal analysis of a case-control study. *Microbiome* 2017;5(1):85. <https://doi.org/10.1186/s40168-017-0302-6>.
 8. van Gils EJM, Hak E, Veenhoven RH, Rodenburg GD, Bogaert D, Bruin JP, et al. Effect of seven-valent pneumococcal conjugate vaccine on *Staphylococcus aureus* colonisation in a randomised controlled trial. *PLoS One* 2011;6(6):e20229. <https://doi.org/10.1371/journal.pone.0020229>.
 9. von Mollendorf C, Mungun T, Ulziibayar M, Nguyen CD, Batsaikhan P, Suuri B, et al. Effect of pneumococcal conjugate vaccination on pneumococcal carriage in hospitalised children aged 2-59 months in Mongolia: an active pneumonia surveillance programme. *Lancet Microbe* 2024;5(12):100929. [https://doi.org/10.1016/S2666-5247\(24\)00171-X](https://doi.org/10.1016/S2666-5247(24)00171-X).
 10. Chan KCC, Subramanian R, Chong P, Nelson EAS, Lam HS, Li AM, et al. Pneumococcal carriage in young children after introduction of PCV13 in Hong Kong. *Vaccine* 2016;34(33):3867 – 74. <https://doi.org/10.1016/j.vaccine.2016.05.047>.
 11. Li Y, Wang SJ, Hong L, Xin LJ, Wang F, Zhou YB. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* in China among children under 14 years of age post-implementation of the PCV13: a systematic review and meta-analysis (2017-2024). *Pneumonia (Nathan)* 2024;16(1):18. <https://doi.org/10.1186/s41479-024-00141-z>.
 12. Chan J, Mungun T, Batsaikhan P, Ulziibayar M, Suuri B, Otgonbayar D, et al. Direct and indirect effects of 13-valent pneumococcal conjugate vaccine on pneumococcal carriage in children hospitalised with pneumonia from formal and informal settlements in Mongolia: an observational study. *Lancet Reg Health West Pac* 2021;15:100231. <https://doi.org/10.1016/j.lanwpc.2021.100231>.

SUPPLEMENTARY MATERIAL

The targeted next-generation sequencing (tNGS) assay was performed following standardized procedures, including nucleic acid extraction and library preparation.

Nucleic Acid Extraction

From each pharyngeal swab sample, 1.3 mL of specimen was aliquoted and mixed with 13 µL of exogenous internal control RNA, followed by centrifugation. The resulting pellet was collected, and nucleic acids were extracted using a commercial nucleic acid extraction kit (KS118-BYTQ, Guangzhou, China) according to the manufacturer's instructions.

Library Preparation and Sequencing

An appropriate volume of nucleic acid was transferred into a 96-well plate, with 2 µL of synthesis primers and synthesis buffer added to each well, for a final reaction volume of 20 µL. First-strand cDNA synthesis was performed under the following conditions: incubation at 25 °C for 5 min, reaction at 37 °C for 45 min, denaturation at 85 °C for 5 s, and maintenance at 4 °C. The cDNA products were then subjected to multiplex PCR for target enrichment using a reaction mixture containing multiplex PCR premix and 11 µL of cDNA template. The amplification program was as follows: 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 1 min. PCR products were purified using magnetic beads, air-dried at room temperature, and eluted in 13.5 µL RNase-free water.

For library amplification, 11.5 µL of purified product was added to the library amplification premix. Following PCR amplification and a second round of magnetic bead purification, libraries were eluted in 20 µL. Library concentration was quantified using a Qubit fluorometer. Qualified libraries showed a distinct peak within the 250–350 bp range, and libraries lacking a peak or with <20% representation were considered failed. Qualified libraries were pooled in equimolar amounts, purified with magnetic beads, and assessed for fragment size distribution using a Qsep100 nucleic acid analyzer. The pooled library was diluted to 1 pmol/L, denatured with NaOH, and loaded onto the MiniSeqDx-CN sequencing platform.

Bioinformatics Analysis

Raw sequencing data were analyzed using the Proprietary Pathogen Data Analysis Management System (KingCreate, Guangzhou, China). Quality control was performed, and only datasets meeting the following criteria were included: sequencing quality score Q30 $\geq$ 75% and a minimum of 50,000 raw reads per sample. High-quality reads were subsequently aligned to a reference database, and normalized read counts were applied to determine pathogen positivity. A pathogen was considered positive if at least one specific target region produced a normalized read count $\geq$ 10.5; otherwise, it was classified as negative.

SUPPLEMENTARY TABLE S1. Baseline demographic characteristics of the participants at the recruitment.

Characteristics	Participants included in the analysis (N=579)	All enrolled children (N=796)	P
Infant sex, n (%)			0.910
Female	269 (46.5)	373 (46.9)	
Male	310 (53.5)	422 (53.1)	
Infant age, median (IQR), days	71.0 (66.0, 78.0)	71.0 (66.0, 77.3)	0.757
Infant height, median (IQR), cm	60.0 (59.0, 62.0)	60.0 (59.0, 62.0)	0.073
Infant weight, median (IQR), kg	6.3 (5.8, 6.8)	6.3 (5.8, 6.8)	0.585
Infant birth length, median (IQR), cm	50.0 (50.0, 50.0)	50.0 (50.0, 50.0)	0.113
Infant birth weight, median (IQR), kg	3.4 (3.2, 3.7)	3.4 (3.2, 3.7)	0.346
Infant disease history*, n (%)			0.831
Yes	18 (3.1)	22 (2.8)	
No	561 (96.9)	774 (97.2)	

Continued

Characteristics	Participants included in the analysis (N=579)	All enrolled children (N=796)	P
Maternal mode of delivery, n (%)			>0.999
Full-term vaginal delivery	291 (50.3)	399 (50.1)	
Cesarean delivery	288 (49.7)	397 (49.9)	
Maternal feeding pattern, n (%)			0.415
Breastfeeding	523 (90.3)	701 (88.1)	
Not breastfeeding after birth	47 (8.1)	80 (10.1)	
Mixed feeding	9 (1.6)	15 (1.9)	
Maternal age, n (%), years			0.822
<35	503 (86.9)	687 (86.3)	
≥35	76 (13.1)	109 (13.7)	
Maternal education, n (%)			0.854
Middle school or below	162 (28.0)	210 (26.4)	
High school or vocational school	100 (17.3)	141 (17.7)	
Junior college	152 (26.3)	223 (28.0)	
Bachelor degree or above	165 (28.5)	222 (27.9)	
Maternal monthly income, n (%), CNY			0.800
<1,000	245 (42.3)	329 (41.3)	
1,000–6,999	270 (46.6)	370 (46.5)	
≥7,000	64 (11.1)	97 (12.2)	
No. of household members, n (%)			0.835
≤4	421 (72.7)	584 (73.4)	
>4	158 (27.3)	212 (26.6)	
With household members <18 years of age, n (%)			0.513
Yes	329 (56.8)	437 (54.9)	
No	250 (43.2)	359 (45.1)	
Monthly household income, n (%), CNY			0.904
<10,000	175 (30.2)	237 (29.8)	
≥10,000	404 (69.8)	559 (70.2)	
Baseline <i>S. pneumoniae</i> carriage			>0.999
Yes	21 (3.6)	29 (3.6)	
No	553 (95.5)	761 (95.6)	
Missing	5 (0.9)	6 (0.8)	
Received first dose of PCV13 before enrollment, n (%)			0.781
Yes	54 (9.3)	79 (9.9)	
No	525 (90.7)	717 (90.1)	

Abbreviation: PCV13=13-valent pneumococcal conjugate vaccine; CNY=Chinese Yuan; COVID-19=coronavirus disease 2019.

* Infant disease history includes pneumonia, jaundice, COVID-19, and lactose intolerance.