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RESEARCH ARTICLE

Impact of COVID-19 Nonpharmaceutical Interventions on Pneumococcal Carriage Prevalence and Density in Vietnam

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ABSTRACT Nonpharmaceutical interventions (NPIs) implemented to contain SARS-CoV-2 have decreased invasive pneumococcal disease. Previous studies have proposed the decline is due to reduced pneumococcal transmission or suppression of respiratory viruses, but the mechanism remains unclear. We undertook a secondary analysis of data collected from a clinical trial to evaluate the impact of NPIs on pneumococcal carriage and density, drivers of transmission and disease, during the COVID-19 pandemic in Ho Chi Minh City, Vietnam. Nasopharyngeal samples from children aged 24 months were assessed in three periods — one pre-COVID-19 period (n = 1,537) and two periods where NPIs were implemented with increasing stringency (NPI period 1 [NPI-1, n = 307], and NPI period 2 [NPI-2, n = 262]). Pneumococci were quantified using *lytA* quantitative PCR and serotyped by DNA microarray. Overall, capsular, and nonencapsulated pneumococcal carriage and density were assessed in each NPI period compared with the pre-COVID-19 period using unadjusted log-binomial and linear regression. Pneumococcal carriage was generally stable after the implementation of NPIs. In contrast, overall pneumococcal carriage density decreased by 0.44 $\log_{10}$ genome equivalents/mL (95% confidence interval [CI]: 0.19 to 0.69) in NPI-1 and by 0.84 $\log_{10}$ genome equivalents/mL (95% CI: 0.55 to 1.13) in NPI-2 compared with the pre-COVID-19 period. Reductions in overall pneumococcal density were driven by reductions in capsular pneumococci, with no corresponding reduction in nonencapsulated density. As higher pneumococcal density is a risk factor for disease, the decline in density provides a plausible explanation for the reductions in invasive pneumococcal disease that have been observed in many countries in the absence of a substantive reduction in pneumococcal carriage.

IMPORTANCE The pneumococcus is a major cause of mortality globally. Implementation of NPIs during the COVID-19 pandemic led to reductions in invasive pneumococcal disease in many countries. However, no studies have conducted a fully quantitative assessment on the impact of NPIs on pneumococcal carriage density, which could explain this reduction. We evaluated the impact of COVID-19 NPIs on pneumococcal carriage prevalence and density in 2,106 children aged 24 months in Vietnam and found pneumococcal carriage density decreased up to 91.5% after NPI introduction compared with the pre-COVID-19 period, which was mainly attributed to capsular pneumococci. Only a minor effect on carriage prevalence was observed. As respiratory viruses are known to increase

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pneumococcal carriage density, transmission, and disease, this work suggests that interventions targeting respiratory viruses may have the added benefit of reducing invasive pneumococcal disease and explain the reductions observed following NPI implementation.

KEYWORDS *Streptococcus pneumoniae*, density, carriage, pneumococcal, Vietnam, COVID-19, nonpharmaceutical intervention

Streptococcus pneumoniae (the pneumococcus) is a major cause of disease, including pneumonia, meningitis, and sepsis. The bacterium commonly colonizes the nasopharynx and carriage is a prerequisite for pneumococcal transmission and disease (1). During the COVID-19 pandemic, the implementation of nonpharmaceutical interventions (NPIs) such as mask wearing, stay-at-home orders, and physical distancing have been critical for the containment of SARS-CoV-2. Introduction of NPIs have been associated with declines in other respiratory pathogens and infectious diseases, including influenza, respiratory syncytial virus (RSV), pneumonia, and invasive pneumococcal disease (IPD) (2–4). It has been proposed that the reduction in IPD may be due to reduced pneumococcal transmission (3), but there are limited data to support this. Four studies have examined the impact of NPIs on pneumococcal carriage prevalence, with varying results (4–7). A study in Belgium found no difference in carriage prevalence pre- and postimplementation of NPIs in children attending daycare. A study in an outpatient health care facility in Serbia found an increase in pneumococcal carriage in children during the COVID-19 pandemic compared with the carriage prevalence in the early pandemic period (7). In contrast, a study in Israel found a reduction in pneumococcal carriage prevalence in one of the two NPI periods assessed, along with a decline in the prevalence of respiratory viruses that was temporally associated with a decline in IPD (4). A study in France found that pneumococcal carriage rates remained stable but a reduction in influenza-like illness and RSV was associated with a 63% decline in IPD (6). Furthermore, the study in France estimated that 93% of the decrease in IPD incidence was associated with decreases in the number of influenza and RSV cases. Collectively, these studies suggest pneumococcal carriage has generally remained stable during the COVID-19 pandemic, while there has been a decline in some circulating respiratory viruses.

Some respiratory viruses increase pneumococcal carriage density, which contributes to transmission and disease (8–13). Therefore, we hypothesized that reductions in IPD associated with NPIs may be due to reductions in pneumococcal carriage density rather than prevalence. To date, no studies have conducted a fully quantitative assessment of the impact of NPIs on pneumococcal carriage density, which may provide the missing link between temporal reductions in circulating respiratory viruses and IPD. Here, we aim to assess the impact of NPIs during the COVID-19 pandemic on pneumococcal carriage prevalence and density in children in Vietnam.

RESULTS

Of the 2,501 participants enrolled in the trial, 318 withdrew and do not contribute data to this analysis (Fig. 1). Between 25 December 2018 and 18 June 2020, 2,183 nasopharyngeal samples were collected from children aged approximately 24 months old. Over the three periods, 2,106 nasopharyngeal samples were included in this analysis (n = 1,537 in pre-COVID-19, n = 307 in NPI-1, and n = 262 in NPI-2). Seventy-seven samples were collected between 1 January and 2 February 2020 and were excluded from analysis as preventative measures may have been implemented before such policies were officially introduced. Eighteen samples were excluded from serotype-specific analyses as no serotyping data were available.

Participant characteristics were similar across the three periods for sex, current breastfeeding, household size, the number of children under 5 years of age in the household (including the participant), number of pneumococcal conjugate vaccine (PCV) doses received, and the type of PCV received (Table 1). Children were older, reported fewer respiratory symptoms, and used fewer antibiotics in both NPI periods compared with the pre-

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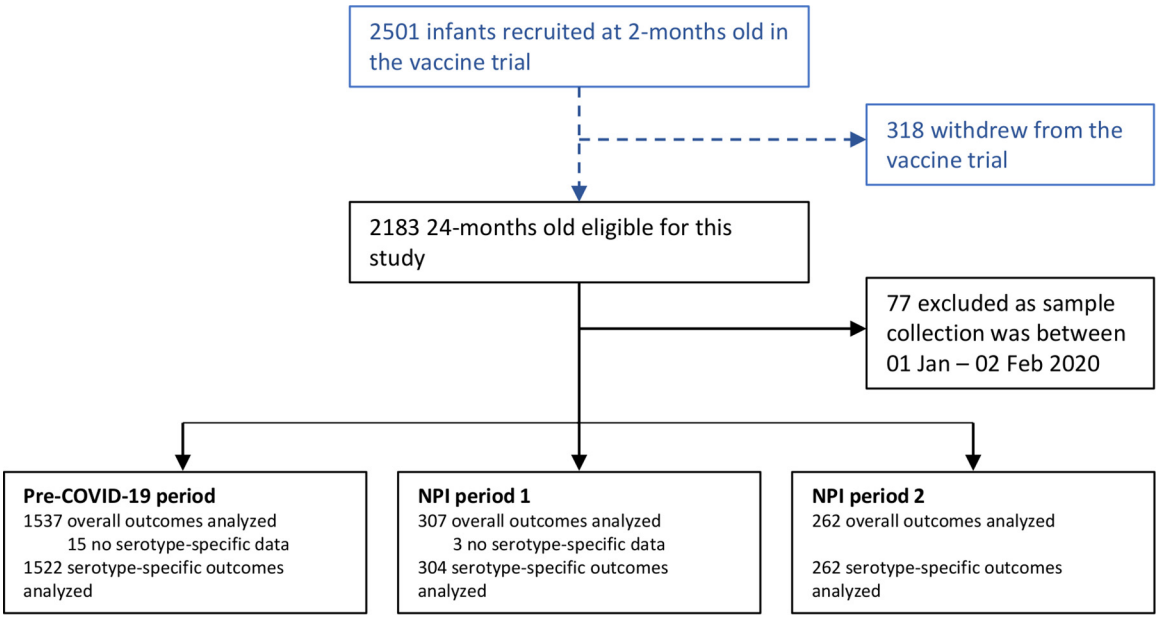

FIG 1 Participant flow diagram. Participants were excluded from all analyses if samples were collected between 1 January to 2 February 2020, as preventative measures may have been implemented before the nonpharmaceutical interventions (NPI) were officially introduced (n = 77) or were excluded from serotype-specific analyses if serotype-specific data were not available (n = 18).

COVID-19 period. Samples were collected across both seasons in the pre-COVID-19 period, while all samples in NPI period 1 were collected in the dry season and all samples in NPI period 2 were collected in the rainy season. There were differences in the proportion of participants from each district, as recruitment commenced at different times in the districts.

Of the 2,106 children assessed, 481 (22.8%) carried pneumococci. Multiple serotype carriage was infrequent (39/2088; 1.9%) and was similar across the three periods (25/1522 (1.6%) in the pre-COVID-19 period, 8/304 (2.6%) in NPI-1, and 6/262 (2.3%) in NPI-2).

Overall pneumococcal carriage prevalence was 23.6% (363/1,537), 22.8% (70/307), and 18.3% (48/262) in the pre-COVID-19, NPI-1, and NPI-2 periods, respectively (Table 2). There was no difference in overall pneumococcal carriage prevalence in NPI-1 compared with the pre-COVID-19 period (prevalence ratio [PR]: 0.97; 95% confidence interval [CI]: 0.77 to 1.21) (Table 2 and Fig. S2 in the supplemental material). There was some evidence of a reduction in overall carriage prevalence in NPI-2 compared with the pre-COVID-19 period (PR: 0.78; 95% CI: 0.59 to 1.02; P value = 0.066). This was driven by a lower point estimate for nonencapsulated carriage prevalence, with wide confidence intervals and a small number of observations. Neither NPI period was associated with changes in capsular carriage prevalence compared with the pre-COVID-19 period.

Overall and capsular pneumococcal carriage density decreased from pre-COVID-19 to NPI-1, with a further reduction in NPI-2 (Fig. 2 and Table 3). Overall pneumococcal carriage density decreased by 63.7% (reduction of 0.44 $\log_{10}$ genome equivalents per mL [GE/mL]; 95% CI: 0.19 to 0.69) in NPI-1 and by 85.5% (reduction of 0.84 $\log_{10}$ GE/mL; 95% CI: 0.55 to 1.13) in NPI-2 compared with the pre-COVID-19 period (Table 3 and Fig. S2). Reductions in overall pneumococcal carriage density were driven by decreases in capsular pneumococcal carriage density, which decreased by 70.5% from pre-COVID-19 to NPI-1 (reduction of 0.53 $\log_{10}$ GE/mL; 95% CI: 0.23 to 0.83), and by 91.5% from pre-COVID-19 to NPI-2 (reduction of 1.07 $\log_{10}$ GE/mL; 95% CI: 0.74 to 1.41). There was no corresponding reduction in nonencapsulated pneumococcal carriage density in NPI-1 (percentage change = −48.7%; difference in means = −0.29 $\log_{10}$ GE/mL; 95% CI: 0.64 to 0.05) or NPI-2 (percentage change = −38.3%; difference in means = −0.21 $\log_{10}$ GE/mL; 95% CI: −0.68 to 0.26) compared with the pre-COVID-19 period.

Overall, we found no clear patterns of serotype-specific changes in pneumococcal carriage

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TABLE 1 Participant characteristics at the 24-month visit[a]

Characteristics	Pre-COVID-19 *n* = 1,537	NPI period 1 *n* = 307	NPI period 2 *n* = 262	*P* value[b]
Age in months (median [range])	24.1 (24.0–28.5)	24.1 (24.0–28.7)	25.0 (24.0–30.8)	<0.001
Sex, female	740 (48.1%)	148 (48.2%)	131 (50.0%)	0.860
District				<0.001
4	663 (43.1%)	88 (28.7%)	74 (28.2%)	
7	381 (24.8%)	104 (33.9%)	68 (26.0%)	
8	493 (32.1%)	115 (37.5%)	120 (45.8%)	
Household size (median [range])[c]	6 (3–34)	6 (3–26)	6 (3–18)	1.000
No. of children under 5 in household[c,d]				0.671
1	897 (58.5%)	193 (62.9%)	153 (58.4%)	
2	497 (32.4%)	91 (29.6%)	84 (32.1%)	
≥3	140 (9.1%)	23 (7.5%)	25 (9.5%)	
Season[e]				<0.001
Dry	513 (33.4%)	307 (100%)	0 (0%)	
Rainy	1,024 (66.6%)	0 (0%)	262 (100%)	
Current breastfeeding	120 (7.8%)	19 (6.2%)	14 (5.3%)	0.268
Current URTI symptoms	250 (16.3%)	14 (4.6%)	7 (2.7%)	<0.001
Antibiotic use in past 2 wks	175 (11.4%)	8 (2.6%)	2 (0.8%)	<0.001
Current antibiotic use	62 (4.0%)	5 (1.6%)	0 (0%)	0.001
Doses of PCV received[f]				0.820
0	540 (35.1%)	110 (35.8%)	87 (33.2%)	
1	498 (32.4%)	91 (29.6%)	89 (34.0%)	
2	499 (32.5%)	106 (34.5%)	86 (32.8%)	
If PCV received, PCV10	486 (48.7%)	101 (51.3%)	88 (50.3%)	0.780

[a]Data are *n* (%) unless specified. URTI, upper respiratory tract infection; PCV, pneumococcal conjugate vaccine; PCV10, 10-valent PCV.
[b]*P* values are comparisons across all time periods based on chi-squared test (for comparisons of proportions), or quantile regression with bootstrapped standard errors (for comparisons of medians).
[c]Missing data for *n* = 3 participants in the pre-COVID-19 period.
[d]Including the participant.
[e]Ho Chi Minh City has a Tropical Savannah climate (27) consisting of a dry season (November to April) and a rainy season (May to October).
[f]Participants received either PCV10 or PCV13.

prevalence by NPI period (Fig. S3A). Pneumococcal carriage density was generally lower in NPI-2 for most serotypes but as numbers were small, we did not conduct formal analyses comparing individual serotypes (Fig. S3B).

Similar patterns were observed in the direct analyses adjusting for the intermediate variables district of residence and season (Table S1), when analyses were restricted to participants without URTI symptoms (Table S2), and when analyses were restricted to unvaccinated participants (Table S3). There was little evidence of a reduction in pneumococcal carriage prevalence in NPI-2 compared with the pre-COVID-19 period in any of the additional analyses.

DISCUSSION

Following the implementation of NPIs for the containment of SARS-CoV-2, a decline in disease caused by other respiratory pathogens, including pneumococci, has been observed

TABLE 2 Pneumococcal carriage prevalence and prevalence ratios in periods with nonpharmaceutical interventions (NPIs) compared with the pre-COVID-19 period[a]

	Prevalence			NPI-1 vs pre-COVID-19		NPI-2 vs pre-COVID-19	
Carriage	Pre-COVID-19	NPI-1	NPI-2	Prevalence ratio (95% CI)	*P* value	Prevalence ratio (95% CI)	*P* value
Overall	363/1,537 (23.6%)	70/307 (22.8%)	48/262 (18.3%)	0.97 (0.77 to 1.21)	0.759	0.78 (0.59 to 1.02)	0.066
Capsular[b]	269/1,522 (17.7%)	52/304 (17.1%)	40/262 (15.3%)	0.97 (0.74 to 1.27)	0.812	0.86 (0.64 to 1.17)	0.347
Nonencapsulated[b]	95/1,522 (6.2%)	20/304 (6.6%)	10/262 (3.8%)	1.05 (0.66 to 1.68)	0.825	0.61 (0.32 to 1.16)	0.131

[a]COVID-19, coronavirus disease 2019; NPI-1, nonpharmaceutical intervention period 1; NPI-2, nonpharmaceutical intervention period 2; 95% CI, 95% confidence interval.
[b]Serotype-specific data were not available for *n* = 18 samples (*n* = 15 pre-COVID-19, *n* = 3 NPI-1).

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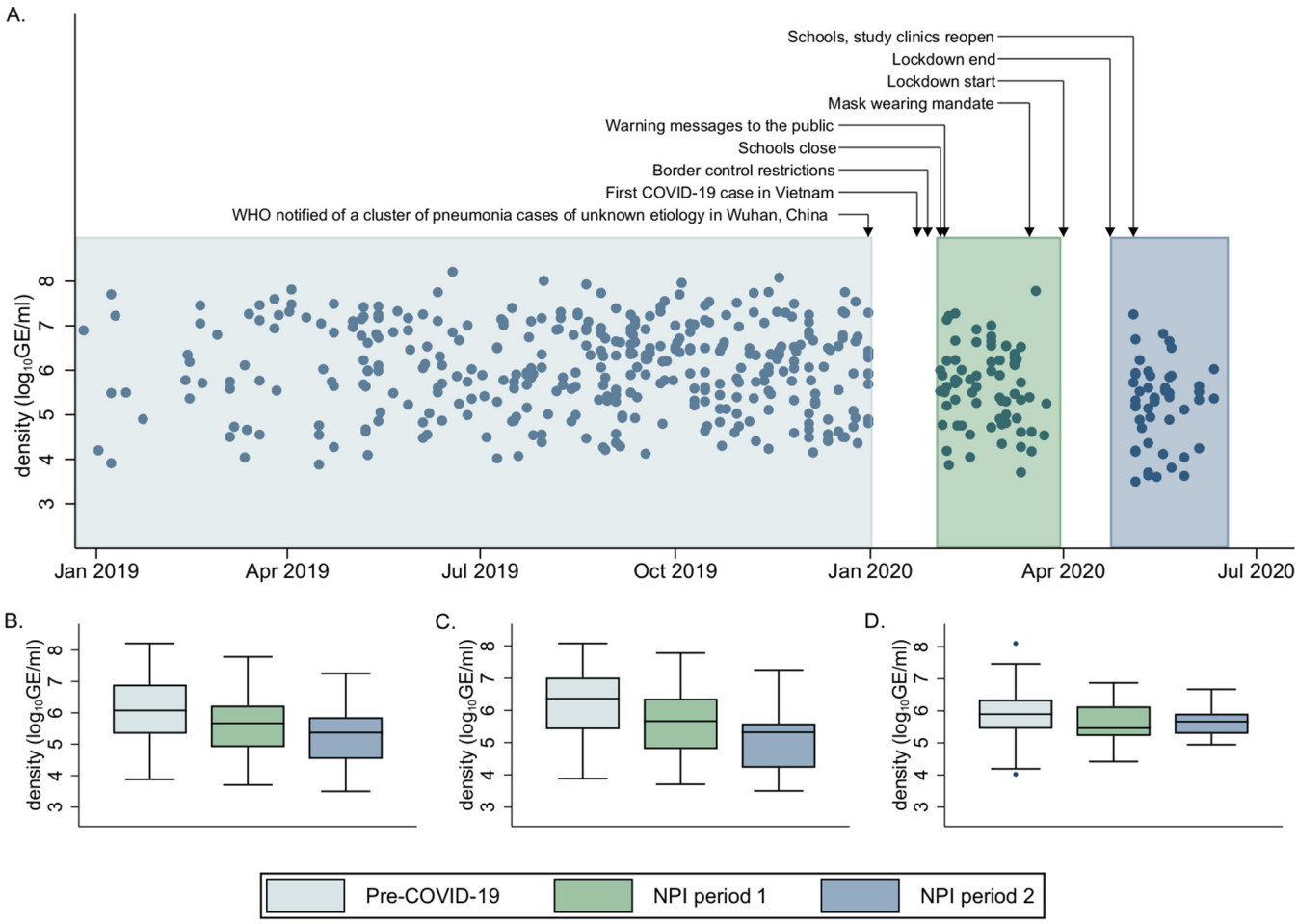

FIG 2 Nasopharyngeal carriage density (log_{10} genome equivalents per mL) among pneumococcal carriers. (A) Overall pneumococcal density from 2019 to 2020 with key events and nonpharmaceutical interventions (NPIs) indicated. Box plots of (B) overall, (C) capsular, and (D) nonencapsulated pneumococcal density. Boxes depict interquartile range (IQR) with a central line for the median. Data points further than the 25th/75th percentile plus 1.5 times the IQR are plotted as individual points. COVID-19 = coronavirus disease 2019.

in multiple countries. The decline in IPD has been attributed to reductions in pneumococcal transmission (3); however, four studies (including this one) have found pneumococcal carriage prevalence was generally stable after the implementation of NPIs (4–6). We found some evidence of a 22% reduction in overall pneumococcal carriage prevalence in one of the periods after NPI implementation, which was driven by a decrease in the prevalence of nonencapsulated pneumococci. As nonencapsulated pneumococci are infrequently implicated in IPD (14), it is unlikely that a change in nonencapsulated carriage prevalence would translate to a substantial decline in IPD as observed in many countries. Studies in Belgium and France found no substantive reductions in pneumococcal carriage pre- and post-NPIs, although similarly to our study,

TABLE 3 Pneumococcal carriage density means and difference in means in periods with nonpharmaceutical interventions (NPIs) compared with the pre-COVID-19 period[a]

		Mean (SD)			NPI-1 vs pre-COVID-19		NPI-2 vs pre-COVID-19	
Density[b]	*n*	Pre-COVID-19	NPI-1	NPI-2	Difference in means (95% CI)	*P* value	Difference in means (95% CI)	*P* value
Overall	481	6.07 (0.99)	5.63 (0.90)	5.23 (0.92)	−0.44 (−0.69 to −0.19)	0.001	−0.84 (−1.13 to −0.55)	<0.001
Capsular[c]	361	6.18 (1.01)	5.65 (0.95)	5.11 (0.95)	−0.53 (−0.83 to −0.23)	0.001	−1.07 (−1.41 to −0.74)	<0.001
Nonencapsulated[c]	125	5.90 (0.74)	5.60 (0.63)	5.69 (0.52)	−0.29 (−0.64 to 0.05)	0.098	−0.21 (−0.68 to 0.26)	0.381

[a]NPI-1, nonpharmaceutical intervention period 1; NPI-2, nonpharmaceutical intervention period 2; COVID-19, coronavirus disease 2019; SD, standard deviation; 95% CI, 95% confidence interval.
[b]Assessed in pneumococcal carriers only and reported in log_{10} genome equivalents per mL.
[c]Serotype-specific data were not available for *n* = 18 samples (pre-COVID-19, *n* = 15; NPI-1, *n* = 3).

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the study in Israel found pneumococcal carriage prevalence was lower in one of the two time periods assessed (4–6). Moreover, using interrupted time-series analyses, the study in France estimated that most of the reduction in IPD was due to reductions in influenza and RSV, with no association with pneumococcal carriage rates.

Importantly, we observed a substantial decline in overall pneumococcal carriage density after the implementation of NPIs, which was driven by reductions in capsular pneumococcal carriage density. There was no corresponding reduction in nonencapsulated pneumococcal carriage density. Capsular pneumococcal carriage density decreased by approximately 70 to 90% in the two periods with NPIs compared with the pre-COVID-19 period. In contrast, the only other study to evaluate the impact of NPIs on pneumococcal carriage density found no difference pre- and post-NPI implementation in Israel using a semiquantitative plate scoring method (4). These differences may be partially explained by the use of the more sensitive quantitative PCR method in our study, as well as differences in study settings such as NPI stringency. It is unclear why there was no reduction in nonencapsulated carriage density in our study. Interestingly, we have previously found that coinfection of infant mice with a murine analogue of RSV increased the carriage density of capsulated pneumococci, but this increase was not observed in nonencapsulated pneumococci (15). Given the reductions in circulating respiratory viruses detected in many settings, it is possible that nonencapsulated pneumococcal carriage density was less affected if respiratory viruses (that act to increase pneumococcal carriage density) largely act on capsulated pneumococci.

A major strength of this study is the use of carriage data from healthy children. Many studies investigating the impact of NPIs on non-SARS-CoV-2 pathogens rely on disease data, which can be influenced by behavioral changes such as reluctance to seek medical treatment for fear of contracting COVID-19. The use of sensitive, fully quantitative, serotyping methods (16) enabled us to quantify the impact of NPIs on pneumococci, and to evaluate differences in capsular and nonencapsulated pneumococci which has not been investigated previously. This study was not able to evaluate the impact of NPIs on pneumococcal carriage acquisition given the cross-sectional study design. Longitudinal carriage studies conducted during the COVID-19 pandemic could provide important data on the impact of NPIs on pneumococcal carriage acquisition and any associated impact on pneumococcal carriage density. In the absence of pneumococcal carriage acquisition data, assessment of multiple serotype carriage may provide insight into changes in pneumococcal acquisition. We found multiple serotype carriage was infrequent and similar across all periods, indicating little evidence of a change in acquisition in this study. Additionally, other temporal changes may have occurred during the study that were not accounted for.

The implementation of NPIs has been associated with declines in IPD (3), which has been temporally associated with the suppression of RSV, influenza, and human metapneumovirus (4, 6). In this study, there was a reduction in symptoms of upper respiratory tract infection and a decline in the use of antibiotics during the NPI periods compared with the pre-COVID period, which is consistent with a reduction in respiratory infections. Respiratory viruses are associated with increased pneumococcal carriage density (8, 9, 17), which is a risk factor for disease (9, 13, 18). The decline in pneumococcal carriage density associated with NPI implementation therefore provides a plausible explanation for the reductions in IPD that have been observed elsewhere in the absence of a substantive reduction in pneumococcal carriage prevalence. For example, NPI implementation may result in reductions in circulating respiratory viruses that act to increase pneumococcal carriage density. In the absence of these viruses, pneumococcal carriage density would be lower, leading to a reduced chance of developing IPD. Interventions that target respiratory viruses therefore have the potential to reduce IPD, increasing the cost-effectiveness and impact of viral prevention and treatment strategies. Further research examining the links between respiratory viruses and pneumococcal carriage acquisition, carriage prevalence, and carriage density is needed to fully elucidate the impact NPIs have on IPD.

MATERIALS AND METHODS

Study design and participants. Nasopharyngeal samples were collected from children aged 24 months between 25 December 2018 and 18 June 2020 as part of a trial investigating reduced-dose schedules of

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pneumococcal conjugate vaccines (PCV) in Ho Chi Minh City, Vietnam (19). In the trial, healthy children from the community who were born at ≥36 weeks gestation and had no significant maternal or perinatal history were enrolled at 2 months of age and followed up to 24 months of age. Further details on inclusion and exclusion criteria are provided in the trial protocol (19). Participants were randomized to one of five groups and received PCV10 at 12 months (0 + 1) or at 2 and 12 months (1 + 1), PCV13 at 12 months (0 + 1) or at 2 and 12 months (1 + 1), or a single dose of PCV10 at 24 months after the 24-month sample collection (unvaccinated comparator). Nasopharyngeal sample collection for the 24-month time point occurred before and after the emergence of SARS-CoV-2, enabling the impact of NPIs on pneumococcal carriage prevalence and density to be evaluated. All children with a 24-month sample were eligible for inclusion in this secondary analysis.

Ethics statements. Ethical approval was obtained for the vaccine trial from the Human Research Ethics Committee of the Royal Children's Hospital Melbourne, the Institutional Review Board at the Pasteur Institute of Ho Chi Minh City, and the Vietnam Ministry of Health Ethical Review Committee for Biomedical Research. The vaccine trial is registered at clinicaltrials.gov (NCT03098628). Written informed consent was obtained from participants.

All participants gave consent for results to be published in scientific journals as part of the Participant Information and Consent form completed during enrollment into the vaccine trial.

Laboratory analyses and carriage outcomes. Swabs were collected and stored according to WHO guidelines (20). Pneumococcal carriage prevalence and density were determined by quantitative PCR (qPCR) targeting the *lytA* gene and molecular serotyping using Senti-SPv1.5 DNA microarrays (BUGS Bioscience), with analysis using a custom web-based software (19, 21).

Serotype 11F-like was reported as 11A, and serotypes 15B and 15C were reported as 15B/C (22, 23). Overall pneumococcal carriage prevalence was defined as carriage of any pneumococci. Capsular carriage included carriage of any serotype excluding nonencapsulated pneumococci. Nonencapsulated carriage included carriage of previously described nonencapsulated pneumococci, including NT2, NT3b, NT4a, and NT4b (24). Any sample containing pneumococci was considered positive. For example, a sample containing both capsular and nonencapsulated serotypes was considered positive for overall, capsular, and nonencapsulated carriage prevalence.

NPI periods. Three time periods were defined based on key events and the implementation of NPIs in Vietnam (25). The pre-COVID-19 period was defined from the start of the 24-month sample collection until the World Health Organization was notified of a cluster of cases in Wuhan, China (25 December 2018 to 31 December 2019). The first NPI period included school closures, warning messages to the public, restrictions on nonessential businesses, and a mask wearing mandate (NPI-1, 03 February 2020 to 31 March 2020). A 3-week lockdown was implemented in Ho Chi Minh City from 01 April to 22 April 2020, during which time study clinics were closed and no samples were collected. The second NPI period started after the lockdown and concluded at the end of sample collection (NPI-2, 23 April 2020 to 18 June 2020). During NPI-2, the mask wearing mandate continued and there was a staggered reopening of nonessential businesses (25, 26). Samples collected between 1 January 2020 and 2 February 2020 were excluded from analyses, as preventative measures (such as mask wearing, hand hygiene, and self-isolation) may have been implemented before such policies were officially introduced.

Statistical analyses. We determined the prevalence and density of overall, capsular, and nonencapsulated pneumococcal carriage, and plotted serotype-specific pneumococcal carriage prevalence and density. Participant characteristics were compared between time periods using the chi-squared test for comparisons of proportions, or quantile regression with bootstrapped standard errors for comparisons of medians. Potential confounders were assessed using a directed acyclic graph, with no confounders identified (see Fig. S1 in the supplemental material). Pneumococcal carriage density data were $\log_{10}$-transformed and are expressed as $\log_{10}$ genome equivalents per mL ($\log_{10}$ GE/mL). Serotype-specific carriage density was calculated by multiplying the overall pneumococcal load (as determined by qPCR) by the corresponding relative abundance of the serotype (as determined by DNA microarray).

In the primary analyses, pneumococcal carriage prevalence and density in each NPI period were compared with the pre-COVID-19 period using unadjusted log-binomial and linear regression. Log-binomial regression was used to compare carriage prevalence across time periods, with results reported as prevalence ratios, 95% confidence intervals (CIs) and *P* values. Linear regression was used to compare $\log_{10}$-transformed pneumococcal carriage density across time periods in pneumococcal carriers, with results reported as differences in means, 95% CIs, and *P* values. Percentage changes in pneumococcal carriage density were calculated by $(10^{\hat{\beta}} - 1) \times 100\%$, where $\hat{\beta}$ is the estimate of the linear regression coefficient, representing the difference in means of $\log_{10}$-transformed pneumococcal carriage density in the NPI period compared with the pre-COVID-19 period. Regression analyses were conducted for overall, capsular, and nonencapsulated pneumococcal carriage prevalence and density outcomes.

Three additional analyses were conducted: (i) to evaluate the direct effect of the NPI period on pneumococcal carriage prevalence and density (i.e., the effect not via intermediate variables), an analysis was conducted including district and season in the models; (ii) to evaluate the potential impact of including children with symptoms of upper respiratory tract infection (URTI) in the primary analysis, an analysis was conducted restricting to children without any self-reported symptoms of URTI; (iii) to evaluate whether any associations observed in the primary analysis were independent of the PCV trial intervention, an analysis was conducted restricting to unvaccinated children alone. Statistical analyses were conducted using Stata v16.0 (StataCorp LLC).

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Data availability. Data will be stored in the Bill and Melinda Gates Foundation Knowledge Integration (KI) repository.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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C.S. and M.L.N. conceived this study with input from B.T. and S.M. B.D.O. and A.C.W.-H. conducted the laboratory analyses with oversight from C.S. J.H. contributed to the interpretation of microarray data. M.L.N. conducted the data analyses with input from C.S., B.T., and C.D.N. M.L.N., C.S., and S.M. prepared the original manuscript. L.T.T.V., T.L.P., and D.Y.U. coordinated the trial sites, with oversight from H.P.T. K.M. and T.V.N. are principal investigators of the vaccine trial, with contributions from B.T., H.P.T., K.B., V.T.T.D., C.S., H.S.-V., D.Y.U., J.B., and C.D.N. All authors reviewed and approved the final manuscript.

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