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Pandemic Social Distancing and Declines in Nasopharyngeal Carriage of Pneumococcus and Related Antimicrobial-Resistant Genes: Evidence From Household-Based Cohort Studies in Lima, Peru

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Background. Peru implemented restrictive social distancing policies during the COVID-19 pandemic. Few studies have examined patterns of nasopharyngeal pneumococcal carriage and related antimicrobial-resistant genes (ARGs), particularly in community-based settings with intense social distancing policies.

Methods. Nasopharyngeal swabs were systematically obtained weekly from children and adults enrolled in 2 household-based prospective cohorts in Lima, Peru: prepandemic (2019–2020) and pandemic (2020–2021). Selected samples underwent sequencing-based detection of *Streptococcus pneumoniae* and related ARGs. We compared detections of pneumococcus and *erm* genes between cohorts during the same 7 epidemiologic weeks in 2020 and 2021 while accounting for relevant covariates and household clustering by mixed effects logistic regression models.

Results. An overall 663 specimens from 114 individuals were included: 407 and 256 samples from the prepandemic and pandemic cohorts, respectively. Carriage of pneumococcus was lower in the pandemic cohort (3.5%) as compared with the prepandemic cohort (23.6%; adjusted odds ratio, 0.09; 95% CI, .02–.32). Similarly, *erm* gene carriage was lower in the pandemic cohort (28.1%) as compared with the prepandemic cohort (58.7%; adjusted odds ratio, 0.21; 95% CI, .10–.46).

Conclusions. We observed a substantial reduction in the carriage of *S pneumoniae* and related ARGs in community-dwelling individuals during a pandemic period with intense social distancing in Lima, Peru.

Keywords. antibiotic resistance genes; colonization; COVID-19 pandemic; next-generation sequencing; pneumococcus.

Streptococcus pneumoniae (pneumococcus) is a leading bacterial cause of global morbidity and mortality. Upper respiratory tract carriage with pneumococcus is an important prerequisite step in the development of mucosal and invasive pneumococcal disease [1]. Pneumococcus commonly colonizes the nasopharynx of young children [2, 3]. Colonizing pneumococci spread through respiratory secretions among people in close contact, and children play an important role as reservoirs, spreading the bacteria to others, including household members and persons in congregate settings such as schools and day cares [4–6].

Pneumococcal isolates often exhibit resistance to common antibiotics. In a previous study conducted in several Latin American countries, >50% of disease-associated isolates were nonsusceptible to penicillin and >60% had resistance to erythromycin, but resistance varied widely by country [7]. Similarly, a study of Peruvian children found pneumococcal resistance to penicillin in 34% of colonizing isolates and to erythromycin in 22.4% [8]. Pneumococcal resistance to these antibiotics is commonly associated with bacterial genes. Erythromycin ribosome methylase (*erm*) genes confer resistance by methylation of 23SrRNA to prevent macrolide binding. Penicillin-binding protein (*pbp*) genes reduce binding affinity of β-lactam antibiotics to their target binding site, the penicillin-binding protein. These antimicrobial-resistant genes (ARGs) are typically detected in the nasopharynx of healthy children, although their presence is not always associated with concurrent

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pneumococcal detection [9]. Transmission of pneumococcus between individuals may also lead to transmission of the ARGs that they harbor, though there may be other pneumococcal-independent mechanisms for gene transmission—for example, from additional commensal organisms or exposure to ARGs in the environment. The prevalence of individual ARG carriage in healthy individuals is understudied.

During the COVID-19 pandemic, strategies such as social distancing and masking were recommended to curtail SARS-CoV-2 spread. While these were adopted in many parts of the world, restrictions and uptake by the community were variable, with some regions implementing strict and prolonged restrictions and others applying more relaxed measures and limited enforcement. These interventions have been generally associated with changes in the typical epidemiology of several respiratory pathogens, including pneumococcus. Some studies have reported relatively stable pneumococcal carriage [10–13] during periods of social distancing, which coincided with a decline in invasive pneumococcal disease during the pandemic [14–17]. Nevertheless, adoption of social distancing has been widely variable [18–21], and few studies have evaluated changes in pneumococcal carriage during the pandemic in settings with strict social distancing.

SARS-CoV-2 was first detected in Peru in March 2020. Shortly thereafter, strict lockdowns were implemented by the government also in March 2020. Peru was among the first countries to implement government-imposed lockdowns, including school closures, cancelations of large public gatherings, and stay-at-home orders [22]. Schools were closed within weeks of the first case detection and remained closed for >2 years, fully reopening in March 2022. Peru had one of the most strict and longest lockdown responses to the pandemic, relative to many other countries where rollout of mitigation strategies was limited, delayed, or variably enforced [23].

We prospectively enrolled 2 household-based cohorts from the same district of Lima, Peru. One cohort was enrolled prior to the COVID-19 pandemic (December 2019–March 2020), and the other was enrolled during the pandemic (November 2020–February 2021) in a period of strict lockdown. We compared the prevalence of nasopharyngeal carriage of pneumococcus and related ARGs (*erm*, *pbp*) between the cohorts.

METHODS

Study Design and Setting

We prospectively enrolled 2 household-based cohorts in San Juan de Lurigancho, a densely populated periurban district in Lima, Peru. In the study area, most basic services are available to enrolled homes, including piped potable water, sewage, and electricity [24]. Enrollment of the first cohort began in December 2019 and continued until March 2020, when the study was stopped due to the start of the COVID-19 pandemic. We then resumed enrollment of a new cohort from the same study area from November 2020 to February 2021 using a similar approach to our prepandemic cohort, although with modified selection criteria [24]. After informed consent was obtained, household members were enrolled and followed through twice-weekly household visits to assess the presence of acute respiratory illness and obtain respiratory samples.

Enrollment and Follow-up of the 2 Study Cohorts

For both cohorts, potentially eligible households were identified by a community-wide census within the same study district prior to the study. Households were excluded if they had plans to move outside the study area and would not be available for follow-up. Follow-up began at enrollment and continued through the end of study, voluntary withdrawal from the study, loss to follow-up, or death of any household family member.

Selection Criteria and Study Population

For the prepandemic cohort, households were eligible for inclusion if they contained at least 1 child aged 5 to 60 months and at least 1 other household member willing to enroll. Follow-up stopped in March 2020 immediately after the first case of SARS-CoV-2 was detected in Peru. The pandemic cohort began enrollment in November 2020 and continued follow-up through March 2021, during the COVID-19 pandemic, with modifications to household eligibility criteria. For the pandemic cohort, households with at least 1 child (<18 years of age), 1 younger adult (18–50 years), and 1 older adult (>50 years) were eligible for enrollment. Three members from each household of varying sizes were enrolled into the prepandemic and pandemic cohorts. Both cohorts were enrolled from the same study area approximately 1 year apart.

Selection of Subset of Households

From all enrolled households, a subset was selected for inclusion in this analysis. As part of another study assessing the prevalence and incidence of SARS-CoV-2 in this population, we first selected a convenience sample of households from the pandemic cohort for sequencing if a SARS-CoV-2 infection occurred in any family member. A corresponding household from the prepandemic cohort was then selected by household composition and calendar period of observation to account for seasonal variation in viral activity. Some households from the prepandemic and pandemic cohorts had occurrence of acute respiratory illness but not all.

Data Collection

Trained fieldworkers visited households twice weekly to assess for the presence of respiratory symptoms and use of antibiotics for each day of surveillance. A onetime structured questionnaire was administered to assess basic demographics, medical history including vaccination status, and patterns of social interaction among participating household members, within

and outside the home. COVID-19 vaccines became available in the region in February 2021 and were offered only to health care staff and frontline pandemic control workers at that time. No COVID-19 vaccination occurred in our population during the pandemic study period.

Specimen Collection

During surveillance, weekly nasopharyngeal swabs were collected from each enrolled household member, regardless of respiratory symptoms, for assessment of bacterial and ARG carriage. Nasopharyngeal swabs were collected via rayon swabs and placed in skim milk–tryptone–glucose–glycerin media [25], stored in cold boxes, and transported to a local laboratory where they were frozen at −80 °C. Frozen samples from selected households were then shipped from Peru to Vanderbilt University Medical Center on dry ice and then stored at −80 °C until sequencing.

Sequencing

The selection process described here yielded 830 samples from individuals in selected households. These samples underwent identification of colonizing bacteria and ARGs per the Respiratory Pathogen ID/AMR Panel (20047050; Illumina). Briefly, DNA and RNA were extracted from aliquots of skim milk–tryptone–glucose–glycerin, and pathogen samples in human background were reverse transcribed into cDNA by the RNA Prep With Enrichment Kit (Illumina). Sequencing libraries were prepared with the RNA Prep With Enrichment Kit and DNA/RNA unique dual indexes, combining cDNA and DNA samples into a single tube. Libraries were enriched as 1-plex reactions via the Respiratory Pathogen ID/AMR Panel. Libraries were denatured and diluted to a final loading concentration of 2 pM and sequenced on the NovaSeq 6000 sequencing instrument at PE150 read lengths. Reads were trimmed to 75 base pairs. FASTQ sequencing data files were input to the IDbyDNA Respiratory Pathogen ID/AMR Platform for detection of pneumococcus and related ARGs. We defined detection of an *erm* gene as any detection of *ermC*, *erm36*, *ermB*, *ermX*, *ermF*, or *ermA*. We defined detection of a *pbp* gene as any detection including *pbp1a* or *pbp2X*.

Statistical Analysis

We calculated the crude prevalence of pneumococcal carriage, *erm* gene, and *pbp* gene detections and compared them between the cohorts during overlapping epidemiologic weeks (weeks 1–7). The overlapping 7 epidemiologic weeks were 2 January 2020 to 14 February 2020 and 4 January 2021 to 19 February 2021 for the prepandemic and pandemic groups, respectively. The comparisons were made for the overall population and stratified by age group (0–4, 5–17, 18–44, >44–75 years).

We used mixed effects logistic regression models to compare the detection of pneumococcus and *erm* genes between the cohorts, adjusting for age, sex, and epidemiologic week. Our primary analysis was conducted by restricting the study population to individuals who were followed during epidemiologic weeks 1 to 7 and were <75 years old. This was done to ensure that the observation time and age distribution were comparable between the cohorts. Additionally, we fit a logistic regression model for the cohort indicator adjusting for age, sex, epidemiologic week, and household size to evaluate the overall overlap of propensity scores between study cohorts derived from the model. Due to the scarcity of *pbp* detections during the pandemic period, *pbp* was not included in multivariable regression analyses. Random intercepts were used to account for clustering at individual and household levels, while fixed effects included cohort, sex, and B-spline functions of epidemiologic week (knots at week 4) and age (knots at 5, 18, and 45 years). To demonstrate the impact of age and epidemiologic week, we plotted the estimated probabilities of detecting pneumococcal carriage and *erm* genes against age (for females at epidemiologic week 3) and epidemiologic week (for females at a median age of 22.7 years) for the prepandemic and pandemic cohorts. The bootstrap method for clustered data was used to construct the 95% CIs based on 2.5% and 97.5% quantiles from 5000 bootstrap samples [26]. A sensitivity analysis was performed including adults >75 years of age and nonoverlapping epidemiologic weeks. All statistical tests were performed in Stata and R software (version 4.3.3.) with packages *lme4* and *splines*. The resulting 2-sided *P* values <.05 were considered statistically significant.

RESULTS

Description of Study Participants

For this study, there were 120 households containing 395 individuals enrolled in the prepandemic cohort, with 90% retention during the follow-up period, and 44 households containing 132 number of individuals enrolled in the pandemic cohort, with 86% retention during the follow-up period. Median household size was 4 (IQR, 2.8–6.3) in the prepandemic cohort and 5 (IQR, 4.0–6.3) in the pandemic cohort. For sequencing, we selected 830 specimens (prepandemic, 414; pandemic, 416) from 32 households including 114 unique individuals: 63 and 51 from the prepandemic and pandemic cohorts, respectively (Figure 1).

After exclusion of observations outside the 7 epidemiologic weeks of interest and individuals aged >75 years, there were 663 samples included in statistical analysis: 407 samples in the prepandemic cohort and 256 in the pandemic cohort (Figure 1). Sociodemographic characteristics of the cohorts are summarized in Table 1. Even after exclusion, there remained notable differences in sociodemographic

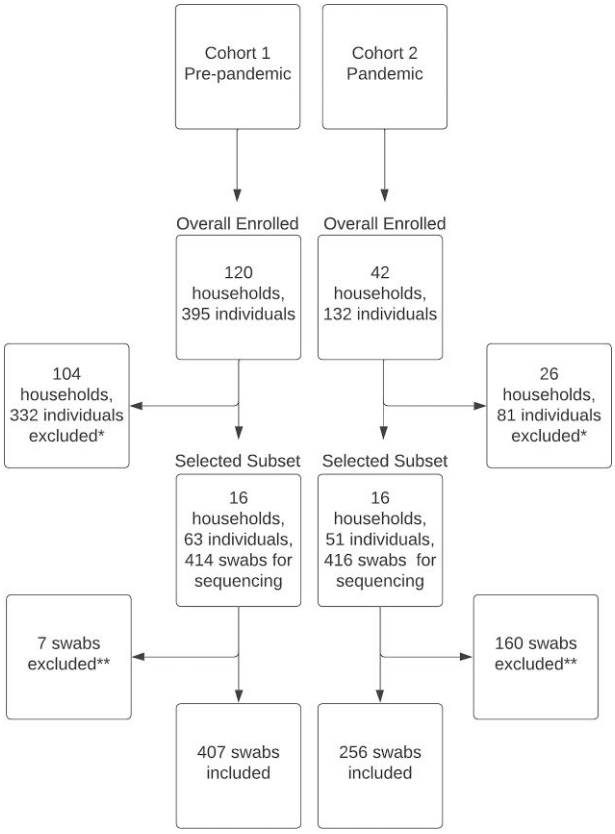

Figure 1. Flow diagram of prepandemic and pandemic cohorts enrolled into the study. *Swabs were included if they met selection criteria of similar household composition or overlapping calendar weeks. **Swabs were excluded if they contributed during nonoverlapping epidemiologic weeks and from individuals >75 years of age.

characteristics between cohorts. However, the data exhibited substantial overlap, a point supported by the density plot of the propensity score (Supplementary Figure 1). We adjusted for those confounders in the multivariable analysis.

Nasopharyngeal Carriage of Pneumococcus and *erm* and *pbp* Genes

In the unadjusted analysis, carriage of pneumococcus was 96 of 407 (23.6%) in the prepandemic cohort and 9 of 256 (3.5%) in the pandemic cohort. An *erm* gene was detected in 239 of 407 (58.72%) samples in the prepandemic cohort and 72 of 256 (28.13%) in the pandemic cohort. A *pbp* gene was detected in 29 of 407 (7.13%) samples in the prepandemic cohort and 1 of 256 (0.39%) in the pandemic cohort (Table 2). The prevalence of pneumococcus, *erm* genes, and *pbp* genes stratified by age group is displayed in Figure 2. As expected, *pbp* and *erm* detections were not restricted to observations with pneumococcal detections. An *erm* gene was detected in 152 of 311 (48.9%) without pneumococcal detection in the prepandemic cohort and 64 of 247 (25.9%) in the pandemic cohort.

In the multivariable analysis, after adjusting for sex and epidemiologic week and controlling for household and individual cluster, detection of pneumococcus (adjusted odds ratio, 0.09; 95% CI, .02–.32) and detection of any *erm* gene (adjusted odds ratio, 0.21; 95% CI, .10–.46) were significantly lower during the pandemic as compared with the prepandemic period. The predicted probabilities of pneumococcus and *erm* gene detection by epidemiologic week and age are shown in Figure 3 for the prepandemic and pandemic cohorts.

Sensitivity analysis that included adults >75 years of age and nonoverlapping epidemiologic weeks led to similar conclusions.

DISCUSSION

In these prospective household-based cohorts in Peru, we demonstrated that nasopharyngeal carriage of pneumococcus was significantly lower during the COVID-19 pandemic as compared with a prepandemic period, which coincided with strict implementation of social distancing measures and prolonged school closures. The largest reductions were observed in children <5 years of age. We similarly observed declines in macrolide-associated ARG detection during the pandemic period. *Erm* gene detections were commonly detected prepandemic but significantly less during the pandemic when accounting for age, sex, and household clustering. *Pbp* gene detections were very low during the pandemic period, precluding evaluation in a multivariable regression model.

There is variability in pneumococcal carriage prevalence estimates across studies, as many studies have different designs or use different detection methods, but in general, the prevalence of pneumococcal carriage is higher in young children and adults in close contact with young children and lower in older adults. Specific prevalence estimates may depend on the pneumococcal detection methods employed. One systematic review and meta-analysis of pneumococcal carriage in children <5 years of age found a pooled prevalence estimate of 47.8% (95% CI, 44.7%–50.8%) in lower- and middle-income countries [27, 28]. The frequency of pneumococcal carriage that we observed in the prepandemic cohort is similar to what has been previously reported, with higher prevalence in young children and lower prevalence in older populations [27, 29–32].

The decline in pneumococcal carriage that we observed during the COVID-19 pandemic contrasts with studies that have reported relatively stable prevalence of pneumococcus during the pandemic. Danino et al described stable rates of pneumococcus carriage in healthy children <3 years of age using culture-based methods in Israel, despite early full COVID-19 lockdowns, followed by several partial lockdowns when schools were intermittently open [14]. In a study conducted in Belgium, Willen et al found no difference in pneumococcal carriage among children attending day care centers during the

Table 1. Characteristics of Prepandemic (2020) and COVID-19 Pandemic (2021) Household-Based Cohorts: San Juan de Lurigancho, Lima, Peru

	Prepandemic	COVID-19 Pandemic	Test Statistic (*P* Value)
No. of participants	407	256	
Epidemiologic week			$F_{1,661} = 17.55$ (<.01)
Median [range]	4 [1–7]	5 [1–7]	
Mean ± SD	3.94 ± 1.97	4.58 ± 1.75	
Household size, median (IQR)	4 (2.42–5.17)	5 (4.0–6.58)	$F_{1,30} = 4.55$ (<.01)
Age, y, median (IQR) [range]	13.3 (4.5–36.8) [0.7–72.4]	26.9 (4.6–55.2) [2.1–74.1]	$F_{1,661} = 22.20$ (<.01)
Age group, y, No. (%)			
0–4	123 (30.22)	79 (30.86)	
5–17	109 (26.78)	20 (7.81)	
18–44	123 (30.22)	68 (26.56)	
≥45	52 (12.78)	89 (34.77)	
Female sex, No. (%)	276 (67.81)	206 (80.47)	$\chi^2 = 12.68$, $df = 1$ (<.01)

Table 2. Sequencing Results From Prepandemic (2020) and COVID-19 Pandemic (2021) Household-Based Cohorts: San Juan de Lurigancho, Lima, Peru[a]

	Prepandemic	COVID-19 Pandemic	χ^2, $df = 1$ (*P* Value)
No. of nasopharyngeal swabs	407	256	
Streptococcus pneumoniae	96 (23.59)	9 (3.52)	47.50 (<.01)
Any *pbp* gene	29 (7.00)	1 (0.24)	16.50 (<.01)
pbp1a	17 (4.18)	0 (0.00)	10.97 (<.01)
pbp2X	13 (3.19)	1 (0.39)	5.98 (.01)
Any *erm* gene	239 (58.72)	72 (28.13)	59.08 (<.01)
ermC	123 (30.22)	34 (13.28)	24.95 (<.01)
erm36	29 (7.13)	3 (1.17)	12.13 (<.01)
ermB	31 (7.62)	0 (0.00)	20.46 (<.01)
ermX	43 (10.57)	21 (8.20)	1.01 (.32)
ermF	6 (1.47)	4 (1.56)	0.01 (.93)
ermA	7 (1.72)	10 (3.90)	3.01 (.08)

[a]Data are presented as No. (%). Detections were obtained from Illumina sequencing of nasopharyngeal swabs collected during the same 7 epidemiologic weeks before and during the COVID-19 pandemic. All prevalence estimates are adjusted by age, sex, and household clustering.

pandemic, though notably day care centers in Belgium remained open during this time, which may have contributed to the sustained high carriage rates observed [15]. Several other studies with varying methodologies reported relatively stable pneumococcal colonization rates during the pandemic [13, 16, 17]. In these studies, nonpharmaceutical interventions were not as intense or did not last as long as in Peru. Our study population is unique in that Peru experienced more strict and prolonged community lockdowns, including prolonged closure of schools and day cares, when compared with other countries. One possible contributor to these observed differences is the intensity or focus of social mitigation measures, with some countries focused more intensely on community-wide restrictions and others more on restricting adults rather than children by keeping schools and day cares open. During the pandemic weeks in our study location, community movement was extremely limited, with strict enforcement and schools and day cares closed for a prolonged period relative to other countries. The prolonged closure of schools and day cares in Peru likely disrupted transmission of colonizing pneumococci, particularly in younger children, who are known to be frequent distributors [12, 33]. The reduction in colonizing pneumococci that we observed may have been a secondary benefit of social mitigation measures and disruption in typical socializing behaviors. Differing methods of pneumococcus detection may have also contributed to the observed differences, as we applied culture-independent next-generation sequencing in contrast to the culture-based methods applied in other studies.

The frequency of nasopharyngeal carriage of ARGs, independent of bacterial detections, is not well known, particularly among healthy individuals in the community, though some studies have shown that carriage with ARGs can be high in young children [9]. A US study by our group assessed carriage rates of bacteria and abundance of antibiotic resistance genes commonly associated with these bacteria in the nasopharynx of young children by quantitative polymerase chain reaction before and during short courses of antibiotics. Carriage of at least 2 genes associated with *S pneumoniae* resistance to penicillins (*pbp2b*) or macrolides (*mef*, *ermB*) occurred in every child, even prior to prescription antibiotic exposure. Following a short course of antibiotics, pneumococcal carriage was nearly eradicated, yet abundance of certain ARGs increased, specifically *erm* genes [9]. In this study, we describe patterns of *erm* and *pbp* gene carriage in healthy individuals of all ages, independent of concurrent bacterial detections. As expected, *erm* genes were frequently observed with pneumococcus. However, these genes were also detected in the absence of pneumococcal codetection, suggesting that other commensal bacteria, including other streptococci, may harbor these genes [9].

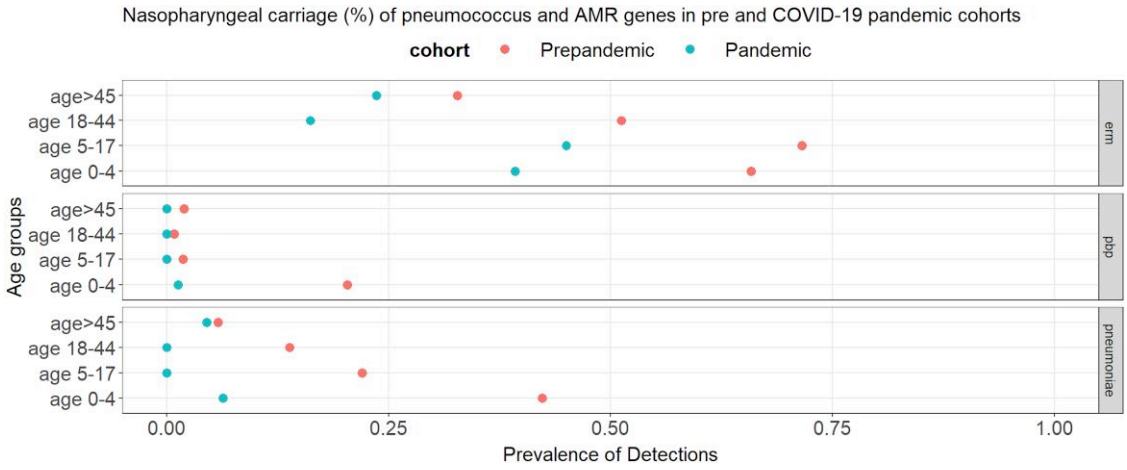

Figure 2. Unadjusted prevalence of nasopharyngeal carriage (percentage) of pneumococcus and *erm* and *pbp* genes stratified by age group in the prepandemic and pandemic cohorts. AMR, antimicrobial resistant.

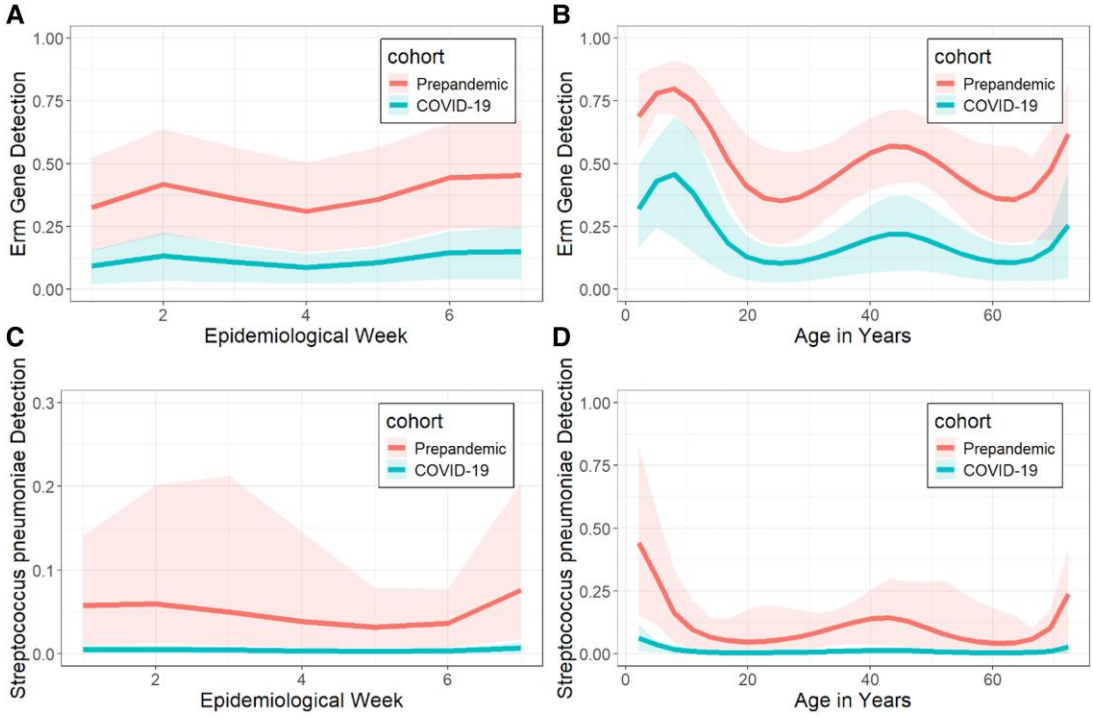

Figure 3. Estimated probability of pneumococcal and *erm* carriage by cohort by epidemiologic week and age. Detections were obtained from Illumina sequencing of nasopharyngeal swabs collected during the same 7 epidemiologic weeks before and during the COVID-19 pandemic. All predicted probabilities were obtained by mixed effect logistic regression models accounting for age, epidemiologic week, and clustering effects from individuals and household. *A* and *C*, Estimated probabilities for females at the median age of 22.7 years. *B* and *D*, Estimated probabilities for females at epidemiologic week 3. Shading indicates 95% CI.

There are several strengths of this study. We examined well-characterized cohorts of healthy children and adults. Since both cohorts were assessed by almost identical design and methods in the same community and over similar calendar periods, we are uniquely able to make comparisons between cohorts. Because both cohorts were enrolled from the same study area, we expect them to be of similar composition, including socioeconomic demographics. Unique to our study is examination of ARGs related to pneumococcus. Unlike other studies, our study encompassed community-dwelling individuals

of all ages, typically from multigenerational households, which allowed us to examine the association of age and pneumococcal and ARG detections. Additionally, we relied on culture-independent next-generation sequencing techniques for detection of bacteria and ARG carriage. Traditional culture-based methods alone may have lower sensitivity for detecting pneumococcus. This approach also revealed ARG carriage irrespective of bacterial detection.

Our study also has several limitations. First, while our 2 cohorts were enrolled from the same study area and households were very similar in composition, each cohort comprised different households, making direct comparison more challenging due to the possibility of unmeasured confounders. Our pandemic cohort had overrepresentation of female sex and was older. This was likely due to different enrollment criteria for the pandemic cohort and the need to be available during the workweek for study visits and sample collection. Despite this, the 2 cohorts had the same distribution of preschool age children, who are known to have the highest pneumococcal carriage [5], and in our adjusted analysis we accounted for age. Second, the use of next-generation sequencing is not the gold standard for detecting colonizing pneumococci, which makes comparison with other studies using different methods challenging [34]. However, our systematic application of this approach may have led to nondifferential misclassification of pneumococcal carriage, making it harder to detect differences between cohorts. Third, there are confounding variables that we were unable to account for, including antibiotic usage and the presence of viral infection or acute respiratory illness symptoms, both of which may have differed between our cohorts [35, 36]. Antibiotic utilization in Peru is among the highest of Latin American countries [37], and usage may have increased during the pandemic [38, 39], although we were unable to account for this potential difference between cohorts. The epidemiology of several respiratory viruses changed during the pandemic, with large reductions in respiratory viral infections and disruption of regular seasonal patterns of influenza and respiratory syncytial virus in most regions that implemented nonpharmaceutical interventions [40, 41]. While ARIs occurred in both cohorts, we did not account for the specific type of viral infection, which may have differed between the cohorts. The enrollment period, however, occurred outside the regular influenza season in Peru, and we did not observe any influenza cases in either cohort. Last, our findings apply directly to the study population with 1 period subject to particular social distancing restrictions and policies; thus, observations may not be directly applicable to other settings with less strict implementation of restrictions.

In summary, we demonstrated lower prevalence of nasopharyngeal pneumococcal carriage during the COVID-19 pandemic as compared with a prepandemic period in a periurban district of Lima, Peru. Similarly, we observed lower prevalence of *erm* genes and low overall detection of *pbp* genes. The decline in carriage was observed during periods of intense COVID-19 pandemic–related social distancing restrictions. These findings provide further evidence that close social and physical interactions, especially among children, may facilitate the spread of pneumococcus and that social mitigation strategies such as masking, school closures, and physical distancing may decrease carriage and/or transmission of pneumococcus and related ARGs.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. C. E. C., L. M. H., and C. G. G. conceptualized and designed the current study, drafted the initial manuscript, and reviewed and revised the manuscript. Q. C. and H. C. performed the statistical analysis. A. J. and K. B. carried out the sequencing. C. F. L., L. M. H., C. G. G., A. I. G., L. E., S. R., and R. C. designed and conducted the original cohort studies, and A. I. G., M. O., B. P., and O. F. managed all study samples at the study site. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Patient consent statement. This study was approved by the institutional review board at Vanderbilt University Medical Center and the Instituto de Investigacion Nutricional ethics committee. Individuals enrolled voluntarily and received no compensation for their participation. Caregivers provided written informed consent for children.

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