MAJOR ARTICLE

The Role of Influenza and Parainfluenza Infections in Nasopharyngeal Pneumococcal Acquisition Among Young Children

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Background. Animal models suggest that influenza infection favors nasopharyngeal acquisition of pneumococci. We assessed this relationship with influenza and other respiratory viruses in young children.

Methods. A case-control study was nested within a prospective cohort study of acute respiratory illness (ARI) in Andean children <3 years of age (RESPIRA-PERU study). Weekly household visits were made to identify ARI and obtain nasal swabs for viral detection using real-time reverse-transcription polymerase chain reaction. Monthly nasopharyngeal (NP) samples were obtained to assess pneumococcal colonization. We determined whether specific respiratory viral ARI episodes occurring within the interval between NP samples increased the risk of NP acquisition of new pneumococcal serotypes.

Results. A total of 729 children contributed 2128 episodes of observation, including 681 pneumococcal acquisition episodes (new serotype, not detected in prior sample), 1029 nonacquisition episodes (no colonization or persistent colonization with the same serotype as the prior sample), and 418 indeterminate episodes. The risk of pneumococcal acquisition increased following influenza-ARI (adjusted odds ratio [AOR], 2.19; 95% confidence interval [CI], 1.02–4.69) and parainfluenza-ARI (AOR, 1.86; 95% CI, 1.15–3.01), when compared with episodes without ARI. Other viral infections (respiratory syncytial virus, human metapneumovirus, human rhinovirus, and adenovirus) were not associated with acquisition.

Conclusions. Influenza and parainfluenza ARIs appeared to facilitate pneumococcal acquisition among young children. As acquisition increases the risk of pneumococcal diseases, these observations are pivotal in our attempts to prevent pneumococcal disease.

Keywords. influenza; parainfluenza; pneumococcal colonization; Peru; children.

Severe pneumonia is usually caused by bacteria, most commonly *Streptococcus pneumoniae* [1]. *Streptococcus pneumoniae* can colonize the human nasopharynx, and children are particularly susceptible to colonization [2]. Although most nasopharyngeal colonization does not result in disease, the acquisition of a new serotype is a critical step in disease pathogenesis [2–4].

Several lines of evidence indirectly support a pathogenic synergism between influenza virus and pneumococcus. First, the prevalence of pneumococcal colonization increases during winter, when influenza and other respiratory viruses circulate [5–8], and more specifically, during episodes of acute respiratory disease in both children and adults [9, 10]. Second, cases of pneumococcal pneumonia increase during influenza epidemics [11, 12]. Third, pneumococcal pneumonia during an influenza episode appears to be more severe [11, 13].

In an efficacy trial of an experimental 9-valent pneumococcal conjugate vaccine (PCV9) in South African children, the incidence of pneumonia during concurrent influenza infections was reduced by 45% in the

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PCV9-vaccinated group compared with controls [14]. Significant pneumonia reductions were also observed for concurrent infections with parainfluenza viruses (PIVs) and human metapneumovirus (MPV) [14, 15]. However, a smaller randomized trial of a similar 11-valent vaccine in the Philippines failed to replicate these observations [16].

Experimental pneumococcal infection of animals previously infected with influenza results in severe disease and death [13]. Whether virulence factors common to both pathogens (ie, neuraminidase) [13] and/or other immunological host factors (eg, impairment of macrophages and neutrophil function) [17–20] mediate this interaction remains unclear. Animal studies also suggest that influenza infection facilitates the transmission of some pneumococcal serotypes [21, 22]. A study of experimental influenza infection in human adults failed to demonstrate a significant increase in pneumococcal colonization; however, the sample size was small and the colonization assessment used oropharyngeal rather than nasopharyngeal samples [23]. Similar studies have not been conducted in children, and whether influenza or other viral infections facilitate nasopharyngeal pneumococcal acquisition remains unknown.

We sought to test the hypothesis that infection with specific respiratory viruses, including influenza virus, increases the risk of acquisition of a new pneumococcal serotype in a cohort of young children living in the Peruvian Andes.

METHODS

This was a case-control study nested within the prospective study of respiratory infections in Peruvian Andean children (RESPIRA-PERU) [24, 25]. Children <3 years of age were enrolled and followed through weekly household visits. Symptoms of acute respiratory illness (ARI) were recorded during each visit, and nasal swabs were obtained during episodes of ARI for identification of respiratory viruses. Pneumococcal colonization was determined through routine monthly collection of nasopharyngeal (NP) swabs without regard to respiratory symptoms [24].

Study Setting

The study was conducted in the Province of San Marcos, Department of Cajamarca, Peru. The Province of San Marcos includes areas of variable altitude ranging from approximately 1500 to 4000 meters above sea level. This rural population has low income, low educational level, and limited access to healthcare services. The median per capita income per month in 2007 (last census) was US $71.10, ranking at the 36th percentile for Peru; 74% of the population is considered poor and 34% live in extreme poverty [24].

Study Cohort Assembly

After institutional review board approvals from Vanderbilt University and the Instituto de Investigacion Nutricional (Lima, Peru) were obtained, study personnel contacted local community authorities from each community in the enrollment area to discuss the nature of the study and request approval. After approval was obtained, a complete enumeration of eligible population in the study catchment area was obtained through a local census.

Selection Criteria

To assure a representative sample of the study communities, broad selection criteria were used to identify study children: (1) families with children aged <3 years (including newborns), and (2) intention to remain in the study area for the next year.

Study Conduct

After informed consent, trained field-workers obtained baseline demographic and socioeconomic information and data on health services utilization patterns and information on known risk factors for ARI, including history of vaccination. Permission was also obtained to access enrollees' medical and vaccination records at the local health centers throughout the study.

Cohort members were followed through weekly household visits by field-workers who visited the home of each enrolled child and interviewed the parent or guardian about signs/symptoms of ARI over the preceding week, recording the date of onset, duration, and specific symptoms. Field-workers were trained in data and sample collection and in the recognition of respiratory signs and symptoms through workshops and reviews of educational material prepared for training in the Integrated Management of Childhood Illnesses (IMCI) World Health Organization protocol [26].

Follow-up started in May 2009 and continued through either the third birthday, withdrawal of consent, loss to follow-up (travel outside the area or inability to locate), death, or the end of the study (30 September 2011). To maintain the size of the study cohort relatively constant, additional newborns were enrolled as they were identified. The study employed a physician to provide care to children identified with IMCI danger signs [24].

Colonization Episodes and Study Period

Consecutive NP swabs were compared to determine acquisition of pneumococcal colonization. Collected NP samples were placed in skim milk, tryptone, glucose, and glycerine media [27], transported to the local research laboratory, and stored at −70°C (as recommended for long-term storage) [28] until shipment to Emory University for the identification of *S. pneumoniae* using conventional bacteriological cultures and quantitative polymerase chain reaction (PCR). *Streptococcus pneumoniae* serotypes were identified using a multiplex PCR approach [29]. NP testing has been completed for years 2009 and 2011 to allow the assessment of changes in pneumococcal

serotypes during the introduction of pneumococcal conjugate vaccines in the population. Thus, the current study was restricted to colonization data from those years (approximately 3600 NP tested).

A *new acquisition episode* was defined as the identification of a *S. pneumoniae* serotype that was not present in the previous NP sample, and included those episodes with pneumococcal colonization detected after having none detected in the previous sample. The date of the nasopharyngeal sample collection that determined the acquisition was termed the "index date."

A *nonacquisition episode* was defined as 2 consecutive swabs in which there was no pneumococcal colonization in the second swab or in which the same serotype was identified in both swabs.

Indeterminate episodes included those in which both swabs were nontypable or in which serogroups could not be completely distinguished.

Exposures

For both pneumococcal acquisition and nonacquisition episodes, study exposures (viral ARI) were measured during the risk window between the index date and the date of the previous nasopharyngeal sample (approximately 1 month apart). Specific viral ARIs were categorized in the following hierarchy: influenza, PIV, respiratory syncytial virus, MPV, human rhinovirus (HRV), and adenovirus.

Identification of Viral Respiratory Infections

Nasal swab specimens were obtained during episodes of ARI, placed in virus transport medium, and transported to the local laboratory where lysis buffer was added before storing at −70° C until shipment to Vanderbilt University for long-term storage at −70°C [30, 31], and for molecular testing with monoplex real-time reverse-transcription PCR. To determine the integrity of the samples, random subsets were also tested for RNase P [24].

Statistical Analysis

We first evaluated the association between pneumococcal acquisition and study covariates including demographics, socioeconomic, and other environmental factors and exposure to viral ARI. The association between any ARI or a specific respiratory viral ARI and pneumococcal acquisition was assessed using a multivariate random-effects logistic regression model that accounted for those covariates associated with pneumococcal acquisition. Because children were allowed to contribute >1 episode of observation, all analyses accounted for the correlation introduced by the clustering of observations at the subject level (individual study identifiers were used to identify the clusters in the regression models). Acquisition of a new pneumococcal serotype was the model outcome, and ARIs or specific viral ARIs were the main study exposures. Absence of an ARI during the observation episode was used as the reference for all comparisons. Secondary analyses included a separate evaluation of the different types of pneumococcal acquisition: from no colonization to colonization, and from colonization to colonization with a new serotype; and an evaluation of individual respiratory viruses, grouping coinfections in a separate exposure category. As routine vaccination with pneumococcal conjugate vaccine was initiated in the study communities during the conduct of the study, subgroup analyses based on calendar year and pneumococcal vaccination status were also conducted. As some viral infections may affect predominantly young children, separate subgroup analyses stratified by age (ie, infants vs older children) and by daycare attendance were also conducted. Furthermore, to explore whether viral infections may facilitate pneumococcal colonization through the elimination of existing colonizing pneumococcus, we assessed the transition from pneumococcal colonization to no colonization among exposure groups. All statistical analyses were performed in Stata 12.1 (StataCorp, College Station, Texas).

RESULTS

This nested case-control study included data from 729 children or 82% of the RESPIRA-PERU prospective cohort [24]. There were 2128 observation episodes in which 2 consecutive monthly NP samples were collected. The median duration of the observation episodes was 28 days (interquartile range, 28–35 days). Among the observation episodes, we identified 681 (32%) acquisition episodes, 1029 (48%) nonacquisition episodes and 418 (20%) indeterminate episodes.

A total of 349 (51%) pneumococcal acquisition episodes represented acquisition after initial NP samples tested negative for pneumococcus, 268 (39%) represented acquisition of a new serotype/serogroup after having been initially colonized with a different serotype/serogroup, and 64 (9%) represented acquisition of a new serotype/serogroup after being initially colonized with a nontypable pneumococcus. Among acquisition episodes the most frequently identified pneumococcal serotypes were 23F, 19F, 6B, 6C, 11A, and 10A (see Supplementary Data 1 for additional details on the serotype distributions by type of episodes).

Characteristics of Acquisition and Nonacquisition Episodes

There were no significant differences in age or sex between acquisition and nonacquisition episodes. Other socioeconomic and environmental variables were equally distributed between acquisition and nonacquisition episodes, except that children with acquisition episodes had more young children in their households and were more likely to attend a daycare equivalent (ie, "wawawasi"). Approximately 45% of children had received at least 2 doses of pneumococcal conjugate vaccine by their index date. Overall, the study characteristics reflect the rural, economically disadvantaged study population (Table 1). The

Table 1. Characteristics of Study Episodes by Pneumococcal Acquisition, San Marcos, Peru, 2009–2011

Characteristic	No Pneumococcal Acquisition (n = 1029)	Pneumococcal Acquisition (n = 681)	*P* Value
Demographic			
Age, mo, median (IQR)	17 (10–27)	18 (11–28)	.114
Female sex, No.	48.2	50.7	.320
No. of household members, median (IQR)	5 (4–6)	5 (4–6)	**.074**
No. of household members aged <5 y, median (IQR)	1 (1–2)	1 (1–2)[a]	.004
Socioeconomic			
No. of rooms in house, median (IQR)	2 (1–3)	2 (1–3)	.841
No. of bedrooms in house, median (IQR)	1 (1–2)	1 (1–2)	.540
Child sleeps with another household member	96.1	96.6	.918
House floor: dirt	89.8	88.8	.529
House walls: adobe (mud bricks)	99.2	99.7	.217
House roof: tile	95.5	96.9	.150
House/lot has a pipeline for water service	81.6	84.1	.293
House has own bathroom	85.4	83.8	.599
Candlelight used at night	57.3	57.3	.999
Agriculture main occupation of head of household	65.5	62.7	.538
Mother's education: up to elementary	70.5	70.3	.447
Environmental exposures			
Open fire/traditional stove for cooking	67.1	65.6	.712
Use wood for the stove	93.2	92.4	.818
At least 1 dose of influenza vaccine	26.7	26.4	.893
At least 2 doses of pneumococcal conjugate vaccine	46.3	43.2	.209
Child attends daycare	5.5	8.1	**.040**
At least 1 smoker at home	8.8	10.4	.275
Exposure to ARI[b]			
No ARI	51.4	47.7	Reference
ARI no virus detected	12.8	12.2	.882
Influenza ARI	1.2	2.4	**.046**
Parainfluenza ARI	3.5	5.9	**.014**
Respiratory syncytial virus ARI	4.6	5.3	.343
Human metapneumovirus ARI	2.6	2.8	.659
Human rhinovirus ARI	19.0	20.7	.214
Adenovirus ARI	3.2	1.9	.185

Values indicate percentages, unless otherwise specified. Values in boldface indicate statistically significant *P* values.

Abbreviations: ARI, acute respiratory illness; IQR, interquartile range.

[a] Mean higher among acquisition episodes.

[b] All counts, including viral coinfections and based on hierarchy.

prevalence of influenza and parainfluenza viruses was higher among pneumococcal acquisition episodes than among nonacquisition episodes. For influenza, 25 (89%) of infections were due to the A(H1N1)pdm strain, whereas for parainfluenza, 73 (96%) of infections were due to PIV3.

Pneumococcal Acquisition and ARI

The risk of acquisition of a new pneumococcal serotype increased significantly following exposure to influenza and PIV ARI when compared with no ARI exposure (adjusted odds ratio [AOR], 2.19; 95% confidence interval [CI], 1.02–4.69 and AOR, 1.86; 95% CI, 1.15–3.01, respectively). In contrast, the risk of pneumococcal acquisition following an ARI associated with other study respiratory viruses was not significantly different from episodes with no ARI (Figure 1). Exposure to any study viral ARI (AOR, 1.22; 95% CI, .99–1.51) or any ARI (AOR, 1.16; 95% CI, .95–1.41) did not significantly increase pneumococcal acquisition risk when compared with no ARI.

The secondary evaluation by types of pneumococcal acquisition indicated that influenza and PIV infections were strongly

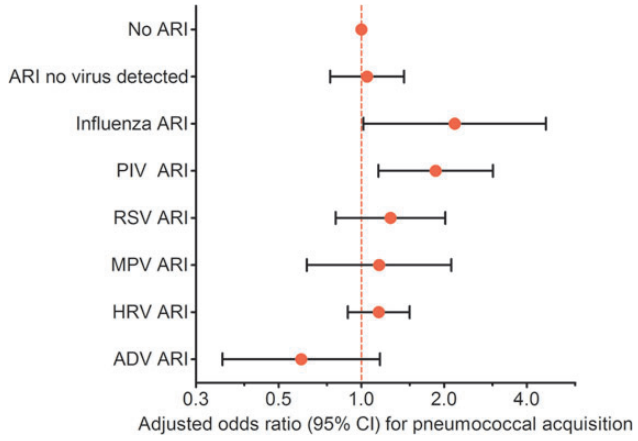

Figure 1. Association between acquisition of a new pneumococcal serotype and previous acute respiratory infection, San Marcos, Peru, 2009–2011. All virus exposures are mutually exclusive. Adjusted odds ratios were obtained from a random-effects logistic regression model that controlled for age, presence of young children at the household, and daycare attendance and accounted for clustering of episodes at the child level. Abbreviations: ADV, adenovirus; ARI, acute respiratory illness; CI, confidence interval; HRV, human rhinovirus; MPV, human metapneumovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

associated with acquisition of a new pneumococcal serotype among children who were already colonized. There was no significant association between any respiratory viral infection and pneumococcal acquisition among children who were not initially colonized (Figure 2). The median age was 17 months in both pneumococcal acquisition groups.

Other secondary and subgroup analyses by year, pneumococcal conjugate vaccination status and daycare attendance showed results consistent with the main findings reported here, but some subgroups had limited power due to small numbers. Of note, the evaluation of individual respiratory viruses, grouping coinfections in a separate exposure category, indicated that ARI with viral coinfections (134 [82%] involved HRV and 38 [23%] influenza or parainfluenza) were also significantly associated with pneumococcal acquisition. The stratified analysis by age group suggested a stronger association with influenza and an attenuated association with PIV among infants, but estimates had limited precision due to small numbers available for these exploratory comparisons (Supplementary Data 2). The stratified analyses of children with 1 or more vaccine doses suggested a relatively consistent association between influenza and PIV infections and pneumococcal acquisition, but there were no influenza infections to explore this specific association among children with >2 vaccine doses (Supplementary Data 2).

The risk of transitioning from pneumococcal colonization to no colonization during the episodes of observation was similar among the exposure groups (Supplementary Data 3).

DISCUSSION

Our study of Andean children provides unique and novel human evidence that laboratory-confirmed influenza and PIV infections significantly increase the risk of acquiring a new pneumococcal serotype. This association seemed to be especially strong for pneumococcal acquisition among children who

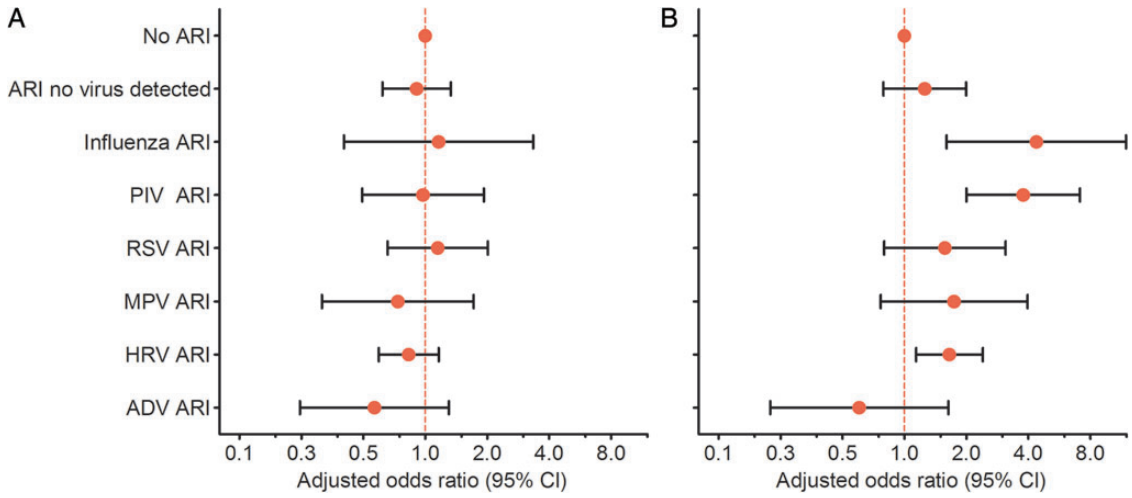

Figure 2. Association between acquisition of a new pneumococcal serotype and previous acute respiratory infections by acquisition type, San Marcos, Peru, 2009–2011. *A*, From no colonization to colonization. *B*, From colonization to colonization with a different serotype. All virus exposures are mutually exclusive. Adjusted odds ratios were obtained from a random-effects logistic regression model that controlled for age, presence of young children at the household, and daycare attendance and accounted for clustering of episodes at the child level. Abbreviations: ADV, adenovirus; ARI, acute respiratory illness; CI, confidence interval; HRV, human rhinovirus; MPV, human metapneumovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

were already colonized with pneumococcus. Because nasopharyngeal acquisition of a new serotype increases the risk of pneumococcal diseases [2, 3], these viral infections likely facilitate the development of pneumococcal diseases.

In 1958, investigators reported that acquisition of a new pneumococcal serotype followed episodes of coryza among young British children [32]. Nevertheless, whether those episodes were due to viral infections was not determined in that study. Another seminal study published in 1975 indicated that acquisition of new pneumococcal serotypes appeared to be associated with ARIs, and the potential role of HRV was examined. However, the study was limited by a small number of pneumococcal acquisition episodes and the lack of molecular laboratory methods; thus, no conclusive association between rhinovirus and pneumococcus was demonstrated [33].

Our findings must be interpreted in light of some limitations. First, other viruses not evaluated in our study might be implicated in acquisition of *S. pneumoniae* serotypes. Although we identified several common respiratory viruses in our samples, we did not test for all respiratory viruses, such as coronavirus, that could be implicated in pneumococcal acquisitions. Second, even though the point estimates of our subgroup analyses were largely consistent with our main findings, we had a limited number of influenza infections detected during the study period, and these were largely restricted to a single respiratory season. A larger number of infections from multiple seasons would have been useful to increase the precision of some estimates. Third, NP samples were collected periodically and it was not possible to identify the exact moment of pneumococcal acquisition. Therefore, we cannot establish the precise temporal association between ARI and pneumococcal acquisition during the observation episodes. However, our study scheduled weekly visits and monthly NP collections uninformed by the presence of ARI or respiratory symptoms. Thus, any potential misclassification in the time of colonization ascertainment is likely nondifferential between acquisition and nonacquisition groups and would favor the null hypothesis. Finally, although we performed intensive surveillance through weekly household visits for detection of ARI, some studies have used more frequent home visits (eg, twice per week) attempting to minimize recall issues. We considered more frequent home visits, but the substantial increase in resources required was prohibitive. Furthermore, such an intensive strategy may have led families to opt out of the study or to modify their behaviors.

There are several hypotheses to explain the apparent synergism between respiratory viruses and pneumococcus. It was postulated that by increasing respiratory secretions, acute respiratory infections could facilitate transmission and acquisition of both viruses and pneumococcus [33, 34]. Other studies that longitudinally collected nasal and nasopharyngeal samples have suggested that during the winter season or episodes of virus-induced acute respiratory disease, the nasopharynx mucous surface colonized with pneumococcus extended forward toward the nasal cavities and downward toward the trachea, facilitating colonization, transmission, and pneumococcal disease [35]. Previous studies using animal models suggested that expression of neuraminidase, an enzyme that cleaves sialic acid on the cell surface and that is produced by influenza, PIV, and pneumococcus, but not the other respiratory viruses tested, could be a common factor that facilitates bacterial adherence, NP pneumococcal colonization, and thus pneumococcal diseases [2–4, 13]. Nevertheless, pneumococcal acquisition and disease occurs even in the absence of influenza or PIV infections, and the precise mechanism for the observed synergism between influenza, PIV, and pneumococcus is unclear.

Building on previous observations from animal models and ecologic studies, a recent study used a compartmental simulation model and administrative databases to explore the nature of the interaction between influenza and pneumococcal pneumonia. Investigators concluded that the most likely nature of this interaction was an enhanced susceptibility to pneumococcal pneumonia within the first week following an influenza infection [12]. The study also acknowledged the crucial role of NP carriage and indicated that the overall impact of this interaction may be difficult to appreciate using aggregated population-level data. These findings are consistent with a previous assessment of the role of influenza on invasive pneumococcal diseases detected through laboratory-based surveillance [36]. Our study complements these previous studies by using prospectively collected individual-level data, systematic collection of respiratory samples, and laboratory-confirmed viral detection and pneumococcal colonization. Furthermore, we focused on pneumococcal acquisition, a more proximal outcome and a necessary step in the pathogenesis of pneumococcal diseases.

Our secondary analysis by type of pneumococcal acquisition suggests that the role of influenza and PIV infections on pneumococcal colonization was restricted to children who were already colonized. There were no age differences between these children and those children who acquired pneumococcus after not being colonized, suggesting that age-related immunity (eg, passive mother-derived immunity) may not explain this observation. Facilitating acquisition of new serotypes through elimination of existing colonizing pneumococcus would not explain our findings, as elimination was similar across exposure groups. Previous experimental studies in animal models have largely focused on new pneumococcal colonization, but there is limited information on the risk of acquisition by replacing colonizing serotype(s) [2, 21, 22]. Although pneumococcal conjugate vaccines prevent acquisition of vaccine serotypes [37], vaccine use started during the study years in our population and was overall relatively low. This allowed us to explore the natural synergism between respiratory viruses and the

pneumococcus in subgroup analysis of unvaccinated children. Finally, the risk of pneumococcal acquisition is modulated by immune and other environmental factors, including the intensity of exposure to the new pneumococcal serotype [2–4]. However, these factors were not measured in our study, and additional research, including other populations, is warranted to clarify this intriguing observation.

In summary, this study assessed the role of influenza and other common respiratory viruses, including PIV, respiratory syncytial virus, MPV, HRV, and adenovirus on pneumococcal acquisition. Exposure to an influenza or PIV ARI facilitated acquisition of new pneumococcal serotypes in young children, whereas exposure to other study respiratory viruses did not. This association seemed to be especially strong among children who were already colonized with pneumococcus. Our study findings suggest a selective synergism between certain respiratory viruses and pneumococcus. Further research into the specific mechanisms of pneumococcal interaction with influenza and PIV is warranted. Because pneumococcal acquisition increases the risk of pneumococcal diseases, these observations are pivotal to developing strategies for disease prevention.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. C. G. G., M. R. G., K. M. E., and C. F. L. designed the study. C. G. G., M. R. G., K. M. E., J. V. W., A. I. G., H. V., S. M. H., and C. F. L. supervised field-work activities and data collection. J. V. W. conducted the viral analyses. J. E. V. and K. P. K. conducted the pneumococcal analyses. C. G. G. conducted the data analyses and drafted the paper. C. G. G., M. R. G., K. M. E., J. V. W., A. I. G., H. V., S. M. H., J. E. V., K. P. K., and C. F. L. reviewed the paper and provided intellectual content. All authors approved the submission of the paper for publication.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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