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Risk Factors for Bacterial Load of Pneumococcal Colonization among Peruvian Children

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Risk Factors for Bacterial Load of Pneumococcal Colonization among Peruvian Children

By

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Bachelor of Science
University of Kentucky
2009

Thesis Committee Chair: Keith Klugman, MD, PhD

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Abstract

Risk Factors for Bacterial Load of Pneumococcal Colonization among Peruvian Children
By Catherine Bozio

Background: *Streptococcus pneumoniae*, and its more than 93 serotypes, is a major cause of child mortality worldwide. Previous studies have established that environmental, biological, social and behavioral factors are important for colonization by *S. pneumoniae*, especially in the developing world. However, no studies have addressed the influence of these risk factors on pneumococcal carriage density. In this thesis, we quantified *S. pneumoniae* present in the nasopharynx of healthy children from a rural community in Peru and investigated the influence of risk factors on bacterial density.

Methods: This was a case-control study. Nasopharyngeal (NP) swabs (N=259) were collected from healthy Peruvian children (<3 years old). *S. pneumoniae* strains were isolated by culture and serotypes were identified. To quantify bacterial load, DNA extracted from NP swabs was analyzed using a *lyt*A-based qPCR assay. Information about risk factors was collected using a questionnaire administered to the children's mothers. To identify risk factors implicated in density, two regression models were constructed: a logistic model that dichotomized the continuous qPCR data into lower and higher bacterial loads and an ordinal logistic regression model that accounted for both colonization status and degree of bacterial load.

Results: The prevalence of pneumococcal colonization among this population was 75.3%, as detected by the qPCR assay. The most prevalent serogroups identified were 6A/B, 15B/C, 23F, 19F, 19A, and 9V/A. In the logistic model, having at least one sibling was a risk factor for density of bacterial colonization (OR=2.79 (95%CI: 1.10-7.06)). In the ordinal logistic model, cooking breakfast for at least 45 minutes (OR=3.24 (95%CI: 1.76-5.98)) and having at least one sibling (OR=1.97 (95%CI: 1.05-3.68)) were also risk factors for pneumococcal density.

Conclusion: Carriage rates of *S. pneumoniae* in children within this community are very high. Risk factors implicated in *S. pneumoniae* density include having at least one sibling and cooking breakfast for at least 45 minutes.

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I. INTRODUCTION

S. pneumoniae is a major cause of disease, including pneumonia, otitis media, meningitis, and sepsis. While these diseases also have other causative agents, *S. pneumoniae* is the primary bacterial cause of pneumonia, which is one of the leading causes of death in children aged less than 5 years of age worldwide. Pneumonia kills an estimated 1.6 million children every year - more than AIDS, malaria and tuberculosis combined [1]. While children under five years of age and the elderly have an increased risk for pneumococcal diseases, the highest burdens of pneumococcal diseases lie in Sub-Saharan Africa and South Asia. Similarly, developing countries have higher incidence and mortality rates due to *S. pneumoniae* [2].

S. pneumoniae, along with other respiratory pathogens, normally resides in the human nasopharynx as a commensal organism in the normal flora. However, not all colonization results in disease [3]. Even when colonized, the host remains asymptomatic until either clearance of the colonized organism occurs or the carrier state progresses to disease. Nasopharyngeal colonization of *S. pneumoniae* is primarily driven by age. Overall, there is a consensus of an increased risk of colonization for children under five years of age, with a particular emphasis on those under three years of age [4-9]. Pneumococcal carriage rates are highest within the first three years of life [3, 10, 11] and decline after that time period. Likewise, in children, pneumococcal colonization can also be detected for 1-4 months, which contrasts with the 2-4 week duration of colonization for adults. Thus, duration of colonization is inversely related with age [9, 12].

In addition to age, there are other factors that influence colonization. A majority of epidemiological studies have focused on the individual predictors for pneumococcal

colonization, including more biologically oriented factors and those of a more social/socioeconomic basis. Note that patterns of colonization differ greatly by region, with rapid/early colonization occurring frequently in most native/aboriginal populations. Regarding gender, a majority of studies found that colonization rates did not differ significantly between girls and boys [7, 13].

Aside from age, health status is also important as a potential risk factor for children. Studies have shown evidence for increased risk of colonization due to chronic illness [5], lower/upper respiratory infections [5, 8], diminished host defenses through viral infections, malnutrition, and/or immune deficiency [7, 9, 14], and/or otitis media [8, 15, 16]. For otitis media, early colonization may be a risk factor for the first occurrence and for recurrent disease as well and this is linked with the direct association between frequency of colonization and episodes of otitis media [17].

Social and behavioral factors are also associated with an increased risk of pneumococcal colonization. For example, daycare centers or orphanages is a well-established risk factor; such crowded environments facilitate horizontal transmission particularly among children as well as within a family's household, and/or within a community [8, 12, 14, 18, 19]. Despite the likelihood that daycare centers, particularly those with more pediatric attendees, are where young children first become exposed to the pneumococcus, the role of family remains an important one in the transmission to and from other siblings and parents/caregivers. Hill et al. provided evidence that the household spread starting most commonly in children, followed by spread to adults [20]. Person-to-person transmission of *S. pneumoniae*, particularly drug-resistant strains, has been demonstrated among siblings with acute otitis media [21]. However, age of the

young child in conjunction with the family composition will affect the likelihood of becoming colonized. For example, Coles et al. showed how infants having at least two siblings under the age of five were at significantly increased risk of carriage at age four months [22] whereas the presence of a family member carrying the same serogroup was a predictor of carriage for children older than six months [4].

Linked with family composition, crowding also tends to be associated with low socioeconomic status, which is linked to poor and cramped housing. Socioeconomic status can also manifest such increased risk through more individual behaviors, such as lower access to health care [12], and more communal characteristics. Huang et al. identified community-level predictors, including living in socioeconomically disadvantaged census tracts and living in a census tract with a median household income of less than $35,000, as contributions to an increased risk of pneumococcal carriage [8]. Along with low socioeconomic status, low level of education has also been demonstrated to be a risk factor [18]; more specifically, this risk is evident in 2 month old infants with mothers who have had less than one year of schooling [22].

Aside from more community and household factors, there are factors that are related to behaviors in the household. Despite overall being beneficial to nursing young, breastfeeding does not appear to substantially influence nasopharyngeal colonization. Coles et al. found that 2-month-old infants who were fed colostrum were more likely to be colonized than those who did not receive colostrum [22]. One study demonstrated that there was no decrease in carriage in infants when the mother received the pneumococcal polysaccharide vaccine [23].

In addition to potentially protecting infants and children through maternal immunization, antibiotic therapy has also been demonstrated to decrease the risk of carriage, particularly therapy taken presently or during the preceding week of the NP swab collection [4, 7]. While such treatment appears protective for typical pneumococcal colonization, antibiotics are a risk factor for antibiotic-resistant *S. pneumoniae* [24].

Evidence indicates that seasonality can also play a role in increased carriage. While *S. pneumoniae* and other respiratory pathogens can be recovered from the nasopharynx throughout the year, the rate of colonization increases in the midwinter [25]. While this is likely due to increased interpersonal contact, decreased ventilation, viral infections, and antibiotic use, reasons remain unclear [12].

In addition to such biological, social, and behavioral risk factors, environmental factors also play a role on the acquisition and carriage of the pneumococcus as well as the development of respiratory disease. In developing countries, cooking and heating solid fuels on open fire or stoves without ventilation mechanisms (i.e., chimney) is common and leads to indoor air pollution. However, the health effects are determined by both the pollution itself as well as the level of exposure. Women and children typically have higher levels of exposure as women tend to prepare and cook meals and to perform domestic duties, especially near the open fire or stove, with children carried on their backs. Because of these simply designed stoves, the incomplete combustion tends to result in substantial emissions, which in conjunction with poor ventilation, produces high levels of air pollution [26]. Inhaling such particulate matters can be hazardous for any person given a certain amount of time; this is especially true for young children whose lungs and airways continue to mature. A randomized trial with improved stoves in

Guatemala supports this with evidence that such predictors for children's carbon monoxide level (the primary pollutant of interest) were stove/fuel type and observed position of the child [27]. Consequently, 56% of all indoor air pollution-attributable deaths occur in children under five years of age [28]. Likewise, children's exposure to passive cigarette smoke can result in an increased risk for colonization of respiratory pathogens and respiratory infections. It is believed that, inhalation of hazardous pollutants from the stove or cigarettes can damage epithelial cells in the respiratory airways and make children more prone to more frequent and severe respiratory infections [29]. Thus, household indoor air pollution from solid fuels can be reduced by improving stoves and ventilation and using more combustible fuels [30]. However, as far as we can tell, there are no studies that have examined the association between indoor air pollution and pneumococcal carriage.

Despite such well-established risk factors for pneumococcal colonization, limited information is available in examining pneumococcal load, particularly any association between nasopharyngeal carriage and pediatric pneumonia [31, 32]. Some recent evidence has demonstrated an association between nasopharyngeal pneumococcal load with viral respiratory tract infections and with pneumococcal serotype [33].

In this prevalent case-control study, we identified significant risk factors for bacterial load of pneumococcal colonization among children less than 3 years of age living in the Andes Mountains in northern Peru.

II. METHODS

Study Design

This case-control study was conducted in the San Marcos Province, Department of Cajamarca, Peru. This study's population of two hundred and fifty-nine healthy children less than three years of age is a subset of Vanderbilt University's parent study of a prospective cohort study nested within a cluster randomized community trial.

For the enrollment of the cohort, initially community/authority approval was obtained and then a complete enumeration of the population in the study catchment area was performed. Field workers visited all houses in the area and interviewed family members using a standardized form. Using information from this local census, fifty-four rural communities were identified, representing approximately 600 households which met the inclusion criteria: 1) the household uses wood for cooking, 2) the household does not have access to potable water, 3) the household is not connected to a public sewage system, 4) there is at least one child aged 0-35 months, 5) there is no intention/plan to move out of the study area for the next year and 6) the eligible child has no chronic medical conditions/congenital defects.

An agreement was signed with all 54 community leaders to participate in the parent study. These communities underwent stratified randomization, based on the size of the land owned; using information collected during the census phase, to receive either the intervention (improved cooking stove) or the control package (educational material and toys, appropriate for each 3-month of age window, during a 12 month period). This study was approved by the Institutional Review Board at Emory University and at

Vanderbilt University and by the ethics committee at the Instituto de Investigacion Nutricional in Lima, Peru.

Study Population

Our study population was selected based on the children who contributed NP swabs to the first 500 samples collected. Thus, two hundred and fifty-nine Andean children between the ages of 0 and 36 months were included in this thesis. These children live in rural communities in and surrounding the town of San Marcos in the Andes Mountains with altitudes between 1700 to 3800 meters above sea level. In this area, a majority of the households (95% in this study population) use wood for cooking and have either untreated piped water delivered to their homes or use natural streams or wells to get their water. Their main activity is agriculture; very few complemented by raising guinea pigs or cattle.

Among this study population, 164 children were defined as cases - subjects who were colonized with *Streptococcus pneumoniae* as detected by qPCR in their first NP swab; sixty-four children were designated as controls - those who were not colonized with *S. pneumoniae* in their first NP swab.

Data Collection

After the initiation of the study and application of the intervention, all cohort members were followed up for one year through weekly household visits in which demographic, socio-economic, and other information on known risk factors for ARIs/pneumonia was collected. At the same time these data were collected, routine nasopharyngeal (NP) swabs for bacterial colonization were collected on a regular monthly basis for a total of one and a half years of follow-up.

Questionnaires

For part of the study population, a more condensed questionnaire addressing the same demographic, socio-economic, and other information was administered. Both questionnaires included topics of ownership of material goods and livestock, household structure, attendance in a wawa-wasi (conceptually similar to a daycare center), indoor air pollution, and breastfeeding. A few topics were covered by one, but not both, questionnaires, including antibiotic use, vaccination history (for PCV, Hib, and measles) and use of "vaso de leche fresca or bronca" (non-pasteurized milk rich in proteins, carbohydrates, vitamins, and minerals).

Specimen collection and storage

Following WHO recommendations, NP samples were collected using Dacron polyester swabs that are then immediately placed in 0.5-1 mL of STGG, a mixture containing skim milk, tryptone, glucose, and glycerin. The excess wire was cut off from the swab, leaving the swab in the transport medium. The specimen was transported to the field site in San Marcos, where it was vortex for 10-20 seconds and frozen with the swab in the medium at -70°C. Subsequently, it was shipped to Lima and then to Dr. Klugman's laboratory in Atlanta, GA in order to identify and quantify *S. pneumoniae* in these samples.

Laboratory Analysis

Five hundred NP swabs were collected from the 259 children between May and August 2009; however, for this study, only the first NP swab collected from each child was analyzed. Once thawed to room temperature from -80°C, 200 μL NP-STGG specimen were transferred to 5 mL of Todd Hewitt broth containing 0.5% yeast extract

(THY) and 1 mL of rabbit serum. After this mixture had been incubated for 6 hours at 37°C in a 5% CO_2 atmosphere, one loop of the THY enriched culture was streaked onto a blood agar plate (with 5% sheep blood) (BAP) and was then incubated for 18-24 hours at 37°C in a 5% C02 atmosphere. Additionally, 1 mL of the THY enriched growth was transferred into a 1.5 ml vial and stored at -70ºC for further DNA extraction and then, if needed, detection of *S. pneumoniae* strains and/or other microorganisms (i.e., *S. aureus* or *H. influenzae*) using a *lyt*A-based qPCR assay.

qPCR

Targeting the *lyt*A gene, a qPCR assay was performed in all NP samples [34]. Briefly, 200 µl of an NP sample were added to 100 µl of TE (10mM Tris-HCl, 1 mM EDTA, pH 8.0) buffer containing 0.04 g/ml lysozyme and 75 U/ml of mutanolysin; this mixture was then incubated for 1 hour at 37°C in a water bath. Subsequent steps were followed from the QIAGEN DNA Mini protocol. Once DNA was eluted in 100 µl of elution buffer, it was stored at -70°C. To prepare qPCR standards, DNA was also extracted from the reference strains *S. pneumoniae* ATCC 33400 or TIGR4 [35] from overnight cultures in THY broth, and then quantified using the nanodrop method. The qPCR assay was performed [36] using the following primers and probe lytA-CDC forward 5-ACG CAA TCT AGC AGA TGA AGC A-3, lytA-CDC reverse 5-TGT GCG TTT TAA TTC CAG CT-3, lytA-CDC probe 5-FAM-TGC CGA AAA CGC TTG ATA CAG GG AG-3-BHQ1. Quantitative PCR reactions were carried out to a final 25 µl volume and performed using the EXPRESS qPCR Supermix Universal (Invitrogen), with 2.5 µl of sample DNA and 200 nM of each primer and probe. Every run included a no-template control. To quantify the number of genome copies of *S. pneumoniae* in each

sample, a standard curve using 10^2 through 10^8 copies of the *S. pneumoniae* genome (ATCC 33400 or TIGR4) were run in parallel. Because *S. pneumoniae* encodes only one copy of the *lyt*A gene, the number of copies detected in the samples is equivalent to the bacterial load in each sample. For each cycle, the parameters were 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Samples with cycle threshold (Ct) values greater than 40 were defined as being negative samples. Positive samples were defined with a Ct value of less than or equal to 35. For any sample with Ct values between 35 and 39, DNA extraction and qPCR were repeated.

Multiplex PCR

A multiplex PCR approach was used to detect most of the pneumococcal capsular serotypes. DNA was extracted from *S. pneumoniae* isolates using the quelex method and 5 µl were used as templates in multiplex PCR reactions. This multiplex PCR approach included eight sequential reactions; each reaction contained 5 pairs of primers and an internal control that targets the *cps*4A locus that is present in all *S. pneumoniae* isolates. The reactions sequentially target the most prevalent capsular serotypes found in Latin America [37, 38]. As multiplex PCR controls, DNA was extracted, as mentioned below, from all *S. pneumoniae* serotypes and was run along with multiplex PCR reactions. To prepare template DNA from *S. pneumoniae* isolates, bacterial cells were suspended in 200 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), added with 20 µl of proteinase K and incubated 56°C for 1 hour. Subsequent steps were followed as per the QIAGEN DNA Mini protocol booklet, for isolation of bacterial DNA. With final volumes of 25 µl, the multiplex PCR reactions contained 2.5 µl of DNA-containing bacterial lysate, 1X PCR master mix [for multiplex PCRs, Qiagen Multiplex PCR kit; for

*cps*4A PCR, New England Biolab (NEB) PCR mix] and the specific set of primers as reported [37, 38]. Thermal cycles were as follows: 94°C for 15 minutes, then 35 amplification cycle of 94°C for 1 minute, 54°C for 1.5 minutes, and 72°C for 1 minute. PCR products were run in 2% agarose gels and visualized under UV light after ethidium bromide staining.

Statistical Analysis

Initially, a univariate analysis was performed to look at the distribution of the bacterial load (using qPCR data). Because of the non-normal distribution, this continuous outcome of density was log-transformed and resulted in a normal distribution. Because this study population is a subset of the initial randomized community trial, we also looked at the balance of the original randomization. One hundred and five families were randomized into the control group who has traditional or tulpia stoves and 112 families were randomized into the intervention group who has the improved stove from the Instituto de Investigacion Nutricional headquartered in Lima, Peru.

Due to the nature of the research question, the applied model strategy was highly sensitive and less specific. As a result, a backwards elimination approach and a forward building approach were applied to two regression models that aimed to identify risk factors for bacterial load of pneumococcal colonization. Ultimately, the forward building approach was more appropriate for the data analysis.

For the first model, the multivariate logistic regression model dichotomized the log-transformed continuous outcome on the mean qPCR value into a binary outcome of lower and higher bacterial load. To only focus on colonized children, this model included only 195 children. Secondly, the ordinal logistic regression model was

constructed to account for all 259 children's colonization status (colonized vs. not colonized) and, among those colonized, the degree of bacterial load (lower vs. higher bacterial load). Thus, the outcome for this model was categorized into three groups: children who were not colonized, colonized children with a lower pneumococcal load, and colonized children with a higher pneumococcal load. For this model, the proportional odds assumption was assessed and met. For each of these models, collinearity was assessed. P-values less than 0.05 were considered statistically significant.

III. RESULTS

Out of the 259 children from whom NP swabs were collected, 195 (75.3%) were positive for *S. pneumoniae*, using a qPCR assay. While we have 259 NP swabs analyzed for colonization status and density, 10 mothers declined to respond to our questionnaire. Tables 1-4 describe variables and characteristics of those children whose mothers replied to the questionnaire.

With the aim of identifying significant risk factors for density of pneumococcal colonization, a multivariate logistic regression model was built. For the logistic outcome, the qPCR data was dichotomized at the mean into higher and lower bacterial load. For this model shown in Table 5, the only significant predictor of pneumococcal density was having at least one sibling as compared to those who did not have siblings (OR=2.79 (95%CI: 1.10-7.06)). The univariate analysis is shown in Table 6.

An additional ordinal logistic regression was constructed to account for a child's colonization status (whether or not they were colonized) and, if colonized, degree of density (lower or higher density of colonization). The degree of density was defined as it

was in the logistic model. The proportional odds assumption was met (p=0.3261). As seen in Table 7, the significant risk factors of density of pneumococcal colonization were cooking breakfast for at least 45 minutes (OR=3.24 (95%CI: 1.76-5.98)) and having at least one sibling (OR=1.97 (95%CI=1.05-3.68)). Having a sibling as the child's primary playmate was a borderline significant risk factor (OR=1.94 (95%CI: 1.00-3.76)). The univariate analysis is displayed in Table 8.

IV. DISCUSSION

Among rural Andean children, 195 (75.3%) were colonized with *S. pneumoniae*, as detected by the qPCR assay. From the two regression models, the significant risk factors identified for density of pneumococcal colonization were having at least one sibling and cooking breakfast for at least 45 minutes.

Having at least one sibling is consistent with known risk factors for pneumococcal colonization. Evidence from other studies indicated the importance of family in pneumococcal transmission to parents/caregivers [20]; alternatively, support has also been provided that the presence of a family member carrying the same serogroup was a predictor for carriage in children older than six months [4]. However, a stronger association between colonization and having siblings has been demonstrated in studies in which person-to-person transmission of *S. pneumoniae* occurred among siblings [21]. Coles et al. showed that four-month old infants having at least two siblings under the age of five years were at a significantly increased risk of carriage [22]. In this study, the increased risk could be attributable to the siblings' presence in the household or the infants' exposure to their siblings' respiratory secretions when playing with shared toys. Unfortunately, with limited power, we could not further analyze how many siblings or

what age of the siblings would increase an infant's risk of pneumococcal density. Regardless, having siblings, especially those who attend day care, are likely to introduce the pneumococcus and/or other respiratory pathogens to younger siblings, which likely increases density of pneumococcal colonization.

One study looking at vaccination in day care attendees indicated that vaccinating older siblings would decrease colonization of vaccine serotypes in younger siblings [39]. Evidence has shown that vaccinating the youngest does not reduce transmission to older children [40, 41]. In a group randomized, controlled trial focused on the Navajo and Apache reservations, Millar et al. concluded that having a siblings colonized with the pneumococcus was associated with an increased risk of carriage [41]. While the older siblings likely expose their younger siblings to any pathogens, the younger siblings are also likely to transmit the pathogen(s) back to their older counterparts. With PCV7 use, studies have demonstrated the decrease in vaccine-type serotypes and an increase in non-vaccine-type serotypes. A cross-sectional study in Navajo and Apache reservations showed that the community-wide PCV7 vaccination reduced the prevalence of vaccine-type carriage and increases the prevalence of non-vaccine-type carriage through at least 3 years of age [40]. Thus, if younger siblings are protected from vaccine-type serotypes and are more likely to carry non-vaccine-type serotypes, their older siblings likely also have that increased risk of carrying non-vaccine-type serotypes.

Despite the lack of evidence in the literature, cooking breakfast for at least 45 minutes makes sense as a risk factor for bacterial load of pneumococcal colonization. A positive association of spending more time in front of a stove and bacterial load is consistent with the fact that women, especially those responsible for cooking, and their

children may be more exposed to the pollutants from the stove [26]. O'Dempsey et al. provide evidence of an increased risk of the child being carried on the mother's back while cooking on pneumococcal colonization [42]; however, there was no mention of any differential risk dependent on the amount of time spent cooking. Despite that this cut-point of 45 minutes was determined by the distribution of the mothers' response, this result provides more specific evidence about the relationship between the mother and child's exposures to the stove's pollutants and pneumococcal density.

Aside from this study that focused on risk factors of pneumococcal density, a study in Vietnam also investigated associations with pneumococcal density and demonstrated that an increased load of *S. pneumoniae* in the nasopharynx was associated with viral coinfection [33]. Unfortunately, we did not have such data to look at any association between viral coinfection and pneumococcal load.

To the best of our knowledge, this study is the first to examine pneumococcal density and its risk factors in Peru and in South America, whereas other studies have focused on pneumococcal colonization. Additionally, we studied household construction and information about indoor air pollution. However, there were limitations to this study. First, this study had a small sample size and only focused on one NP sample per child. Additionally, this study only detected prevalent cases of colonization. Further, with the nature of the research question of identifying risk factors, we have high sensitivity, yet low specificity for the results found. Consequently, we run the risk of having false positive results due to the multiple comparisons.

Regardless of such limitations, this study presented some public health implications. While the population was a subset of a randomized community trial

focusing on improved stoves, this case-control study could not show the benefits of the intervention on pneumococcal density of carriage. Aside from the intervention of an improved stove, this study did provide some evidence of the exposure to cooking as a risk for pneumococcal carriage density, which could lead to other interventions to reduce horizontal transmission of *S. pneumoniae*.

From this study, there are many future directions that we would like to further explore. Given the topography of the study area, it would be interesting to consider varying altitude on pneumococcal density as well as pneumococcal colonization. Because the heptavalent pneumococcal conjugate vaccine (PCV7) was introduced after the collection of these NP samples, an investigation is warranted to examine the impact of PCV7 on pneumococcal colonization, pneumococcal density, and prevalence of vaccine and non-vaccine serotypes in the population. As demonstrated in this study, the prevalence of vaccine serotypes was lower compared to what they were prior to the introduction of PCV7 in the United States. More specifically, we would also like to examine any association with pneumococcal serotype and pneumococcal load though we were unable to do so in this study. Aside from this, further investigations could also examine the relationships between bacterial load of pneumococcal colonization prior to and during viral and/or bacterial respiratory infections. Finally, more studies could explore if and how seasonality affects pneumococcal density by collecting samples over the course of at least a year.

In summary, this case-control study aimed to identify risk factors of density of pneumococcal colonization among Peruvian infants. In this study population, the prevalence of pneumococcal colonization was 75.3%. With many factors contributing to

this high prevalence, data were collected on social, behavioral, and environmental factors and analyzed in two multivariate regression models. From such models, significant predictors were having at least one sibling and cooking breakfast for at least 45 minutes. Despite concerns of multiple comparisons, our results provide evidence that the increased risk of pneumococcal density is partially attributable to some behavioral and household factors, one of which may be linked to indoor air pollution.

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VI. TABLES

Table 1: Frequencies of variables regarding indoor air pollution		
	No.	%
Age of stove		
At least 3 years old	75	28.96
Less than 3 years old	166	64.09
Have blinds on windows		
Yes	12	4.63
No	234	90.35
Type of cooking fuel used		
Alternate fuel (i.e., electricity, gas, carbon)	3	1.16
Wood	245	94.59
Have glass windows		
Yes	10	3.86
No	235	90.73
Type of stove		
Improved stove	142	54.83
Traditional stove/tulpia	105	40.54
Have tight windows		
Yes	9	3.47
No	237	91.51
Presence of ventilation mechanism		
Yes	126	48.65
No	121	46.72
Have wooden windows		
Yes	22	8.49
No	224	86.49

Table 2: Frequencies of variables regarding household characteristics		
	No.	%
Number of individuals in the household		
At least 6 family members	69	26.64
Less than 6 family members	180	69.5
Number of siblings		
At least one sibling	88	33.98
No siblings	159	61.39

Number of total rooms in house		
At least 4 rooms	37	14.29
Less than 4 rooms	211	81.47
Father's education		
Education from technical institution or university	10	3.86
Primary or secondary education	221	85.33
No education or homeschooled	13	5.02
Mother's education		
Education from technical institution or university	11	4.25
Primary or secondary education	190	73.36
No education or homeschooled	28	10.81

Table 3: Frequencies of individual and household behaviors

	No.	%
Mother breastfed child		
Yes	111	42.86
No	137	52.9
Wawa-wasi attendance		
Yes	6	2.32
No	242	93.44
Time spent cooking breakfast		
At least 45 minutes	73	28.19
Less than 45 minutes	174	67.18
Time spent cooking lunch		
More than 60 minutes	66	25.48
60 minutes or less	182	70.27
Time spent cooking dinner		
At least 45 minutes	77	29.73
Less than 45 minutes	182	70.27
Antibiotic use 7 days prior to NP sample collection		
Yes	7	2.7
No	243	93.82
Receipt of heptavalent pneumococcal conjugate vaccine (PCV7)		
Yes	57	22.01
No	202	77.99

Child consumed Vaso de leche		
Yes	196	75.68
No	41	15.83
Child's primary playmate		
Cousin or friend	28	10.81
Sibling	153	59.07
Parent/caregiver	64	24.71
Child has toys		
Yes	214	82.63
No	33	12.74
Whether child shares toys		
Yes	164	63.32
No	63	24.32
Whether child sleeps alone or with someone		
Alone	6	2.32
With someone	242	93.44
People who sleep with the child		
Siblings	12	4.63
Parent/Parents/Caregivers	231	89.19

Table 4: Frequencies of biological characteristics

	No.	%
Colonized children		
Yes	195	75.29
No	64	24.71
Degree of density		
Higher density	128	65.64
Lower density	67	34.36
Colonized pneumococcal serotype		
6A/B/C	30	11.58
23F	17	6.56
19F	11	4.25
19A	7	2.7
11A/11D	7	2.7
10A	6	2.32
18A/B/C	5	1.93
15B/C	5	1.93
9V/9A	5	1.93
23A	4	1.54

22F/A	4	1.54
15B	4	1.54
35B	3	1.16
15A	3	1.16
35F/47F	2	0.77
35A/C/42	2	0.77
17F	2	0.77
35F/47F	1	0.39
24A/B/F	1	0.39
22F	1	0.39
16F	1	0.39
7C/7B/40	1	0.39
6A	1	0.39
Non-typeable	21	8.11
Child's age		
<12 months	72	27.8
12-23 months	98	37.84
≥24 months	89	34.36
Child's gender		
Male	128	49.42
Female	121	46.72
Had an ARI episode within 7 days of NP sample collection		
Yes	32	12.36
No	227	87.64

Table 5: Significant Risk Factor for Pneumococcal Density in a Multivariate Logistic Regression Model

Variable	OR	95% CI	P-value
Having no siblings	1.00		
Having at least one sibling	2.79	1.10-7.06	0.0309

Table 6: Univariate Analysis of Significant Risk Factors in Multivariate Logistic Regression Model

		No.	%
Density			
Higher	Siblings	33	51.6
	No Siblings	31	48.4
Lower	Siblings	41	33.3
	No Siblings	82	66.7

8 were missing

Table 7: Significant Risk Factors for Pneumococcal Density in an Ordinal Logistic Regression Model

Variable	OR	95% CI	P-value
Cooking breakfast in less than 45 minutes	1.00		
Cooking breakfast for at least 45 minutes	3.24	1.76-5.98	0.0002
Having no siblings	1.00		
Having at least one sibling	1.97	1.05-3.68	0.0337
Child's primary playmate			
Parent/caregiver	1.00		
Sibling	1.94	1.00-3.76	0.0503
Cousin or friend	1.97	0.75-5.18	0.1712

Table 8: Univariate Analysis of Significant Risk Factors in an Ordinal Logistic Regression Model

		No.	%
Density			
Higher	Siblings	33	51.6
	No Siblings	31	48.4
Lower	Siblings	41	33.3
	No Siblings	82	66.7
Not Colonized	Siblings	14	23.3
	No Siblings	46	76.7
	12 were missing		
Density			
Higher	At least 45 minutes spent cooking breakfast	27	41.5
	Less than 45 minutes spent cooking breakfast	38	58.5
Lower	At least 45 minutes spent cooking breakfast	39	31.7
	Less than 45 minutes spent cooking breakfast	84	68.3
Not Colonized	At least 45 minutes spent cooking breakfast	7	11.9
	Less than 45 minutes spent cooking breakfast	52	88.1
	12 were missing		