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Differences in Bacterial Colonization and Mucosal Responses Between High and Low SES Children in Indonesia

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Background: Increased nasopharyngeal carriage of pathogenic bacteria is found in low socioeconomic status (SES) settings. How SES affects local immune responses, important for controlling colonization, is currently unknown.

Objective: Examining bacterial colonization and cytokine response in the nasal mucosa of children from high and low SES.

Methods: Nasosorption samples were collected in October 2019 from 48 high SES and 50 low SES schoolchildren, in a cross-sectional study in Makassar, Indonesia. Twenty-five cytokines were measured in nasal fluid. Quantitative polymerase chain reaction was performed to determine carriage and density of Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis and Staphylococcus aureus. Data were analyzed using multivariate regression.

Results: H. influenzae and S. pneumoniae densities were increased in low SES settings compared to the high SES settings (P = 0.011, P = 0.086), with 6 and 6 times higher median densities, respectively. Densities of H. influenzae and S. pneumoniae were positively associated with levels of IL-1beta and IL-6. After correcting for bacterial density, IL-6 levels were higher in colonized children from high SES than low SES for H. influenzae and S. pneumoniae (both P = 0.039).

Conclusion: Increased densities of H. influenzae and S. pneumoniae were observed in low SES children, whereas IL-6 levels associated with colonization were reduced in these children, indicating that immune responses to bacterial colonization were altered by SES.

Key Words: Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, IL-1beta, IL-6, IL-1RA, socioeconomic status, upper respiratory tract colonization.

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METHODS

Data Collection

Data were obtained as part of a larger study into high and low SES school-age children in Makassar, Indonesia. The main objective of this larger study was to study the effect of SES on the immune response.
responses.15 This study had a cross-sectional design and included children attending 2 primary schools in Makassar, the capital of South Sulawesi. These schools were selected based on the socio-economic background of the children attending the school. One of the schools, SD Athirah, is a privately funded school with extensive facilities and therefore attended by children from high SES families. The other school, SD Baraya, is a public school, has very limited facilities and is predominantly attended by low SES children. Both schools are located in the center of Makassar, 2 km apart. Within the selected schools and selected grades, all children were invited to participate in the study and all children with parental/guardian informed consent were included in the study. We excluded children that were using antihistamines or corticosteroids. From this large study including 360 children in total, nasosorption samples were collected from a subgroup of 98 children for this current study, based on order of children’s availability indicated by teachers.

In the current study, we aimed to compare (1) microbial presence and density and (2) mucosal responses with microbes between low SES and high SES. We expected the microbial prevalence in our study population to be 20%-50%, including 10–25 children per SES group for a given microbe.

Samples were collected in October 2019. Informed written consent was obtained from primary caregivers. Ethical approval was obtained from the Health Research Ethical Committee, Faculty of Medicine, Hasanuddin University (no:703/H4.6.4.531/PP36/2019).

Socio-demographic information was obtained from all participants via questionnaires. To determine skin reactivity to house dust mite, a common aeroallergen, a skin prick test (SPT) was performed in children using histamine chloride (10 mg/ml) as a positive control, saline as a negative control and 2 extracts: Dermatophagoides pteronyssinus and D. farinae (HAL Allergen BV, Leiden). Skin test reactivity was considered positive if the longest diameter was greater than or equal to 5 mm larger as measured 15 minutes after application. Finally, all children were asked to fill the stool container (Sarstedt Inc, Nümbrecht, Germany), and the Kato-Katz methods were used to quantify the presence and density and (2) mucosal responses with microbes.

Nasosorption Samples Preparation

Nasosorption samples were collected by inserting an absorptive matrix strip (Nasosorption, Hunt Developments) into one nostril and pushed against the nasal lining for 30–60 seconds.16 These strips were then placed on ice until storage at −80°C within 8 hours after collection. The nasosorption samples were eluted by adding 100 µL sterilized phosphate buffered saline with 1% bovine serum albumin+0.05% Triton-X100 to the filter and spun down at 3600 × g for 10 minutes at 4°C. The supernatant was transferred to a new tube and centrifuged at 16,000 × g, 4°C, 10 minutes. The supernatant was moved to a new tube for cytokine analysis and the pellet was stored at −20°C until DNA extraction.

Cytokine Concentration Measurement

Of the supernatant, 12 µl was used to measure the concentration of 25 cytokines by the Human Cytokine 25-plex ProcartaPlex Panel (Invitrogen, ThermoFisher No:EXP250-12166-901) according to manufacturer's instructions, but additional washing steps and alcohol flushes were performed to account for the mucus present in such nasal samples, which can cause beads to aggregate. The samples were measured by the Luminox200 device at a normal RP1 target and concentrations were obtained by using the xPonent3.1 software in pg/mL. Children for whom >90% of the cytokines could not be measured in their nasosorption sample were excluded from further analysis. This was the case for 15% of the children: 6 high and 9 low SES children.

Generation of Standard Curves

DNA concentrations of S. aureus (American Type Culture Collection 43300), H. influenzae (American Type Culture Collection 49766), S. pneumoniae 6305 and clinical isolate of M. catarrhalis were determined spectrophotometrically with the NanoDrop1000 (Thermo scientific). The copy numbers of template were calculated using the genome length computed with the Technology Networks (https://www.genomeworks.com/tnt/tools/copynumbercalculator). A standard curve was generated of a 10-fold serial dilution scheme ranging from 10^7 to 10^1 copy/mL.

Bacterial Colonization Measurement

To determine nasal bacterial colonization, quantitative polymerase chain reaction (qPCR) was performed on the bacterial pellet. DNA extraction was performed using magnetic beads, as previously published.17 To determine the colonization of S. pneumoniae and H. influenzae, qPCR was performed using the LytA and IgA1 gene, respectively, as previously described.17,18 qPCR for the copB gene was performed to determine colonization of M. catarrhalis, and for S. aureus, the nuc gene was used as previously described.14 Using the standard curves, the genome length and Avogradro’s number, the number of genome copies/µl was determined in the samples per bacterium. Children negative for all 4 bacteria and with 16S Ct values that did not exceed the background signal17 were excluded from further analysis. This excluded 4 children from high SES and 4 children from low SES. Details regarding the primers and probes utilized in this study can be found in Table 1.

| Target gene | Sequence (5′ - 3′) | Bacteria/Target | Reference |
|-------------|-------------------|----------------|----------|
| lytA        | Forward ACGCAATCTAGCAGATGAAGCA | Streptococcus pneumoniae | 17 |
|             | Reverse TCGGCTTTTATGCCAGCT | | |
|             | Probe FAM-TGCCGAAAAAGCTTGTACAGGGAG-BHQ-1 | | |
| IgA1        | Forward CAAAATTGCCAAGATTTAAGTTTCTTTAGGCA | Hemophilus influenza | 18 |
|             | Reverse TCGTCCATCTACTGGCAA | | |
|             | Probe FAM-CCTGCGGTAGACC-MGB | | |
| CopB        | Forward CGTTTTGACGTGGTTTTGCTTTT | Moraxella catarrhalis | 6 |
|             | Reverse TAGATTTAGGTACCCTGCTACG | | |
|             | Probe HEX-ACCGACAGATAACCCAAAGCTTTGG-BHQ1 | | |
| Nuc         | Forward GTGCTTATGGTATTGATGTTGATGTTG | Staphylocooccus aureus | 19 |
|             | Reverse AAAAGCGTTGAGGTCTTATGTTGTATGTTG | | |
|             | Probe HEX-AAGTCCTAAGTGCACACGAAATGCGA-BHQ1 | 18SrRNA | |
| 16S         | Forward CGAAAGCGTGGGGAGGAG | | |
|             | Reverse GTGCTTACTCCCGAGGGG | | |
|             | Probe ATTAGATACCCTGGTAGTCCA | | |
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Statistical Analysis

The standardized z-scores of the body mass index (z-BMI) were determined according to the WHO guidelines. To obtain approximately normally distributed data, cytokine concentrations and bacterial loads were log10-transformed. Student’s t test was performed for continuous data, and for binary and categorical data, Pearson’s χ² was used. Pearson’s correlation was used to examine the correlation of 2 continuous variables. To assess the main drivers of the correlation between bacterial colonization and cytokines, we performed a canonical correlation analysis. Similarly to principal component analysis, canonical correlation allows the integration of 2 multidimensional datasets to find common drivers of variation maximizing covariance between the datasets, allowing us to compare bacteria and cytokines on a global level. To investigate the effect of SES on the bacterial loads and to estimate the effect of bacterial density and SES on cytokine levels,
a regression model was performed. For all regression models in this study, age, sex and z-BMI were considered a priori founders and adjustment was performed accordingly. To identify the main drivers of the correlation between bacterial density and cytokine concentrations, a canonical correlation analysis was performed by using Wilk’s Lambdas, including the density of the 4 bacteria and the concentrations of all widely measurable cytokines. The 2 bacteria and 2 cytokines that showed the largest coefficient in the canonical correlation analysis were selected for further analysis. P-values smaller than 0.05 were considered statistically significant. Data were analyzed and visualized using RStudio and R software.

**RESULTS**

**Bacterial Density of \textit{H. influenzae} and \textit{S. pneumoniae} Increased in Low SES**

A total of 98 school-age children were included: 50 children attended the low SES school and 48 children the high SES school (Table 2). There was no significant difference in age or sex, but z-BMI and parental income were between lower in low SES compared with high SES children. Finally, the presence of some of the factors that could affect the bacterial colonization and cytokine levels was examined. However, only 2 children, both high SES, had self-reported atopic asthma and 3 children, all low SES, had a...
current helminth infection; thus, these factors were not taken along in the rest of the analysis.

The carriage rate was highest for *M. catarrhalis* (81.1%, 73/90), more than 50% for *H. influenzae* (66.7%, 60/90) and *S. pneumoniae* (52.2%, 47/90), lowest for *S. aureus* (42.2%, 38/90) and most common was a combination of 2 (30.0%, 27/90) or 3 (30.0%, 27/90). Neither the carriage rates of the bacteria nor the number of co-colonizing bacteria differed significantly between high and low SES.

Although carriage rates and combinations did not differ between high and low SES, differences in the densities of bacteria were observed (Fig. 1). The median bacterial load of *H. influenzae* was 59,075 copies per nasosorption sample in low (IQR: 6330–381,417) and 9804 in high SES (IQR: 960–41,675), indicating a 6 times higher median density. For *S. pneumoniae*, the median bacterial load was 97,422 in the low (IQR: 580–1,008,292) and 1446 in the high SES (IQR: 366–87,704), corresponding to a 67 times higher median density. No significant differences were observed in the bacterial loads of *M. catarrhalis* and *S. aureus* between high and low SES. To further examine the relation between the bacterial load and SES, a multivariate regression analysis adjusted for *a priori* confounders was performed. These results showed that SES significantly affected the *H. influenzae* (β_{SES} = −1.19, *P* = 0.006) and *S. pneumoniae* density (β_{SES} = −1.25, *P* = 0.026).

**FIGURE 3.** Nasal cytokine levels in high and low SES school-age children. All children for which cytokines could be measured were included, thus n = 79 of which 39 low SES (blue) and 40 high SES (red) children. The boxes represent the interquartile range and the line within represents the median. Individuals are depicted by circles. The whiskers represent the 1.5 IQ of the upper and lower quartile. SES: Socio-economic status.
Correlation Between Densities of the Different Bacteria

Correlation analysis showed that *H. influenzae* density positively correlated with *M. catarrhalis* density ($\beta = 0.53, P < 0.001$) and the bacterial load of *S. pneumoniae* negatively associated with *S. aureus* ($\beta = -0.50, P = 0.04$) (Fig. 2). Furthermore, there was a trend for a positive association between *H. influenzae* and *S. pneumoniae* density ($\beta = 0.34, P = 0.07$). No association was found between the other combinations of bacteria.

IL-1beta Concentrations are Increased in the Low SES Compared with the High SES

To understand whether impaired mucosal responses might be responsible for the higher densities of colonized bacteria in low SES children, the concentration of 25 cytokines in nasal fluid was analyzed using a multiplex assay. Nine of these cytokines were detectable in the majority of samples: IL-18, IL-1alpha, IL-1beta, IL-1RA, IL-27, IL-4, IL-6, IL-7 and TNF-alpha. The other cytokines were below the limit of reliable detection in most samples (Table 3). Comparison of cytokine levels between high and low SES showed that IL-1beta levels were significantly increased in the low SES, with a geometric mean concentration of 338.8 (95% CI: 15.6–7.226) in low SES and 102.3 (95% CI: 4.7–2.194) in high SES after adjusting for confounders ($\beta_{\text{SES}} = -0.518, P = 0.048$) (Fig. 3, Table 4).

IL-6 Concentrations are Increased in High SES Children Colonized by *H. influenzae* or *S. pneumoniae*

The densities of *H. influenzae* and *S. pneumoniae* and IL-1beta and IL-6 concentrations were identified as the main drivers of the correlation between bacteria and cytokines (Table 5). Indeed both *H. influenzae* and *S. pneumoniae* densities were positively associated with IL-1beta and IL-6 levels (Fig. 4A–D). To quantify these correlations, a regression model including *a priori* confounders was used. The results showed that *H. influenzae* and *S. pneumoniae* densities are positively associated with IL-1beta ($\beta_{\text{bacteria}} = 0.294, P = 0.002, \beta_{\text{SES}} = 0.221, P = 0.008$, respectively) and IL-6 ($\beta_{\text{bacteria}} = 0.190, P < 0.001$ and $\beta_{\text{bacteria}} = 0.136, P = 0.006$, respectively) (Fig. 4E). After adjusting for *a priori* confounders and the bacterial densities, IL-6 levels were increased in high compared with low SES in children colonized by *H. influenzae* ($\beta_{\text{SES}} = 0.360, P = 0.039$) and *S. pneumoniae* ($\beta_{\text{SES}} = 0.366, P = 0.039$), indicating that IL-6 levels were increased in high compared with low SES at any given density of these bacteria. To examine whether SES affected the strength and direction of this association, a regression model, including the interaction between these bacterial density and cytokine levels was performed, which showed that this was not significant (Table 6). Thus, children from both high and low SES had dose-dependent cytokine responses to *H. influenzae* or *S. pneumoniae* colonization, but these responses were stronger in high SES children for IL-6.

**TABLE 4.** Results of regression models cytokine levels adjusted for *a priori* confounders

| Mean cytokine concentration | Coefficient SES(95% CI) | $P$ value |
|----------------------------|-------------------------|-----------|
| IL-18                      |                         |           |
| Low SES                    | 741.3                   | Reference |
| High SES                   | 468.8                   | -0.199 (-0.442 to 0.0439) | 0.113 |
| IL-1alpha                  |                         |           |
| Low SES                    | 9.984                   | Reference |
| High SES                   | 6.18                    | -0.186 (-0.605 to 0.234) | 0.389 |
| IL-1beta                   |                         |           |
| Low SES                    | 338.8                   | Reference |
| High SES                   | 102.3                   | -0.518 (-1.02 to -0.0149) | 0.048 |
| IL-1RA                     |                         |           |
| Low SES                    | 75,857                  | Reference |
| High SES                   | 67,608                  | -0.0494 (-0.374 to 0.275) | 0.766 |
| IL-27                      |                         |           |
| Low SES                    | 42.66                   | Reference |
| High SES                   | 49.32                   | 0.0626 (-0.0360 to 0.161) | 0.218 |
| IL-4                       |                         |           |
| Low SES                    | 2.455                   | Reference |
| High SES                   | 2.786                   | 0.0553 (-0.377 to 0.488) | 0.803 |
| IL-6                       |                         |           |
| Low SES                    | 85.11                   | Reference |
| High SES                   | 116.95                  | 0.138 (-0.164 to 0.439) | 0.374 |
| IL-7                       |                         |           |
| Low SES                    | 16.0                    | Reference |
| High SES                   | 21.18                   | 0.106421 (-0.0852 to 0.298) | 0.28 |
| TNF-alpha                  |                         |           |
| Low SES                    | 53.7                    | Reference |
| High SES                   | 64.41                   | 0.0786 (-0.119 to 0.276) | 0.437 |

SES, Socio-economic status.

All children for which cytokines could be measured were included, thus n = 79 of which 39 low SES and 40 high SES children. Mean cytokine concentration is the geometric mean in high and low SES after adjusted for *a priori* confounders including age (in years), sex and z-BMI.
cytokine levels at the mucosa. In the current study, we examined the association between the cytokine levels and bacterial colonization, a local factor that is thought to play a significant role. Indeed, densities of *H. influenzae* and *S. pneumoniae* were positively associated with IL-1beta and IL-6 levels. The importance of the IL-1 cytokine signaling in the clearance of *S. pneumoniae* has been shown in mice and reduced IL-1 responses have been suggested to be permissive for persistent colonization during infancy. Moreover, high levels of IL-6 and IL-1beta were found in response to non-typeable *H. influenzae* in individuals suffering from chronic suppurative lung disease. Other local factors that might be of importance are co-infections. Co-infections with viruses in the nasal cavity are common and are strongly associated with increased *S. pneumoniae* nasopharyngeal load and invasive disease in children. In addition, co-infection with intestinal helminths has also been shown to increase pneumococcal carriage density and induce the development of invasive disease. Intestinal helminths are known to modulate the immune system and induce a more tolerogenic response to favor chronicity of infections, which also affects the response to other pathogens and allergens. In the current study, helminth infections were assessed, but only a limited number of infections were detected, due to a recent deworming program implemented. However, light infections might have been present as the Kato-Katz method used in this study has limited sensitivity. 

After adjusting for bacterial densities, increased IL-6 levels were observed in children colonized by *H. influenzae* or *S. pneumoniae* from high compared with low SES, whereas this was not observed for IL-1beta. This might be explained by the role of IL-6 in the IL-1 signaling pathway and its capacity to promote the differentiation of Th17-cells. Although IL-1beta primarily initiates IL-1 signaling, IL-6 production is one of the numerous downstream effects of the IL-1 signaling pathway. Furthermore, high levels of IL-6 are known to promote differentiation of CD4+ cells to form Th17-cells, a cell type that has been shown to be essential for the clearance of *S. pneumoniae* in mice. A study analyzing the Th17-cells and cytokines in adenoidal tissue from *S. pneumoniae*-positive and *S. pneumoniae*-negative children found an increased number of Th17-cells and higher levels of IL-17A and IL-6 in *S. pneumoniae* positive and *S. pneumoniae*-negative children. In the current study, increased IL-6 levels were observed in the high SES, whereas the density of *H. influenzae* and *S. pneumoniae* was reduced, supporting the role of IL-6 in controlling these bacteria. The IL-17A levels were below the level of detection in most samples collected in this study; however, very low levels of IL-17A might be enough to activate local T-cells and therefore be involved in the bacterial clearance.

Since a limited number of children were colonized with *S. aureus* and the presence and density was not associated with that of the other bacteria, responses to colonization by *S. aureus* were not likely to be identified by the canonical correlation analysis. Therefore, the relation between *S. aureus* carriage status and cytokine concentrations was further examined, whereby IL-1 cytokines were of main interest since these cytokines were shown previously to be important for *S. aureus* control. In the current study in the low SES, increased IL-1beta and IL-1RA levels were observed in *S. aureus* carriers compared with noncarriers, whereas in the high SES, decreased levels were found in carriers. The ratio between IL-1RA and IL-1beta was not affected by the carrier status but tended to be decreased in low compared with high SES after adjusting for *S. aureus* carriage. A human nasal inoculation with *S. aureus* showed that IL-1beta was upregulated after inoculation in individuals able to clear the bacteria compared with individuals but found no difference in IL-1RA levels. Furthermore, they showed that the IL-1RA/IL-1beta ratio was significantly decreased in individuals with persistent *S. aureus* and proposed this ratio as metric for the

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**DISCUSSION**

To the best of our knowledge, this is the first study evaluating the nasal cytokine response in relation to bacterial colonization in high and low SES children. This study showed increased densities of *H. influenzae* and *S. pneumoniae* in low compared with high SES. The densities of these bacteria were positively associated with IL-1beta and IL-6 levels. After correcting for bacterial density, IL-6 levels were increased in high SES, indicating that IL-6 levels were observed in high compared with low SES (βSES = 0.465, P = 0.078) (Table 7). The pattern observed for IL-1beta and IL-1RA did not exist for other IL-1 cytokines, including IL-1alpha (see Figure, Supplemental Digital Content 1; http://links.lww.com/INF/E697).

**TABLE 5. Canonical correlation analysis of bacterial loads**

| Bacterial load | Standardized coefficients |
|---------------|---------------------------|
| *H. influenzae* | -0.877 |
| *S. pneumoniae* | -0.240 |
| *M. catarrhalis* | 0.109 |
| *S. aureus* | -0.016 |

| Cytokine concentration | Standardized coefficients |
|------------------------|---------------------------|
| IL-1alpha              | 0.097 |
| IL-1beta               | -0.791 |
| IL-1RA                 | 0.037 |
| IL-4                   | 0.080 |
| IL-6                   | -0.311 |
| IL-7                   | -0.028 |
| IL-18                  | 0.209 |
| IL-27                  | 0.173 |
| TNF-alpha              | -0.141 |

P = 0.059) (Table 7). Since IL-1beta and IL-1RA compete for the same receptor and the activity of IL-1beta is affected by the levels of IL-1RA, the ratio between IL-1RA and IL-1beta was determined (Fig. 5C). The interaction between *S. aureus* carriage and SES was not seen for the ratio between these cytokines (βinteraction = 0.074, P = 0.859), but the IL-1RA/IL-1beta ratio tended to be higher in high compared with low SES (βSES = 0.465, P = 0.078) (Table 7). The results of the current study are in line with these previous findings; however, in contrast to the study Fadlyana et al, we included children from different SES within 1 urban center, thereby providing unique insights into the role of SES on nasopharyngeal bacterial carriage and densities in children. Finally, the associations between different microbial species have been described in earlier studies and our results are therefore in line with literature. 

Without taking bacterial colonization status into account, only IL-1beta was significantly different between high and low SES, whereas there was a large dynamic range in cytokine levels observed, indicating that local factors are important for driving

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Clearance of *S. aureus* in the nasal mucosa rather than the expression of individual cytokines. In the current study, the IL-1RA/IL-1beta ratio tended to be increased in high compared with low SES, indicating that the response to *S. aureus* in low SES children might be reduced. No significant differences in the IL-1RA/IL-1beta ratio between carriers and noncarriers were found; however, it should be kept in mind that the duration of the colonization was not measured in the current study and some of the carriers would be able to clear *S. aureus* over time. To elucidate the relation between SES and *S. aureus* persistence, longitudinal studies are thus needed.

A factor that can affect the bacterial colonization and cytokine responses and which can be impacted by SES is the vaccination status against pneumococcal and *H. influenzae* type B (Hib) diseases. Unfortunately, we were not able to obtain reliable information regarding the vaccination status for the children in this study. The children in our study were not vaccinated through their national childhood vaccination program. However, in a private clinic on payment additional vaccines can be administered, which predominantly involves the Hib vaccine. Although vaccination status might differ between SES, its effect on the bacterial colonization and cytokine responses needs to be further investigated.

### FIGURE 4

**Association between *H. influenzae* (A and B) or *S. pneumoniae* concentration (C and D) and IL-1beta and IL-6 concentration in high and low SES school-age children and (E) the quantification of this association by multivariate regression.**

For all models with *H. influenzae* only children colonized and with cytokine data are included thus n = 49, which includes 27 high SES children and 22 low SES children. For all models with *S. pneumoniae* only children colonized and with cytokine data are included thus n = 36, which includes 15 high SES children and 21 low SES children. SES: Socio-economic status.
load detected here is probably limited since the (sero)types prevalent in Indonesia only partially match with the available vaccines. A cross-sectional study among 302 young children in Indonesia in 2016, thus after Hib vaccine was incorporated in the national program, but before the pneumococcal conjugate vaccine (PCV13) was introduced, showed a carriage rate of 27.5% of *H. influenzae*, but none of these isolates was type B.\(^{30}\) Moreover, before the introduction of Hib vaccination, it was shown that Hib vaccination would not have an effect on the pneumonia incidence in Indonesia, whereas it significantly did in Africa and South America, indicating that in Indonesia other respiratory pathogens are responsible for LRTIs.\(^{31}\) Moreover, a study by Dunne et al showed that only 46% of the pneumococcal isolates obtained from Indonesian children are covered by the PCV13.\(^{30}\) While location-specific serotype circulation is of course possible, one can expect that the isolates found in our study are also (sero)types that will only partly be covered by the vaccine, which limits the expected potential effect of differences in vaccination status between high and low SES on the results.

One of the limitations of the current study is that only 4 pathogenic bacteria were measured in a cross-sectional manner, whereas it is known that viral coinfections and microbiota in general are also important in shaping responses. Moreover, future studies should assess changes and responses longitudinally. In this study, sampling took place in a single season, dry season. There are indications that season can impact the bacterial colonization.\(^{32}\) Although in the current study sampling for both high and low SES took place in the same season and city, and children from both groups were sampled at the same time, the effect of season on the main outcome cannot be ruled out. Furthermore, the small sample size for some analyses limits our analysis. In addition, while school-going children are important drivers of transmission within communities, most childhood pneumonia occurs before the age of 5 years; therefore, it would also be of interest to study mucosal immune responses in infants in future studies.

In conclusion, the results of the current study indicate that the local immune response to nasopharyngeal bacterial colonization

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**FIGURE 5.** The IL-1RA and IL-1Beta concentrations in *S. Aureus* carrier and non-carrier in high and low SES. All children for which both cytokines and bacterial colonization could be measured were included, thus n = 73 of which 36 low SES and 37 high SES children. The boxes represent the interquartile range and the line within represents the median. The whiskers represent the 1.5 IQ of the upper and lower quartile concentration of the cytokine. SES, Socio-economic status.

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**TABLE 6.** Regression model *H. influenzae* and *S. pneumoniae* load and the IL-1beta or IL-6 concentrations with interaction term

|                   | Intercept (95% CI) | Coefficient Bacterial Load (95% CI) | P value | Coefficient SES (95% CI) | P value | Coefficient Interaction (95% CI) | P value |
|-------------------|-------------------|-------------------------------------|---------|--------------------------|---------|----------------------------------|---------|
| *H. influenzae*   |                   |                                     |         |                          |         |                                  |         |
| IL-1beta          | 1.01 (−0.951 to 2.96) | 0.316 (0.089–0.542) | 0.009   | 0.105 (−1.58 to 1.71)   | 0.899   | −0.05 (−0.393 to 0.288)          | 0.764   |
| IL-6              | 1.12 (0.076–2.17)  | 0.194 (0.073–0.314) | 0.003   | 0.398 (−0.461 to 1.26)  | 0.369   | −0.01 (−0.191 to 0.173)          | 0.924   |
| *S. pneumonia*    |                   |                                     |         |                          |         |                                  |         |
| IL-1beta          | 1.46 (−0.554 to 3.47) | 0.228 (0.05–0.402) | 0.012   | −0.056 (−1.40 to 1.29)  | 0.935   | −0.025 (−0.330 to 0.280)         | 0.875   |
| IL-6              | 1.10 (−0.076 to 2.28) | 0.144 (0.042–0.246) | 0.010   | 0.486 (−0.300 to 1.27)  | 0.236   | −0.030 (−0.209 to 0.148)         | 0.743   |

Adjusted for *a priori* confounders age (in years), sex and z-BMI. Both bacterial loads and cytokine concentrations are log10 transformed. For all models with *H. influenzae* only children colonized are included thus n = 49, which includes 27 high SES children and 22 low SES children. For all models with *S. pneumonia* only children colonized are included thus n = 36, which includes 15 high SES children and 21 low SES children.
TABLE 7. Relation between S. Aureus carrier status and the IL-1beta or IL-1RA concentrations with interaction term

|                  | Intercept (95% CI) | Coefficient Carrier Status (95% CI) | P-value Carrier Status | Coefficient SES (95% CI) | P-value SES | Coefficient Interaction (95% CI) | P-value Interaction |
|------------------|--------------------|------------------------------------|------------------------|--------------------------|-------------|---------------------------------|--------------------|
| IL-1RA           | 4.59 (3.75–4.44)   | 0.199 (−0.144 to 0.543)            | 0.26                   | 0.228 (−0.150 to 0.605)  | 0.242       | −0.700 (−1.19 to −0.207)        | 0.007              |
| IL-1beta         | 2.21 (0.65–3.76)   | 0.251 (−0.300 to 0.801)            | 0.376                  | −0.206 (−0.811 to −0.340) | 0.508       | −0.772 (−1.56 to 0.016)         | 0.059              |
| Ratio IL-1RA/IL-1beta * | 2.39 (1.00–3.78)   | −0.051 (−0.615 to 0.513)           | 0.86                   | 0.433 (−0.187 to 1.053)  | 0.175       | 0.074 (−0.733 to 0.881)         | 0.859              |
| Ratio IL-1RA/IL-1beta † | 2.36 (1.02–3.69)   | −0.017 (−0.432 to 0.399)           | 0.937                  | 0.465 (−0.044 to 0.975)  | 0.078       |                                 |                    |

* log10 transformed without interaction term.
† log10 transformed with interaction term.

Adjusted for a priori confounders age (in years), sex and z-BMI. Cytokine concentrations are log10 transformed.

is altered by SES. Since nasopharyngeal carriage of these bacteria precedes disease and local immune responses are important in controlling potential pathogenic bacteria, elucidating the relation between the bacterial colonization and local cytokine response is an important first step in understanding the bacteria-host relationship. Insights in this relationship are important for the development of vaccine strategies and treatment options and will eventually lead to reduced LRTIs worldwide.

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