Density of Upper Respiratory Colonization With Streptococcus pneumoniae and Its Role in the Diagnosis of Pneumococcal Pneumonia Among Children Aged <5 Years in the PERCH Study

Henry C. Baggett,1,2 Nora L. Watson,3 Maria Deloria Knoll,4 W. Abdullah Brooks,5,6 Daniel R. Feikin,7,8 Laura L. Hammitt,9,10 Stephen R. C. Howie,9,10,11 Karen L. Kolotro,12 Orin S. Levine,13,14 Shabir A. Madhi,6,15 David R. Murdoch,16,17 J. Anthony G. Scott,18,19 Donald M. Thea,20,21 Martin Antonio,20,21 Juliet O. Awori,22,23 Vicky L. Baillie,24,25 Andrea N. DeLuca,26 Amanda J. Driscoll,27 Julie Duncan,28,29 Bernard E. Ebruke,30 Doli Goswami,31 Melissa M. Higdon,4 Ruth A. Karron,32,33 David P. Moore,32,34,35 Susan C. Morphet,32,35,36 Justin M. Mulindwa,32,36 Daniel E. Park,33,37 Wantaena Paveenkittiporn,38 Barameh Piraalam,39 Christine Prosperi,4,40 Samba O. Sow,41 Milagritos D. Tapia,42 Khaleq Zaman,43 Scott L. Zeger,44 and Katherine L. O’Brien45 for the PERCH Study Group

1Global Disease Detection Center, Thailand Ministry of Public Health-US Centers for Disease Control and Prevention Collaboration, Nonthaburi; 2Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia; 3Emmes Corporation, Rockville, Maryland; 4International Vaccine Access Center, and 5Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; 6International Centre for Diarrhoeal Disease Research, Bangladesh (icddr.b), Dhaka and Matlab; 7Division of Viral Diseases, National Center for Immunizations and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; 8Kenya Medical Research Institute-Wellcome Trust Research Programme, Kilifi, Kenya; 9Medical Research Council Unit, Basse, The Gambia; 10Department of Paediatrics University of Auckland, and 11Centre for International Health, University of Otago, Dunedin, New Zealand; 12Division of Infectious Disease and Tropical Pediatrics, Department of Pediatrics, Center for Vaccine Development, Institute of Global Health, University of Maryland School of Medicine, Baltimore, Maryland; 13Bill & Melinda Gates Foundation, Seattle, Washington; 14Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, and 15Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases Unit, University of the Witwatersrand, Johannesburg, South Africa; 16Department of Pathology, University of Otago, and 17Microbiology Unit, Canterbury Health Laboratories, Christchurch, New Zealand; 18Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, United Kingdom; 19Center for Global Health and Development, Boston University School of Public Health, Massachusetts; 20Microbiology and Infection Unit, Warwick Medical School, University of Warwick, Coventry, and 21Department of Pathogen Molecular Biology, London School of Hygiene & Tropical Medicine, United Kingdom; 22Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; 23University Teaching Hospital, Lusaka, Zambia; 24Department of International Health, Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; 25Department of Paediatrics & Child Health, Chris Hani Baragwanath Academic Hospital and University of the Witwatersrand, South Africa; 26Microbiology Laboratory, Middlemore Hospital, Counties Manukau District Health Board, Auckland, New Zealand and 27Milken Institute School of Public Health, Department of Epidemiology and Biostatistics, George Washington University, District Columbia; 28National Institute of Health, Ministry of Public Health, Nonthaburi, and 29Nakhon Phrom Provincial Health Office, Nakhon Phrom, Thailand; 30Centre pour le Développement des Vaccins (CVD-Mail), Barnako, Mali; and 31Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

Background. Previous studies suggested an association between upper airway pneumococcal colonization density and pneumococcal pneumonia, but data in children are limited. Using data from the Pneumonia Etiology Research for Child Health (PERCH) study, we assessed this potential association.

Methods. PERCH is a case-control study in 7 countries: Bangladesh, The Gambia, Kenya, Mali, South Africa, Thailand, and Zambia. Cases were children aged 1–59 months hospitalized with World Health Organization–defined severe or very severe pneumonia. Controls were randomly selected from the community. Microbiologically confirmed pneumococcal pneumonia (MCP) was confirmed by detection of pneumococcus in a relevant normally sterile body fluid. Colonization density was calculated with quantitative polymerase chain reaction analysis of nasopharyngeal/oropharyngeal specimens.

Results. Median colonization density among 56 cases with MCP (MCP cases; 17.28 × 10^5 copies/mL) exceeded that of cases without MCP (non-MCP cases; 0.75 × 10^5) and controls (0.60 × 10^5) (each P < .001). The optimal density for discriminating MCP cases from controls using the Youden index was >6.9 log_{10} copies/mL; overall, the sensitivity was 64% and the specificity 92%, with variable performance by site. The threshold was lower (≥4.4 log_{10} copies/mL) when MCP cases were distinguished from controls who received antibiotics before specimen collection. Among the 4035 non-MCP cases, 500 (12%) had pneumococcal colonization density >6.9 log_{10} copies/mL; above this cutoff was associated with alveolar consolidation at chest radiography, very severe pneumonia, oxygen saturation <92%, C-reactive protein ≥40 mg/L, and lack of antibiotic pretreatment (all P < .001).

Conclusions. Pneumococcal colonization density >6.9 log_{10} copies/mL was strongly associated with MCP and could be used to improve estimates of pneumococcal pneumonia prevalence in childhood pneumonia studies. Our findings do not support its use for individual diagnosis in a clinical setting.

Keywords. pneumococcus; colonization; pneumonia; children; etiology.
High density of pneumococcal colonization (ie, high bacterial density in the nasopharynx) has been proposed as a more sensitive marker for pneumococcal pneumonia than blood culture [7].

Previous studies, mostly in adults, have demonstrated an association between the density of pneumococcal colonization and pneumococcal pneumonia [8–11]. Several other studies among children evaluated the use of pneumococcal colonization density as a marker of pneumococcal pneumonia [12–14], but these studies used surrogate end points (ie, radiographic pneumonia) for true pneumococcal pneumonia rather than confirmed pneumococcal pneumonia cases for the density evaluation. Although these studies suggest the use of pneumococcal nasopharyngeal (NP) density as a potential diagnostic tool for pneumococcal pneumonia, additional data among children are needed to confirm the association and identify a density threshold with acceptable diagnostic accuracy. Therefore, we evaluated the utility of upper respiratory tract colonization density as a diagnostic tool for pneumococcal pneumonia in a large study of childhood pneumonia.

METHODS

Study Design and Case Definitions

The Pneumonia Etiology Research for Child Health (PERCH) study is a multicountry, standardized case-control evaluation of the etiologic agents causing severe and very severe pneumonia among children in developing countries [15]. Enrollment occurred for 24 months between August 2011 and January 2014 at each of 9 study sites in 7 countries: Dhaka and Matlab, Bangladesh; Basse, The Gambia; Kilifi, Kenya; Bamako, Mali; Soweto, South Africa; Nakhon Phanom and Sa Kaeo, Thailand; and Lusaka, Zambia. Identification and selection of cases and controls have been described elsewhere [16].

Cases were hospitalized children aged 1–59 months with World Health Organization–defined severe or very severe pneumonia [17]. Severe pneumonia was defined as the presence of cough or difficulty breathing and lower chest wall indrawing; very severe pneumonia, as cough or difficulty breathing and ≥1 of the following: central cyanosis, difficulty breastfeeding/drinking, vomiting everything, convulsions, lethargy, unconsciousness, or head nodding. Exclusion criteria for cases were hospitalization within the previous 14 days, discharge as a PERCH case within the past 30 days, residence outside the study catchment area, or resolution of lower chest wall indrawing after bronchodilator therapy for children with wheezing.

Controls were randomly selected children from the community without severe or very severe pneumonia, were enrolled year round, and were frequency matched to cases by age group [16]. Controls were also matched for human immunodeficiency virus (HIV) status at the 2 sites (Zambia and South Africa) with high HIV prevalence. Controls with acute respiratory illness or other mild illnesses were included only if they did not have severe or very severe pneumonia.

Pneumococcal Conjugate Vaccine

Pneumococcal conjugate vaccine (PCV) was in use for the entire enrollment period in The Gambia, Kenya, Mali, and South Africa. PCV was introduced in July 2013 in Zambia, 18 months after enrollment started. In Bangladesh and Thailand, PCV was available only on the private market during the study period with almost no usage in the study areas.

Specimen Collection and Laboratory Testing

All laboratory methods were standardized across sites [18]. A flocked NP swab (flexible minitip; Copan) and a rayon oropharyngeal (OP) swab specimen were collected from each case and control and were placed into the same vial. The NP/OP specimen was tested for pneumococcus (lytA gene target) as part of a multiplex real-time polymerase chain reaction (PCR) assay (FTD Respiratory Pathogens 33; Fast-track Diagnostics) performed using an Applied Biosystems 7500 (ABI-7500) platform. Standard curves for quantification were generated on an approximately 3-monthly basis and were used to calculate pathogen density (in copies per milliliter) from the sample cycle threshold values. Densities <10^4 or >10^8 copies/mL were outside the linear range of the PCR assay, limiting precise density estimation.

A second NP specimen for S. pneumoniae culture was collected simultaneously with the first swab specimen; pneumococcal isolates were serotyped using Quellung reaction or latex agglutination, as described elsewhere [18]. Testing was performed at each site, and all sites participated in external quality assurance programs for both pneumococcal PCR and serotyping [18].

Cases, but not controls, had blood collected for culture. Some sites (Bangladesh, The Gambia, Mali, and South Africa) collected lung aspirates from children with consolidation on chest radiographs (CXRs) who met clinical and radiologic criteria for the procedure [19]. Pleural fluid was collected from cases when clinically indicated. Lung aspirate and pleural fluid specimens were tested for pneumococcus by means of culture and PCR; pleural fluid was also tested for pneumococcal antigen (Binax NOW; Alere).

Definitions

Antibiotic pre-exposure was defined as either a positive serum bioassay result (cases and controls) or documentation of antibiotics administered at the referral or study hospital before specimen collection (cases only) [20]. Microbiologically confirmed pneumococcal pneumonia (MCPP) was defined, in PERCH cases, as detection of pneumococcus from a culture of blood, lung aspirate, or pleural fluid; by PCR of lung aspirate or pleural fluid; or by detection of pneumococcal antigen in pleural fluid. A control was considered to have a respiratory tract illness (RTI) if cough or runny nose were reported. RTI was also considered present if a child had (1) ear discharge, wheezing, or difficulty breathing and (2) either fever (temperature ≥38.0°C or reported fever in the past 48 hours) or sore throat.
CXRs were obtained at admission for cases, and each digital image was assessed by 2 members of a panel of 14 radiologists and pediatricians trained in the standardized interpretation of pediatric CXRs; films with discordant conclusions were adjudicated [21, 22]. Clinical characteristics, including oxygen saturation, were assessed on the day of enrollment. Case mortality was assessed at hospital discharge and by contact 30 days after discharge.

**Statistical Analysis**

Demographic, clinical and laboratory characteristics were compared by subject group using the χ² test. Median pneumococcal colonization density was compared across groups with the Kruskal-Wallis test. Density histograms and comparisons by subject group were repeated among strata defined by antibiotic exposure before NP/OP specimen collection.

An optimal density threshold for discriminating cases with MCPP (MCPP cases) from all controls was identified using the Youden index [23]. The optimal density threshold was also calculated for MCPP cases versus the subset of controls without RTI (non-RTI controls), and among children who were HIV negative. To guard against bias in the estimates of sensitivity owing to a small number of MCPP cases, the Youden index was calculated using leave-one-out cross-validation. To characterize a potential trend in risk associated with increasing pneumococcal density, we used logistic regression models adjusted for age, sex, and site to evaluate associations of pneumococcal density categories with clinical and CXR indicators of pneumonia, and with case severity measures.

To evaluate whether elevated colonization density may identify cases with pneumococcal pneumonia among those without MCPP, we compared known clinical and laboratory correlates of bacterial pneumonia among cases without MCPP (non-MCPP cases) with colonization density above versus below the identified optimal threshold. The association of elevated pneumococcal colonization density with known correlates of pneumonia was evaluated using separate logistic regression models adjusted for age, sex, and site. Analyses were repeated to extend comparison of characteristics among non-MCPP cases with density above the threshold versus all MCPP cases, and among MCPP cases above versus below the threshold.

**Ethical Considerations**

The PERCH study protocol was approved by the institutional review board or ethical review committee at each of the study site institutions and at The Johns Hopkins Bloomberg School of Public Health. Parents or guardians of all participants provided written informed consent.

**RESULTS**

Of 4232 cases enrolled in the PERCH study, 4136 had available *S. pneumoniae* colonization and density data. Of those, data on MCPP status were available on 4091 cases (56 MCPP and 4035 non-MCPP cases); 45 cases were excluded owing to missing data required to define MCPP status. Of 5325 controls, the analysis included 1226 controls with and 3962 without RTI in whom *S. pneumoniae* colonization and density were measured by PCR analysis of the NP/OP specimen. An additional 3 MCPP cases, 82 non-MCPP cases, 11 cases with unknown MCPP status, and 137 controls did not have analyzable NP/OP PCR results because of missing or insufficient samples (2.3%).

Among the 56 MCPP cases, 21% were aged 1–5 months, 23% were 6–11 months, 30% were 12–23 months, and 25% were 24–59 months; 52% were male. Age and sex distribution were similar across MCPP, non-MCPP, and control groups (mean age, 14 months), except that a higher proportion of non-MCPP cases (41%) were aged <6 months compared with MCPP cases (21%). Cases with MCPP were identified at all 5 African sites (15 in The Gambia, 5 in Kenya, 24 in Mali, 5 in South Africa, and 7 in and Zambia) but at neither of the 2 Asian sites (Bangladesh and Thailand) (Table 1).

MCPP cases were more likely to be colonized with *S. pneumoniae* (by culture or PCR, 100% [56 of 56]) compared with non-MCPP cases (75.7% [3055 of 4035]), all controls (81.4% [4224 of 5188]), controls with RTI (85.5% [1048 of 1226]), and controls without RTI (80.2% [3176 of 3962]), and were more likely to be HIV infected (23.2%) than non-MCPP cases (5.6%) (P ≤ .01 for each). Non-MCPP cases were more likely than those with MCPP to have received antibiotics before NP/OP specimen collection (46% vs 29%; P < .01). Antibiotic use before NP/OP specimen collection occurred in 3 of 14 MCPP cases in The Gambia (data missing for 1), 2 of 5 in Kenya, 3 of 24 in Mali, 5 of 5 in South Africa and 3 of 6 in Zambia (data missing for 1).

Among children who had a positive density value, median *S. pneumoniae* colonization density was highest in MCPP cases (17.28 × 10⁶ copies/mL) relative to non-MCPP cases (0.75 × 10⁶) and controls (0.60 × 10⁶) (P < .001 for each) (Table 1). However, in South Africa, the only site where all MCPP cases had received prior antibiotics, MCPP cases had lower median density (0.25 × 10⁶) than both non-MCPP cases (0.70 × 10⁶) and controls (0.77 × 10⁶), although differences were not statistically significant. For each case and control group, the median colonization density was lower in children with prior antibiotic use than in those without, and lower in those with NP culture negative versus positive for *S. pneumoniae* (Table 1).

Density among MCPP cases varied by site (Table 1 and Figure 1; P < .001); median density differed by >100-fold between the site with the highest density, Mali (35.81 × 10⁶ copies/mL), which that also had the highest proportion of MCPP cases (3.6%), and the sites with the lowest density, Kenya and South Africa (0.35 and 0.25 × 10⁶ copies/mL), both with 5 MCPP cases (<0.2%). Among non-MCPP cases and controls, density distributions were similar across sites (Figure 1). Median densities were lowest in Thailand in all groups. The all-site density distribution curves were shifted toward higher densities in MCPP cases versus controls, but the distributions...
| Characteristic | MCPP Cases | Non-MCPP Cases | All Controls | RTI Controls | Non-RTI Controls |
|---------------|------------|----------------|-------------|--------------|-----------------|
| No. (%)       | No. (%)    | No. (%)        | No. (%)     | No. (%)      | No. (%)         |
| Overall       | 56         | 55 (96.2)      | 17.28       | 4035         | 2892 (71.7)     |
| Age, mo       |            |                |             |              |                 |
| 1–5           | 12         | 11 (91.7)      | 22.27       | 1660         | 1129 (68.0)     |
| 6–11          | 13         | 13 (100.0)     | 9.11        | 920          | 684 (74.3)      |
| 12–23         | 17         | 17 (100.0)     | 26.42       | 894          | 651 (72.8)      |
| 24–59         | 14         | 14 (100.0)     | 13.02       | 561          | 428 (76.3)      |
| Sex           |            |                |             |              |                 |
| Male          | 29         | 28 (96.6)      | 14.82       | 2311         | 1655 (71.6)     |
| Female        | 27         | 27 (100.0)     | 18.71       | 1724         | 1237 (71.8)     |
| HIV infected<sup>a</sup> | 13 | 13 (100.0) | 28.58 | 225 | 160 (71.1) |
| PCV vaccinated<sup>c</sup> | 36 | 36 (100) | 17.46 | 2050 | 1525 (74.4) |
| Prior antibiotic use | 12 | 11 (91.7) | 32 | 608 | 402 (66.1) |
| NP culture positive for pneumococcus | 44 | 43 (97.7) | 20.38 | 2099 | 1936 (92.2) |
| Pneumococcus colonized (culture or PCR positive) | 56 | 55 (98.2) | 17.28 | 3055 | 2892 (84.7) |
| PERCH site    |            |                |             |              |                 |
| The Gambia    | 15         | 14 (93.3)      | 14.9        | 591          | 503 (85.1)      |
| Kenya         | 5          | 5 (100.0)      | 0.35        | 626          | 461 (73.6)      |
| Mali          | 24         | 24 (100.0)     | 35.81       | 647          | 477 (73.7)      |
| South Africa  | 5          | 5 (100.0)      | 0.25        | 908          | 577 (63.5)      |
| Zambia        | 7          | 7 (100.0)      | 5.37        | 542          | 418 (77.1)      |
| Bangladesh    | 0          | 0 (0.0)        | NA          | 499          | 335 (67.1)      |
| Thailand      | 0          | 0 (0.0)        | NA          | 222          | 121 (54.5)      |

Abbreviations: HIV, human immunodeficiency virus; MCPP, microbiologically confirmed pneumococcal pneumonia; NA, not applicable; NP, nasopharyngeal; OP, oropharyngeal; PCR, polymerase chain reaction; PCV, pneumococcal conjugate vaccine; PERCH, Pneumonia Etiology Research for Child Health; RTI, respiratory tract illness.

<sup>a</sup>MCPP cases were confirmed by the following tests: blood culture (n = 44), PCR of lung aspirates (n = 6) or pleural fluid (n = 5), lung aspirate culture (n = 4), pneumococcal antigen in pleural fluid (n = 3), and pleural fluid culture (n = 1); several cases were confirmed by ≥1 test. Median density was defined as the median NP/OP pneumococcal density, calculated by PCR for the lta gene among children with PCRP/positive NP/OP specimens.

<sup>c</sup>Controls were matched for HIV status at the 2 sites (South Africa and Zambia) with high HIV prevalence.

<sup>c</sup>PCV vaccinated was defined as ≥1 dose (restricted to Kenya, Gambia, Mali, and South Africa).
of these groups overlapped substantially (Figure 2). The colonization density distribution among MCPP cases pretreated with antibiotics was shifted toward lower densities compared with MCPP cases without antibiotics before NP specimen collection.

The optimal colonization density threshold for discriminating MCPP cases from controls was >6.9 log₁₀ copies/mL (sensitivity, 64.3%; specificity, 92.2%; age-, sex-, and site-adjusted odds ratio, 17.9 [95% confidence interval 9.9–32.4]). The threshold was unchanged when restricted to controls without RTI and when limiting the comparison to HIV-negative children. When restricted to those MCPP cases (n = 40) and controls (n = 5074) without prior use of antibiotics, the optimal threshold was 6.6 log₁₀ copies/mL (sensitivity, 77.5%; specificity, 85.3%), and it was 4.4 log₁₀ copies/mL when restricted to MCPP cases (n = 16) and controls (n = 114) exposed to antibiotics (sensitivity, 100%; specificity, 52.6%).

The proportion of cases and controls with densities >6.9 log₁₀ copies/mL among those positive varied by site (Figure 3), sex, HIV status, antibiotic pre-exposure, and pneumococcal culture positivity (Table 2). The proportion of MCPP cases with density >6.9 log₁₀ copies/mL ranged from 0 of 5 in Kenya to 21 of 24 (87.5%) in Mali. Across sites, this proportion was lower among MCPP cases who received antibiotic pretreatment than in those who did not (P = .04). The proportion of controls with density >6.9 log₁₀ copies/mL ranged from 1.2% in Thailand to 15.6% in Mali.

Among all PERCH cases, high colonization density was associated with clinical and severity measures considered suggestive of bacterial pneumonia (Table 3). Increasing density was associated

Figure 1. Pneumococcal colonization density by case and control group and Pneumonia Etiology Research for Child Health (PERCH) site; density was calculated by means of polymerase chain reaction (PCR) for the lytA gene performed on nasopharyngeal/oropharyngeal specimens from PCR-positive children. Diamonds represent group means; horizontal lines through boxes, group medians; dashed lines, areas outside the linear range of the assay for calculation of pneumococcal density from cycle threshold values, where there is a greater degree of uncertainty in density calculations. Boxes extend to the 25th and 75th percentiles and whiskers to minimum and maximum values. MCPP, microbiologically confirmed pneumococcal pneumonia; non-RTI, without respiratory tract illness.

Figure 2. Pneumococcal colonization density distribution among cases with microbiologically confirmed pneumococcal pneumonia (MCPP) and controls (left) and among cases with MCPP by prior antibiotic use (right); density was calculated by means of polymerase chain reaction for the lytA gene performed on nasopharyngeal/oropharyngeal specimens. Dashed lines (densities less than 4 log₁₀ copies/mL and greater than 8 log₁₀ copies/mL) represent areas outside the linear range of the assay for calculation of pneumococcal density from cycle threshold values, where there is a greater degree of uncertainty in density calculations.
in a dose-dependent manner with very severe pneumonia, white blood cell count >15/μL, C-reactive protein (CRP) ≥40 mg/L, and coinfection with any virus for which testing was performed. CXR-confirmed pneumonia, consolidation on CXR, HIV infection, oxygen saturation <92% with room air, and respiratory syncytial virus coinfection were all associated with density >6.9 log_{10} copies/mL, but without clear evidence of increasing strength of association with increasing densities.

Compared with MCPP cases with density ≤6.9 log_{10} copies/mL, those with density >6.9 log_{10} copies/mL had higher frequencies of very severe pneumonia and fatal outcome, and lower frequencies of prior antibiotic use, CXR-confirmed pneumonia, and consolidation on CXR (Table 4), but these differences were not statistically significant. Among non-MCPP cases, those with density >6.9 log_{10} copies/mL (n = 500; 12.4%) were more likely than those below the threshold to have very severe pneumonia, CXR-confirmed pneumonia, consolidation on CXR, oxygen saturation <92%, HIV infection, CRP ≥40 mg/L, or any virus coinfection, and they were less likely to have been previously treated with antibiotics. MCPP cases, regardless of colonization density, were similar to non-MCPP cases with density >6.9 log_{10} copies/mL, for frequency of elevated white blood cell count, oxygen saturation <92%, prior antibiotic use, or any virus coinfection, but they were more likely to be HIV positive or to have very severe pneumonia, CXR-confirmed pneumonia, alveolar consolidation on CXR, CRP ≥40 mg/L, or fatal outcomes, after adjustment for age, sex, and site.

The serotype of the invasive pneumococcal isolate was available for 46 (98%) of 47 culture-positive MCPP cases, and that of the NP isolate was available for all 44 NP culture-positive MCPP cases. One MCPP case infected with serotype 18C, although NP culture-positive, was PCR negative for pneumococcus, so density could not be determined. Of 43 with serotype data for both the NP and invasive isolate and PCR data, 32 (72.7%) had matching invasive and NP serotypes, with 18 serotypes represented, including both vaccine and nonvaccine serotypes (Figure 4). Although the number of MCPP cases with each identified serotype was small (1–4 per serotype), the distribution of colonization densities seemed similar by serotype. However, the 2 MCPP cases infected with serotype 13 and serotype 14 had colonization densities ≤6.9 log_{10} copies/mL; neither had received prior antibiotics. For serotypes identified in ≥10 controls, the percentages of controls with density >6.9 log_{10} copies/mL were similar across serotypes and ranged from 2.3% to 15.6% (Figure 4), equivalent to 84.4% to 97.7% specificity.

**DISCUSSION**

In the PERCH study, pneumococcal colonization density was significantly higher among children with MCPP than among other pneumonia cases or community controls. The strength of the association increased with increasing colonization density, with an optimal density threshold of >6.9 log_{10} copies/mL (64% sensitivity, 92% specificity) to distinguish MCPP cases from controls, but performance varied by site. The optimal threshold was lower (≥4.4 log_{10} copies/mL; 100% sensitivity, 52.6% specificity) for children treated with antibiotics before specimen collection. Pneumococcal colonization density was associated in a dose-dependent manner with characteristics regarded as suggestive of bacterial pneumonia (alveolar consolidation on CXR, very severe pneumonia, and elevated CRP levels).

Pneumococcal colonization density was also found to divide PERCH cases along a spectrum of disease severity from MCPP cases; MCPP cases with density >6.9 log_{10} copies/mL had the greatest proportion with very severe pneumonia and
### Table 2. Proportion of Children With NP/OP Pneumococcal Colonization Density >6.9 Log10 copies/mL by Case and Control Group and Characteristics

| Characteristic                          | MCPP Cases | Non-MCPP Cases | All Controls | RTI Controls | Non-RTI Controls |
|-----------------------------------------|------------|----------------|--------------|--------------|------------------|
|                                         | Density >6.9 Log10 Copies/mL, No. (%) | Density >6.9 Log10 Copies/mL, No. (%) | Density >6.9 Log10 Copies/mL, No. (%) | Density >6.9 Log10 Copies/mL, No. (%) | Density >6.9 Log10 Copies/mL, No. (%) |
| Overall                                 | 56 (64.3)  | 4035 (12.4)    | 5188 (7.8)   | 1226 (9.8)   | 3962 (7.2)       |
| Age, mo                                 |            |                |              |              |                  |
| 1–5                                     | 12 (75.0)  | 1660 (12.0)    | 1619 (8.5)   | 304 (10.9)   | 1315 (8.0)       |
| 6–11                                    | 8 (61.5)   | 920 (13.0)     | 1240 (7.4)   | 319 (10.7)   | 921 (8.3)        |
| 12–23                                   | 17 (70.6)  | 884 (13.8)     | 1288 (6.9)   | 345 (7.2)    | 923 (6.7)        |
| 24–59                                   | 14 (50.0)  | 561 (10.3)     | 1061 (8.2)   | 258 (10.9)   | 803 (59.3)       |
| Sex                                     |            |                |              |              |                  |
| Male                                    | 29 (58.6)  | 2311 (11.5)    | 2602 (7.4)   | 617 (9.4)    | 1985 (6.8)       |
| Female                                  | 27 (70.4)  | 1724 (13.6)    | 2585 (8.2)   | 609 (12.0)   | 1976 (7.5)       |
| HIV infected                            |            |                |              |              |                  |
| Yes                                     | 13 (69.2)  | 225 (18.7)     | 212 (11.8)   | 45 (13.3)    | 167 (11.4)       |
| No                                      | 35 (62.9)  | 3453 (11.3)    | 4388 (6.8)   | 981 (8.0)    | 3407 (6.5)       |
| PCV vaccinated                          |            |                |              |              |                  |
| Yes                                     | 36 (66.7)  | 2050 (13.2)    | 2562 (8.4)   | 575 (11.1)   | 1987 (7.5)       |
| No                                      | 12 (66.7)  | 608 (16.3)     | 482 (10.6)   | 127 (16.5)   | 355 (8.5)        |
| Prior antibiotic use                    |            |                |              |              |                  |
| Yes                                     | 16 (43.8)  | 1861 (75)      | 114 (4.4)    | 32 (13.1)    | 82 (4.9)         |
| No                                      | 38 (71.1)  | 2038 (170)     | 4648 (8.1)   | 1082 (10.3)  | 3566 (7.4)       |
| NP culture positive for pneumococcus    |            |                |              |              |                  |
| Yes                                     | 44 (75.0)  | 2099 (20.8)    | 3559 (10.3)  | 908 (11.2)   | 2651 (9.7)       |
| No                                      | 12 (25.0)  | 1894 (3.0)     | 1585 (1.8)   | 301 (5.7)    | 1284 (2.8)       |
| Pneumococcus colonized (culture or PCR positive) | 56 (64.3)  | 3055 (16.4)    | 4224 (9.6)   | 1048 (11.5)  | 3176 (8.9)       |

Abbreviations: HIV, human immunodeficiency virus; MCPP, microbiologically confirmed pneumococcal pneumonia; NP, nasopharyngeal; OP, oropharyngeal; PCR, polymerase chain reaction for lytA gene; PCV, pneumococcal conjugate vaccine; RTI, respiratory tract illness.

*P < .05 for comparison of proportion with pneumococcal colonization density >6.9 log10 copies/mL by sex (non-MCPP case group), HIV (non-MCPP case group, all controls, and non-RTI controls), prior antibiotic use (non-MCPP case group), and NP culture positive (MCPP and non-MCPP case groups, all controls, RTI controls, and non-RTI controls).

Fatal outcomes, followed by MCPP cases with density ≤6.9 log10 copies/mL, non-MCPP cases with density >6.9 log10 copies/mL, and non-MCPP cases ≤6.9 log10 copies/mL who had the lowest proportion with these characteristics. The association of colonization density with disease severity was observed in a previous study among HIV-infected adults with pneumonia in South Africa [24] but has not been reported among children.

Viral infections, especially influenza, have previously been associated with pneumococcal pneumonia and invasive pneumococcal disease in human studies [25, 26] and animal models [27, 28]. We found that high pneumococcal colonization density was associated with virus detection in the upper respiratory tract, and this finding was explained in part by respiratory syncytial virus coinfection. This finding may indicate that upper respiratory infection with viral pathogens enhances the density of pneumococcal colonization, but it does not directly address whether these copathogen infections are themselves related to the lower respiratory tract disease. Our finding is consistent with a recent study in South Africa among hospitalized adults and children with acute lower respiratory tract infection (LRTI), which showed that pneumococcal colonization density was associated with the presence of respiratory viruses [10]. In a case-control study such as the PERCH study, we cannot assess the potential causal role of viral infection increasing pneumococcal density or even whether viral infection preceded pneumococcal colonization. Longitudinal cohort studies, such as the Drakenstein study [29], are more suited to address this question.

Our findings in children are similar to the reported association between pneumococcal colonization density and confirmed pneumococcal pneumonia in adults [8, 9, 11, 30]. Studies among children have found that higher colonization density was associated with alveolar consolidation on CXR [12–14], a proxy for pneumococcal pneumonia. In a study among 550 children hospitalized with LRTI in Vietnam [12], cases with consolidation on CXR had higher median NP pneumococcal density at PCR (6.1 log10 copies/mL) than others with LRTI (6.1 log10 copies/mL) and community controls (5.9 log10 copies/mL). These studies did not identify a colonization density threshold that reliably predicted radiographically confirmed pneumonia.
Table 3. Associations of Increasing Pneumococcal Colonization Density With Clinical and Severity Measures Among All Cases*  

| Outcome                        | Density, log_{10} Copies/mL | Adjusted OR (95% CI)* | P Value* |
|--------------------------------|-----------------------------|-----------------------|---------|
| **CXR positive**               |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 0.89 (0.68–1.16)            | 0.39                  |         |
| 4 to ≤6.9                     | 1.09 (0.92–1.28)            | 0.32                  |         |
| >6.9                          | 1.53 (1.19–1.97)            | <0.01                 |         |
| **Consolidation on CXR**       |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 0.86 (0.62–1.20)            | 0.38                  |         |
| 4 to ≤6.9                     | 1.13 (0.92–1.39)            | 0.23                  |         |
| >6.9                          | 1.62 (1.27–2.07)            | <0.001                |         |
| **Very severe pneumonia**     |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 1.26 (0.97–1.64)            | 0.09                  |         |
| 4 to ≤6.9                     | 1.20 (1.01–1.42)            | 0.03                  |         |
| >6.9                          | 1.62 (1.47–1.85)            | <0.001                |         |
| **HIV infected**              |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 1.01 (0.60–1.70)            | 0.96                  |         |
| 4 to ≤6.9                     | 0.94 (0.67–1.31)            | 0.72                  |         |
| >6.9                          | 2.01 (1.30–3.10)            | <0.01                 |         |
| **WBC count >15/μL**          |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 1.02 (0.79–1.32)            | 0.88                  |         |
| 4 to ≤6.9                     | 1.32 (1.13–1.55)            | <0.001                |         |
| >6.9                          | 1.45 (1.14–1.85)            | <0.01                 |         |
| **CRP ≥40 mg/L**              |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 0.91 (0.66–1.27)            | 0.59                  |         |
| 4 to ≤6.9                     | 1.74 (1.43–2.12)            | <0.001                |         |
| >6.9                          | 3.59 (2.74–4.71)            | <0.001                |         |
| **Oxygen saturation <92% with room air** |                   |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 1.02 (0.75–1.39)            | 0.88                  |         |
| 4 to ≤6.9                     | 1.02 (0.84–1.24)            | 0.84                  |         |
| >6.9                          | 1.51 (1.14–2.02)            | <0.01                 |         |
| **Death**                     |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 0.75 (0.49–1.16)            | 0.20                  |         |
| 4 to ≤6.9                     | 0.54 (0.41–0.72)            | <0.001                |         |
| >6.9                          | 0.95 (0.66–1.38)            | 0.80                  |         |
| **Virus coinfection**          |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 1.18 (0.83–1.69)            | 0.36                  |         |
| 4 to ≤6.9                     | 1.44 (1.15–1.80)            | <0.01                 |         |
| >6.9                          | 1.92 (1.27–2.89)            | <0.01                 |         |
| **RSV coinfection**            |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 1.24 (0.97–1.60)            | 0.09                  |         |
| 4 to ≤6.9                     | 0.86 (0.74–1.00)            | 0.05                  |         |
| >6.9                          | 1.30 (1.03–1.65)            | 0.03                  |         |
| **Influenza coinfection**      |                             |                       |         |
| 0                              | 1.00                        |                       | ...     |
| 1 to <4                       | 1.90 (1.26–2.87)            | <0.01                 |         |
| 4 to ≤6.9                     | 1.10 (0.82–1.48)            | 0.52                  |         |
| >6.9                          | 1.06 (0.66–1.71)            | 0.81                  |         |

Abbreviations: CI, confidence interval; CRP, C-reactive protein; CXR, chest radiograph; HIV, human immunodeficiency virus; OR, odds ratio; RSV, respiratory syncytial virus; WBC, white blood cell;  
*Pneumococcal colonization density calculated from polymerase chain reaction (PCR) for the lytA gene on nasopharyngeal/oropharyngeal specimens.  
*ORs and P values calculated from a multivariable logistic regression model of categorical density as a predictor of each outcome, with adjustment for age, sex, and site.  
*CXR positive defined as radiographic evidence of pneumonia (consolidation and/or other sterile body fluid).  
*Virus coinfection defined as positive for any virus tested by PCR of the nasopharyngeal/oropharyngeal specimens including influenza A, B, or C; parainfluenza viruses 1, 2, 3, or 4; coronavirus NL63, 229E, OC43, or HKU1; human metapneumovirus A or B; human rhinovirus; RSV A or B; adenovirus; enterovirus/parechovirus; human bocavirus; and cytomegalovirus.  
*Influenza A, B, or C.  

A study among children and adults hospitalized with acute LRTI in South Africa found that invasive pneumococcal pneumonia was associated with increased colonization density; cases with density >1000 copies/mL had 18 times greater odds of invasive pneumococcal pneumonia than colonized cases with density <1000 copies/mL [10]. The South African study defined invasive pneumococcal pneumonia by detection of S. pneumoniae by PCR in the blood, a diagnostic not used in our study owing to poor specificity [31, 32]. We found that the best-performing threshold (6.9 log_{10} [10^{6.9}] copies/mL) was much higher than that suggested by the South African study, but comparison of density thresholds between studies is limited by methodologic differences.

The association of pneumococcal colonization density with MCPP does not indicate its utility for patient care. Even in a population with a relatively high prevalence of pneumococcal disease (eg, children hospitalized with pneumonia), the positive predictive value would probably be too low to influence clinical decision making. In settings with lower pneumococcal disease prevalence (eg, countries using PCV), the positive predictive value would be even lower. Although the negative predictive value may be relatively high, it would not be high enough to justify withholding antibiotics in hospitalized children with clinical or radiographic evidence suggestive of bacterial pneumonia. Furthermore, to be useful in a clinical setting, local data on the pneumococcal colonization density distribution would be needed, and patient assessment would have to account for antibiotic pretreatment.

Although our findings are strengthened by the large study size, 7 country sites, and systematic enrollment of well-characterized cases and controls using standardized clinical criteria and laboratory procedures, there were limitations. The number of MCPP cases limited stratified analyses by study site and pneumococcal serotype and prevented calculation of site-specific density thresholds. The findings were largely driven by cases from the 3 sites with the most MCPP cases (The Gambia, Mali, and Zambia). Despite previous evidence of substantial pneumococcal disease burden in children in Bangladesh [33, 34] and Thailand [35, 36], no MCPP cases were identified among enrolled PERCH cases in either of those sites, limiting the evaluation of this threshold at those sites. However, Bangladesh and Thailand did have cases with colonization density above the threshold, the proportion of which in Bangladesh exceeded that in Kenya and Zambia.

The association between pneumococcal pneumonia and colonization density was derived using MCPP cases, but the potential application as a diagnostic assay would be most important to identify cases without pneumococcal detection from blood or other sterile body fluid, which represent the majority of cases with pneumococcal pneumonia [6]. Therefore, the sensitivity of the 6.9 log_{10} copies/mL threshold for detecting pneumococcal pneumonia may be lower than we estimated based on the...
MCPP cases. Finally, our study design did not allow assessment of the temporal relationship of colonization density with MCPP. Our analysis aimed not to assess causality but rather to identify a diagnostic adjunct to improve pneumococcal case detection over detection from invasive specimens alone. In addition to study limitations, there are limitations inherent to the measurement of pneumococcal colonization density. Although the PERCH study made great efforts to standardize specimen collection [37] there was no way to standardize the specimen volume taken from the NP/OP space. Higher specimen volume resulting from, for example, coryza could increase measured colonization density.

Our findings provide strong evidence for the relationship between pneumococcal colonization density and pneumococcal pneumonia in children. Pneumococcal colonization density seems to improve detection of pneumococcal pneumonia beyond blood culture, which though highly specific, is insensitive and available only in settings with good microbiology.
capacity. However, the sensitivity of colonization density remains suboptimal, limiting its utility in clinical settings at the individual case level.

Notes

Author contributions. H. C. B. led analysis and interpretation and drafted manuscript. N. L. W. performed analyses and interpretation of results. M. D. K., D. R. F., L. H., D. R. M., D. E. P., S. L. Z., and K. L. O. assisted with interpretation of results and drafting of manuscript. H. C. B., M. D. K., W. A. B., D. R. F., L. H., S. R. C. H., K. L. K., O. S. L., S. A. M., D. R. M., J. A. G. S., D. M. T., R. A. K., and K. L. O. conceived and designed the study and supervised study conduct. M. A., J. O. A., S. O. S., M. D. T., and K. Z. were involved in study conduct, data collection, and/or data management. All authors reviewed and approved the manuscript. H. C. B. had final responsibility for the decision to submit for publication.

Acknowledgments. We offer sincere thanks to the patients and families who participated in this study. We recognize the efforts of the following groups during the development, study conduct, and analysis phases (see Supplemental Materials for full list of names): Pneumonia Methods Working Group, PERCH Expert Group, PERCH contributors, and the PERCH Chest Radiograph Reading Panel. This article is published with the permission of the director of the Kenya Medical Research Institute.

PERCH Study Group. Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland: K. L. O. (principal investigator [PI]), O. S. L. (former PI; current affiliation, Bill & Melinda Gates Foundation, Seattle, Washington), M. D. K. (co-PI), D. R. F. (joint affiliation with Centers for Disease Control and Prevention, Atlanta, Georgia), A. N. D., A. J. D., Nicholas Fancourt, Wei Fu, L. H., M. M. H., E. Wangeci Kagucia, R. A. K., Mengying Li, D. E. P., C. P., Zhenke Wu, S. L. Z.; The Emmes Corporation, Rockville, Maryland: N. L. W.; Nuffield Department of Clinical Medicine, University of Oxford, United Kingdom: Jane Crawley; University of Otago, Christchurch, New Zealand: D. R. M.; International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka and Matlab, Bangladesh: W. A. B. (site PI), Hubert P. Endtz, K. Z., D. G., Lokman Hossain, Yasmin Jahan, Hasan Ashraf; Medical Research Council, Basse, The Gambia: S. R. C. H. (site PI), B. E. E., M. A., Jessica McLellan, Eunice Machuka, Arifin Shamsul, Syed M. A. Zaman, Grant Mackenzie; KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya: J. A. G. S. (site PI and PERCH co-PI), J. O. A., S. C. M., Alice Kamau, Sidi Kazungu, Micah Sliba Ominde; Division of Infectious Disease and Tropical Pediatrics, Department of Pediatrics, Center for Vaccine Development, Institute of Global Health, University of Maryland School of Medicine, Baltimore, and Centre pour le Développement des Vaccins (CVD-Mali), Bamako, Mali: K. L. K. (site PI), M. D. T., S. O. S., Mamadou Sylla, Boubou Tamboura, Uma Onwuchekwa, Nana Kourouma, Aliou Toure; Respiratory and Meningeal Pathogens Research Unit, University of the Witwatersrand, Johannesburg, South Africa: S. A. M. (site PI), D. P. M., Peter V. Adrian, V. L. B., Locadiah Kuwanda, Azwifarwi Mudau, Michelle J. Groome, Nasreen Mahomed; Thailand Ministry of Public Health – US CDC Collaboration, Nonthaburi: H. C. B. (site PI), Somsak Thamthitiwat, Susan A. Maloney (former site PI), Charatdao Bunthi, Julia Rhodes, Pongpun Sawatwong, Pasakorn Akarasewi (site co-PI, Ministry of Public Health); Boston University School of Public Health, Massachusetts, and University Teaching Hospital, Lusaka, Zambia: D. M. T. (site PI), Lawrence Mwananyanda, James Chipeta, Phil Seidenberg, James Mwansa, Somwe wa Somwe, Geoffrey Kwenda; Canterbury Health Laboratory, Christchurch, New Zealand: Trevor P. Anderson, Joanne Mitchell.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, the US Department of Health and Human Services, or the US government.

Financial support. This work was supported by the Bill & Melinda Gates Foundation (grant 48968 to the International Vaccine Access Center, Department of International Health, Johns Hopkins Bloomberg School of Public Health, for the PERCH study) and the Wellcome Trust of Great Britain (clinical fellowship 098532 to J. A. G. S.).
**Supplement sponsorship.** This article appears as part of the supplement “Pneumonia Etiology Research for Child Health (PERCH): Foundational Basis for the Primary Etiology Results,” sponsored by a grant from the Bill & Melinda Gates Foundation to the PERCH study of Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

**Potential conflicts of interest.** K. L. O. has received grant funding from GSK and Pfizer and participates on technical advisory boards for Merck, Sanofi Pasteur, PATH, Affinivax, and ClearPath. M. D. K. has received funding for consultations from Merck, Pfizer, and Novartis, and grant funding from Merck. L. L. H. has received grant funding from Pfizer and GlaxoSmithKline (GSK). K. L. K. has received grant funding from Merck Sharp & Dohme. S. A. M. has received honoraria for advisory board participation from Bill & Melinda Gates Foundation, Pfizer, Medimmune, and Novartis and institutional grants from GSK, Novartis, Pfizer, MinervaX, and Bill & Melinda Gates Foundation and has served on speakers bureau for Sanofi Pasteur and GSK. All other authors: No reported conflicts. 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.

**References**

1. Gray BM, Converse GM 3rd, Dillon HC Jr. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis 1980; 142:923–33.

2. Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004; 4:44–54.

3. Simel B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O’Brien KL. Pneumococcal Carriage Group. The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines 2012; 11:841–55.

4. Cutts FT, Zaman SM, Enwere G, et al; Gambian Pneumococcal Vaccine Trial Group. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. Lancet 2005; 365:1139–46.

5. Black SB, Shinefield HR, Ling S, et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. Pediatr Infect Dis J 2002; 21:810–5.

6. Madhi SA, Kuwanda L, Cutland C, Klugman KP. The impact of a 9-valent pneumococcal conjugate vaccine on the public health burden of pneumonia in HIV-infected and -uninfected children. Clin Infect Dis 2005; 40:1511–8.

7. Klugman KP, Madhi SA, Albrich WC. Novel approaches to the identification of *Streptococcus pneumoniae* as the cause of community-acquired pneumonia. Clin Infect Dis 2008; 47(suppl 3):S202–6.

8. Strålin K, Herrmann B, Abdeldaim G, Olćen P, Holmberg H, Mölling P. Comparison of sputum and nasopharyngeal aspirate samples and of the PCR gene targets hlyA and Spn9802 for quantitative PCR for rapid detection of pneumococcal pneumonia. J Clin Microbiol 2014; 52:83–9.

9. Albrich WC, Madhi SA, Adrian PV, et al. Use of a rapid test of pneumococcal colonization density to diagnose pneumococcal disease. Clin Infect Dis 2012; 54:601–9.

10. Wolter N, Tempia S, Cohen C, et al. High nasopharyngeal pneumococcal density, increased by viral coinfection, is associated with invasive pneumococcal pneumonia. J Infect Dis 2014; 210:1649–57.

11. Alpkvist H, Athlin S, Naucuir P, et al. Clinical and microbiological factors associated with high nasopharyngeal pneumococcal density in patients with pneumococcal pneumonia. PLoS One 2015; 10:e0140112.

12. Yu HT, Yoshida LM, Suzuki M, et al. Association between nasopharyngeal load of *Streptococcus pneumoniae*, viral coinfection, and radiologically confirmed pneumonia in Vietnamese children. Pediatr Infect Dis J 2011; 30:1–8.

13. Anh DH, Huong P, T, Watanabe K, et al. Increased rates of intensive nasopharyngeal bacterial colonization of Vietnamese children with radiological pneumonia. Tohoku J Exp Med 2007; 213:167–72.

14. Esposito S, Zampiero A, Terranova L, et al. Pneumococcal bacterial load colonization as a marker of mixed infection in children with alveolar community-acquired pneumonia and respiratory syncytial virus or rhinovirus infection. Pediatr Infect Dis J 2013; 32:1199–204.

15. Levine OS, O’Brien KL, Deloria-Knoll M, et al. The Pneumonia Etiology Research for Child Health project: a 21st century childhood pneumonia etiology study. Clin Infect Dis 2012; 54(suppl 2):S93–101.

16. Deloria-Knoll M, Feikin DR, Scott JA, et al; Pneumonia Methods Working Group. Identification and selection of cases and controls in the Pneumonia Etiology Research for Child Health project. Clin Infect Dis 2012; 54(suppl 2):S117–23.

17. World Health Organization (WHO). Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. Geneva, Switzerland: World Health Organization, 2005.

18. Driscoll AJ, Karron RA, Morpeth SC, et al. Standardization of laboratory methods for the Pneumonia Etiology Research For Child Health study. Clin Infect Dis 2017; 64(suppl 3):S245–52.

19. Falade AG, Mulolland EK, Adegbola RA, Greenwood BM. Bacterial isolates from blood and lung aspirate cultures in Gambian children with lobar pneumonia. Ann Trop Paediatr 1997; 17:315–9.

20. Driscoll AJ, Deloria Knoll M, Hammitt LL, et al. The effect of antibiotic exposure and specimen volume on the detection of bacterial pathogens in children with pneumonia. Clin Infect Dis 2017; 64(suppl 3):S368–77.

21. Cherian T, Mulolland EK, Carlin JB, et al. Standardized interpretation of paediatric chest radiographs for the diagnosis of pneumonia in epidemiological studies. Bull World Health Organ 2005; 83:353–9.

22. Fancourt N, Deloria Knoll M, Barger-Kamate B, et al. Standardized interpretation of chest radiographs in cases of pediatric pneumonia from the PERCH study. Clin Infect Dis 2017; 64(suppl 3):S253–61.

23. Youden WI. Index for rating diagnostic tests. Cancer 1950; 3:325–5.

24. Albrich WC, Madhi SA, Adrian PV, et al. Pneumococcal colonisation density: a new marker for disease severity in HIV-infected adults with pneumonia. BMJ Open 2014; 4:e005953.

25. Walter ND, Taylor TH, Shay DK, et al; Active Bacterial Core Surveillance Team. Influenza circulation and the burden of invasive pneumococcal pneumonia during a non-pandemic period in the United States. Clin Infect Dis 2015; 50:175–83.

26. Talbot TR, Poehlking KA, Hartert TV, et al. Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. Am J Med 2005; 118:285–91.

27. McCullers JA. Insights into the interaction between influenza virus and pneumococcus. Clin Microbiol Rev 2006; 19:571–82.

28. McCullers JA, Rehg JE. Lethal synergism between influenza virus and *Streptococcus pneumoniae* results in formation of a new pathologic entity and the role of platelet-activating factor receptor. J Infect Dis 2002; 186:341–50.

29. le Roux DM, Myer L, Nicol MP, Zar HI. Incidence and severity of childhood pneumonia in the first year of life in a South African birth cohort: the Drakenstein Child Health Study. Lancet Glob Health 2015; 3:e95–e103.

30. Werno AM, Anderson TP, Murdoch DR. Association between pneumococcal load and disease severity in adults with pneumonia. J Med Microbiol 2012; 61(pt 8):1129–35.

31. Morpeth SC, Deloria Knoll M, Watson NL, et al. Detection of pneumococcal DNA in blood by PCR for diagnosing pneumococcal pneumonia in young children from low and middle income countries. Clin Infect Dis 2017; 64(suppl 3):S347–56.

32. Deloria Knoll M, Morpeth SC, Watson NL, et al. Evaluation of pneumococcal load in blood by PCR for the diagnosis of pneumococcal pneumonia in young children in the Pneumonia Etiology Research for Child Health (PERCH) study. Clin Infect Dis 2017; 64(suppl 3):S357–67.

33. Arifeen SE, Saha SK, Rahman S, et al. Invasive pneumococcal disease among children in rural Bangladesh: Results from a population-based surveillance. Clin Infect Dis 2009; 48:103–13.

34. Saha SK, Al Emran HM, Hossain B, et al; Pneumococcal Study Group. *Streptococcus pneumoniae* serotype-2 childhood meningoitis in Bangladesh: a newly recognized pneumococcal infection threat. PLoS One 2012; 7:e32134.

35. Baggett HC, Peruski LF, Olsen SJ, et al. Incidence of pneumococcal bacteremia requiring hospitalization in rural Thailand. Clin Infect Dis 2009; 48(suppl 2):S65–74.

36. Rhodes J, Desjeuillette S, Maloney SA, et al. Pneumococcal bacteremia requiring hospitalization in rural Thailand: an update on incidence, clinical characteristics, serotype distribution, and antimicrobial susceptibility, 2005–2010. PLoS One 2013; 8:e66038.

37. Crawley J, Prosperi C, Baggett HC, et al. Standardization of clinical assessment and sample collection across all PERCH study sites. Clin Infect Dis 2017; 64(suppl 3):S228–37.