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SUPPLEMENT ARTICLE

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

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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 (MCPP) 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 MCPP (MCPP cases; 17.28×10^6 copies/mL) exceeded that of cases without MCPP (non-MCPP cases; 0.75×10^6) and controls (0.60×10^6) (each $P < .001$). The optimal density for discriminating MCPP 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 MCPP cases were distinguished from controls who received antibiotics before specimen collection. Among the 4035 non-MCPP 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 MCPP 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.

Streptococcus pneumoniae colonization of the nasopharynx precedes and is necessary for development of pneumococcal pneumonia and invasive pneumococcal disease [1, 2] but most commonly resolves without progression to disease [3]. Diagnosis of pneumococcal pneumonia in children currently relies on blood culture, which demonstrated only 5%–15% of cases [4–6].

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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, discharged 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 (*lyt*A 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 χ^2 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 of density above versus below the threshold as a predictor of each characteristic, with adjustment 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%) ($P \leq .01$ 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 (17.28×10^6 copies/mL) relative to non-MCPP cases (0.75×10^6) and controls (0.60×10^6) ($P < .001$ 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 (0.25×10^6) than both non-MCPP cases (0.70×10^6) and controls (0.77×10^6), 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 (35.81×10^6 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 0.25×10^6 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

Table 1. NP/OP Pneumococcal PCR Positivity and Density by Case and Control Group and by Characteristic[a]

	MCPP Cases			Non-MCPP Cases			All Controls			RTI Controls			Non-RTI Controls		
Characteristic	No.	PCR Positive, No. (%)	Median Density, 10^6 Copies/mL	No.	PCR Positive, No. (%)	Median Density, 10^6 Copies/mL	No.	PCR Positive, No. (%)	Median Density, 10^6 Copies/mL	No.	PCR Positive, No. (%)	Median Density, 10^6 Copies/mL	No.	PCR Positive, No. (%)	Median Density, 10^6 Copies/mL
Overall	56	55 (98.2)	17.28	4035	2892 (71.7)	0.75	5188	3975 (76.6)	0.60	1226	998 (81.4)	0.85	3962	2977 (75.2)	0.53
Age, mo															
1–5	12	11 (91.7)	22.27	1660	1129 (68.0)	0.98	1619	1141 (70.5)	0.95	304	236 (77.6)	1.29	1315	905 (68.8)	0.86
6–11	13	13 (100.0)	9.11	920	684 (74.3)	0.69	1240	1001 (80.7)	0.55	319	266 (83.4)	0.74	921	735 (79.8)	0.48
12–23	17	17 (100.0)	26.42	894	651 (72.8)	0.81	1268	985 (77.7)	0.46	345	273 (79.1)	0.85	923	712 (77.1)	0.39
24–59	14	14 (100.0)	13.02	561	428 (76.3)	0.34	1061	849 (80.0)	0.34	258	223 (86.4)	0.61	803	626 (78.0)	0.31
Sex															
Male	29	28 (96.6)	14.82	2311	1655 (71.6)	0.68	2602	2014 (77.4)	0.55	617	499 (80.9)	0.73	1985	1515 (76.3)	0.48
Female	27	27 (100.0)	18.71	1724	1237 (71.8)	0.85	2585	1961 (75.9)	0.65	609	499 (81.9)	1.01	1976	1462 (74.0)	0.58
HIV infected[b]															
Yes	13	13 (100.0)	28.58	225	160 (71.1)	1.83	212	133 (62.7)	1.18	45	32 (71.1)	1.13	167	101 (60.5)	1.18
No	35	34 (97.1)	14.9	3453	2474 (71.6)	0.65	4388	3388 (77.2)	0.58	981	804 (82.0)	0.77	3407	2584 (75.8)	0.53
PCV vaccinated[c]															
Yes	36	36 (100)	17.46	2050	1525 (74.4)	0.76	2562	2027 (79.1)	0.6	575	493 (85.7)	0.84	1987	1534 (77.2)	0.52
No	12	11 (91.7)	32	608	402 (66.1)	1.87	482	341 (70.7)	0.8	127	109 (85.8)	1.69	355	232 (65.4)	0.6
Prior antibiotic use															
Yes	16	16 (100.0)	1.69	1861	1294 (69.5)	0.33	114	69 (60.5)	0.33	32	23 (71.9)	0.77	82	46 (56.1)	0.3
No	38	37 (97.4)	20.38	2038	1501 (73.7)	1.62	4648	3590 (77.2)	0.62	1082	893 (82.5)	0.84	3566	2697 (75.6)	0.56
NP culture positive for pneumococcus															
Yes	44	43 (97.7)	20.38	2099	1936 (92.2)	1.69	3559	3311 (93.0)	0.75	908	858 (94.5)	1.03	2651	2453 (92.5)	0.68
No	12	12 (100.0)	0.23	1894	928 (49.0)	0.08	1585	631 (39.8)	0.08	301	124 (41.2)	0.07	1284	507 (39.5)	0.08
Pneumococcus colonized (culture or PCR positive)	56	55 (98.2)	17.28	3055	2892 (94.7)	0.75	4224	3976 (94.1)	0.6	1048	998 (95.2)	0.85	3176	2978 (93.8)	0.53
PERCH site															
The Gambia	15	14 (93.3)	14.9	591	503 (85.1)	1.74	624	553 (88.6)	0.67	156	142 (91.0)	0.66	468	411 (87.8)	0.68
Kenya	5	5 (100.0)	0.35	626	461 (73.6)	0.25	857	684 (79.8)	0.26	211	178 (84.4)	0.36	646	506 (78.3)	0.23
Mali	24	24 (100.0)	35.81	647	477 (73.7)	2.83	724	573 (79.1)	1.15	298	256 (85.9)	2.3	426	317 (74.4)	0.79
South Africa	5	5 (100.0)	0.25	908	577 (63.5)	0.7	959	647 (67.5)	0.77	53	41 (77.4)	0.56	906	606 (66.9)	0.78
Zambia	7	7 (100.0)	5.37	542	418 (77.1)	0.46	606	482 (79.5)	0.58	89	74 (83.1)	0.53	517	408 (78.9)	0.58
Bangladesh	0	0 (0.0)	NA	499	335 (67.1)	1.19	768	631 (82.2)	0.99	169	135 (79.9)	1.45	599	496 (82.8)	0.88
Thailand	0	0 (0.0)	NA	222	121 (54.5)	0.04	650	406 (62.5)	0.21	250	172 (68.8)	0.51	400	234 (58.5)	0.11

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.

[a]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 *lyt*A gene among children with PCR-positive NP/OP specimens.

[b]Controls were matched for HIV status at the 2 sites (South Africa and Zambia) with high HIV prevalence.

[c]PCV vaccinated was defined as ≥1 dose (restricted to Kenya, Gambia, Mali, and South Africa).

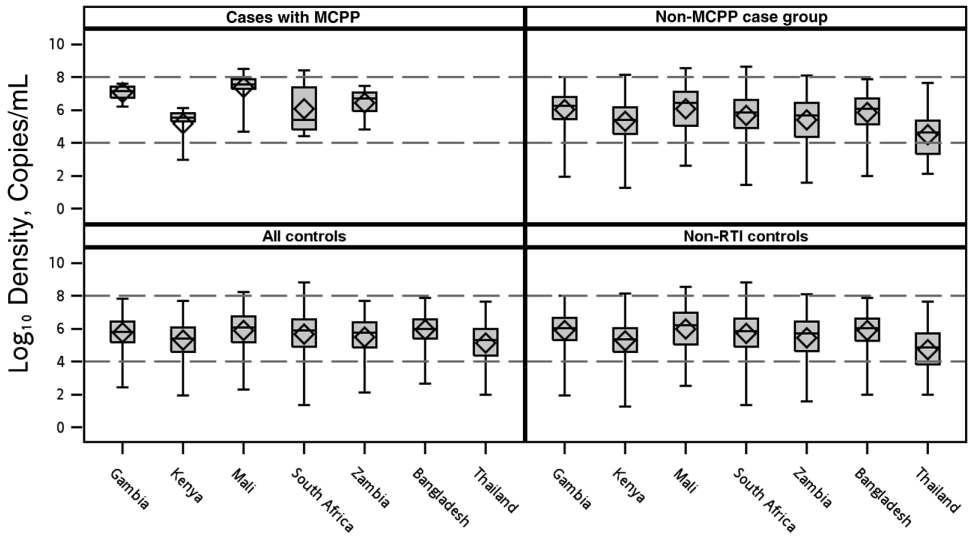

Figure 1. Pneumococcal colonization density by case and control group and Pneumonia Etiology Research for Child Health (PERCH) site; density was calculated by means of polymerase chain reaction (PCR) for the *lytA* gene performed on nasopharyngeal/oropharyngeal specimens from PCR-positive children. Diamonds 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.

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 $\log_{10}$ 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 $\log_{10}$ copies/mL (sensitivity, 77.5%; specificity, 85.3%), and it was 4.4 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ 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

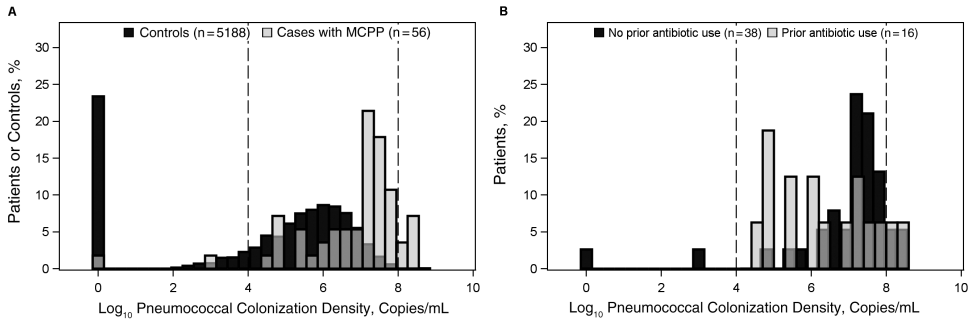

Figure 2. Pneumococcal colonization density distribution among cases with microbiologically confirmed pneumococcal pneumonia (MCPP) and controls (*left*) and among cases with MCPP by prior antibiotic use (*right*); density was calculated by means of polymerase chain reaction for the *lytA* gene performed on nasopharyngeal/oropharyngeal specimens. Dashed lines (densities less than 4 $\log_{10}$ copies/ml and greater than 8 $\log_{10}$ 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.

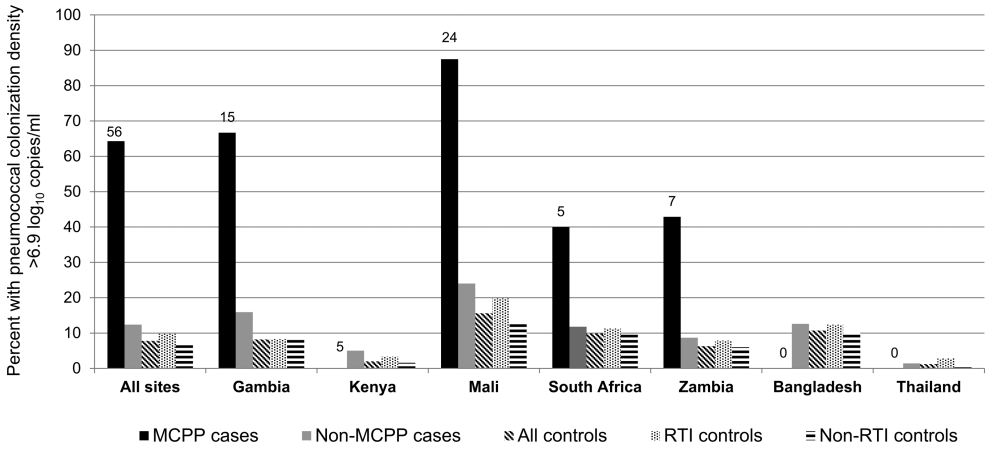

Figure 3. Percentage of children with nasopharyngeal/oropharyngeal pneumococcal colonization density >6.9 $\log_{10}$ copies/mL among positives, by site and case and control group; density was calculated by means of polymerase chain reaction for the *lytA* gene performed on nasopharyngeal/oropharyngeal specimens. Numbers above bars represent the number of microbiologically confirmed pneumococcal pneumonia (MCPP) cases at the site. RTI, respiratory tract illness.

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 was 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 Log_{10} Copies/mL by Case and Control Group and Characteristics[a]

	MCPP Cases		Non-MCPP Cases		All Controls		RTI Controls		Non-RTI Controls	
Characteristic	No.	Density >6.9 Log_{10} Copies/mL, No. (%)	No.	Density >6.9 Log_{10} Copies/mL, No. (%)	No.	Density >6.9 Log_{10} Copies/mL, No. (%)	No.	Density >6.9 Log_{10} Copies/mL, No. (%)	No.	Density >6.9 Log_{10} Copies/mL, No. (%)
Overall	56	36 (64.3)	4035	500 (12.4)	5188	404 (7.8)	1226	120 (9.8)	3962	284 (7.2)
Age, mo										
1–5	12	9 (75.0)	1660	199 (12.0)	1619	138 (8.5)	304	33 (10.9)	1315	105 (8.0)
6–11	13	8 (61.5)	920	120 (13.0)	1240	92 (7.4)	319	34 (10.7)	921	58 (6.3)
12–23	17	12 (70.6)	894	123 (13.8)	1268	87 (6.9)	345	25 (7.2)	923	62 (6.7)
24–59	14	7 (50.0)	561	58 (10.3)	1061	87 (8.2)	258	28 (10.9)	803	59 (7.3)
Sex										
Male	29	17 (58.6)	2311	265 (11.5)[b]	2602	193 (7.4)	617	58 (9.4)	1985	135 (6.8)
Female	27	19 (70.4)	1724	235 (13.6)[b]	2585	211 (8.2)	609	62 (10.2)	1976	149 (7.5)
HIV infected										
Yes	13	9 (69.2)	225	42 (18.7)[b]	212	25 (11.8)[b]	45	6 (13.3)	167	19 (11.4)[b]
No	35	22 (62.9)	3453	389 (11.3)[b]	4388	300 (6.8)[b]	981	78 (8.0)	3407	222 (6.5)[b]
PCV vaccinated[c]										
Yes	36	24 (66.7)	2050	270 (13.2)	2562	214 (8.4)	575	64 (11.1)	1987	150 (7.5)
No	12	8 (66.7)	608	99 (16.3)	482	51 (10.6)	127	21 (16.5)	355	30 (8.5)
Prior antibiotic use										
Yes	16	7 (43.8)	1861	140 (7.5)[b]	114	5 (4.4)	32	1 (3.1)	82	4 (4.9)
No	38	27 (71.1)	2038	347 (17.0)[b]	4648	376 (8.1)	1082	111 (10.3)	3566	265 (7.4)
NP culture positive for pneumococcus										
Yes	44	33 (75.0)[b]	2099	436 (20.8)[b]	3559	368 (10.3)[b]	908	110 (12.1)[b]	2651	258 (9.7)[b]
No	12	3 (25.0)[b]	1894	56 (3.0)[b]	1585	28 (1.8)[b]	301	5 (1.7)[b]	1284	23 (1.8)[b]
Pneumococcus colonized (culture or PCR positive)	56	36 (64.3)	3055	500 (16.4)	4224	404 (9.6)	1048	120 (11.5)	3176	284 (8.9)

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

[a]Pneumococcal colonization density calculated by PCR for the *lyt*A gene performed on NP/OP specimens in PCR-positive children.

[b]$P < .05$ for comparison of proportion with pneumococcal colonization density ≥6.9 log_{10} 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).

[c]PCV vaccinated was defined as ≥1 dose (restricted to Kenya, Gambia, Mali, and South Africa).

fatal outcomes, followed by MCPP cases with density ≤6.9 log_{10}, non-MCPP cases with density >6.9 log_{10}, and non-MCPP cases ≤6.9 log_{10}, 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 enhanced 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 (6.9 log_{10} copies/mL) than others with LRTI (6.1 log_{10} copies/mL) and community controls (5.9 log_{10} 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[a]

Outcome	Density, Log_{10} Copies/mL	Adjusted OR (95% CI)[b]	*P* Value[b]
CXR positive[c]	0	1.00	…
	1 to <4	0.89 (.68–1.16)	.39
	4 to ≤6.9	1.09 (.92–1.28)	.32
	>6.9	1.53 (1.19–1.97)	<.01
Consolidation on CXR	0	1.00	…
	1 to <4	0.86 (.62–1.20)	.38
	4 to ≤6.9	1.13 (.92–1.39)	.23
	>6.9	1.99 (1.48–2.69)	<.001
Very severe pneumonia	0	1.00	…
	1 to <4	1.26 (.97–1.64)	.09
	4 to ≤6.9	1.20 (1.01–1.42)	.03
	>6.9	1.62 (1.27–2.07)	<.001
HIV infected	0	1.00	…
	1 to <4	1.01 (.60–1.70)	.96
	4 to ≤6.9	0.94 (.67–1.31)	.72
	>6.9	2.01 (1.30–3.10)	<.01
WBC count >15/μL	0	1.00	…
	1 to <4	1.02 (.79–1.32)	.88
	4 to ≤6.9	1.32 (1.13–1.55)	<.001
	>6.9	1.45 (1.14–1.85)	<.01
CRP ≥40 mg/L	0	1.00	…
	1 to <4	0.91 (.66–1.27)	.59
	4 to ≤6.9	1.74 (1.43–2.12)	<.001
	>6.9	3.59 (2.74–4.71)	<.001
Oxygen saturation <92% with room air	0	1.00	…
	1 to <4	1.02 (.75–1.39)	.88
	4 to ≤6.9	1.02 (.84–1.24)	.84
	>6.9	1.51 (1.14–2.02)	<.01
Death	0	1.00	…
	1 to <4	0.75 (.49–1.16)	.20
	4 to ≤6.9	0.54 (.41–.72)	<.001
	>6.9	0.95 (.66–1.38)	.80
Virus coinfection[d]	0	1.00	…
	1 to <4	1.18 (.83–1.69)	.36
	4 to ≤6.9	1.44 (1.15–1.80)	<.01
	>6.9	1.92 (1.27–2.89)	<.01
RSV coinfection	0	1.00	…
	1 to <4	1.24 (.97–1.60)	.09
	4 to ≤6.9	0.86 (.74–1.00)	.05
	>6.9	1.30 (1.03–1.65)	.03
Influenza coinfection[e]	0	1.00	…
	1 to <4	1.90 (1.26–2.87)	<.01
	4 to ≤6.9	1.10 (.82–1.48)	.52
	>6.9	1.06 (.66–1.71)	.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;

[a]Pneumococcal colonization density calculated from polymerase chain reaction (PCR) for the *lyt*A gene on nasopharyngeal/oropharyngeal specimens.

[b]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.

[c]CXR positive defined as radiographic evidence of pneumonia (consolidation and/or other infiltrates).

[d]Virus coinfection defined as positive for any virus tested by PCR of the nasopharyngeal/oropharyngeal specimen (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).

[e]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

Table 4. Characteristics by Pneumococcal Colonization Density Among Cases With or Without MCPP[a]

Characteristic	Cases, No. (%)					Adjusted OR[b] (95% CI)		
	Group A: Non-MCPP ≤6.9 Log_{10} Copies/mL (n = 3535)	Group B: Non-MCPP >6.9 Log_{10} Copies/mL (n = 500)	Group C: All MCCP (n = 56)	Group D: MCPP ≤6.9 Log_{10} Copies/mL (n = 20)	Group E: MCPP >6.9 Log_{10} Copies/mL (n = 36)	Group B vs A (Reference)	Group C vs B (Reference)	Group E vs D (Reference) OR[b] (95% CI)
Age, mo								
1–5	1461 (41)	199 (40)	12 (21)	3 (15)	9 (25)	…	…	…
6–11	800 (23)	120 (24)	13 (23)	5 (25)	8 (22)	…	…	…
12–23	771 (22)	123 (25)	17 (30)	5 (25)	12 (33)	…	…	…
24–59	503 (14)	58 (12)	14 (25)	7 (35)	7 (19)	…	…	…
Male sex	2046 (58)	265 (53)	29 (52)	12 (60)	17 (47)	…	…	…
Site								
Gambia	497 (14)	94 (19)	15 (27)	5 (25)	10 (28)	…	…	…
Kenya	595 (17)	31 (6)	5 (9)	5 (25)	0 (0)	…	…	…
Mali	492 (14)	155 (31)	24 (43)	3 (15)	21 (58)	…	…	…
South Africa	801 (23)	107 (21)	5 (9)	3 (15)	2 (6)	…	…	…
Zambia	495 (14)	47 (9)	7 (13)	4 (20)	3 (8)	…	…	…
Thailand	219 (6)	3 (1)	0 (0)	0 (0)	0 (0)	…	…	…
Bangladesh	436 (12)	63 (13)	0 (0)	0 (0)	0 (0)	…	…	…
Very severe pneumonia	1106 (31)	196 (39)	32 (57)	8 (40)	24 (67)	1.43 (1.16–1.77)	1.95 (1.04–3.65)	3.00 (.97–9.30)
HIV infected	183 (5)	42 (8)	13 (23)	4 (20)	9 (25)	2.00 (1.37–2.91)	3.95 (1.61–9.69)	1.33 (.34–5.20)
CXR positive[c]	1586 (45)	251 (50)	38 (68)	19 (95)	19 (53)	1.44 (1.16–1.78)	4.37 (1.75–10.89)	—
Consolidation vs normal CXR	755 (21)	149 (30)	32 (57)	16 (80)	16 (44)	1.81 (1.40–2.34)	6.30 (2.43–16.35)	—
WBC count >15/μL	1270 (38)	179 (38)	26 (48)	12 (63)	14 (40)	1.17 (.96–1.44)	1.72 (.94–3.13)	0.39 (.12–1.23)
Oxygen saturation <92% with room air	916 (30)	176 (39)	21 (40)	6 (32)	15 (44)	1.55 (1.21–1.99)	1.02 (.52–2.01)	1.71 (.53–5.57)
CRP ≥40 mg/L	754 (25)	191 (44)	40 (82)	16 (84)	24 (80)	2.53 (2.03–3.16)	3.36 (1.52–7.41)	0.75 (.16–3.44)
Prior antibiotic use	1721 (50)	140 (29)	16 (30)	9 (45)	7 (21)	0.46 (.36–.58)	1.16 (.53–2.52)	0.32 (.09–1.06)
Any virus coinfection[d]	3120 (88)	468 (94)	53 (95)	19 (95)	34 (94)	1.71 (1.17–2.50)	1.41 (.39–5.13)	0.90 (.08–10.53)
Death	278 (9)	54 (12)	14 (27)	2 (11)	12 (36)	1.20 (.89–1.68)	2.24 (1.07–4.72)	4.57 (.89–23.37)

Abbreviations: CI, confidence interval; CRP, C-reactive protein; CXR, chest radiograph; HIV, human immunodeficiency virus; OR, odds ratio; WBC, white blood cell.

[a]Pneumococcal colonization density calculated from polymerase chain reaction (PCR) for the *lyt*A gene on nasopharyngeal/oropharyngeal specimens (PCR-negative cases included).

[b]ORs calculated from a logistic regression model of case group as a predictor of each characteristic. All models were adjusted for age, sex, and site, except for the group E vs D comparison, where the sample size was too small for adjustment. ORs are undefined for the group E vs D comparison for CXR positive and consolidation on CXR ($P = .03$ for each; Fisher exact test) and were not calculated for covariates (age, sex, and site).

[c]CXR positive defined as radiographic evidence of pneumonia (consolidation and/or other infiltrates).

[d]Virus coinfection defined as positive for any virus tested by PCR of the nasopharyngeal/oropharyngeal specimen (including influenza A, B, or C; parainfluenza virus 1, 2, 3, or 4; coronavirus NL63, 229E, OC43, or HKU1; human metapneumovirus A and B; human rhinovirus; respiratory syncytial virus A or B; adenovirus; enterovirus/parechovirus; human bocavirus; and cytomegalovirus).

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

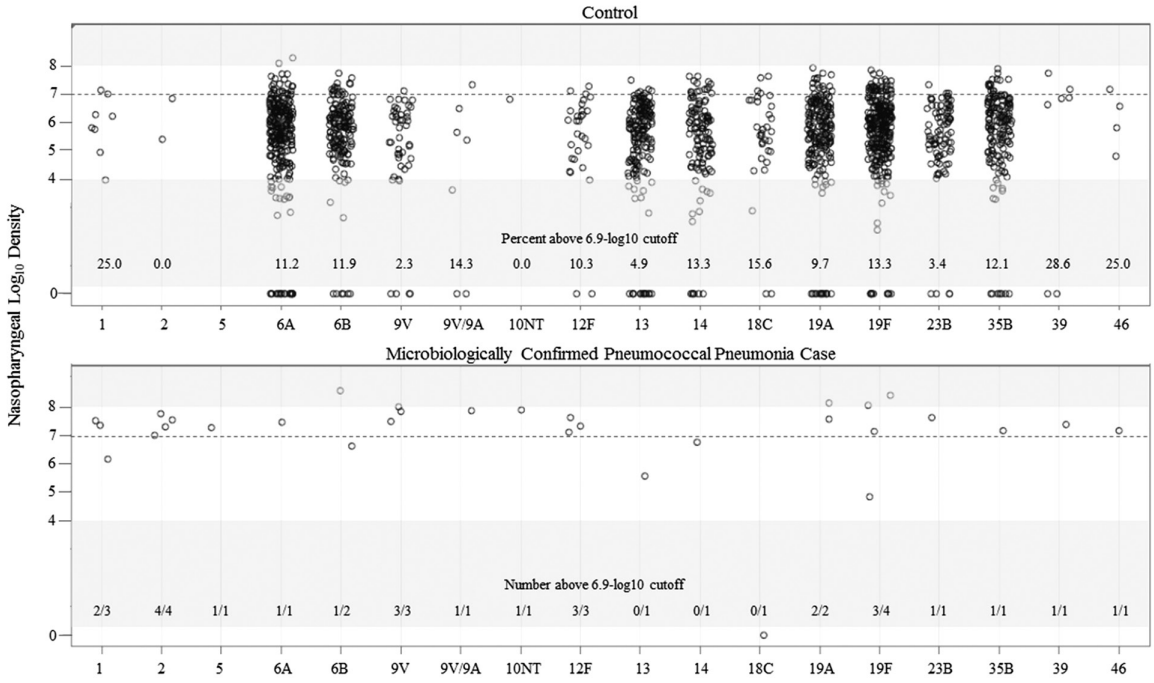

Figure 4. Pneumococcal colonization density by serotype of the invasive isolate among cases with microbiologically confirmed pneumococcal pneumonia (MCPP) or the colonizing isolate among all controls; density calculated by means of polymerase chain reaction for the *lytA* gene (copies/mL) performed on nasopharyngeal/oropharyngeal specimens. MCPP cases are limited to those for which the serotype of the invasive isolate was the same as that of the colonizing isolate. Shaded areas indicate 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.

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. 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. 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., V. L. B., A. N. D., A. J. D., J. D., B. E. E., D. G., M. M. H., D. P. M., S. C. M., J. M. M., D. E. P., W. P., B. P., C. P., 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.

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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**; 4:144–54.
3. Simell 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, Olcén P, Holmberg H, Mölling P. Comparison of sputum and nasopharyngeal aspirate samples and of the PCR gene targets *lytA* 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 pneumonia. 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, Nauclér P, et al. Clinical and microbiological factors associated with high nasopharyngeal pneumococcal density in patients with pneumococcal pneumonia. PLoS One **2015**; 10:e0140112.
12. Vu 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**; 30:11–8.
13. Anh DD, Huong Ple T, Watanabe K, et al. Increased rates of intense 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, Mulholland 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, Mulholland 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 WJ. Index for rating diagnostic tests. Cancer **1950**; 3:32–5.
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 **2010**; 50:175–83.
26. Talbot TR, Poehling 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*: characterization of a mouse model and the role of platelet-activating factor receptor. J Infect Dis **2002**; 186:341–50.
29. le Roux DM, Myer L, Nicol MP, Zar HJ. 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 meningitis 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, Dejsirilert 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.