Murdoch, DR; Morpeth, SC; Hammitt, LL; Driscoll, AJ; Watson, NL; Baggett, HC; Brooks, WA; Deloria Knoll, M; Feikin, DR; Kotloff, KL; +99 more... Levine, OS; Madhi, SA; O’Brien, KL; Scott, JAG; Thea, DM; Ahmed, D; Awori, JO; Deuca, AN; Ebruke, BE; Higdon, MM; Jorakate, P; Karron, RA; Kazungu, S; Kwenda, G; Hossain, L; Makprasant, S; Moore, DP; Mudau, A; Mwaba, J; Panchalingam, S; Park, DE; Prosperi, C; Salaudeen, R; Toure, A; Zeger, SL; Howie, SRC; O’Brien, KL; Levine, OS; Knoll, MD; Feikin, DR; Deluca, AN; Driscoll, AJ; Fancourt, N; Fu, W; Hammitt, LL; Higdon, MM; Kagucia, EW; Karron, RA; LI, M; Park, DE; Prosperi, C; Wu, Z; Zeger, SL; Watson, NL; Crawley, J; Murdoch, DR; Brooks, WA; Endtz, HP; Zaman, K; Goswami, D; Hossain, L; Jahan, Y; Ashraf, H; Howie, SRC; Ebruke, BE; Antonio, M; McLellan, J; MacHuka, E; Shamshul, A; Zaman, SMA; MacKenzie, G; Scott, JAG; Awori, JO; Morpeth, SC; Kamau, A; Kazungu, S; Ominde, MS; Kotloff, KL; Tapia, MD; Sow, SO; Sylla, M; Tamboura, B; Onwuchekwa, U; Kourouna, N; Toure, A; Madhi, SA; Moore, DP; Adrian, PV; Baillie, VL; Kuwanda, L; Mudau, A; Groome, MJ; Mahomed, N; Baggett, HC; Thamthitiwat, S; Maloney, SA; Bunthi, C; Rhodes, J; Sawatwong, P; Akarasewi, P; Thea, DM; Mwananyanda, L; Chipeta, J; Seidenberg, P; Mwansa, J; Wa Somwe, S; Kwenda, G; Anderson, TP; Mitchell, J; (2017) Microscopic Analysis and Quality Assessment of Induced Sputum From Children With Pneumonia in the PERCH Study. Clinical infectious diseases, 64 (suppl.,S271 – S279. ISSN 1058 – 4838DOI : https://doi.org/10.1093/cid/cix083

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Microscopic Analysis and Quality Assessment of Induced Sputum From Children With Pneumonia in the PERCH Study


Background. It is standard practice for laboratories to assess the cellular quality of expectorated sputum specimens to check that they originated from the lower respiratory tract. The presence of low numbers of squamous epithelial cells (SECs) and high numbers of polymorphonuclear (PMN) cells are regarded as indicative of a lower respiratory tract specimen. However, these quality ratings have never been evaluated for induced sputum specimens from children with suspected pneumonia.

Methods. We evaluated induced sputum Gram stain smears and cultures from hospitalized children aged 1–59 months enrolled in a large study of community-acquired pneumonia. We hypothesized that a specimen representative of the lower respiratory tract will contain smaller quantities of oropharyngeal flora and be more likely to have a predominance of potential pathogens compared to a specimen containing mainly saliva. The prevalence of potential pathogens cultured from induced sputum specimens and quantity of oropharyngeal flora were compared for different quantities of SECs and PMNs.

Results. Of 3772 induced sputum specimens, 2608 (69%) had <10 SECs per low-power field (LPF) and 2350 (62%) had >25 PMNs per LPF, measured traditionally associated with specimens from the lower respiratory tract in adults. Using isolation of low numbers of oropharyngeal flora and higher prevalence of potential pathogens as markers of higher quality, <10 SECs per LPF (but not >25 PMNs per LPF) was the microscopic variable most associated with high quality of induced sputum.

Conclusions. Quantity of SECs may be a useful quality measure of induced sputum from young children with pneumonia.

Keywords. pneumonia; induced sputum; quality; children.

Clinical Infectious Diseases • CID 2017:64 (Suppl 3) • S271

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Induced Sputum Quality Assessment • CID 2017:64 (Suppl 3) • S271

Sputum is the lower respiratory specimen most commonly collected from adults as part of the diagnostic workup for community-acquired pneumonia. However, sputum collection is more problematic in children, who typically have difficulty with expectoration [1, 2]. Collection of induced sputum through methods such as hypertonic saline nebulization can potentially overcome this problem.

Specimen quality has a large impact on the interpretation of sputum culture results [3]. Specimens originating from the lower
respiratory tract can be contaminated by upper respiratory secretions during the collection process, and some poorly collected specimens may be entirely composed of upper respiratory secretions. Either situation can lead to the incorrect conclusion that an organism colonizing the upper airways is causing pneumonia. Consequently, it has become standard practice for diagnostic laboratories to assess the quality of an expectorated sputum specimen using indicators that suggest it has been obtained from the lower respiratory tract. This involves assessing the number of squamous epithelial cells (SECs) and polymorphonuclear cells (PMNs) in a Gram-stained smear of the specimen [4, 5]. The presence of low numbers of SECs and high numbers of PMNs per low-power field (LPF) have been traditionally regarded as being indicative of a high-quality specimen [6]. Likewise, sputum specimens with relatively low numbers of PMNs and high numbers of SECs are likely to represent oropharyngeal contamination and are typically rejected for routine culture. These quality systems have been developed for expectorated sputum from adults, but have never been formally evaluated for induced sputum samples from children with suspected pneumonia.

This is the first of 5 companion papers in this supplement on induced sputum analysis from the Pneumonia Etiology Research for Child Health (PERCH) study. This article is focused on the assessment of whether pediatric induced sputum specimens are representative of the lower respiratory tract and does not evaluate the utility of induced sputum for diagnostic testing. A specific objective was to identify a quality measure indicating a lower-respiratory tract source that could be applied to induced sputum specimens from children with pneumonia. Other articles in the supplement focus on the usefulness of induced sputum culture, the added value of testing induced sputum from adults, but have never been formally evaluated for induced sputum samples from children with suspected pneumonia.

**METHODS**

**Participants**

Participants were children aged 1–59 months who were hospitalized with World Health Organization (WHO)-defined severe or very severe pneumonia as part of the PERCH study, a case-control study involving 9 sites in 7 countries from sub-Saharan Africa and South Asia. Details of this study have been described elsewhere [10, 11]. As part of a comprehensive evaluation, induced sputum was collected from cases, ideally before antibiotics were administered.

**Specimen Collection**

Induced sputum was obtained at enrollment by study staff following an established methodology [12, 13]. A β-2 agonist was given by a metered dose inhaler 5 minutes prior to nebulization with sterile hypertonic saline (3%–5% sodium chloride) to minimize the risk of bronchospasm. Saline nebulization occurred for at least 10 minutes using a jet nebulizer with a facemask and mixed oxygen flow at a rate of 5–8 L/minute. Percussion of the chest wall was done in children <24 months of age during nebulization, and in older children in the absence of cough. Each quadrant of the posterior aspect of the chest was tapped gently 5–10 times to mobilize lower respiratory secretions and induce a cough in the child. A sterile mucus extracting catheter attached to a suction device was then inserted through the nose into the posterior nasopharynx and sputum was collected into a sterile trap. Suction was applied only once the catheter was in place and not applied during removal of the catheter to avoid aspirating anterior nasal contents. The catheter was flushed with 5 mL sterile normal saline at the end of the procedure, and the specimen was immediately sent to the laboratory for processing.

**Laboratory Methods**

Gram-stained smears were made from the most visually purulent portion of each induced sputum specimen. The quality of sputum was assessed by determining the numbers of SECs and PMNs within the following categories: <10, 10–25, or >25 cells per representative (×100) LPF. Microorganisms seen in the smear under high power (×1000) were described according to classic Gram stain morphotypes. The most purulent portion of each specimen was inoculated onto sheep or horse blood, chocolate, and MacConkey agars, streaked out using a standard 4-quadrant streaking method, and incubated at 35°C for 48 hours. Cultures were examined at 24 hours and 48 hours, and predominant organisms were identified and quantified according to the furthest quadrant with visible colonies (first quadrant, scanty; second quadrant, 1+; third quadrant, 2+; fourth quadrant, 3+). Background mixed oropharyngeal flora (including viridans streptococci, commensal *Neisseria*, coagulase-negative staphylococci, yeasts [except *Cryptococcus*], diphtheroids, and *Capnocytophaga*) were quantified as a group but not identified further.

Medical laboratory scientists processed the specimens at each site, and efforts were made to standardize these methods across all study sites through uniform standard operating procedures, on-site training, and internal and external quality checks [14] (including participation in the Royal College of Pathologists of Australasia’s Quality Assurance Programme).

**Study Definitions**

Sputum culture results were interpreted using the following 6 increasingly more rigorous interpretive criteria for each organism identified:

1. Organism isolated in any quantity;
2. Organism isolated in any quantity and with compatible Gram stain morphotype;
3. Organism isolated as the predominant organism;
4. Organism isolated as the predominant organism and with compatible Gram stain morphotype;
5. Organism isolated in quantities of 2+ or 3+;
6. Organism isolated in quantities of 2+ or 3+ and with compatible Gram stain morphotype.

Prior antimicrobial therapy was defined as (1) antibiotic activity in serum by bioassay, or (2) documented administration of antibiotics before induced sputum sample collection [15].

Chest radiographs from each child were read by a panel of radiologists and pediatricians trained in the standardized interpretation of pediatric chest radiographs [16]. Chest radiographs were classified as either consolidation, other infiltrate, both consolidation and other infiltrate, normal, or uninterpretable.

**Statistical Analysis**
As there are no suitable gold standards to assess sputum quality, we identified variables that were likely markers of sputum quality. We hypothesized that a specimen representative of the lower respiratory tract will contain smaller quantities of oropharyngeal flora and larger quantities of potential pathogens compared to a poor-quality specimen containing mainly saliva.

The prevalence of potential pathogens cultured from induced sputum specimens was compared across the 6 interpretive criteria and for different quantities of SECs and PMNs. The quantity of oropharyngeal flora was also compared for different quantities of SECs and PMNs.

To characterize potential correlates of poorer-quality specimens, we used logistic regression models of clinical characteristics (prior antimicrobial use, radiographic pneumonia, and human immunodeficiency virus infection), SEC quantity, and PMN quantity as predictors of higher oropharyngeal flora quantities. Associations between each clinical characteristic and oropharyngeal flora quantity were estimated by odds ratios (unadjusted and adjusted for all evaluated characteristics and PERCH site). Oropharyngeal flora quantity was evaluated as quantity greater or equal to 2+ or 3+ vs lower quantity or not present.

**Ethical Considerations**
The study protocol was approved by the institutional review board or ethics committee at each of the 7 institutions and at the Johns Hopkins School of Public Health. Parents or guardians of participants provided written informed consent.

**RESULTS**
Induced sputum culture results were available for analysis from 3772 of 4232 (89.1%) children enrolled in PERCH; 2695 (71.4%) had severe pneumonia and 1077 (28.6%) very severe pneumonia: 518 from Bangladesh, 596 from The Gambia, 592 from Kenya, 544 from Mali, 824 from South Africa, 191 from Thailand, and 507 from Zambia. The median age of the children was 8 months (interquartile range, 3–16 months), and 1579 (41.9%) were female; 2833 (75.1%) had evidence of receipt of antimicrobials before collection of induced sputum.

Table 1 shows the characteristics of the induced sputum specimens by demographic and clinical variables. There was variability in the quality of specimens across study sites, with large numbers of SECs reported in a higher proportion of cases from South Africa. Detection of 4 major potential pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*) was greater in specimens from children without evidence of prior antibiotic use. Otherwise, there was little variation in sputum characteristics for most variables.

Over two-thirds of samples had <10 SECs per LPF and a similar proportion had >25 PMNs per LPF (Table 2), quantities traditionally associated with high-quality sputum samples among adult populations. A similar pattern was observed when the analysis was restricted to cases with chest radiographic changes (Supplementary Table 1A).

Table 3 shows the distribution of organisms cultured from sputum samples using the 6 different interpretive criteria. *Haemophilus influenzae*, *S. pneumoniae*, and *M. catarrhalis* were the predominant organisms isolated. The prevalence of all organisms declined with progressively more rigorous interpretive criteria, as expected.

Figure 1 shows the prevalence of the 5 major organism groupings with differing culture interpretive criteria and with varying quantities of SECs and PMNs. The prevalence of *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* decreased with increasing numbers of SECs. The same relationship was not observed for other gram-negative bacteria or *S. aureus*, for which there was a slight increase in prevalence with increasing numbers of SECs. The prevalence of all organisms remained relatively unchanged with varying numbers of PMNs. These patterns were similar when the analysis was restricted to cases with chest radiographic changes (Supplementary Figure 1A). The findings were also similar when the analysis was stratified by prior antibiotic use, although organism prevalence was lower in cases with prior antibiotic use (Supplementary Figure 1B and 1C).

Quantity of oropharyngeal flora was recorded in 3677 (97%) sputum samples, of which 661 (18%) reported 3+ and 652 (18%) reported no oropharyngeal flora (Table 1). The quantity of oropharyngeal flora increased with the presence of greater numbers of SECs, but there was no clear association with numbers of PMNs (Figure 2). The findings were similar when restricted to radiographic pneumonia cases and when stratified by prior antibiotic use (Supplementary Figure 2A–C).

Table 4 shows the analysis of variables associated with high quantities of oropharyngeal flora. Sputum specimens with fewer SECs were associated with a lower odds of culturing larger quantities of oropharyngeal flora. PMNs >25 per LPF was also associated with an increased odds of culturing larger quantities of oropharyngeal
<table>
<thead>
<tr>
<th>Age</th>
<th>&lt;6 mo</th>
<th>6–11 mo</th>
<th>12–23 mo</th>
<th>&gt;23 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity</td>
<td>Very severe</td>
<td>Severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>Kenya</td>
<td>Gambia</td>
<td>Mali</td>
<td>Zambia</td>
</tr>
<tr>
<td></td>
<td>Bangladesh</td>
<td>South Africa</td>
<td>Thailand</td>
<td></td>
</tr>
<tr>
<td>Prior antibiotics</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Oropharyngeal Flora*</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td>1+</td>
</tr>
<tr>
<td>Any Potential Pathogen by Sputum Interpretive Criteriaa</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
| Denominator for the All row is N = 3772; denominator for subsequent rows corresponds to the number of cases in that subgroup.
| Abbreviations: CXR, chest radiograph; HIV, human immunodeficiency virus; LPF, low-power field; PMNs, polymorphonuclear cells; SECs, squamous epithelial cells.
| aQuantity of oropharyngeal flora was recorded in 367 (97%) sputum samples; no oropharyngeal flora was recorded in 62 (17%) sputum samples.
| bNumber of children with any potential pathogen defined as Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, or Moraxella catarrhalis by sputum interpretive criteria: 1, organism present in any amount; 2, present in any amount with compatible Gram stain morphology; 3, present as the predominant organism; 4, present as the predominant organism with compatible Gram stain morphology; 5, present in quantities ≥2+; 6, present in quantities ≥2+ with compatible Gram stain morphology. Sputum interpretive criteria are not mutually exclusive and children will appear in multiple columns.
| cChest radiograph positive defined as radiographic evidence of pneumonia (consolidation and/or other infiltrates).
flora, but the effect size was smaller and (unlike with SECs) there was not an increasing trend across PMN categories.

**DISCUSSION**

The key finding from this study is that <10 SECs per LPF was the best measure of induced sputum quality in young children with pneumonia, using high quantity of oropharyngeal flora and low prevalence of potential pathogens as markers of poorer quality. It was also notable that a large proportion (69.1%) of induced sputum samples met this criterion for good quality. If this criterion is an accurate marker of good quality, this finding implies that a large proportion of induced sputum specimens in this study were actually obtained from the lower respiratory tract.

Criteria used by diagnostic laboratories to identify microscopically high-quality sputum specimens from adults were derived from expert opinion, supported by limited data using surrogate markers of quality such as the quantity of oropharyngeal flora [4, 5]. Sputum with <10 SECs and >25 PMNs per LPF have long been regarded as ideal [17], although the requirement for large numbers of PMNs has been questioned given that some pneumonias are not necessarily associated with production of purulent sputum [18]. Indeed, the sentinel study by Murray and Washington indicated that <10 SECs was the key quality measure, and that the presence of leukocytes did not influence the quality interpretation when substantial numbers of SECs were present [5]. Our findings support the application of <10 SECs as a quality measure for induced sputum specimens from children as well. The reason for the association between >25 PMNs and increased amounts of oropharyngeal flora is unclear, although the effect size was small.

**Table 2. Comparison of PMN and SEC Quantity in Induced Sputum Samples From Children Aged 1–59 Months With World Health Organization–Defined Severe or Very Severe Pneumonia**

<table>
<thead>
<tr>
<th>No. of SECs per LPF</th>
<th>&gt;25</th>
<th>10–25</th>
<th>&lt;10</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>1553 (41.2)</td>
<td>502 (13.3)</td>
<td>553 (14.7)</td>
<td>2608 (69.1)</td>
</tr>
<tr>
<td>10–25</td>
<td>478 (12.7)</td>
<td>137 (3.6)</td>
<td>125 (3.3)</td>
<td>740 (19.6)</td>
</tr>
<tr>
<td>&gt;25</td>
<td>319 (8.5)</td>
<td>47 (1.2)</td>
<td>58 (1.5)</td>
<td>424 (11.2)</td>
</tr>
<tr>
<td>All</td>
<td>2350 (62.3)</td>
<td>686 (18.2)</td>
<td>736 (19.5)</td>
<td>3772 (100.0)</td>
</tr>
</tbody>
</table>

Data are presented as No. (%). Percentages represent percentage of total specimens among cases in whom induced sputum was collected and had available culture results (N = 3772). Abbreviations: LPF, low-power field; PMNs, polymorphonuclear cells; SECs, squamous epithelial cells.

**Table 3. Prevalence of Bacteria by Sputum Culture Interpretive Criteria in Induced Sputum Samples From Children Aged 1–59 Months With World Health Organization–Defined Severe or Very Severe Pneumonia (N = 3772)**

<table>
<thead>
<tr>
<th>Sputum Culture Interpretive Criteria</th>
<th>Spn</th>
<th>Saur</th>
<th>Oth Strc</th>
<th>Hinf</th>
<th>Mcat</th>
<th>Entrbd</th>
<th>Mgnr</th>
<th>Ognr</th>
<th>Paerc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism present in any amount</td>
<td>1095 (29.0)</td>
<td>387 (10.3)</td>
<td>35 (0.9)</td>
<td>1429 (37.9)</td>
<td>1025 (27.2)</td>
<td>422 (11.2)</td>
<td>165 (4.4)</td>
<td>119 (3.2)</td>
<td>27 (0.7)</td>
</tr>
<tr>
<td>Present in any amount with compatible Gram stain morphotype</td>
<td>947 (25.1)</td>
<td>179 (4.7)</td>
<td>28 (0.7)</td>
<td>1029 (27.3)</td>
<td>781 (20.7)</td>
<td>185 (4.9)</td>
<td>120 (3.2)</td>
<td>58 (1.5)</td>
<td>19 (0.5)</td>
</tr>
<tr>
<td>Present as the predominant organism</td>
<td>860 (22.8)</td>
<td>291 (7.7)</td>
<td>27 (0.7)</td>
<td>1138 (30.2)</td>
<td>825 (21.9)</td>
<td>329 (8.7)</td>
<td>95 (2.5)</td>
<td>94 (2.5)</td>
<td>24 (0.6)</td>
</tr>
<tr>
<td>Present as the predominant organism with compatible Gram stain morphotype</td>
<td>744 (19.7)</td>
<td>141 (3.7)</td>
<td>23 (0.6)</td>
<td>794 (21.0)</td>
<td>629 (16.7)</td>
<td>145 (3.8)</td>
<td>68 (1.8)</td>
<td>47 (1.2)</td>
<td>16 (0.4)</td>
</tr>
<tr>
<td>Present in quantities ≥2+</td>
<td>683 (18.1)</td>
<td>182 (4.8)</td>
<td>0 (0.0)</td>
<td>819 (21.7)</td>
<td>639 (16.9)</td>
<td>91 (2.4)</td>
<td>39 (1.0)</td>
<td>21 (0.6)</td>
<td>16 (0.4)</td>
</tr>
<tr>
<td>Present in quantities ≥2+ with compatible Gram stain morphotype</td>
<td>626 (16.6)</td>
<td>92 (2.4)</td>
<td>0 (0.0)</td>
<td>613 (16.3)</td>
<td>535 (14.2)</td>
<td>50 (1.3)</td>
<td>23 (0.6)</td>
<td>13 (0.3)</td>
<td>11 (0.3)</td>
</tr>
</tbody>
</table>

Abbreviations: Entrb, Enterobacteriaceae; Hinf, Haemophilus influenzae; Mcat, Moraxella catarrhalis; Mgnr, mixed gram-negative rods; Ognr, other nonfermentative gram-negative rods; Oth Str, other streptococci and enterococci; Paer, Pseudomonas aeruginosa; Saur, Staphylococcus aureus; Spn, Streptococcus pneumoniae.

cSputum culture interpretive criteria are not mutually exclusive; children may appear in multiple criteria.

dAll percentages are based on total number of induced sputum specimens (N = 3772).

cOther streptococci and enterococci includes streptococci (other than S. pneumoniae) and enterococci species.

dEnterobacteriaceae includes Escherichia coli, Enterobacter species, Klebsiella species, Citrobacter species, and Serratia species, excluding mixed gram-negative rods.

eOther nonfermentative gram-negative rods includes Acinetobacter species and Pseudomonas species. Pseudomonas aeruginosa also reported separately.
Figure 1. A–E, Prevalence of organisms by induced sputum culture interpretive criteria and induced sputum quality variables in children aged 1–59 months with World Health Organization–defined severe or very severe pneumonia. Sputum interpretive criteria: 1, organism present in any amount; 2, present in any amount with compatible Gram stain morphotype; 3, present as the predominant organism; 4, present as the predominant organism with compatible Gram stain morphotype; 5, present in quantities ≥2+; 6, present in quantities ≥2+ with compatible Gram stain morphotype. Sputum interpretive criteria are not mutually exclusive and children will appear in multiple columns. Other nonfermentative gram-negative rods include Acinetobacter species and Pseudomonas species. Abbreviations: PMNs, polymorphonuclear cells; SECs, squamous epithelial cells.
A large amount of cellular material was obtained from most induced sputum samples in this study. More than two-thirds of specimens had <10 SECs per LPF and a similar proportion had >25 PMNs per LPF (40% had both <10 SECs and >25 PMNs per LPF). These findings are similar to those from other childhood pneumonia studies that collected induced sputa [19, 20], and are similar to that reported for expectorated sputum from adults with pneumonia [21, 22]. Our initial concern that the use of the saline flush in the induced sputum collection process may dilute the specimen is likely unwarranted, and probably mitigated by the use of the most purulent portion of the specimen for making the Gram stain smear.

The study has several limitations. Most importantly, we lacked a gold standard for good-quality sputum obtained from the lower respiratory tract and, instead, relied on surrogate markers such as quantity of background oropharyngeal flora. While specimens from the oropharynx are more likely to contain large amounts of oropharyngeal flora, true lower respiratory specimens will also contain normal commensals from the upper airways through contamination in the collection process. The exact relationship between quantities of oropharyngeal flora in upper and lower airways is unknown. Second, as expected, we found evidence that antibiotic use before specimen collection affects culture findings. We accounted for the influence of antibiotics in the analyses, although our imperfect definition of prior antibiotic use may have failed to identify cases who had received antibiotics [15]. Third, despite efforts to standardize methods across sites through training, uniform standard operating procedures, and internal and external quality checks, there may still be variations in the reporting of sputum cultures and Gram stain

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**Table 4. Associations of Clinical and Induced Sputum Characteristics With 2+/3+ Oropharyngeal Flora in Children Aged 1–59 Months With World Health Organization–Defined Severe or Very Severe Pneumonia**

<table>
<thead>
<tr>
<th>Induced Sputum Characteristic</th>
<th>Unadjusted OR (95% CI)</th>
<th>Multivariable Modela AOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SECs per LPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;25 (reference)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10–25</td>
<td>0.57 (.40–.65)</td>
<td>0.62 (.46–.84)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>0.23 (.19–.29)</td>
<td>0.31 (.23–.41)</td>
</tr>
<tr>
<td>PMNs per LPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 (reference)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10–25</td>
<td>1.05 (.84–1.31)</td>
<td>1.04 (.78–1.38)</td>
</tr>
<tr>
<td>&gt;25</td>
<td>1.60 (1.34–1.91)</td>
<td>1.52 (1.21–1.92)</td>
</tr>
<tr>
<td>Prior antibiotic useb</td>
<td>0.58 (.50–.68)</td>
<td>0.72 (.57–.93)</td>
</tr>
<tr>
<td>CXR positivec</td>
<td>0.94 (.79–1.13)</td>
<td>0.97 (.82–1.15)</td>
</tr>
<tr>
<td>HIV positive</td>
<td>1.19 (.89–1.58)</td>
<td>1.51 (1.02–2.23)</td>
</tr>
</tbody>
</table>

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; CXR, chest radiograph; HIV, human immunodeficiency virus; LPF, low-power field; OR, odds ratio; OROF, oropharyngeal flora; PMNs, polymorphonuclear cells; SECs, squamous epithelial cells.

a Adjusted for all other characteristics included in the model and Pneumonia Etiology Research for Child Health (PERCH) site.

b Prior antibiotic use defined as serum bioassay positive, antibiotics received at referral hospital, or administered before induced sputum specimen collection.

c CXR positive defined as any abnormal CXR result (consolidation and/or other infiltrate).

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Figure 2. A–C, Quantity of oropharyngeal flora in induced sputum in children aged 1–59 months with World Health Organization–defined severe or very severe pneumonia by induced sputum quality variables (N = 3772). Abbreviations: PMNs, polymorphonuclear cells; SECs, squamous epithelial cells.
smears between scientists and between sites. There was vari-
ability in findings between sites (Table 1), and it is uncertain
the degree to which this reflects true differences in the patient
populations and whether there is a contribution from inter-
observer variability.

Despite these limitations, the results of this study indicate that
good-quality sputum specimens can be collected from children
with pneumonia through saline nebulization induction, and
that analysis should be restricted to specimens with <10 SECs
per LPF on Gram stain smear. Although our analysis relied on
culture results, this restriction identifies characteristics of sput-
um specimens most likely to be derived from the lower air-
ways and, therefore, the same criterion could also apply to other
testing methods such as PCR. Subsequent analyses will further
explore the utility of induced sputum in diagnosing pneumonia
etiology [9, 23].

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases
online. Consisting of data provided by the author to benefit the reader,
the posted materials are not copyedited and are the sole responsibili-
y of the author, so questions or comments should be addressed to the corre-
spending author.

Notes
Author contributions. D. R. M. led the analysis, interpreted results,
and drafted the manuscript. N. L. W., S. C. M., D. R. F., L. L. H., and S. R.
C. H. participated in the analysis and interpretation of results and drafting
of the manuscript. O. S. L., K. L. O., D. R. F., D. R. M., M. K. D., L. L. H.,
M., and R. A. K. conceived and designed the study and supervised study
S. M., D. P. M., A. M., J. M., S. P., D. E. P., C. P. R. S., and A. T. were involved
in study conduct, data collection, and/or data management. S. L. Z. pro-
vided expert statistical guidance. All authors reviewed and approved the
manuscript. D. R. M. had final responsibility for the decision to submit for
publication.

Acknowledgments. We are grateful to the members of the original
study teams for running the studies and collecting data. We would also like
to acknowledge members of the following group who contributed to the
study design, conduct, and analysis phases of PERCH (see Supplementary
Materials for full list of names): Pneumonia Methods Working Group,
PERCH Expert Group, PERCH Contributors, and the PERCH Chest
Radiograph Reading Panel. We offer sincere thanks to the patients and fam-
ilies who participated in this study.

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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, Department of Health and Human
Services, or the US government. This article is published with the permis-
sion of the Director of the Kenya Medical Research Institute.

Financial support. PERCH was supported by the Bill & Melinda Gates
Foundation (grant number 48968 to the International Vaccine Access
Center, Department of International Health, Johns Hopkins Bloomberg
School of Public Health). J. A. G. S. was supported by a clinical fellowship
from The Wellcome Trust of Great Britain (award number 098532).

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. M. D. K. has received funding for con-
sultancies from Merck, Pfizer, and Novartis, and grant funding from Merck.
L. L. H. has received grant funding from Pfizer and GlaxoSmithKline. K.
L. O. K. has received grant funding from Merck Sharp & Dohme. S. A. M. has
received honoraria for advisory board membership from the Bill & Melinda
Gates Foundation, Pfizer, Medimmune, and Novartis; has received institu-
tional grants from GSK, Novartis, Pfizer, MinervaX, and the Bill & Melinda
Gates Foundation; and has served on speaker’s bureaus for Sanofi Pasteur
and GSK. 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. All other authors report no potential conflicts.
All authors have submitted the ICMJE Form for Disclosure of Potential
Conflicts of Interest. Conflicts that the editors consider relevant to the con-
tent of the manuscript have been disclosed.

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