

## **The Aetiology of pneumonia from analysis of Lung aspirate and Pleural fluid samples: Findings from the PERCH study**

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### Summary:

Lung (LA) and pleural fluid (PF) aspiration was performed on a subgroup of PERCH pneumonia cases with radiological consolidation or pleural fluid collection. Bacterial pathogens predominated, (*Streptococcus pneumoniae* in LA, *Staphylococcus aureus* in PF), in contrast to overall PERCH findings.

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## Abstract

### Background

An improved understanding of childhood pneumonia aetiology is required to inform prevention and treatment strategies. Lung aspiration is the gold standard specimen for pneumonia diagnostics. We report findings from analyses of lung and pleural aspirates collected in the Pneumonia Etiology Research for Child Health (PERCH) study.

### Methods

The PERCH study enrolled children aged 1–59 months hospitalized with World Health Organization defined severe or very severe pneumonia in 7 countries in Africa and Asia. Percutaneous trans-thoracic lung (LA) and pleural fluid (PF) aspiration was performed on a sample of pneumonia cases with radiological consolidation and/or pleural fluid in 4 countries. Venous blood and nasopharyngeal/oropharyngeal swabs were collected from all cases. Multiplex quantitative PCR and routine microbiologic culture were applied to clinical specimens.

### Results

Of 44 LAs performed within 3 days of admission on 622 eligible cases, 13 (30%) had a pathogen identified by either culture (5/44) or by PCR (11/29). A pathogen was identified in 12/14 (86%) PF specimens tested by either culture (9/14) or PCR (9/11). Bacterial pathogens were identified more frequently than viruses. All but one of the cases with a virus identified were co-infected with bacterial pathogens. *Streptococcus pneumoniae* (9/44 [20%]) and *Staphylococcus aureus* (7/14 [50%]) were the predominant pathogen identified in LA and PF, respectively.

### Conclusions

Bacterial pathogens predominated in this selected subgroup of PERCH participants drawn from those with radiological consolidation or pleural fluid, with *S. pneumoniae* and *S. aureus* the leading pathogens identified.

**Key words:** Lung aspirate, pleural fluid aspirate, PERCH, pneumonia, etiology childhood

## Introduction

Pneumonia remains a leading cause of child mortality, with the overwhelming burden of deaths occurring in developing countries.<sup>1</sup> An improved understanding of childhood pneumonia aetiology is required to inform prevention and treatment strategies.

The Pneumonia Etiology Research for Child Health (PERCH) study aimed to identify the causes and risk factors for severe childhood pneumonia in developing countries in the pneumococcal and *Haemophilus influenzae* type b (Hib) conjugate vaccine era, and is the largest such study since the 1980s.<sup>2,3</sup>

PERCH applied conventional microbiological and molecular diagnostic techniques to a wide range of clinical specimens including percutaneous transthoracic fine needle lung aspirates (LA) and pleural fluid (PF) aspirates, which obtain a specimen directly from the site of infection. Such samples, though rarely obtained in practice, together with the application of molecular diagnostic techniques, have been shown to significantly improve the diagnostic yield in pneumonia aetiology studies.<sup>4,5</sup> This article reports findings from LA and PF specimens collected in the PERCH study.

## Methods

PERCH was a multi-country case control study of severe or very severe pneumonia aetiology carried out in nine sites in seven participating countries (The Gambia, Mali, Kenya, Zambia, South Africa, Bangladesh and Thailand).<sup>6,7</sup> Cases were children aged 1–59 months with World Health Organization (WHO) defined severe or very severe pneumonia (pre-2013 definition).<sup>6</sup> Children with wheeze whose lower chest wall indrawing resolved following bronchodilator therapy were excluded. Each site recruited participants for two years between August 2011 to January 2014. Case enrolment, specimen collection and laboratory procedures were standardized.<sup>7-10</sup> Percutaneous trans-thoracic lung aspiration was performed on eligible cases in the 4 PERCH countries where ethical approvals for LA collection was obtained: The Gambia, South Africa, Mali and Bangladesh. In The Gambia, lung aspirations were done throughout the case enrolment period, but at the 3 other sites the procedure commenced at

various times into the recruitment period based on when ethical approvals were obtained and staff were trained in specimen collection techniques. PF was collected at all sites per local clinical practice guidelines. Pneumococcal conjugate vaccine (PCV) had been introduced prior to the study in Kenya (10-valent), The Gambia, Mali, and South Africa (13-valent).

### ***Ethics***

Ethical approval for the PERCH study, and for the use of fine needle lung aspiration procedure, was obtained from the Johns Hopkins Bloomberg School of Public Health Institutional Review Board (JHSPH IRB) and from local Ethics review boards for each participating site. Written informed consent was obtained from guardians for all procedures undertaken.

### ***Lung Aspiration***

Fine needle percutaneous lung aspiration was used in this study, the details of which are described, and it is important not to confuse this with percutaneous lung biopsy, which has a materially different safety profile.

*Site Preparation:* A standardized protocol for specimen collection procedures was developed from The Gambia site, which has a long history of safety and utility of the procedure.<sup>11</sup> Training and subsequent support for clinical staff at the other participating PERCH sites was provided by experienced clinicians from The Gambia.

*Case Eligibility:* Cases were eligible for lung aspiration if they had a peripheral confluent alveolar consolidation identified on chest radiograph (CXR) by the treating clinician, no contraindications (e.g. presence of pneumatoceles on CXR and post-measles pneumonia), and written informed consent. The procedure was deferred in children who were clinically unstable. Ultimately, the decision to undertake lung aspiration on eligible children rested with the attending clinician and consent by the child's parents/legal guardians.

*Procedural Steps:* As described elsewhere<sup>11</sup>, children were positioned sitting or supine and the site of consolidation seen on chest radiograph was identified clinically by locating an area of maximal dullness to percussion or crepitations on auscultation. Skin over the identified area was sterilized. A standard 21-gauge hypodermic needle attached to a 5ml syringe into which 1ml of sterile 0.9% saline had been aspirated was used for the procedure. The needle was inserted over the superior aspect of the rib and advanced into the identified area of maximal dullness on percussion, strictly avoiding the cardiac area. The area was then aspirated immediately and the needle withdrawn, over about 2 seconds, maintaining maximal suction pressure on the syringe. With the needle used for the lung aspiration procedure still attached, aspirate contents were flushed into a sterile universal specimen container.

*Safety Monitoring:* Safety monitoring for the LA procedure included close observation of cases including vital signs and oximetry for at least 4 hours post-procedure. Adverse events were defined according to the protocol and reported to the study safety monitor.

#### ***Pleural Fluid and Other Clinical Specimens***

Cases with pleural fluid on CXR and/or clinical assessment were considered for aspiration of PF, which was done according to routine clinical practice,<sup>12,13</sup> however this assessment was not standardized across sites. Treating clinicians interpreted CXRs for PF specimen eligibility in real time.

Other specimens including venous blood and nasopharyngeal/oropharyngeal swabs (NP/OP) were collected using standardized methods described previously.<sup>9,10</sup>



### **Laboratory Methods**

Laboratory testing methods used for these analyses have been described separately.<sup>10,14-17</sup> In brief, we used multiplex quantitative PCR (FTD Resp-33 kit; Fast-track Diagnostics, Sliema, Malta) and routine culture to test LA, PF, and NP/OP specimens. Blood was cultured for bacterial pathogens and was also tested for *lytA* (a molecular marker of *S. pneumoniae*) by PCR. Pneumococcal serotypes were determined by Quellung method or PCR; microarray was used to determine serotype for NP/OP-culture negative but PCR-positive specimens.<sup>18</sup> For *H. influenzae*, all culture isolates were serotyped; the FTD panel included an all-serotype *H. influenzae* PCR target and a Hib target.

### **Statistical analysis**

For both LA and PF, only those specimens obtained within three days of enrollment were included in analyses to limit the potential impact of nosocomial infection on findings. A PERCH reading panel provided standardized CXR interpretations independent of clinical care.<sup>19</sup> Selected baseline characteristics were compared between cases with LA and all other cases categorized as having consolidation on CXR as per the PERCH CXR Reading Panel, restricting to the four sites performing lung aspiration.<sup>19</sup> Categorical variables were assessed using logistic regression (adjusted for age and site, where relevant) and the Kruskal-Wallis test was performed for continuous variables. For cases with LA or PF specimens, frequencies and proportions of cases with pathogens identified by culture and by PCR were calculated, restricting analyses to those children with results available for the measurement. We assessed concordance in the pathogens detected between LA and PF specimens compared to NP/OP and blood culture specimens.

## Results

### *Lung Aspirates*

Of 2757 children enrolled at the four sites performing lung aspiration, 622 (23%) had CXR confluent alveolar consolidation, of whom 48 (8%) had LA performed (Figure 1a). Forty-four (92%) of the 48 lung aspirations were performed within 3 days of enrolment (61% on the day of admission) and are included in these analyses. One of the 44 cases had CXR findings judged to be normal by the CXR reading panel and two had uninterpretable CXRs; the remaining 41 cases had a CXR finding of consolidation confirmed by the panel. The main reasons for ineligibility for LA were non-consent by the parent/caregiver and that in the attending clinician's judgement the procedure was not safe to perform, generally because of the location of consolidation or the child's clinical condition. Failure to collect a LA sample from a potentially eligible case was either because the site had not yet had training in the procedure or there were operational barriers that prevented it, such as high workloads and logistical challenges with the LA specimen collection during after-hours or weekends.

LA cases were similar to all other pneumonia cases with CXR consolidation but in whom an LA specimen was not taken with respect to clinical and demographic characteristics such as the median age, pneumonia severity, vaccination status and median duration of hospital stay, but not by site, the majority (24/44, 54.5%) of lung aspirations being performed at The Gambia site (Table 1). The proportion of cases pre-treated with antibiotics prior to LA collection was lowest in The Gambia (9/24, 38%) and was 80% or higher in all other sites (Supplemental Table 1). Cases where a pathogen was detected on LA (culture or PCR) had a higher prevalence of fever (69% vs 25%;  $p<0.001$ ) and lethargy (31% vs 8%;  $p=0.02$ ) and an absence of wheezing (0% vs 27%;  $p=0.02$ ) compared to all other cases with CXR consolidation but in whom no LA sample was obtained (Supplementary Table 2).

All LA samples had culture results available, but only 29/44 (66%) had PCR results available, with missing PCR results due to low specimen volume. Pathogens were identified by culture in 5/44 (11%) cases and by PCR in 11/29 (38%) cases (Table 2a). Thirteen LA samples had a pathogen

detected by either method (13/44, 30%), with 7/13 (54%) positive for multiple pathogens (Table 2a) and 9/13 (69%) from The Gambia (Supplementary Table 3a). Pathogen detection decreased with increasing days between admission and LA collection (Supplementary Table 4). *Streptococcus pneumoniae* was the predominant pathogen (9/44 [20%]; 5/9 identified as single infections) followed by *Haemophilus influenzae* (4/44 [9%], 3 being non-type b and one type b) and *Moraxella catarrhalis* (4/44 [9%]). Of four cases with a virus identified, two were positive for cytomegalovirus (CMV), one for adenovirus, and one for human metapneumovirus (HMPV) (Table 2a); most viral infections on LA (3/4 cases) were identified as co-infections with bacterial pathogens; CMV was the only virus found by itself. Of 9 cases in whom *S. pneumoniae* was identified through culture and/or PCR of LA specimens, 5 (56%) were identified as single infections (Supplemental Table 3a). Six cases had more than one bacterium identified, including 2 cases with *S. pneumoniae* and *H. influenzae* co-infection, and 2 cases with *S. pneumoniae* and *M. catarrhalis* co-infection. *Pneumocystis jirovecii* was identified in one HIV-uninfected case from The Gambia, as a co-infection with *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and CMV (Supplemental Table 3a). Among the LA cases with both culture and PCR results and who had a pathogen identified (N=9), 3 (33%) cases had the same bacteria identified in both culture and PCR, whereas an additional 6 cases had bacteria detected in PCR only (Supplemental Table 5a). Of 42 LA specimens tested for *M. tuberculosis* by culture, none were positive.

In the 13 LA positive cases who had paired blood culture results available, three cases were blood culture positive and two of those had the same bacteria detected in both specimens (Supplemental Table 3a). Of the five LA cultures that grew *S. pneumoniae*, three were PCV13-type serotypes (serotypes 1, 5, 6A) and two were non PCV13-type serotypes (serotypes 12F, 20), and all had received 3 doses of PCV. Four of these cases were *S. pneumoniae*-positive by NP culture and/or blood culture. There was serotype concordance in 3/4 of these cases (Supplemental Tables 3a and 6).

### *Pleural Fluid*

Of 19 cases with pleural fluid confirmed by the adjudication process and PF samples collected, 5 were excluded from analysis, due to collection >3 days after admission (n=4) and unavailable test results (n=1) (Figure 1b). Cases who had PF samples were similar with respect to clinical and demographic characteristics to children who were eligible for the procedure but did not have it performed (Table 3).

A pathogen was identified in 12/14 (86%) PF samples (Table 2b). Of these, 11/12 had only bacteria detected and 1/12 had viral-bacterial co-detection (*S. aureus* and human bocavirus) (Table 2b). The predominant pathogen was *S. aureus* (7/14, 50%) followed by *S. pneumoniae* (5/12, 36%). Of 9 cases with culture and PCR results who had a pathogen identified, 5 (56%) had a pathogen identified by both tests (4/5 being *S. aureus*), and 4 (44%) had a pathogen identified by PCR alone (3/4 being *S. pneumoniae*) (Supplemental Table 5b). Of the 12 PF positive cases, 15 pathogens were detected on PF, of which 10 (67%) were also detected in either of the paired NP/OP or blood culture (Supplemental Table 3b).

Of the five PF cases positive for *S. pneumoniae*, one had a PCV13-type serotype detected (serotype 5) on culture from PF, blood, and NP (a 42-month old child with 1 PCV dose) (Supplemental Table 6); the four remaining cases were positive by PCR or BinaxNOW only and not serotyped.

### **Serious Adverse Events**

There were three SAEs reported among LA cases (two in-hospital deaths not clearly related to the procedure and one transient drop in oxygen saturation following the procedure), and none reported amongst PF cases (details in the Appendix). The 2 deaths in LA cases represented a case fatality rate (4.5%) that was no higher than among those with consolidation on CXR that did not have the procedure (8%) (OR (95% CI): 0.57 (0.13-2.45) (Table 1).

## Discussion:

We have described the range of pathogens identified by culture and PCR of lung and pleural fluid aspirates collected from African and Asian children with severe pneumonia and CXR consolidation in the PCV era. In LA, *S. pneumoniae* was the predominant pathogen identified, followed by *H. influenzae* and *M. catarrhalis*, while in PF *S. aureus* was the predominant pathogen, followed by *S. pneumoniae*. In comparison to culture alone, PCR increased the pathogen yield, from 11% to 38% for LA (Supplementary Table 2a). From LA, *S. pneumoniae* was found as a single pathogen (5/9) as often as it was found in combination with other pathogens (4/9), usually other bacteria (3/9). In contrast, viruses were infrequently detected (n=4), led by CMV (2/4).

Earlier pneumonia etiology studies conducted in the pre-PCV era that applied either standard culture, PCR or both to lung aspirates reported *S. pneumoniae* as the predominant pathogen.<sup>4-5,20-22</sup> In this study, *S. pneumoniae* remained the leading pneumonia pathogen in the sampled group, despite the use of PCV in 3 of the 4 sites performing LA (all except Bangladesh). Viruses were identified infrequently in either LA or PF, in contrast to overarching PERCH etiology findings of 61.4% (95% credible interval: 57.3 - 65.6%) of radiologically confirmed pneumonia attributed to viruses, with RSV the predominant virus identified.<sup>23</sup> Where viruses were identified in LA specimens, they were mostly detected in combination with bacteria (3/4), with no LA specimen positive for RSV. Also, despite the low prevalence of pleural effusions among all radiological pneumonias in this study (40 of 1935), the finding of *S. aureus* and *S. pneumoniae* as the leading pathogens in PF was similar to findings from other studies on pleural effusions, including studies in the post-PCV era.<sup>5, 24-30</sup>

The difference in findings between the overall PERCH study, dominated by viral causes, and aetiology of the LA cases, dominated by bacteria is most likely because LA cases sampled represent a narrow clinical subgroup. Of note, LA cases came mostly from The Gambia, which had the second-highest aetiology fraction for *S. pneumoniae* by site in the overall PERCH results (15.1%).<sup>23</sup> Another

consideration is the difficulty of attributing causality to the pathogens detected; while this appears less problematic for lung or pleural aspirates than for other samples it is still an issue. The relative absence of viruses, for instance, may reflect the inability of even the best clinical samples taken at one point in time to elucidate causality due to a chain of pathogen and immune events. Nevertheless, the findings from the LA group do reinforce the possibility that polymicrobial disease is important.<sup>21</sup> Another possibility is that the PERCH study's overall results underrepresented bacterial causes, although this risk was addressed by comprehensive methodologic and analytic efforts to account for the known difficulty in diagnosing bacterial pneumonia. With all these considerations in view, the findings do emphasise that even in the context of widespread PCV and Hib vaccination, and the increasing contribution of viral aetiologies, bacterial infections remain important.

Having considered the differences of the findings of this study to those from all severe pneumonia cases in the PERCH study, we should also consider how generalizable the findings presented here are to children with consolidation on CXR. With the exception of site, demographic and clinical features were similar between those who underwent LA and those who did not, which is in favour of generalizability. Nevertheless, in the current study, only a small fraction (8%) of cases with CXR consolidation was sampled. Diagnostic yield, by either PCR or culture, for pneumococcus (20%) was lower than has been reported (25-41%) in pneumonia aetiology studies conducted in the pre-PCV era in similar settings.<sup>4,5</sup> This lower diagnostic yield could be due to a number of factors including changing trends in antibiotic use, the impact of PCV, or may reflect sampling issues, bias from the small proportion of potentially-eligible cases sampled, or chance. Rates of antibiotic use prior to LA collection appear considerably higher in PERCH (26/43, 60%) than in a previous Gambian study (18%).<sup>5</sup> The previous Gambian study was conducted in the pre-PCV era whereas PERCH was conducted after PCV introduction in all but one of the countries collecting LA samples, and we would expect to see a lower rate of detection of pneumococcus on LA specimens as a result. Nevertheless, in the current study, of the pneumococcal isolates for which serotype data was available 67% (4/6) are included in current PCVs. Also, 39% of specimens were collected after the day of admission with the

low yield (18%) in this group contributing to the lower-than-expected overall pathogen yield (Supplemental Table 4).

The outcomes of children who underwent the LA procedure in this study are consistent with the overall safety and utility of lung aspiration reported previously, with a serious adverse event rate no higher than in those who did not have the procedure. The procedure is not widely practiced among clinicians for various reasons, including unfamiliarity with the procedure and concerns regarding patient safety. One earlier report of over 25 years' experience of lung aspirations in The Gambia, showed that there were infrequent episodes (less than 3%) of adverse events including minor bleeding and pneumothorax, all of which were transient.<sup>11</sup> The experience of the PERCH study is that clinicians in diverse settings were taught and successfully performed this procedure safely.

This study highlights the continued importance, even in the PCV era, of bacterial aetiologies, prominently *S. pneumoniae* and *S. aureus*, in a select group of children with severe pneumonia associated with clear radiological consolidation or pleural effusion. Despite their limitations and lack of uptake, where appropriate skill and care are applied lung and pleural aspiration samples remain useful diagnostic specimens for such pneumonia cases, worthy of consideration both for research and clinical care. In this study they have provided an indication of possible differences in aetiology between such cases and the majority of severe pneumonia cases, as demonstrated in the difference between the findings of this study and the viral-aetiology dominated findings of the PERCH study overall. This study also highlights the value of molecular diagnostics for increasing the rate of pathogen detection.

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## **Conflicts of Interests**

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**Table 1. Comparison of clinical and demographic characteristics of cases with consolidation on CXR who did and did not have lung aspirates collected (adjusted for age and site<sup>a</sup>)**

	<b>Cases with LA collected</b>	<b>Cases with no LA collected</b>	<b>p-value<sup>a</sup></b>
	n=44	n=574	
<b>Age in months, Median (IQR)</b>	8.5 (3-19)	7 (3-13)	0.18
	n (%)	n (%)	
<b>Age &lt;1 year</b>	26 (59)	396 (69)	0.39
<b>Female</b>	20 (46)	284 (50)	0.69
<b>Site</b>			
The Gambia	24 (54)	81 (14)	<0.0001
Mali	10 (23)	135 (24)	
South Africa	6 (14)	304 (53)	
Bangladesh	4 (9)	54 (9)	
<b>Very severe pneumonia (vs. severe)</b>	14 (32)	187 (33)	0.27
<b>Duration of illness; Median days (IQR)<sup>b</sup></b>	3.5 (3-6)	3 (2-6)	0.90
<b>Hypoxaemic<sup>c</sup></b>	14 (32)	321 (56)	0.93
<b>Tachycardia<sup>d</sup></b>	26 (59)	293 (51)	0.51
<b>Temperature <math>\geq 38^{\circ}\text{C}</math></b>	22 (50)	142 (25)	0.30
<b>Wheeze<sup>e</sup></b>	8 (18)	157 (27)	0.11
<b>Danger signs</b>			
Head nodding	6 (14)	146 (25)	0.80
Central cyanosis	1 (2)	17 (3)	0.77
Inability to feed/drink	2 (4.5)	41 (7)	0.86
Vomiting everything	0 (0)	7 (1)	0.73

Lethargy	8 (18)	43 (7.5)	0.07
Multiple or prolonged convulsions	3 (7)	13 (2)	0.14
<b>Antibiotic pre-treatment<sup>f</sup></b>	11 (25)	260 (45)	0.34
<b>Vaccination</b>			
At least 1 HibCV dose	35 (83)	416 (77)	0.98
At least 1 PCV dose	31 (74)	355 (70)	0.29
<b>Severe wasting<sup>g</sup></b>	6 (14)	100 (18)	0.88
<b>HIV positive</b>	4 (9)	69 (12)	0.29
<b>Died in hospital</b>	2 (4.5)	44 (8)	0.48
<b>RSV NP/OP+</b>	6 (14)	129 (23)	0.85
<b>Parainfluenza 1 NP/OP+</b>	3 (7)	25 (4)	0.50

Abbreviations: LA, lung aspirate; PCV, pneumococcal conjugate vaccine; HibCV, *Haemophilus influenzae* type b conjugate vaccine; NP/OP+, positive by nasopharyngeal/oropharyngeal PCR.

Table restricted to sites where LA specimens were available (Gambia, South Africa, Bangladesh and Mali). Cases with LA specimen collected more than 72 hours after enrollment were excluded from the analysis. Consolidation based on PERCH standardized CXR reading panel, not clinician reading during hospitalization.

- P-values based on a logistic regression model adjusted for age in months and site, with Firth adjustment for categorical variables and Kruskal-Wallis test for continuous variables comparing cases with LA specimen taken to cases with consolidation on CXR but without LA specimen taken.
- The number of days with cough, fever, difficulty breathing, wheeze, or runny nose, whichever symptom was longest.
- Hypoxaemic defined as oxygen saturation at admission <90% at South Africa and <92% at other sites, or oxygen requirement (if on oxygen and room air saturation not available).
- Elevated heart rate at baseline clinical assessment defined as: greater than 160 bpm in infants 0-11 months, greater than 150 bpm in children 12-35 months, greater than 140 bpm in children 36-59 months.
- Presence of audible or auscultatory wheeze at admission.
- Antibiotic pretreatment was defined as having either a positive serum bioassay or documentation of antibiotics administered at the referral or study hospital prior to NP/OP specimen collection.
- Weight-for-height < -3 z-scores.

**Table 2a. Organisms identified by culture and/or PCR of lung aspirate specimens**

	<b>PCR (N=29) n (%)</b>	<b>Culture (N=44) n (%)</b>	<b>Either PCR or Culture (N=44) n (%)</b>
<b>Any positive<sup>a</sup></b>	11 (38)	5 (11)	13 (30)
<i>S. pneumoniae</i>	7 (24)	5 (11)	9 (20)
<i>H. influenzae</i>	4 (14)	1 (2)	4 (9)
<i>C. pneumoniae</i>	1 (3)	0	1 (2)
<i>M. catarrhalis</i>	4 (14)	0	4 (9)
<i>P. jirovecii</i>	1 (3)	0	1 (2)
<b>Adenovirus</b>	1 (3)	N/A	1 (2)
<b>CMV</b>	2 (7)	N/A	2 (4)
<b>HMPV</b>	1 (3)	N/A	1 (2)
<b>Combinations<sup>b</sup></b>			
<i>S. pneumoniae</i> + <i>H. influenzae</i>	--	--	2 (4)
<i>S. pneumoniae</i> + <i>M. catarrhalis</i>	--	--	2 (4)
<b>Adenovirus</b> + <i>C. pneumoniae</i>	--	--	1 (2)
<i>H. influenzae</i> + <i>M. catarrhalis</i> + <i>S. pneumoniae</i> + <i>P. jirovecii</i> + <b>CMV</b>	--	--	1 (2)
<i>H. influenzae</i> + <i>M. catarrhalis</i> + <b>HMPV</b>	--	--	1 (2)

Abbreviations: HMPV, human metapneumovirus; CMV, cytomegalovirus.

<sup>a</sup> Total number of cases with organism identified is not the sum of the number of organisms identified because some cases tested positive for more than one organism.

<sup>b</sup> Combinations presented as detection by either PCR or culture, not split by detection method.

**Table 2b. Organisms identified by culture and/or PCR of pleural fluid specimens**

	PCR (N=11) n (%)	Culture (N=14) n (%)	Either PCR or Culture (N=14) n (%)
<b>Any positive<sup>a</sup></b>	9 (82)	9 (64)	12 (86)
<i>S. pneumoniae</i>	4 (36)	1 (7)	5 (36)
<i>H. influenzae</i>	1 (9)	0 (0)	1 (7)
<i>S. aureus</i>	4 (36)	7 (50)	7 (50)
<i>E. coli</i>	0	1 (7)	1 (7)
<i>Streptococcus Group F</i>	0	1 (7)	1 (7)
<b>HBOV</b>	1 (9)	N/A	1 (7)
<b>Combinations<sup>b</sup></b>			
<i>S. aureus</i> + <b>HBOV</b>	--	--	1 (7)
<i>S. aureus</i> + <i>S. pneumoniae</i> <sup>c</sup>			1 (7)
<i>E. coli</i> + <i>Streptococcus Group F</i> + <i>H. influenzae</i>	--	--	1 (7)

Abbreviations: HBOV, human bocavirus.

<sup>a</sup>Total number of cases with organism identified; is not a sum of the number of organisms identified because some cases have more than one pathogen identified.

<sup>b</sup>Combinations presented as detection by either PCR or culture, not split by detection method.

<sup>c</sup>Of the 5 cases positive for *S. pneumoniae* in pleural fluid, 4 were identified by culture or PCR and 1 additional case was identified using BinaxNOW®, which was also culture+ for *S. aureus* (see Table 4b for details). One PCR+ had a negative BinaxNOW® test result.



**Table 3. Clinical characteristics of cases with pleural fluid<sup>a</sup> on initial CXR comparing those with pleural fluid collected and pleural fluid not collected**

	Cases with PF collected	Cases without PF collected	p-value <sup>b</sup>
	n=14	n=21	
<b>Age in months, Median (IQR)</b>	19 (7-36)	12 (4-27)	0.17
<b>Age &lt;1 year</b>	4 (29)	10 (48)	0.61
<b>Female</b>	10 (71)	9 (43)	0.15
<b>Site</b>			
Kenya	2 (14)	7 (33)	0.82
The Gambia	1 (7)	3 (14)	
Mali	5 (36)	6 (29)	
Zambia	2 (14)	3 (14)	
South Africa	4 (29)	2 (10)	
<b>HIV positive</b>	0 (0)	1 (5)	0.60
<b>Very severe pneumonia (vs. severe)</b>	3 (21)	5 (24)	0.74
<b>Hypoxaemic<sup>c</sup></b>	8 (57)	11 (52)	0.79
<b>Tachycardia<sup>d</sup></b>	9 (64)	13 (62)	0.64
<b>Temperature <math>\geq 38^{\circ}\text{C}</math></b>	7 (50)	14 (67)	0.22
<b>Wheeze<sup>e</sup></b>	0 (0)	3 (14)	0.29
<b>Antibiotic pre-treatment<sup>f</sup></b>	8 (57)	8 (38)	0.87
<b>Vaccination</b>			
At least 1 HibCV dose	11 (79)	15 (88)	0.39
At least 1 PCV dose	8 (67)	11 (65)	0.91
<b>Severe malnutrition<sup>g</sup></b>	0 (0)	2 (10)	0.61
<b>Died in hospital</b>	1 (7)	3 (14)	0.94
<b>RSV NP/OP+</b>	0 (0)	5 (25)	0.11
<b>Parainfluenza NP/OP+</b>	0 (0)	0 (0)	-

Abbreviations: PF, pleural fluid; PCV, pneumococcal conjugate vaccine; HibCV, *Haemophilus influenzae* type b conjugate vaccine; NP/OP+, positive by nasopharyngeal-orpharyngeal PCR.

Table restricted to sites where PF specimens were available (Kenya, The Gambia, Mali, Zambia, and South Africa); no samples collected at the Asian sites. Cases with PF specimens collected more than 72 hours after enrollment were excluded from the analysis.

- Pleural fluid identified by at least two readers or arbitrators in the CXR reading process on the first CXR taken, and confirmed by Gambia PERCH clinicians, regardless of CXR final conclusion based on the PERCH CXR Reading Panel. Only includes cases with pleural fluid seen on CXR from sites where PF specimens were taken.
- P-values based on a logistic regression model adjusted for age and site for categorical variables, and Kruskal-Wallis for continuous variables.
- Hypoxia defined as oxygen saturation at admission <90% at South Africa and Zambia, and <92% at all other sites, or oxygen requirement (if on oxygen and room air saturation not available).
- Elevated heart rate at baseline clinical assessment defined as: greater than 160 bpm in infants 0-11 months, greater than 150 bpm in children 12-35 months, greater than 140 bpm in children 36-59 months.
- Presence of audible or auscultatory wheeze at admission.
- Antibiotic pretreatment was defined as having either a positive serum bioassay or documentation of antibiotics administered at the referral or study hospital prior to NP/OP specimen collection.
- Weight-for-height < -3 z-scores.

## Figure Legends:

### Figure 1a. Enrollment of Lung Aspiration cases (n=44) in 4 PERCH sites performing lung aspiration

<sup>a</sup> Includes two cases the PERCH CXR Reading Panel determined to have normal CXR results and two cases the Panel determined to have uninterpretable results. The decision to perform the procedure was made by the clinical team responsible for care of the patient, based on information available to it at the time of admission.

<sup>b</sup> Lung aspirates not performed due to procedure not yet initiated at site or eligible but not done.

<sup>c</sup> Includes one case in which the PERCH CXR Reading Panel determined to have normal CXR results.

<sup>d</sup> Includes one case in which the PERCH CXR Reading Panel determined to have normal CXR results, and two cases which the Panel determined to have uninterpretable results.

### Figure 1b. Enrollment of Pleural Fluid cases (n=19) in the PERCH study

a. Clinicians from The Gambia PERCH site reviewed all of the CXRs from those cases where two or more standardized readers indicated pleural effusion, or the case had a pleural fluid specimen obtained. The cases confirmed to have presence of any pleural fluid on CXR by the clinical review team were considered confirmed by the adjudication process. See Appendix for more details.

b. An additional three pleural fluid samples were obtained but not captured here because the adjudication process determined that their radiograph did not have evidence of pleural fluid. Two were obtained from children with consolidation on chest radiograph (sample collected on day of admission in one case and on day 3 post-admission in the other) and one with an uninterpretable radiograph (specimen collected on day 6 post-admission).



