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REVIEW ARTICLE

Etiology of Childhood Pneumonia: What We Know, and What We Need to Know!

Based on 5th Dr. IC Verma Excellence Oration Award

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Abstract Childhood community acquired pneumonia continues to be an important clinical problem at the individual, institutional and community levels. Determination of microbial etiology is critical to develop evidence-based management (therapeutic and prophylactic) decisions. For decades, the approach to this relied on culture of lung aspirate specimens obtained from children with radiographically confirmed pneumonia, before administering antibiotics. Such studies revealed the major bacteria associated with pneumonia, prompting the World Health Organization to develop a highly sensitive clinical definition of pneumonia and advocate empiric antibiotic therapy; in order to save lives (focusing on community settings lacking resources for diagnostic tests). However, it spawned research studies conducted in/from/by institutions enrolling children with the relatively non-specific WHO definition of pneumonia. Specificity got further compromised by abandoning lung aspiration and using naso/oro pharyngeal specimens; even in children who had received antibiotics. This led to the recovery of viruses more often than bacteria. The use of highly sensitive molecular based diagnostics (especially PCR) facilitated the detection of multiple organisms (bacteria, viruses, atypical organisms and even fungal species); making it difficult to attribute etiology in individual cases. This challenge was sought to be addressed through the multi-site PERCH Study (Pneumonia Etiology Research for Child Health), designed as a case-control study to conclusively determine the etiology of pneumonia. However, despite a slew of publications, the answer to the central question of etiology has not emerged so far. Since none of the PERCH Study sites was located in India, the Community Acquired Pneumonia Etiology Study (CAPES) was conducted at Chandigarh. This turned out to be the largest single-centre pneumonia etiology study, and generated a wealth of data. This article summarizes the current challenges in pneumonia etiology research; outlines the key observations from the PERCH and CAPES projects, as well as other important studies; and suggests a way forward for pneumonia etiology research in the current era.

Keywords Acute lower respiratory tract infection · Bacteria · Etiology · Polymerase chain reaction

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Introduction

The importance of childhood community acquired pneumonia (CAP) cannot be emphasized enough. It has been, and continues to be, the single most important cause of childhood morbidity and mortality across the world [1]. In the early nineties, over 25% of the annual deaths of children in developing countries before the fifth birthday was attributable to pneumonia [2]. In 2008, it was estimated to be responsible for 1.6 million deaths among <5-y-old children around the world [3]. As recently as 2013, the worldwide Global Burden of Diseases (GBD) analysis suggested that CAP could be responsible for approximately 0.9 million childhood deaths; this translates to over 14% of all childhood deaths [4]. As expected the global burden is borne disproportionately by resource-constrained countries; where the incidence of childhood CAP is estimated to be 15 fold higher than resource rich settings [5]. Some of this is related to higher prevalence of baseline 'risk factors' such as malnutrition, inadequate breast feeding, exposure to household pollution, overcrowding, *etc*.

[6]. Naturally, the addition of other risk factors, such as exposure to HIV in some settings, compounds the problem.

In addition to immense public heath importance, childhood pneumonia also has serious consequences for the individual child. Besides the immediate risks of complications and mortality, emerging data shows that pneumonia in childhood can predispose to long term complications such as decreased lung function, asthma and even chronic obstructive lung disease in later life [7].

Thus, the determination of microbial etiology in childhood pneumonia has considerable importance. At the individual level, it impacts treatment decisions such as whether to use antibiotics (or not), choice of antibiotics, duration of therapy, *etc*. At the institutional level, it determines antibiotic administration policies; and at the community level, these decisions have greater implications including patterns of antimicrobial resistance. From the public health perspective, this apparently simple issue governs vaccination (and/or other prophylaxis) policies, allocation of resources to specific programmes in this direction, and to some extent, the research agenda in childhood pneumonia. Needless to mention, commercial interests (antibiotics, vaccines, delivery programmes, *etc*.) run into billions of dollars.

Determination of Pneumonia Etiology

For almost a whole century, the direct demonstration of organisms by culture (or staining methods) in lung aspirates of children with pneumonia, was the accepted approach to determine microbial etiology, in developed and developing countries [8–11], including India [12–16]. In fact, lung aspiration is regarded the gold standard for determining etiology [17, 18]. The approach was bolstered by the finding that pulmonary aspirates in a small cohort of children without clinical or radiographic pneumonia, were uniformly sterile [9], suggesting that lung aspiration had no false positivity. Some studies focusing on postmortem lung aspiration also contributed valuable data [19]. The main premise with this approach was that the lung was regarded as a sterile tissue; consequently any micro-organism found there, was regarded as pathogenic. Lung aspiration studies demonstrated the major bacteria implicated in childhood pneumonia *viz*. *Streptococcus pneumoniae* and *Haemophilus influenzae*; along with *Staphylococcus aureus* and other bacteria in some studies. A limited number of studies also attempted to identify viruses either by culture or other methods such as immunofluorescence [11, 20–23]. It should be emphasized that many of the lung aspiration analyses were undertaken in children with radiographically confirmed pneumonia and often, before antibiotic administration. Table 1 summarizes the data from an exhaustive review of lung aspirate studies [24]; this shows that developed countries stopped performing lung aspirates by the 1970s, by which time developing countries started such studies and continued till the mid-nineties. More recently, a study in Malawi using PCR in lung aspirate samples of 95 children with radiographic pneumonia, reported that while aspirate culture yielded bacteria in only 2 cases; PCR showed bacteria in 36 cases. Viruses were identified singly or in combination in 24 cases. Altogether lung aspirate PCR could identify organisms in 59 of 95 (62.3%) children [25]. In another small study of 55 children with severe pneumonia; 47 of whom underwent lung aspiration; pathogens were identifiable by a combination of culture and PCR (from lung aspirates or pleural fluid) in over 90% cases [26].

Some of the studies examined blood as a surrogate sample. However studies comparing lung aspirate with blood cultures confirmed the relatively poor sensitivity of the latter. In a review of 9 such series, while lung aspirates could identify organisms in over 50% cases, blood culture could identify the etiology in only 25% [24]. Blood culture was negative in about a third of the cases; and both blood and lung aspirate cultures were positive in less than one-fifth cases.

The identification of bacteria in various studies resulted in two important developments with widespread ramifications. First, the WHO led global efforts to enhance the use of antibiotics, in order to save lives of children with pneumonia, especially in resource limited settings. For this, a highly sensitive clinical definition of pneumonia was evolved whereby *field workers* (note emphasis) in resource constrained settings could identify (and treat) children urgently; with the focus being to prevent mortality. The definition was based on easy to recognize clinical symptoms and signs; and did away with radiographic confirmation [27]. Global experts recognized that while this approach could ensure that no child failed to receive antibiotics, it would result in over-treatment on account of false positive diagnosis. But this trade-off was considered acceptable. An undesirable 'side-effect' was that health-care systems in developing countries with access to resources/ facilities, also started using the highly sensitive definition of pneumonia; both for management of individual children, as well as research studies. This spawned a series of studies on pneumonia etiology wherein unknown proportions of enrolled children probably did not actually have pneumonia.

The second development with enhanced antibiotic usage was that many studies started enrolling children (using the liberal WHO definition) even if they had received antibiotics for varying durations of time. Naturally this decreased the yield of bacteria identified by culture. Around the same time, data from a large multi-centric study (10 sites in developing countries) of acute respiratory tract infection (ARI) etiology, became available [28]. This study relied on blood and pleural fluid culture to identify bacteria; and nasopharyngeal aspirate culture to identify viruses. The study reported that viruses

Table 1 Summary of lung aspirate studies around the world till 2000. Data calculated from Vuori-Holopainen [24]

	Europe	North America	South America	Africa	Asia	Oceania	Total
Pre 1950							
Studies	6	5	0	2	0	0	13
Sample size	2–61	13–405		52–233			1071
Bacteria identified	30–100%	18–100%		78–92%			551 (51.4%)
1951–1970							
Studies	1	4	1	0	3	0	9
Sample size	51	1–32	125		17–25		272
Bacteria identified	65%	0–100%	54%		29–44%		119 (43.8%)
1971–1980							
Studies	0	1	6	5	3	1	16
Sample size		27	21–530	7–88	68–193	18	1321
Bacteria identified		22%	10–57%	17–79%	53–88%	44%	455 (34.4%)
1981–1990							
Studies	0	0	0	5	1	1	7
Sample size				40–108	70	83	402
Bacteria identified				33–67%	51%	61%	220 (54.7%)
Post 1991							
Studies	0	0	0		7	5	12
Sample size					1–99	12–100	669
Bacteria identified					38–100%	16–50%	333 (49.8%)

(especially respiratory syncytial virus) were identified more often than bacteria in children with lower respiratory infection, enrolled from the community as well as hospital (in-patient and out-patient).

In a sense, this opened the floodgates for a variety of research studies examining nasopharyngeal (swab or aspirate), or nasal or even oropharyngeal samples among children with the liberal definition of pneumonia, including those with prior receipt of antibiotics. The non-specificity of these samples *vis* a *vis* lung aspirate samples resulted in several bacteria and viruses being identified; and accorded etiologic status. In fact, this trend has continued till now; with the added layer of advanced molecular techniques such as polymerase chain reaction (PCR) replacing culture (as a faster, albeit more expensive method). PCR being highly sensitive (for detection of microbial footprints) although less specific (for determination of a cause-and-effect relationship) made it possible to detect multiple organisms (pathogenic or otherwise) and label them as etiologic agents. The major problem with this approach (in addition to the non-specific definition of pneumonia) is that almost all the bacterial species implicated in pneumonia causation (*S. pneumoniae*, *H. influenzae*, *S. aureus*, Mycoplasma *etc*) are also found in the nasopharynx of normal children; who get colonized in early life without suffering from an infection [29–32]. In such a setting, the inference that detection (of organisms) implies causality is akin to assuming that anyone detected at the scene of a crime is responsible for it.

The problem is compounded further by other studies in recent years showing that most of the viruses identified in nasopharyngeal secretions of children with so-called pneumonia, are also identifiable in those with upper respiratory infection; and even normal (*i.e.*, asymptomatic) infants and children [33–35]. A recent systematic review of case-control studies suggested that detection of RSV, influenza virus, human metapneumovirus, and parainfluenza virus in nasopharyngeal secretions of cases was associated with lower respiratory tract infection much more often than controls; whereas there was no such association for other viruses such as coronaviruses, bocavirus, or adenovirus [36]. However, this is not entirely helpful for individual children because there will be exceptions to the trend.

A recent publication by Zar et al. [37] succinctly outlined the relative advantages and disadvantages of using various biological specimens (such as blood, naso/oro pharyngeal secretions, blood, broncho-alveolar lavage, lung aspirate) and diverse methods to identify organisms (including culture, PCR, antigen detection, serology studies *etc*). However, there is no single test in any biological sample that can accurately establish etiology in children with pneumonia.

Table 2 summarizes the major challenges in confirming microbial etiology of childhood pneumonia.

Table 2 Challenges in confirming microbial etiology of childhood pneumonia

Challenges with the concept of "pneumonia"

•Pneumonia" is a pathologic diagnosis with clinical and radiographic correlates that can suggest (but not necessarily confirm) its presence. However, histopathologic confirmation is not feasible in individual cases or epidemiologic studies.

•Surrogate definitions compromise either sensitivity (for example radiographic definition) and/or specificity (for example WHO definition) or both (for example clinician diagnosed pneumonia)

•Multiplicity of definitions across studies with difficulty in comparison(s).

•Studies restricted to hospitalized children create a bias associated with health-seeking behavior and/or strong referral systems and/or survival (*i.e*., children who die before reaching the hospital are not included).

•Studies using radiographic inclusion criteria do not always use standardized criteria.

Challenges with the concept of "etiology"

•In modern times, it is highly unlikely that Koch's postulates (for determining etiology) can be fulfilled in any research study.

•The assumption that identification of an organism indicates causality (even from lung aspirates and/or blood samples) is not necessarily correct.

Challenges with biological specimens to determine etiology

•The ideal specimen would be lung tissue *from the area with pneumonia* (note emphasis) obtained at the onset of illness, but this is not feasible in routine clinical practice or research studies.

•The closest to this ideal (radiographically guided lung aspirate/ biopsy; or broncho-alveolar lavage, at the onset of illness or at least at presentation) is difficult for ethical, technical and/or epidemiologic reasons.

•Lung aspirates/biopsy or broncho-alveolar lavage specimens later in the course of disease, or after death; in hospitalized children can create the risk of detecting hospital acquired pathogens (especially in sick children with multiple interventions including intubation).

•The lung is not necessarily a sterile tissue and has a dynamic microbiome that could be influenced by a variety of factors.

•Lung aspiration is also not fool-proof and negative results have been observed despite targeting the correct area and obtaining appropriate representative samples. There are of course risks associated with the technique although it is regarded safe in expert hands.

•Blood has limited sensitivity (and possibly specificity) in childhood pneumonia.

•Nasal swabs/ nasopharyngeal aspirates/ nasopharyngeal swabs/ oropharyngeal swabs do not necessarily reflect the organisms in the lower airways or lungs.

•Sputum is often not produced by infants and young children.

•Induced sputum is a viable alternative, but requires premedication with bronchodilator and has the risk of inducing emesis.

•Gastric aspirate and/or lavage samples are useful only for detecting organisms resistant to gastric acid.

•The volume of blood used for culture alters the results. The ideal volume of blood required may not be obtainable in young infants and children.

•Use of prior antibiotics (rampant in settings with uncontrolled access) compromises findings in blood and to some extent, lung aspirates.

•There are no biomarkers that correlate with microbial etiology

Challenges with processing of biological specimens to determine etiology

•Samples need to be collected, transported to the lab and processed appropriately. Although sample collection is often timely, there are delays in transport and/or processing.

•Culture is the usual gold standard for bacteria but has limited sensitivity. For some organisms, PCR (or other molecular based methods) have higher sensitivity, but it is difficult to distinguish between live organisms, dead organisms, or remnants of organisms.

•Molecular methods to detect organisms such as PCR are generally reported as positive or negative. However, this depends on the limits of detection (which are generally not reported).

•PCR can detect only the organisms that are looked for; in other words, there is an inherent selection bias. This results in missing organisms, that were not searched for and/or novel/unexpected organisms.

•Studies designed to identify one (or a limited number of selected) micro-organisms, are inherently biased.

•Highly sensitive methods often reveal footprints of multiple organisms; but the contributory role of these (in etiology) is unclear.

•Serology based tests for atypical organisms are unreliable for determination of etiology.

•Detection of antigens in urine lacks specificity.

Challenges with interpretation of results

•The detection of one or more organisms in various biological specimens need not mean causality.

•The significance and interpretation of different organisms in different biological specimens of individual cases, is unclear.

•Case control studies can only suggest pathogenicity (in the overall group), but not confirm it (in individual cases or the whole group).

•Case control studies cannot factor in data from multiple specimens as invasive/painful methods are generally not used in controls.

•It is unclear which children (healthy or those with non pneumonia respiratory infections) should serve as controls in case-control studies.

•Statistical methods such as latent class analysis can slot cases into etiology 'classes', but do not confirm etiology in individual cases.

Pneumonia Etiology Research for Child Health (PERCH)

Recognizing these problems, the Bill and Melinda Gates Foundation initiated the multi-centric PERCH (Pneumonia Etiology Research for Child Health) study in seven developing countries (contributing nine sites) to confirm the microbial etiology of childhood pneumonia [3, 38]. This was designed as a case-control study among those less than 5 y (excluding neonates), to identify pathogens in multiple biological samples (*viz*. blood, naso or oro pharyngeal swabs, and induced sputum, in cases) by multiple methods *viz*. culture and PCR. Cases were those hospitalized with WHO defined severe pneumonia and controls were age-frequency matched children (healthy as well as those with non-pneumonia respiratory symptoms). Numerous methodological refinements were introduced to enhance validity of the study including efforts to standardize definitions [39], clinical methods, sample collection [40, 41], laboratory methods [42, 43] and chest radiography (performance and interpretation) [44]. Additional refinements included documenting and studying the effect of prior antibiotic therapy (and also the volume of blood drawn), on bacterial culture results [45, 46]; the relationship between bacterial density in the upper airway and blood culture [47, 48]; and correlation of viral load in the upper airway with pneumonia [49]. In addition, tremendous emphasis has been given to appropriate data management, quality control, data analysis and interpretation [50–53]. These resource intensive (in terms of time, manpower and materials) efforts; foster the assurance of high internal and external validity of the results. A summary of the main results reported till date is shown in Table 3. However, so far the PERCH study has not reported the central question of pneumonia etiology.

Community Acquired Pneumonia Etiology Study (CAPES)

None of the PERCH sites was located in India. However, India reportedly contributes the largest number of childhood pneumonia cases; and greatest mortality with over 400,000 childhood deaths in the under-five age group [63–67]. Further, India has been under tremendous global pressure to initiate the administration of Pneumococcal Conjugate Vaccine (PCV) in the routine immunization programme, although the scientific rationale and evidence base are controversial [68–71]. Despite this, none of the numerous Indian studies on childhood pneumonia etiology have been designed to address the challenges presented in Table 2. Therefore, the Community Acquired Pneumonia Etiology Study (CAPES) was initiated at PGIMER Chandigarh in 2010, in collaboration with Karolinska Institute, Stockholm [72]. The study enrolled children (1 mo to 12 y) with pneumonia (WHO IMNCI criteria) identified through active surveillance in the community and passive surveillance in the hospital (out-patient department as well as Emergency); over a period from April 2011 through December 2014. Overall, approximately 46,000 children were screened and over 4000 enrolled, making it one of the world's largest single-centre studies on pneumonia etiology. Children who had received antibiotics for more than 24 h at presentation were excluded. The laboratory tests included blood and nasopharyngeal aspirate (NPA) bacterial cultures, and serology in duplicate for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*. Multiplex PCR for 25 bacterial/viral species was undertaken in a subgroup selected to represent the entire cohort. In addition, children who required endotracheal intubation had bronchoalveolar lavage (BAL) specimens taken for culture and multiplex PCR. Thus this was a comprehensive study designed to determine microbial etiology. The salient results, conclusions, strengths and limitations (available from data published so far) are briefly presented in Table 4.

The challenge of distinguishing nasopharyngeal colonizing organisms from pneumonia causing pathogens has plagued pneumonia etiology researchers worldwide. In order to better understand the timing and patterns of nasopharyngeal colonization in Indian infants, a longitudinal study was initiated at PGIMER Chandigarh. A cohort of 100 infants was enrolled at birth and nasopharyngeal aspirate samples obtained. The infants were serially followed till 2 y of age (6 visits coinciding with vaccination). At each visit, history of respiratory tract infection (personal and family members) in the preceding two weeks was obtained. Those who were free of symptoms and signs for the preceding 14 d underwent nasopharyngeal aspirate analysis by bacterial culture and viral multiplex PCR. The data are being analysed, but preliminary results suggest early colonization (in some cases at birth) with a wide array of bacteria and viruses; dynamic pattern of organisms, and no relationship to recent (*i.e*., >14 d) upper respiratory tract infection in the infants or family members. This suggests a dynamic microbiome that could impact on the microbial etiology in the event of lower respiratory tract infections.

In order to further address some of the limitations of CAPES, a smaller prospective study (CAPES 2) was undertaken at PGIMER Chandigarh (during 2015–16) wherein only children with severe pneumonia who had received no prior antibiotics were enrolled. In addition to blood and nasopharyngeal aspirate samples, sputum, induced sputum, and where applicable, pleural fluid samples were obtained. In children with non-resolution of symptoms within 72 h, bronchoalveolar lavage, and lung aspirate specimens were obtained. Postmortem lung aspirates were also drawn. The samples were processed for bacterial culture and viral analysis by multiplex PCR (21 organisms), in addition to selected serological tests for atypical organisms. Preliminary analysis provides a rich pool of data. Blood and nasopharyngeal aspirate culture

Table 3 Salient results available from the PERCH study till August 2017

- •PERCH enrolled 4232 cases and 5325 controls from nine sites in seven developing countries. The controls included healthy children as well as those with respiratory symptoms not fulfilling the definition of pneumonia [54].
- •Serum bioassay confirmed prior antibiotic use in over 25% cases and 2.3% controls. Evaluation through combined assessment of history, referral document and bioassay, identified prior antibiotic use in 43.5% cases [45].
- •Prior antibiotic exposure reduced the probability of detecting most bacteria (by culture and PCR), although the effect on *S. aureus* was unclear [45].
- •The volume of blood obtained for culture showed a direct relationship with isolation of bacteria; with highest yield when >4 ml was taken. This effect was consistent for children with and without prior receipt of antibiotics [45].
- •*S. pneumoniae* was identified by culture of blood, pleural fluid, or lung aspirate in only 56 cases. However none of the 4 sites in Asia had a single culture-proven case despite the absence of a Pneumococcal vaccination programme [47].
- •*S. pneumoniae* PCR in blood was positive in 291/3995 (7.3%) cases and also 273/4987 (5.5%) controls. However, only 36 of 56 (64.3%) cases with culture confirmed Pneumococcal bacteremia, were PCR positive. In fact, 243/3832 (6.3%) children without confirmed bacterial infection also were PCR positive. These data suggest that blood PCR may not be a suitable test in Pneumococcal pneumonia [55].
- •Quantitative PCR for Pneumococcus in naso/oro pharyngeal samples showed significantly higher load in cases with culture-proven Pneumococcus (n = 56), compared to cases without Pneumococcus, as well as controls. However, cut-off value >6.9 log10 copies/mL to distinguish confirmed Pneumococcal cases *vs*. controls had sensitivity 64% and specificity 92% [47].
- •Although quantitative load of Pneumococcus (determined by PCR) was higher in culture positive cases than controls, significant overlap precluded accurate differentiation, confirming the limited utility of quantitative Pneumococcal PCR in blood for diagnosing Pneumococcal pneumonia [56].
- •There were only 52 microbiologically confirmed cases with any of the following organisms: *H. influenzae, M. catarrhalis, S. aureus*, or *P. jirovecii*. Only 2 of these were from the 4 Asian sites. Quantitative PCR of naso/oro pharyngeal samples could not reliably distinguish between culture confirmed cases *vs*. controls [48].
- •Almost 90% cases and 80% controls had at least one of the 17 viruses tested for by multiplex PCR in nasopharyngeal/oropharyngeal samples; the respective proportions for 2 viruses were 53% and 40%; and for >3 viruses were 18% and 12% [58].
- •Quantitative estimation of viral load in nasopharyngeal/oropharyngeal samples showed considerable overlap between radiographically confirmed cases and controls. Children with very severe pneumonia and those who died did not have higher viral loads. These findings suggest that quantitative PCR for viruses may not be discriminatory [58].
- •The yield of bacteria and viruses by PCR of induced sputum samples was comparable to that obtained by PCR of naso/oropharyngeal specimens. Quantitative analysis did not provide additional information [57].
- •*Bordetella pertussis* was detected in 53/ 4200 (1.3%) cases and 11/ 5196 (0.2%) controls in naso/oro pharyngeal aspirates, suggesting a possible role in pneumonia. There was disproportionately high mortality among cases [59].
- •Nineteen hundred thirty five of 3587 interpretable chest radiographs (54%) showed abnormality; although consolidation was seen in far fewer children, and there was significant variation across sites. Classic clinical signs of severe pneumonia (hypoxemia, fever, tachypnea, *etc*) were observed more frequently in those with radiographic abnormalities [60].
- •CRP ≥40 mg/L was observed in 77% of 119 HIV-negative cases with bacterial pneumonia (defined by positive blood culture or positive lung aspirate or pleural fluid culture or PCR) compared with 17% of 556 RSV pneumonia cases (defined as nasopharyngeal/oropharyngeal or induced sputum PCR-positive without confirmed/suspected bacterial pneumonia), suggesting utility for distinguishing bacterial *vs*. RSV-associated pneumonia, although not other viruses. However, 30% of 286 children with both bacteria and RSV also had CRP ≥40 mg/L [61].
- •There were wide variations in the results obtained from the 9 sites with significant differences between sites in Asia compared to Africa [45, 47, 48, 55, 57, 60, 61].
- •PERCH has developed a bio-repository of specimens for later testing [62].

were positive in 4% and 39% cases respectively (compared to 2% and 11% in CAPES), confirming the adverse impact of even <24 h antibiotic intake prior to presentation. BAL culture was positive in about one third of specimens, but Acinetobacter dominated, raising the dilemma whether this was acquired before or after hospitalization. Lung aspirates obtained in non-responsive children gave a poor yield of bacteria on culture, but several viruses could be identified by PCR. As expected, a plethora of viruses was identified by PCR of nasopharyngeal aspirates, but the same problems in attributing etiology were encountered. Additional analysis of the data from this cohort are underway.

In 2012–13, the Indian Council of Medical Research (ICMR) took cognizance of the importance of pneumonia etiology in India, and the ongoing CAPES project; and decided to initiate a multi-centric study across India for confirmation of microbial etiology. Five institutions were selected (CMC Vellore, PGIMER Chandigarh, KGMU Lucknow, KEM Pune and NICED Kolkata) to contribute cases (community and hospital based) and controls. A detailed project protocol has been prepared with emphasis on case definitions, clinical protocol, sampling scheme (in cases and controls), laboratory testing (including culture, PCR and advanced molecular techniques). Funding was recently secured and the study is expected to be in initiated in late 2017.

The findings from CAPES and other studies clearly suggest that there are wide variations in the clinical presentation, course and outcome of children with pneumonia even when

Table 4 Salient findings, conclusions, strengths and limitations of the Community Acquired Pneumonia Etiology Study (CAPES)

Salient findings

•Blood culture yielded organisms in approximately 2% children

The most common organism isolated in blood was *Staphyococcus aureus*, not *Streptococcus pneumoniae*.

•Gram negative bacilli (*Klebsiella pneumoniae*, *Acinetobacter* species, *Salmonella typhi*) together outnumbered Pneumococcal isolates.

•Broncho-alveolar lavage culture done in a limited number of cases (but not at presentation) identified organisms in only 10%.

•Corresponding PCR of BAL yielded organisms in 93% samples; however a single organism (bacteria or virus) was found in only 33%. The rest had multiple organisms in different combinations.

•Nasopharyngeal aspirate culture was positive in only about 15% cases with *S. pneumoniae* predominating, followed by *H. influenzae* and *S. aureus*.

•Multiplex PCR of nasopharyngeal aspirate samples yielded multiple bacteria and viruses. Only 1.4% children did not show any of the 25 species looked for. The majority (59%) had multiple organisms, making it impossible to attribute causality.

•A single bacterial species was observed in only 9.8% cases; and a single virus identified in only 6.5% cases.

•Surprisingly, cytomegalovirus (CMV) was the dominant isolate among viruses, followed by RSV, followed by Rhinovirus, Coronavirus, Parainfluenza virus, Influenza virus, *etc*.

•The yield of bacteria on PCR of nasopharyngeal aspirates was several fold higher than culture (76% *vs*. 11% for *S. pneumoniae*; 31% *vs*. 1.3% for *H. influenzae* and 20% *vs*. 0.9% for *S. aureus*).

•The patterns of distribution of organism classes was similar in children with non-severe, severe and very severe pneumonia.

•The distribution of organisms in children who died was not significantly different from survivors.

•Serology tests (done in duplicate) for *M. pneumoniae* and *C. pneumoniae* were positive in 4.3% and 1.1% respectively.

•Even among cases with a single bacterial or viral isolate, analysis of various factors showed that it is impossible to predict bacterial *vs*. viral etiology at presentation.

Salient conclusions

•Nasopharyngeal samples are inappropriate specimens to determine pneumonia etiology.

•The presence of multiple organisms in the majority of broncho-alveolar lavage specimens (albeit taken during the course of illness, rather than presentation) precludes attribution of etiology in most cases.

•It is difficult to determine whether detection of multiple potential pathogens, represents true mixed infection, or whether infection by one organism encourages a harmless colonizer to become pathogenic.

•Blood culture has poor sensitivity for pneumonia etiology, but *Staphylococcus aureus* and Gram negative rods (neither targeted by current vaccines) are important pathogens.

Salient strengths

•Largest single-centre study of childhood pneumonia etiology

•Recruitment of community and hospital cases.

•Standard case definitions of pneumonia and pneumonia severity.

•Standard reporting protocol for chest radiography.

•Sample processing and testing were done in accredited laboratories in India.

Salient limitations

•No tests were done to confirm antibiotic activity in serum; hence results could not be separately analyzed in children with and without prior antibiotic therapy.

•Multiplex PCR could be undertaken in only a subgroup of children representing the whole cohort (on account of financial constraints).

•Serotyping of bacteria (especially *S. pneumoniae*) could not be undertaken due to financial constraints.

•Broncho-alveolar lavage was not performed at presentation (in accordance with the institutional protocol); further doing it in intubated children creates the risk of detecting hospital acquired colonization/infection.

•Lung aspirates were not performed.

•PCR testing was qualitative, and not quantitative (although the limits of detection for each organism were pre-specified).

•Analysis of results, by chest radiograph findings is pending.

the same limited set of organisms are identified from diverse specimens and using various processing methods. This raises the question whether microbes alone (singly or in combination) can be held responsible for pneumonia. It is of course well known that environmental factors such as nutritional status, living conditions, gestational age, breastfeeding patterns *etc*., influence the occurrence, course and outcome of pneumonia in individual cases and the community. However, it is also possible that host responses to colonization, and/or infection could account for individual variations in the course and outcome. In a representative subgroup of the CAPES cohort, we were able to evaluate a panel of 21 cytokines/ chemokines

with the aim of identifying signatures that could serve as potential biomarkers of pneumonia severity [73]. This is probably the largest panel of potential biomarkers studied in a single cohort. There were significant differences in the levels of 5 of the 21 chemokines between children with severe *vs*. non-severe pneumonia, at the time of presentation. Likewise, children who died showed significant differences from survivors in the levels of four chemokines. However, none of these could act as a reliable biomarker to predict either severity or outcome. We are planning additional studies when funding is available.

Pneumonia Etiology Research: What is the Way Forward?

Over the past few decades, pneumonia etiology research has witnessed a shift from studies that focused on specificity, in terms of definition of pneumonia (usually radiological evidence), specimen collection (generally lung aspiration in antibiotic naïve children), and processing (culture for bacteria and viruses); to the current focus on sensitivity (evidenced by a liberal clinical definition of pneumonia, surrogate specimens in the presence of prior antibiotic use, and molecular methods) thereby compromising specificity. This has thrown up a lot of data but the key issue of pin-pointing the etiology in individual cases is still elusive. Paradoxically a statement made 40 y ago, that "a wide variety of viruses and bacteria… are associated with respiratory tract infections, and no specific etiologic agent has been found in a significant proportion of patients" [74], appears true even today, but for entirely different reasons.

Considering this,what kind of pneumonia etiology studies should be undertaken? Data available so far suggest that any new pneumonia etiology study, should attempt to rectify (rather than replicate) the limitations described. Experts such as Shann insist that etiology in childhood pneumonia can be confirmed only through lung aspirate analysis in children who are antibiotic naïve [75]. However, personal experience confirms that it is not easy to convince research colleagues, ethics boards, and funding agencies (and not just children/ families) for lung aspiration, given the small but definite risk of events such as pneumothorax, necessitating enhanced monitoring [17, 24]. In such a situation, bronchoscopic bronchoalveolar lavage specimens obtained at presentation in hospitalized children could be the ideal material. Bronchoscopy has the advantage of targeting the specific area(s) in the lung(s) that can be identified through digital radiography. In the hands of experienced clinicians, it is a safe procedure and can yield a wealth of data, not only for pathogen identification, but also host responses (through analysis of BAL cytology, cytokines, and chemokine fractions). Laboratory processing should continue to use traditional methods (especially bacterial culture) in addition to modern techniques. Naturally, a reasonably large sample size of cases would be required, making such a research study time-consuming and expensive.

Etiology of Childhood Pneumonia: What Do We Need to Know?

On the other hand, it can be argued that the wealth of data available from excellent studies across the globe, makes it clear that determination of microbial etiology in individual cases of pneumonia is a complex (perhaps impossible) task. Even the resource-intensive PERCH study has not so far been able to address the issue, despite complex statistical wizardry. It appears that we can at best identify patterns of organism distributions in communities that are epidemiologically heterogeneous in many respects. Therefore, it is doubtful if a whole country, or even a region within the country, will behave as a single epidemiologic unit. More likely, there will be wide variations even within defined geographic/ political boundaries. Against this backdrop, it is pertinent to wonder whether we really need to invest more (time, money, manpower and resources) to know the precise microbial etiology in individual pneumonia cases on a routine basis. Would it be enough if we could predict reasonably well the likelihood of bacterial (*vs*. viral or atypical infection) infection without actually identifying the organism in each case (exception being tuberculosis); and identify factors that predict adverse outcome (complications, non-response to therapy and mortality)? At a deeper level, why do micro-organism(s) with pathogenic potential create disease (with varying severities) in some children and leave others unaffected? In other words, should the emphasis of pneumonia etiology research shift from efforts to merely identify pathogens, to a more holistic approach that embraces the epidemiological triad comprising agent factors (*i.e.*, what causes an organism to become pathogenic in individual children), host factors (both at the macro level such as putative risk factors as well as micro level such as individual immune/inflammatory responses) and environmental factors (again at the macro and micro levels); and the complex interplay among these?

Compliance with Ethical Standards

Conflict of Interest None.

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References

1. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385:430–40.

2. Berman S. Epidemiology of acute respiratory infections in children of developing countries. Rev Infect Dis. 1991;13:S454–62.
3. Adegbola RA. Childhood pneumonia as a global health priority and the strategic interest of the Bill & Melinda Gates Foundation. Clin Infect Dis. 2012;54:S89–92.
4. Global Burden of Disease Pediatrics Collaboration. Global and national burden of diseases and injuries among children and adolescents between 1990 and 2013: findings from the global burden of disease 2013 study. JAMA Pediatr. 2016;170:267–87.
5. Rudan I, O'Brien KL, Nair H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health. 2013;3:010401.
6. Jackson S, Mathews KH, Pulanic D, et al. Risk factors for severe acute lower respiratory infections in children: a systematic review and meta-analysis. Croat Med J. 2013;54:110–21.
7. Chan JY, Stern DA, Guerra S, Wright AL, Morgan WJ, Martinez FD. Pneumonia in childhood and impaired lung function in adults: a longitudinal study. Pediatrics. 2015;135:607–16.
8. Cecil R, Baldwin H, Larsen N. Clinical and bacteriologic study of two thousand typed cases of lobar pneumonia. Trans Assoc Am Phys. 1926;41:208–23.
9. Mimica I, Donoso E, Howard JE, Ledermann GW. Lung puncture in the etiological diagnosis of pneumonia. Am J Dis Child. 1971;122:278–82.
10. Shann F, Gratten M, Germer S, Linnemann V, Hazlett D, Payne R. Aetiology of pneumonia in children in Goroka hospital, Papua New Guinea. Lancet. 1984;2:537–41.
11. Escobar JA, Dover AS, Dueñas A, et al. Etiology of respiratory tract infections in children in Cali, Colombia. Pediatrics. 1976;57:123–30.
12. Kalra SK, Sasidharan T, Vatwani V, Sarkar P. Lung puncture: a diagnostic aid in childhood pneumonia. Indian Pediatr. 1981;18:727–30.
13. Patwari AK, Bisht S, Srinivasan A, Deb M, Chattopadhya D. Aetiology of pneumonia in hospitalized children. J Trop Pediatr. 1996;42:15–20.
14. Prakash J, Agarval DK, Agarval KN, Kulati AK. Etiologic diagnosis of pneumonia in under-five children. Indian Pediatr. 1996;33:329–31.
15. Tewari AD, Sen R, Mittal KK, Saini R, Sen J. Lung puncture aspiration in the diagnosis of acute pneumonias. Indian Pediatr. 1991;28:647–52.
16. Misra S, Bhakoo ON, Ayyagiri A, Katariya S. Clinical and bacteriologicalprofile of neonatal pneumonia. Indian J Med Res. 1991;93:366–70.
17. Vuori-Holopainen E, Salo E, Saxen H, et al. Etiological diagnosis of childhood pneumonia by use of transthoracic needle aspiration and modern microbiological methods. Clin Infect Dis. 2002;34:583–90.
18. Shann F. Bacterial pneumonia: commoner than perceived. Lancet. 2001;357:2070–2.
19. Shann F. Etiology of severe pneumonia in children in developing countries. Pediatr Infect Dis. 1986;5:247–52.
20. Dover AS, Escobar JA, Duenas AL, Leal EC. Pneumonia associated with measles. JAMA. 1975;234:612–4.
21. Gratten M, Montgomery J. The bacteriology of acute pneumonia and meningitis in children in Papua New Guinea: assumptions, facts and technical strategies. PNG Med J. 1991;34:185–98.
22. Hughes JR, Sinha DP, Cooper MR, Shah KV, Bose SK. Lung tap in childhood: bacteria, viruses, and mycoplasma in acute lower respiratory tract infections. Pediatrics. 1969;44:477–85.
23. Forgie IM, O'Neill KP, Lloyd-Evans N, et al. Etiology of acute lower respiratory tract infections in Gambian children: II. Acute lower respiratory tract infection in children ages one to nine years presenting at the hospital. Pediatr Infect Dis J. 1991;10:42–7.
24. Vuori-Holopainen E, Peltola H. Reappraisal of lung tap: review of an old method for better etiologic diagnosis of childhood pneumonia. Clin Infect Dis. 2001;32:715–26.
25. Carrol ED, Mankhambo LA, Guiver M, Banda DL, The IPD Study Group, et al. PCR improves diagnostic yield from lung aspiration in malawianchildren with radiologically confirmed pneumonia. PLoS ONE. 2011;6:e21042.
26. Howie SR, Morris GA, Tokarz R, et al. Etiology of severe childhood pneumonia in the Gambia, West Africa, determined by conventional and molecular microbiological analyses of lung and pleural aspirate samples. Clin Infect Dis. 2014;59:682–5.
27. World Health Organization. Technical bases for the WHO recommendations on the management of pneumonia in children at first-level health facilities: Programme for the control of acute respiratory infections. Geneva: World Health Organization; 1991.
28. Selwyn BJ. The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. Coordinated data group of BOSTID researchers. Rev Infect Dis. 1990;12:S870–88.
29. Kumar KL, Ashok V, Ganaie F, Ramesh AC. Nasopharyngeal carriage, antibiogram & serotype distribution of Streptococcus pneumoniae among healthy under five children. Indian J Med Res. 2014;140:216–20.
30. Tenenbaum T, Franz A, Neuhausen N, et al. Clinical characteristics of children with lower respiratory tract infections are dependent on the carriage of specific pathogens in the nasopharynx. Eur J Clin Microbiol Infect Dis. 2012;31:3173–82.
31. van den Bergh MR, Biesbroek G, Rossen JW, et al. Associations between pathogens in the upper respiratory tract of young children: interplay between viruses and bacteria. PLoS One. 2012;7:e47711.
32. Spuesens EB, Fraaij PL, Visser EG, et al. Carriage of mycoplasma pneumoniae in the upper respiratory tract of symptomatic and asymptomatic children: an observational study. PLoS Med. 2013;10:e1001444.
33. Rhedin S, Lindstrand A. Rotzén–Östlund M, et al. clinical utility of PCR for common viruses in acute respiratory illness. Pediatrics. 2014;133:e538–45.
34. Lambert SB, Allen KM, Druce JD, et al. Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy preschool–aged children using parent–collected specimens. Pediatrics. 2007;120:e929–37.
35. García-García ML, Calvo C, Pozo F, et al. Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. Pediatr Infect Dis J. 2008;27:358–60.
36. Shi T, McLean K, Campbell H, et al. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: a systematic review and meta-analysis. J Glob Health. 2015;5:010408.
37. Zar HJ, Andronikou S, Nicol MP. Advances in the diagnosis of pneumonia in children. BMJ. 2017;358:j2739.
38. 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:S93–101.
39. Scott JA, Wonodi C, Moïsi JC, et al. The definition of pneumonia, the assessment of severity, and clinical standardization in the pneumonia etiology research for child health study. Clin Infect Dis. 2012;54:S109–16.
40. 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:S228–37.
41. Grant LR, Hammitt LL, Murdoch DR, O'Brien KL, Scott JA. Procedures for collection of induced sputum specimens from children. Clin Infect Dis. 2012;54:S140–5.
42. Driscoll AJ, Karron RA, Morpeth SC, et al. Standardization of laboratory methods for the PERCH study. Clin Infect Dis. 2017;64:S245–52.

43. Murdoch DR, Morpeth SC, Hammitt LL, et al. Microscopic analysis and quality assessment of induced sputum from children with pneumonia in the PERCH study. Clin Infect Dis. 2017;64:S271–9.
44. 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:S253–61.
45. 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:S368–77.
46. Driscoll AJ, Bhat N, Karron RA, O'Brien KL, Murdoch DR. Disk diffusion bioassays for the detection of antibiotic activity in body fluids: applications for the pneumonia etiology research for child health project. Clin Infect Dis. 2012;54:S159–64.
47. Baggett HC, Watson NL, Deloria Knoll M, et al. 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. Clin Infect Dis. 2017;64:S317–27.
48. Park DE, Baggett HC, Howie SRC, Shi Q, Watson NL, Brooks WA. Colonization density of the upper respiratory tract as a predictor of pneumonia—Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, and Pneumocystis jirovecii. Clin Infect Dis. 2017;64:S328–36.
49. Feikin DR, Fu W, Park DE, et al. Is higher viral load in the upper respiratory tract associated with severe pneumonia? Findings from the PERCH study. Clin Infect Dis. 2017;64:S337–46.
50. Hammitt LL, Feikin DR, Scott JAG, et al. Addressing the analytic challenges of cross-sectional pediatric pneumonia etiology data. Clin Infect Dis. 2017;64:S197–204.
51. Watson NL, Prosperi C, Driscoll AJ, et al. Data management and data quality in PERCH, a large international case-control study of severe childhood pneumonia. Clin Infect Dis. 2017;64:S238–44.
52. Deloria Knoll M, Fu W, Shi Q, et al. Bayesian estimation of pneumonia etiology: epidemiologic considerations and applications to the pneumonia etiology research for child health study. Clin Infect Dis. 2017;64:S213–27.
53. Wu Z, Deloria-Knoll M, Zeger SL. Nested partially latent class models for dependent binary data; estimating disease etiology. Biostatistics. 2017;18:200–13.
54. Higdon MM, Hammitt LL, Deloria Knoll M, et al. Should controls with respiratory symptoms be excluded from case-control studies of pneumonia etiology? Reflections from the PERCH study. Clin Infect Dis. 2017;64:S205–12.
55. Morpeth SC, Knoll MD, Scott JAG, Park DE, Watson NL, Baggett HC. Detection of pneumococcal DNA in blood by polymerase chain reaction for diagnosing pneumococcal pneumonia in young children from low- and middle-income countries. Clin Infect Dis. 2017;64:S347–56.
56. Deloria Knoll M, Morpeth SC, Scott JAG, Watson NL, Park DE, Baggett HC. Evaluation of pneumococcal load in blood by polymerase chain reaction for the diagnosis of pneumococcal pneumonia in young children in the PERCH study. Clin Infect Dis. 2017;64:S357–67.
57. Thea DM, Seidenberg P, Park DE, et al. Limited utility of polymerase chain reaction in induced sputum specimens for determining the causes of childhood pneumonia in resource-poor settings: findings from the pneumonia etiology research for child health (PERCH) study. Clin Infect Dis. 2017;64:S289–300.
58. Feikin DR, Fu W, Park DE, Shi Q, Higdon MM, Baggett HC. Is higher viral load in the upper respiratory tract associated with severe pneumonia? Findings from the PERCH study. Clin Infect Dis. 2017;64:S337–46.
59. Barger-Kamate B, Deloria Knoll M, Kagucia EW, et al. Pertussis-associated pneumonia in infants and children from low- and middle-income countries participating in the PERCH study. Clin Infect Dis. 2016;63:S187–96.
60. Fancourt N, Deloria Knoll M, Baggett HC, et al. Chest radiograph findings in childhood pneumonia cases from the multisite PERCH study. Clin Infect Dis. 2017;64:S262–70.
61. Higdon MM, Le T, O'Brien KL, et al. Association of C-reactive protein with bacterial and respiratory syncytial virus-associated pneumonia among children aged <5 years in the PERCH study. Clin Infect Dis. 2017;64:S378–86.
62. Klugman KP, Rodgers GL. PERCH in perspective: what can it teach us about pneumonia etiology in children? Clin Infect Dis. 2017;64:S185–7.
63. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5mortality in 2000–15: an updated systematic analysis with implications for the sustainable development goals. Lancet. 2016;388:3027–35.
64. Pneumonia: the forgotten killer of children. The United Nations Children's Fund (UNICEF)/World Health Organization (WHO), 2006.
65. World Health Statistics. Geneva: WHO; 2007. Available at: http://www.who.int/whosis/whostat2007.pdf. Accessed on 24 Aug 2010.
66. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull WHO. 2008;86:408–16.
67. Mathew JL, Patwari AK, Gupta P, et al. Acute respiratory infection and pneumonia in India: a systematic review of literature for advocacy and action: UNICEF–PHFI series on newborn and child health, India. Indian Pediatr. 2011;48:191–218.
68. Levine OS, Cherian T. Pneumococcal vaccination for Indian children. Indian Pediatr. 2007;44:491–6.
69. Mathew JL. Universal pneumococcal vaccination in India. Indian Pediatr. 2008;45:160–1.
70. Mathew JL. Pneumococcal vaccination in developing countries: where does science end and commerce begin? Vaccine. 2009;27:4247–51.
71. Mathew JL, Singhi S. Pneumococcal disease in India: the dilemma continues. Indian J Med Res. 2014;140:165–6.
72. Mathew JL, Singhi S, Ray P, et al. Etiology of community acquired pneumonia among children in India: prospective, cohort study. J Glob Health. 2015;5:050418.
73. Saghafian-Hedengren S, Mathew JL, Hagel E, et al. Assessment of cytokine and chemokine signatures as potential biomarkers of childhood community-acquired pneumonia severity: a nested cohort study in India. Pediatr Infect Dis J. 2017;36:102–8.
74. Walsh JA, Warren KS. Selective primary health care: an interim strategy for disease control in developing countries. N Engl J Med. 1979;301:967–74.
75. Shann F. Pneumonia in children in developing countries. In: Curtis N, Finn A, Pollard A, editors. Hot Topics in Infection and Immunity in Children VII. Available at: https://link.springer.com/chapter/10.1007%2F978-1-4419-7185-2_5. Accessed on 10th Aug 2017.