Acute lower respiratory infections are among the most common problems in children worldwide. In developing countries, they constitute the most common cause of mortality in children aged less than five years, leading to an estimated 1.9 million deaths annually, most of which are thought to be due to pneumonia (1). Despite the frequency and severity of this condition, the burden and epidemiology of disease have been difficult to describe with sufficient accuracy because of the lack of an adequate definition of pneumonia for epidemiological purposes and of the lack of sensitive and specific tests to establish bacterial aetiology. Some of these problems, which still remain unresolved, are evident in the study reported by Nacul et al. in this issue of the Journal (2).

Defining pneumonia

Conventionally, pneumonia is clinically diagnosed based on a combination of clinical signs and symptoms and confirmed by findings of chest radiography. Simple clinical signs, such as tachypnoea and lower chest in-drawings, have been shown to be reliable signs of pneumonia (3). While these signs are appropriate for case management in primary healthcare programmes where high sensitivity is important, they are not sufficiently specific to accurately estimate the burden of disease and give low efficacy estimates for interventions that are aimed specifically at preventing bacterial pneumonia.

Auscultatory findings, such as crepitations and bronchial breath sounds, have been conventionally used by physicians in the clinical diagnosis of pneumonia. However, these findings are subjective and highly dependent on the skill of the observer. As a result, the diagnosis of pneumonia using auscultatory findings has been difficult to standardize, and inter-observer agreement regarding the presence and absence of these signs has been poor (4).

Radiography is considered to be the gold standard for the diagnosis of pneumonia. However, different studies have varied in the radiological findings used to define pneumonia. While some studies have classified only cases with alveolar consolidation as pneumonia, others have considered the presence of any pulmonary parenchymal infiltrates as constituting pneumonia. Furthermore, there is relatively poor agreement even between radiologists on the presence or absence of infiltrates in paediatric chest radiographs. This variability persists even when standard definitions and reporting forms are used. Analysis of the readings of four trained paediatric radiologists who read chest radiographs from two different studies using common definitions and reporting forms showed that while there was reasonable agreement for alveolar consolidation, agreement was low for many other findings (Weber M. Personal communication, 2004). Despite these limitations, radiography still remains the best available tool to diagnose pneumonia. Since bacterial pneumonia is thought to account for the majority of pneumonia-related deaths and since current interventions, such as vaccines and case management, focus on bacterial disease, the World Health Organization (WHO) initiated a process to improve the inter-observer agreement for categorizing pneumonia with alveolar consolidation, the finding that is most representative of this condition. A simplified definition, coupled with a training programme, and the system of two independent readings with an arbitration reading of those images where the two primary readings were discordant, was developed and tested (5). The use of the definitions and methods resulted in reasonably good agreement in categorizing pneumonia with alveolar consolidation and has been used in Haemophilus influenzae type b (Hib) and pneumococcal vaccine trials to determine the effect of these vaccines on pneumonia (6). The
same definitions are also currently being used for documenting the burden of pneumonia in several studies in developing countries. Available data suggest that, while the radiological definitions of WHO are enriched for the diagnosis of bacterial pneumonia, they are still not optimally sensitive and specific (7-12).

**Laboratory determination of bacterial aetiology**

Although certain radiological patterns are more common with bacterial pneumonia, similar patterns may be seen with non-bacterial infections. On the other hand, bacterial pneumonia may not always produce the classical radiological patterns. The evolution of the characteristic radiological changes may lag behind the onset and progression of clinical disease with changes not visible early in the course of disease and persisting for a period after clinical improvement. Judging by the efficacy estimates of Hib and pneumococcal vaccines against pneumonia, it appears that the radiological definitions of WHO may perform differently under different conditions, presumably as a result of differences in methods for case ascertainment and the timing of chest radiography in the course of illness (7-12). Thus, there is a need for other measures which identify bacterial pneumonia with greater sensitivity and specificity either by themselves or as adjuncts to radiography. A number of laboratory tests, including non-specific markers of bacterial disease or tests to identify specific bacterial pathogens, have been evaluated. However, the evaluation of the sensitivity and specificity of such tests has been difficult because of the lack of an optimal 'gold standard' for diagnosing bacterial pneumonia. Co-infection with bacterial and viral pathogens are common in developing countries, further complicating the matter (13,14).

**Non-specific laboratory tests**

A number of laboratory tests are known to be abnormal more often with bacterial infection and have been evaluated for the diagnosis of bacterial pneumonia. These include: (i) total leucocyte and granulocyte counts; (ii) erythrocyte sedimentation rate (ESR); (iii) serum C-reactive protein (CRP); and (4) serum procalcitonin (PCT).

Studies have shown that there are no significant differences in the leucocyte and granulocyte counts between those with alveolar and interstitial pneumonia, or between those classified as having pneumonia of bacterial, viral, mixed or unknown aetiologies (15). Greater proportions of those diagnosed to have bacterial pneumonia have a white blood cell count greater than 10,000/cu.mm or a granulocyte count greater than 5,000/cu.mm but with considerable overlap. While the specificity of leucocyte and granulocyte counts in diagnosing bacterial pneumonia is reasonably high depending on the break-point chosen, the sensitivity is low (16).

Like leucocyte counts, the ESR is higher in children who have pneumonia with alveolar consolidation compared to those with interstitial pneumonia (15). However, there is considerable overlap in values between the two groups. Moreover, the timing of obtaining the sample in relation to onset of illness affects the ESR (17). This makes it difficult to define a cut-off that would make it an useful adjunct to radiological criteria for diagnosing bacterial pneumonia.

CRP has been shown to be significantly higher in patients with alveolar pneumonia compared to those with interstitial pneumonia (15). It has also been shown to be significantly higher in those with bacterial pneumonia compared to those with non-bacterial pneumonia. As with the other tests, there is a degree of overlap. CRP is the highest on the second day of illness with levels dropping after that making the timing of specimen collection an important factor. When used in conjunction with radiological findings, a CRP level of >80 mg/L had a sensitivity and specificity of 0.46 and 0.78 respectively in differentiating between bacterial and non-bacterial community-acquired pneumonia (18).

Circulating levels of calcitonin precursors are known to increase several thousand folds with microbial infections and various forms of severe systemic inflammation (19). Serum procalcitonin has been shown to be helpful in predicting the presence of serious bacterial infection in patients with fever without localizing signs. In pneumonia, there is a significant difference between the levels of procalcitonin in those with 'bacterial' and 'viral' pneumonia, although there was overlap in the lower ranges of values. Using a cut-off value of 1 ng/L, serum procalcitonin demonstrated a sensitivity and specificity of 0.86 and 0.88 respectively in one study (20). However, malaria may substantially increase serum procalcitonin levels. This may limit the use of the test in malaria-endemic populations given the overlap in clinical manifestations of pneumonia and malaria (21). The use of serum PCT for the diagnosis of pneumonia has not yet been adequately evaluated in developing countries.
Laboratory tests to identify specific bacterial pathogens of pneumonia

The use of conventional bacteriological culture to establish the aetiology of pneumonia has been limited because of the lack of an optimal specimen to culture. Blood culture is specific but lacks sensitivity because, bacteraemia is only observed in a few cases of bacterial pneumonia. Furthermore, low pathogen concentrations and sample volumes, prior antibiotic use, and autolysis may render cultures negative even in those with bacteraemia. Culture of sputum and nasopharyngeal swabs are non-specific in that they do not differentiate between lower respiratory infection and carriage in the upper respiratory tract, especially in children in whom asymptomatic carriage is very common. Lung aspirates are considered to provide the best sample for culture but is an invasive procedure that is limited to those who have a large area of consolidation that is accessible to percutaneous needle aspiration. In one study in Kenya, 30% of lung aspirate specimens did not contain human DNA, suggesting the aspirate contained only the injected saline and air (22). Thus, an improperly-collected lung aspirate specimen may not be very much superior to blood culture.

A number of non-culture laboratory methods, including antigen detection, polymerase chain reaction (PCR), and serological tests for antibody, have been evaluated for the diagnosis of bacterial pneumonia. While initial evaluation of these tests was promising, in most instances, large studies with appropriate controls have failed to confirm the utility of these assays.

**Urinary antigen tests:** The use of antigen detection tests to see pneumococcal capsular polysaccharide in the urine has been evaluated in several studies. Children with nasopharyngeal carriage of pneumococcus may excrete capsular polysaccharide in the urine, and hence these tests have not been found to be useful in populations with high rates of carriage (23,24).

**Polymerase chain reaction:** Several PCR assays using different target genes of pneumococcus, including pneumolysin, autolysin, DNA polymerase 1, PBP 2B, and PsaA, have been evaluated. Sensitivities of the tests have varied between 33% and 100% and specificity between 55% and 100%. Diverse patient characteristics, type of specimens, timing of sample acquisition in relation to antibiotic use, choice of primers and techniques, management of potential PCR inhibitors, conditions of storage of specimens, and in-vitro sensitivity and specificity are factors that may account for the discrepancies between the test results. A study from Israel showed that a PCR assay on sera with pneumolysin as target was positive in all patients with pneumococcal bacteraemia and meningitis, and 38% of patients with lobar or segmental pneumonia. However, the test also gave a positive test in 17% of 202 healthy controls. In healthy controls, the test was more likely to be positive in those with nasopharyngeal carriage of pneumococcus (25). In another study using whole blood, plasma, or buffy coat with pneumolysin as the target, buffy coat was the most sensitive in identifying blood or pleural fluid culture-positive cases (11/12, 92%) (26). However, 12 of 40 healthy controls also had a positive buffy-coat PCR. Unlike in the previous study, no association was found between positive PCR and nasopharyngeal carriage in the control group.

**Antibody assays:** Antibodies to a number of pneumococcal antigens or antigen-antibody complexes have been evaluated as potential tests for the diagnosis of pneumococcal pneumonia. These include antibody to pneumolysin, C-polysaccharide, capsular polysaccharide, and PsaA (27,28). The first three have been shown to have low sensitivity (28). A two-fold or greater increase in PsaA antibody in paired sera has demonstrated higher sensitivity and specificity, but the timing of collection of the sera during convalescence may be critical in ensuring optimal sensitivity and specificity (27).

**Vaccine-probe approach**

Because of the limitations of the existing laboratory methods in determining the aetiology of pneumonia, data from trials on vaccine efficacy have been used for determining the burden of bacterial pneumonia. This approach, termed as the ‘vaccine-probe approach,’ assumes that the vaccine has almost 100% efficacy and estimates the burden of pathogen-specific pneumonia on the basis of reduction of disease incidence in the vaccinated group compared to the control group. This approach was first used for the determination of the burden of pneumonia due to Hib (7,8). Although this approach indicated that the burden of Hib-associated pneumonia was far greater than that estimated by the use of conventional laboratory methods, the estimation of burden is subject to errors. The burden estimate is often described as the proportion of disease prevented by the vaccine, i.e. the estimated vaccine efficacy against pneumonia. Since the efficacy estimate is highly dependent on the sensitivity and speci-
ficity of the outcome measure, these estimates may be misleading for pneumonia. Furthermore, the herd effect of the vaccine is not taken into account, leading to an underestimation of the burden. With pneumococcal pneumonia, the multiplicity of serotypes causing pneumonia, only some of which are represented in the vaccine, the variation in efficacy of the different serotypes in the vaccine and the potential for replacement disease are additional factors that make burden estimation complicated. Nevertheless, the use of this approach remains the only method to estimate the burden of vaccine-preventable pneumonia.

Despite the limitations of the current tools to accurately diagnose bacterial pneumonia, current evidence clearly indicates that this is a major cause of childhood morbidity and mortality. Better methods to accurately measure the burden and to describe the responsible pathogens are required to develop and evaluate suitable interventions to reduce mortality.

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