EDITORIAL COMMENTARY

Expanding the Diagnosis of Pediatric Bacteremic Pneumococcal Pneumonia from Blood Cultures to Molecular Methods: Advantages and Caveats

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(See the article by Resti et al, on pages 1042–1049.)

Pneumonia is among the leading causes of childhood morbidity and mortality worldwide. Multiple microbial agents can be responsible for this infection, dominated by *Streptococcus pneumoniae* (pneumococcus), which is believed to be the main cause of severe pneumonia in the developing world. However, the etiologic diagnosis remains challenging, and novel approaches are needed to identify the pathogens that cause pneumonia, to guide treatment and prevention policies. Blood cultures provide unambiguous evidence of the etiology of the infection and allow antimicrobial susceptibility testing with the optimization of antibiotic therapy. The availability of bacterial isolates also affords an opportunity for their epidemiologic characterization, and in the case of pneumococci this includes serotyping, which is critical for guiding vaccination policies. However, the sensitivity of blood cultures in community-acquired pneumonia is low, and this is aggravated when antibiotics have been administered previously. Moreover, positive results are available only 24–36 h after blood collection, a delay that will be reflected in patient management.

Work on alternative diagnostic methods to rapidly establish the pneumococcal cause of pneumonia has been ongoing. Although it may be unrealistic to expect that new methods replace blood cultures, at least for years to come, they may be important complementary tools to improve patient care. A step forward occurred with the availability of a rapid immunochromatographic test that detects soluble polysaccharide antigen in urine, which produces positive results in 70%–87% of adult patients with pneumonia [1]. However, this test is of little use in pediatric patients because it does not distinguish children with pneumococcal pneumonia from asymptomatic carriers [2, 3].

Molecular methods that could reliably detect *S. pneumoniae* in blood would allow a more rapid identification of bacteremic pneumonia, greatly enhancing diagnosis and the optimization of antimicrobial treatment of patients. The promise of these methods seems to have not quite been realized yet because a recent systematic review and meta-analysis of the published literature concluded that "currently available methods for PCR [polymerase chain reaction] with blood samples for the diagnosis of IPD [invasive pneumococcal disease] lack the sensitivity and specificity necessary for clinical practice" [1, p 489]. In this context, the study by Resti et al [4], reported in this issue of *Clinical Infectious Diseases,* provides new data on how we can improve the diagnosis while simultaneously allowing the documentation of the serotypes causing community-acquired bacteremic pneumococcal pneumonia in children.

The authors compare their method with blood cultures, the gold standard criterion for the diagnosis of bacteremic pneumococcal pneumonia. Bacteria were generally assumed to be present in higher concentrations in the blood of children than in adults, but Kellogg et al [5] showed that 60% of children from birth to age 15 years had low-level bacteremia (≤10 colony-forming units per mL) and 23% had extremely low pathogen concentrations (≤1 colony-forming units per mL). The volume of cultured blood is the most important variable influencing the yield of this method. Culture positivity increases with the amount of blood, and when the volume is inadequate, negative blood cultures can be misleading in falsely excluding bacteremia [6, 7]. The recommended total volume of blood suggested for reliable identification of bacteremia from in-

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fants and children who weigh ≤1 to >36.3 kg (aged 0–15 years) varies from 2 to 40–60 mL [5, 8]. However, these values are not always met in practice, and some authors report that more than one-half of the blood cultures from children contained an inadequately small volume of blood, precluding the detection of even high-level bacteremia [6].

Resti et al included children aged 0–16 years in their study, taking 4–6 mL of blood (up to 3 sets) for culture. Although the lower value in the range is appropriate for newborns, the higher value would only be considered adequate for children up to 12.7 kg. Because the median age was >4 years, this suggests that for a large fraction of the population the cultured blood volume was suboptimal. As a consequence, the ability of the gold standard criterion standard to detect bacteremia may have been compromised, inflating the comparative efficiency of the molecular method. In addition, whole-blood samples for real-time polymerase chain reaction (RT-PCR) were obtained from all patients, but clinicians were allowed to choose when to also request blood cultures. As a result, 753 patients were tested by RT-PCR, but only 292 were tested by both RT-PCR and culture, and the authors indicate that blood cultures were performed more frequently in patients with more severe pneumonia. Considering only a fraction of the patients may have focused on specific groups of patients, which may not reflect the global population. Another point of debate is the opinion expressed by the authors that this selection bias favors culture sensitivity because severe pneumonia is not necessarily associated with higher rates of positive cultures for *S. pneumoniae* [9]. Furthermore, the data presented regarding pneumococcal DNA quantitative analysis did not find any difference between complicated and noncomplicated pneumonia, suggesting that in this study the bacterial load would be the same in both conditions, making it equally likely to obtain a positive culture from patients in both groups.

A critical point in any molecular method is its specificity, and the study authors focus their discussion on the specificity of the targets, primers, and DNA probes used to identify *S. pneumoniae*. This is always an important point but more so in the case of *S. pneumoniae,* which is notoriously difficult to distinguish from other closely related viridans streptococci. The 2 classic phenotypic tests used to identify *S. pneumoniae,* bile solubility and optochin susceptibility, have been shown to occasionally fail to provide correct identifications. Conversely, the presence of the genes encoding pneumolysin (*ply*) or the major autolysin (*lytA*), 2 important pneumococcal virulence factors, was also shown in other viridans streptococci [10] and in the recently described species *Streptococcus pseudopneumoniae* [11]. However, the pathogenicity of these strains has been questioned, and certainly they are much less frequent causes of infection than *S. pneumoniae,* minimizing these problems. In addition to the *lytA* gene, Resti et al amplified the *cpsA* gene, a conserved gene in the capsular polysaccharide biosynthesis locus, further strengthening the pneumococcal identification because neither of the alternatives is expected to be encapsulated.

The specificity of the assay is also conditioned by how well it correlates with a diagnosis of bacteremic pneumococcal pneumonia. The possibility of false-positive results due to asymptomatic carriage is always a potential confounder, particularly in the context of pediatric infections because of a high proportion of carriers among children. Almost all the studies reviewed by Avni et al [1] describe the presence of circulating pneumococcal DNA in individuals not suspected of having pneumococcal infection. However, most of these studies were performed in adults, in whom pneumococcal carriage is lower than in children. Two studies have specifically addressed the effect of carriage in children in the detection of circulating DNA, both targeting the *ply* gene [12, 13], but only the study by Dagan et al evaluated healthy control persons. A high rate of positivity (24%) was noted among healthy controls, indicating that circulating pneumococcal DNA could be the result of carriage alone. Although Resti et al refer to unpublished work by their own group that suggests that their method would not have detected colonization, more work is needed to clarify this point.

The study also offers the additional information of the serotypes that cause the infections, and this could be used to obtain clues to whether the results reflect colonization or infection due to the different serotype distribution in these 2 bacterial populations. The dominance of serotype 1 and its association with older children and complicated infections suggests that indeed the authors were detecting infection, because this serotype is rarely found in carriage and its association with empyemas has been demonstrated. In fact, the serotype distribution was, with a few exceptions, similar to the one we found recently in Portugal [14], further supporting the identification of clinically relevant isolates.

The work by Resti et al is another step toward the development of much needed improved diagnostic tools for pneumococcal infections. However, additional work is needed to establish the sensitivity and specificity of these techniques before their universal adoption, in particular in the case of pediatric infections. The costs associated with these technologies may also defer their use in developing countries, where the burden of pneumococcal infection is highest. It is hard to argue for the cost benefits of these methods, compared with culture methods, in the diagnosis of pneumococcal infection. Even considering the costs of traditional serotyping, the large investments in equipment and expertise necessary to perform these techniques will inevitably make them harder to implement. RT-PCR methods for the detection of pneumococcal bacteremia will probably be widely used outside specialized research laboratories, but there are still a number of issues that need clarification before their routine use in diagnosis.

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