The Remaining Challenges to Laboratory-based Surveillance of Invasive Pneumococcal Disease

Microbiologist's Perspective

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Limited information is available on the outline of invasive diseases caused by *Streptococcus pneumoniae* among the underprivileged children in India. Estimation of Invasive Pneumococcal Disease (IPD) and Pneumonia depends mainly on hospital-based clinical surveillance data. Data on serotype prevalence and antimicrobial resistance of IPD have been documented in some studies in India [1-4]. Due to national inaccessibility of a central surveillance system and appropriate laboratory facilities, the estimate of the disease burden is more intriguing.

The challenges in the laboratory isolation of *S. pneumoniae* include the scarcity of standard culture media and improper sampling [5]. In addition, its isolation by conventional culture methods is often hindered by prior antibiotic intake [6]. Isolation of fastidious *S. pneumoniae* requires suitable culture media enriched with sheep blood, but many laboratories use human blood – that has passed its date of expiry – which does not support the growth of pneumococci, leading to lower isolation rates. Minimum transportation time for culture sample has a direct effect on the yield of the pathogen in diseased conditions. Cerebro spinal fluid (CSF) and blood should be transported to the laboratory preferably within 1-2 hours of drawing the sample. Published reports state fewer than 10% of patients with clinical diagnosis of pneumonia yield a positive blood culture [5,7,8].

Presence of more than 1000 colony forming units/mL of the organism in a sample is required for a positive antigen detection test such as latex agglutination or counter immune electrophoresis; therefore such assays are of limited value in detecting pneumococci in culture negative samples [6]. In the current issue of *Indian Pediatrics*, Nisarga, *et al*. [9] found that 56 culture negative samples were positive for *S. pneumoniae* by polymerase chain reaction (PCR) using *lytA* as target gene. Serotyping information of these 56 PCR positive and culture negative samples would have provided additional information. It should be noted that various targets have been recommended for identification of pneumococci from cultures isolates, but these have not been tested directly on culture negative specimens [10]. Amplification of *lytA* gene has been evaluated and expected to be present in all the virulent isolates while few studies have demonstrated the presence of *lytA* and *ply* gene in members of the *S. mitis* group [10]. Therefore, the presence of these genes seems to be non-specific, and it cannot be presumed that the other members of the *S.mitis* group do not possess it or may have a facsimile of gene. Hence, *lyt A* and *ply*-based PCR does not appear to be clinically useful and should be used cautiously to eliminate false positives from blood and fluid samples [10,11]. Among the culture positive specimens, only 11 were tested by PCR; of these six showed the presence of *lytA* gene. It would have been desirable that all the culture positive clinical samples were tested for *lytA gene* in this study. Nucleic acid amplification tests such as PCR do not require viable bacteria for a positive assay, and are generally considered to be highly sensitive in comparison to culture. However, the presence of PCR inhibitors in clinical specimens can compromise the sensitivity [12], and might explain the reason for five negative PCR among culture positives in this study. In this study, 44 % isolates of *S. pneumoniae* (16/36) were resistant to trimethoprim/ sulfamethoxazole; this number is quite less compared to 87% as reported in a recent article from India [4]. It is to be mentioned here that a study at Christian Medical College and Hospital (CMC) in Vellore, India, has documented the presence of serotypes 3, 6A, and 19A during 2007-2011 [4]. Inclusion of molecular serotyping by sequential multiplex PCR in addition to conventional antisera-based Quellung reaction would afford a reliable and inexpensive way to serotype four isolates and 56 clinical samples which could not be typed in this study. It is far most important to know serotype of all *S. pneumonaie* strains for

implementation of pneumococcal conjugate vaccines, and to monitor circulating strains to consider vaccine effectiveness and subsequent substitution of serotypes.

This study over two years has rendered useful information on the incidence, clinical spectrum and serogroups of IPD in children. Pneumococcal meningitis still remains to be a serious problem globally in spite of potent antibiotic usage and adjunct therapy. This study can serve as a touchstone for successful surveillance in India since further amelioration in therapy might be futile.

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Invasive Pneumococcal Disease and India

Pediatrician's Perspective

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In this issue of *Indian Pediatrics*, Nisarga and colleagues have surveyed for the pneumococcal disease in Bangalore through the Pneumonet Programme [1], and have attempted to curtail the knowledge gap regarding the burden of invasive pneumococcal disease and distribution of pneumococcal serotypes in India. Many other surveillance programmes like SAPNA, INCLEN, IBIS and ASAP are also working to assess the invasive pneumococcal disease burden in India. Knowing disease burden is important to make decisions regarding the introduction of pneumococcal vaccine in National immunization program. Many multi-centric [2] and