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Original Article

Pan India distribution of pneumococcal serotypes (PIDOPS) causing invasive pneumococcal disease and pneumonia in children between 6 weeks and 5 years and their antimicrobial resistance – Phase I

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ABSTRACT

Analysis of the published pneumococcal surveillance reports reveals variability and lack of long-term studies from India. The variability relates to case definition, characterization, surveillance, and laboratory methods. In the backdrop of scarcity of data, a multisite surveillance network study, PIDOPS Phase I, was launched in the year 2013 at 7 institutional and 51 sentinel sites.

During the course of the study, novel molecular techniques were developed and standardized for rapid, accurate detection and typing. 1504 serum samples from IPD pediatric subjects were analyzed with automated blood culture and qmPCR. 108 isolates and 456 positive serum samples were serotyped by Quellung and PCRSeqTyping, respectively. The isolates were tested for their MIC and Multilocus Sequence Typing.

The strength of this study was the use of advanced techniques providing comprehensive surveillance data and development of pan India referral system before the introduction of pneumococcal conjugate vaccine in the national immunization program.

1. Introduction

Infectious diseases are responsible for millions of deaths worldwide and claim the lives of more than five million children under five each year, making their prevention key to meeting Millennium Development Goal 4 (MDG4).[1] Five countries where 44% of the world's children aged less than 5 years live (India, China, Pakistan, Bangladesh, Indonesia, and Nigeria) contribute more than half of the new pneumonia cases annually.[2] The data available on the causative organisms causing childhood pneumonia have identified *Streptococcus pneumonia*, *Haemophilus influenza*, and viruses, such as Respiratory Syncytial Virus (RSV), Influenza, Para-influenza, and Adenoviruses, as major pathogens. Diseases caused by *S. pneumonia* constitute a major public health problem in India.

Habib Farooqui et al. in their recent publication statistically estimated that in year 2010, 3.6 million (3.3–3.9 million) episodes of severe pneumonia and 0.35 million (0.3–0.4 million) pneumonia deaths (all-cause) occurred in children younger than 5 years in India. They estimated that annual severe pneumonia incidence varied greatly from one state to another. The top five contributors to India's pneumococcal pneumonia burden in terms of number of cases and deaths were Uttar Pradesh, Bihar, Madhya Pradesh, Rajasthan, and Jharkhand. Reports of excessive burden of infection, high prevalence of drug resistance, and IPD are documented in several other Indian studies.[3–6]

Out of 94 known serotypes of *S. pneumoniae*, relatively few of them are responsible for most serious disease.[7] Antibodies to the capsular polysaccharide confer protection against the disease; however, this protection is serotype specific. Thus the country's serotype specific data play an important role in directing the strategies in the control and prevention of *S. pneumonia* infections.

2. Rationale

Surveillance for invasive pneumococcal disease in children is needed for:

- Characterization of national and local trends.
- Establishing a baseline for monitoring IPD trends and seasonality.
- Monitoring impact of vaccines on disease.

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- Tracking emerging strains and serotype replacement.
- Tracing antimicrobial resistance and temporal changes in the prevalence of pneumococcal isolates.
- Reducing unnecessary use of antimicrobial agents.
- Informing future vaccine development
- Research and policy development and raising awareness among clinicians and the general public.[8]

Currently, there is a pressing need to know the most prevailing organisms, their serotypes, and disease burden in children to decide upon the suitability and choice of vaccine. Recognizing the requirement, PIDOPS PROJECT PHASE I was launched in the year 2013. The study initiated in 5 sites was expanded to include 2 additional sites and 51 sentinel centers. Infrastructure and laboratory capacities developed during the PNEUMONET study were integrated and newer facilities were added.

3. Ethical clearance

Ethical clearance was obtained from prime site and institutional centers. Written informed consent of the parent/guardian was taken before filling the CRF and sample collection.

4. Methodology

The study was a prospective, observational, multicenter activity. It consisted of screening and identifying children between the age groups of 6 weeks and 5 years with clinically suspected IPD or pneumonia meeting WHO/IMNCI clinical criteria or with radiographic evidence. Children with raised CBC, CRP, and procalcitonin were recruited for the study. Sample collection and processing were done as detailed in the line diagram. Collected serum samples were stored at −80 °C/−20 °C at the institutional sites and transferred to KIMS lab on dry ice in batches of 25 numbers. Pneumococcal isolates were subcultured on to blood agar plates and STGG transport media and transferred to the prime site within 24–48 h of isolation (Figs. 1 and 2).

Conventional culture, antibiogram, and serotyping of the isolates were performed according to CLSI and WHO guidelines.[9]

5. Molecular methods

5.1. Quantitative multiplex real-time PCR-

Validation and standardization of the methodology involved the use of 4 oligonucleotide primer-probe sets (pneumolysin [ply], autolysin [lytA], pneumococcal surface adhesion A [psaA], and Spn9802 [DNA fragment]) in a single-reaction mixture. The assay was validated with a panel of 43 *S. pneumoniae*, 29 non-pneumococcal isolates, 20 culture-positive, 26 culture-negative, and 30 spiked serum samples. Using *S. pneumoniae* ATCC 49619 strain, a standard curve was obtained and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous internal control. The lower limit of detection was equivalent to 4 genome copies/μl.[10]

5.2. PCRSeqTyping

Molecular identification and typing in culture-negative PCR-positive serum samples was performed by PCR amplification of the CPS region followed by the Sanger sequencing of the amplicon. 59 serotypes were identified accurately from 1st PCR and Sequencing. 32 homologous serotypes categorized into 10 groups were identified uniquely by 2nd PCR and sequencing.[11]

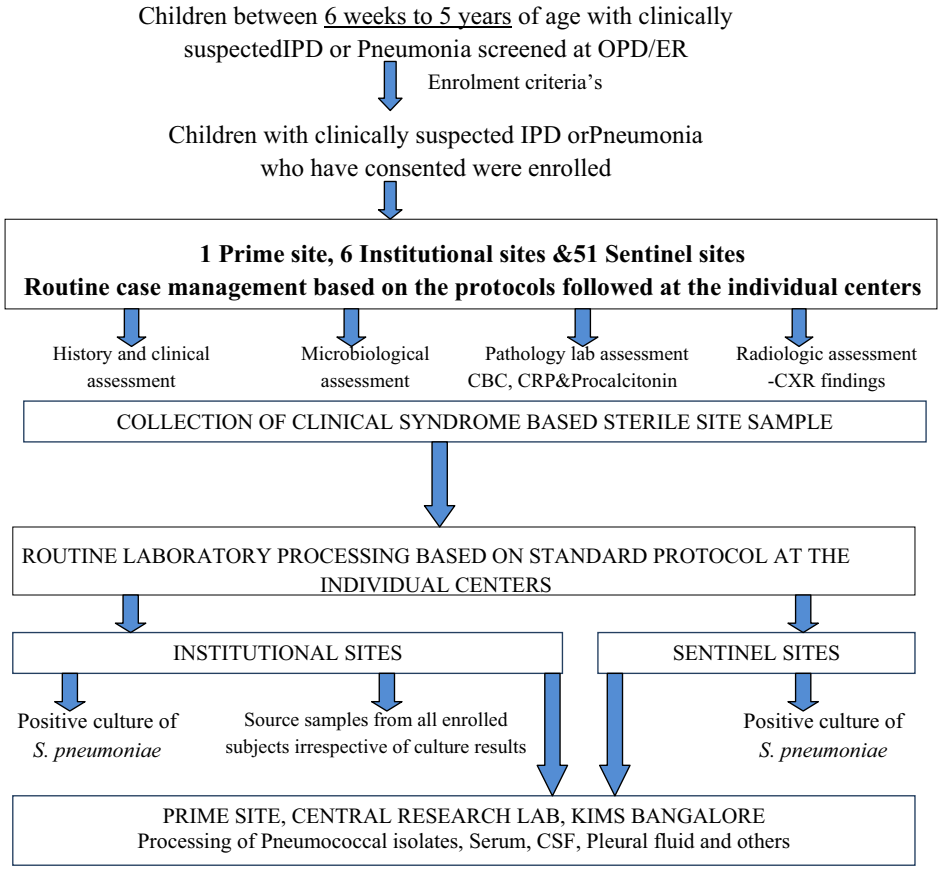

Fig. 1. Sample collection algorithm.