DETECTION OF PNEUMOCOCCAL SEROTYPES WITHIN SEROGROUPS 24 & 38/25: PCR SEROTYPING FOR CULTURE-NEGATIVE SPECIMENS

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BACKGROUND

Culture has long been used as a gold standard for confirming bacterial infection. Despite having the gold standard, developing countries like Bangladesh etiology detection is obscured due to the administration of antibiotic prior to specimen collection. This critical condition hamper disease diagnosis along with surveillance that leads to policy decision like vaccine introduction or follow up study after vaccine introduction. To overcome this situation PCR is method of choice which has been regularly used since 2007 in our country for culture negative CSF specimens to detect etiology and serotypes from meningitis suspected cases. In Bangladesh, annually 21,000 deaths occur from pneumococcal disease. Its serotype diversity and varied geographic distribution make it more difficult to comprehend and eradicate it by vaccination. Because currently available vaccine contains antigens from specific serotypes which is mostly predominant in developed countries, whereas the burden of disease is higher in developing countries like Bangladesh with more serotype diversity and less serotype coverage for vaccine (Figure 1). In March 2015, Bangladesh has introduced PCV 10 in EPI program, it is expected to reduce pneumococcal infections caused by vaccine serotypes. Recently, an increase of serogroups 24 and 38/25 has been noticed, leading to fear of serotype replacement in the post-vaccine era. However, currently available conventional PCR primers cannot distinguish the serotypes within serogroups 24 and 38/25. Therefore, we designed primers to detect these serotypes in culture-negative samples.

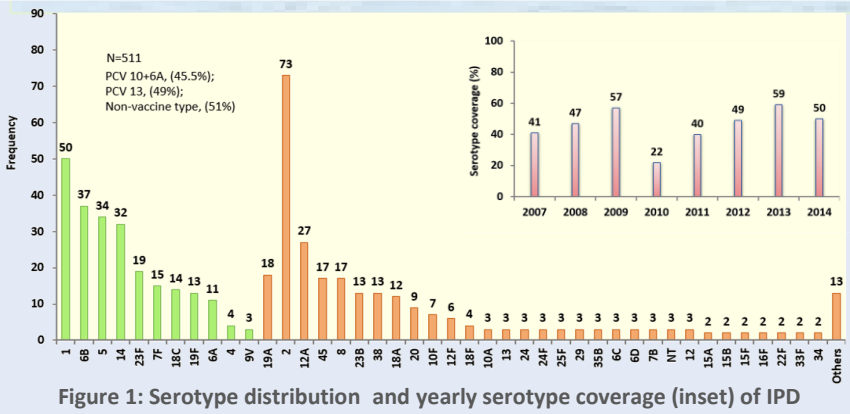

Figure 1: Serotype distribution and yearly serotype coverage (inset) of IPD cases during 2007-14

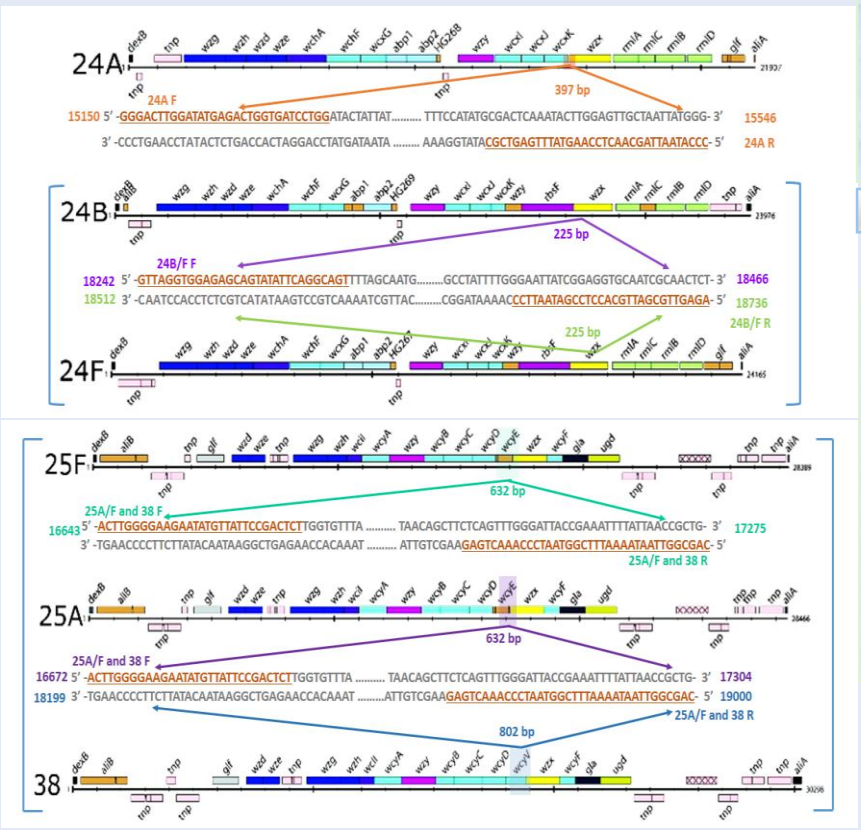

Figure 2: Schematic diagram of cps locus (Bently et al) with primer sequence, designing site and product size.

METHOD

Six multiplex-compatible primers were designed manually from published sequences of capsular polysaccharide (cps) loci (Figure 2). Blastn was used to verify their specificity and OligoAnalyzer version 3.1 for physical properties. Specificity of the primers were confirmed using Quellung-confirmed isolates of serotypes 24A, 24B, 24F, 25A, 25F and 38. Cross-reactivity with 45 different predominant serotypes from invasive cases and human DNA were checked. Finally, their utilization was tested on culture-negative serogroup confirmed (24 and 38/25) clinical specimens.

RESULTS

These primers distinguish serotypes by yielding bands of different sizes for 24A (397bp), 24B/F (225bp), 25A/F (632bp) and 38 (802bp) (Figure 3). Primers to separate 24B/F and 25A/F could not be designed due to high (99%) sequence similarity. These primers successfully differentiated three serogroup 24-confirmed culture negative cases into 24B/F and four serogroup 38/25-confirmed cases into three serotype 25A/F and one serotype 38. No cross reactivity has been noticed with other serotypes and human DNA.

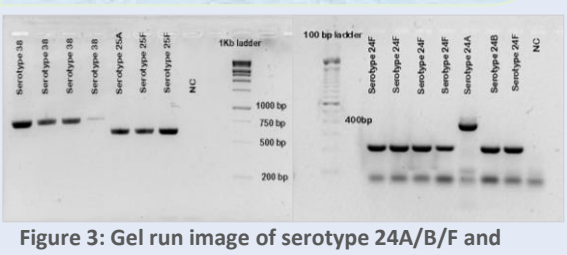

Figure 3: Gel run image of serotype 24A/B/F and serotype 25A/F/38

CONCLUSION

Accurate serotyping data of *Streptococcus pneumoniae* in particular geographical area from both pre and post vaccine period is important. Many studies have reported emergence of non-vaccine type in post PCV period. Our data from pre vaccine period (2007-2013) reported 50 different serotypes. Therefore, here is much more opportunity to fill up the vacuum after wiping away the vaccine type. This work can contribute to generate better serotype data (by discerning serotypes 24A, 24B/F, 25A/F and 38) for pneumococcal surveillance and impact studies, especially in prior antibiotic prone area, where Quellung is not helpful.

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