ONE ASSAY FOR ALL: A NOVEL MICROARRAY ASSAY FOR SEROTYPING OF STREPTOCOCCUS PNEUMONIAE IN CULTURE NEGATIVE QMPCR POSITIVE SERUM SPECIMENS

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BACKGROUND AND AIMS

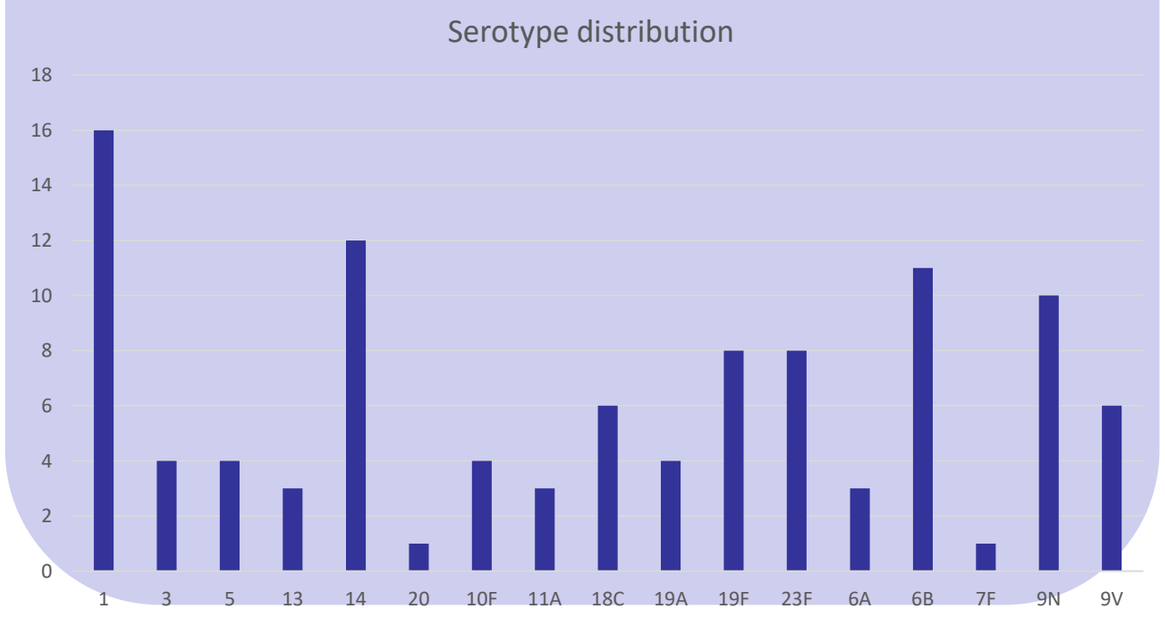
- Current pneumococcal serotyping methods relies on culturing which is often negative.
- Serum provides a convenient and widely available source of pathogen detection
- The difficulty in differentiating homologous strains and presence of host DNA are of hindrance for the adoption of molecular techniques
- This study aimed to evaluate a microarray method to detect and serotype *S. pneumonia* directly from serum specimens

METHODS

- Probes were designed and added to custom KIMS SP CPS v2.0 array using Sure design software to identify and serotyping of *S. pneumoniae* from serum samples
- Unique probes (identified by multiple sequence alignment technique) and modified algorithms were used to identify homologous strains. Microbiome DNA enrichment kit was used to selectively increase bacterial DNA from Serum specimens.
- 96 culture negative qmPCR positive, 8 culture positive and qmPCR positive serum samples were processed with in-house optimized microarray protocol using CY3 and CY5 dyes.
- Species specific probes were designed and added to custom chip to detect respiratory pathogens and antibiotic resistance genes

RESULTS

- The custom KIMS SP-CPS v2.0 chip identified serotype information accurately for serum specimens and homologous strains.



RESULTS

- Among 102 serum samples tested, Serotype 1 was the most predominant serotype followed by 14, 6B, 19F and 23F.
- Excellent concordance with established serotyping methods (Sequetyping) was seen with additional advantage of multiple serotype carriage and their relative abundance levels.

Sample ID	Serotype	Spiked Serotype /Organism	Multiple serotype detection by Microarray	Serotype relative abundance levels quantification
K-01	19F	1	19F and 1	19F (50%), 1 (50%)
K-03	6B	13	6B and 13	6B (50%), 13 (50%)
K-09	19A	3	19A and 3	19A (50%), 3 (50%)
K-17	18C	5	18C and 5	18C (50%), 5 (50%)
K-20	23F	13	23F and 13	23F (50%), 13 (50%)
K-33	6A	3	6A and 3	6A (50%), 3 (50%)
K-35	1	Neisseria meningitidis	1 and Neisseria meningitidis	1 (50%), Neisseria meningitidis (50%)
K-40	19A	Neisseria meningitidis	19A and Neisseria meningitidis	19A (50%), Neisseria meningitidis(50%)

Table:1 : Multiple serotype carriage detection and relative abundance levels quantification

- The custom pneumococcal chip has detected spiked pathogens DNA accurately however failed to detect mutations in antibiotic resistance genes. Optimized protocol with the use of two dyes, low running cost per sample was achieved.

CONCLUSION

- Preliminary analysis suggest that our customized Pneumococcal microarray is a robust assay for identification and typing of *S. pneumoniae* from culture negative serum specimens, which can be used as a diagnostic tool in clinical setting

REFERENCES

1. Feehery GR, Yigit E, Oyola SO, Langhorst BW et al. A method for selectively enriching microbial DNA from contaminating vertebrate host DNA. PLoS One. 2013;8:e76096

2. Bentley SD, Aanensen DM, Mavroidi A, Saunders D et al. Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes. PLoS Genet. 2006;2:e31.