

COMPARISON OF PNEUMOCOCCAL SEROTYPING METHODS FOR APPLICATION TO VACCINE IMPACT STUDIES IN NEPAL

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INTRODUCTION

PCV10 was introduced in Nepal in 2015 and projects were in place to assess multiple aspects of vaccine impact. This study compared established in-country capacity for conventional serotyping with molecular serotyping by microarray to investigate potential benefits in carriage studies.

METHODS

In the 2014-15 pre-vaccine era, nasopharyngeal swabs were collected from 1,904 community-dwelling children aged 6 months to <5 years from an urban area (Kathmandu).

Swabs were transported in STGG to the laboratory and cultured on 5% sheep blood agar with 5% gentamicin for microbiological isolation.

Isolated organisms were identified as the pneumococcus by optochin sensitivity test and in-country conventional serotyping by Quellung (Q) was performed using pneumococcal typing antisera.

Duplicate aliquots of the STGG were stored at -80°C prior to shipment to the UK for molecular serotyping by microarray (M).

RESULTS

1,241 pneumococcal-positive swabs were analysed by Quellung and microarray. Overall concordance was 87% for detection of the same serotype (1,077/1,241).

For the 13% samples with discordant results (164/1,241):

- 5% were non-typeable (NT) by Quellung (59/1,241) but typed by microarray as either pneumococcal serotypes (14/1,241), related *Streptococcus* spp. (29/1,241) or a mixture of both of these (16/1,241).
- 8% were serotyped by Quellung (105/1,241) but typed by microarray as either out of serogroup (40/1,241), within serogroup (51/1,241) or a related *Streptococcus* spp. (14/1,241).

Carriage of multiple pneumococcal serotypes was detected in 1% samples by Quellung (11/1,241) and 24% samples by microarray (296/1,241). Due to multiple serotype carriage, 32% more PCV10 VTs were detected in these samples by microarray than Quellung (Q:376, M:508), indicating VT carriage in 21% more children (Q:375, M:453).

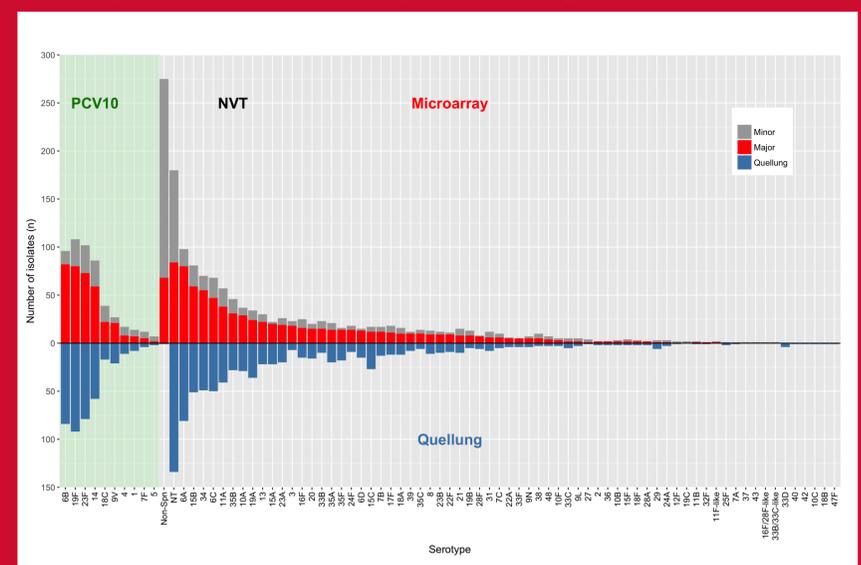
Microarray detected multiple carriage of pneumococcal serotypes, non-typeables and/or related species in 45% of samples (554/1,241) and was able to discriminate non-encapsulated non-typeable pneumococcal lineages from related *Streptococcus* spp.

Antimicrobial resistance genes were detected by microarray in 65% samples (810/1,241) to provide a proxy for potential resistance circulating in the pneumococcal population.

In addition to serotype, genotype was also assessed by microarray through arrayCGH analysis of the pneumococcal genome to reveal pneumococcal lineages and population structure within these samples.

FIGURE 1

Serotype distribution by Quellung and microarray



Frequency of serotypes detected in samples by Quellung and microarray, highlighting PCV10 serotypes and indicating whether the major or a minor abundant serotype by microarray analysis

CONCLUSION

Concordance between the two serotyping methods was good, ensuring comparable trends across studies regardless of method used. In some cases, discordance was due to differences in using phenotypic and molecular methods or the presence of closely related *Streptococcus* spp. in samples. In other cases, the nature of the discordance was less expected and would require follow-up to investigate further.

The sensitive detection of multiple pneumococcal serotype carriage by microarray increases the power to assess vaccine impact upon circulating PCV10 VTs. In this study, 32% more PCV10 VTs were detected in the same samples as previously analysed by Quellung, equating to detection of PCV10 VTs in 21% more children. This may have importance when assessing the more subtle indirect effects of vaccine impact required for herd immunity.

This dataset presents a solid pre-vaccine baseline against which to compare post-vaccine results. Microarray analysis of post-vaccine era samples from the same urban area is planned as part of the wider vaccine impact study. Further insights into antimicrobial resistance and pneumococcal population structure by microarray provide a more comprehensive analysis of the samples beyond simply serotyping.

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