

## PNEUMOCOCCAL POPULATION STRUCTURE IN NEPALESE CHILDREN PRIOR TO PCV INTRODUCTION

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## BACKGROUND

- We aimed to evaluate the molecular epidemiology of pneumococci in Nepal from nasopharyngeal carriage in healthy young children and relate that to the pneumococci isolated from the nasopharynx of children with pneumonia or invasive pneumococcal disease (IPD) prior to pneumococcal vaccine introduction.

## METHODS

- Pneumococcal nasopharyngeal carriage isolates were collected from healthy community carriers (CC) (in three time periods: 2009, 2012, and 2014) and paediatric inpatients with clinician diagnosed pneumonia (IP) in 2014.
- IPD isolates were collected from sterile-site cultures between 2005 and 2014.
- Extracted DNA underwent whole-genome-sequencing. Population structure was defined by clustering genomes on sequence similarity, initially into primary clusters which were then sub-divided into secondary clusters, using hierBAPS.

**Table 1. Demographics of Nepalese children from whom pneumococcus was isolated**

Cohort	Time period of collection	Mean age	%Male	N samples analysed
IPD	Jan 2005 – Jul 2014	2.96	50.72	68
Inpatient Pneumonia Carriage 2014	Mar 2014 – Oct 2014	1.5	62.32	69
Healthy Carriage 2014	Apr 2014 – Oct 2014	1.2	54.07	134
Healthy Carriage 2012	May 2012 – Oct 2012	0.8	46.51	84
Healthy Carriage 2009	Feb 2009 – Apr 2009	0.8	49.07	107

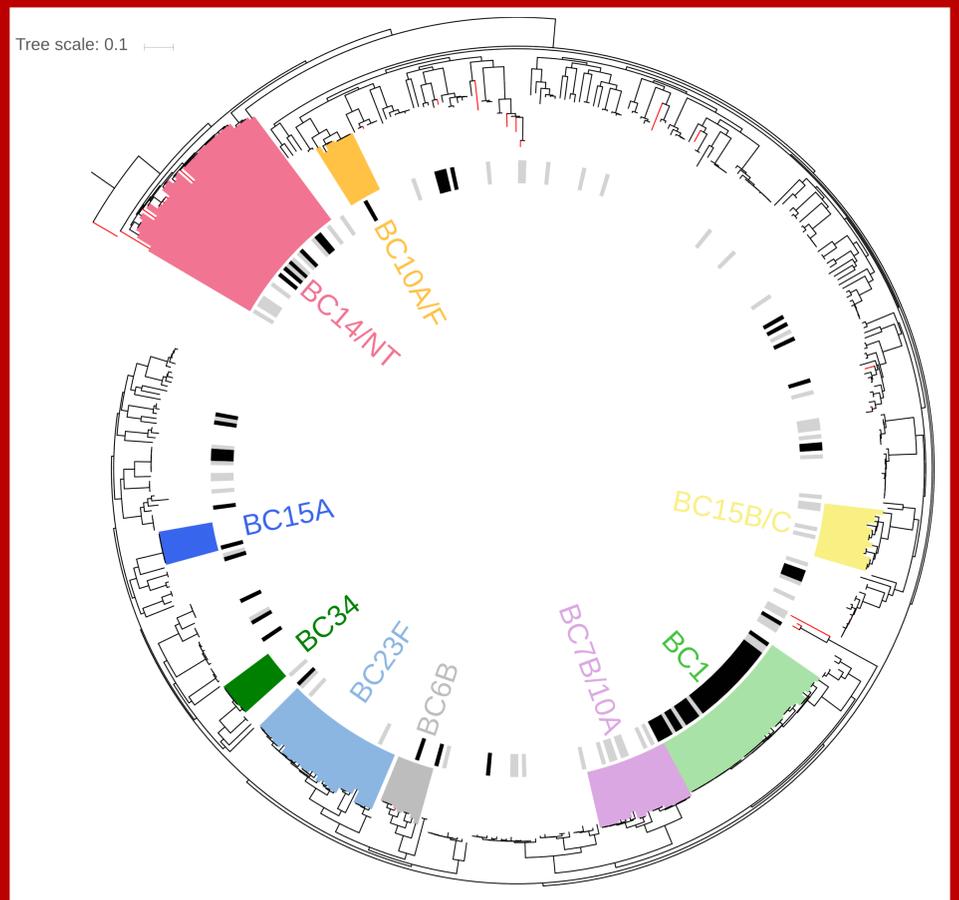
## RESULTS

- From a total of 462 pneumococci, 13 primary and 45 secondary BAPS clusters were defined.
- Evidence of serotype expansion was exemplified by a significant increase in proportion of healthy carriers of serotype 14 within a single primary cluster from 2009 compared with 2014 ( $p=0.0027$ ).
- Of the secondary clusters 167/462 (36.15%) isolates were classified into the 'bin' (polyphyletic) cluster.
- 31, 26, and 16 secondary clusters were defined in the CC, IP, and IPD groups respectively (CC vs IPD,  $p=0.0029$ ).
- 22, 20, and 26 secondary clusters were defined for healthy carriers in 2009, 2012, and 2014 respectively.
- Non-typeable isolates had the greatest amount of cluster diversity.

### FUNDING

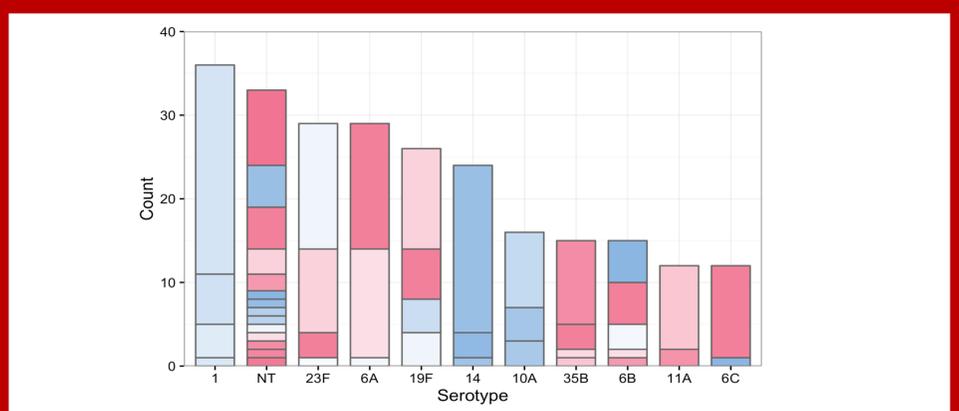
- This study was supported by the Wellcome Trust Sanger Institute sequencing facility and funded by the Bill and Melinda Gates Foundation (grant code OPP1034556). Collection of samples from the 2014 inpatient pneumonia and healthy carriage cohorts was funded by Gavi, The Vaccine Alliance.

## PNEUMOCOCCAL POPULATION STRUCTURE PRIOR TO PCV INTRODUCTION IN NEPALESE CHILDREN



**Figure 1.** Maximum likelihood whole-genome SNP based phylogeny of pneumococcal isolates from Nepalese children prior to PCV introduction. The taxa categorised into the nine dominant BAPS clusters are coloured and all non-typeable isolates represented by red branches. Coloured bars on the inner circle indicate whether the isolate was from IPD (black), carriage during pneumonia (gray) or healthy carriers (white). Visualisation created using itol.embl.de

## SEROTYPE DISTRIBUTION OF SECONDARY BAPS CLUSTERS



**Figure 2.** The frequency of secondary BAPS clusters per serotype for the most prevalent serotypes, demonstrates a high level of diversity within non-typeable pneumococci and contrastingly low diversity across serotypes 1 and 14.

## CONCLUSIONS

- Pneumococci found in nasopharyngeal carriage among healthy Nepalese children are highly diverse but there is evidence of significant expansion of clusters of certain related genotypes over time in the absence of vaccine pressure.
- IPD isolates were less diverse and dominated by strains with serotype 1 capsule expression.