HOTSPOTS OF RECOMBINATION IN PNEUMOCOCCI ISOLATED FROM NEPALESE CHILDREN PRIOR TO PCV INTRODUCTION.

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GPS
Global Pneumococcal Sequencing Project

INTRODUCTION

- Environmental influences such as human immune responses and antibiotic use are thought to elicit selective pressure on the genome of pneumococcus.
- These selective pressures are evident by the frequency of recombination in affected genes. We aimed to identify the recombination patterns of pneumococci isolated from Nepalese children prior to PCV introduction.

METHODS

- DNA from 458 (388 carriage and 70 invasive) pneumococcal isolates from Nepalese children, collected prior to national PCV introduction, underwent whole-genome-sequencing on the Wellcome Trust Sanger Institutes core sequencing pipeline.
- Population clusters was defined by sequence similarity using hierBAPS. Regions of recombination within clusters were determined using Gubbins with hotspots defined as regions with a recombination frequency above the 95th centile.
- BLASTn comparison of these regions with *Streptococcus pneumoniae* ATCC700669 was then used to identify the genes within hotspots.

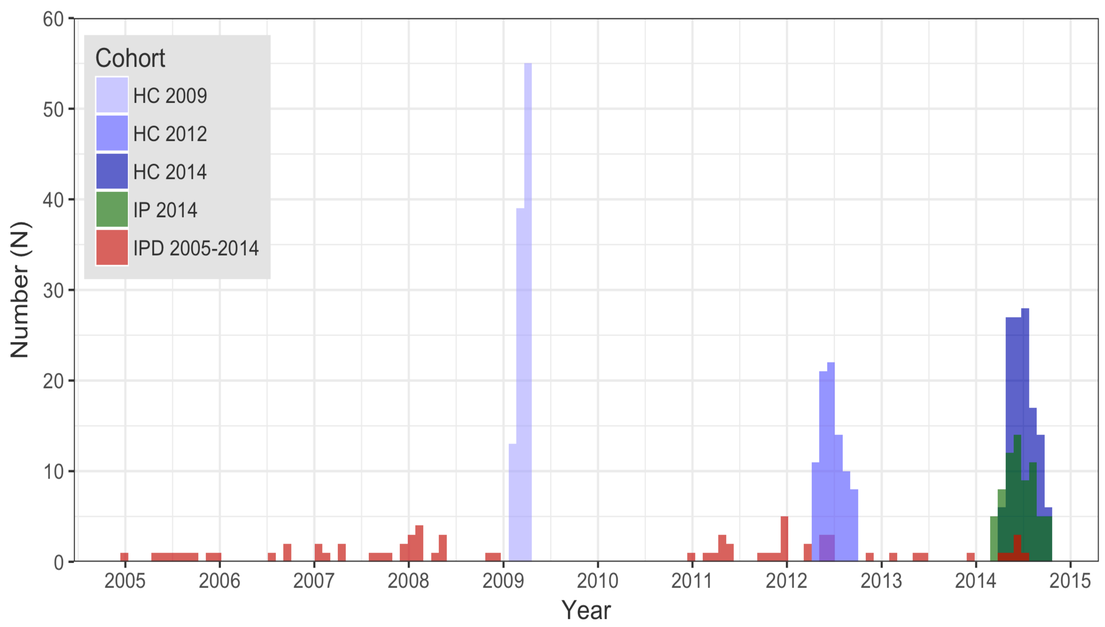

Figure 1. Number of sequenced pneumococcal isolates collected from Nepalese children at Patan Hospital, Kathmandu, Nepal between 2005 and 2014.

Three cohorts were studies where isolates were collected from healthy children in three separate time-periods (HC 2009, HC 2012, and HC 2014). One cohort was a study where carriage samples were collected from children admitted to the hospital with pneumonia (IP 2014). In addition, isolates collected from sterile site cultures of children attending the hospital were analysed (IPD 2005-2014). PCV10 was introduced into the Kathmandu region in August 2015.

RESULTS

- 281 described genes were found to be present in a hotspot in more than one of the population clusters.
- The genes which were in hotspots of recombination across the most clusters were *pspA*, SPN23F03020, SPN23F03030, *mraW*, *ftsL*, *pbp2x*, *arcA*, and *pspC*.
- Interestingly, the two surface proteins *pspA* and *pspC* were identified as hotspots in the same group of five population clusters.

Table 1. Characteristics of population clusters of pneumococci isolated from Nepalese children prior to PCV introduction.

Cluster name	Number of isolates, n (%)	Serotypes present (n)
BC6D	23 (5)	6D (8), 34 (8), and 15A (7)
BC23F-1	11 (2.4)	23F (10), and NT (1)
BC6A	25 (5.5)	6A (12), 17F(6), 21(4), 9N (1), 19A (1), and NT (1)
bin	233 (50.9)	multiple
BC19F	15 (3.3)	19F (12), NT (2), and 19A (1)
BC15B	13 (2.8)	15B (8) and 15C (5)
BC35B	11 (2.4)	35B (10) and NT (1)
BC14	31 (6.8)	14 (25) and NT (6)
BC23F-2	21 (4.6)	23F (16), 19F (4), and 23B (1)
BC1	37 (8.1)	1 (37)
BC11A	11 (2.4)	11A (11)
BCNT-1	3 (0.7)	NT (2) and 17F (1)
BC10A	17 (3.7)	10A (7), 7B (6), 19F (3), and 19A (1)
BCNT-2	7 (1.5)	NT (7)

***Table 2. Described genes most frequently detected in population cluster hotspots of recombination*.**

Coding DNA sequence	Product	Hotspot clusters
pspA	pneumococcal surface protein A	BC10A, BC14, BC15B, BC19F, BC23F-1
SPN23F03020	LacI family regulatory protein	BC6A, BC14, BC15B, BC23F-1, BC23F-2
SPN23F03030	membrane protein	BC6A, BC14, BC15B, BC23F-1, BC23F-2
mraW	S-adenosyl-methyltransferase MraW	BC6A, BC14, BC15B, BC23F-1, BC23F-2
ftsL	cell division protein	BC6A, BC14, BC15B, BC23F-1, BC23F-2
pbp2x	penicillin binding protein 2x	BC6A, BC14, BC15B, BC23F-1, BC23F-2
arcA	arginine deiminase	BC1, BC6A, BC6D, BC15B, BC19F
pspC	pneumococcal surface protein C	BC10A, BC14, BC15B, BC19F, BC23F-1

CONCLUSION

- Host immunity and antibiotic use may influence genetic plasticity, given the number of population cluster recombination hotspots that contained genes such as *pspA*, *pspC*, and *pbp2x*.

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