

# Pherotype diversity is not affected by conjugate vaccine-induced serotype changes

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## Background and goals

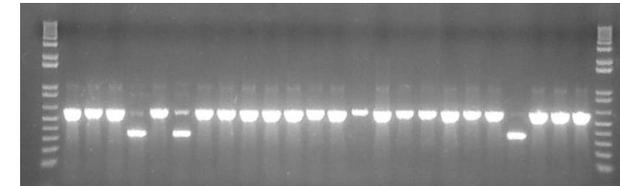
Recombination through transformation is the main mechanism of genetic transfer in *Streptococcus pneumoniae*. It occurs when bacteria become competent, which is triggered when the competence-stimulating peptide (CSP) reaches a critical extracellular concentration. There are two major variants of this 17 aa peptide in the pneumococcal population and any given strain presents only one type of CSP and is not able to respond to the other variant. Hence, this determines the pherotype of the strains, which can be CSP1 or CSP2. We previously reported that pherotype is driving genetic differentiation within *S. pneumoniae* [1].

It is being seen worldwide that the introduction of the conjugate vaccines changes the serotype distribution of pneumococcus causing invasive pneumococcal disease (IPD). After PCV7 introduction in 2001 in Portugal, the serotypes 4, 6B, 14 and 23F decreased while 19A serotype increased [2]. The introduction of PCV13 in early 2010 caused the decrease of serotypes 1 and 19A, while there was a small rise in non-vaccine serotypes [3].

The aim of this work was to evaluate the effect of these vaccine induced changes in the pherotype distribution of the invasive pneumococcal population.

## Methods

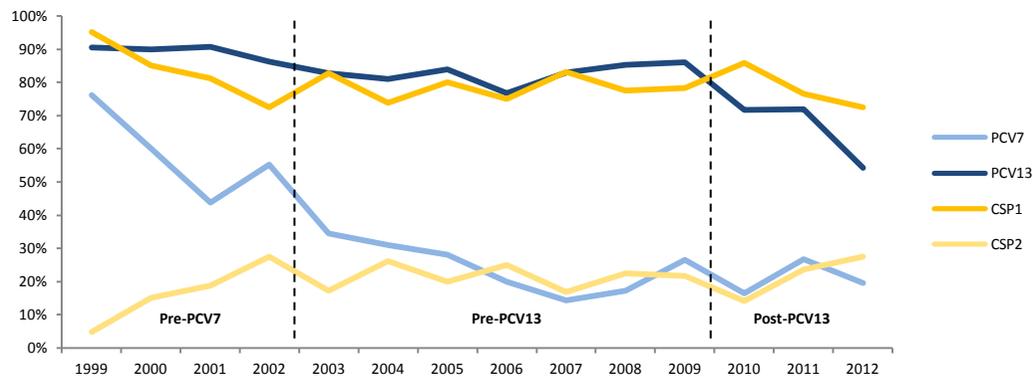
We determined pherotype by PCR and serotype by Quellung reaction of a collection of 386 strains causing IPD in children in Portugal between 2009 and 2012. We also compared our results with data from the periods before PCV7 and PCV13 introduction (1999 to 2008).



**Figure 1 – Pherotype identification by PCR.** Example gel photograph. CSP1 and CSP2 strains present a 620 bp and 340 bp fragments, respectively.

## Results

### Vaccine-induced serotype changes did not alter pherotype proportion



**Figure 2 – Evolution of PCV7 and PCV13 serotypes and CSP1 and CSP2 proportions over the years.** Vertical dashed lines indicate vaccine iavaolability: Pre-PCV7, Pre-PCV13 and Post-PCV13. Comparing these periods, PCV7 serotypes decreased from 57% to 22% and PCV13 serotypes from 83% to 66% after the introduction of the respective vaccines. However, the proportion of pherotypes did not change: CSP1 strains represented 83% of the isolates in Pre-PCV7 period and 79% in both Pre-PCV13 and Post-PCV13 periods.

### Serotypes remained associated with the same pherotype

**Table 1 – Association between serotype and pherotype by vaccination period.** Only serotypes significantly associated ( $p < 0.05$ ) are presented.

NS: not significantly associated ( $p > 0.05$ ).  
Asterisk (\*):  $p < 0.05$  after Fisher's exact test but not significant after false discovery rate correction.

Serotype	Pre-PCV7 (nr. CSP1/CSP2)	Pre-PCV13 (nr. CSP1/CSP2)	Post-PCV13 (nr. CSP1/CSP2)
1	NS (15/0)	CSP1 (140/1)	CSP1 (35/0)
7F	NS (4/0)	CSP1 (66/0)	CSP1 (23/0)
14	NS (22/2)	CSP1 (53/0)	CSP1* (15/0)
6A	CSP2* (0/2)	CSP2 (2/14)	CSP2 (0/8)
9N	NS (0/1)	CSP2 (0/8)	CSP2* (0/2)
10A	NS (1/1)	NS (1/2)	CSP2 (1/7)
19A	NS (3/3)	CSP2 (72/57)	CSP2 (17/13)
19F	NS (3/0)	CSP2 (6/16)	NS (5/3)
24F	- (0/0)	CSP2 (0/7)	CSP2 (1/5)
Total nr.	102 (84/18)	625 (493/132)	222 (175/47)

## References and acknowledgements

- 1 – Carrolo M, Pinto FR, Melo-Cristino J and Ramirez M. 2009. Pherotypes are driving genetic differentiation within *Streptococcus pneumoniae*. BMC Microbiol, 9:191.
- 2 – Aguiar, S. I., I. Serrano, F. R. Pinto, J. Melo-Cristino, and M. Ramirez. 2008. Changes in *Streptococcus pneumoniae* serotypes causing invasive disease with non-universal vaccination coverage of the seven-valent conjugate vaccine. Clin Microbiol Infect 14:835-43.
- 3 – Aguiar, S. I., M. J. Brito, J. Gonçalo-Marques, J. Melo-Cristino, and M. Ramirez. 2010. Serotypes 1, 7F and 19A became the leading causes of pediatric invasive pneumococcal infections in Portugal after 7 years of heptavalent conjugate vaccine use. Vaccine 28:5167-73.

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## Conclusions

Surprisingly, the dramatic changes in the serotype distribution of the population causing IPD in children brought about by conjugate vaccines, did not alter the proportion of CSP variants. This suggests that pherotype may play an important role in the pneumococcal population, although its precise function remains elusive.