Delineating the PCV13 perturbation to the *Streptococcus pneumoniae* carriage population in Cambodia

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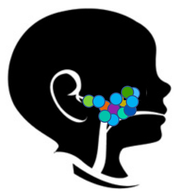
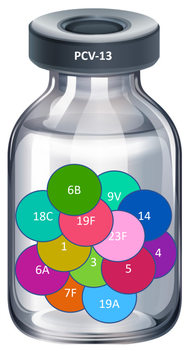
Streptococcus pneumoniae background



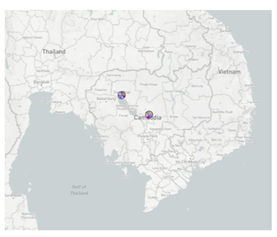
Colonized the nasopharynx of ~60% of Cambodian children prior to the 2015 pneumococcal conjugate vaccine (PCV) introduction and is a leading cause of lower respiratory infection mortality globally[1,2].



Streptococcus pneumoniae is very diverse with approximately 800 Global Pneumococcal Sequence Clusters (GPSCs). These comprised >100 serotypes which can be swapped between GPSCs[3].

A 13 valent Pneumococcal Conjugate Vaccine (PCV13) was broadly introduced in 2015 in Cambodia. PCV introduction is known to alter the ecology in a region – often with an expansion of non-vaccine types[4].

Analysis pipeline



Isolate collection: A total of 690 carriage isolates of *Streptococcus pneumoniae* were collected from healthy participants in Siem Reap, Cambodia from January 2013 until February 2017 (4 were excluded due to discordance with the metadata indicative of a sample swap).

Summary of samples: After filtering 686 were included in and comprised Pre/peri-PCV13 (01/2013–12/2015, N=258) and the post-PCV13 nasopharyngeal carriage isolates (01/2016-02/2017, N=432) . The sample population had a mean age of 18 months and 46.2% were female.

Sequencing and assembly: Isolates were sequenced at the Sanger Institute on an Illumina HiSeq platform. Sequences were assembled (velvet) and annotated as part of the Global Pneumococcal Sequencing project (GPS).

In Silico Classification: Strain and serotype classification employed PopPUNK[3] and SeroBA[5]. The CDC-AMR pipeline was employed for in silico drug resistance screening. Gene presence absence was elucidated using Panaroo[6]. Trees were constructed using FastTree[7].

Statistical Analysis: All statistical analysis was conducted using R v3.6.0 and included Simpsons diversity index, Welch's t-test, and Fishers exact test for determining shifts in the composition and structure of the post-PCV13 populations.

Results

- PCV13 serotypes significantly decreased (p=0.002 [95% Confidence interval 0.26-0.90], OR 0.61) while non-PCV13 serotype significantly increased (p=0.002[1.19-2.27], OR 1.64) in the post-PCV13 populations.
- There was a significant increase in Simpsons diversity index for both serotype (p=0.006) and strain (p=0.023) in the post PCV13 population
- Concordance between phenotypic and in silico drug resistance predictions exceeded 94% for all antimicrobials evaluated.

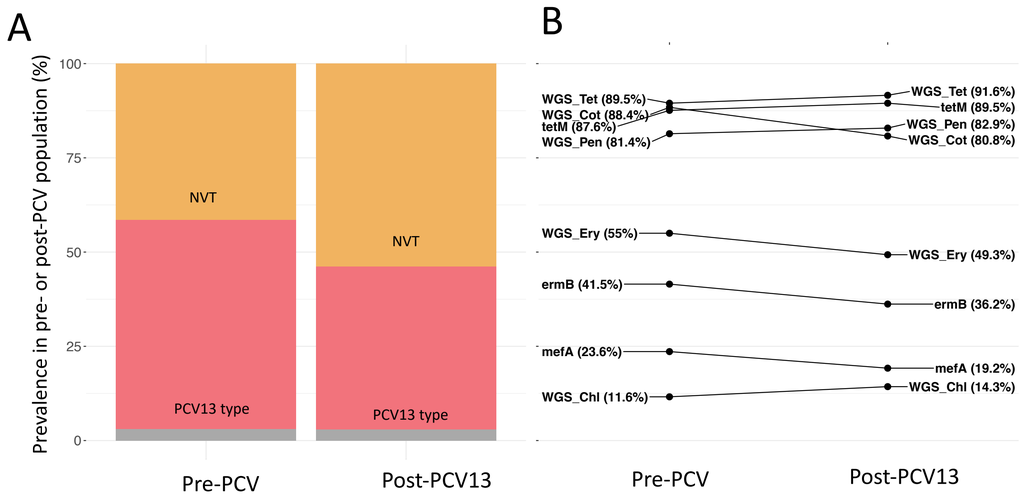


Figure 1. **Comparisons of vaccine status and drug resistance in the pre and post-PCV13 populations. A)** Prevalence of PCV13 type (PCV13; pink) and non-vaccine type (NVT; orange) serotypes B) Prevalence of in silico drug resistance and plasmids from the pre- to the post-PCV13 populations. Pre-PCV13 population N=283; post-PCV13 populations N=428. WGS: Whole genome sequencing; Tet: tetracycline; Pen: penicillin; Cot: co-trimoxazole; Ery: erythromycin; Chl: chloramphenicol. Plasmids: tetM, ermB, mefA, cat.

Results (cont.)

- Significant changes in prevalence were detected in the post-PCV13 populations of serotypes 19F, 23A, 34, and 6D.
- A gene for a zeta toxin, pezT_3, was identified as having significantly increasing prevalence among the non-vaccine serotypes in the post-PCV13 population (p=0.0003 [1.49-4.13], OR 2.46).
- A tetracycline resistance gene *tet(*M) increased significantly among NVT 23A (N=26, GPSC626, p= 0.03[1.39-9.69], OR 2.84) and 6D (N = 9, GPSC16, p = 0.03[1.19-inf], OR - Inf), and decreased significantly in vaccine type 19F (N=52, 98.1% GPSC1; p = 0.02[0.26-0.88], OR 0.48)

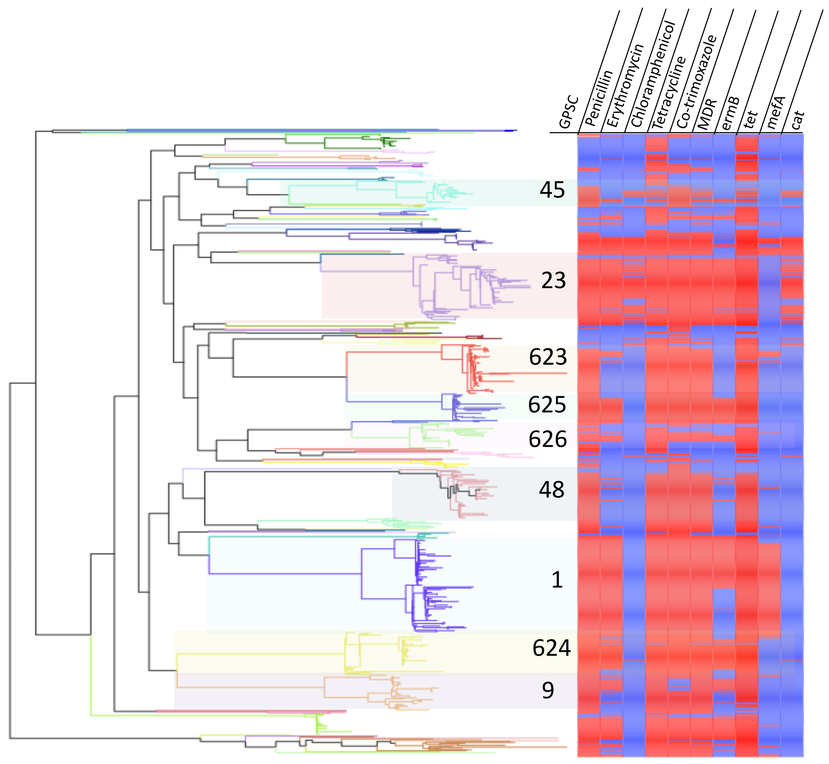


Figure 2. **Phylogeny of Streptococcus pneumoniae isolates (N=686) in Cambodia**. Only GPSCs comprising >50% of the total population are labeled. Edges are coloured by distinct GPSCs. Drug resistance was determined using the CDC AMR pipeline. Penicillin drug resistance was defined as MIC >= 0.12ug/ml.

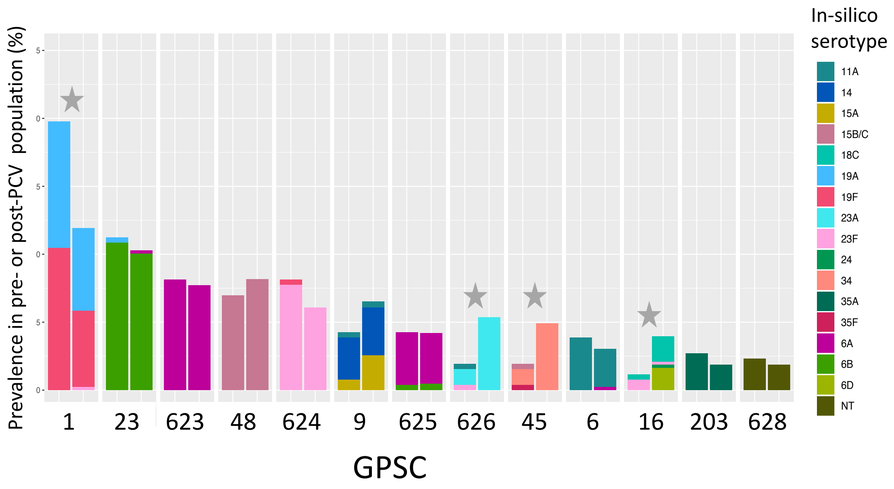


Figure 3. GPSC prevalence change of *S. pneumoniae* carriage isolates from pre- to post-PCV13. Prevalence of GPSC stratified by pre- and post- PCV13 population colored by serotypes pre (left bar) and post-PCV13 (right bar) filled by serotype. Only GPSCs together comprising >75% total population are included.

Table 1. **Serotypes 19F, 23A, 34, and 6D significantly changed prevalence from the pre- to the post- population.** These correspond with significantly changing GPSCs (Figure 3). Calculated using the Fishers exact test.

Serotype (N)	OR	p-value (95% CI)	Predominant GPSC	Direction of change
19F (52)*	0.48	0.02(0.26-0.89)	1 (98.1%)	Decrease
23A (27)	2.84	0.03(1.04-9.69)	626 (96.3%)	Increase
34 (24)	4.55	0.01(1.35-24)	45 (100%)	Increase
6D (9)	∞	0.03(1.19-∞)	16 (87.5%)	Increase

*Included in PCV13

Conclusion

- The strain population in Cambodia has been perturbed by the vaccine but had not yet reached equilibrium 24 months following PCV13 introduction.
- The change in frequency of *tet(*M) and pezT within GPSCs and serotypes may reflect overall prevalence change or be genetic drivers of expansion of NVTs. Monitoring and further evaluating genetic signatures of perturbation could support evaluation of vaccine impact.
- Additional isolate collection is ongoing for detection of trends towards equilibrium post-PCV13 in this population.
- Next steps include evaluating Cambodia in the context of surrounding countries and determining if the perturbation results in similar serotype expansion.

References

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