Comparative Genomic Analysis of Samples Collected Pre- and Post-introduction of Pneumococcal Conjugate Vaccine (PCV7) in Hong Kong

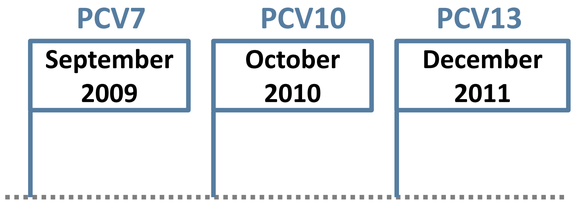
P.Y.T. Law[1], H. Cao[1], M.C. Liu[1], S.S. Chiu[2], S.D. Bentley[3], R.F. Breiman[4], L. McGee[5], R.A. Gladstone[3], S.W. Lo[3], and P.L. Ho[1] and The Global Pneumococcal Sequencing Consortium[6]*

[1] The University of Hong Kong, Microbiology and Carol Yu Centre for Infection, Pokfulam, Hong Kong SAR; [2] The University of Hong Kong, Paediatrics and Adolescent Medicine, Pokfulam, Hong Kong SAR; [3] The Wellcome Trust Sanger Institute, Infection Genomics, Cambridge, United Kingdom; [4] Emory University, Hubert Department of Global Health, Atlanta, USA; [5] Centers for Disease Control and Prevention, Respiratory Diseases Branch, Atlanta, USA; [6] http://www.pneumogen.net/gps/

GPS
Global Pneumococcal Sequencing Project

Introduction

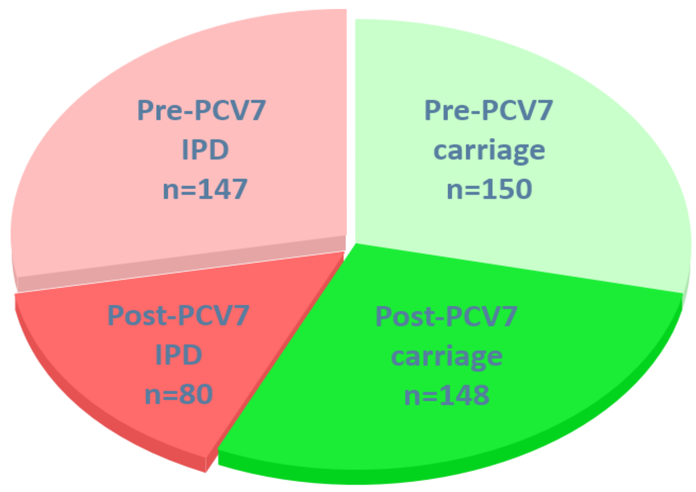
Since the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) into the childhood immunization program in Hong Kong in September 2009, and replacement of higher valent PCV10 and PCV13 in 2010 and 2011, a local epidemiological study conducted in 2010-2013 revealed changes in carriage prevalence and serotype distribution in young children in the post-PCV7 era (Ho *et al.*, 2015). Our study aimed to provide a high-resolution picture of the changes in the pre- and post-vaccine era by whole genome sequencing (WGS) of pneumococci collected in Hong Kong.



Methodology

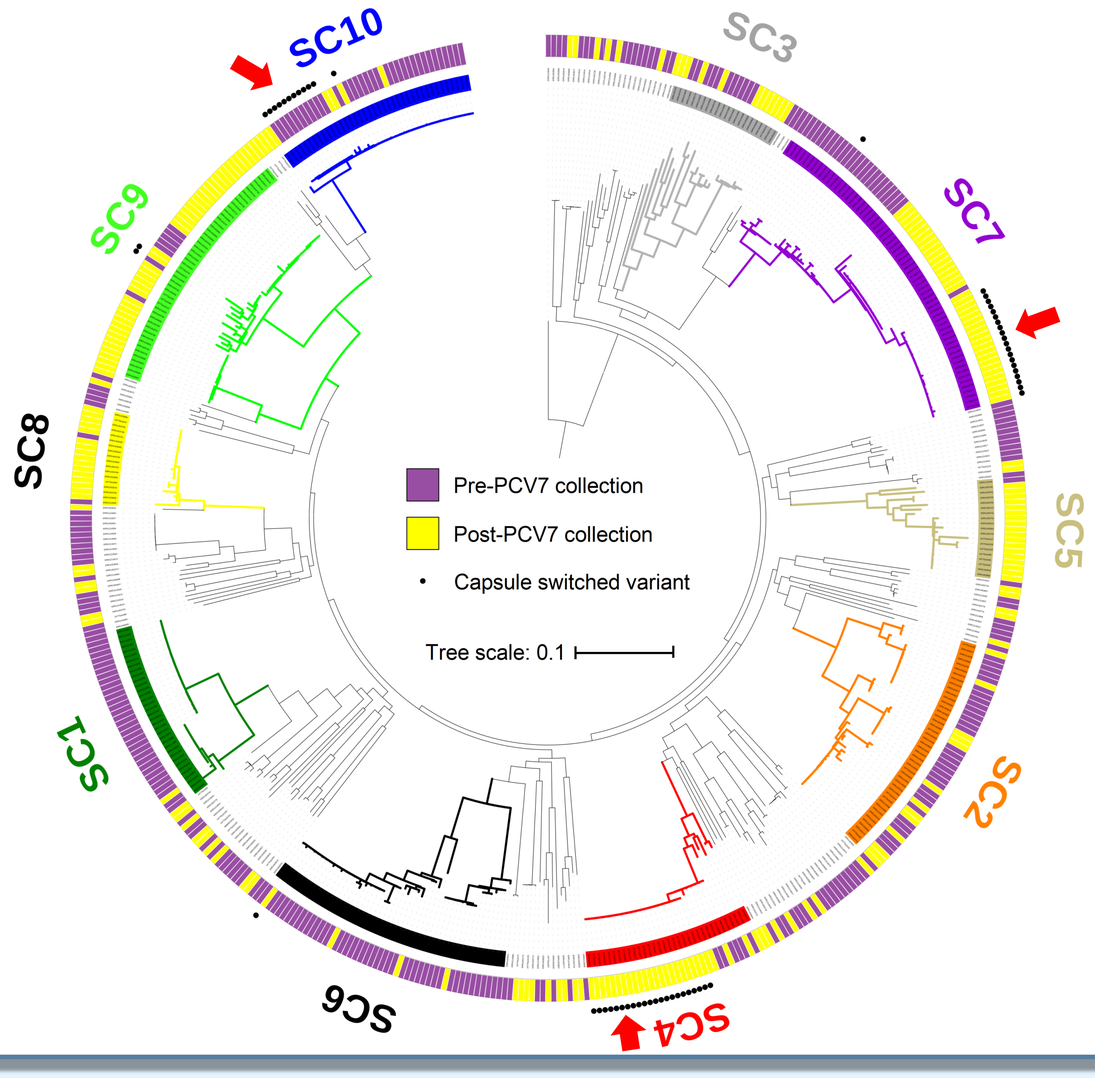
A total of 525 isolates collected during 1995-2017 from Hong Kong were selected and sent for WGS to represent the pre- (1995-2008) and post-PCV7 (2009-2015) implementation eras for both carriage Invasive Pneumococcal Disease (IPD, n=227) population as presented in Figure 1.

Figure 1 Composition of whole genome sequenced collection



WGS data and quality control were obtained from the Sanger Institute as part of the Global Pneumococcal Sequencing (GPS) project. Software employed for bioinformatics analyses included Velvet, RAxML, pneumoCaT, Roary, and Gubbins. Sequence clusters (SC) were defined based on maximum-likelihood (ML) phylogeny. Penicillin susceptibilities were determined with E-Test.

Figure 2. Population structure of pre- and post-PCV7 collections of *Streptococcus pneumoniae* in Hong Kong.



Results

Population structure

Ten sequence clusters (SCs) were identified amongst 525 sequenced isolates from Hong Kong. Of which, SC4 (serotype 3), SC5 (serotype 15A), SC7 (serotype 19A/F), SC8 (serotype 15A), and SC9 (serotype 15A) were predominantly associated with post-PCV7 collection as presented in Figure 2. SC1 (serotype 14) and SC4 (serotype 3) had the highest invasive odds ratio (OR) of 3.56 and 6.57 respectively. Other characteristics are presented in Table 1.

Capsular switching events

A total of 8 capsular switching events were detected, represented as black dots (•). Three of which resulted in a clonal expansion in SC4, SC7 and SC10, indicated with red arrows (➔). Two of these expansions were observed in the post-PCV7 era, these are SC4 (serotype 9V → 3) and SC7 (serotype 19F → 19A). The capsular switching region of them were estimated to be 18.8kb and 29.6kb in length, switching all genes involved in the production of capsular polysaccharides.

Table 1 Characteristics of sequence clusters of Hong Kong pneumococcal population structure

SC	No. of isolates (pre- / post- PCV7)	No. of isolates Carriage / IPD	Invasive odds ratio	Predominant§ serotype	Predominant§ MLST	PMEN* clones
SC 1	35 (34 / 1)	10 / 25	**3.56**	14	ST15	England^{14}-9
SC 2	45 (32 / 13)	21 / 24	1.56	3	ST180	Netherlands^{3}-31
SC 3	22 (11 / 11)	20 / 2	**0.12**	23A	ST338	Colombia^{23F}-26
SC 4	33 (6 / 27)	6 / 27	**6.57**	3	ST6011	None
SC 5	19 (1 / 18)	13 / 6	0.6	15B/C	ST3397	None
SC 6	48 (44 / 4)	24 / 24	1.35	6B	ST90	Hungary^{19A}-6
SC 7	64 (25 / 39)	32 / 32	1.36	19F	ST236	Taiwan^{19F}-14
SC 8	18 (2 / 16)	15 / 3	**0.25**	15A	ST63	Sweden^{15A}-25
SC 9	49 (7 / 42)	43 / 6	**0.16**	15B/C	ST199	Netherlands^{15B}-37
SC10	39 (34 / 5)	21 / 18	1.14	23F	ST81	Spain^{23F}-1

§ Predominant serotypes and MLSTs were determined by the majority of the isolates in that sequence cluster.
* PMEN, Pneumococcal Molecular Epidemiology Network.
Invasive odds ratios (OR) were determined by the equation a·d / b·c, where a and b are number of IPD and carriage isolates in the cluster respectively, and c and d are the total number of IPD and carriage isolates not in the cluster, respectively.
OR larger than 2 are indicated in **red**, and OR less than 0.5 are indicated in **green**.

Results (con't)

Penicillin non-susceptibility acquisition

Two independent capsular switching events had resulted in penicillin non-susceptible isolates (MIC > 0.06 μg/ml) from an otherwise penicillin susceptible sequence cluster (SC9, serotype 15B/C →14).

Conclusion

We observed changes in the prevalence of lineages in the pre- and post-PCV7 collections. Two of the capsular switching variants from PCV7 serotypes to non-PCV7 serotypes were followed by clonal expansions in the post-PCV7 era. The expansion of capsular switched variant in SC4 is particularly worrisome owing to the lineage's highly invasive OR.

Acknowledgement

The presenting author is a recipient of the **ISPPD-11 Travel Grant**. This study was supported by grants from the **Health and Medical Research Fund** of the Food and Health Bureau of the Hong Kong Special Administrative Region. This project is conducted in collaboration with **the Global Pneumococcal Sequencing (GPS) Project**, funded by **The Bill & Melinda Gates Foundation**.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.