EPIDEMIOLOGY OF SEROGROUP 6 *Streptococcus pneumoniae* CARRIAGE AND INVASIVE DISEASE IN KILIFI, KENYA, AND EFFECT OF THE 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV10).

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1. Introduction

- Serogroup 6 *Streptococcus pneumoniae* traditionally comprised of serotypes 6A and 6B.
- Serotypes 6A/6B were classically identified by the quellung test using factor antisera 6b/6c respectively (**Table 1**).
- Serotypes 6C/6D were discovered in 2007/2009.
- Serotypes 6C/6D cross-react serologically with factor antisera 6b/6c respectively, and have previously been misclassified as serotypes 6A/6B.
- Cross-reactivity caused by the lack of sufficiently discriminatory antisera can potentially lead to the misreporting of the prevalence of the serogroup 6 serotypes.
- In 2009, the Statens Serum Institute (SSI) commercially availed a new 6A-specific factor antiserum named "6b*" , that does not cross-react with serotype 6C.
- In 2010, the new factor antiserum 6d was introduced by the SSI. Factor 6d reacts with serotypes 6C/6D and distinguishes 6A/6B from 6C/6D.
- Kenya introduced the serotype 6B containing PCV10 vaccine in January 2011.
- We re-typed archived carriage and invasive pneumococcal isolates using factor antisera 6b*,6c and 6d to examine the prevalence of 6C/6D before vaccine introduction, and the impact of PCV10 on all serogroup 6 isolates.

Factor antisera	SEROTYPES			
	6A	6B	6C	6D
6b	+	-	+	-
6b*	+	-	-	-
6c	-	+	-	+
6d	-	-	+	+

Table1:Serologic properties of serogroup 6 isolates using quelling reagents

2. Methods

This was a retrospective study of 2,133 serogroup 6 carriage isolates recovered from previous nasopharyngeal carriage studies across all ages conducted from 2004-2015, in the Kilifi Health and Demographic Surveillance System (KHDSS); three of which have been published (**See references**); and 128 serogroup 6 invasive isolates recovered from subjects presenting with suspected invasive bacterial disease to the Kilifi County Hospital (KCH) from 2002-2015 (**Table 3**). The first post-vaccine carriage survey was conducted 6 months after PCV10 introduction.

The isolates had been previously identified by α-hemolysis, optochin susceptibility, bile solubility and serotyped by the quellung method. The isolates were retrieved from storage at -80˚C, cultured in blood agar and incubated in 5% CO_2 , 37˚C for 18-24 hours . Retyping was done both by the quellung method using factor antisera 6b*, 6c and 6d. Two conventional PCRs were used for serotype confirmation. The first differentiated 6A/C from 6B/D and the second distinguished serotypes 6C/6D from serotype 6A/6B respectively. PCR primers specific for serotypes 6A, 6B, 6C and 6D adapted from Jin et al, 2009 (**Figure 1**) were run separately but concurrently in each respective PCR.

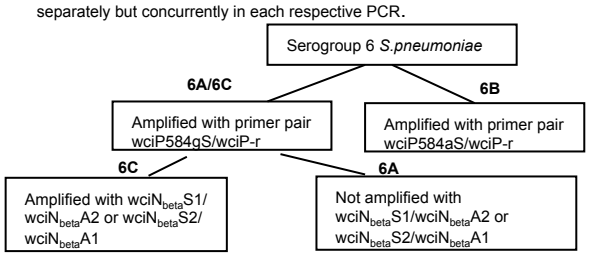

Figure 1: Algorithm for identification of serogroup 6 serotypes by PCR. (Jin et al., 2009)

Year	Carriage			Invasive Disease		
	All SPN	Serogroup 6		All SPN	Serogroup 6	
	N	n	%	N	n	%
2002	-	-		81	12	14.8
2003	-	-		107	16	15.0
2004	192	46	24.0	92	18	19.6
2005	520	120	23.1	71	12	16.9
2006	280	65	23.2	51	9	17.6
2007	861	187	21.7	95	13	13.7
2008	944	215	22.8	58	7	12.1
2009	269	58	21.6	64	8	12.5
2010	201	37	18.4	114	9	7.9
2011	180	27	15.0	52	9	17.3
2012	250	29	11.6	36	8	22.2
2013	204	15	7.4	29	2	6.9
2014	205	12	5.9	49	1	2.0
2015	213	17	8.0	27	4	14.8
Total	4319	828	19.2	926	128	13.8

Table 2: Annual Number of all serotype and serogroup 6 pneumococcal isolates collected from carriage (2004-2015) and disease (2002-2015) within the KHDSS and KCH respectively

3. Statistical Analyses

3.1 Nasopharyngeal carriage study analysis

Nasopharyngeal carriage prevalence estimates from 2004–2015, across all ages, were calculated with exact confidence intervals. A chi-square test for trend was used to assess changes in prevalence of group 6 carriage from 2010, the last survey before PCV10 introduction, and 2015.

3.2 Invasive pneumococcal disease (IPD) incidence

For patients across all ages residing within the KHDSS, crude incidence rates were calculated by dividing the number of laboratory confirmed cases of IPD by person-time at risk and are presented per 100,000 person years with 95% confidence intervals (95% CIs). Incidence rate ratios (IRRs) were estimated by Poisson regression for the pre- and post-vaccine eras (2002-2010 vs 2011-2015). We adjusted for secular trends in IPD incidence across the whole study period using calendar year as the adjustment variable.

4. Results

4.1 Concordance between original and retyped quellung results

- There was a 96% concordance rate between the original and retyped quellung results.
- Two serotype 6A isolates collected in 2009, one from carriage and the other invasive disease were found to be serotype 6C after retyping.
- The remaining discordant isolates after retyping were of non-serogroup 6 serotypes.

4.2 Concordance between retyped quellung and PCR results

- There was a 99% concordance between the second quellung and PCR results.
- Discordant isolates were untypeable by the second quellung but typeable by PCR.
- Five 6A and four 6B invasive isolates identified by the original quellung were untypeable by the second quellung. PCR serotyping showed that these were six 6A and three 6B isolates.

4.3 Nasopharyngeal carriage prevalence of serogroup 6 *S. pneumoniae* 2004-2015

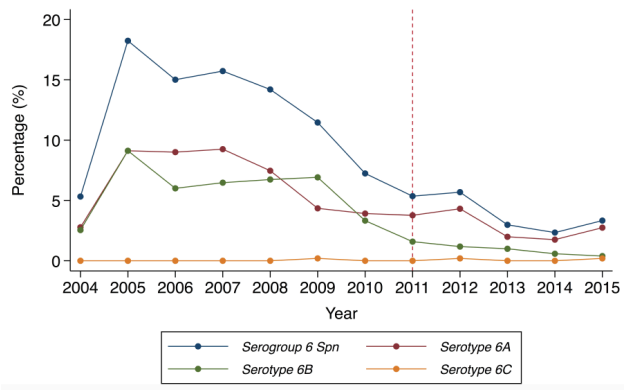

Figure 2 : Annual carriage prevalence rates of serogroup 6 *S. pneumoniae* 2004-2015

4.3.1 Chi-square test for trend results

- The prevalence of 6B carriage declined from 3.3% in 2010 to 0.4% in 2015 (chi-square test for trend, p<0.001).
- The prevalence of 6A carriage declined from 3.9 % in 2010 to 2.7% in 2015 (p=0.026).

4.4 Incidence of serogroup 6 invasive pneumococcal disease 2002-2015

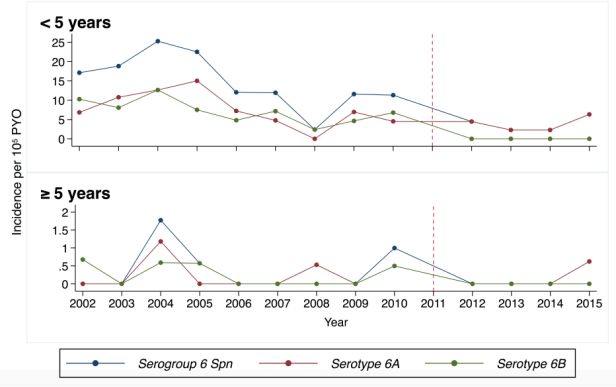

Figure 3: Annual IPD incidence rates of serogroup 6 *S.pneumoniae* for patients < 5 and ≥ 5 years 2002-2015

Serogroup 6 serotypes	IRR (95% CII)	p-value
Serotype 6A	1.26 (0.29, 5.43)	0.76
Serotype 6B	0.00 (not estimatable)	0.99
Total serogroup 6	0.61 (0.18, 1.99)	0.41

Table 3:IRRs for serogroup 6 serotypes in the pre-and post- vaccine periods for patients < 5 years of age.

Serogroup 6 serotypes	IRR (95% CII)	p-value
Serotype 6A	0.25 (0.01, 7.30)	0.42
Serotype 6B	0.00 (not estimatable)	1.00
Total serogroup 6	0.29 (0.02, 5.09)	0.40

Table 3:IRRs for serogroup 6 serotypes in the pre-and post- vaccine periods for patients ≥ 5 years of age.

5. Conclusions

- The inclusion of the new factors 6b* and 6d to our serotyping panel together with the use of serotype-specific PCR improved distinction between serotypes 6A/B, 6A/C, 6B/D.
- 6B carriage prevalence in all age groups reduced considerably after vaccine introduction.
- 6B IPD reduced by 100% in all age groups after 2012.
- There was relatively little change in 6A carriage and IPD in all ages post-vaccination.
- Serotype 6C is rare in Kilifi and does not contribute significantly to carriage or disease.
- Serotype 6D was not identified in Kilifi in carriage or IPD.

References

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