

Molecular characterisation of pneumococcal serotype 16F: Established predominant carriage and otitis media serotype in the 7vPCV era

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Abstract

Young Australian Aboriginal children in remote communities experience very high rates of pneumococcal carriage and otitis media. Prior to introduction of the 7-valent pneumococcal conjugate vaccine (7vPCV, Prevenar), serotype 16F was an important type found in nasal and ear discharge swabs. Since commencement of pneumococcal immunisation for Aboriginal infants in 2001, 16F has become the predominant established serotype in carriage and otitis media in young Aboriginal children. BOX typing and multi-locus sequence typing revealed a diverse population of serotype 16F strains, and evidence of potential capsule switching from a vaccine serotype 4 to a serotype 16F.

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1. Introduction

An intensive program of pneumococcal vaccination began in 2001. Aboriginal infants received pneumococcal conjugate vaccine (7vPCV, Prevenar) at 2, 4, and 6 months of age, followed by 23 valent polysaccharide vaccine (23vPPV, Pneumovax) at 18 months of age. Older children received catch-up vaccines. Longitudinal studies of pneumococcal carriage and otitis media in Aboriginal infants in three Northern Territory communities demonstrated no significant difference in the proportion of vaccinated compared to non-vaccinated infants who experienced middle ear effusion, acute otitis media or tympanic membrane perforation in the first year of life [1]. Nasopharyngeal pneumococcal carriage remained at approximately 80%, however, 7vPCV type carriage dropped from 56% to 25% in 12–17 month olds. Serotype 16F was the

dominant replacement strain which rose in prevalence from 10% to 24% and in rank from fourth to the most common. Furthermore, a cross sectional survey in 29 remote communities across the Northern Territory (NT) in 2003 found that an average of 80% of 644 children were nasopharyngeal carriers of pneumococcus. Serotype 16F was the dominant serotype overall, accounting for 18% of 574 carriage isolates (16% children) and 24% of 34 ear discharge isolates.

Serotype 16F is not included in any current vaccine, however it is mentioned as a potential cause of Invasive Pneumococcal Disease (IPD) in some countries [2–4] and high risk populations [5] including indigenous Australians in Queensland [6] and the Northern Territory [7] since introduction of conjugate vaccine.

The aim of this study was to characterise, by molecular type and antibiogram, pneumococcal serotype 16F isolates collected before and after the introduction of 7vPCV in order to understand the clonal diversity of this important pathogen during a period of increasing prevalence in the population. For this analysis we compared 16F isolates from the three communities with pre-7vPCV and 7vPCV era data.

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Table 1
BOX type for serotype 16F isolates

	1996	2000	2003–2004
Number of children	7	19	24
Number of isolates	8	22	37
BOX type	Isolates, <i>n</i> (%)		
A.1	1 (13%)	4 (18%)	2 (5%)
A.2		14 (64%)	30 (81%)
C		4 (18%)	1 (3%)
D	1 (13%)		
E	1 (13%)		
F	2 (25%)		1 (3%)
J			2 (5%)
K	3 (36%)		
L			1 (3%)

2. Methods

Sixty-seven serotype 16F nasal or nasopharyngeal isolates were cultured from Aboriginal children in the three study communities in 1996, 2000, and 2003/2004 (Table 1). Longitudinal studies of otitis media and carriage had been ongoing in these three remote Aboriginal communities since 1992. The pre-7vPCV isolates were collected in 1996 and 2000 as part of a randomised controlled trial of amoxicillin versus placebo for treatment of otitis media. The 7vPCV era isolates were collected in 2003 and 2004 during longitudinal studies of otitis media and carriage in the same three communities. Only the first serotype 16F isolate from each child was included in this analysis, unless differences in antibiotic susceptibility profile had been identified in sequential 16F isolates from the same child.

Screening for antibiotic non-susceptibility was determined by disk diffusion (oxacillin, penicillin G, erythromycin, cotrimoxazole, tetracycline and chloramphenicol), and penicillin MIC was determined by E-Test. The 67 isolates were BOX typed [8]. Twenty-seven isolates were then selected for multi-locus sequence typing (MLST); isolates were grouped by year, BOX typing profile, and antibiotic susceptibility, and one to eight rep-

resentative isolates of each group were selected at random (Table 2).

3. Results

BOX typing results for all 67 isolates are summarised in Table 1. Nine BOX types were identified, and up to six BOX types were present during a single study period.

MLST analysis of 27 representative isolates (based on year, the antibiotic susceptibility and BOX typing profile) is summarised in Table 2. Seven MLST sequence types (STs) were detected and six of these were new to the international MLST database (www.mlst.net). ST246 was already present in the database, but had only previously been reported from serotype 4 isolates. This suggests that capsule switching has occurred, with a serotype 4 isolate acquiring a serotype 16F capsule.

BOX type A.1 had multiple MLST types (double locus variants) associated with it—ST2041 in 1996 and ST2044 in subsequent years. Interestingly, BOX type A.2, the dominant clone from 2000 onwards, was also ST2044.

No isolates were macrolide resistant, and none had penicillin MICs ≥ 0.1 $\mu\text{g/mL}$.

4. Discussion

Serotype 16F has been an important serotype associated with nasopharyngeal carriage and tympanic membrane perforation in this population for some years. Since commencement of universal infant immunisation serotype 16F has become the predominant serotype in carriage and in ear discharge. Serotype 16F IPD cases have increased in the Northern Territory following universal infant 7vPCV program with catch-up, and 16F is now the second most common IPD serotype [7].

Non-vaccine serotypes emerging as predominant in the 7vPCV-era include 19A in IPD [9], and 15, 11 and 29 in carriage, in the USA [10]. Few developing countries have universal 7vPCV immunisation, and serotype 16F appears to be rare

Table 2
Multi-locus sequence type and BOX type for representative 16F isolates

	1996	2000	2003–2004
Number of isolates	8	22	37
Number of isolates selected for MLST	6	7	14
MLST	BOX type (number of isolates selected for MLST analysis)		
2041	A.1 (1)		
2042	F (2), K (1)		F (1)
2043	D (1)		
2044		A.1 (1), A.2 (5)	A.1 (2), A.2 (8), L (1)
2045		C (1)	C (1)
2196			J (1)
246	E (1)		

in IPD serotype surveillance in such regions. Serogroup 16 has recently been reported as an important carriage serotype with long carriage times in non-vaccinated Gambian infants [11]. Whether serotype 16F emerges in developing country regions as 7vPCV is increasingly used remains to be seen.

The key findings from this molecular analysis of serotype 16F support our view that this serotype is established and not an outbreak or epidemic strain with a short lifespan in the community. The diversity of MLST present in 1996 is indicative of it being an established serotype in this population rather than a clonal expansion with potentially short duration.

We provide evidence of potential capsule switching from a vaccine serotype 4 to a serotype 16F. Capsular switching is thought to be a potential mechanism for pneumococci to evade high levels of vaccine-induced antibody, and has been described in several other studies. We believe capsular switching may occur at higher rates in populations with high carriage of multiple serotypes, but we have not yet systematically looked for this using MLST analyses.

In conclusion, we report serotype 16F as the predominant pneumococcal serotype in carriage and otitis media with tympanic membrane perforation in Australian Aboriginal children in remote communities since implementation of infant 7vPCV immunisation. We believe that the diversity of serotype 16F is evidence of its success as an established pathogen in this population at high risk of pneumococcal disease, and propose 16F as a candidate for higher valency pneumococcal conjugate vaccines.

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