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Long-term impact of pneumococcal polysaccharide vaccination on nasopharyngeal carriage in children following a reduced dose pneumococcal conjugate vaccine primary series in infancy

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ABSTRACT

Previously, the Fiji Pneumococcal Project (FiPP) evaluated reduced dose immunization schedules that incorporated pneumococcal protein conjugate and/or polysaccharide vaccine (PCV7 and 23vPPV, respectively). Immune hyporesponsiveness was observed in children vaccinated with 23vPPV at 12 months of age compared with children who did not receive 23vPPV.

Here we assess the long-term impact of 23vPPV vaccination on nasopharyngeal carriage rates and densities of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Moraxella catarrhalis*. Nasopharyngeal swabs ($n = 194$) were obtained from healthy children who participated in FiPP (now aged 5–7 years). *S. pneumoniae* were isolated and identified by standard culture-based methods, and serotyped using latex agglutination and the Quellung reaction. Carriage rates and densities of *S. pneumoniae*, *H. influenzae*, *S. aureus* and *M. catarrhalis* were determined using real-time quantitative PCR.

There were no differences in the rate or density of *S. pneumoniae*, *H. influenzae* or *M. catarrhalis* carriage by PCV7 dose or 23vPPV vaccination in the vaccinated participants overall. However, differences were observed between the two main ethnic groups: Fijian children of Indian descent (Indo-Fijian) were less likely to carry *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, and there was evidence of a higher carriage rate of *S. aureus* compared with indigenous Fijian (iTaukei) children. Polysaccharide vaccination appeared to have effects that varied between ethnic groups, with 23vPPV vaccination associated with a higher carriage rate of *S. aureus* in iTaukei children, while there was a lower carriage rate of *S. pneumoniae* associated with 23vPPV vaccination in Indo-Fijian children.

Overall, polysaccharide vaccination had no long-term impact on pneumococcal carriage, but may have impacted on *S. aureus* carriage and have varying effects in ethnic groups, suggesting current WHO vaccine schedule recommendations against the use of 23vPPV in children under two years of age are appropriate.

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1. Introduction

Streptococcus pneumoniae (the pneumococcus) is the most common cause of pneumonia, which is responsible for an estimated 1.3 million deaths annually in children under five years of age [1]. There are over 90 different serotypes of *S. pneumoniae* [2,3]. Prior to the introduction of pneumococcal conjugate vaccines (PCVs), the vast majority of pneumococcal disease was caused by a limited number of serotypes [4]. Nasopharyngeal carriage of *S. pneumoniae* is considered a pre-requisite for the development of pneumococcal disease [5]. The introduction of PCVs has led to a dramatic reduction in invasive pneumococcal disease caused by vaccine-type *S. pneumoniae* [6–8]. However, in many settings, there has been little change in overall *S. pneumoniae* carriage due to replacement of vaccine type *S. pneumoniae* with non-vaccine types (serotype replacement) [9,10]. The potential for species replacement following pneumococcal vaccination is also of concern, particularly given that some studies have found a negative relationship between vaccine-type *S. pneumoniae* and *Staphylococcus aureus* carriage [11,12]. In some settings, pneumococcal vaccination has also affected colonization of the respiratory pathogens *Moraxella catarrhalis* and *Haemophilus influenzae* [13,14].

The Fiji Pneumococcal Project (FiPP) was a single-blind, open-labelled randomized Phase II vaccine trial conducted in Suva, Fiji, designed to identify a pneumococcal vaccination schedule more suited to resource-poor countries. Specifically, FiPP evaluated a reduced dose 7-valent pneumococcal conjugate vaccine (PCV7) primary series in infancy, followed by the 23-valent pneumococcal polysaccharide vaccine (23vPPV) booster at 12 months of age [15]. One of the key findings was immune hyporesponsiveness in 23vPPV-vaccinated children compared with children not vaccinated with 23vPPV, observed following challenge with a micro-dose (20%) of 23vPPV at 17 months of age [16]. Nasopharyngeal carriage rates of *S. pneumoniae* were unaffected by 23vPPV vaccination at the same time-point [17].

FiPP also highlighted the differences in carriage rates between the two main ethnicities of Fiji, indigenous Fijians (iTaukei) and Fijians of Indian descent (Indo-Fijians) [18], consistent with an earlier carriage survey in the same setting [19]. In FiPP, carriage of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* was higher in iTaukei children than Indo-Fijian children, and higher *M. catarrhalis* densities were found in iTaukei children vaccinated with 23vPPV compared with iTaukei children who were not vaccinated with 23vPPV [18].

In the follow-up study to FiPP, a subset of children were enrolled to investigate the long-term impact of 23vPPV vaccine on carriage, immunity and clinical outcomes. Here we report the long-term effect of pneumococcal vaccination on nasopharyngeal carriage of *S. pneumoniae* and other respiratory pathogens, and examine differences in carriage between the two main ethnicities of Fijian children.

2. Materials and methods

2.1. Study design

The original FiPP study design is described elsewhere [15]. In brief, healthy Fijian infants ($n = 552$) were randomized to receive either 0, 1, 2 or 3 doses of PCV7 (*Prevnar*®, Pfizer Inc., USA) at 6, 10 and/or 14 weeks, with or without a 12 month dose of 23vPPV (*Pneumovax*®, Merck & Co., Inc., USA). Children who were randomized to receive 0 or 1 dose of PCV7 were given a catch-up dose of this vaccine at 2 years of age. The long-term implications of the immune hyporesponsiveness observed at 18 months in children receiving 23vPPV were investigated in the Fiji follow-up study by enrolling all FiPP participants that were contactable ($n = 195$), now aged 5–7 years old. One hundred and ninety five nasopharyngeal swabs were collected, of which 194 were available for microbiological analysis (Table 1). The characteristics of this subset, such as ethnicity, gender and number of children in each group, were similar to that of FiPP (see Supplemental Table S1 and Table 2 in Russell et al., 2010 [17]), and were similar when considering each vaccine alone in this study (see Supplemental Table S2)—although children who received 3 doses of PCV7 were slightly older than children who received 2 doses of PCV7 (median age 6.21 years vs. 5.92 years, $p = 0.017$). The geometric mean antibody levels in this study for 23vPPV vaccinated and 23vPPV unvaccinated children were similar to those observed in these children at 18 months of age in FiPP (data not shown).

This study was approved by the Human Research Ethics Committee, Royal Children's Hospital, Melbourne and the Fiji National Research Ethics Review Committee.

2.2. Nasopharyngeal swabs

Buffered cotton nasopharyngeal swabs (Sarstedt, Australia) were collected and transported as described previously [17], in line with World Health Organization guidelines [20,21]. Swabs were stored frozen in 1 ml of skim milk tryptone glucose glycerol (STGG) medium, and later transported on dry ice to the Pneumococcal Research laboratory at the Murdoch Childrens Research Institute in Melbourne, where they were stored at −80 °C until processing.

2.3. Culture, identification and serotyping

Samples were cultured on Columbia horse blood agar plates containing 5 μg/ml of gentamicin (gHBA; Oxoid brand, Thermo Fisher Scientific, Australia) and incubated for 36–44 h (37 °C, ~5% CO_2) [22]. Two α-hemolytic colonies were randomly selected for subculture using a method previously described [22]. These colonies, plus any additional morphologically distinct α-hemolytic colonies, were subcultured onto Columbia horse blood agar plates (HBA; Oxoid brand, Thermo Fisher Scientific, Australia) and incubated for 24 h (37 °C, ~5% CO_2).

Colonies that were optochin sensitive were presumptively identified as *S. pneumoniae*. Other α-hemolytic colonies that were non-susceptible to optochin (intermediate or resistant) were tested for bile solubility and with the Phadebact® Pneumococcus test (Boule Diagnostics AB, Huddinge, Sweden) to enable identification.

Presumptive pneumococci were serotyped by latex agglutination using a combination of commercial reagents (Denka-Seiken Co., Ltd., Japan) and reagents produced in-house [23,24] using Statens Serum Institute antisera (SSI, Copenhagen, Denmark). Equivocal reactions were confirmed using the Quellung reaction with antisera from SSI [25]. Isolates that were non-typeable were tested for the presence of the *lytA* gene by real-time PCR [26]. *lytA*-positive non-typeable isolates were further examined by multilocus sequencing typing (MLST) [27]. *lytA*-negative non-typeable isolates were excluded from further analysis. MLST allelic profiles were submitted to the *S. pneumoniae* MLST database (http://pubmlst.org/spneumoniae/) sited at the University of Oxford [28] where new sequence types (STs) were assigned for those isolates with allelic profiles not matching any existing ST. A serotype 14-specific PCR [29] was used to test non-typeable isolates that had MLST STs associated with serotype 14.

2.4. Quantitative PCR

Genomic DNA was extracted from 100 μl STGG using the QIAmp DNA Minikit (Qiagen) as previously described [18]. *S. pneumoniae*,

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