Vaccine 33 (2015) 628–634

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Acquisition of *Streptococcus pneumoniae* in South African children vaccinated with 7-valent pneumococcal conjugate vaccine at 6, 14 and 40 weeks of age

Marta C. Nunes[a,b], Stephanie A. Jones[a,b], Michelle J. Groome[a,b], Locadiah Kuwanda[a,b], Nadia Van Niekerk[a,b], Anne von Gottberg[a,c], Linda de Gouveia[a,c], Peter V. Adrian[a,b], Shabir A. Madhi[a,b,c,*]

[a] *Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[b] *Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, University of the Witwatersrand, Johannesburg, South Africa*
[c] *National Institute for Communicable Diseases: A Division of National Health Laboratory Service, Centre for Vaccines and Immunology, Johannesburg, South Africa*

ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form
24 November 2014
Accepted 10 December 2014
Available online 23 December 2014

Keywords:
Pneumococcal conjugate vaccine
Colonization
Carriage
2 + 1
Streptococcus pneumoniae

ABSTRACT

Background: Seven-valent pneumococcal conjugate vaccine (PCV7) was introduced into the South African immunization program using 6, 14 and 40 weeks dosing schedule (2 + 1), with no catch-up in older children since April 2009. We investigated pneumococcal colonization acquisition in children who received this schedule and also compared it to historical cohorts of PCV-naïve children (n = 123 in 2007) and children who received a 3 + 1 PCV7 schedule (n = 124 in 2005/06).
Methods: Two hundred and fifty children aged 6–12 weeks were enrolled from December 2009 to April 2010. Participants had nasopharyngeal swabs collected on eight occasions between enrolment and 2-years of age. Standard methods were undertaken for bacterial culture and *Streptococcus pneumoniae* were serotyped using the Quellung method. Pneumococcal and *Staphylococcus aureus* colonization in the present study was compared to colonization in two historical longitudinal cohorts.
Results: S. pneumoniae was identified in 1081 (61.4%) of 1761 swabs collected in the current cohort. Pneumococcal colonization peaked at 41-weeks of age (76.8%) and decreased to 62.8% by 2-years of age (p = 0.002); PCV7-serotype colonization decreased during the same period from 28.6% to 15.6% (p = 0.001). Children from the current cohort compared to PCV-naïve children were less likely to be colonized by PCV7-serotypes from 40-weeks to 2-years of age and acquired PCV7-serotypes less frequently. No differences in overall pneumococcal, PCV7-serotype and non-PCV7-serotype colonization or new serotype acquisitions were detected comparing the current cohort to the historical cohort who received the 3 + 1 PCV7 schedule. *Staphylococcus aureus* colonization was similar in all three cohorts.
Conclusion: A 2 + 1 PCV7 schedule implemented in South Africa was temporally associated with reduced risk of vaccine-serotype colonization compared to historically unvaccinated children. Also, vaccine-serotype acquisition rate using the 2 + 1 schedule was similar to that in the 3 + 1 dosing cohort, suggesting that similar indirect protection against pneumococcal disease could be derived from either schedule in South Africa.

Abbreviations: EPI, expanded program on immunization; HibCV, *Haemophilus influenzae* type b conjugate vaccine; NICD, National Institute for Communicable Diseases; NP, nasopharyngeal; NPS, nasopharyngeal swabs; NVT, pneumococcal non-vaccine serotype; PCV, pneumococcal conjugate vaccine; PCV7, seven-valent pneumococcal conjugate vaccine; SD, standard deviations; VT, pneumococcal vaccine serotypes.

* Corresponding author at: Respiratory and Meningeal Pathogens Research Unit, Chris Hani Road, Chris Hani-Baragwanath Hospital, New Nurses Residence-1st Floor West Wing, Bertsham, Gauteng 2013, South Africa. Tel.: +27 11 386 6137; fax: +27 11 882 1872.
E-mail address: shabirm@nicd.ac.za (S.A. Madhi).

http://dx.doi.org/10.1016/j.vaccine.2014.12.023
0264-410X/

1. Introduction

Streptococcus pneumoniae together with other bacteria are part of the human commensal flora of the respiratory tract and are generally associated with asymptomatic colonization. Pneumococcal colonization is, however, a pre-requisite for developing pneumococcal disease, which usually occurs within two-months of acquisition of new virulent serotypes [1–3]. Pneumococcal colonization in young children is also the main source for transmission of the bacterium in the community, hence preventing nasopharyngeal (NP) colonization in children is regarded as a strategy to prevent disease in the children as well as in other older individuals [4]. Pneumococcal conjugate vaccines (PCV) decrease the incidence of pneumococcal disease caused by the serotypes included in the vaccine (VT), by inducing protective antibodies against invasive disease in vaccinated individuals, as well as by reducing the rate of VT acquisitions and/or decrease in density of colonization [5,6]. The reduction in VT-NP colonization in PCV-vaccinated children is usually associated with an increase in non-vaccine pneumococcal serotype (NVT) colonization [5,7,8]. Moreover studying the effect that immunization with PCV has on pneumococcal colonization in young children, the most important source of transmission in the community, could inform whether indirect-protection (i.e. herd protection) against pneumococcal disease among unvaccinated individuals at the population level could be achieved [4,6].

The direct and indirect impact of PCV-vaccination on pneumococcal disease and colonization has been assessed in clinical and observational trials that used different immunization schedules (for review see [9–11]). There are, however, limited data on the effect of a 2 + 1 dosing schedule on colonization and even less data from settings with a high prevalence of pneumococcal colonization during early life. Two prospective studies in high income settings that used the seven-valent PCV (PCV7) in 2 + 1 (vaccination at 2, 4, and 11 months or at 4, 6, 12 months of age) immunization schedules, reported reductions in VT-NP carriage in the first and second year of life [12,13].

Seven-valent PCV (Prevenar®) was introduced into the South African national public immunization program as of April 2009, with infants scheduled to receive three doses at 6, 14 and 40-weeks of age and no catch-up campaign in older children [14]. This study aimed to measure the prevalence of pneumococcal colonization and the rate of new serotype acquisition in young children receiving PCV7 as part of the immunization program (PCV-2 + 1 cohort). We also compared colonization prevalence and acquisition of VT and NVT to two historical cohorts who had received a 3 + 1 PCV7 dosing schedule (PCV7-3 + 1 cohort) and another which was PCV-naïve.

2. Methods

2.1. Study participants and sample collection

This longitudinal, prospective study enrolled 250 children at age 6–12 weeks between December 2009 and April 2010. The details of the study population and study design have been described [15]. Briefly, infants born to HIV-uninfected women received the primary series of PCV7 at enrolment and 12–24 weeks of age, followed by a third dose at 38–42 weeks. PCV7 was given concurrently with other recommended childhood vaccines scheduled at the time. At enrolment (visit-1), after receiving the second dose of PCV7 and on six subsequent occasions (visit-2 to visit-8), nasopharyngeal swabs (NPS) were collected by trained nurses as described [8]. Dacron-tipped swabs on a flexible aluminum shaft (Cat# 151D, Medical Wire Equipment Co. Ltd.; Wiltshire, England) were inserted gently though a nostril and then inoculated into skim milk tryptone-glucose-glycerol transport media (STGG) and stored at −70 °C until processing at the National Institute for Communicable Diseases (NICD) laboratory as described [16].

Colonization in the present study was compared to colonization in two historical longitudinal cohorts of HIV-unexposed-uninfected infants. One cohort was enrolled in 2007 and consisted of 123 PCV-naïve children [17]; the other was enrolled in 2005/2006 and involved 124 children who received PCV7 on a 3 + 1 schedule at 6, 10 and 14-weeks of age after which children were randomized to receive either a booster dose of PCV7 or *Haemophilus influenzae* type b conjugate vaccine (HibCV) at 64–76 weeks [18]; Table 1.

2.2. Laboratory assays and methods

Standard microbiologic tests were used for culture and identification of *S. pneumoniae* and *S. aureus* as described [16]. The same laboratory methods were used in the present study and in the two historical cohorts. *Pneumococcus* serotyping was undertaken by the Quellung method with specific antisera (Statens Serum Institute, Copenhagen, Denmark). Serotype-6C was distinguished from serotype-6A by PCR [19], serotypes 25A and 38 were undistinguishable and are represented as 25A/38. If pneumococcal colonies presented with different morphologies on the same plate, one colony representative of each was sub-cultured and serotyped. PCV7-serotypes included 4, 6B, 9V, 14, 18C, 19F and 23F; PCV13-serotypes were PCV7-serotypes plus 1, 3, 5, 6A, 7F and 19A.

2.3. Statistical analysis

A new acquisition was defined when a serotype(s) not identified at the previous visit was isolated from a child. Detection of multiple new serotypes simultaneously was considered as independent new acquisitions. Pneumococci present at the first study-visit were regarded as acquisition episodes. Only children who received their PCV7-vaccinations within the protocol defined window-periods and attended the schedule-visits within the following intervals: 3–6 weeks post-second PCV7 dose (visit-2), 38–42 weeks of age (visit-3), 1–3 weeks post-second PCV7 dose (visit-4), 52–59 weeks of age (visit-5), 65–80 weeks of age (visit-6), and 100–110 weeks of age (visit-8) were included in the analysis; Table 1. Comparisons with the two historical longitudinal cohorts were performed at visit-2, visit-3, visit-5 (PCV-naïve cohort only), visit-6 and visit-8. Age at the time of visit was described using means and standard deviations (SD). Chi-square or Fisher exact tests were used to compare the distribution of colonization prevalence. Multivariate logistic regression controlled for season of sample collection (June/July/August as winter, September/October/November as spring, December/January/February as summer and March/April/May as fall) and age (as continuous variable) was used to compare colonization prevalence between the current cohort and the two historical cohorts at age-appropriate visits for which all three cohorts had available colonization data. Kaplan–Meier estimates of pneumococcal first acquisition were calculated for the current PCV7-2 + 1 cohort and PCV-naïve children from age 20-weeks onwards, restricted to children with colonization data available for all the five common study-visits; and for PCV7-2 + 1 and the PCV7-3 + 1 cohorts from age 20-weeks onwards restricted to children with colonization data available for visit-2, visit-3, visit-6 and visit-8 (limited to children randomized to receive a booster PCV7 dose in the PCV7-3 + 1 cohort). Between-cohort differences were compared by log-rank test. *P*-Values <0.05 were considered significant. Analyses were performed using STATA version 12.1 (College Station, Texas).