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Longitudinal study on *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus* nasopharyngeal colonization in HIV-infected and -uninfected infants vaccinated with pneumococcal conjugate vaccine☆,☆☆

Shabir A. Madhi[a,b,c,*], Alane Izu[b,c], Marta C. Nunes[b,c], Avye Violari[d], Mark F. Cotton[e], Patrick Jean-Philippe[f], Keith P. Klugman[c], Anne von Gottberg[a,c], Nadia van Niekerk[b,c], Peter V. Adrian[b,c], on behalf of the CIPRA 4 team[1]

[a] *National Institute for Communicable Diseases – A Division of National Health Laboratory Service, Sandringham, South Africa*
[b] *Department of Science/National Research Foundation: Vaccine Preventable Diseases, University of the Witwatersrand, Faculty of Health Science, Johannesburg, South Africa*
[c] *Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, Johannesburg, South Africa*
[d] *University of Witwatersrand, Perinatal HIV Research Unit, Johannesburg, South Africa*
[e] *Children's Infectious Diseases Clinical Research Unit, Department of Paediatrics & Child Health, Faculty Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa*
[f] *Henry Jackson Foundation, Division of AIDS (HJF-DAIDS), A Division of The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Contractor to NIAID, NIH, DHHS, Bethesda, MD, United States*

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ABSTRACT

Background: Streptococcus pneumoniae, *Haemophilus influenzae* and *Staphylococcus aureus* are all potentially pathogenic, which frequently colonize the nasopharynx (NP) prior to causing disease.

We studied bacterial NP-colonization in 321 HIV-infected and 243 HIV-uninfected children vaccinated with 7-valent pneumococcal conjugate vaccine (PCV7) at 6, 10 and 14 weeks of age.

Methods: HIV-uninfected infants included those born to HIV-uninfected (HUU) and HIV-infected women (HEU); HIV-infected children with CD4+ lymphocyte ≥25% were randomized to initiate antiretroviral therapy immediately (ART-Immed) or when clinically indicated (ART-Def). Nasopharyngeal swabs for bacterial culture were taken prior to each PCV7 dose (Visits 1–3) and at 20, 39, 47 and 67 weeks of age (Visits 4–7). Swabs were cultured by standard methods and pneumococcal serotyping done by the Quellung method.

Results: Colonization patterns for pneumococcus, *H. influenzae* and *S. aureus* did not differ between HUU and HEU children; and were also generally similar between ART-Def and ART-Immed children. Prevalence of PCV7-serotype colonization was similar between HIV-infected and HIV-uninfected children, however, overall pneumococcal and specifically non-vaccine serotype colonization tended to be lower in HIV-infected children. HIV-infected children also had a 44% lower prevalence of *S. aureus* colonization at Visit-1 ($p = 0.010$); and *H. influenzae* colonization was also lower among HIV-infected than HIV-uninfected children at Visit-2, Visit-3, Visit-6 and Visit-7.

Conclusion: Vaccine-serotype colonization is similar in PCV-immunized HIV-infected and HIV-uninfected children. We, however, identified a lower prevalence of overall-pneumococcal and *H. influenzae* colonization in HIV-infected children post-PCV vaccination, the clinical-relevance of which warrants further study.

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* Corresponding author at: National Institute for Communicable Diseases/National Health Laboratory Service, 1 Modderfontein Road, Sandringham, Gauteng 2131, South Africa. Tel.: +27 11 3866137; fax: +27 11 8821872.
E-mail address: madhis@rmpru.co.za (S.A. Madhi).

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1. Background

Nasopharyngeal colonization by *Streptococcus pneumoniae* (pneumococcus) and other potentially pathogenic bacteria such as *Haemophilus influenzae* and *Staphylococcus aureus* is a pre-requisite for developing disease [1]. There are limited comparative studies on the effect of HIV infection or HIV-exposure on pneumococcal [2–9], *H. influenzae* or *S. aureus* nasopharyngeal colonization [7]. Furthermore, past studies in HIV-infected children were mainly cross-sectional, involving children above one year of age, usually not receiving antiretroviral treatment (ART), nor immunized with pneumococcal conjugate vaccine (PCV) [2–6,9].

The prevalence of pneumococcal nasopharyngeal colonization in HIV-infected children in previous studies was between 20% and 81% [4,7,8], and similar to HIV-uninfected children [3–6]. A longitudinal cohort study by Gill et al. [2] enrolling mainly HIV-uninfected children born to HIV-infected mothers (HEU), suggested that HEU children were 1.4-fold (95% confidence interval, [95% CI]: 1.0–1.9) more likely to be colonized by *S. pneumoniae* than children born to HIV-uninfected mothers (HIV-unexposed). Furthermore, cotrimoxazole prophylaxis has been associated with modest reduction (7%) of pneumococcal colonization without affecting any specific serotypes [3–6]. Previous studies among HIV-infected children mainly focused on pneumococcal nasopharyngeal colonization, excluding other potentially pathogenic bacteria such as *H. influenzae* and *S. aureus.*

PCV reduces the risk of nasopharyngeal colonization acquisition by vaccine-serotypes in healthy HIV-uninfected children, whilst increasing colonization by non-vaccine serotypes [10]. PCV immunization is associated with a temporal increase in *S. aureus* colonization among children [11]. HIV-infected children have a 40-fold greater risk of invasive pneumococcal disease and 98-fold greater risk of invasive *S. aureus* disease than uninfected children [12–14]. Therefore, comparing nasopharyngeal bacterial ecology in PCV-vaccinated HIV-infected and uninfected children could inform the relative role of colonization in predisposing to developing invasive disease.

The aim of this study was to determine the effect of HIV exposure, HIV infection and timing of ART initiation on the dynamics of *S. pneumoniae*, *S. aureus* and *H. influenzae* nasopharyngeal (NP) colonization in infants vaccinated with 7-valent PCV (PCV7).

2. Methods

This study addressed a secondary-objective in a cohort of HIV-infected and HIV-uninfected children in whom the immunogenicity of a three dose primary series of PCV7 (i.e. Prevnar®; Wyeth Vaccines, NJ, USA), administered at 6, 10 and 14 weeks of age was evaluated [15,16]. Briefly, PCV7 was administered concurrently with other recommended childhood vaccines in South Africa, including *H. influenzae* type-b conjugate vaccine (HibCV). Infants were enrolled in Soweto (Gauteng) and Tygerberg (Western Cape) in South Africa from April 2005 to June 2006. Participants included HIV-infected infants with CD4+ T-lymphocyte cells ≥25% randomized to initiate ART immediately (ART-Immed); or when clinically (CDC Stage C or investigator-selected severe Stage B) and/or immunologically indicated as per then WHO recommendations for CD4+ depletion (ART-Def). A convenience sample of HIV-infected children with CD4+ percentage <25% at enrolment and initiated on immediate ART (HIV+/CD4 < 25%), were also included. All HIV-infected children were co-enrolled in the Children with HIV Early Antiretroviral (CHER) study, in which the first line ART regimen used was zidovudine, lamivudine and lopinavir/ritonavir [17]. Children in the ART-Immed group were randomized to interrupt ART if clinically and immunologically stable at either one- or two-years of age [18].

Furthermore, 125 infants born to HIV-infected mothers who tested negative for HIV (HEU) and 125 born to HIV-uninfected mothers (HIV-unexposed) were also enrolled. Details of verification of the HIV-infection status of the infants and mothers have been described, including repeat testing of the HEU to confirm their HIV-uninfected status [15]. HIV-infected children received daily trimethoprim-sulfamethoxazole (cotrimoxazole) prophylaxis until at least 12 months age and HEU infants until 6 months of age. Adherence to cotrimoxazole prophylaxis was monitored at scheduled study-visits.

Nasopharyngeal swabs were collected prior to each of the three doses of PCV, which were scheduled for between 6–12 weeks (Visit-1), 9–18 weeks (Visit-2) and 12–24 weeks (Visit-3), respectively. Thereafter, swabs were repeated at 3–6 weeks after the third PCV dose (Visit-4) at 38–42 weeks (Visit-5), 44–52 weeks (Visit-6) and at 64–76 weeks of age (Visit-7). 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 [7]. Standard microbiologic tests were used for culture and identification of *S. pneumoniae*, *H. influenzae* and *S. aureus* as described [7]. Serotyping was performed with the Quellung method (Statens Serum Institute, Copenhagen, Denmark) and serotypes 4, 6B, 9V, 14, 18C, 19F or 23F categorized as PCV7 vaccine-serotypes and the rest as non-vaccine serotypes. Serotype 6A isolates were further tested by polymerase chain reaction to discriminate between serotype 6A and 6C as described [19].

2.1. Statistical analysis

Comparisons and adjusted odds ratios of colonization between groups were performed using logistic regression adjusted for study center, gender, race, receiving cotrimoxazole prophylaxis and for study-group when comparing HIV-infected (including ART-Def, ART-Immed and HIV+/CD4 < 25% groups) to HIV-uninfected children. To minimize confounding between the groups, children were only included in the analysis at the analyzed time-point if PCV vaccination and the study-visits occurred within the protocol defined window periods. To assess statistical significance, *p*-values are adjusted for multiple comparisons using a method which controls the false discovery rate. Adjusted *p*-values are reported and compared to alpha = 0.05 [20]. All analyses were performed in R (R Foundation for Statistical Computing; Vienna, Austria).

2.2. Ethics considerations

The study was approved by the Human Subjects Research Committees of the University of the Witwatersrand, Stellenbosch University, the Medicine Control Council of South Africa and Clinical Science Review Committee of the Division of AIDS. Signed informed consent was obtained from the parents of the children. The clinical trials registry reference number for the study is ClinicalTrials.gov NCT00099658.

3. Results

Overall, 527 infants were enrolled, including 244 (46.3%) males and 480 (91.1%) Black-Africans. The mean age at time of NP swabs did not differ between groups, being 7.4 (Standard Deviation; S.D.: 1.2), 11.4 (S.D.: 1.2), 15.5 (S.D.: 1.4), 19.5 (S.D.: 1.3), 39.4 (S.D.: 1.1), 47.4 (S.D.: 1.8) and 67.4 (S.D.: 2.0) weeks at Visit-1 though to

Table 1
Demographic features at various study visits when analyzed for nasopharyngeal bacterial colonization in HIV-infected and -uninfected children vaccinated with pneumococcal conjugate vaccine.

Variable	Overall	HUU[a]	HEU[b]	ART-Immed[c]	ART-Def[d]	HIV+/CD4 < 25%[e]
Total number of subjects[f]	527	114	122	193	88	10
Male sex (%)	244 (46.3)	62 (54.4)	66 (54.1)	80 (41.5)	32 (36.4)	4 (40.0)
Race: Black African (%)	480 (91.1)	85 (74.6)	114 (93.4)	185 (95.9)	87 (98.9)	9 (90.0)
Mixed ancestry (%)	47 (9.9)	29 (25.4)	8 (6.8)	8 (4.1)	1 (1.1)	1 (10.0)
Mean age (SD) weeks at Visit-1; *n*	7.4 (1.2); *N* = 527	7.0(1.0); *n* = 114	7.4 (1.0); *n* = 122	7.5 (1.2); *n* = 193	7.3 (1.2); *n* = 88	8.6 (1.6); *n* = 10
Mean age (SD) weeks at Visit-2; *n*	11.4 (1.2); *N* = 524	11.1 (1.1); *n* = 116	11.5 (1.0); *n* = 120	11.6 (1.3); *n* = 192	11.4 (1.3); *n* = 86	12.7 (1.8); *n* = 10
Mean age (SD) weeks at Visit-3; *n*	15.5 (1.4); *N* = 518	15.2 (1.2); *n* = 114	15.6 (1.1); *n* = 121	15.7 (1.6); *n* = 189	15.5 (1.3); *n* = 84	16.6 (1.7); *n* = 10
Mean age (SD) week at Vist-4 [1-month post 3rd PCV dose][f]; *n*	19.5 (1.3); *N* = 483	19.2 (1.2); *n* = 113	19.5 (1.0); *n* = 119	19.6 (1.3); *n* = 170	19.8 (1.5); *n* = 71	20.6 (1.6); *n* = 10
Mean age (SD) weeks at Visit-5 [38–42 weeks age][f]; *n*	39.4 (1.1); *N* = 446	38.8 (0.8); *n* = 108	39.2 (0.9); *n* = 115	39.5 (1.2); *n* = 154	39.9 (1.3); *n* = 61	40.3 (1.4); *n* = 8
Mean age (SD) weeks at Visit-6 [44–52 weeks][f]; *n*	47.4 (1.8); *N* = 402	46.9 (1.9); *n* = 100	47.3 (1.9); *n* = 96	47.6 (1.4); *n* = 150	47.8 (1.8); *n* = 49	48.1 (1.4); *n* = 7
Mean age (SD) weeks at Visit-7 [64–76 weeks][f]; *n*	67.4 (2.0); *N* = 447	66.6 (1.4); *n* = 108	67.1 (2.2); *n* = 114	67.8 (1.8); *n* = 153	68.4 (2.6); *n* = 63	68.0 (1.5); *n* = 9

[a] HUU: HIV-uninfected children born to HIV-uninfected mothers.
[b] HEU: HIV-uninfected children born to HIV-infected mothers.
[c] ART-Immed: HIV-infected children with CD4+ cell count ≥25% started on antiretroviral treatment (ART) at time of first dose of PCV7.
[d] ART-Def: HIV-infected children with CD4+ cell count ≥25% at time of first dose of PCV7 that were started on ART based on clinical or immunological indications.
[e] HIV+/CD4+ <25%: HIV-infected children with CD4+ <25% at enrolment and initiated on immediate ART.
[f] Include subjects who received all three PCV doses within protocol-defined window periods.

Visit-7, respectively (Table 1). The first three study visits coincided with PCV7 vaccination.

The median CD4+ T-lymphocyte cell count and percent in HIV-infected infants at Visits 1, 3–7 are indicated in Supplementary Table 1. Generally, CD4+ cell count and CD4+ percent were higher in the ART-Immed compared to ART-Def group from Visit-3 until Vist-6, whilst similar at Visit-7. The proportion of children receiving cotrimoxazole prophylaxis ranged from 95% to100% in ART-Immed and ART-Def children between Visit-1 and Visit-5 and decreased subsequently to 52–56% by Visit-7 (Supplementary Table 1). Also, 44% to 76% of HEU were receiving cotrimoxazole prophylaxis between Visit-1 and Visit-4, decreasing to 24% and less from Visit-5 onward.

3.1. Colonization in HIV-unexposed compared to HEU children

Among HEU children, being on cotrimoxazole prophylaxis was not associated with any difference in prevalence of colonizing organisms throughout the study (data not shown). The prevalence of overall pneumococcal, vaccine-serotype and non-vaccine serotype colonization did not differ between HEU and HIV-unexposed children, throughout the study; Table 2. Vaccine-serotype colonization increased from 6.4% among the composite group of HIV-uninfected children (i.e. HIV-unexposed and HEU) at Visit-1 to 14.7% one-month following the third dose of PCV (Visit-4; $p = 0.008$) and remained similar thereafter (range from 17.6% to 21.4%); Fig. 1 and Supplementary Table 2. Colonization by non-vaccine serotypes was at least two-fold greater than vaccine-serotype colonization in HIV-uninfected children ($p < 0.002$ for all time-points), including being 18.9% at Visit-1 and increasing to approximately 40% from Visit-5 (9 months of age) onward (Fig. 1 and Supplementary Table 2).

The prevalence of *H. influenzae* colonization did not differ between HIV-unexposed and HEU children at any time-point (Table 3). *S. aureus* prevalence was higher in HIV-unexposed infants at Visit-2 through Visit-4 (44.3%, 43.0% and 34.8%, respectively) compared to HEU infants (25.0%, 23.1%, 17.9%, respectively; $p = 0.002$ for all), albeit similar thereafter (Table 3). The higher prevalence of *S. aureus* colonization in HIV-unexposed compared to HEU infants at Visits-3 and 4 was evident independent of whether the HEU infants were receiving cotrimoxazole prophylaxis (data not shown). Among the composite group of HIV-uninfected children, the prevalence of *H. influenzae* colonization increased steadily from 27.1% at Visit-1 to 59.5% at Visit-7 (chi square for trend $p < 0.001$), whilst conversely the prevalence of *S. aureus* declined from 52.8% at Vist-1 to 12.7% at Visit-7 ($p < 0.001$) (Fig. 2 and Supplementary Table 2).

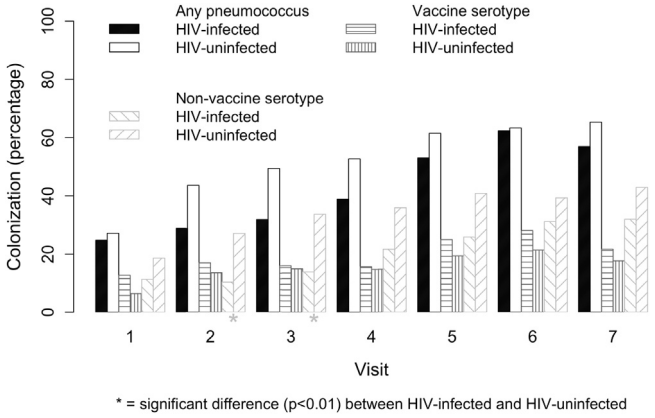

Fig. 1. Pneumococcal colonization prevalence in HIV-infected and HIV-uninfected infants during the first 18 months of life.

3.2. Colonization in HIV-infected children managed with either early or deferred antiretroviral treatment

Colonization patterns for pneumococcus, *H. influenzae* and *S. aureus* were similar between ART-Def and ART-Immed children; Tables 2 and 3. The only exceptions being a lower overall pneumococcal colonization at Visit-6 in the ART-Def (46.7%) compared to ART-Immed group (69.4%; $p = 0.042$), due to a lower prevalence of non-vaccine serotype colonization (15.6% vs. 37.4%, respectively; $p = 0.023$), Table 2. Cotrimoxazole prophylaxis was not associated with any difference in colonization in ART-Def and ART-Immed infants at Visit-6 and Visit-7 (Supplementary Table 3).

Table 2
Prevalence of nasopharyngeal (NP) colonization by *Streptococcus pneumoniae* stratified by vaccine-serotypes and non-vaccine serotypes in HIV-infected and -uninfected children vaccinated with pneumococcal conjugate vaccine.

Study time-point NP swab analyzed for pneumococcal colonization	HIV-uninfected children			HIV-infected children				
	HUU[a]	HEU[b]	*p*-Value[c]	ART-Def[d]	*p*-Value[e]	ART-Immed[f]	*p*-Value[g]	*p*-Value[h]
Visit-1	***N* = 114**	***N* = 122**		***N* = 88**		***N* = 193**		
Overall Pnc[i]	33;28.9% (21.4–37.9)	31;25.4% (18.5–33.8)	0.826	22;25% (17.1–35)	0.834	49;25.4% (19.8–32.0)	0.826	0.93
VT-Pnc[j]	7;6.1% (3.0–12.1)	8;6.6% (3.4–12.4)	0.982	9;10.2% (5.5–18.3)	0.555	27;14% (9.8–19.6)	0.150	0.560
NVT-Pnc[k]	23;20.2% (13.8–28.5)	21;17.2% (11.5–24.9)	0.876	11;12.5% (7.1–21.0)	0.426	22;11.4% (7.6–16.7)	0.181	0.888
Visit-2	***N* = 116**	***N* = 120**		***N* = 86**		***N* = 192**		
Overall Pnc	54;46.6% (37.7–55.6)	49;40.8% (32.5–49.8)	0.698	21;24.4% (16.6–34.5)	0.014	61;31.8% (25.6–38.7)	0.063	0.446
VT-Pnc	17;14.7% (9.4–22.2)	15;12.5% (7.7–19.6)	0.816	12;14% (8.2–22.8)	0.982	36;18.8% (13.9–24.9)	0.560	0.570
NVT-Pnc	34;29.3% (21.8–38.2)	30;25.0% (18.1–33.4)	0.816	8;9.3.0% (4.8–17.3)	0.010	22;11.5% (7.7–16.7)	0.003	0.826
Visit-3	***N* = 114**	***N* = 121**		***N* = 84**		***N* = 188**		
Overall Pnc	61;53.5% (44.4–62.4)	55;45.5% (36.9–54.3)	0.543	25;29.8% (21–40.2)	0.018	64;34.0% (27.6–41.1)	0.018	0.724
VT-Pnc	18;15.8% (10.2–23.6)	17;14% (9.0–21.4)	0.828	14;16.7% (10.2–26.1)	0.888	30;16.0% (11.4–21.9)	0.970	0.876
NVT-Pnc	42;36.8% (28.6–46.0)	37;30.6% (23.1–39.3)	0.610	9;10.7% (5.7–19.1)	0.002	30;16.0% (11.4–21.9)	0.002	0.364
Visit-4	***N* = 113**	***N* = 119**		***N* = 70**		***N* = 170**		
Overall Pnc	63;55.8% (46.6–64.6)	59;49.6% (40.8–58.4)	0.626	25;35.7% (25.5–47.4)	0.058	68;40.0% (32.9–47.5)	0.056	0.826
VT-Pnc	17;15% (9.6–22.8)	17;14.3% (9.1–21.7)	0.826	8;11.4% (5.9–21)	0.682	29;17.1% (12.1–23.4)	0.876	0.555
NVT-Pnc	45;39.8% (31.3–49)	38;31.9% (24.2–40.8)	0.560	15;21.4% (13.4–32.4)	0.071	37;21.8% (16.2–28.6)	0.014	0.993
Visit-5	***N* = 108**	***N* = 115**		***N* = 59**		***N* = 154**		
Overall Pnc	70;64.8% (55.4–73.2)	67;58.3% (49.1–66.9)	0.762	28;47.5% (35.3–60)	0.198	88;57.1% (49.2–64.7)	0.610	0.481
VT-Pnc	24;22.2% (15.4–30.9)	19;16.5% (10.8–24.4)	0.570	15;25.4% (16.1–37.8)	0.727	40;26.0% (19.7–33.4)	0.621	0.993
NVT-Pnc	44;40.7% (31.9–50.2)	47;40.9% (32.3–50.0)	0.888	12;20.3% (12–32.3)	0.060	44;28.6% (22–36.2)	0.187	0.481
Visit-6	***N* = 100**	***N* = 96**		***N* = 45**		***N* = 147**		
Overall Pnc	69;69.0% (59.4–77.2)	55;57.3% (47.3–66.7)	0.221	21;46.7% (32.9–60.9)	0.056	102;69.4% (61.5–76.3)	0.982	0.042
VT-Pnc	24;24.0% (16.7–33.2)	18;18.8% (12.2–27.7)	0.680	11;24.4% (14.2–38.7)	0.910	44;29.9% (23.1–37.8)	0.481	0.834
NVT-Pnc	41;41.0% (31.9–50.8)	36;37.5% (28.5–47.5)	0.632	7;15.6% (7.7–28.8)	0.012	55;37.4% (30.0–45.5)	0.603	0.023
Visit-7	***N* = 108**	***N* = 114**		***N* = 54**		***N* = 150**		
Overall Pnc	70;64.8% (55.4–73.2)	75;65.8% (56.7–73.9)	0.826	34;63.0% (49.6–74.6)	0.960	84;56.0% (48.0–63.7)	0.560	0.555
VT-Pnc	18;16.7% (10.8–24.8)	21;18.4% (12.4–26.5)	0.888	14;25.9% (16.1–38.9)	0.371	30;20.0% (14.4–27.1)	0.689	0.624
NVT-Pnc	47;43.5% (34.6–52.9)	48;42.1% (33.4–51.3)	0.982	16;29.6% (19.1–42.8)	0.289	51;34.0% (26.9–41.9)	0.364	0.820

[a] HUU: HIV-unexposed, uninfected children.
[b] HEU: HIV-uninfected infant born to HIV-infected mother.
[c] *p*-Value comparing HEU to HUU.
[d] ART-Def: HIV-infected children with CD4+ cell count ≥25% at time of first PCV-7 dose that were started on antiretroviral treatment (ART) based on clinical or immunological indications.
[e] *p*-value: comparing ART-Def to HUU.
[f] ART-Immed: HIV-infected children with CD4+ cell count ≥25% started on ART at the time of first dose of PCV-7.
[g] *p*-Value: comparing ART-Immed to HUU.
[h] *p*-Value: comparing ART-Def to ART-Immed.
[i] Overall Pnc: all *Streptococcus pneumoniae* serotypes.
[j] VT-Pnc: 7-valent pneumococcal conjugate vaccine serotypes (i.e. 4, 6B, 9V, 14, 18C, 19F, 23F).
[k] NVT-Pnc: serotypes not included in pneumococcal conjugate vaccine. Number in the cell refers to number of positive observations, percentage of the total and value in parenthesis are 95% confidence intervals. All *p*-values have been adjusted for multiple comparisons.

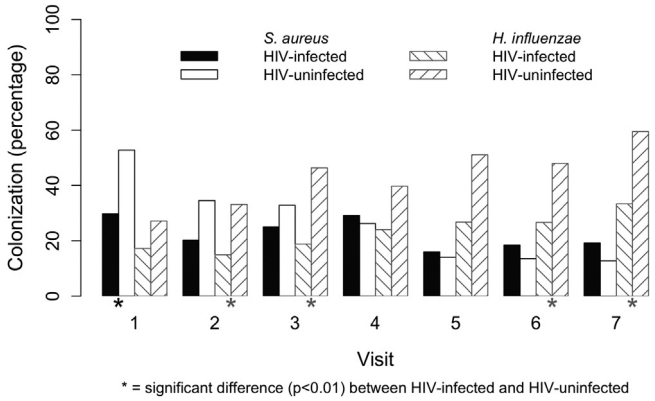

Fig. 2. *Staphylococcus aureus* and *Haemophilus influenzae* colonization prevalence in HIV-infected and HIV-uninfected infants during the first 18 months of life.

3.3. Pneumococcal colonization in HIV-infected compared to HIV-uninfected children

Compared to HIV-unexposed children, ART-Def and ART-Immed infants had a lower prevalence of overall pneumococcal colonization from Visit-3 to Visit-4 (as well as Visit-2 and Visit-6 in ART-Def group), which was specifically evident for non-vaccine serotypes; Table 2. At Visit 6, the lower prevalence of non-vaccine serotype colonization among ART-Def children was driven by receipt of cotrimoazole prophylaxis (aOR: 0.177; $p = 0.001$), however, this was not evident in the ART-Immed group (Supplementary Table 3).

Among the composite group of all HIV-infected children, the prevalence of vaccine-serotype colonization increased from 12.7% at Visit-1 to 24.9% at 9-months of age (Visit-5; chi square for trend $p < 0.001$) and remained similar thereafter up until Visit-7 (chi square for trend $p = 0.197$). There was a similar prevalence of vaccine-serotype compared to non-vaccine serotype colonization among HIV-infected children from Visit-1 through Visit-6, although this trended to being lower for vaccine-serotype at Visit-7 (21.6% vs. 31.9%; $p = 0.059$) (Fig. 1 and Supplementary Table 2). The prevalence

Table 3
Prevalence of nasopharyngeal colonization by *Staphylococcus aureus* and *Haemophilus influenzae* in HIV-infected and -uninfected children vaccinated with pneumococcal conjugate vaccine.

Study time-point and organism	HIV-uninfected children			HIV-infected children				
	HUU[a]	HEU[b]	*p*-value[c]	ART-Def[d]	*p*-value[e]	ART-Immed[f]	*p*-value[g]	*p*-value[h]
Visit-1	***N*=114**	***N*=122**		***N*=88**		***N*=193**		
S. aureus[i]	63;55.8% (46.6–64.6); *N*=113	61;50% (41.3–58.7)	0.269	30;34.5% (25.3–44.9); *N*=87	0.005	49;25.4% (19.8–32)	<0.001	0.198
H. influenzae[j]	38;33.3% (25.3–42.4)	26;21.3% (15.0–29.4)	0.187	14;15.9% (9.7–25.0)	0.059	36;18.7% (13.8–24.7)	0.056	0.820
Visit-2	***N*=116**	***N*=120**		***N*=86**		***N*=192**		
S. aureus	51;44.3% (35.6–53.5); *N*=115	30;25.0% (18.1–33.4)	0.002	17;19.8% (12.7–29.4)	0.001	35;18.3% (13.5–24.4); *N*=191	<0.001	0.820
H. influenzae	41;35.3% (27.2–44.4)	37;30.8% (23.3–39.6)	0.809	14;16.3% (10.0–25.5)	0.027	28;14.6% (10.3–20.3)	0.002	0.834
Visit-3	***N*=114**	***N*=121**		***N*=84**		***N*=188**		
S. aureus	49;43.0% (34.3–52.2)	28;23.1% (16.5–31.4)	0.002	20;24.1% (16.2–34.3); *N*=83	0.006	47;25.5% (19.8–32.3); *N*=184	0.002	0.876
H. influenzae	55;48.2% (39.3–57.3)	54;44.6% (36.1–53.5)	0.950	17;20.2% (13.0–30.0)	0.006	34;18.1% (13.2–24.2)	<0.001	0.826
Visit-4	***N*=113**	***N*=119**		***N*=70**		***N*=170**		
S. aureus	39;34.8% (26.6–44.0); *N*=112	21;17.9% (12.0–25.9); *N*=117	0.002	24;34.8% (24.6–46.6); *N*=69	0.481	43;27.2% (20.9–34.6); *N*=158	0.067	0.560
H. influenzae	50;44.2% (35.4–53.4)	42;35.3% (27.3–44.2)	0.446	21;30.0% (20.5–41.5)	0.187	37;21.8% (16.2–28.6)	0.002	0.452
Visit-5	***N*=108**	***N*=115**		***N*=59**		***N*=154**		
S. aureus	18;16.8% (10.9–25.0); *N*=107	13;11.4% (6.8–18.5); *N*=114	0.342	13;22.0% (13.4–34.1)	0.706	18;12.3% (7.9–18.6); *N*=146	0.496	0.125
H. influenzae	56;51.9% (42.5–61.0)	58;50.4% (41.4–59.4)	0.940	13;22.0% (13.4–34.1)	0.005	41;26.6% (20.3–34.1)	0.002	0.866
Visit-6	***N*=100**	***N*=96**		***N*=45**		***N*=147**		
S. aureus	12;12.2% (7.1–20.2); *N*=98	14;14.7% (9.0–23.2); *N*=95	0.783	13;26.5% (16.2–40.3); *N*=49	0.080	23;15.9% (10.8–22.7); *N*=145	0.557	0.173
H. influenzae	49;49.0% (39.4–58.7)	45;46.9% (37.2–56.8)	0.930	8;17.8% (9.3–31.3)	0.010	45;30.6% (23.7–38.5)	0.047	0.165
Visit-7	***N*=108**	***N*=114**		***N*=54**		***N*=150**		
S. aureus	15;14.0% (8.7–21.8); *N*=107	13;11.5% (6.8–18.7); *N*=113	0.528	12;19.7% (11.6–31.3); *N*=61	0.834	27;18.1% (12.8–25.1); *N*=149	0.878	0.910
H. influenzae	64;59.3% (49.8–68.1)	68;59.6% (50.5–68.2)	0.834	17;31.5% (20.7–44.7)	0.018	54;36.0% (28.8–43.9)	0.011	0.762

[a] HUU: HIV-unexposed, uninfected children.
[b] HEU: HIV-uninfected infant born to HIV-infected mother.
[c] *p*-Value: comparing HEU to HUU.
[d] ART-Def: HIV-infected children with CD4+ cell count ≥25% at time of first PCV-7 dose that were started on antiretroviral treatment (ART) based on clinical or immunological indications.
[e] *p*-Value: comparing ART-Def to HUU.
[f] ART-Immed: HIV-infected children with CD4+ cell count ≥25% started on ART at the time of first dose of PCV-7.
[g] *p*-Value: comparing ART-Immed to HUU.
[h] *p*-Value: comparing ART-Def to ART-Immed.
[i] *S. aureus*: *Staphylococcus aureus.*
[j] *H. influenzae*: *Haemophilus influenzae.* Number in the cell refers to number of positive observations, percentage of the total and value in parenthesis are 95% confidence intervals. All *p*-values have been adjusted for multiple comparisons.

of vaccine-serotype colonization was similar between HIV-infected and HIV-uninfected children (Fig. 1 and Supplementary Table 2). Non-vaccine serotype colonization was generally higher in HIV-uninfected than HIV-infected children from Visit-2 to Visit-3, and a similar trend observed thereafter (Fig. 1 and Supplementary Table 2).

3.4. S. aureus *colonization in HIV-infected and HIV-uninfected children*

Compared to HIV-unexposed children, ART-Def and ART-Immed infants had a lower prevalence of colonization by *S. aureus* from Visit-1 to Visit-3, which was however similar thereafter, Table 3. Overall, HIV-infected children had a lower prevalence of *S. aureus* colonization (29.7%) than HIV-uninfected children (52.8%; $p=0.010$) at Visit-1, which did not however differ significantly thereafter (Fig. 2; Supplementary Table 2). At Visit-6 and Visit-7, the prevalence of *S. aureus* colonization in both groups of HIV-infected children on cotrimoxazole prophylaxis was similar to those not on prophylaxis; as well as compared to HIV-unexposed children (Supplementary Table 2).

3.5. H. influenzae *colonization in HIV-infected compared to HIV-uninfected children*

The prevalence of *H. influenzae* colonization was consistently lower in ART-Def and ART-Immed children from Visit-1 up until Visit-7, compared to HIV-unexposed infants, with the exception of Visit-4 in ART-Def; Table 3. The prevalence of *H. influenzae* colonization in HIV-infected children steadily increased from 17.2% at Visit-1 to 33.3% (chi square for trend $p<0.001$) at Visit-7. *H. influenzae* colonization was, however, greater in the overall groups of HIV-uninfected than HIV-infected children at Visit-2, Visit-3 Visit-6 and Visit-7, with a similar trend observed at the other visits (Supplementary Table 2). At visits 5 and 6, the lower prevalence of *H. influenzae* colonization among the HIV-infected groups compare to HIV-unexposed children trended to be more evident in those who remained on cotrimoxazole prophylaxis, than in those in whom prophylaxis had been discontinued (Supplementary Table 3).

4. Discussion

To our knowledge, this is the first longitudinal study to detail the dynamics of nasopharyngeal colonization by three potentially

pathogenic bacteria in HIV-infected and HEU children vaccinated with PCV. Our findings suggests that a three dose primary series of PCV have a similar effect against vaccine-serotype colonization in HEU, HIV-infected and HIV-unexposed children in the first 18 months of life. There were, however, differences observed in nasopharyngeal ecology between HIV-infected and HIV-uninfected children. There was a lower overall prevalence of pneumococcal colonization among HIV-infected children predominantly attributable to a lower prevalence of non-vaccine serotype colonization. There was also a lower prevalence of *H. influenzae* from Visit-2 onward in HIV-infected compared to HIV-uninfected children; as well as a lower prevalence of *S. aureus* colonization in HIV-infected children at Visit-1.

The HIV-infected children in our study differ from previous studies on bacterial colonization interactions [7,21], as they were either initiated on antiretroviral treatment immediately upon diagnosis of HIV infection or within the first year of life. Previous studies in ART naïve HIV-infected children suggested that the synergistic relationship observed among healthy children between pneumococcal and *H. influenzae* colonization and negative association between pneumococcal and *S. aureus* colonization [22,23], were not evident among HIV-infected children [7,21]. In contrast, in our study, the lower overall prevalence of pneumococcal colonization was associated with a lower prevalence of *H. influenzae* and higher prevalence of *S. aureus* colonization in HIV-infected than HIV-uninfected children. Therefore, while host adaptive immune factors may have contributed to the lack of association between these bacteria in the earlier studies on HIV-infected children, we observed these interactions in the relatively immunocompetent HIV-infected children in our study.

The biological mechanism for the interactions between pneumococcus, *H. influenzae* and *S. aureus* and possibly other bacteria, as well as respiratory viruses was recently reviewed by Bosch et al. [1]. Whilst the complexity of bacterial–bacterial, viral–bacterial and viral–viral interactions is increasingly being explored, a number of mechanisms have been proposed for the interactions between *S. pneumoniae*, *H. influenzae* and *S. aureus*. This includes *S. pneumoniae* producing hydrogen peroxide which inhibits *S. aureus* and *H. influenzae* colonization and immune-mediated cross-reactive antibody between *S. pneumoniae* and *S. aureus* [24–26]. Also, the expression of phosphorylcholine by both *S. pneumoniae* and *H. influenzae* and neuraminidase produced by *S. pneumoniae* may contribute to competitive in vitro effects between these species [1]. Furthermore, pilus-island containing pneumococcal species has been negatively associated with *S. aureus* colonization [27].

The complexity of this bacterial–bacterial interplay is, however, further manifest in that while in vitro studies indicate a negative association between *S. pneumoniae* and *H. influenzae* colonization, an opposite synergistic association was observed in healthy children but not so among immunocompromized HIV-infected children [7,28]. This underscores, the possible additional role of the host, including adaptive immune factors, which may contribute to the interaction of potentially pathogenic bacteria and possibly commensal bacteria in the nasopharynx. Recent observations at our site support adaptive immune factors as synergistic interaction between *S. pneumoniae* and *H. influenzae* and negative competitive association between *S. pneumoniae* or *H. influenzae* and *S. aureus* observed in children, were not evident in adults [28].

We speculate that the reason for the differences observed in prevalence of pneumococcal and *H. influenzae* colonization between the PCV-vaccinated HIV-infected and HIV-uninfected children in our study, may be due to receipt of cotrimoxazole prophylaxis in HIV-infected children. Previous studies in HIV-infected individuals have documented a lower prevalence of pneumococcal colonization in those on cotrimoxazole prophylaxis [3–6], which in turn could result in lower prevalence of *H. influenzae* (which acts synergistically with *S. pneumoniae* colonization) and higher prevalence of *S. aureus* colonization (for which negative association with pneumococcal colonization has been established). Our study was, however, limited in exploring whether cotrimoxazole prophylaxis was responsible for the lower prevalence of non-vaccine serotype and *H. influenzae* colonization during the earlier study visits (Visits 1–5), as the majority of HIV-infected children were on prophylaxis. Also, whereas there was a trend toward stronger association of the ART-Def an ART-Immed children who were on cotrimoxazole prophylaxis being less likely to be colonized by *H. influenzae* at Visit-6 and Visit-7, for the non-vaccine serotypes this was only evident among the ART-Def group. Similarly, there was a lower prevalence of *S. aureus* colonization among the HIV-infected groups compared to HIV-unexposed children from Visit-1 to Visit 3, which again we were unable to evaluate if this was due to cotrimoxazole prophylaxis since the majority of HIV-infected children were on cotrimoxazole at this stage.

The lower prevalence of colonization by non-vaccine serotypes and *H. influenzae*, nevertheless bode well for HIV-infected children, premised on their disease-causing potential being similar in HIV-uninfected children when early ART is initiated. Also, the similarity in *S. aureus* colonization prevalence between HIV-infected and HIV-uninfected children in our study, indicates that they could be less at risk of severe *S. aureus* invasive disease compared to that previously observed in ART naïve HIV-infected children [12,14].

Previous studies in healthy children have attributed an increase in *S. aureus* colonization to PCV immunization [11], having also established an inverse association between colonization by vaccine-serotypes (but not non-vaccine serotypes) and *S. aureus* [29]. Although our study did not include a PCV unvaccinated control group of unvaccinated children, the prevalence of *S. aureus* colonization at 9 and 15 months of age was similar to that observed in a separate parallel cohort of PCV-unvaccinated HIV-uninfected children [28].

Limitations of our study include the absence of PCV-unvaccinated control groups, as PCV was already licensed in South Africa at the time of the study, although not yet available in the public immunization program. Consequently, we compared the dynamics of bacterial nasopharyngeal colonization in our study to a referent group of HUU PCV-vaccinated infants. An earlier study in the same community had previously observed a 50% reduction in vaccine-serotype colonization among HIV-uninfected children 6-months (approximately equivalent to Visit-5 in our study) following the primary three-dose series of PCV [30]. Also, our study has not yet analyzed for antibiotic susceptibility of the colonizing isolates, to determine what effect this may have on colonization patterns in the context of HEU and HIV-infected infants receiving cotrimoxazole prophylaxis.

Although bacterial nasopharyngeal colonization is not a measure of disease, it is increasingly advocated as an additional measure of the potential efficacy of different PCV formulations [31]. Also, there is a strong association between the dominant pneumococcal serotypes colonizing the nasopharynx, detected by culture method, and those serotypes causing pneumococcal disease. Furthermore, examining the ecology of the nasopharynx may also contribute to understanding whether the increased risk of invasive disease in immunocompromized individuals, including HIV-infected children, is a function of innate impairment of immunity or because of an increased risk of nasopharyngeal acquisition and colonization by specific bacteria.

Our findings indicate that the increased risk of pneumococcal disease in HIV-infected children including in the era of PCV immunization [32], is unlikely due to an increase in prevalence of pneumococcal colonization, but rather due to other immunosuppressive mediators which predispose to pneumococcal disease.

Disclaimer

The content of this publication does not necessarily reflect the views or policies of NIAID, nor does mention of trade names, commercial projects, or organizations imply endorsement by the US Government.

Conflict of interest

Receipt of research funding, honoraria and consultancy from Pfizer (SAM). Receipt of research grants, consultancies and honoraria from GSK (SAM, KPK). Receipt of research funding from Pfizer (AvG). Prevenar was donated by Wyeth Vaccines and Pediatrics (now Pfizer).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.04.024

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