HUMAN VACCINES & IMMUNOTHERAPEUTICS
2020, VOL. 16, NO. 8, 1918–1922
https://doi.org/10.1080/21645515.2019.1706411

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SHORT REPORT

Impact of pneumococcal conjugate vaccine on the carriage density of *Streptococcus pneumoniae* and *Staphylococcus aureus* in children living with HIV: a nested case–control study

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ABSTRACT

Nasopharyngeal colonization density of *Streptococcus pneumoniae* (pneumococcus) is associated with disease severity and transmission. Little is known about the density of pneumococcal carriage in children with HIV (CLH). Pneumococcal vaccines may impact the density of pneumococcus and competing microbes within the nasopharynx. We examined the impact of one dose of PCV13 on carriage density of pneumococcus and *Staphylococcus aureus*, in CLH, HIV-uninfected children (HUC), and their unvaccinated parents. We conducted a pilot-nested case–control study, within a larger prospective cohort study, on the impact of PCV13, in families in West Bengal India. Quantitative real-time PCR was run on 147 nasopharyngeal swabs from 27 CLH and 23 HUC, and their parents, before and after PCV13 immunization. CLH had higher median pneumococcal carriage density, compared to HUC: 6.28×10^8 copies/mL vs. 2.11×10^5 copies/mL ($p = .005$). Following one dose of PCV13, pneumococcal densities dropped in both groups, with an increase in *S. aureus* carriage to 80% from 48% in CLH, and to 60% in HUC from 25%. While limited in sample size, this pilot study shows that CLH carried higher densities of pneumococcus. PCV13 was associated with a decrease in pneumococcal density and a temporal increase in *S. aureus* carriage regardless of HIV status.

ARTICLE HISTORY
Received 2 September 2019
Revised 10 December 2019
Accepted 13 December 2019

KEYWORDS
Streptococcus pneumoniae; *Staphylococcus aureus*; HIV; colonization density; PCV13; nasopharynx microbiology

Nasopharyngeal (NP) colonization is a prerequisite for invasive disease from *Streptococcus pneumoniae* (pneumococcus) and *Staphylococcus aureus* (*S. aureus)*. The density of colonization within the nasopharynx, determines transmission, pneumonia susceptibility, and disease severity, specifically in children, and HIV-infected individuals.[1–4]

HIV infection in children increases the risk of invasive pneumococcal disease (IPD), and invasive disease due to *S. aureus*, by 40 to 100 times.[5,6] Typically, there is a negative association between pneumococcus and *S. aureus* carriage in healthy individuals;[7–11] however, in children with HIV, this may disappear.[9,12–14]

Access to pneumococcal conjugate vaccines (PCV) globally have significantly decreased vaccine serotype (VT) IPD and carriage. With the decrease in VT pneumococcus in the nasopharynx, a temporary increase in *S. aureus* carriage has been observed in young children.[10,15,16] PCV13 is being introduced in India in a phased manner, but not yet in programs for children with HIV. We looked at the impact of one dose of PCV13 catch-up immunization on pneumococcal and *S. aureus* carriage density, in vaccinated HIV infected and uninfected Indian children, and their unvaccinated parents, before and after PCV13.

We conducted a nested case–control study, within a larger prospective cohort study on pneumonia prevention in HIV-infected children in rural West Bengal, carried out from March 2012 to September 2014.[17–19] Children living with HIV (CLH) and HIV-uninfected children (HUC), received one dose of 13-valent pneumococcal conjugate vaccine (PCV13, *Prevnar 13*®, Pfizer) as catch-up immunization. Over 1800 nasopharyngeal (NP) calcium alginate swabs were collected in 1 mL of skim milk tryptone glucose glycerin (STGG) from children, and one of their unvaccinated parents, at multiple time points before and after vaccination, and banked at −80°C. As part of this study, quantitative real-time polymerase chain reaction (PCR) was run on nasopharyngeal swabs from a random selection of 147 NP swabs collected at baseline, and 2-months post-PCV13 immunization, from children five and under, and their parents. Twenty-seven children had HIV (CLH) and 23 did not (HUC). A small number of swabs were investigated because this was an exploratory pilot study. The *Institutional Ethical Committee*, of the Indian Institute of Technology-Kharagpur approved the study. Written informed consent was obtained from all study participants.

DNA was extracted from swabs using the RTP pathogen kit (Stratec®). We ran quantitative PCR for *S. pneumoniae* (*LytA)* and *S. aureus* [*sensor histidine kinase* (vicK)], using the FTD Respiratory pathogens 21 plus kit (FTD Diagnostics®). Positive samples had cycle threshold (Ct) value ≤38. The Ct value is defined as the number of amplification cycles required for the detection of the target organism nucleic acid; thus, higher Ct values indicate the lower density of bacterial DNA. The colonization density in copies/mL was calculated using reference standards.

Weight-for-age z-scores (WAZ) were calculated using EpiInfo™7. Children with Z scores between −2 and −3, fell into the moderately malnourished category; those below −3, were categorized as severely malnourished.[20] CLH were categorized

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into immunologic categories based on CD4 counts.[21] χ^2 or Fisher's exact test were used for categorical variables, and Wilcoxon rank-sum test and two-sample t test, for Ct value and density. The risk for carriage was calculated by logistic regression and for carriage density by linear regression. All data analysis was performed using Stata 13 (Stata Corp.).

The 147 swabs came from 27 CLH and their parents, and 23 HUC and their parents, collected at two time points (Table 1). Quantitative real-time PCR was run on nasopharyngeal swabs from 23 CLH, 20 HUC, 17 PCLH, and 15 PHUC before PCV-13 immunization, and 20 children and 16 parents of each group after PCV-13 immunization. The median age of CLH was 3.9 years at baseline, and 3.1 for HUC. Stunting and wasting were more prevalent in CLH. The median WAZ and HAZ scores were lower in CLH (WAZ −2.33; HAZ −1.96) compared to HUC (WAZ −1.33; HAZ −0.92) (p = .003; p = .0028). All PCLH were HIV-infected, and 78% of them were on antiretroviral treatment (ART). Mothers contributed 89% of all parental swabs.

Twenty-six percent of CLH were on ART for a median duration of 2 months at baseline. Most CLH were in immune stage 2 (48%) or 3 (29%) at baseline. The median CD4 count was 677 cells/mm^3 throughout the study.

The pneumococcal carriage rates were similar in CLH (9/23, 39%) and HUC (8/20, 40%) at baseline (p = .95) (Table 2). Children with stage 2 and 3 HIV disease had 88% carriage compared to children with stage 1 HIV (11%) (p = <0.001). Carriage rates did not change following immunization in either CLH or HUC [7/20 (35%) in both p = 1.0]. Post-PCV13 in CLH, carriage was found only in children with stage 2 (4/7, 57%) and 3 disease (3/7, 42%), and mainly in those not on ART (4/7, 57%).

Overall, pneumococcus was more frequently detected in children (31/83; 37%) than in parents (13/64; 20%), regardless of HIV or immunization status (p = .025). Parents were 8 times more likely to carry pneumococcus if their child was colonized (p = .006), suggesting familial carriage. No difference was noted in the carriage in either group of parents before [PCLH (11%); PHUC (20%) p = .52], and after their child received PCV13 [PCLH (31%), PHUC (18%)] (p = .41)] (Table 2).

The median density of pneumococcal carriage was higher in CLH as compared to HUC: 6.28×10^8 copies/mL vs. 2.11×10^5 copies/mL (p = .009) at baseline (Table 2). Most CLH (8/9, 88%) had carriage density ≥1x10^6 copies/mL; in contrast, only one HUC had a density ≥10^6 at baseline. This was reflected in the differences in Ct values of CLH compared to HUC (p = .0053) (Table 2). The pneumococcal density in children had a strong association with HIV in child [Regression coefficient (Coeff) 4.89; 95% CI 1.984–7.79; p = .002] and HIV in mothers [Coeff 4.22, 95% CI 1.17–7.27; p = .008].

Following PCV13, there was a decline in pneumococcal densities in both CLH (3.77x10^7 copies/mL; p = .11) and in HUC (4.94x10^4 copies/mL; p = .70), but neither achieved statistical significance. There was, however, a significant increase in the Ct values in both groups, reflecting a reduction in bacterial load (CLH [20.44], HUC [32.22]; p = .018). Post-PCV13, no child in the HIV group carried ≥10^9 copies, and (4/7) 57% of carriers in the HUC had <10^5 copies.

We also looked at the *S. aureus* carriage in children and their parents pre- and post-PCV13. At baseline, 48% (11/23) of CLH had *S. aureus* carriage compared to 25% (5/20) in HUC (p = .12) (Table 3). Ninety percent (10/11) of CLH having *S. aureus* carriage at baseline had stage 2 or 3 HIV disease; and 63% (7/11) were not on ART. Following PCV13, *S. aureus* carriage increased to 80% (16/20) (p = .029) in CLH, and 60% (12/20) (p = .025) in HUC. There was a trend toward an increase in carriage densities of *S. aureus* following PCV-13 in CLH, but not in HUC.

S. aureus carriage did not differ between the parents of either group at baseline (PCLH 29%, PHUC 27%; p = .86) (Table 3). Following PCV13, parents also had a significant increase in *S. aureus*. Carriage increased to 62% in PCLH (p = .05) and to 93% in PHUC (p = .0001). *S. aureus* carriage in the child increased risk of carriage in parents, 6.6 times in PCLH (95% CI: 1.22–35.43, p = .028) and 3.3 times in PHUC (95% CI: 0.68–16.3, p = .13). Overall, post-PCV13 *S. aureus* carriage increased in children by 3.93 times (95% CI 1.57–9.84, p = .003), regardless of their HIV status.

CLH are at 20–40 times increased risk of IPD, even when on ART.[22] Pneumococcal conjugate vaccines have significantly decreased IPD and pneumonia in CLH, in multiple settings.[23] We conducted a single dose catch-up PCV immunization study in children >2 years of age, and looked at carriage 2-months post-immunization. Other studies have also looked at carriage 2-months post-PCV.[24,25] The CDC catch-up schedule for PCV recommends one dose for healthy children over 2 years, and two doses separated by 8 weeks for CLH under-18 years.[26] The timing and number of doses of PCV for CLH is still not clear in developing countries, where

Table 1. Demographic characteristic of study participants by HIV status.

Characteristic	Overall (n = 50)	Children with HIV (n = 27)	Children without HIV (n = 23)
Child's age baseline years, median (IQR25, IQR75)	3.52 (2.91, 4.04)	3.91 (3.36, 4.83)	3.11 (2.66, 3.74)
Female child, n (%)	26 (52%)	13 (48.2%)	13 (56.52%)
Number of Children in house, median (IQR25, IQR75)	2 (1, 2)	2 (2, 2)	1 (1, 2)
Family income median (IQR25, IQR75)	9478 (7594, 11362)	11362 (9478, 11362)	7594 (7594, 9478)
Children			
HAZ, median (IQR25, IQR75)			
Baseline	−1.53 (2.54, −.62)	−1.96 (−3.05, −1.22)	−0.92 (−1.8, −.44)
Post-PCV	−1.36 (−2.07, −0.84)	−1.76 (−2.32, −1.09)	−1.03 (−1.58, −0.06)
WAZ, median (IQR25, IQR75)			
Baseline	−1.94 (−2.52, −1.1)	−2.33 (−3.91, −1.91)	−1.33 (−1.97, −0.72)
Post-PCV	−2.25 (−2.57, −1.26)	−2.50 (−3.24, −2.28)	−1.26 (−2.08, −0.61)
Parent			
Parent on ART during study	78%	78%	-
Mothers age at baseline, median years (IQR25, IQR75)	26 (23, 30)	30 (25,33)	25 (21, 26)
Mother Education (median years, IQR25, IQR75)	8 (6, 10)	6.5 (3, 9)	9 (7, 12)
Mothers Previously on TB treatment	5 (10.2%)	5 (19.23%)	0

Table 2. Pneumococcal colonization in children and parents before and after PCV13 immunization in the child.

	Pre-PCV13 (A)	Post-PCV13 (B)	P1	P2	P3
Pneumococcus colonization in children					
HIV infected, n/x (%)	9/23 (39.13%)	7/20 (35%)	1	0.95	1
HIV uninfected, n/x (%)	8/20 (40%)	7/20 (35%)	1		
Ct value (Median, IQR25, IQR75)					
HIV infected	16.1 (12.93, 24.98)	20.44 (19.42, 27.79)	0.07	**0.005**	**0.018**
HIV uninfected	28.36 (27.73, 32.52)	32.22 (22.15, 33.31)	0.57		
Carriage Density (copies/mL) (median, IQR25, IQR75)					
HIV infected	6.28e+08 (5.69e+06, 3.05e+09)	3.77e+07 (2.15e+05, 6.62e+07)	0.11	**0.009**	0.14
HIV uninfected	2.1e+05 (3.2e+04, 3.75e+05)	4.94e+04 (9.48e+03, 4.15e+07)	0.70		
Pneumococcus colonization in parent					
HIV infected	2/17 (11.76%)	5/16 (31.25%)	0.17	0.52	0.41
HIV uninfected	3/15 (20%)	3/16 (18.7%)	0.93		

n/x; n = number of swabs positive; x = total number of swabs tested.
P1 = p values comparing column A (pre) and column B (post-PCV) by χ^2 test for colonization and two-sample t test for Ct and carriage density.
P2 = p values comparing HIV infected and uninfected groups, pre-PCV by χ^2 test for colonization and Wilcoxon rank sum test for continuous variables (Ct and carriage density).
P3 = p values comparing HIV infected and uninfected groups, post-PCV by χ^2 test for colonization and Wilcoxon rank sum test for continuous variables (Ct and carriage density).

Table 3. *Staphylococcus aureus* colonization in children and their parents before and after PCV13.

	Pre-PCV13 (A)	Post-PCV13 (B)	P1	P2	P3
S. aureus colonization in children					
HIV-infected, n/x (%)	11/23 (47.8%)	16/20 (80%)	**0.029**	0.12	0.16
HIV uninfected, n/x (%)	5/20 (25%)	12/20 (60%)	**0.025**		
Ct value (Median, IQR25, IQR75)					
HIV-infected	27.35 (18.12, 33.82)	19.73 (13.77, 29.45)	0.24	0.69	0.22
HIV uninfected	20.97 (19.76, 32.72)	30.22 (13.77, 29.45)	0.77		
Density (Copies/mL)(median, IQR25, IQR75)					
HIV-infected	2.98e+05 (1.09e+03, 5.22e+08)	1.51e+08 (4.2e+04, 2.15e+10)	0.14	0.69	0.12
HIV uninfected	1.90e+07 (3.5e+03, 4.61e+07	3.1e+04 (1.0e+04, 2.86e+09)	0.20		
S. aureus colonization in parent					
HIV-infected	5/17 (29.1%)	10/16 (62.5%)	0.056	0.86	0.032
HIV uninfected	4/15 (26.67%)	15/16 (93.3%)	0.0001		

n/x;n = number of swabs positive; x = total number of swabs tested.
P1 = p values comparing column A (pre) and column B (post-PCV) by χ^2 test for colonization and two-sample t test for Ct and carriage density.
P2 = p values comparing HIV infected and uninfected groups, pre-PCV by χ^2 test for colonization and Wilcoxon rank sum test for continuous variables (Ct and carriage density).
P3 = p values comparing HIV infected and uninfected groups, post-PCV by χ^2 test for colonization and Wilcoxon rank sum test for continuous variables (Ct and carriage density).

access to ART has been delayed.[22] In India, at this time no PCV is offered to CLH. We found that CLH had higher pneumococcal carriage densities before and after one dose of PCV-13, and, unfortunately most children were not on ART, which puts these children at high risk of severe IPD. Also, we found that CLH were living in households with adults with HIV who are also at increased risk for IPD, and clearly increased carriage in children predicted carriage in parents in this group.

Several culture-based studies have reported similar pneumococcal carriage rates in CLH versus HUC[27–31], but culture-based studies do not typically comment on the density of carriage.[19] Quantitative PCR is gaining recognition to study carriage and the impact of PCVs, particularly on the dynamics of serotype-specific carriage density.[32,33] The pneumococcal carriage density determines disease severity in HIV-infected adults[3] and is associated with transmission.[2,4] Through qPCR, we could quantify the density of carriage and the detection limit increased.[19] A recent study using qPCR, showed increased pneumococcal density in CLH compared to HUC in Mozambique, both before and after the country rolled-out PCV-10.[34] The impact of the introduction of PCV into childhood immunization programs on IPD and carriage in unvaccinated adults with HIV is an area of active investigation throughout sub-Saharan Africa.[35] Increased carriage density in the context of HIV could be due to a decrease in pneumococcus specific CD4-T lymphocytes, particularly the CD154 response, which is known to clear pneumococcus.[32,36]

The impact of PCVs on the density of VT versus NVT serotypes is less clear.[32,34,37–39] We found an overall drop in pneumococcal density post-PCV13. This warrants further investigation in high-risk families.

Typically, a negative correlation exists between pneumococcus and *S. aureus* in young children;[7,9–11] possibly mediated by hydrogen peroxide produced by pneumococcus,[40] or cross-reactive antibodies[41] or by pilus.[42] VT strains in particular are negatively correlated with *S. aureus*.[10,11] HIV infection impacts this association. Co-colonization of pneumococcus and *S. aureus* has been seen in CLH by us[12] and others[9,13] and in HIV-infected adults.[14]

Pneumococcal immunization may influence the colonization of competing microbes. Some population-based studies have observed an increase in *S. aureus* carriage following PCV-7 vaccination during infancy,[15,16,33,43,44] and some have not.[45,46] Gambian children had an *S. aureus* carriage increase 4 months after PCV-7.[47] In South Africa, children had an initial increase in *S. aureus* carriage post-PCV-7 immunization that then

normalized 16 months post-immunization.[33] It is difficult to make comparisons with other studies as they differ in age, size, methodology, immunization doses, and the time point post-immunization. An increase in *S. aureus* among parents could be from the familial transmission.

The major strength of this study is the use of qPCR to examine the density of colonization of pneumococcus in high-risk HIV-infected Indian children. This is the first study that examines the nasopharyngeal ecology in Indian families with respect to PCVs.

The major limitation lies in the small sample size, lack of serotype-specific density data, and short time-point post-vaccination. The *S. aureus* increase needs validation in larger cohorts of high-risk children. Future larger longitudinal studies of serotype-specific carriage density and immunogenicity in CLH are required to draw final conclusions on the dosing and timing of PCV in these children, living in low and middle-income countries.

This descriptive pilot study is important in the Indian context, due to the ongoing-phased roll-out of PCVs in India. India has a huge burden of HIV and pneumococcal disease. But still, CLH are not yet prioritized to receive pneumococcal vaccines. This study provides insights on the increased risk of CLH in carrying the high density of pneumococcus in families where both children and adults are affected by HIV. Population-based PCV immunization, may provide indirect protection to unvaccinated high-risk adults.

This study demonstrates that the pneumococcal and *S. aureus* colonization interactions exist within the nasopharyngeal space of CLH and HUC. Pneumococcal vaccines interrupt these interactions in vaccinated children.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the Robert Austrian Research Awards Committee 2016 for pneumococcal vaccinology under Grant [Robert Austrian Research Award 2016].

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