Effect of Introduction of Pneumococcal Conjugate Vaccine Immunization on Nasopharyngeal Colonization of *Streptococcus pneumoniae* in South Africa

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Declaration

I, Susan Audrey Nzenze, declare that this thesis is my own work. It is being submitted for the degree of Doctor in Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university

Signature: 

Date: 19 November 2015

Publications directly contributing to this PhD and role of the student in these studies

I designed the study protocol and questionnaires for the colonization projects. I also collected the data, including swabs on my own in the first year and in later years with the help of trained nurses. Swabs were processed at the Center for Respiratory Diseases and Meningitis (National Institute for Communicable Disease, Johannesburg, South Africa). I guided the data entry and cleaned the data myself. I also performed the data analysis with input from my co-authors and wrote up the first draft. I also edited all subsequent drafts including revisions from journals.

1. **Nzenze S**A, Shiri T, Nunes MC, Klugman KP, Kahn K, Twine R, de Gouveia L, von Gottberg A and Madhi SA. Temporal changes in pneumococcal colonization in a rural-African community with high HIV-prevalence following routine infant pneumococcal-immunization. 2013. Pediatric Infectious Disease Journal. 32 (11):1270–1278.

2. **Nzenze S.A**, Shiri T, Nunes MC, Klugman KP, Kahn K, Twine R, de Gouveia L, von Gottberg A and Madhi SA, Temporal association of infant immunization with pneumococcal conjugate vaccine on the ecology of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus* nasopharyngeal colonization in a rural South African community. 2014. Vaccine 32(2014) 5520-5530.

3. **Nzenze, SA**, von Gottberg A, Shiri T, van Niekerk N, de Gouveia L, Violari A, Nunes M and Madhi SA. Temporal changes in pneumococcal colonization in HIV-infected and HIV-uninfected mother-child pairs following transitioning from 7-valent to 13-valent pneumococcal conjugate vaccine, Soweto, South Africa. Journal of Infectious Diseases.2015 Oct 1; 212(7); 1082-92, first published online March 17, 2015 doi:10.1093/infdis/jiv167

4. **Nzenze SA,** Madhi SA, Shiri T, Nunes MC, Klugman KP, de Gouveia L, Moore D, Karstaedt AS, Tempia S and von Gottberg A, Pneumococcal vaccine in infants: Trends on community-wide effect on invasive disease and associations with colonization, Soweto, South Africa." draft.

Other work

1. Shiri T, Auranen K, van Niekerk N, von Gottberg A, **Nzenze SA**, Madhi SA, Trends in pneumococcal transmission in rural South African households after pneumococcal conjugate vaccine introduction, submitted

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Abstract

Introduction

Pneumococcal conjugate vaccine (PCV) immunization of children decreases their risk of nasopharyngeal acquisition of vaccine serotypes and concurrently reduces the transmission thereof to PCV unvaccinated age groups. We studied the impact of routine infant PCV immunization at population level, on the epidemiology of nasopharyngeal pneumococcal colonization in a rural (Agincourt) and an urban (Soweto) South African community with high prevalence of HIV-infection. Furthermore, we delineated the effect of infant PCV immunization on bacterial interactions of *Streptococcus pneumoniae* with *Haemophilus influenzae* and *Staphylococcus aureus* at the population level. Lastly, we assessed the utility of colonization data to predict the impact of childhood PCV immunization on the direct and indirect effect against invasive pneumococcal disease (IPD).

Materials and Methods

A series of cross sectional colonization surveys were undertaken (in Agincourt and Soweto) between 2009 and 2012. These years were representative of the pre- or early-PCV-era and PCV-era years. The seven valent PCV (PCV7) was introduced into the South African national immunization program in April 2009, using a 6, 14 and 40 weeks of age dosing schedule with no catch up campaign of older children. Subsequently, PCV7 was replaced by 13-valent PCV (PCV13) in May 2011, with a limited catch up campaign.

Nasopharyngeal swabs were collected among household members and mother-infant pairs and processed for *S. pneumoniae, H. influenzae* and *S. aureus* using standard microbiologic techniques. Additionally, the trends in incidence of IPD for 2005 to 2012 were evaluated. Multivariate logistic regressions were performed to assess the impact of PCV on carriage in different age groups. Adjusted risk ratios (aRR) or adjusted odds ratios (aOR) are reported as measures of impact and association. We compared the predicted changes in IPD among children and their mothers, stratified by HIV, using a theoretical model and compared this to the observed changes in IPD.

Results

Among rural households, the prevalence of PCV7 serotype colonization among all ages decreased from 18.3% in 2009 to 11.4% in 2011; p<0.0001. This included reductions (adjusted risk ratio; aRR) of 50% (95% Confidence Interval [95%CI]:0.42-0.59), 34% (95%CI: 0.48-0.92) and 64% (95%CI: 0.18-0.74) in age groups <2 years, 6-12 years and adults, respectively. The prevalence of PCV7 serotype colonization among primary caregivers decreased from 10.2% in 2009, to 5.4% in 2011, (p<0.001). Non-vaccine serotype colonization prevalence increased by 35% (95%CI: 1.17-1.56) among children <2 year of age in 2011, however, it declined by 45%-54% among adolescents and adults.

In urban mother-infant pairs, PCV13serotype colonization decreased from 2010 compared to 2012 among HIV-uninfected (aOR: 0.32; 95%CI: 0.25-0.40) and HIV-infected children (aOR: 0.37; 95%CI: 0.28-0.49), whilst there was an increase in non-vaccine serotype colonization. Decreases in PCV13 serotype colonization were also

observed in HIV-uninfected women (aOR: 0.44; 95%CI: 0.23-0.81); with a similar trend in HIV-infected women. Non PCV13 serotype colonization declined in 2012 compared to in 2010 among HIV-infected women (aOR: 0.69, 95%CI: 0.48-0.99). HIV-infected compared to HIV–uninfected women had higher prevalence of overall (20.5% vs. 9.7% in 2010; 13.8% vs. 9.7% in 2012) and PCV13 serotype colonization (8.7% vs. 5.4% in 2010; 4.8% vs. 2.0% in 2012) in both sampling periods; $p<0.04$ for all observations.

For bacterial associations in the rural population, from 2009 to 2011 in children 0-2 years and 3-12 years of age, the prevalence of overall *S. pneumoniae* colonization decreased from 74.9% to 67.0% ($p<0.001$). Although there was also a decrease in prevalence of *H. influenzae* colonization in the 3-12 year age group (55.1% to 45.3%, $p<0.001$), this was not evident among those <2 years of age. The prevalence of *S. aureus* colonization remained unchanged in all childhood age groups. In individuals older than 12 years of age, the prevalence of colonization decreased for all studied bacteria including *S. pneumoniae* (11.2% vs 6.8%), *H. influenzae* (16.7% vs. 8.8%) and *S. aureus* (31.2% vs. 23.7%); $p<0.001$ for all comparisons.

Analysing the colonization and IPD findings, between the pre PCV era (2007-2009) and the PCV13 era (2012), we observed reductions in vaccine serotype colonization and IPD due to PCV7 serotypes and the additional six serotypes included in PCV13 among children and women. Using the changes in vaccine serotype colonization over time, the hypothetical model accurately predicted changes in vaccine serotype IPD incidence compared to the observed changes in PCV-unvaccinated HIV-infected and HIV-

uninfected adults; and among children too old to have been immunized. The model, however, underestimated the reduction in vaccine serotype IPD among the child age-group targeted for immunization. The model was, however, not useful in predicting the changes that occurred for non-vaccine serotypes either among PCV-vaccinated or PCV-unvaccinated age-groups.

Discussion and Conclusion

Infant PCV immunization resulted in population wide decreases in vaccine serotype colonization of *S. pneumoniae* including among HIV-infected adults in both rural and urban settings. Surveillance of colonization prior and following childhood PCV immunization can be used to infer indirect effects against vaccine serotype IPD in the community even in high HIV-prevalence settings such ours.

List of Abbreviations

aOR	Adjusted Odds Ratio
aRR	Adjusted Risk Ratio
ART	Antiretroviral therapy
HIV	Human Immunodeficiency Virus
IPD	Invasive Pneumococcal Disease
NP	Nasopharyngeal
NIP	National Immunization Program
OR	Odds ratio
PCV	Pneumococcal conjugate vaccine
PCV5	5-valent pneumococcal conjugate vaccine
Pnc-T	4-valent pneumococcal conjugate vaccine conjugated to tetanus toxoid
Pnc-D	4-valent pneumococcal conjugate vaccine conjugated to diphtheria toxoid
PCV7	7-valent pneumococcal conjugate vaccine
PCV10	10-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PnCOM-PCV7	7-valent pneumococcal conjugate vaccine conjugated to meningococcal outer membrane protein complex
RR	Risk ratio
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
95%CI	95% confidence interval

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Chapter 1

1.1 Background

Streptococcus pneumoniae is the leading cause of death in children (1, 2) and the commonest cause of community acquired pneumonia among adults (3). An estimated 826,000 deaths due to pneumococcal disease occurred in children aged 1-59 months in 2000, including 91,300 deaths among HIV-infected children. Most of these deaths were attributable to pneumonia (90%), followed by meningitis (7%)(4). By 2013, the annual number of deaths due to *S. pneumoniae* had declined to 436,000, nevertheless, the majority (>90%) of these deaths still occurred in low-income countries, where childhood immunization with pneumococcal conjugate vaccine (PCV) was yet to be introduced (5).

Immunization of children with PCV offers the opportunity to reduce pneumococcal morbidity and mortality, particularly among children with limited access to curative health-care where the risk of mortality is likely the greatest (6). In 2007, the World Health Organization (WHO) recommended vaccination of children with PCV in all countries with under-five mortality rate greater than 50 per 1,000 live births, where greater than 50,000 children die annually and in countries with a high HIV prevalence (7).

The introduction of seven-valent PCV (PCV7) into childhood immunization programs of high-income countries, first initiated in USA in 2000, has been associated with reduced nasopharyngeal (NP)-acquisition of the vaccine serotypes among vaccinated-children.

This has also resulted in reduced transmission of these serotypes to unvaccinated-individuals, (8, 9) which has consequently decreased the incidence of vaccine serotype invasive pneumococcal disease (IPD) among PCV-vaccinated and -unvaccinated individuals (i.e. indirect effect) (10-12). The indirect effect has also benefited young infants who were too young to be immunized (13), and PCV-unvaccinated HIV-infected adults in USA (14, 15). In the USA, the number of IPD cases prevented through the indirect effect of PCV is almost 2.5 fold greater than the number prevented among the vaccinated childhood population (11). Similarly, childhood PCV immunization has been associated with reduction in all-cause pneumonia among age-groups targeted for vaccination, and with even greater number of cases being prevented among PCV-unvaccinated adults in USA (16). Although, an increase in non-vaccine serotype IPD has partly offset the decrease in vaccine serotype IPD, overall IPD rates remain significantly lower than in the pre-PCV era (10).

PCV uptake in low and middle income countries has lagged behind high-income countries. Among the WHO member states, 60% of members from the Americas region, 50% Western Mediterranean region, 41% African region, 33% Western Pacific region and none from Asia had introduced PCV in December 2012 (17). Global efforts to accelerate the introduction of PCV in low-income countries through GAVI supported 42 countries to introduce PCV into their national immunisation programs (18). To date 116 countries have introduced PCV into the NIPs, including widespread private market in Portugal. Despite this progress 59% of the world's children live in 43 nations that are yet to make a decision regarding PCV introduction (18).

Beginning in April 2009, South Africa implemented PCV7 immunization for infants through its national immunization program. Vaccination was targeted specifically at infants who had yet to receive their first dose of other vaccines given at 6 weeks of age, with no catch-up campaign of older children. The PCV dosing schedule adopted in South Africa was a two dose primary series at 6 and 14 weeks of age, followed by a booster dose at 9 months of age (19, 20).

This thesis reports on the impact of infant PCV immunization on the bacterial ecology of the nasopharynx among PCV-vaccinated and -unvaccinated age-groups, in rural and urban South African communities, and where there is a high prevalence of HIV infection.

1.2 Nasopharyngeal carriage of *S. pneumoniae*

S. pneumoniae is a normal commensal of the upper respiratory tract acquired from early on in life. Most serotypes of pneumococci that cause disease in children are also identified as colonizing healthy carriers. Although NP pneumococcal colonization does not always result in pneumococcal disease, the risk for developing disease is greatest within the first 1-2 months of acquisition of a new serotype (21). Consequently, there is a close association between the prevailing colonizing pneumococci and those causing disease (21-23). This is corroborated by animal-model studies in which nasal inoculation of *S. pneumoniae* leads to otitis media or invasive disease (24-28). Furthermore in longitudinal cohort studies in children less than two years age, a recent acquisition (less

than two months) of a new serotype was associated with acute otitis media or respiratory infection. Thus, there is >90% concordance between disease-causing serotype and NP serotype at time of illness (21-23, 29). This is further corroborated by >90% of children with IPD simultaneously being colonized with the homotypic serotype, compared to a prevalence of 52% among healthy controls (30, 31).

Serotypes commonly isolated from the nasopharynx of children prior to PCV immunization programs were from serogroups 6, 14, 19 and 23 (21, 32-34). All of these serogroups are represented by at least one serotype in the 7-valent (PCV7), 10-valent (PCV10) and 13-valent (PCV13) PCV formulations. These vaccine serotypes are less commonly isolated from adults, among whom there is a higher prevalence among women and particularly those infected by HIV (35). Additional PCV serotypes included in PCV10 and PCV13 are serotypes 1 and 5. Although these serotypes are rarely associated with NP colonization in healthy individuals, they cause a significant burden (15-20%) of pneumococcal disease in Africa and other low-middle income countries (36). These serotypes are also commonly identified in the nasopharynx of individuals with pneumococcal disease due to the homotypic serotype, as well as among household contacts of cases during outbreaks (30, 37).

Young children colonized by pneumococci, are the major source of transmission of pneumococci in the community (33). This is further corroborated by children without siblings acquiring the pneumococcus later in life and being colonized by fewer serotypes than children with siblings (21). Also, there is heightened pneumococcal transmission

and acquisition in environments where many children are concentrated, such as in day-care centers (8, 21, 32, 38). Day-care attendance by children is also associated with transmission of these acquired serotypes to community contacts not attending day care (39). Both younger children and adults in households where there are children attending day-care, have a higher prevalence of pneumococcal colonization (38, 40). This observation is independent of the longer duration of pneumococcal colonization among young children compared to adults (41). Although day-care attendance may be less common in low-income African settings, an analogous situation in these countries is overcrowding in the household, as well as many children from households playing together in groups.

There are notable differences regarding age of first pneumococcal acquisition and prevalence of colonization during childhood and in adults in low-income countries (and high-risk populations such as American Indians, Alaskan Natives and Australian Aboriginals) compared to those in high-income countries (33). Colonization by the pneumococcus is acquired in the first few weeks of life in low-income countries. As an example in The Gambia, the mean age of first acquisition of the pneumococcus was 33 days, while >80% of children were colonized by pneumococcus at three-months of age (42). Similarly in Papua New Guinea, all children had acquired the pneumococcus by 3 months of age, (43) while in Bangladesh 50% and 90% of the infants had at least one acquisition of the pneumococcus at 8 and 21 weeks respectively (44).

In contrast, colonization of infants by pneumococci in high-income countries occurs later in childhood. A study in USA reported the mean age of first colonization at 6 months of age, with an upper range of 18 months (21). Similarly, most Finnish children only acquired pneumococci between 12 to 24 months of age (45). Colonization prevalence of 40 to 50% among children in high-income settings may only be reached by two years of age, which is then followed by a reduction in prevalence of NP colonization around five years of age to adult levels of approximately 10% (33). The prevalence of pneumococcal colonization may, however, persist at high levels into adulthood in low-income settings, e.g. 50% among adults > 40 years in The Gambia (46).

Considering the differences in dynamics of pneumococcal colonization among children and adults from high- and low-income countries, it is unclear whether the indirect effects on colonization and pneumococcal disease of childhood PCV immunization observed in high-income countries will materialize in low-income settings. The high prevalence of pneumococcal colonization among adults in low-income countries in general, could serve as a source of pneumococcal transmission in the community. This may be even more pertinent in settings with a high prevalence of adult HIV-infection, since HIV-infection (and particularly among women) is associated with a higher prevalence of PCV7 serotype colonization (47). As such, in settings with a high prevalence of adult HIV-infection, these individuals could theoretically serve as an additional reservoir of colonization and transmission of PCV7 serotypes.

1.3 Pneumococcal conjugate vaccines and dosing schedule

Based on the polysaccharide composition of the capsular wall of *S. pneumoniae,* 42 serogroups have been characterized, which are further classified into at least 93 distinct serotypes (48). Only a limited number of these 93 serotypes, however, are responsible for the majority of IPD cases (49), most of which have been incorporated into PCV formulations. The PCV7 was first licensed in 2000 in USA for use in children under two years of age. This formulation includes serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. The PCV7 serotypes accounted for 80% of IPD in young children in the USA, Canada and Australia, at least 50% of IPD in Africa, Europe and Latin America and 30% of IPD in Asia prior to the PCV-era (50). Globally, since 2010, PCV7 has been replaced with the 10-valent PCV (PCV10; which includes serotypes 1, 5 and 7F) and the 13-valent PCV (PCV13; which has additional serotypes 3, 6A and 19A). Surveillance data from South Africa prior to the PCV-era, reported 70% of IPD were caused by PCV7 serotypes +serotype 6A, whereas 77% and 86% of childhood IPD cases were caused by PCV10- and PCV13-serotypes, respectively (51).

There has been varying dosing schedules used for PCV childhood immunisation between countries. These include two doses during infancy followed by a booster dose in the second year of life (2 + 1 schedule), three doses in infancy (3 + 0 schedule), and three doses in infancy followed by a booster dose in the second year of life (3 + 1 schedule). There has also been variability between national immunization programs as to whether PCV vaccine introduction was coupled with a catch up campaign of older children, who

would otherwise not be included in the adopted vaccine schedule for younger children. The catch-up campaign of older childhood age groups is targeted at providing protection to these children, and has also been postulated to accelerate the interruption of pneumococcal transmission and hence expedite the time to when indirect protection would materialise in communities (52).

The WHO recommends that countries should consider local epidemiological factors when deciding on the PCV schedule, (7) with an implied preference for a 3 dose primary series (3+0),without a booster dose (due to cost constrains), premised on the efficacy trials undertaken in Africa (53, 54). Alternately, WHO recommends a 2 dose primary series followed by a booster dose in the second year of life (2+1) schedule (55). The 2+1 dosing schedule has been widely adopted in many European countries (56-59). A recent systematic review and meta-analysis, dominated by data from high-income countries, suggest there to be little difference in the effectiveness of different dosing schedules against vaccine serotype colonization, all-cause pneumonia or vaccine serotype IPD (56). Also, there has been similar levels of indirect protection experienced in countries which implemented 2+1 compared to those with a 3+1 dosing schedules(60-64). The relevance of dosing schedules on the long-term and community wide effects of pneumococcal transmission dynamics and disease, however, remain to be fully elaborated in low-middle income settings (56).

The rationale for South Africa choosing a modified 2+1 dosing schedule (6, 14 and 40 weeks age), included aiming at improving the persistence of protection among HIV-

infected children. This was important since waning of vaccine efficacy was observed following 5 years of follow-up in HIV-infected children when provided with a 3-dose primary series (6, 10 and 14 weeks) without a booster dose of vaccine (65, 66). The public-health relevance of needing to extend the duration of protection against IPD in HIV-infected infants (19) was motivated by them contributing to 75% of IPD cases and deaths in South Africa, despite only constituting 5-6% of the childhood population (67). The 2+1 dosing schedule was demonstrated to have similar immunogenicity one month following the primary series of two doses, compared to after the third dose at 14 weeks age in a historical cohort who received PCV at 6, 10 and 14 weeks (20). Furthermore, the geometric mean concentrations (GMCs) following the third dose at 9 months of age in the 2+1 schedule resulted in much higher GMC compared to after the third dose in the 3+0 schedule (20). This indicates the potential of more prolonged immunity with the 2+1 compared to 3+0 schedule in South Africa (20). To date, South Africa remains the only country to have adopted this dosing schedule (6, 14 and 40 weeks age). Hence, delineating the direct and indirect effect of this PCV dosing schedule on pneumococcal colonization, IPD and pneumonia, is important to determine the potential utility of such a schedule in other low-middle income countries.

1.4 Effect of PCV vaccination on pneumococcal colonization

PCV vaccination has been shown to be effective in reducing vaccine serotype carriage in a variety of settings. A selection of such studies are summarised in Tables 1.1, 1.2 and 1.3. Early clinical trials, reported that a three-dose PCV primary series reduced vaccine

serotype carriage in vaccinated children compared to controls by 9-12 months of age (8, 9, 68, 69). Furthermore, there have been randomized trials which reported that household contacts of day-care attending PCV-vaccinated children, were less likely to be colonized by the vaccine serotypes compared to household members in which the children received placebo(8, 33, 70-73) . This included lower prevalence of colonization among older unvaccinated siblings and adults sharing household with PCV-vaccinated children. Concomitantly, an increase in non-vaccine serotype (NVT) among PCV-vaccinees and their PCV-unvaccinated contacts has also been reported (8, 68, 69, 73, 74) .

Following PCV introduction in national immunization programs, population level effects on colonization of vaccine introduction have been described in a variety of settings. Observational data from several countries, including The Gambia, Kenya, Australia, the United Kingdom, The Netherlands, France, the USA and Colombia consistently shows decreases in vaccine serotype colonization in PCV-vaccinated and -unvaccinated age-groups, with varying degrees of non-vaccine serotype replacement (14, 59, 75-84). In the USA, whereas vaccine serotype colonization had decreased by >97% seven years following PCV7 introduction, there was a near complete replacement of carriage by non-vaccine serotypes (85). Various degrees of non-vaccine serotype increases have also been observed in various settings from within 2 years of PCV introduction, including in Portugal where the PCV was introduced into the private market (14, 59, 75-77, 81-83, 85-89). Differences in the extent of non-vaccine serotype replacement between settings may vary depending on timing of the survey after vaccine introduction, extent of

immunization coverage of targeted age-group, the dosing schedule used and whether there was a catch-up campaign of older children.

Table 1.1: Randomised Controlled Trials on the effect of PCV on colonization by WHO regions

Author	Study site	Study population	Vaccine formulation	Dose	Age when colonization evaluated	Vaccine serotypes: OR of colonization in vaccinees compared to controls	Non-vaccine serotypes: OR of colonization in vaccinees compared to controls
Africa region							
Matched case control studies							
(Obaro 1996)(69)	The Gambia	PCV5 recipients matched to PCV-unvaccinated controls according to age and place of residence	PCV5-CRM197 [1]	3 doses at 2, 3 and 4 months,OR two doses at 2 and 3 months followed by PPSV23 at 18 months	24 months	3 dose arm: OR 0.11(95%CI: 0.04-0.31) 2 dose arm: OR 0.22 (95%CI: 0.08-0.61).	3 dose arm: OR 4.51(95% CI:1.62-14.37), 2 dose group, OR 0.65 (95% CI: 0.29-1.42).
Randomised controlled trials							
Mbelle 1999 (68)	South Africa	Children enrolled and given vaccine at 6, 10 and 14 weeks	CRM-9[2]	3 doses at 6, 10 and 14 weeks	9 months	Decreased among vaccinees, 18% compared to 36% in controls OR: 0.38 (95% CI: 0.25-0.58)	Increased among vaccinees 36% versus 25% in controls OR: 1.75 (95% CI: 1.18-2.60)
Obaro 2000 (90)	The Gambia	Children that received vaccine or placebo had carriage measured at 5 and 9 months of age	CRM-9	3 doses at 2, 3 and 4 months	5 and 9 months	Decreased from 68% in controls to 58% in vaccinees to OR 0.84 (95% CI: 0.47 to 1.50)	Increased from 35% to 49% , OR 1.8 (95% CI: 1.04-3.9)
Cheung, 2009 (74)	The Gambia	Vaccine recipients and controls enrolled at 6 to 51 weeks and their younger siblings	CRM-9	3 doses from 6 to 51 weeks	9-15 and 21-27 months	VT carriage at 9-15 months and at 21-27 months less in vaccine recipients , OR 0.44 (95% CI: 0.36-0.53) and OR 0.47 (95% CI: 0.39-0.58), respectively	Carriage at 9- 15 months and at 21-17 months increased in vaccines compared to controls, OR 2.03 (95% CI: 1.69-2.44) and OR 1.92 (95% CI: 1.57-2.33)

							respectively
Eastern Mediterranean							
Dagan, 1996(70)	Israel	Children 12-18 months old	PnCOM PC-7[3]	One dose vs two doses vs PPSV23	13-21 months	One dose: OR 0.35 (95% CI: 0.16-0.78); 25% to 9% at 3 months after vaccination Decrease from 25% to 7% , OR 0.29 (95% CI: 0.12-0.74) at 1 month after vaccination in two dose arm No change in PPSV23 recipients	No change
Dagan 1997(91)	Israel	Children aged 2, 4 and 6 months	Pnc-T[4] Or Pnc-D[5]	3 doses at 2, 4 and 6 months followed by PPSV23 at 18 months		Decrease from 27% to 10%, OR 0.13 (95% CI: 0.04-0.49) for Pnc-D Decrease from 27% to 10%, OR 0.23 (95% CI: 0.11-0.74) for Pnc-T	Non-significant increase
Dagan 2002 (8)	Israel	Toddlers attending day care age 12-17months and 18-35 months	CRM-9	2 doses at least 2-3months apart for those aged 12-17months 1 dose for those aged 18-35 months	24-35 and 36-47 months	Decreases observed among children 24-35 months OR 0.40 (95% CI: 0.26-0.60) and among children 36-47 months OR 0.57 (95% CI: 0.38-0.87)	Increase from 7% to 10% among children $\leq$ 36months
Europe							
Kilpi 2001(72)	Finland	Infants enrolled at 2months	PnCOM PC-7	2, 4, 6 and 12 months	12 and 18 months	At 12 months VT declined by 17% (95%CI: -9 to 37%) At 18 months VT declined by 41% (95% CI: 33 to	Not reported

						55%)	
Van Gills 2009 (63)	Netherlands	Healthy new borns followed up to 24 months of age and their parents	CRM-7[6]	2 doses vs 2+1 doses vs control	12, 18 and 24 months	At 12months decrease from 38% to 25% in 2 dose, OR 0.52 (95% CI, 0.38-0.74) and from 38% to 20% in 2+1 schedule , OR 0.40 (95% CI 0.28-0.57), Further decreases to 16% and 14% carriage at 18 and 24 months respectively in 2+1 schedule	NVT increased at 12 months in 2 dose arm and 2+1 dose arm from 29% to 38%, OR 1.48 (1.07-2.06) Similar results at 18 and 24 months for both groups

[1]PCV-5 serotypes 6B, 14, 18C, 19F and 23F, [2] PCV-9 serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F conjugated to CRM, [3]PnCOMPC-7, serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to meningococcal outer membrane protein complex, [4]Pnc-T: PCV4 serotypes 6B, 14, 19F and 23F conjugated to tetanus toxoid, [5]Pnc-D: PCV4 serotypes 6B, 14, 19F and 23F conjugated to diphtheria toxoid, [6]PCV-7 serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to CRM

Table 1.2: Community studies evaluating the impact of childhood PCV immunization on direct and indirect effect

Author	Study site	Study population	Vaccine formulation	Dose	Years between vaccine introduction and study	Vaccine serotypes: OR of colonization in vaccinees compared to controls	Non-vaccine serotypes: OR of colonization in vaccinees compared to controls
Americas							
O'Brien 2007 (9)	Navajo	American Indian infants and their unvaccinated contacts	CRM-7[1]	3+1 schedule at 2, 4, 6 and 12-15months	1month, 6-9months, 12-15 months	At one month post PCV vaccination, infants had lower odds of carrying VT (OR, 0.40: 95% CI: 0.23-0.67) Older siblings in vaccinated communities had lower carriage , OR 0.27 (95% CI: 0.07-1.07)	Increased 6 months after booster dose (OR, 1.67: 95% CI: 1.02-2.78)
Millar 2008 (73)	Navajo	Households of children vaccinated with PCV or placebo	CRM-7	3+1 doses at least 2months apart for infant aged 6weeks to 7 months; 2+1 doses for infants aged 7to11months; 2 doses for infants12-23months	3-15 months after completion of trial	Vaccinated children and adults living in the same communities were less likely to be colonised, OR, 0.57(95% CI: 0.26-0.96)	Children living in vaccinated communities were more likely to be colonized, OR, 2.26 (95% CI: 1.22-4.20)
Africa							
Roca 2014 (92)	The Gambia	Infants immunized with PCV13 and their mothers compared to infants immunized with PCV-7	CRM-7 vs CRM-13[2]	Three doses at 2, 3 and 4 months Children aged 2-11months received 3 doses one month	1year	Carriage of PCV7 serotypes lower in PCV13 recipients (4.9%) compared to PCV7 recipients (9.4%),	Significant increase in non-typable isolates in PCV13 recipients vs PCV7 recipients (6.6% vs 0.6%, p=0.005)

				apart Children aged 12-30 months received 2 doses one months apart Older than 30 months received one dose PCV (in vaccinated villages) or Meningococcal vaccine (unvaccinated villages)		p=0.025 Carriage of PCV13 serotypes was lower in PCV13 recipients (18.0%) compared to PCV7 recipients (33.3%), p<0.001 Carriage prevalence of six additional serotypes in PCV13 was lower (13.4%) in PCV13 recipients compared to PCV7 recipients (23.9%), p=0.001	
Roca 2011 (79)	The Gambia	Household members in vaccinated and unvaccinated villages Baseline survey: Dec 2003 to May 2004	CRM-7	Infants received 3 doses at 2, 3 and 4 months Children aged 2-11months received 3 doses one month apart Children aged 12-30 months received 2 doses one months apart Older than 30 months received one dose PCV (in vaccinated villages) or Meningococcal	CSS[3]1: 4-6months after vaccination CSS2: 12 months after vaccination CSS3: 22 months after vaccination	Decrease between CSS1 and CSS2 in vaccinated villages Among 5 to 15 year age-group: OR 0.15 (95%CI: 0.04-0.57) and >15 year age-group: OR 0.32 (95% CI: 0.10-0.98) Decrease between CSS1 and CSS3 among 5-15years, OR 0.37: (95% CI: 0.15 to 0.90) and among >15 years, OR 0.37 (95% CI: 0.15 to 0.90)	Transient increase noted at CSS1 among vaccinated adults, OR 1.50 (95% CI: 1.03-2.18) Decrease at CSS1 in both villages among adults , OR 0.46 (95% CI: 0.26-0.82) And at CSS3, OR 0.53 (95% CI: 0.31-0.92)

				vaccine (unvaccinated villages)			

[1]PCV-7 serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to CRM
[2]PCV13, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F conjugated to CRM
[3]CSS, cross sectional survey

Table 1.3: Ecological studies evaluating the direct and indirect effect of PCV immunization in different settings by WHO regions

Author	Study Site	Study Population	Vaccine	Dose	Years between vaccine introduction and study	VT	NVT
Americas							
Huang 2009 (85)	Massachusetts, USA	Children 3 months to <7 years seen in primary care practises	CRM-7[1]	3+1 schedule at 2, 4, 6 and 12-15 months in national EPI, from 2000 , with catch-up	7	Decreased from 36% in 2001 to 3% in 2007 , OR 0.05 (95% CI: 0.03-0.11)	Increased across the survey years from 15% in 2001, 19% in 2004 and 29% in 2007, OR 2.3 (95% CI1.8-3.0)
Pelton 2004 (87)	Massachusetts, USA Oct 2000-Sept 2003	Children aged 2-24 months presenting for well child visits and children with acute otitis media	CRM-7	3+1 schedule at 2, 4, 6 and 12-15 months in national EPI, from 2000, with catch up	3	VT decreased from 22% to 2%, OR 0.13 (95% CI 0.03-0.63)	NVT increased from 7% to 16%, OR 2.2 (95% CI, 0.63-7.64)
Gounder 2014 (75)	Alaska	Children aged <5 years and adults >18 years	CRM-13[2]	3+1 schedule at 2,4 , 6 months and 12-15months, introduced 2010 to replace PCV-7	2	Among children <5 years PCV13 serotypes declined to 4% from 13%, p<0.01 Among adults >18 years VT13 serotypes decreased to 1% from 4%, p<0.01	Not reported
Hammitt 2006 (84)	Alaska	Households in 8 Alaskan villages	CRM-7	3+1 schedule at 2, 4, 6 and 12-15months with catch-up	4	Carriage decreased among children <5years from 55% to 5% , OR 0.09 (95% CI: 0.04-0.16))	Increased among adults >18 from 59.2% to 89.9%, OR 1.52 (95% CI: 1.37-1.68

						Among adults carriage decreases from 28% to 4.5%, p<0.001dults 0.16 (95% CI: 0.10-0.26)	
Europe							
Van Hoek 2014 (83)	England	Children aged 1-5 years and their household members.	CRM-13	2+ 1 dose at 2, 4 and 12/13 months, PCV 7 introduced in 2006 and replaced by PCV13 in April 2010	6	Continued decrease PCV-7 serotype from 2.9% in 2001/2002 to only 0.3% in 2012/2013, OR 0.02 (95% CI: 0.00-0.07 Decrease in additional VT13 serotypes for all age group, from 6.3% in 2001/2 to 0.6% in 2012/2013 , OR 0.12 (0.04-0.34)	Increase from 5.2% to 23.6%, OR 5.37 (95% CI: 3.78-7.65)
Flasche 2011 (59)	England	Households with children eligible to have received routine or catch up PCV	CRM-7	2 +1 schedule at 2, 4 and 12-13months, with catch-up for children up to 2 years old, since 2006	2-3	Among children < 5yrs decrease from 31.9% to 3.6%, OR 0.06 (95% CI: 0.03-0.16) Among individuals aged 5-20 years decrease 9.9% to 0% Among adults >20 years decrease from 4.1% to 2.3% OR for > 5 years, 0.31 (95% CI: 0.04-2.49)	Among children < 5 years increased from 15.3% to 45.3%, OR 4.25 (95% CI: 2.81-6.43) Among individuals 5-20 years increased from 9.1% to 26.3% Among adults >20 years increased from 3.3% to 7.5% OR for >5 years, 5.16 (95% CI: 1.95-13.66_
Rodrigues 2009 (88)	Portugal	Children attending day care	CRM-7	2+1 at 2, 4, 12 months of age, Non-universal coverage since 2001,	6	Decreased in children with >1 dose of PCV from 43.7 % to 13.7%,	Increased among children who received >1 dose form 46.5% to 78.2 % < OR 4.13

				introduced nationally 2015		OR 0.20 (95% CI: 0.11-0.37)	(95% CI 2.36-7.23)
Sa-Leao 2009 (81)	Portugal	Children in day care centre	CRM-7	2+1 at 2, 4, 12, Non-universal coverage since 2001, introduced nationally 2015	5	Decrease from 53.1% to 11.2% (p<0.001)	Increased from 46.9% to 88.8%(p<0.001)
Cohen 2010 (14)	France	Children 6 to 24 months with acute otitis media	CRM-7	3+0 at 2, 4 and 11 months of age	3	Decrease from 44.3% to 17.3%, OR 0.39 (0.32-0.47)	Increase from 9.6% to 23.5%, OR 2.45 (95% CI: 1.88-3.20)
Dunais 2008 (86)	France	Children in day care centres	CRM-7	2+ 1 at 2, 4 and 11 months of age from 2003	3	Decreased from 76.5% to 21.0%, OR 0.08 (95% CI, 0.05-0.14) comparing between 1999 and 2006	Increased from 9.8% to 37.4% , OR 2.14 (95% CI, 1.08-4.26) between 1999 and 2006
Spijkerman 2011 (82)	Netherlands	Children aged 11-24 months and their parents	CRM-7	3 doses at 11 months and 4 doses at 24 months with no catch up	3	Children aged 11months: decrease from 38% to 8%, aOR 0.14 (95% CI: 0.09- 0.23) Children aged 24 months: decrease in VT from 36% to 4%, aOR 0.08 (95% CI: 0.05 - 0.15) Parents: decrease in VT from 8% to 1%, aOR 0.06 (95% CI: 0.01-0.26)	In children aged 11 months increase in NVT from 29% to 39% , aOR 1.64 (1.15-2.32) In children aged 24 months increase in NVT from 30% to 45%, aOR 2.01(1.43-2.84) In parents increase in NVT from 8% to 15%, aOR 1.98 (1.16-3.36)
Steens 2014(89)	Norway	Children attending day care centres	CRM-7 introduced in 2006 and replaced by	2 + 1 schedule at 3, 5 and 12 months of age	2 and 4 (2006 vs 2008 vs 2012)	Decreased from 54% to31.6 % , OR 0.58 (95% CI: 0.52-0.65) by 2008 and further declined to 9.3% , OR 0.17 (95% CI: 0.14-0.21)	Increased from 33.6% to 60. 60.6% , OR 1.8 (95% CI: 1.63-1.99) in 2008 and ti 56.9% in 2012 , OR 1.69 (95% CI: 1.53-1.88)

			CRM-13 in 2011				
Western Pacific							
Mackenzie 2007 (78)	Australia	Unimmunised children and adults living in three remote Australian villages	CRM-7	3 +1 dose schedule at 2, 4 , 6 months with booster dose of PPSV-23 at 23 months of age, since 2001 with catch up for children up to 23 months of age	12 and 30 months after vaccine introduction	No change at 12 and 30 months after vaccine introduction.	No change at 12 and 30 months after vaccine introduction
Africa							
Hammit 2014 (76)	Kenya	Household data	PHiD-CV10[3]	3 + 0 schedule at 6, 10 and 14 weeks, also all infants catch up, 3 doses at least 4 weeks apart Additionally, catch up campaign for children aged 12-59 months, at least 2 doses of vaccine	2	Decrease in children <5 years from 34% to 13%. Prevalence ratio 0.36 (95% CI, 0.26-0.51) and inn individuals > 5 years prevalence ratio 0.34 (0.18-0.62)	Increased in children < 5 years from 41% to 57%, adjusted prevalence ratio 1.37 (1.13 to 1.65). And among individuals >5 years NVT were unchanged 1.13 (0.92 to 1.38)
Kim 2014 (77)	Kenya	Children <5 years	PHiD-CV10	3+0 schedule at 6, 10 and 14 weeks of age, introduced 2011 with no catch-up	3 (2009 vs 2012)	Among all children decrease from 38% in 2009 to 17% in 2012 ($p<0.001$) In infants <12 months declined by 57%, $p<0.001$ In children 1to4 years old 59% decrease, $p<0.001$ Also in unvaccinated children 1to4years 61% decrease, $p<0.0001$	Not reported

[1]PCV7 serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to diphtheria carrier protein
[2]PCV13, serotypes, 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) which are conjugated to diphtheria carrier protein,
[3]PHiD-CV10 serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F, conjugated to *Haemophilus Influenzae* protein D.

With the exception of the Australian study in a remote region where at 30 months there was no change in neither vaccine serotype nor non-vaccine serotype colonization in unimmunised children and adults (78) the studies in Table 1.3 above demonstrate varying degrees in vaccine and non-vaccine serotype change relative to childhood PCV immunization. Whilst the published studies are informative from a diversity of settings, very few of these studies were undertaken at a community-wide level and across a spectrum of age-groups. Furthermore, none of the studies were undertaken in a setting with a high prevalence of HIV-infection.

In this thesis temporal changes in pneumococcal colonization, following infant PCV-immunization introduction in South Africa, are described in relation to age-group and HIV infections status Chapters 3 and 4.

1.5 Bacterial interactions in the nasopharynx

Besides *S. pneumonia*e, the nasopharynx is an ecological niche for other organisms, including *Haemophilus influenzae* and *Staphylococcus aureus* (22). Changes in pneumococcal colonization induced by PCV immunization could theoretically lead to changes in the ecology of the nasopharynx and patterns of colonization and disease by other colonizing bacteria. Prior to PCV introduction, an inverse associations was shown observed between *S. pneumoniae* and *S. aureus* colonization in HIV-uninfected children (65, 93-96) but not in HIV-infected children (65, 97). In some studies, this association was strongest for PCV7 serotypes (94), whereas no such associations were observed by others (98). The negative association between *S. aureus* and *S. pneumoniae* colonization (93, 94) is thought to be due to inhibition of S. aureus by hydrogen peroxide produced by the pneumococcus (93, 99). Failure of the adaptive immunity to develop in HIV-infected children has also been proposed

as impacting on this relationship and this is supported by data from studies in HIV-infected children which failed to show a negative relationship between *S. pneumoniae* and *S. aureus* (65, 97).

Conversely, a synergistic association has been observed between *H. influenzae* and *S. pneumoniae* nasopharyngeal colonization in HIV-uninfected children, (65, 100). This synergistic association between *H. influenzae* and *S. pneumoniae* also extends to nasopharyngeal densities, that is, a high density of *H. influenzae* is associated with high density of S. *pneumoniae* and similarly, a low density of *H. influenzae* is associated with a low density of *S. pneumoniae* (96). This equilibrium is, however, disrupted in disease and a negative association between *H. influenzae* and *S. pneumoniae* has been described in children with upper respiratory tract infection (101).

There are limited data on bacterial interactions in adults, among whom prior to the PCV-era no association was observed between these bacteria in HIV-uninfected or HIV-infected individuals (102-105), An increase in the prevalence of *S. aureus* colonization has been documented following PCV introduction in some settings among vaccinated children and their parents (82, 103). However, this has not been consistently observed (65, 95, 106, 107). Bacterial associations in the nasopharynx may be strain/serotype specific (93), for example following PCV immunization there was a concomitant increase in non-vaccine serotype and *H. influenzae* colonization in the Netherlands among vaccinated children and their parents at 11 and 24 months after PCV7 vaccination (108). This is despite a decrease in pneumococcal vaccine serotype colonization observed during the same period.

There has been no published report on the association of childhood PCV-immunization on the nasopharygeal bacterial ecology either at a community level, or specifically in settings with a high prevalence of HIV-infection or in HIV-infected individuals. In Chapter, 5 of this thesis we describe the temporal association of infant PCV immunization on nasopharyngeal ecology of *S. pneumoniae*, *S.aureus* and *H. influenzae* by age-group in a setting with a high prevalence of HIV infection among adults.

1.6 Effect of PCV on invasive pneumococcal disease (IPD)

Surveillance data for IPD in the USA indicates that between 1998/99 and 2003 there was a 75% decline in IPD caused by all serotypes and a 94% decrease in IPD PCV7-disease in the general population (10). This reduction was greatest in children less than two years of age. In addition, a notable reduction in IPD was evident among those older than 5 years, and particularly among the elderly (>65 years age), supporting an indirect effect of childhood PCV-vaccination (10, 11, 73, 109-111). In South Africa, the greatest burden of IPD among adults is in HIV-infected adults aged 18-45 years (112). While increased anti-retroviral therapy (ART) management showed a clear decrease in IPD among children (113), no change was observed among adults in the same period. Demonstration of an indirect protection among adults could provide an additional public-health benefit of infant PCV immunization in a setting of high HIV prevalence such as ours.

The observed decrease in IPD in USA was offset by a concomitant increase in non-vaccine serotype disease (10). Although non-vaccine serotypes are less commonly associated with disease, these serotypes may acquire virulence factors associated with increased invasive potential. Also, non-vaccine serotype replacement disease may be of particular concern

among immunocompromised individuals, such as those with HIV. In the USA, the incidence of IPD due to non-PCV7 serotypes, in particular 19A, increased by 58% among those aged less than 2 years and by 135% in those 2 to 4 years of age, within three years of PCV7 introduction (114, 115). An increase in disease due to non-vaccines serotypes 19A and 7F was also described following PCV introduction in Portugal,even though there was only 43% vaccine coverage in children at the time (116). Increase in non-vaccine serotype IPD may, however, also be independent of PCV-immunization and solely due to natural temporal fluctuations (117-120). One such example was from South Korea where the increase in serotype 19A IPD occurred prior to PCV immunization,(117) but concurrently with the increase which was being observed in countries where PCV had been introduced (114, 115, 121).

1.7 Use of IPD or colonization in surveillance

IPD surveillance may be challenging for countries with poor infrastructure and limited resources. Since pneumococcal nasopharyngeal colonization is a precursor for pneumococcal disease, the impact of PCV immunization against IPD may be imputable by undertaking ecological pre- and PCV-era studies on the changes in serotype-specific colonization (122). Since studies on nasopharyngeal colonization are fairly easy to undertake, requiring much less infrastructure compared to IPD surveillance, it could be a useful tool to evaluate vaccine effectiveness against IPD in low-middle income countries. Evaluating the effect of new formulations of PCV against pneumococcal colonization is also advocated as an additional measure for vaccine licensure as well as post vaccine introduction surveillance (123). An improved understanding of the link between colonization and IPD is, however, required to help use changes in colonization as a proxy for PCV effect against IPD (124).

Theoretically, this could be achieved by incorporating the invasive potential, i.e. measure of expected IPD for a given prevalence of colonization in health and disease by individual serotypes (122). Imprecise estimates of IPD post-vaccine introduction were, however, obtained when using individual serotype data, compared to a simplified model that grouped PCV-serotypes and non-vaccine serotypes (122). This model was applied to six populations, rural Alaska, urban Alaska, England, Massachusetts, the Netherland and the Navajo. With the exception of England, the model correctly predicted within 95% confidence interval the measured incidence of IPD post-PCV introduction (122).

There is limited data from low to middle-income countries to support the use of colonization data as a proxy of the direct and indirect effectiveness of PCV against IPD. Furthermore, the high HIV prevalence and other concurrent interventions targeted against HIV, which may also impact on incidence of IPD, adds a further complexity in using temporal changes in PCV-serotype colonization to estimate vaccine effectiveness against IPD (122). In this thesis we aim to address this by describing concurrent changes in IPD and NP colonization in mother-infant pairs stratified by HIV-status in Chapter 6.

1.8 Study aims and objectives

This study evaluated the effects of the introduction of PCV into the childhood immunization program in South Africa on pneumococcal colonization in urban and rural communities with a high prevalence of HIV-infection. Furthermore, we explored the association of temporal changes in vaccine and non-vaccine serotype colonization and IPD in an urban black-African community.

The specific objectives of the study included:

i) To determine the effect of infant PCV-immunization on the epidemiology of nasopharyngeal colonization in different age groups in a rural African community with high prevalence of HIV-infection;

ii) To evaluate the effect of routine infant PCV immunization following transitioning from PCV7 in 2011 to PCV13 in 2013, on the prevalence of vaccine serotype and non-PCV13 serotype colonization in HIV-infected and HIV-uninfected mother-child pairs in South Africa at a community level;

iii) To determine the effect of infant PCV7 immunization on the prevalence of nasopharyngeal colonization and interaction between *S. pneumoniae*, *S. aureus* and *H. influenzae* among children and older individuals in a rural South African community with a high prevalence of HIV-infection;

iv) To analyse the direct and indirect effectiveness of infant PCV immunization on invasive pneumococcal disease by HIV-status in Soweto, South Africa;

v) To evaluate the fitness of a proposed model by Weinberger et al. for predicting the changes in IPD from serial colonization studies compared to that observed for vaccine serotype and non-vaccine serotype IPD.

The reporting format in this thesis includes:

i. In chapter 3 we describe the effect of introduction of PCV by age-group on nasopharyngeal colonization of *S. pneumoniae* in a rural community with high HIV prevalence.
ii. In chapter 4, we explore the temporal association of infant PCV immunization on the dynamics of pneumococcal colonization in mother-child pairs, and also elucidate the effect of HIV on the transmission.

iii. In chapter 5, we detail the association of PCV vaccination on colonization prevalence by *S. aureus* and *H. influenzae,* and whether these interactions differed in the pre-PCV compared to the PCV-era.

iv. In chapter 6 we report on the effect of infant PCV vaccination on trends of IPD in children and adults, stratified by HIV-status, in an urban black-African setting. Furthermore, we analyzed how changes in PCV-serotype and non-vaccine serotype colonization compared to changes in incidence of IPD in the same population as well as the robustness of the model proposed by Weinberger et al. in our setting.

Finally, the results are summarized in chapter 7, and future research work is described.

Chapter 2 Methods

2.1 Study design

The different data sources contributing to the individual study objectives are summarized in table 2.1.

Table 2.1: Summary of different data collection points and papers in which data is reported

Study site	Year					Paper
	2005-2008	2009	2010	2011	2012	
	Pre-PCV era	PCV7 introduced	PCV7-ear	PCV-7 replaced by PCV13	PCV13-era	
Agincourt		Cross-sectional survey 1: Agincourt (household study)		Cross-sectional survey 2: Agincourt (household study)		Paper 1 and 2
Soweto	Longitudinal carriage survey among mother infant pairs		Cross-sectional survey 1: Soweto (mother-infant pairs)		Cross-sectional survey 2: Soweto (mother-infant pairs)	Paper 3 and Paper 4
Soweto	Pre-vaccine IPD data	IPD data	IPD data	IPD data	IPD data	Paper 4

2.2 Study population

The series of studies were undertaken in two study populations, including a rural community in Mpumalanga Province and an urban population in Soweto, Gauteng Province, South Africa. The characteristics of these two populations are detailed below.

2.2.1 Agincourt, Mpumalanga, South Africa

The Agincourt sub-district in Mpumalanga Province, South Africa, has a population of approximately 84,000 people in 14,000 households and 26 villages. It covers an approximate area of 402 km² in rural northeast South Africa, close to the Mozambique border and a third of the population consists of immigrants of Mozambican descent. The Agincourt sub-district is one of two socio-demographic surveillance sites in South Africa and has been under constant surveillance by the MRC/Wits Rural Public Health and Health Transitions Research Unit since 1992 (125).

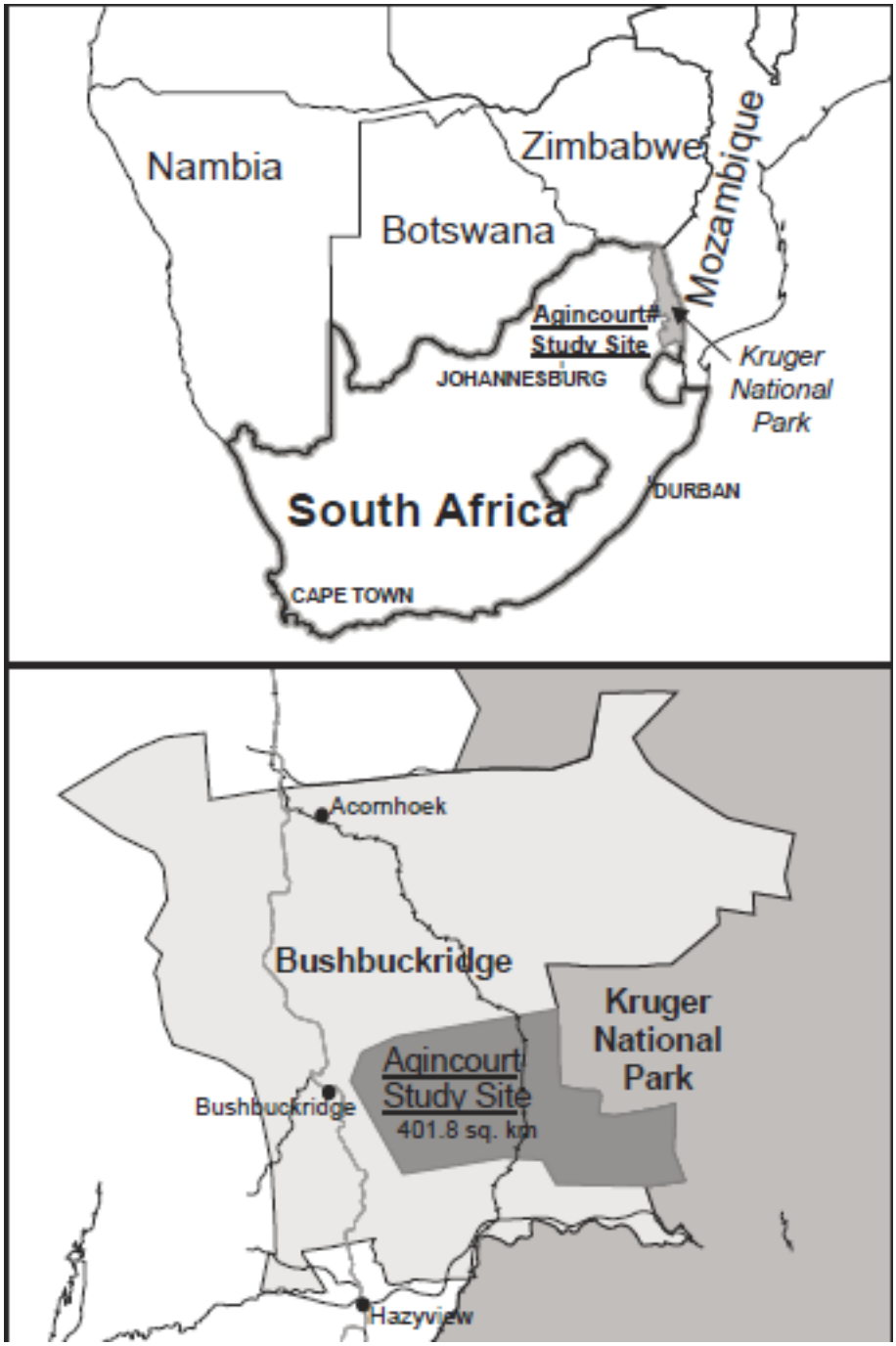


Figure 2.1: Map of Agincourt subdistrict

Despite improvement in electricity and water supply the area has poor infrastructure. It offers limited local employment and subsistence farming opportunities. Consequently, most adults seek work in industrial, mining or agricultural centers away from home, with 60% of men 30–54 years age, and 25% of women age 25–49 years being migrant labourers. A health center with five satellite clinics services the health care needs of the community. The health

center refers complicated cases for further management to one of three district hospitals that are 25 to 60 kilometres from Agincourt. Demographic transitions have included sustained decrease in fertility since the mid-1970s, which has steadied at near replacement level with little recent change in population size. Decreases in mortality rates were ongoing until the mid-1990s, following which it increased due to the HIV/AIDS epidemic, with major reversal in mortality in young adults and children under-5 years age. By 2005, life expectancy at birth had fallen by 12 years for women and by 14 years for men (125). In 2009, females were expected to live to 64.4 years while males had a lower life expectancy of 55.7years (126).

2.2.2 Soweto, Johannesburg, South Africa

Soweto is the most populous black township in Johannesburg with an approximate population of 1.4 million and a birth cohort of 28 000 per year (127). The community under surveillance is a low-income, urbanized black-African community of various tribal ethnicities. The prevalence of HIV infection in among adults in Gauteng Province (in which Soweto is based) was 16% in 2005 and 30% in pregnant women attending antenatal clinics in Soweto since 2005 (ANOVA-unpublished data). These prevalences have remained largely unchanged by 2013 (ANOVA-unpublished data). The vertical mother-to-child transmission (MTCT) rate of HIV has, however, declined from 8-12% in 2007 to <2% by 2010 due to more aggressive antiretroviral therapy (ART) regimens targeted at pregnant women and aimed at preventing vertical HIV transmission (128). HIV-infected adults and children are provided with free ART through established HIV clinics in Soweto, including at CHBAH. This includes triple ART for all HIV-infected pregnant women irrespective of their CD4+ counts, with a coverage rate of approximately 75%. HIV testing of infants born to HIV-infected mothers is undertaken through the established MTCT program at 6 weeks of age. HIV testing is also undertaken in the majority of children hospitalized in the general wards at Chris Hani

Baragwanath Academic hospital, the only public facility hospital serving the community of Soweto at the time of this study.

Children admitted at CHBAH are provided free state-funded health care, including costs related to laboratory investigations and clinical management. Similarly, adults are expected to pay a nominal administrative fee which is inclusive of all hospital costs. Patients admitted with suspected sepsis and pneumonia have blood cultures undertaken as part of standard of care, and similarly lumbar punctures for cerebrospinal fluid microbiologic evaluation are undertaken in those suspected to have meningitis. CHBAH has been a sentinel surveillance site for IPD surveillance undertaken by the National Institute for Communicable Diseases (NICD) since 1999, and the only site able to provide serial measurement of incidence of PCV-serotype and non-vaccine serotype IPD stratified by HIV status. It is therefore possible to correlate colonization data with IPD data for this population.

2.3 PCV introduction in South Africa

South Africa introduced PCV7 (Prevnar™, Pfizer, New York, US) into the national, public-funded childhood immunization program from April 2009. The vaccine is administered in a two-dose primary series at ages 6 and 14 weeks, followed by a third dose at age 9 months. No catch up campaign of any child older than 6 weeks of age, at the time of initiation of the PCV-immunization program, was offered during the course of this study, if they had already received any other childhood-vaccine recommended from 6 weeks of age onward. Since May 2011, PCV7 has replaced by a 13-valent PCV (PCV13; Prevenar-13 ™, Pfizer, Pearl River, New York, NY, USA), which was coupled to a limited catch-up campaign targeting children up to 3 years of age, and high risk-groups (including HIV-infected children) up to 6 years of

age. The immunization coverage for three doses of PCV in Gauteng was reportedly 12.3% in 2009, 86.3% in 2010 and 99% in 2011 and 2012 (19, 129).

2.4 Determination of bacterial colonization

Carriage of pneumococcus is more common in the winter months (32). We undertook serial cross sectional surveys in the winter months (May to September) each year to account for nay seasonal variation. Sampling of the pharynx was undertaken by trained medical study staff at the households. Nasopharyngeal swabs were performed using an aluminium shafted, Dacron swab (MW and E, Medical Wire and Equipment Co. Ltd., Corsham, Wiltshire, England) as described (130). Additionally, an oropharyngeal swab was collected in adults. Specimens were placed in skimmed milk, tryptose, glycerol and glucose (STGG) broth transport media, transported in a cooler box and then stored at -70°C within 6-hours of sampling. Samples were shipped intermittently to the Centre for Respiratory Diseases and Meningitis (CRDM) laboratory at the National Institute for Communicable Diseases (NICD) in Johannesburg on dry ice and stored at -70°C until processed by routine microbiological methodologies. Serotyping was undertaken by the Quellung method using serogroup and factor-specific antisera (Statens Serum Institute, Copenhagen, Denmark). Presumptive pneumococcal isolates which did not exhibit the Quellung reaction with serogroup-specific, factor-specific and all-pooled anti-sera were categorized as non-typeable once pneumococcal identification was confirmed with *lyt*A PCR.(131) Where >1 distinct morphological colony type was present, each colony was serotyped. Serotypes 6A, 6B, 6C and 6D were distinguished only using the relevant antisera and without any additional molecular typing.

2.5 Ethical issues

The colonization studies study protocols were approved by the University of Witwatersrand, Human Research Ethics Committee (Medical), Ethics numbers M090015 and M090114. Additionally, specific ethics approval for this PhD was also obtained (Reference: M110530).

2.6 Informed consent

Informed consent was obtained for all adults; and a parent/legal guardian/primary care-giver of a child signed consent on behalf of children less than 18 years of age. Also, verbal assent was obtained from children older than 8 years of age.

Chapter 3: Temporal changes in pneumococcal colonization in a rural African community with high HIV-prevalence following routine infant pneumococcal immunization

3.1 Introduction

Colonization of the nasopharynx by *S. pneumoniae*, though usually asymptomatic, may be associated with pneumococcal disease particularly within the first two months of acquisition of new pathogenic serotypes (21). At the time of illness, the same serotype colonizing the nasopharynx is identifiable from diseased sites (21, 29).

Immunization of children with PCV, decreases their risk of acquisition of vaccine serotypes, particularly serotypes traditionally associated with a high prevalence of asymptomatic NP colonization during childhood; i.e. 6A, 6B, 9V, 19A, 19F and 23F (21, 32, 33). Routine PCV7 childhood immunization in industrialized settings has been associated with reduced NP-acquisition of the vaccine serotypes among vaccinated children, which in-turn has resulted in reduced transmission of these serotypes to unvaccinated individuals (8, 9). Consequently, reductions in incidence of vaccine serotype IPD among vaccinated and unvaccinated individuals have been observed in settings with low prevalence of HIV infection (10-12). A decline in vaccine serotype IPD has also been observed among PCV-unvaccinated HIV-infected adults following childhood PCV7 immunization in USA,(14, 15) which has partly been offset by an increase in non-vaccine serotype IPD of greater magnitude compared to the general population of adults (14, 15).

In Africa, a study from The Gambia reported significant decline in vaccine serotype colonization among unvaccinated adolescents and adults following childhood PCV7

immunization, which included a targeted catch-up campaign of children aged up to three years (80). This study finding may have been confounded by the prolonged time period (3 years) between when the pre-vaccination survey was undertaken relative to when PCV immunization was initiated. Also, the possibility of natural temporal fluctuations of circulating pneumococcal serotypes may have contributed to differences in serotype-specific pneumococcal colonization, including the lower prevalence of non-vaccine serotype colonization, following childhood immunization with PCV (80).

In this study we aimed to determine the effect of infant PCV immunization on the epidemiology of nasopharyngeal colonization in different age-groups in a rural African community with high prevalence of HIV-infection.

3.2 Methods

3.2.1 Study setting and study population

Two cross-sectional studies, spaced two years apart were undertaken in a rural setting which is a health and socio-demographic surveillance site (HDSS), in the Agincourt sub-district in Mpumalanga province, northeast South Africa. Details of the Agincourt HDSS have been described in Chapter 2. Details of the surveyed population are summarized in Table 3.1.

Table 3.1: Characteristics of households and individuals enrolled into the study and population characteristics for Agincourt

Characteristic	Period-1 (N=577)	Period-2 (N= 1079)	p value
Household structure			
Children ≤ 5years (mean ± SD)	1.83 ± 1.0	1.96 ± (1.1)	0.02
Individuals 6 - 18years (mean ± SD)	2.4 ± 1.8	2.2 ± 1.7	0.03
Individuals >18 years (mean ± SD)	4.5 ± 2.4	4.7 ± 2.6	0.13
Individuals in household, (mean ± SD)	8.73 ± 4.1	8.8 ± 4.2	0.74
Child in house that attends daycare, n/N (%)	164/572 (28.7%)	325/982 (33.1%)	0.07
Number of rooms used for sleeping, (mean ± SD)	3.15 ± 1.5	3.2 ± 1.7	0.56
Fuel used for cooking			
Coal/Wood (%)	394 (68.3)	685 (63.5)	0.11
Paraffin/Gas (%)	11 (1.9)	17 (1.6)	0.64
Electricity (%)	168 (29.3)	365 (34.2)	0.03
Participants age > 12years			
No of participants	994	1781	
Female	815 (82.1)	1538 (86.3)	<0.001
Mean age ± SD	34.01 ± 16.5	33.4 ± 16.3	0.35
HIV-infected (self-reporting)	40/505 (7.9)	135/1320 (10.2)	0.11
Participants age, ≤ 12 years			
No of participants	1016	1877	
Female	515 (50.7)	964 (51.4)	0.73
Mean age ± SD	4.32 ± 3.4	3.80 ± 3.4	<.001
HIV-infected (self-reported)	5/165 (3.0)	20/631 (3.1)	0.93
Population characteristics[1]			
Total population	87359	90036	
Percentage <5 years	12.3	11.9	
Percentage < 15 years	22.2	21.8	
Percentage ≥ 65 years	4.7	4.6	
Life expectancy at birth (females) –years	62	66[2]	
Life expectancy at birth (males)-years	54	56[2]	
Infant mortality rate per 1000 live births	33	35	
Under-5 mortality per 1000 live births (females)	57	41	
HIV prevalence among adults (19-46)	Not available	37.4%[3]	
Total number of households	15455	16091	
Mean household size	5.64	5.59	
Percentage using coal/woof for cooking	56.2%	47.2%	
Vaccine coverage in children DPT3	72%	Not available	
Vaccine coverage measles 1 (age at PCV3 vaccination	85%	Not available	

Those characteristics which could have influenced the prevalence of pneumococcal NP-colonization and which differed between the two sampling periods at a significance level of

[1] Population characteristics calculated as of 1st May 2009 for Period 1 and 1st of May 2011 for Period 2 based on data from the Agincourt Health and Demographic Surveillance System (AHDSSS) database.
[2] Life expectancy for Period 2 based on data for 2010, the most recent complete years data.
[3] Ref (Gomez-Olive et al, 2012 based on data from an HIV sentinel survey carried out in the Agincourt area in 2011)

p-value <0.1 were adjusted when estimating the relative risk of pneumococcal carriage in Period 1 and Period 2.

3.2.2 Study sampling framework and procedure

The initial survey (Period 1) was undertaken from May to October 2009, i.e. immediately upon introduction of PCV into the immunization program. A subsequent (Period 2) survey was undertaken between May to October 2011. Based on the annual census update of the 27 villages in the HDSS, children <2 years of age were identified and linked to their parent household for potential study participation in each village. Villages were then randomly selected for order of sampling, with the number of villages approached continuing until the target sample size was achieved. A household was included if in addition to the listed child there was at least one individual older than 12 years who agreed to participate in the study. The Agincourt HDSS definition for a household, also used in this study, was "a group of people who resided and ate together including temporary migrants, (i.e. a household member who is away for more than 6 months per year but retains a significant link to the rural/sending household) who ate with them on return". Meetings were held with community members, traditional and civic leaders to explain the study. A questionnaire was administered at the time of swab collection. No HIV testing was performed but self-reported HIV infection status was collected.

3.2.3 Sample size calculation

Since there were no prior data on the prevalence of pneumococcal NP-colonization in this setting, the estimated prevalence of vaccine serotype colonization among adults in 2009 was

based on data from Navajo Indian populations (4%) (73) and an African population in Malawi (132) in the absence of PCV immunization. Vaccine serotype colonization in South African adults was expected to be greater than in Navajo Indians, since a higher prevalence of colonization had been reported among HIV-infected Malawian women (11.4% vs. 5.9% in HIV-uninfected women) (132). We postulated that the prevalence of vaccine serotype colonization would be 10% among adults in 2009. We calculated the need to enrol 611 adults in each survey-period to detect at least a 45% reduction in PCV7 serotype colonization between Period 1 and Period 2 with 80% power. Based on the actual observed prevalence of PCV7 serotype colonization among adults in Period 1 (3.0%), we recalculated the sample size for Period 2 and targeted enrolling at least 1783 adults to provide 80% power to detect a 50% reduction in PCV7 vaccine serotype colonization compared to Period 1.

3.2.4 Determination of bacterial colonization

Sampling procedures and sample processing as described in section 2.4.

3.2.5 Definitions and statistical analysis

For all statistical analysis, individuals older than 12 years were categorized as adolescents/adults and those ≤12 years as children. Multiple, simultaneous serotype colonization in the same individual were considered as independent events when measuring the prevalence of colonization if they differed in stratification into PCV7 serotype (4, 6B, 9V, 14, 18C, 19F and 23F), non PCV7 serotype or being non-typeable. Non PCV7 serotypes refer to any other pneumococcal serotypes (including serotype 6A) and excluded non-typeable isolates. Comparison of the prevalence of pneumococcal NP colonization and serotype

distribution between the two periods were performed using chi-squared or Fisher's exact tests where appropriate. Log binomial regression models were used to estimate the risk ratios for pneumococcal carriage in Period 1 versus Period 2, adjusting for potential risk factors for colonization including age, gender, fuel used for cooking in the household, presence of a child attending day care in the household and household structure, i.e. the presence of any child below 5 years and any individual between 6 and 18 years of age in the household. In addition, generalized linear mixed models were developed to adjust for potential correlation among subjects from the same household plus all the other risk factors used in the log binominal regression models. Statistical analyses were performed with STATA 12 (Statacorp, Texas, USA) and SAS version 9.2 software (SAS Institute, Inc., NC, USA).

3.3 Results

A total of 577 and 1079 households were recruited in Period 1 and Period 2, respectively. This included 994 and 1781 adults/adolescents and 1016 and 1877 children, in the two respective periods. Characteristics of the sampled households are summarized in Table 3.1. Differences between households in the two periods included a higher proportion who used electricity for cooking in Period-2 (34.2% vs. 29.3%; p=0.032); higher mean number of children <5 years of age within households in Period 2 and consequently an older mean age of children enrolled in Period 1(Table 3.1).

3.3.1 Temporal changes in prevalence of pneumococcal colonization

In Period 1, 83.0% of children <2 years of age were colonized, including 28 who carried two serotypes, resulting in a total of 359 pneumococcal isolates (Table 3.2). The prevalence of PCV7 serotype colonization was 45.1% in children age <2 years in Period 1 (Table 3.2). In 2 to 5 year old children, overall colonization was 80.2%, including 17 children who were colonized by two serotypes which resulted in 236 isolates. The prevalence of PCV7 serotype and non PCV7 serotype colonization in the 2 to 5 year age-group was 35.5% and 45.8%, respectively in Period 1, (Table 3.2).

Among 6 to 12 year-old children, 60.0% were colonized, including 13 with two serotypes, resulting in 199 pneumococcal isolates identified. The prevalence of PCV7 serotype was 19.0% among the 6 to 12 year age group, which was lower than in the <2 year age-group (p <0.0001), while non PCV7 serotype colonization prevalence (42.6%) was similar to that in the youngest age group (p=0.32; Table 3.2. Overall, the prevalence of colonization in adolescents and adults was 22.8%, 10.7% and 5.1% among the 13-18 years, 19 to 45 years and >45 year age groups, respectively; Table 3.2. Additionally, the prevalence of PCV7 serotype colonization was 5.7%, 3.0% and 1.7% in the respective above specified adolescent and adult age groups; Table 3.2. Females who were primary caregivers to the children, had similar overall prevalence of pneumococcal colonization (compared to other adolescents/adults (10.2% [49/478] vs. 8.4% [19/225; p=0.45) but higher prevalence for PCV7 serotypes (3.8% [18/478] vs. 1.3% [3/225]; p=0.06) in Period 1.

Table 3.2 Comparison of prevalence of all pneumococci, PCV7 serotype, non PCV7 serotype and non-typeable serotypes by age and gender in Period 1 (2009) and Period 2 (2011).

	Total per group N[1]		Overall prevalence colonisation n (%)			PCV7 serotypes[3] (VT) n (%)			Non PCV7 serotypes n (%)			Non-typeable serotypes		
Age group	Period 1	Period 2	Period 1	Period 2	p-value	Period 1	Period 2	p-value	Period 1	Period 2	p-value	Period 1	Period 2	p-value
<2years														
Female	196	456	161(82.1)	336(73.7)	0.02	84(42.9)	111(24.3)	<0.0001	76(38.8)	223(48.9)	0.02	7(3.6)	14(3.1)	0.74
Male	203	452	170(83.7)	330(73.5)	0.003	96(47.3)	102(22.6)	<0.0001	79(38.9)	233(51.6)	0.003	5(2.5)	8(1.8)	0.55
Total (%)	399(19.9)	908(24.8)	331(83.0)	666(73.4)	0.0002	180(45.1)	213(23.5)	<0.0001	155(38.9)	456(50.2)	0.0001	12(3.0)	22(2.4)	0.54
ARR[2]			**0.89(0.84,0.94)**			**0.50(0.42,0.59)**			**1.35(1.17,1.56)**			**0.81(0.38,1.72)**		
2-5years														
Female	142	209	111(78.2)	147(70.3)	0.10	43(30.3)	55(26.3)	0.42	69(48.6)	92(44.0)	0.40	1(0.7)	2(1.0)	-
Male	131	213	108(82.4)	160(75.1)	0.11	54(41.2)	66(31.0)	0.05	56(42.8)	95(44.6)	0.74	5(3.8)	6(2.8)	0.61
Total (%)	273(13.6)	422(11.5)	219(80.2)	307(72.8)	0.02	97(35.5)	121(28.7)	0.06	125(45.8)	187(44.3)	0.70	6(2.2)	8(1.9)	0.78
ARR[2]			**0.96(0.88,1.04)**			**0.79(0.63,0.99)**			**0.99(0.84,1.18)**			**0.83(0.29,2.34)**		
6-12years														
Female	156	266	95(60.9)	133(50.0)	0.03	31(19.9)	32(12.0)	0.03	65(41.7)	102(38.4)	0.50	2(1.3)	1(0.4)	-
Male	154	218	91(59.1)	109(50.0)	0.08	28(18.2)	29(13.3)	0.20	67(43.5)	80(36.7)	0.19	0(0.0)	3(1.4)	-
Total (%)	310(15.4)	484(13.2)	186(60.0)	242(50.0)	0.006	59(19.0)	61(12.6)	0.01	132(42.6)	182(37.6)	0.16	2(0.7)	4(0.8)	0.77[4]
ARR[2]			**0.83(0.74,0.95)**			**0.66(0.48,0.92)**			**0.87(0.73,1.04)**			**1.40(0.26,7.66)**		
13-18years														
Female	92	170	20(21.7)	19(11.2)	0.02	5(5.4)	3(1.8)	0.08[4]	15(16.3)	15(8.8)	0.07	0(0.0)	1(0.6)	-
Male	66	115	16(24.2)	16(13.9)	0.08	4(6.1)	3(2.6)	0.25[4]	13(19.7)	13(11.3)	0.12	0(0.0)	0(0.0)	-
Total (%)	158(7.9)	285(7.8)	36(22.8)	35(12.3)	0.004	9(5.7)	6(2.1)	0.05	28(17.7)	28(9.8)	0.02	0(0.0)	1(0.3)	-
ARR[2]			**0.54(0.35,0.81)**			**0.49(0.17,1.39)**			**0.55(0.34,0.89)**			**-**		
19-45years														
Female	541	1069	63(11.7)	61(5.7)	<0.0001	18(3.3)	11(1.0)	0.001	47(8.7)	46(4.3)	0.0004	1(0.2)	7(0.7)	-
Male	92	119	5(5.4)	8(6.7)	0.70	1(1.1)	2(1.7)	-	4(4.4)	6(5.0)	0.25[4]	0(0.0)	0(0.0)	-
Total (%)	633(31.5)	1188(32.5)	68(10.7)	69(5.8)	0.0001	19(3.0)	13(1.1)	0.003	51(8.1)	52(4.4)	0.001	1(0.2)	7(0.6)	-
ARR[2]			**0.48(0.34,0.67)**			**0.36(0.18,0.74)**			**0.46(0.31,0.68)**			**-**		
>45years														
Female	195	324	12(6.2)	20(6.2)	0.99	4(2.1)	3(0.9)	0.17[4]	8(4.1)	15(4.6)	0.78	0(0.0)	3(0.9)	-

Male	42	48	0(0.0)	2(4.2)	-	0(0.0)	1(2.1)	-	0(0.0)	1(2.1)	-	0(0.0)	0(0.0)	-
Total (%)	237(11.8)	372(10.2)	12(5.1)	22(5.9)	0.66	4(1.7)	4(1.1)	0.52[4]	8(3.4)	16(4.3)	0.57	0(0.0)	3(0.8)	-
ARR[2]			**1.15(0.55,2.38)**			**0.63(0.16,2.49)**			**1.15(0.48,2.72)**			**-**		
OVERALL														
Female	1322(65.8)	2494(68.2)	462(35.0)	716(28.7)	<0.0001	185(14.0)	215(8.6)	<0.0001	280(21.2)	493(19.8)	0.30	11(0.8)	28(1.1)	0.40
Male	688(34.2)	1165(31.8)	390(56.7)	625(53.7)	0.20	183(26.6)	203(17.4)	<0.0001	219(31.8)	428(36.7)	0.03	10(1.5)	17(1.5)	0.99
Total (%)	2010(100)	3659(100)	852(42.4)	1341(36.7)	<0.0001	368(18.3)	418(11.4)	<0.0001	499(24.8)	921(25.2)	0.77	21(1.0)	45(1.2)	0.53
ARR[2]			**0.87(0.83,0.91)**			**0.57(0.51,0.65)**			**1.03(0.95,1.13)**			**1.18(0.69,2.03)**		

NB: In Period-1, 2010 subjects were enrolled of whom 852 were colonized, including 64 among whom two serotypes were identified. In Period-2, 3659 participants were enrolled, of whom 1341 were colonized, including 91 with two-serotypes. In cases of simultaneous detection of multiple serotype colonization in the same individual, the serotypes were considered as independent events when measuring the prevalence of colonization if they differed in stratification by vaccine serotype, non-vaccine serotype or being non-typeable.

[1]Total number of participants in each age group
[2]ARR (adjusted risk ratio), risk ratio adjusted for age, gender, household structure (i.e. presence of a child less than 5 years and presence of child between 6 and 18 years), any child attending day care and fuel used for cooking in the household.
[3] PCV7- serotypes 4, 6B, 9V, 14, 18C, 19F and 23F
[4] Fisher's exact test was used to compare the two groups

In Period 2, 666 (73.4%) children <2 years of age were colonized, including 47 in whom two serotypes were identified, which resulted in 713 pneumococcal isolates. The overall prevalence of pneumococcal colonization was 11.6% lower in Period 2 compared to Period 1 (p=0.0002) in the <2 year age-group (Table 3.2). This was dominantly due to a 50.0% reduction in PCV7 serotype colonization (ARR 0.50, 95%CI): 0.42-0.59), whilst there was a 35.0% increase in prevalence of non PCV7 serotype colonization by Period 2; p=0.0001. Among 2 to 5 year-old children, although there was a 21% reduction in PCV7 serotype colonization, the prevalence of overall and non PCV7 serotype colonization remained unchanged in Period 2 compared to Period 1; Table 3.2.

Among older age groups, lower prevalence of PCV7 serotype colonization in Period 2 compared to Period 1 were evident in children age 6 to12 years (ARR 0.66, 95%CI: 0.48-0.92), a similar trend was observed in the 13 to 18 year age-group (ARR 0.49, 95%CI: 0.17-1.39) and significantly lower prevalence was observed in the 19 to45 year age-group (ARR 0.36, 95%CI: 0.18-0.74). The prevalence of non PCV7 serotype colonization remained unchanged comparing Period 2 to Period 1 in the 6 to 12 year age-group, whereas 45.0% and 54.0% reduction was observed in the 13 to 18 and 19 to 45 year age-groups, respectively; Table 3.2. Overall pneumococcal colonization declined by 17%, 46% and 52% among the age-groups 6 to 12 years, 13 to 18 years and 19 to 45 years, respectively. The prevalence of pneumococcal colonization among the primary caregivers declined to 5.4% (58/1067; p= 0.001) overall, 0.94% (10/1067; p=<0.001) for PCV7 serotypes but remained unchanged among non-caregivers [8.3% (58/696 overall and 2.01% for PCV7 serotypes in Period 2; p=0.9 and 0.4 respectively].

Limiting the analysis to those villages only sampled during both periods resulted in similar findings compared to when analyzing the entire study cohorts, Table 3.3. Generalized linear

mixed models adjusting for potential intra-household clustering had no effect on the observed results, Table 3.4

Table 3.3 Comparison of prevalence of all pneumococci, PCV7 serotype, non PCV7 serotype and non-typeable serotypes by age and gender in Period 1 and Period 2 for nine villages sampled in Period 2 that matched those sampled in Period 1.

	Total per group		Overall prevalence colonisation			PCV7 serotypes (VT)[4]			Non PCV7 serotypes			Non-typeable serotypes		
	N[1]		n (%)			n (%)			n (%)					
Age group	Period 1	Period 2	Period 1	Period 2	p-value	Period 1	Period 2	p-value	Period 1	Period 2	p-value	Period 1	Period 2	p-value
<2years														
Female	205	261	170(82.9)	206(78.9)	0.28	85(41.5)	70(26.8)	<.001	78(38.1)	126(48.3)	0.03	7(3.4)	10(3.8)	0.81
Male	222	270	188(84.7)	205(75.9)	0.02	99(44.6)	65(24.1)	<.0001	84(37.8)	134(49.6)	0.01	5(2.3)	6(2.2)	0.98
Total (%)	427(20.6)	531(27.4)	358(83.8)	411(77.4)	0.01	184(43.1)	135(25.4)	<.0001	162(37.9)	260(49.0)	<.001	12(2.8)	16(3.0)	0.85
ARR[2]			**7.7% (1.6 to 13.6%)**			**41.0% (27.0 to 54.7%)**			**-29.1% (-45.3 to -12.4 %)**			**-7.2% (-84.9 to 76%)**		
2-5years														
Female	150	98	119(79.3)	66(67.4)	0.03	43(28.7)	26(26.5)	0.71	75(50.0)	38(38.8)	0.08	1(0.7)	2(2.0)	-
Male	140	109	117(83.6)	82(75.2)	0.10	55(39.3)	37(33.9)	0.39	57(40.7)	45(41.3)	0.93	5(3.6)	0(0.0)	-
Total (%)	290(14.0)	207(10.7)	236(81.4)	148(71.5)	0.01	98(33.8)	63(30.4)	0.43	132(45.5)	83(40.1)	0.23	6(2.1)	2(1.0)	0.34[5]
ARR[2]			**12.1% (2.9 to 21.6%)**			**9.9% (-14.9 to 34.0%)**			**11.9% (-7.5 to 30.9%)**			**53.3% (-78.5 to 1.73%)**		
6-12years														
Female	161	130	100(62.1)	73(56.2)	0.30	31(19.3)	15(11.5)	0.07	67(41.6)	57(43.9)	0.70	2(1.2)	1(0.8)	-
Male	162	97	99(61.1)	53(54.6)	0.31	28(17.3)	13(13.4)	0.41	71(43.8)	39(40.2)	0.57	0(0.0)	1(1.0)	-
Total (%)	323(15.6)	227(11.7)	199(61.6)	126(55.5)	0.15	59(18.3)	28(12.3)	0.06	138(42.7)	96(42.3)	0.92	2(0.6)	2(0.9)	0.72[5]
ARR[2]			**9.9% (-3.6 to 23.4%)**			**32.5% (-1.7 to 64.6%)**			**1.0% (-18.6 to 20.4%)**				**-**	
13-18years														
Female	92	83	20(21.7)	7(8.4)	0.02	5(5.4)	0(0.0)	-	15(16.3)	7(8.4)	0.12	0(0.0)	0(0.0)	-
Male	67	50	17(25.4)	6(12.0)	0.07	4(6.0)	0(0.0)	-	13(19.4)	6(12.0)	0.28	0(0.0)	0(0.0)	-
Total (%)	159(7.7)	133(6.9)	37(23.3)	13(9.8)	0.002	9(5.7)	0(0.0)	-	28(17.6)	13(9.8)	0.05	0(0.0)	0(0.0)	-
ARR[2]			**58.0% (21.2 to 93.2%)**				**-**		**44.5% (-1.4 to 88.6%)**					
19-45years														
Female	546	579	68(12.5)	37(6.4)	0.001	18(3.3)	9(1.6)	0.06	49(9.0)	24(4.2)	0.001	1(0.2)	4(0.7)	0.20
Male	92	65	5(5.4)	5(7.7)	0.57	1(1.1)	1(1.5)	0.80	4(4.4)	4(6.3)	0.61	0(0.0)	0(0.0)	-
Total (%)	638(30.8)	644(33.2)	73(11.4)	42(6.5)	0.002	19(2.98)	10(1.6)	0.09	53(8.3)	28(4.4)	0.004	1(0.2)	4(0.6)	0.18[5]
ARR[2]			**43.0% (15.7 to 70.7%)**			**47.9% (-8.1 to 107.4%)**			**47.7% (15.6 to 80.6%)**				**-**	
>45years														
Female	195	175	11(5.6)	13(7.4)	0.49	4(2.1)	2(1.1)	0.49	7(3.6)	11(6.3)	0.23	0(0.0)	0(0.0)	-

Male	42	24	0(0.0)	1(4.2)	-	0(0.0)	1(4.2)	-	0(0.0)	0 (0.0)	-	0(0.0)	0(0.0)	-
Total (%)	237(11.4)	199(10.3)	11(4.6)	14(7.0)	0.28	4(1.7)	3(1.5)	0.88[5]	7(3.0)	11(5.5)	0.18	0(0.0)	0	-
ARR[2]			**-51.6% (-156.5 to 44.6 %)**			**10.7% (-167.8 to 174.0%)**			**-87.2% (-235.1 to 43.6%)**					
OVERALL														
Female	1349	1326	488(36.2)	402(30.3)	0.001	187(13.9)	122(9.2)	<.001	291(21.6)	263(19.8)	0.27	11(0.8)	17(1.3)	0.24
Male	725	615	426(58.8)	352(57.2)	0.57	186(25.7)	117(19.0)	0.004	229(31.6)	228(37.1)	0.03	10(1.4)	7(1.1)	0.69[5]
Total (%)	2074(100)	1941(100)	914(44.1)	754(38.9)	<.001	373(18.0)	239(12.3)	<.0001	520(25.1)	491(25.3)	0.87	21(1.01)	24(1.2)	0.50
ARR[2]			**11.9% (4.9 to 18.7%)**			**31.5% (19.2 to 43.8%)**			**-0.9% (-11.6 to 9.8%)**			**-22.1% (-90.4 to 43.8%)**		

NB Summary of all pneumococci, vaccine type (VT), non-vaccine type (NVT) and non-typeable pneumococcal colonization by age and gender. In Period 1, number of subjects N=2010 of which 852 were colonized and 64 had dual carriage, making a total of 2074 with 916 pneumococcal isolates. Period 2, N=1892 of which 705 were colonized and 49 had dual carriage, making a total of 1941 with 754 positive swabs.

[1]Total number of participants swabbed, if double colonization detected swab was counted twice

[2]Total number of pneumococcal isolates detected

[3]ARR (adjusted risk ratio)

[4]PCV7 serotypes, serotypes 4, 6B, 9V, 14, 18C, 19F and 23F

[5]means that Fisher's exact test was used to compare the two groups

Table 3.4 Comparison of two models, one that assumes that individuals in the same household are independent (logistic) and the second that assumes intra-household clustering (random effects or generalized linear mixed model).

Group	Dependant Variable	Adjusted odds ratios (Period 1 vs. Period 2)		ICC
		Logistic model[1]	Random effects model	
<2 years	Overall colonization	0.58(0.42-0.78)	0.53(0.37-0.77)	0.23
	VT[2]	0.35(0.27-0.46)	0.32(0.23-0.44)	0.11
	NVT[3]	1.67(1.30-2.14)	1.73(1.32-2.27)	0.08
2-5years	Overall colonization	0.66(0.45-0.96)	0.61(0.38-0.96)	0.21
	VT	0.71(0.51-0.99)	0.68(0.47-0.99)	0.08
	NVT	0.97(0.71-1.33)	0.98(0.66-1.45)	0.24
6-12years	Overall colonization	0.65(0.48-0.88)	0.63(0.44-0.89)	0.13
	VT	0.60(0.40-0.90)	0.58(0.13-2.65)	0.98
	NVT	0.79(0.58-1.07)	0.76(0.53-1.10)	0.18
13-18years	Overall colonization	0.46(0.27-0.79)	0.46(0.26-0.81)	0.01
	VT	0.45(0.15-1.30)	0.31(0.13-0.72)	0.02
	NVT	0.46(0.25-0.83)	0.40(0.18-0.88)	0.23
19-45years	Overall colonization	0.45(0.31-0.64)	0.42(0.27-0.64)	0.21
	VT	0.36(0.17-0.75)	0.23(0.13-0.40)	0.01
	NVT	0.43(0.28-0.66)	0.35(0.23-0.55)	0.03
>45years	Overall colonization	1.11(0.53-2.35)	0.63(0.32-1.24)	0.01
	VT	0.70(0.17-2.88)	0.31(0.15-0.64)	0.01
	NVT	1.14(0.46-2.81)	0.40(0.20-0.79)	0.02
Overall	Overall colonization	0.68(0.59-0.78)	0.66(0.56-0.78)	0.13
	VT	0.52(0.44-0.62)	0.46(0.37-0.57)	0.24
	NVT	1.01(0.88-1.16)	1.02(0.86-1.22)	0.17

ICC – intra class correlation coefficient

[1]The logistic model gives the same adjusted relative risks given in Table 3.2 (if the log binomial is used) – it assumes that subjects from the same household are independent. The hierarchical model aka the random effects model or Generalized Linear Mixed Model assumes that subjects from the same household are correlated. The model fitted here is Y_ij = b_i + Beta0 + Beta1*Period_ij + other factors such as age, gender, day care attendance, where b_i ~ N(0, sigma^2). It is the value of sigma that tells us how much clustering is in the data. The Intra-Class Correlation (ICC where class is the household) tells us the amount of clustering in the data ICC=sigma^2/(sigma^2+(pi^2)/3).

[2]VT, refers to PCV7 serotypes: 4, 6B, 9V, 14, 18C, 19F and 23F

[3]NVT, excluding PCV7 serotypes

3.3.2 Serotype specific colonization by age group over two periods

Among children aged 0-12 years, the dominant colonizing serotypes in Period 1 were 19F (14.2%), 6B (10.2%), 6A (9.3%), 23F (8.9%), 15 (7.6%), 19A (5.0%) and 14 (4.3%) in decreasing order of frequency (Table 3.5). The prevalence of colonization was significantly lower in Period 2 among children <2 years for serotypes 6B, 18C, 19F and 23F. There were, however, no significant changes in prevalence of colonization by individual PCV7 serotype in older children and particularly in the 2 to 5 years of age where PCV7 serotypes remained dominant (Table 3.5).

Non PCV7 serotypes which showed significant increase among children age <2 years from Period 1 compared to Period 2 included serogroup 15 (i.e. serotypes 15A, 15B, 15C combined) which increased from 5.8% to 12.2% ($p<0.0001$), serotypes 16F and serotypes 11A; Table 3.5. The dominant serotypes in descending order among colonized children <2 years in Period 2 included 6A (12.3%), 15B (7.6%), 19A (6.3%), 16F (5.8%), 11A (3.2%) and 15A (2.8%). The prevalence of colonization by serotypes 6A and 19A remained unchanged between the study-periods in children and adults. Among the individuals >12 years, a significant increase in serogroup 15 from 3.3% to 10.6% ($p=0.02$) was also observed (Table 3.5).

Table 3.5 Serotype distribution by age group in each of the survey Periods.

	<2 years			2 to 5 years			6 to 12 years			All adults (>12years)		
Serotype	**Period 1** (N=359)	**Period 2** (N=713)[2]	p-value	**Period 1** (N=236)[3]	**Period 2** (N=333)[4]	p-value	**Period 1** (N=199)[5]	**Period 2** (N=254)[6]	p-value	**Period 1** (N=122)[7]	**Period 2** (N=132)[8]	p-value
PCV7 Serotypes[9]												
4	3 (0.8)	5 (0.7)	0.54	2 (0.8)	5 (1.5)	0.39	9 (4.5)	3 (1.2)	0.03	5 (4.1)	2 (1.5)	0.19
6B	52 (14.5)	40 (5.6)	<0.0001	16 (6.8)	24 (7.2)	0.84	13 (6.5)	10 (3.9)	0.21	5 (4.1)	3 (2.3)	0.32
9V	7 (1.9)	6 (0.8)	0.12	6 (2.5)	7 (2.1)	0.73	5 (2.5)	5 (2.0)	0.70	3 (2.5)	3 (2.3)	0.62
14	20 (5.6)	31 (4.3)	0.38	9 (3.8)	11 (3.3)	0.75	4 (2.0)	9 (3.5)	0.25	3 (2.5)	3 (2.3)	0.62
18C	5 (1.4)	2 (0.3)	0.05	5 (2.1)	3 (0.9)	0.20	1 (0.5)	2 (0.8)	0.59	4 (3.3)	1 (0.8)	0.16
19F	63 (17.5)	87 (12.2)	0.02	37 (15.7)	47 (14.1)	0.60	13 (6.5)	16 (6.3)	0.92	6 (4.9)	8 (6.1)	0.69
23F	34 (9.5)	44 (6.2)	0.05	23 (9.7)	28 (8.4)	0.58	14 (7.0)	16 (6.3)	0.76	6 (4.9)	3 (2.3)	0.21
Subtotal	**184 (51.3)**	**215 (30.2)**	**<0.0001**	**98 (41.5)**	**125 (37.5)**	**0.34**	**59 (29.6)**	**61 (24.0)**	**0.18**	**32 (26.2)**	**23 (17.4)**	**0.09**
Additional serotypes included in PCV10 [10]												
1	1 (0.3)	1 (0.1)	0.56	2 (0.8)	0	-	0	1 (0.4)	-	0	0	-
5	0	0	-	2 (0.8)	0	-	0	0	-	0	0	-
7	4 (1.1)	7 (1.0)	0.53	2 (0.8)	4 (1.2)	0.51	3 (1.5)	9 (3.5)	0.15	5(4.1)	0	-
Subtotal	**5 (1.4)**	**8 (1.1)**	**0.70**	**6 (2.5)**	**4 (1.2)**	**0.19**	**3 (1.5)**	**10 (3.9)**	**0.10**	**5 (4.1)**	**0**	**-**
Additional serotypes included in PCV 13[11]												
3	1 (0.3)	6 (0.8)	0.26	8 (3.4)	7 (2.1)	0.35	12 (6.0)	14 (5.5)	0.82	10 (8.2)	11 (8.3)	0.97
6A	37 (10.3)	88 (12.3)	0.33	23 (9.7)	42 (12.6)	0.29	14 (7.0)	28 (11.0)	0.15	5 (4.1)	8 (6.1)	0.48
19A	23 (6.4)	45 (6.3)	0.95	8 (3.4)	16 (4.8)	0.41	9 (4.5)	8 (3.1)	0.45	7 (5.7)	2 (1.5)	0.07
Subtotal	**61 (17.0)**	**139 (19.5)**	**0.32**	**39 (16.5)**	**65 (19.5)**	**0.36**	**35 (17.6)**	**50 (19.7)**	**0.60**	**22 (18.0)**	**21 (15.9)**	**0.65**
Dominant non PCV13 serotypes[12]												
15A	2 (0.6)	20 (2.8)	0.01	2 (0.8)	8 (2.4)	0.14	0	5 (2.0)	-	1 (0.8)	6 (4.5)	0.07
15B	13 (3.6)	54 (7.6)	0.01	15 (6.4)	16 (4.8)	0.42	12 (6.0)	3 (1.2)	0.004	1 (0.8)	3 (2.3)	0.34
15C	6 (1.7)	13 (1.8)	0.86	8 (3.4)	4 (1.2)	0.07	2 (1.0)	7 (2.8)	0.16	2 (1.6)	5 (3.8)	0.26
Total sero-group 15	21 (5.8)	87 (12.2)	0.001	25 (10.6)	28 (8.4)	0.38	14 (7.0)	15 (5.9)	0.63	4 (3.3)	14 (10.6)	0.02
16F	4 (1.1)	41 (5.8)	0.0001	1 (0.4)	13 (3.9)	0.01	8 (4.0)	13 (5.1)	0.58	4 (3.3)	7 (5.3)	0.32
34	7 (1.9)	29 (4.1)	0.07	11 (4.7)	15 (4.5)	0.93	9 (4.5)	8 (3.1)	0.45	6 (4.9)	5 (3.8)	0.66

11A	4 (1.1)	23 (3.2)	0.03	11 (4.7)	5 (1.5)	0.03	10 (5.0)	2 (0.8)	0.01	4 (3.3)	5 (3.8)	0.55
Other	73 (20.3)	171 (24.0)	0.18	45 (19.1)	78 (23.4)	0.21	61 (30.7)	95 (37.4)	0.13	45 (36.9)	57 (43.2)	0.31
Subtotal	**109 (30.4)**	**351 (49.2)**	**<0.0001**	**93 (39.4)**	**139 (41.7)**	**0.58**	**102 (51.3)**	**133 (52.4)**	**0.82**	**63 (51.6)**	**88 (66.7)**	**0.02**
Total	**359 (100%)**	**713(100%)**		**236(100%)**	**333(100%)**		**199(100%)**	**254(100%)**		**122(100%)**	**132(100%)**	

331 children aged < 2years were colonised in Period 1 including 28 with double colonization, [2]666 children aged < 2years were colonised in Period 2 including 47 with double colonization, [3]219 children aged 2-5 years were colonized in Period 1 including 17 with double colonization [4]307 children aged 2-5 years were colonized in Period 2 including 26 with double colonization, [5]199 children aged 6-12 years were colonized in Period 1 including 13 with double colonization, [6]242 children aged 6-12 years were colonized in Period 2 including 12 with double colonization [7]116 adolescents/adults were colonized in Period 1 including 6 with double colonization, [8]126 adolescents/adults were colonized in Period 2 including 6 with double colonization

[9]PCV7 serotypes: serotypes 4, 6B, 9V, 14, 18C, 19F and 23F

[10]Additional PCV10 serotypes: serotypes 1, 5 and 7F

[11]Additional PCV13 serotype: serotypes 3, 6A and 19A

[12]Non-PCV 13 serotypes: excluding serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F

3.3.3 HIV and pneumococcal colonization

HIV-infection status in Period 1 and Period 2 was volunteered by 505 and 1320 adults and 165 and 631 children, respectively, see table 3.6. In Period 1, adolescent/adults who self-reported as being HIV-infected had a trend towards higher prevalence of overall colonization (8/40, 20.0%) compared to HIV-uninfected individuals (55/465, 11.8%; p=0.13). This difference was significant in Period 2 when 10.4% (14/135) of self-reported HIV-infected individuals were colonized compared to 5.1% (60/1185) of reportedly HIV-uninfected adolescents/adults; p=0.01. PCV7 serotype colonization was also greater in adolescents/adults reported to be HIV-infected compared to HIV-uninfected individuals in Period 1 (10.0% [4/40] vs. 3.2% [15/465]; p=0.054) and Period 2 (3.0% [4/135] vs. 1.0% [12/1185], p=0.011). There were no differences in prevalence of overall or PCV7 serotype colonization among children aged <12 years in Period 1 or Period 2 when stratified by self-reporting HIV-status.

Table 3.6 Prevalence of pneumococcus in adults and children who confirmed their HIV status

		Adults (n/N), %			Children (n/N), %		
		HIV positive	**HIV negative**	p-value[1]	**HIV positive**	**HIV negative**	p-value[1]
All pneumococci	**Period 1**	8/40 (20%)	55/467 (11.8%)	0.13	5/5 (100%)	116/160 (72.5%)	0.21
	Period 2	14/135 (10%)	60/1185 (5%)	0.011*	15/20 (75%)	415/611 (68%)	0.50
	p-value[2]	0.107	<0.0001*		0.29	0.27	
PCV7 Serotypes[3]	**Period 1**	4/40 (10%)	15/467 (3%)	0.05*	2/5 (40%)	52/160 (32.5%)	0.53
	Period 2	4/135 (3%)	12/1185 (1%)	0.07	5/20 (25%)	140/611 (22.9%)	0.79
	p-value[2]	0.08	0.001*		0.44	0.013*	

[1]: comparison between different HIV status in the same Period
[2]: comparison between Periods of same HIV status
[3]PCV7 serotypes: serotypes 4, 6B, 9V, 14, 18C, 19F and 23F

3.3.4 Effect of the number of PCV doses received on carriage in vaccine recipients

A total of 386 (51.5%) of 749 children, <2 years of age in Period 2 who had verifiable immunisation records at hand, were fully vaccinated. Fifty percent (347/692) of the children age 9 months to 2 years were fully vaccinated with three doses of PCV7, while 31.9% (221/692) had received two doses and 17.9% (124/692) had only received a single dose. Fully-vaccinated 9 months to 2 year old children had a lower prevalence of PCV7 serotype colonisation (59/347; 17.0%) compared to those who had only received two (56/221; 25.3%, p=0.02) or a single dose (37/124; 29.8%, p = 0.002).

Table 3.7 Number of children sampled in period-2 who received PCV7

		Number of doses, n (%)		
Age group	**Total**	**1**	**2**	**3**
<3 months	5	5 (100)		
3 – <9 months	52	18 (34.7)	32 (61.5)	2 (3.8)
9 months- 2 years	692	124 (17.9)	221 (31.9)	347 (50.2)
Total	749	147 (19.6)	253 (33.8)	349 (46.6)

In 3-9 month old children, there was no difference in PCV7 serotype colonization between partially vaccinated (i.e. only received one PCV7 dose; 27.8%) compared to those who had received both scheduled PCV7 doses; 28.1%, p=0.979. Overall, in a logistic regression model, adjusting for age, there was an inverse relationship between the number of doses of PCV7 received and PCV7 serotype colonization [OR 0.69, 95%CI: 0.56-0.87], indicating that incompletely vaccinated children were more likely to carry vaccine serotypes compared to fully vaccinated children.

Table 3.8 Odds ratios for carriage in vaccine recipients with increasing number of vaccine doses received.

	Effects of the number of doses received and age on the odds of carriage in vaccinee	
Carriage	**Age**	**Number of doses received**
Any pneumococci	1.02(0.71-1.46)	0.83(0.67-1.02)
PCV7[1]	0.97(0.67-1.40)	0.69(0.56-0.87)

NB: Adjusted odds ratio and 95% confidence intervals are given
[1]PCV7 serotypes: serotypes 4, 6B, 9V, 14, 18C, 19F and 23F

3.4 Discussion

We report on the temporal association of infant PCV7 immunization on the epidemiology of nasopharyngeal pneumococcal colonization among children and adults in a rural African setting with high HIV-prevalence. Our study indicated the prevalence of PCV7 serotype colonization in the <2 and 2-5 year age groups were almost similar at the time of initiation of the PCV immunization program, nevertheless, a vaccination program limited to immunization at 6, 14 weeks and 9 months of age was associated with reduction in vaccine serotype colonization in all study age-groups, including the 2 to 5 years old unvaccinated children. Notably, the reduction in PCV7 serotype colonization was observed with 51.5% of the targeted population having been fully vaccinated based on their age group. This suggests that despite a high prevalence of vaccine serotype colonization among older children in resource poor, rural settings, children <2 years of age are likely the primary source of transmission of vaccine serotypes in the community, and that an indirect effect of PCV immunization can be realized within two years of initiating the PCV immunization program, even without a catch-up campaign of older children and with a fairly modest level of vaccine coverage of the target population. A higher level of vaccine coverage, may have been

associated with even a greater magnitude of reduction of colonization by vaccine serotypes among unvaccinated individuals compared to what was observed in our setting.

The findings of our study corroborate that of clinical trials in which the direct and indirect effect of PCV vaccinantion of children reduced colonization among vaccinated and unvaccinated individuals (9, 68, 71, 73, 74, 133-135). There are also few observational ecological studies where this has now been reported including from The Gambia, (80) United Kingdom,(136) The Netherlands,(82) USA among Native Americans, Alaska (84) and the general USA population (87, 137) and in Australasia (78). The results of our study, nevertheless, are unique in so far as we evaluated a national immunisation program with no catch-up campaign of older children as undertaken elsewhere, (78, 80, 82, 87, 136, 137) and also it was undertaken in a setting of high underlying HIV-positivity among adults and with only modest levels of age-appropraite PCV7 coverage.

Pneumococcal colonization prevalence was generally high in our study population, which is similar to that reported from a few other African countries (46, 80). Notably, the prevalence of pneumococcal colonization in children <2 years of age (83.8%) was comparable to 3 to 5 year old children (81.4%) and also high (60%) in the 6 to 12 year age-group at the time of PCV7 introduction. In contrast, the prevalence of pneumococcal colonization in children from developed countries peaks at 40 to 50% at approximately two years of age and is subsequently followed with reduction from around five years of age to adult levels of 10% or less (33). While our study suggests that young infants are the main source of pneumococcal transmission to adolescents and older adults, the more modest reduction (21%) in vaccine serotype colonization in the 2 to 5 year age group possibly indicates some ongoing

transmission of vaccine serotypes among this age group, who are also more likely to intermingle in the community and in play groups or day care centres. Although 2 to 5 year old children are at lower risk of IPD compared to <2 year old children, (138) they do however remain at risk of developing acute otitis media and other mucosal infections which itself may warrant catch-up campaigns in this age-group in the presence of only modest reduction in PCV7 serotype colonization with immunization only directed at young infants. Our study is unable to establish whether there is any added benefit of a PCV catch up campaign of older children in accelerating the timing and magnitude of indirect effect as postulated (52). Nevertheless, we identified an indirect-effect on PCV7 serotype NP colonization materialized within similar timelines and magnitude, in the absence of a catch up campaign, compared to settings where catch up campaigns have been implemented (80).

There is limited information on pneumococcal NP colonization in settings with high prevalence of HIV, including conflicting data on whether infants born to HIV-infected mothers have higher rates of pneumococcal NP colonization compared to children born to HIV-uninfected mothers (139, 140). Also, no difference in prevalence of pneumococcal colonization has been observed between HIV-infected compared to HIV-uninfected PCV unvaccinated children, (141, 142) similar to that identified in our study. Although limited to self reports , the prevalence of HIV-positivity among children in our study (3%) was similar to that recorded in a separate HIV survey undertaken in the area in 2007 (4.4%; 95% CI 2.79 to 5.97) (143). HIV-infected women reportedly have a higher prevalence and rate of overall and PCV7 serotype pneumococcal colonization, (35, 132, 140) as well as an increased risk of PCV7 serotype IPD (33). The heightened risk of PCV7 serotype colonization in HIV-infected women was partly corroborated by our study. This, despite probable under reporting of HIV-positivity status among adults in our study, i.e.10% compared to 23.9% for the general adult population in 2011 HIV survey (144). Such under-reporting in our study would

have biased our results toward under-estimating the effect of HIV-positivity on the prevalence of pneumococcal colonization.

The likelihood of infant PCV immunization having an indirect protective effect against vaccines-serotype IPD among HIV-infected women, including in high HIV prevalence settings such as ours, was alluded to by the decrease of PCV7 serotype colonization among 19-45 year old HIV-infected women which was of a greater magnitude compared to reportedly HIV-uninfected individuals. This reduction in PCV7 serotype colonization among HIV-infected adults also lessened an earlier theoretical concern of ours, that HIV-infected women could remain a potential reservoir for PCV7 serotype colonization and transmission in settings such as ours despite PCV vaccination of children. Caregivers, which included mothers, had higher PCV7 serotype carriage than non-caregivers in Period 1, which declined significantly in Period 2. The close contact between caregivers and young children may explain the high carriage among caregivers in Period 1 (38, 40) and the early indirect effect among caregivers in Period 2.

While the increase in non PCV7 serotype colonization was anticipated among the age-group eligible to have been vaccinated, i.e. <2 years of age (8, 9, 68, 69) there was an unexpected decrease in non PCV7 serotype colonization among age-groups >12 years old. The reasons for this decline in non PCV7 serotype colonization following introduction of infant PCV immunization remains to be explored further. To our knowledge, there is only one similar observation of decline in non PCV serotype and overall colonization among adults which was reported from The Gambia (80). The only other studies, to our knowledge, which evaluated the effect of childhood PCV immunization on the epidemiology of NP colonization among adults was among Navajo Indians in USA,(73, 145) and Alaskan-natives (84). Among Navajo Indians, 1 year following implementation of childhood PCV immunization, no

changes were observed in non-vaccine serotype colonization among adults post introduction of childhood PCV7 immunization, despite an increase among childhood age groups who had been targeted for vaccination (73). In contrast among Alaskan natives there was an increase in non-vaccine serotype colonization among adults observed within 4 years of childhood PCV introduction (84). The lack of an increase in non-vaccine serotype colonization among adults in the era of childhood PCV-immunization may relate to most non-vaccine serotype strains being less efficient in colonizing healthy adults. Also, there may be a time lag before non-vaccine serotype increases are observed among adults as our study was done within 2 years of vaccine implementation.

Possible reasons as to why non-vaccine serotype colonization may have declined among individuals >12 years in our setting remains unclear. This may reflect co-incidental temporal changes in circulation of these non-vaccine serotypes, although a similar decline would then possibly have been expected among younger children, unless the longer duration of pneumococcal colonization in children had masked this (21). A further possible explanation for the decline in non PCV7 serotype colonization among adults may include an increase in ART coverage in HIV-infected adults with AIDS, which has previously been associated with a lower risk of pneumococcal colonization following at least one year of ART (35). Other hypothetical reasons include that increased transmission of non-vaccine serotypes to adults may result in robust anamnestic responses against the non-vaccine serotypes. This could possibly reduce the acquisition and/or duration and subsequently likelihood of detection of these non-vaccine serotypes in adults. Despite the decrease in overall non-vaccine serotype among adults, serogroup 15 was an exception in having increased in prevalence of colonization in children (5.8% vs. 12.2%) as well as in adolescents/adults (3.3% vs. 10.6%). This increase in serogroup 15 colonization, dominantly associated with serotype 15B,

warrants ongoing monitoring in relation to changes in invasive disease due to this serogroup, especially in settings such as ours where serogroup 15 had contributed to 2-3% IPD among children and adults prior to PCV-introduction (146-148). Also, a trend toward a higher incidence of serotype 15A IPD in placebo-recipients was observed in the phase III efficacy trial in South Africa, particularly among HIV-infected children (54). The importance of serogroup 15, which is not targeted in either the 10- or 13-valent licensed PCV or a 15-valent PCV currently being developed, in causing IPD associated with antibiotic-resistant strains in the era of PCV immunization has also been reported in Canada (149).

Other common non PCV7 serotypes in Period 2, which were associated with a high proportion of IPD prior to infant PCV immunization in South Africa, were serotypes 6A and 19A. Although PCV7 has been associated with cross-protection against serotype 6A IPD, (150) we did not identify any changes in the overall proportion of individuals colonized by serotype 6A between Period 1 and Period 2 among children or older age groups in our study. We also distinguished between serotype 6A and 6C, as cross-protection associated with 6B inclusion in PCV has been shown for 6A but not for 6C (150). Decline in serotype 6A colonization among children has, however, been reported in Massachusetts-USA, although, this was only identified 6 years post PCV7 introduction and was associated with concomitant increase in 6C colonization (151). Serotype 19A colonization (and IPD) which increased in the USA and other countries post PCV introduction, (85) did not increase in our setting; and is unlikely to increase since the immunization program had subsequently transitioned from PCV7 to PCV13 in 2011. Serotype 3 was the only serotype more common among adults than children and its prevalence did not change across periods. In South Africa, serotype 3 is an important cause of IPD (147, 152) and among HIV-infected patients in Uganda, serotype 3 was the most prevalent serotype identified from oropharyngeal swabs (153).

Limitations specific to this study included the reliance on self-reporting of HIV-infection status. This was predicated by resource constraints in undertaking informed testing for all study participants, as well as attempting to minimize any stigma being associated with the study if perceived to be HIV related. Our study was not powered to detect changes in prevalence of individual serotypes. Nevertheless, significant decreases were observed in colonization by serotypes 6B, 18C, 19F and 23F among children <2 year old. In addition, our study only spanned a two year period, which may under-estimate the longer term effects of childhood PCV immunization on the epidemiology of pneumococcal colonization in the community. Furthermore, we do not have any data on IPD from the same community, to determine what effect the direct and indirect changes associated with vaccine- and non-vaccine serotype colonization may have had on the incidence of IPD.

In conclusion, this study demonstrates indirect protection against PCV7 serotype colonisation among adolescents and adults in a community with high underlying HIV-infection, which was induced by only modest levels of PCV7 immunization coverage that was targeted solely at infants as part of a routine immunization program. Although, our study was done within two years of implementation of the PCV7-immunization program, the findings are strongly suggestive that PCV7 immunization targeted at only infants reduces transmission of the targeted vaccine serotypes in high-risk, rural African populations such as ours.

Chapter 4: Temporal changes in pneumococcal colonization in HIV-infected and HIV-uninfected mother-child pairs following transitioning from 7-valent to 13-valent pneumococcal conjugate vaccine, Soweto, South Africa

4.1 Introduction

HIV-infected individuals have an 8-40 fold greater risk of developing IPD, including when on anti-retroviral treatment (35, 152, 154). Furthermore, HIV-infected women are more predisposed to IPD due to "pediatric serotypes" than HIV-infected men (47). A few studies have shown that pneumococcal colonization prevalence is similar between HIV-infected and HIV-uninfected children (142, 155). Although there are limited data on the effect of HIV-infection on pneumococcal colonization in adults (140, 156, 157), HIV-infected women have a higher prevalence of colonization by serotypes commonly associated with IPD in children, many of which are included in PCV7 (35, 140, 158). Consequently, the indirect effect of infant PCV immunization in the prevention of vaccine serotype pneumococcal disease may be attenuated in settings with a high prevalence of HIV among adults, who could serve as an additional reservoir of vaccine serotype colonization.

PCV immunization directly decreases vaccine serotype colonization in the immunized children and indirectly in healthy unvaccinated children and adults, however, there are limited data on the direct and indirect effect of routine childhood PCV-immunization on nasopharyngeal carriage of *Streptococcus pneumoniae* among HIV-infected children and adults (65, 159). An earlier cross-sectional study in our setting reported no difference in colonization prevalence by either vaccine serotype or non-vaccine serotypes between vaccinated and –unvaccinated HIV-infected children five years following receipt of three doses of an investigational 9-valent PCV during infancy, however, that study was performed

prior to routine PCV immunization or management of HIV-infected children with antiretroviral therapy (65).

The aim of this component of the study was to evaluate the effect of routine infant PCV immunization, following transitioning from PCV7 in 2011 to PCV13 in 2013, on the prevalence of PCV13 additional vaccine serotype and non PCV13 serotype colonization in HIV-infected and HIV-uninfected mother-child pairs in South Africa at a community level. This evaluation included age groups that would have been eligible for PCV immunization and age-groups, including HIV-infected women, who would not have been immunized against pneumococcus with any pneumococcal vaccine.

4.2 Methods

4.2.1 Study population

The study was undertaken in Soweto (Gauteng, South Africa); details of the study population are described in Chapter 2. In April 2009, PCV7 was introduced into the national public immunization program and was subsequently changed to PCV13 in May 2011, as previously described in Chapter 2.

4.2.2 Study participants

We enrolled HIV-infected and HIV-uninfected mother-child dyads between May 2010 and February 2011 (early PCV7 era; period 1) and again from May 2012 to April 2013 (PCV13-era; period 2). Children were aged between 0 and 12 years. In period 1, we targeted enrolling 700 mother-child pairs with concordant HIV-status in each arm. Individuals were included in this study if they had confirmed HIV status and provided written informed consent. HIV discordant pairs were excluded from the study. Individuals with a contra-indication to

nasopharyngeal swabbing, e.g, bleeding diathesis and current symptomatic disease were also excluded from the study.

Based on the PCV7 serotype colonization prevalence among mothers during period 1, we planned on enrolling 602 HIV-infected and 1,234 HIV-uninfected mother-child pairs in period 2, to detect at least a 50% reduction in PCV7 serotype colonization in period 2 compared to period 1 in the women, with 80% power. The study was also sufficiently powered (90%) to detect at least a 50% decrease in all PCV13 serotype colonization between period 1 and period 2 in both groups of women. Mothers with more than one child were evaluated as multiple mother-child pairs.

HIV-infected mother-child pairs were recruited from two established HIV clinics at Chris Hani Baragwanath Academic Hospital, where the majority of HIV-infected children in Soweto received their routine care during the study period. HIV-uninfected mother-child pairs were recruited from wellness baby clinics. The HIV infection status of women without a documented HIV seronegative test in the previous six months was determined, following counselling and consenting, using a rapid HIV test (Determine–Alere International Limited, Ballybrit, Galway, Ireland). Children of HIV-uninfected women were presumed to be HIV-uninfected and children of HIV-infected mothers were tested per age-dependent criteria if they had not been previously tested. Overall >98% of mothers who were invited to take part in the study agreed to participate.

Demographic and risk factors for colonization were evaluated in participants at the time of swab collection. Child's risk factors assessed included day care attendance, rhinitis at time of sampling, breastfeeding history, underlying tuberculosis, hospitalization in preceding 3-months, current antibiotic treatment, use of anti-retroviral treatment for HIV-infected children; and among mothers age, alcohol-intake history, current antibiotic therapy, cigarette

smoking, presence of rhinitis, previous tuberculosis treatment, hospitalization in preceding 3-months and use of anti-retroviral treatment for HIV-infected mothers.

4.2.3 Determination of bacterial colonization

Swab collection and sample collection as described earlier in section 2.4.

4.2.4 Definitions and statistical analysis

To determine the impact of infant PCV immunization on the prevalence of overall pneumococcus, vaccine serotype and non-vaccine serotype colonization, children were stratified into four age groups according to the probability of having been vaccinated, i.e. children likely to be incompletely vaccinated (<9 months of age), those eligible to have been fully vaccinated in both study periods (9 to 24 months of age), those likely to have been fully vaccinated only in period 2 (>24 to 48 months of age) and those unlikely to have received PCV at all (>48 to 144 months of age).

We compared colonization prevalence in children and adults between the two study periods, including stratification by HIV infection status. Differences in the demographic and clinical characteristics between the populations in the two study periods were addressed by controlling for possible confounding factors on colonization. Univariate logistic regression analysis was conducted and those characteristics with a p-value <0.1 were included in a multivariable analysis to calculate adjusted odds ratio (adjusted OR) and corresponding 95% confidence intervals (95%CI) for colonization between the study periods. Similar analyses

were implemented for comparison of colonization between HIV-infected and –uninfected groups.

Regression analyses

For the regression analysis we analysed the data by age category as detailed above, HIV-status and colonization status to reduce the complexity embedded in the data. For each age class (HIV-uninfected or HIV-infected or combined), we implemented a model of carriage (any pneumococcus or PCV7 serotypes or PCV13 additional 6 serotypes or PCV13 serotypes or non-vaccine serotypes) with the independent variable vaccination period (dichotomized: 0 for period 1 and 1 for period 2), adjusted for other variables that were deemed potential determinants for carriage by a univariate analysis. Only variables that produced a p-value of <0.1 were used in the multivariate model for pneumococcal carriage. Multivariable analysis was necessitated by significant differences in demographic and clinical variables between the populations in the two periods to offset possible confounding by these factors.

We did not include the time from vaccination but only considered the vaccination period, i.e., PCV7 era or PCV13 era as we were not aiming for risk factors for carriage but only the difference of carriage between two periods. No weighting of these characteristics included in the multivariable analyses was used as sometimes this could potentially increase the random error in the estimates. Similarly, for each age strata, we also investigated the association between carriage and HIV-infection status, i.e., carriage as a dependent variable, HIV status as an explanatory variable plus other factors based on the relative importance in univariate analyses, i.e., those with a p-value <0.1 in the univariate analysis. Again no weighting was undertaken for the different characteristics.

Furthermore, we did not adjust the number of tests, as we undertook a priori planned analysis. Nevertheless, we only considered a p-value <0.01 as significant, to offset any chance findings based on the multiple comparisons undertaken. Comparison of serotype prevalence between period 1 and period 2 was performed using chi-squared or Fisher's exact tests where appropriate.

4.3 Results

4.3.1 Study participants

We enrolled 1,376 (including 704 HIV-infected) and 1,556 (608 HIV-infected) women in period 1 and period 2, respectively, Table 4.1, together with 1,411 (713 HIV-infected) and 1,649 (616 HIV-infected) of their children in the respectively periods; Table 4.2. This included 35 and 93 women in period 1 and period 2, respectively, who had more than one child enrolled concurrently. None of the mother-child pairs were enrolled in both study periods.

Table 4.1 Demographic characteristic of mothers enrolled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era) in Soweto, South Africa

Characteristic	**Period 1** (PCV7 era) **N=1376**	**Period 2** (PCV13 era) **N=1556**	**p-value**
Number of HIV-infected	704 (51.2)	608 (39.1)	
Mean age, years ±SD[1]	30.4±6.50	29.1±6.6	<0.001
Smoker, n[2]/N[3](%)	80/1375 (5.8%)	92/1556 (5.9%)	0.91
Takes snuff, n/N (%)	78/1372 (5.7%)	88/1553 (5.7%)	0.98
Drinks alcohol, n/N (%)	240/1375 (17.4%)	418/1547 (27.0%)	<0.001
Suffers from a chronic illness, n/N (%)	115/1369 (8.4%)	124/ 1421 (8.0%)	0.76
HIV-infected and on ART[4], n/N (%)	299/697(42.9%)	330/608 (54.3%)	<0.001
Currently on TB[5] treatment, n/N (%)	20/1371 (1.5%)	16/1538 (1.0%)	0.31
Treated for TB in past year, n/N (%)	80/1357 (5.9%)	83/1532 (5.4%)	0.58
Currently on antibiotic treatment, n/N (%)	79/1368 (5.8%)	20/1540 (1.3%)	<0.001
Hospitalized in the last 3 months, n/N (%)	25/1369 (1.8%)	19/ 1527 (1.2%)	0.21

[1]standard deviation;
[2]Number of individuals with the investigated outcome;
[3]Total number of individuals with available information on the characteristic;
[4]Anti-retroviral therapy;
[5]Tuberculosis

Table 4.2 Demographic characteristics of children enrolled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era) in Soweto, South Africa

Characteristic		All children			HIV-infected			HIV-uninfected		
		Period 1 (PCV7 era)	**Period 2** (PCV13 era)	**p-value**	**Period 1** (PCV7 era)	**Period 2** (PCV13 era)	**p-value**	**Period 1** (PCV7 era)	**Period 2** (PCV13 era)	**p-value**
All children enrolled; n, mean age in years ±SD[1]		1411; 2.7 ± 1.98	1649; 2.3 ± 2.05	<0.001	713; 3.3± 2.1	616; 3.8 ± 2.12	<0.001	698; 2.03±1.6	1033; 1.4 ±1.37	<0.001
<9 months; n[2], mean age (SD) months;		230; 4.8± 1.8	396; 4.8 ± 2.3	<0.001	60; 4.9 ± 2.0	52; 5.0 ± 2.0	0.76	170; 4.8 ± 1.3	344; 4.8 ± 2.2	0.99
9-24 months; n, mean age in years ±SD		408; 1.22 ± 0.4	539; 1.18 ± 0.34	0.99	155; 1.41 ± 0.37	92; 1.23 ± 0.39	<0.001	253; 1.07 ± 0.35	447; 1.16 ± 0.34	<0.001
>24- 48 months; n, mean in age years ±SD		449; 3.2 ±0.55	348; 2.9 ± 0.54	0.10	262; 3.01 ± 0.60	182; 2.98 ± 0.58	0.60	187; 3.53 ± 0.25	166; 2.87 ± 0.54	<0.001
>48-144 months; n, mean in age years ±SD		324; 5.5 ± 1.43	366; 5.6± 1.15	0.31	236; 5.72 ± 1.51	290; 5.66 ± 1.14	0.60	88; 4.7 ± 0.82	76; 5.63 ± 1.14	<0.001
Currently breastfed, n/N[3] (%)		319/1410 (22.6)	565/1645 (34.4)	<0.001	18/713 (2.5)	45/566 (7.5)	<0.001	301/697 (43.2)	501/973 (51.5)	0.002
Ever breastfed, n/N (%)		499/1060 (47.1)	628/1056 (59.5)	<0.001	148/665 (22.3)	213/549 (38.8)	<0.001	337/378 (89.2)	389/465 (83.7)	0.02
Attendance at day-care, n/N (%)		623/1410 (44.2)	560/1632 (34.3)	<0.001	379/712 (53.2)	325/605 (53.7)	0.86	232/678 (34.2)	216/969 (22.3)	<0.001
Currently on TB[4] treatment/prophylaxis, n/N (%)		60/1396 (4.2)	67/1643 (4.1)	0.76	57/700 (8.14)	59/608 (9.7)	0.32	3/676 (0.44)	7/975 (0.7)	0.36
Treated for TB in the past year, n/N (%)		116/1349 (8.6)	166/1608 (10.3)	0.11	122/660 (17.0)	150/583 (25.7)	0.002	4/669 (0.6)	9/966 (0.93)	0.33
Currently taking antibiotics, n/N (%)		120/1396 (8.6)	90/1642 (5.7)	0.001	108/704 (15.3)	49/609 (8.1)	<0.001	12/672 (1.8)	39/973 (4.0)	0.01
Hospitalized in the last 3 months, n/N (%)		63/1395 (4.5)	109/1634 (6.7)	0.01	42/702 (6.0)	75/607 (12.4)	<0.001	21/673 (3.1)	31/968 (3.2)	0.93
Pneumococcal vaccine receipt[5]										
<9 months	at least one dose	196/230 (85.2)	320/396 (80.8)	0.31	30/60 (50.0)	18/52 (34.6)	0.10	166/170 (97.6)	302/344 (87.8)	0.22
	At least 2 doses	172/230 (74.8)	306/396 (77.3)	0.48	16/60 (26.7)	11/52 (21.1)	0.50	156/170 (91.8)	295/344 (85.8)	0.05

9 -24months	At least one dose	259/408 (63.5)	400/539 (74.2)	<0.001	35/155 (22.6)	15/92 (16.3)	0.24	224/253 (88.5)	385/447 (86.1)	0.36
	At least 2doses	255/408 (62.5)	395/539 (73.3)	0.001	34/155 (21.9)	15/92 (16.3)	0.28	221/253 (87.4)	380/447 (85.0)	0.39
	At least 3 doses	231/408 (56.6)	355/539 (65.9)	0.004	27/155 (17.4)	13/92 (14.1)	0.50	204/253 (80.6)	342/447 (76.5)	0.21
>24-48 months	At least one dose	0/449	145/348 (41.7)	NA[6]	0/262	14/182 (7.7)	NA	0/187	131/166 (78.9)	NA
	At least 2 doses	0/449	143/348 (41.1)	NA	0/262	14/182 (7.7)	NA	0/187	129/166 (77.7)	NA
	At least 3 doses	8/449 (1.8)	135/348 (38.8)	<0.001	8/262 (3.1)	13/182 (7.1)	0.046	0/187	122/166 (73.5)	NA
>48-144months	At least one dose	0/324	9/366 (2.5)	NA	0/236	3/290 (0.7)	NA	0/88	6/76 (7.9)	NA
	At least 2 doses	0/324	7/366 (1.9)	NA	0/236	1/290 (0.3)	NA	0/88	6/76 (7.9)	NA
	At least 3 doses	15/324 (11.7)	6/366 (1.6)	0.022	15/236 (6.4)	1/290 (0.3)	0.005	0/88	5/76 (6.6)	NA

[1]Standard deviation
[2]Number of individuals with investigated outcome,
[3]Total number of individuals with available information on the characteristic
[4]Tuberculosis,
[5]only for individuals with available vaccination records at time of interview
[6]NA: Not done due to limited number of observations in one group

Generally, there was no difference in the prevalence of PCV13 serotype or non-vaccine serotype colonization in HIV-infected individuals on antiretroviral therapy compared to those not on antiretroviral therapy among women or children in either study period (Table 4.3). As such, no further stratifications were undertaken for antiretroviral therapy usage.

Table 4.3 Association of antiretroviral treatment on prevalence of pneumococcal colonization in HIV-infected (HIV+) children and mothers sampled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era) in Soweto, South Africa.

Period	Age group	Total N[1]		All pneumococci n[2]			PCV7 serotypes[5] n			PCV13 serotypes[6] n			Non PCV13 serotypes[7] n		
		ART[3]	No ART	ART	No ART	p-value	ART	No ART	p-value	ART	No ART	p-value	ART	No ART	p-value
Period 1	All HIV+ children	592	59	415	34	0.048	157	12	0.302	232	20	0.426	198	15	0.210
	%			70.1	57.6		20.3	26.5		39.2	33.9		33.5	25.4	
	<9 months	46	7	23	3	0.774	7	0	NA[4]	7	1	0.949	16	2	0.322
	%			50.0	42.9		15.2	0.0		15.2	14.3		34.8	28.6	
	9-24 months	126	4	82	2	0.873	40	0	NA	56	0	NA	28	2	0.228
	%			65.1	50.0		31.7	0.0		44.4	0.0		22.2	50.0	
	>24-48 months	226	20	162	13	0.527	66	6	0.940	94	10	0.466	72	3	0.135
	%			71.7	65.0		29.2	30.0		41.6	50.0		31.9	15.0	
	>48-144 months	194	28	148	16	0.031	44	6	0.882	75	9	0.506	82	8	0.168
	%			76.3	57.1		22.7	21.4		38.7	32.1		42.3	28.6	
	HIV+ Mothers	299	398	66	76	0.334	13	19	0.790	28	32	0.537	41	45	0.339
	%			22.1	19.1		4.4	4.8		9.4	8.0		13.7	11.3	
Period 2	All HIV+ children	518	34	315	19	0.569	57	8	0.028	101	9	0.324	217	10	0.152
	%			60.8	55.9		11.0	23.5		19.5	26.5		41.9	29.4	
	<9 months	34	10	10	6	0.077	3	3	0.120	3	3	0.120	7	3	0.532
	%			29.4	60.0		8.8	30.0		8.8	30.0		20.6	30.0	
	9-24 months	73	8	39	4	0.854	10	2	0.338	14	2	0.654	25	2	0.712
	%			53.4	50.0		13.7	25.0		19.2	25.0		34.3	25.0	
	>24-48 months	154	2	104	1	0.548	17	0	NA	27	0	NA	79	1	0.971
	%			67.5	50.0		11.0	0.0		17.5	0.0		51.3	50.0	
	>48-144 months	257	14	162	8	0.657	27	3	0.192	57	4	0.577	106	4	0.347
	%			63.0	57.1		10.5	21.4		22.2	28.6		41.3	28.6	
	HIV+ Mothers	330	279	46	39	0.989	10	3	0.059	19	10	0.210	27	29	0.347
	%			13.9	14.0		3.0	1.1		5.8	3.6		8.2	10.4	

[1]N is the total in the group [2]n is the number that was colonized; [3]ART: Antiretroviral therapy, [4]NA: Not applicable due to limited observations, [5]PCV7 serotypes: serotypes 4, 6B, 9V, 14, 18C, 19F and 23F; [6]PCV13 serotypes, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, [7]non-PCV13 serotypes: excluding serotypes in PCV13

The proportion of mother-child pairs who were concurrently colonized on the day of sampling by any pneumococcus declined from 11.0% (155 of 1411 pairs) in period 1 to 6.8% (112 of 1649 pairs) in period 2, p<0.001; Figure 4.1 and Table 4.4. This was evident among HIV-uninfected pairs (declined from 6.7 % (47/698) in period 1 to 4.6% (47/1033) in period 2, p=0.049); as well as among HIV-infected pairs (declined from 15.2% (108/713) to 10.6% (65/616), p=0.013. Similar significant decreases were observed when serotypes were grouped as PCV13 serotypes.

Table 4.4 The proportion of mother-child pairs sampled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era) from Soweto, South Africa who were concurrently colonized by any pneumococcus, same serotype, PCV13 serotype and non-PCV13 serotype

Age group	Total isolates		Any pneumococcus			Same serotype			PCV13 serotypes[2]			Non-PCV13 serotypes[3]		
	Period 1	Period 2	Period 1	Period 2	p-value[1]	Period 1	Period 2	p-value	Period 1	Period 2	p-value	Period 1	Period 2	p-value
All children	1411	1649	155(11.0)	112(6.8)	**<0.001**	56(4.0)	45(2.7)	0.056	34(2.4)	13(0.8)	**<0.001**	22(1.6)	32(1.9)	0.424
HIV-uninfected	698	1033	47(6.7)	47(4.6)	**0.049**	18(2.6)	30(2.9)	0.686	13(1.9)	5(0.5)	**0.005**	5(0.7)	25(2.4)	**0.008**
HIV-infected	713	616	108(15.2)	65(10.6)	**0.013**	38(5.3)	15(2.4)	**0.007**	21(3.0)	8(1.3)	**0.040**	17(2.4)	7(1.1)	0.088
<9 months														
All	230	396	20(8.7)	26(6.6)	0.325	10(4.4)	18(4.6)	0.908	8(3.5)	4(1.0)	**0.033**	2(0.9)	14(3.5)	**0.032**
HIV-uninfected	170	344	11(6.5)	23(6.7)	0.926	6(3.5)	16(4.7)	0.554	6(3.5)	4(1.2)	0.072	0	12(3.5)	-
HIV-infected	60	52	9(15.0)	3(5.8)	0.101	4(6.7)	2(3.9)	0.410	2(3.3)	0	-	2(3.3)	2(3.9)	0.635
9-24 months														
All	408	539	46(11.3)	29(5.4)	**0.001**	21(5.2)	10(1.9)	**0.005**	14(3.4)	2(0.4)	**<0.001**	7(1.7)	8(1.5)	0.778
HIV-uninfected	253	447	23(9.1)	21(4.7)	**0.021**	10(4.0)	9(2.0)	0.129	6(2.4)	1(0.2)	**0.010**	0	8(1.8)	-
HIV-infected	155	92	23(14.8)	8(8.7)	0.159	11(7.1)	1(1.1)	**0.027**	8(5.2)	1(1.1)	0.092	7(4.5)	0	-
>24-48 months														
All	449	348	46(10.2)	28(8.1)	0.289	13(2.9)	9(2.6)	0.792	6(1.3)	2(0.6)	0.243	7(1.6)	7(2.0)	0.630
HIV-uninfected	187	166	10(5.4)	16(9.6)	0.123	1(0.5)	5(3.0)	0.082	1(0.5)	0	-	0	5(3.0)	-
HIV-infected	262	182	36(13.7)	12(6.6)	**0.017**	12(4.6)	4(2.2)	0.143	5(1.9)	2(1.1)	0.397	7(2.7)	2(1.1)	0.211
>48-144 months														
All	324	366	43(13.3)	29(7.9)	**0.022**	12(3.7)	8(2.2)	0.236	6(1.9)	5(1.4)	0.611	6(1.9)	3(0.8)	0.196
HIV-uninfected	88	76	3(3.4)	5(6.6)	0.282	1(1.1)	0	-	0	0	-	1(1.1)	0	-
HIV-infected	236	290	40(17.0)	248.3)	**0.002**	11(4.7)	8(2.8)	0.245	6(2.5)	5(1.7)	0.514	5(2.1)	3(1.0)	0.256

[1]Significant differences, i.e., *P <0.05*, are in bold type font; [2]PCV13 serotypes, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F; [3]non-PCV13 serotypes: excluding serotypes in PCV13

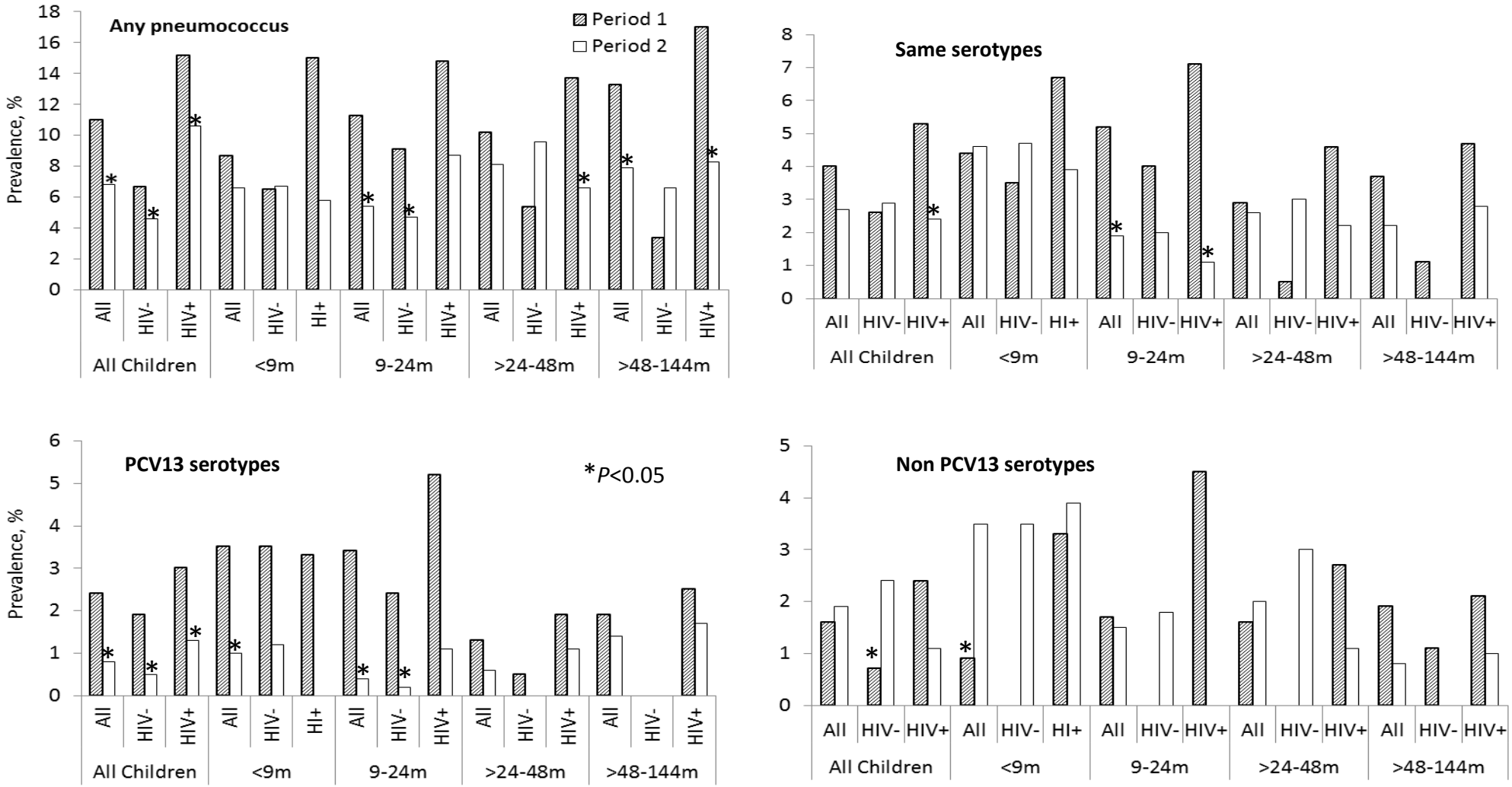


Figure 4.1: The proportion of mother-child pairs sampled in 2010 (Period-1; PCV7 era) and 2012 (Period-2; PCV13 era) from Soweto, South Africa who were concurrently colonized by any pneumococcus, same serotype, PCV13 serotype and non-PCV13 serotype.

PCV13 serotypes, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F; non-PCV13 serotypes: excluding serotypes in PCV13

4.3.2 Temporal changes in pneumococcal colonization prevalence in children

Overall among children, the prevalence of any pneumococcal serotype, PCV7 serotypes, PCV13 additional serotypes and any of the PCV13 serotypes were higher in period 1 compared to period 2, Figure 4.2 and Table 4.5. In contrast, the prevalence of non-vaccine serotype colonization increased in period 2 (30.8% vs. 42.7%, $p<0.0001$). Reduction in the prevalence of PCV13 serotype colonization was evident in all age groups, whilst a concomitant increase in the prevalence of non-vaccine serotype colonization was also observed in all age groups, albeit not significant in infants <9 months age.

Table 4.5 Comparison of pneumococcal colonization by HIV-status in children and mothers enrolled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era), in Soweto, South Africa.

	Total per group		All Pneumococci		PCV7 serotypes[3]		Additional PCV13 serotypes[4]		PCV13 serotypes[5]		Non PCV13 serotypes[6]	
	N		n (%)		n (%)		n (%)		n (%)		n (%)	
Group	**Period 1[1]**	**Period 2[2]**	**Period 1**	**Period 2**	**Period 1**	**Period 2**	**Period 1**	**Period 2**	**Period 1**	**Period 2**	**Period 1**	**Period 2**
All children												
Total	1411	1649	930(65.9)	966(58.6)	343(24.3)	173(10.5)	187(13.3)	97(5.9)	530(37.6)	270(16.4)	435(30.8)	704(42.7)
AOR[1] (95% CI)			0.76(0.65, 0.89)		0.35(0.27, 0.46)		0.43(0.33, 0.57)		0.34(0.28, 0.40)		1.77(1.51, 2.06)	
HIV-uninfected	698	1033	444(63.6)	597(57.8)	159(22.8)	99(9.58)	98(14.0)	49(4.7)	257(36.8)	148(13.3)	206(29.5)	454(44.0)
AOR (95% CI)			0.85(0.69, 1.04)		0.39(0.29, 0.51)		0.32(0.23, 0.47)		0.32(0.25, 0.40)		1.93(1.56, 2.39)	
HIV-infected	713	616	486(68.2)	369(59.9)	184(25.8)	74(12.0)	89(12.4)	48(7.7)	273(38.3)	122(19.8)	229(32.1)	250(40.6)
AOR (95% CI)			0.72(0.55, 0.93)		0.38(0.28, 0.52)		0.61(0.42, 0.89)		0.37(0.28, 0.49)		1.55(1.21, 2.00)	
<9months												
Total	230	396	125(54.4)	193(48.7)	38(16.5)	40(10.1)	20 (8.7)	14 (3.5)	58(25.2)	54(13.6)	69(30.0)	140(35.4)
AOR (95% CI)			0.94(0.47, 1.86)		0.66(0.40, 1.08)		0.40(0.20, 0.82)		0.52(0.34, 0.79)		1.63(0.80, 3.31)	
HIV-uninfected	170	344	98 (57.7)	175(50.9)	31 (18.2)	33(9.6)	18 (10.6)	14 (4.1)	49 (28.8)	47 (13.7)	51 (30.0)	129 (37.5)
AOR (95% CI)			0.73(0.50, 1.06)		0.57(0.33, 0.99)		0.36(0.17, 0.75)		0.43(0.27, 0.68)		1.29(0.86, 1.94)	
HIV-infected	60	52	27 (45.0)	18 (34.6)	7 (11.7)	7 (13.5)	2 (3.3)	0 (0.0)	9 (15.0)	7 (13.5)	18 (30.0)	11 (21.2)
AOR (95% CI)			0.78(0.35, 1.73)		0.86(0.19, 3.85)		-		0.53(0.13, 2.14)		0.82(0.32, 2.07)	
9-24 months												
Total	408	539	267(65.4)	322(59.7)	116(28.4)	56(10.4)	56 (13.7)	24 (4.5)	172(42.2)	80(14.8)	105(25.7)	245(45.5)
AOR (95% CI)			0.73(0.55, 0.96)		0.31(0.18,0.53)		0.46(0.23, 0.93)		0.26(0.16, 0.41)		2.02(1.36, 2.99)	
HIV-uninfected	253	447	169(66.8)	273(61.1)	70(27.7)	43(9.62)	39 (15.4)	19 (4.3)	109(43.1)	62(13.9)	68(26.9)	214(47.9)
AOR (95% CI)			0.74(0.53, 1.03)		0.28(0.18, 0.43)		0.25(0.14, 0.45)		0.23(0.16, 0.33)		2.51(1.79, 3.52)	
HIV-infected	155	92	98(63.2)	49(53.2)	46(29.7)	13(14.1)	17(11.0)	5(5.4)	63(40.7)	18(19.6)	37(23.9)	31(33.7)
AOR (95% CI)			0.94(0.50, 1.77)		0.35(0.16, 0.80)		0.40(0.11, 1.53)		0.31(0.14, 0.67)		1.85(1.01, 3.37)	
>24-48 months												
Total	449	348	316(70.4)	227(65.2)	123(27.4)	31(8.91)	66 (14.7)	23 (3.5)	189(42.1)	54(15.5)	137(30.5)	175(50.3)
AOR (95% CI)			0.88(0.64, 1.20)		0.21(0.13, 0.34)		0.42(0.26, 0.69)		0.25(0.17, 0.36)		2.52(1.86, 3.42)	
HIV-uninfected	187	166	131(70.1)	110(66.3)	45(24.1)	12(7.23)	32 (17.1)	11 (6.7)	77(41.2)	23(13.9)	60(32.1)	87(52.4)
AOR (95% CI)			1.02(0.63, 1.64)		0.24(0.12, 0.49)		0.33(0.16, 0.71)		0.26(0.14, 0.45)		2.37(1.52, 3.69)	

HIV-infected	262	182	185(70.6)	117(64.3)	78(29.8)	19(10.4)	34 (13.0)	12 (6.6)	112(42.8)	31(17.0)	77(29.4)	88(48.4)
AOR (95% CI)			0.83(0.54, 1.27)		0.28(0.16, 0.49)		0.48(0.24, 0.96)		0.30(0.19, 0.47)		2.18(1.46, 3.26)	
>48-144 months												
Total	324	366	222(68.5)	224(61.2)	66(20.4)	46(12.6)	45(13.9)	36(9.8)	111(34.3)	82(22.4)	124(38.3)	144(39.3)
AOR (95% CI)			0.75(0.55, 1.04)		0.53(0.35, 0.81)		0.70(0.43, 1.12)		0.52(0.37, 0.75)		1.12(0.82, 1.54)	
HIV-uninfected	88	76	46(52.3)	39(51.3)	13(14.8)	11(14.5)	9 (10.2)	5 (6.5)	22(25.0)	16(21.1)	27(30.7)	24(31.6)
AOR (95% CI)			0.96(0.52, 1.78)		0.98(0.41, 2.33)		0.62(0.20, 1.93)		0.80(0.38, 1.66)		1.05(0.53, 2.08	
HIV-infected	236	290	176(74.6)	185(63.8)	53(22.5)	35(12.1)	36 (15.3)	31(10.7)	89(37.7)	66(22.8)	97(41.1)	120(41.4)
AOR (95% CI)			0.62(0.42, 0.91)		0.43(0.26, 0.70)		0.70(0.41, 1.18)		0.45(0.29,0.71)		0.97(0.66, 1.42)	
Mothers												
Total	1376	1556	209(15.2)	176(11.3)	52(3.78)	18(1.15)	45(3.3)	30(1.9)	97(7.05)	48(3.08)	117(8.50)	128(8.21)
AOR (95% CI)			0.71(0.57, 0.89)		0.38(0.21, 0.68)		0.69(0.42, 1.13)		0.53(0.36, 0.78)		0.83(0.63, 1.09)	
HIV-uninfected	672	948	65(9.67)	92(9.67)	20(2.98)	5(0.53)	16(2.3)	14(1.5)	36(5.36)	19(2.00)	30(4.46)	73(7.68)
AOR (95% CI)			1.02(0.73, 1.43)		0.22(0.07, 0.68)		0.65(0.30, 1.42)		0.44(0.23, 0.81)		1.58(1.00, 2.49)	
HIV-infected	704	608	144(20.5)	84(13.8)	32(4.55)	13(2.13)	29(4.1)	16(0.2)	61(8.66)	29(4.76)	87(12.4)	55(9.03)
AOR (95% CI)			0.61(0.45, 0.82)		0.56(0.28, 1.11)		0.72(0.38, 1.37)		0.63(0.39, 1.02)		0.69(0.48, 0.99)	

[1]PCV7 era; [2]PCV13 era

[3]PCV7 serotypes: serotypes included in the seven-valent pneumococcal conjugate vaccine, i.e. 4, 6B, 9V, 14, 18C, 19F, 23F;

[4]Additional PCV13 serotypes: serotypes 1, 3, 5, 6A, 7F and 19A; [5]PCV13-serotypes: combination of PCV7 and additional PCV13-serotypes; [6]Non PCV13serotypes: serotypes not included in the PCV13 including the non-typeable serotypes. AOR: adjusted odds ratio; 95% CI: 95% Confidence Interval;

In children, variables considered in the models are: child attending day care, child having a running nose, mother being colonized, whether the child was ever breast fed, whether the child was infected with tuberculosis, child ever hospitalized and child on antibiotics. In HIV-infected children, we also included the use of antiretroviral therapy (ART). In women, we considered characteristics such as whether the mother was a smoker, mother drinks alcohol, mother with a running nose; mother takes snuff, mother ever hospitalized and whether the child was colonized. In addition, in HIV-infected mothers, in the multivariate model, we included the use of ART, antibiotics use and whether the mother was previously treated for tuberculosis. All the variables were modelled as categorical variables.

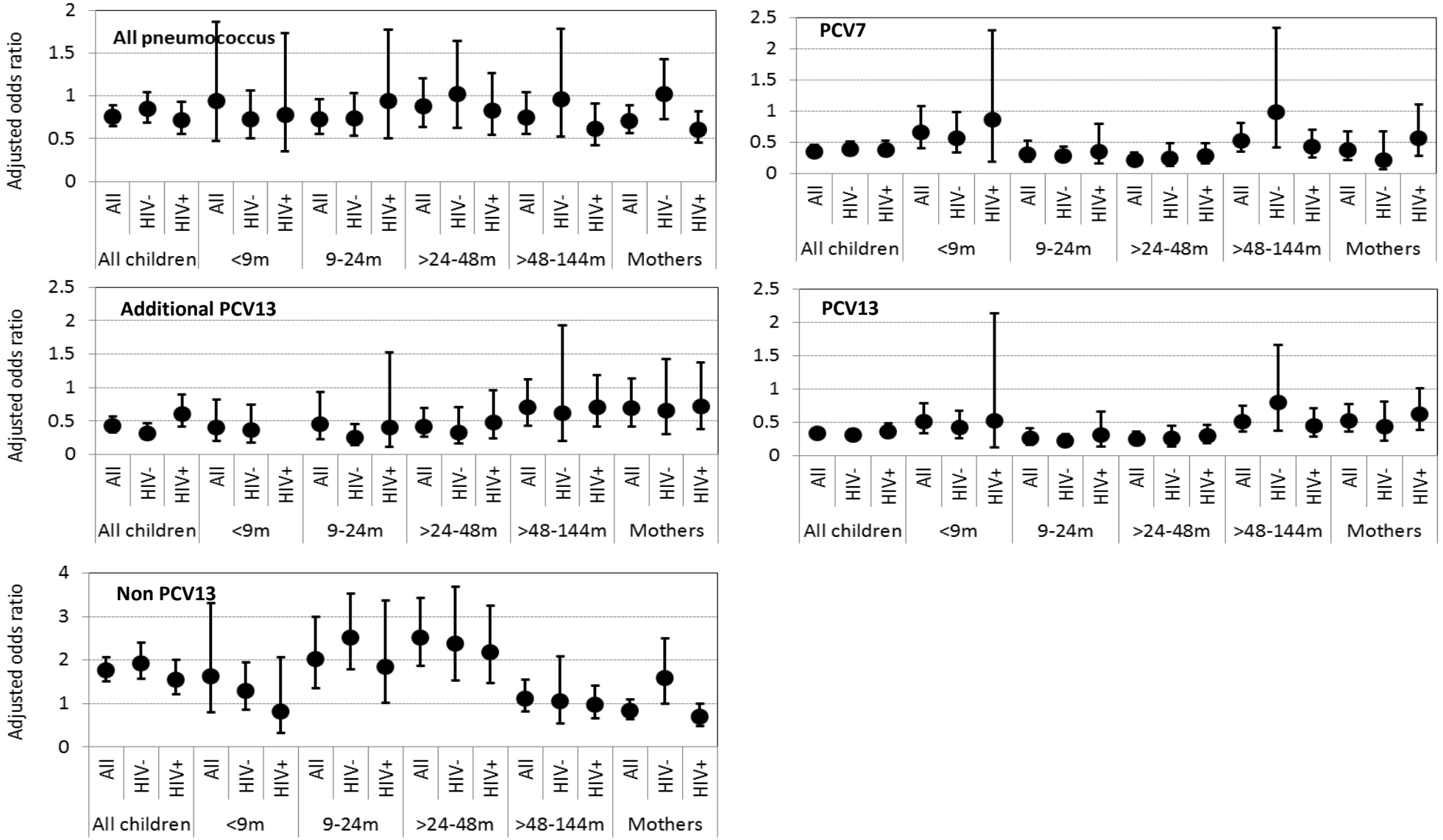


Figure 4.2 Comparison of pneumococcal colonization by HIV-status in children and mothers enrolled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era), in Soweto, South Africa. PCV7 serotypes: serotypes 4, 6B, 9V, 14, 18C, 19F, 23F; Additional PCV13

serotypes: serotypes 1, 3, 5, 6A, 7F and 19A; PCV13 serotypes: combination of PCV7 and additional PCV13 serotypes; Non PCV13 serotypes: serotypes not include in the PCV-13 including the non-typeable serotypes

Among HIV-uninfected children, reductions in PCV7 serotype colonization from period 1 to period 2 was evident among all age groups except those >48 months age. A similar trend for lower prevalence of PCV13 additional serotypes colonization was observed in these age groups; Figure 2. On the other hand, the prevalence of non PCV13 serotype colonization was greater in period-2 (44.0%) than period-1 (29.5%), p<0.0001. This was evident in children age 9-24 months and >24-48 months, with a similar trend also observed among infants <9 months age, whereas it remained unchanged among those >48 months age.

Overall among HIV-infected children, the prevalence of overall pneumococcal colonization trended to being higher in period 1 compared to period 2 (68.2 vs 59.9%, respectively; p=0.012); including for PCV7 serotypes (25.8 vs 12.0%; p<0.0001 and any PCV13 serotype (38.3 vs 19.8%; p<0.0001). Conversely, there was a higher prevalence of non PCV13 serotype colonization in period 2 (40.6%) compared to period 1 (32.1%; p=0.001). The decline in prevalence of PCV13 serotype colonization was detected among all HIV-infected age groups >9 months old, whilst there was a limited number of evaluable children <9 months old; Table 4.5. An increase in prevalence of non PCV13 serotype colonization between period 1 to period 2 among HIV-infected children was only apparent in in the age group >24-48 months (29.4 vs 48.4%; p=0.0001).

The prevalence of individual serotype colonization in period 1 and period 2 among all the children and when stratified by age group are reported in Figure 4.3. Overall, declines in colonization were observed for serotypes 3 (3.3% to 0.8% (p=0.03), 6A (6.0% vs. 1.9%, p<0.001), 6B (5.2% vs. 1.7%, p<0.001), 14 (3.1% vs. 0.9%, p<0.001), 19A (5.0% vs. 2.7%, p=0.001), 19F (8.0% vs. 4.5%, p<0.001) and 23F (6.2% vs. 2.2%, p<0.001); Figure 4.3a.

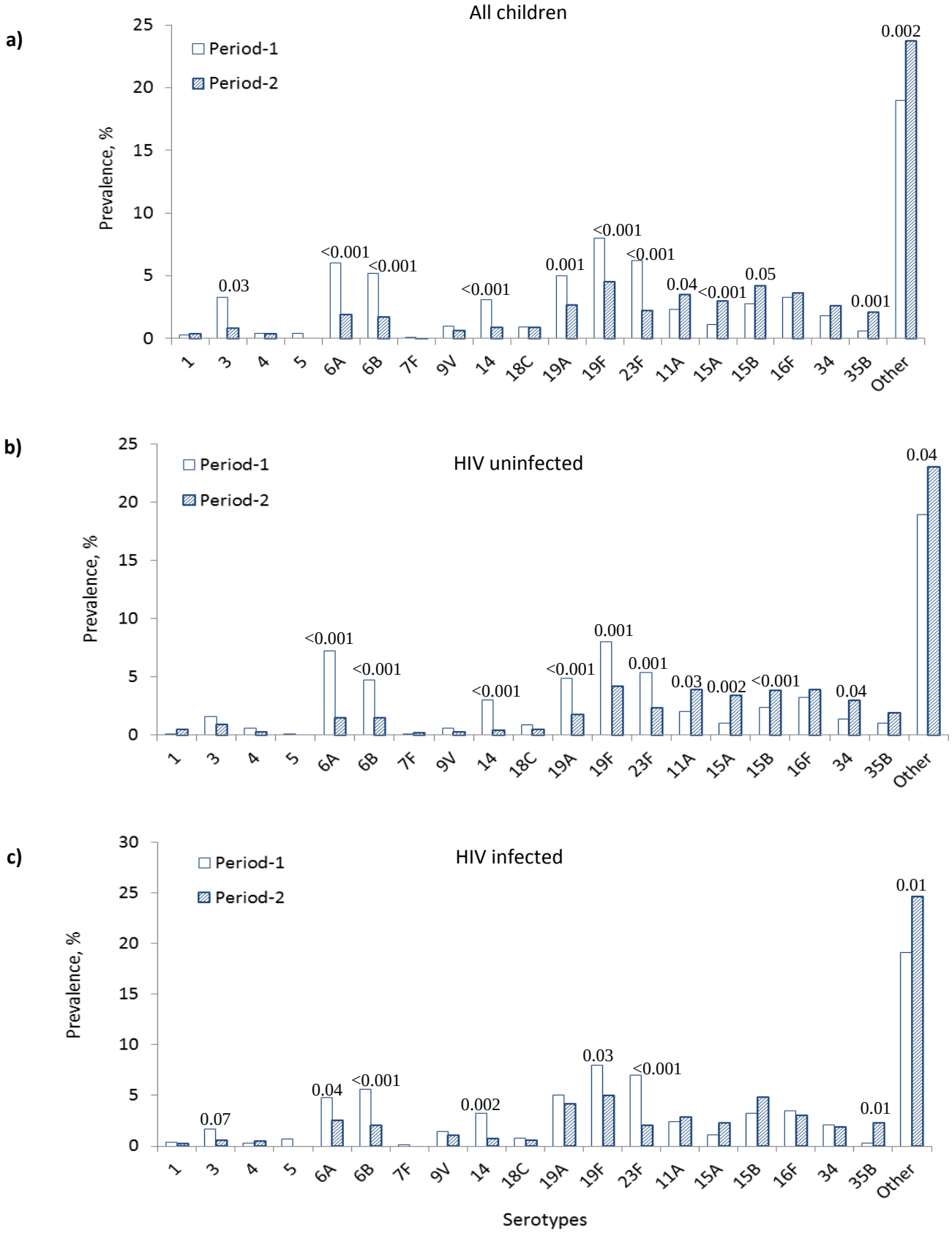


Figure 4.3 Prevalence of common serotypes in all children (A), HIV-uninfected children (B) and HIV-infected children (C) observed in 2010 (Period 1; PCV7 [serotypes 4, 6B, 9V, 14, 18C, 19F, 23F] era) and 2012 (Period 2; PCV13 [serotypes 1, 3, 4, 5, 6A, 6B,7F, 9V, 14, 18C, 19A, 19F, 23F] era).

Decline in prevalence of colonization by all these serotypes were evident in both HIV-infected and HIV-uninfected children, albeit not significant for serotype 3 in both groups and for 19A among the HIV-infected children (Figure 4.3b and 4.3c). Serotypes 1, 4, 5, 7F, 9V and 18C were uncommon (≤2%) in both periods.

Overall, the most common non PCV13 serotypes in period 2 included 11A (3.5%, n=58), 15A (3.0%, n=49), 15B (4.2%, n=69), 16F (3.6%, n=59), 34 (2.6%, n=43) and 35B (2.1%, n=34); Figure 4.3. The prevalence of colonization by each of the above serotypes increased between period 1 to period 2, except for 16F and 34. Among HIV-uninfected children, an increase in non-vaccine serotype colonization was observed specifically for serotypes 11A (2.0 vs 3.9%; p=0.04), 15A (1.0 vs 3.4%; p=0.002), 15B (2.4 vs 3.8%; p<0.0001) and 34 (1.4 vs 3.0%, p=0.04). Although similar trends were evident in HIV-infected children, this was only significant for serotype 35B (0.3% to 2.3%; p=0.01).

4.3.3 Temporal changes in pneumococcal colonization prevalence in women

Among women, there was a decline in overall pneumococcal colonization prevalence from period 1 (15.2%) to period 2 (11.3%; p=0.003), including PCV7 serotype (3.8% vs 1.2%; p=0.001) and PCV13 serotype (7.1% vs 3.1%; p=0.001) colonization; Figure 4.2. The decline in PCV13 serotype colonization between period 1 and period 2 was significant among HIV-uninfected women with a similar trend observed in HIV-infected women. Similar findings were observed when analyses were limited to the PCV7 serotypes.

The overall prevalence of pneumococcal colonization remained unchanged among HIV-uninfected women, with a non-significant increase in non-vaccine serotype colonization in period 2 compared to period 1 (4.5 vs 7.7%; p=0.05). In contrast, there was a reduction in

colonization by any serotype among HIV-infected women between period 1 (20.5%) and period 2 (13.8%; p=0.001), due to the lower PCV13 serotype colonization.

Among women, the most frequently colonizing vaccine serotypes in period 1, were 19F (1.3%, n=18), 23F (0.9%, n=13), 6A (0.8%, n=11) and 6B (0.5%, n=7), the prevalence of which declined to 0.4 % (p=0.006), 0.2% (p=0.005), 0.3% (p=0.04) and 0.1% (p=0.05) in period 2, respectively; Figure 4.4a. Serotype 3 colonization was more prevalent in HIV-infected (2.0%) than HIV-uninfected women (0.6%; p=0.02) in period 1, with a non-significant decrease observed between period 1 and period 2 in HIV-infected women (2.0% vs 1.2%; p=0.23); Figure 4.4b and 4.4c.

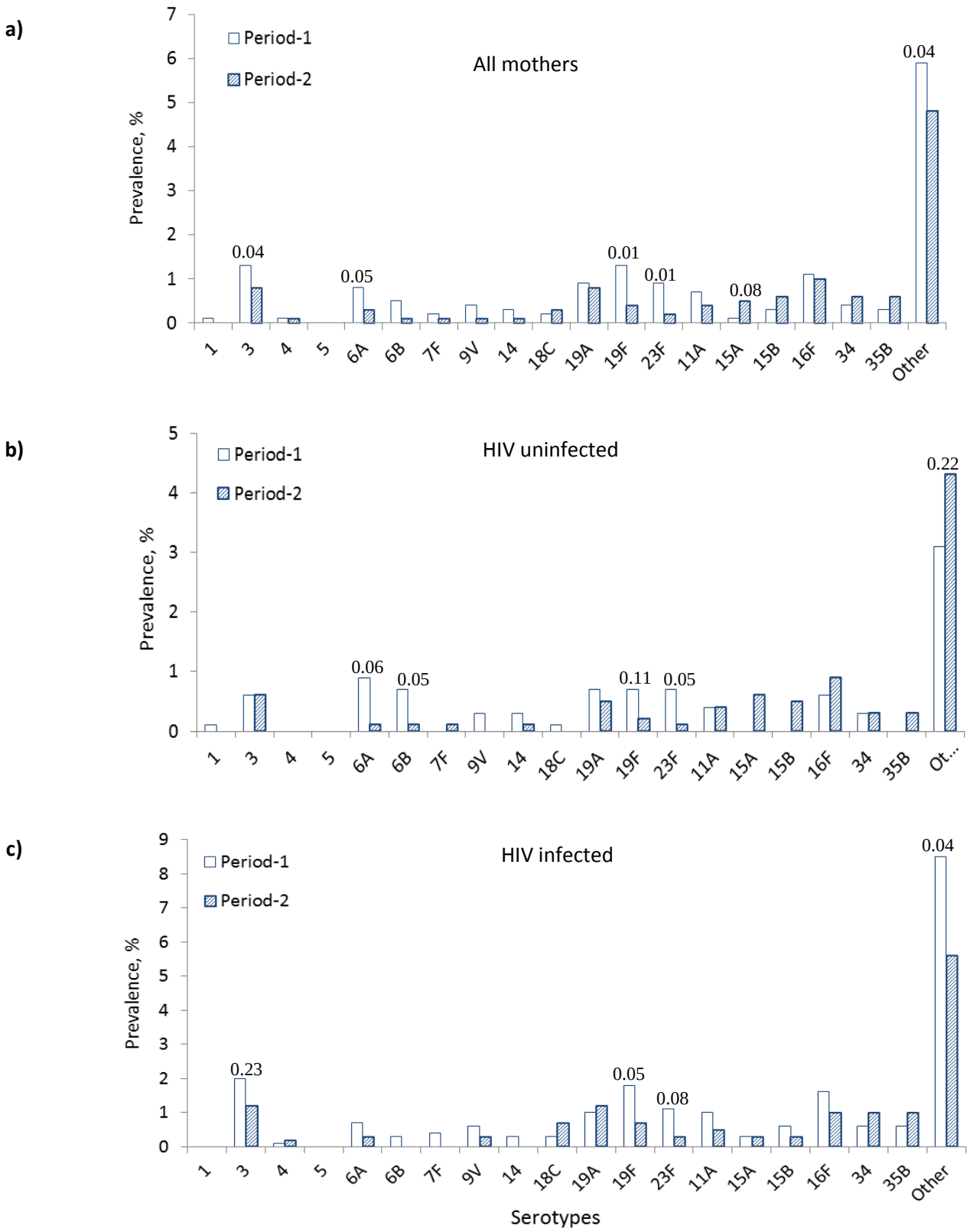


Figure 4.4 Prevalence of common serotypes in all women (A), HIV-uninfected women (B) and HIV-infected women (C) observed in 2010 (Period 1) and 2012 (Period 2)

4.3.4 Differences in carriage between HIV-infected and HIV-uninfected groups

The demographic characteristics differed significantly between HIV-infected and HIV-uninfected children in both study periods; Table 4.6. After adjusting for these differences, no differences in carriage of overall pneumococcus and PCV13 serotypes were observed in either study period between HIV-uninfected and HIV-infected children; Figure 4.5 and Table 4.7.

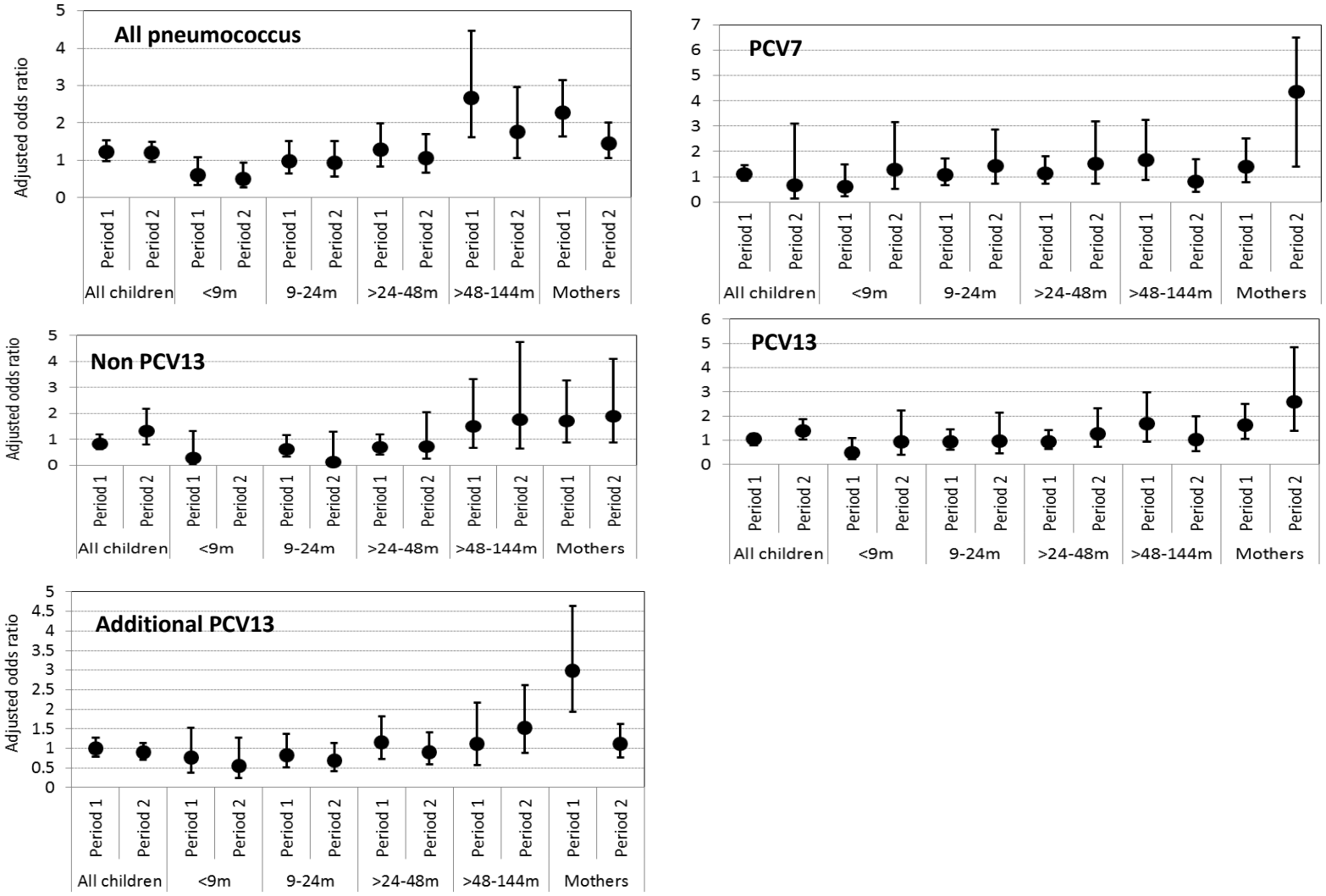


Figure 4.5 Comparison of pneumococcal colonization in HIV-uninfected and HIV infected children (mothers) sampled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era) in Soweto, South Africa.

Table 4.6 Comparison of demographic characteristics between HIV-infected and HIV-uninfected children enrolled in 2010 (Period 1; PCV era) and 2012 (Period 2; PCV13 era) in Soweto, South Africa

		Period 1 (PCV7 era)			**Period 2** (PCV13 era)		
Characteristic		**HIV-infected**	**HIV-uninfected**	**p-value**	**HIV-infected**	**HIV-uninfected**	**p-value**
All children enrolled; N, mean age in years ±SD[1]		713, 3.3± 2.1	698,2.03±1.6	<0.001	616, 3.8±2.12	1033,1.4 ±1.37	<0.001
<9 months; [2]n, mean age (SD) months;		60, 0.41 ± 0.17	170,0.40 ± 0.11	0.60	52, 0.42± 0.17	344,0.40 ± 0.18	0.26
9-24 months; n, mean age in years ±SD		155, 1.41 ± 0.37	253, 1.07 ± 0.35	<0.001	92, 1.23±0.39	447,1.16 ± 0.34	0.08
24.1- 48 months; n, mean in age years ±SD		262, 3.01 ± 0.60	187, 3.53 ± 0.25	<0.001	182, 2.98±0.58	166,2.87 ± 0.54	0.07
48.1-144 months; n, mean in age years ±SD		236, 5.72 ± 1.51	88, 4.7 ± 0.82	<0.001	29, 5.66±1.14	76,5.63 ± 1.14	0.83
Currently breastfed, n/N[3] (%)		18/713 (2.5)	301/697 (43.2)	<0.001	45/566 (7.5)	501/973(51.5)	<0.001
Ever breastfed, n/N (%)		148/665 (22.3)	337/378(89.2)	<0.001	213/549 (38.8)	389/465(83.7)	<0.001
Attendance at day-care, n/N (%)		379/712 (53.2)	232/678(34.2)	<0.001	325/605 (53.7)	216/969(22.3)	<0.001
Currently on TB[4] treatment/prophylaxis, n/N (%)		57/700(8.14)	3/676(0.44)	<0.001	59/608 (9.7)	7/975 (0.7)	<0.001
Treated for TB in the past year, n/N (%)		122/660 (17.0)	4/669(0.6)	<0.001	150/583 (25.7)	9/966 (0.93)	<0.001
Currently taking antibiotics, n/N (%)		108/704(15.3)	12/672 (1.8)	<0.001	49/609 (8.1)	39/973 (4.0)	0.001
Hospitalized in the last 3 months, n/N (%)		42/702(6.0)	21/673(3.1)	0.011	75/607 (12.4)	31/968 (3.2)	<0.001
Pneumococcal vaccine receipt[5]							
<9 months	At least one dose	30/60 (50.0)	166/170 (97.6)	<0.001	18/52 (34.6)	302/344 (87.8)	<0.001
	At least 2 doses	16/60 (26.7)	156/170 (91.8)	<0.001	11/52 (21.1)	295/344 (85.8)	<0.001
9 -24months	At least one dose	35/155 (22.6)	224/253 (88.5)	<0.001	15/92 (16.3)	385/447 (86.1)	<0.001

	At least 2 doses	34/155 (21.9)	221/253 (87.4)	<0.001	15/92 (16.3)	380/447 (85.0)	<0.001
	At least 3 doses	27/155 (17.4)	204/253 (80.6)	<0.001	13/92 (14.1)	342/447 (76.5)	<0.001
>24-48 months	At least one dose	0/262	0/187	NA[6]	14/182(7.7)	131/166 (78.9)	<0.001
	At least 2 doses	0/262	0/187	NA	14/182(7.7)	129/166 (77.7)	<0.001
	At least 3 doses	8/262 (3.1)	0/187	NA	13/182(7.1)	122/166 (73.5)	<0.001
>48-144 months	At least one dose	0/236	0/88	NA	3/290 (0.7)	6/76 (7.9)	0.003
	At least 2 doses	0/236	0/88	NA	1/290 (0.3)	6/76 (7.9)	<0.001
	At least 3 doses	15/236 (6.4)	0/88	NA	1/290 (0.3)	5/76 (7.9)	0.002

[1]Standard deviation;
[2]Number of individuals with investigated outcome
[3]Total number of individuals with available information on the characteristic
[4]Tuberculosis;
[5]only for children with available vaccination records at time of interview
[6]NA: Not done due to limited number of observations in one group

Table 4.7 Comparison of pneumococcal colonization in HIV-uninfected (HIV-) and HIV-infected (HIV+) children (mothers) sampled in 2010 (Period 1; PCV7 era) and 2012 (Period 2; PCV13 era) in Soweto, South Africa.

	Total per group		**All Pneumococci**		**PCV7 serotypes**[1]		**Additional PCV13 serotypes**[2]		**PCV13 serotypes**[3]		**Non PCV13 serotypes**[4]	
	N		n (%)		n (%)		n (%)		n (%)		n (%)	
Group	**HIV-**	**HIV+**	**HIV-**	**HIV+**	**HIV-**	**HIV+**	**HIV-**	**HIV+**	**HIV-**	**HIV+**	**HIV-**	**HIV+**
All children												
Period 1	698	713	444(63.6)	486(68.2)	159(22.8)	184(25.8)	98(14.0)	89(12.4)	257(36.8)	273(38.3)	206(29.5)	229(32.1)
AOR[5] (95% CI)[6]			1.22(0.98,1.53)		1.11(0.85,1.45)		0.84(0.61,1.18)		1.06(0.78,1.27)		1.00(0.79,1.27)	
Period 2	1033	616	597(57.8)	369(59.9)	99(9.58)	74(12.0)	49(4.7)	48(7.7)	148(13.3)	122(19.8)	454(44.0)	250(40.6)
AOR (95% CI)			1.20(0.95, 1.50)		0.66(0.14, 3.09)		1.32(0.79, 2.19)		**1.38(1.02, 1.86)**		0.90(0.71, 1.13)	
<9months												
Period 1	170	60	98 (57.7)	27 (45.0)	31 (18.2)	7 (11.7)	18 (10.6)	2 (3.3)	49 (28.8)	9 (15.0)	51 (30.0)	18 (30.0)
AOR (95% CI)			0.60(0.33, 1.09)		0.60(0.24, 1.49)		0.29(0.06, 1.31)		0.49(0.22, 1.10)		0.76(0.38, 1.52)	
Period 2	344	52	175(50.9)	18 (34.6)	33(9.6)	7 (13.5)	14 (4.1)	0 (0.0)	47 (13.7)	7 (13.5)	129 (37.5)	11 (21.2)
AOR (95% CI)			**0.51(0.28, 0.94)**		1.27(0.51, 3.16)		-		0.94(0.39, 2.23)		0.56(0.25, 1.27)	
9-24 months												
Period 1	253	155	169(66.8)	98(63.2)	70(27.7)	46(29.7)	39 (15.4)	17(11.0)	109(43.1)	63(40.7)	68(26.9)	37(23.9)
AOR (95% CI)			0.98(0.64, 1.51)		1.07(0.66, 1.73)		0.63(0.34, 1.17)		0.94(0.61, 1.44)		0.83(0.51, 1.37)	
Period 2	447	92	273(61.1)	49(53.2)	43(9.62)	13(14.1)	19 (4.3)	5(5.4)	62(13.9)	18(19.6)	214(47.9)	31(33.7)
AOR (95% CI)			0.93(0.57, 1.51)		1.43(0.71, 2.85)		0.13(0.01, 1.29)		0.98(0.45, 2.13)		0.69(0.42, 1.14)	
>24-48 months												
Period 1	187	262	131(70.1)	185(70.6)	45(24.1)	78(29.8)	32 (17.1)	34 (13.0)	77(41.2)	112(42.8)	60(32.1)	77(29.4)
AOR (95% CI)			1.29(0.83, 1.99)		1.14(0.72, 1.81)		0.69(0.41, 1.18)		0.95(0.63, 1.42)		1.15(0.73, 1.81)	
Period 2	166	182	110(66.3)	117(64.3)	12(7.23)	19(10.4)	11 (6.7)	12 (6.6)	23(13.9)	31(17.0)	87(52.4)	88(48.4)
AOR (95% CI)			1.07(0.67, 1.71)		1.51(0.71, 3.20)		0.72(0.25, 2.04)		1.28(0.72, 2.31)		0.91(0.59, 1.40)	
>48-144 months												
Period 1	88	236	46(52.3)	176(74.6)	13(14.8)	53(22.5)	9 (10.2)	36 (15.3)	22(25.0)	89(37.7)	27(30.7)	97(41.1)
AOR (95% CI)			**2.68(1.61, 4.46)**		1.67(0.86, 3.24)		1.51(0.68, 3.31)		1.68(0.94, 2.99)		1.11(0.57, 2.17)	

Period 2	76	290	39(51.3)	185(63.8)	11(14.5)	35(12.1)	5 (6.5)	31(10.7)	16(21.1)	66(22.8)	24(31.6)	120(41.4)
AOR (95% CI)			**1.77(1.06, 2.97)**		0.81(0.39, 1.68)		1.75(0.64, 4.75)		1.04(0.55, 1.98)		1.53(0.89, 2.62)	
Mothers												
Period 1	672	704	65(9.67)	144(20.5)	20(2.98)	32(4.55)	16(2.3)	29(4.1)	36(5.36)	61(8.66)	30(4.46)	87(12.4)
AOR (95% CI)			**2.27(1.64, 3.15)**		1.40(0.79, 2.51)		1.71(0.89, 3.27)		**1.63(1.05, 2.51)**		**2.99(1.93, 4.64)**	
Period 2	948	608	92(9.67)	84(13.8)	5(0.53)	13(2.13)	14(1.5)	16(0.2)	19(2.00)	29(4.76)	73(7.68)	55(9.03)
AOR (95% CI)			**1.46(1.06, 2.02)**		**4.37(1.39,13.7)**		1.90(0.88, 4.09)		**2.59(1.38, 4.85)**		1.12(0.77, 1.63)	

[1]PCV7 serotypes: serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F;
[2]Additional PCV13 serotypes: serotypes 1, 3, 5, 6A, 7F and 19A
[3]PCV13 serotypes: combination of PCV7 and additional PCV13-serotypes;
[4]Non PCV13 serotypes: serotypes not included in the PCV13 including the non-typeable serotypes.
[5]AOR: adjusted odds ratio; [6]95% CI: 95% Confidence Interval;

In children, variables included in the models include child attending day care, child having a running nose, mother being colonized, whether the child was ever breast fed, whether the child was infected with tuberculosis, child ever hospitalized and child on antibiotics. In women, included characteristics such as whether the mother was a smoker, mother drinks alcohol, mother with a running nose, mother takes snuff, mother ever hospitalized and whether the child was colonized. All the variables were modelled as categorical variables.

There were more HIV-infected women who had suffered a chronic illness, were currently on tuberculosis treatment, who had been treated for tuberculosis in the previous year, or were currently on antibiotics than HIV-uninfected women in both study periods; see Table 4.8. HIV-infected women compared to HIV-uninfected individuals had higher prevalence of any-serotype colonization in period-1 (20.5% vs. 9.7%, $p<0.001$) as well as PCV13-serotype colonization (4.8% vs. 2.0% in period-2; $p=0.003$), Table 4.7.

Table 4.7 Comparison of demographic characteristics between HIV-infected and HIV-uninfected women in Period 1 and Period 2, in Soweto, South Africa

Characteristic	Period 1 N=1376			Period 2 N=1556		p-value
	HIV-infected	HIV-uninfected	p-value	HIV-infected	HIV-uninfected	p-value
Number enrolled	704 (51.2)	672 (48.8)		608 (39.1)	948 (60.9)	
Smoker, n[1]/N[2] (%)	40/704 (5.7)	38/672 (5.7)	0.938	45/608 (7.4)	46/948 (4.9)	0.037
Takes snuff, n/N (%)	50/704 (7.1)	27/672 (4.0)	0.013	61/608 (10.0)	26/948 (2.7)	<0.001
Drinks alcohol, n/N (%)	110/704 (15.6)	127/672 (18.9)	0.108	159/608(26.2)	250/948 (26.4)	0.923
Suffers from a chronic illness, n/N (%)	70/704 (9.9)	60/672 (8.9)	0.212	59/608 (9.7)	55/948 (5.8)	0.004
Currently on TB[3] treatment, n/N (%)	17/704 (2.4)	3/672 (0.4)	0.002	15/608 (2.5)	1/948 (0.1)	<0.001
Treated for TB in past year, n/N (%)	76/704 (10.8)	4/672 (0.6)	<0.001	74/608 (12.2)	7/948 (0.7)	<0.001
Currently on antibiotic treatment, n/N (%)	76/704 (10.8)	3/672 (0.4)	<0.001	12/608 (2.0)	8/948 (0.8)	0.054
Hospitalized in the last 3 months, n/N (%)	12/704 (1.7)	13/672 (1.9)	0.750	8/608 (1.3)	10/948 (1.1)	0.639

[1]Number of individuals with investigated outcome

[2]Total number of individuals with available information on the characteristic

[3]Tuberculosis

4.4 Discussion

The targeted immunization of young South African infants with three doses of PCV at 6, 4 and 40 weeks of life was temporally associated with a decline in vaccine serotype colonization among HIV-infected and HIV-uninfected individuals, including among individuals such as HIV-infected women who were not targeted for immunization. This was observed during a period of time when the immunization program transitioned from use of PCV7 since April 2009 to PCV13 in May 2011, and in the absence of any substantive catch-up campaign of older children.

Similar to previous reports, HIV-infected adults in our study, even in the presence of infant PCV immunization, had a higher prevalence of overall and PCV13 serotype colonization than HIV-uninfected adults (47, 140, 153, 159, 160), implying that HIV-infected adults are still at increased risk of IPD due to these serotypes. Recently, it has been shown that the indirect effects of childhood PCV immunization in South Africa on adult IPD were similar in HIV-infected and in HIV-uninfected adults, nonetheless incidence of IPD remained 36-fold higher in HIV-infected adults in the PCV era (112). Specifically, post PCV introduction, HIV-infected adults aged 25-44 years had a higher incidence of overall, PCV7 serotype, 6A, PCV13 serotype and non-vaccine serotype IPD compared to HIV-uninfected adults (112). Despite the heightened IPD risk, currently there is no national recommendation to vaccinate HIV-infected adults with PCV, although the PCV has been shown to be 75% efficacious against vaccine serotype IPD in HIV-infected adults for a limited period of time (161). As such, the indirect effect realised from vaccinating infants in South Africa is likely to have contributed to the indirect effect of protection against vaccine serotype IPD even in HIV-infected individuals (112). This likely culminated through reduced community transmission of these vaccine serotypes from young children to older unvaccinated individuals, including HIV-infected children.

In partially vaccinated HIV-infected children, i.e. age group 0-9 months, no change was observed from Period 1 to Period 2 in vaccine serotype or non-vaccine serotype colonization. This may be attributable to the small numbers of children in this age group or may suggest that two doses of PCV given at 6 and 14 weeks are inadequate to protect against vaccine serotype colonization (162), especially among HIV-infected children at these early stages of the PCV immunization program. We expect a reduced risk of exposure to vaccine serotypes over time which will likely result in reduction of vaccine serotype colonization among this age group (13). An indirect effect against vaccine serotype colonization has also been reported among young children not yet eligible for PCV vaccination in The Gambia, following vaccination of individuals across all age groups in selected villages (163).The decrease in PCV7 serotypes, but not in the PCV13 additional serotypes in HIV-uninfected children <9 months is an encouraging evidence that younger children are also benefitting from indirect protection as the immunization programme continues. This is also corroborated by decreases in PCV7 serotype IPD of 78%, among children younger than 10 weeks of age in South Africa (112).

The reduction in vaccine serotype colonization from period 1 to period 2 in fully vaccinated children was, however, partially offset by the increase in non PV13 serotype colonization that resulted in the overall pneumococcal colonization remaining unchanged among this group. On the other hand, non PCV13 serotype colonization among HIV-infected women did not differ significantly between the two study periods, which was also corroborated by no increase in non PCV13 serotype IPD among HIV-infected adults since the introduction of PCV into the South African immunization program (112).

Among children >48 months of age, the overall prevalence of pneumococcal colonization remained unchanged in HIV-uninfected children in period 2 compared to period 1, although a decline in PCV13 serotype colonization was detected among HIV-infected children.

Similarly, in a rural South African community with high HIV-prevalence, there was no change in colonization among children aged >3 to 12 years, two years post PCV7 introduction (162). Although unlikely, the decrease in PCV13 serotype colonization in older HIV-infected children might have been due to the catch up immunization campaign targeted at this age-group at the time of PCV13 introduction. In The Gambia, decreases in colonization among older children were observed in villages that had additional catch up vaccination 12 months after catch up was initiated (80). The high colonization prevalence among older children in settings such as ours and other low-middle income countries, suggests that catch up campaigns aimed at older children, could possibly accelerate and improve the indirect effects of PCV immunization compared to only targeting young infants for immunization (52). Nevertheless, even with no initial catch up campaign in our setting, an indirect effect was detected within three years of PCV introduction into our public immunization program, indicating young children likely to have been the most important source of transmission of these vaccine serotypes prior to the PCV immunization program. The reduction in vaccine serotype colonization among HIV-infected children in the era of PCV immunization, is in contrast to our earlier randomized controlled trial of an investigational 9 valent PCV, in which no difference was observed in prevalence of vaccine serotype colonization between PCV vaccinated and unvaccinated HIV-infected children at 5 years post vaccination (65). This earlier study only measured direct protection at the individual level, whereas the current study is evaluating the community wide effect of the infant PCV immunization program, and measures the composite of the direct and indirect effects among vaccinated and indirect effect among unvaccinated age groups.

Our study was not powered to detect changes in individual serotypes, especially when stratifying by HIV-status or age groups, nevertheless, decreases in individual vaccine serotypes were observed among fully vaccinated children for serotypes 6A, 6B, 19F and 23F.

Furthermore, largely due to the success of the prevention of mother-to-child transmission program in South Africa, we were unable to recruit similar numbers of young HIV-infected and HIV-uninfected children; however, statistical power was achieved to demonstrate changes in older age-groups. Another limitation of our study is that it is cross-sectional and we cannot exclude the fact that the observed changes can be purely temporally driven.

In conclusion, our study suggests that PCV13 immunization of infants, vaccinated at 6, 10 and 40 weeks of age, was associated with a reduction in vaccine serotype colonization among HIV-infected and HIV-uninfected individuals, including among age groups not targeted for vaccination. Although HIV-infected women are disproportionately affected by disease caused by predominantly "pediatric-serotypes" included in PCV7, in whom the burden remains 40-fold greater than the general adult population even in the presence of antiretroviral therapy (15), the indirect effect against PCV13 serotype colonization in HIV-infected women is likely to reduce the burden of vaccine serotype IPD in this group.

Chapter 5: Temporal association of infant immunization with pneumococcal conjugate vaccine on the ecology of *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus* nasopharyngeal colonization in a rural South African community

5. 1 Introduction

The nasopharynx is an ecological niche for pathogens, including bacteria such as *Streptococcus pneumonia*e, *Haemophilus influenzae* and *Staphylococcus aureus*. Typically these bacteria exist in a commensal state of equilibrium with other nasopharyngeal flora, but may occasionally cause respiratory mucosal and invasive diseases(164). Pneumococcal and *H. influenzae* type b polysaccharide protein conjugate vaccines have been introduced into childhood immunization programmes in many countries.

Pneumococcal conjugate vaccines (PCV) are directed against a limited number of pneumococcal serotypes, and reduce nasopharyngeal acquisition of the vaccine serotypes in immunized children, (8, 68, 69, 71) which could affect the equilibrium of colonization by other bacteria (103, 104). Furthermore, reduced nasopharyngeal colonization by pneumococci in young children may interrupt the transmission thereof to older PCV unvaccinated community members (73), and this may consequently alter the nasopharyngeal flora of the community in general. The decline in colonization by PCV serotypes has, however, generally been associated with a concomitant increase in non-vaccine serotypes colonization, resulting in the overall prevalence of pneumococcal colonization remaining unchanged among PCV vaccinated and unvaccinated individuals (68, 69, 71, 74, 133, 135).

In addition to changes in pneumococcal colonization due to childhood PCV immunization, it has been hypothesised that nasopharyngeal colonization by bacteria such as *H. influenzae* and *S. aureus* could also be affected (165-167). Interactions of non-vaccine serotype pneumococci with either *H. influenzae* or *S. aureus* in children and adults are largely

unknown. Previously described associations in PCV unvaccinated populations include an inverse relationship between *S. pneumoniae* colonization, particularly by those serotypes included in PCV7 and *S. aureus* (93, 94). This raised a theoretical concern that PCV immunization could increase the prevalence of colonization by *S. aureus*, which may lead to an increase in *S. aureus* disease (93, 168). Conversely, a synergistic association has been observed between *H. influenzae* and *S. pneumoniae* nasopharyngeal colonization in HIV-uninfected children, (65, 100) suggesting that reduction in colonization by one may reduce colonization and consequently disease by the other.

The aim of this component of my studies was to determine the effect of infant PCV7 immunization on the prevalence of nasopharyngeal colonization and interaction between *S. pneumoniae*, *S. aureus* and *H. influenzae* among children and older individuals in a rural South African community with a high prevalence of HIV-infection. This analysis expands on our previous analysis of the same study cohort in which we described the effect of PCV7 immunization on pneumococcal colonization in different child and adult age groups as described in Chapter 3 (162).

5.2 Methods

Detailed methods of the study and the study population have previously been published(162). Briefly, two cross sectional surveys were conducted in rural Mpumalanga province (in the Agincourt Health Socio-Demographic Surveillance Site- Agincourt HDSS) from May to October in 2009 (Period-1) and May to October in 2011 (Period-2), in which nasopharyngeal swabs were collected from children (≤12 years) and adolescents/adults (>12 years). *S. pneumoniae*, *H. influenzae* and *S. aureus* were isolated from nasopharyngeal swabs and processed by standard microbiologic methods as previously described (65). PCV7 was

introduced in to the national immunization program from May 2009 and substituted with PCV13 in May 2011 at 6 and 14 weeks of age, followed by a booster dose at 9 months.

5.2.1 Definitions and statistical analyses

PCV7serotypes included serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. All other serotypes were classified as non PCV7 serotypes. Comparison of the prevalence of *S. pneumoniae*, *S. aureus* and *H. influenzae* nasopharyngeal colonization between the two study periods and bacterial interactions were analysed using repeated measures multivariate logistic regression models. Other risk factors for colonization such as attendance at day care center, fossil fuel usage for cooking and/or heating, age of participant and household structure, were added to the models to adjust for possible confounding. To explore the effects of covariates on each of the 3 pathogens, colonization of each pathogen was modelled separately by using the remaining 2 pathogens as predictors including an interaction term and other covariates. All statistical analyses were performed with STATA 12 (Statacorp, Texas, USA) and SAS version 9.2 software (SAS Institute, Inc., NC, USA).

5.3 Results

In period 1 and period 2, 1016 and 1877 children and 994 and 1781 adolescents/adults from 577 and 1079 households respectively, were enrolled. Detailed demographic characteristics of the participants and households sampled are presented in section 3.1. Differences observed in period 1 compared to period 2 among study participants included older median age for children and higher proportion of children who were breastfed, these and other characteristics are shown in Table 3.1.

5.3.1 Temporal changes in prevalence of nasopharyngeal bacterial colonization

In period 1, the prevalence of colonization among children was 74.9% for *S. pneumoniae*, compared to 55.7% for *H. influenzae* (p<0.0001) and 12.3% for *S. aureus* (p<0.0001); Table 5.1 . Among adults sampled in period 1, colonization by *S. aureus* (31.2%) was more common than by *H. influenzae* (16.7%, p<0.0001) or by *S. pneumoniae* (11.2%, p<0.0001), similar trends were observed in period 2. The frequency of individuals who were not colonized by any of the three bacteria increased significantly in both children (13.8% vs. 19.2%; p<0.0001) and adolescents/adults (51.3% vs. 67.0%; p<0.0001) from period 1 compared to period 2, respectively; Table 5.1.

Details of *S. pneumoniae* colonization prevalence have been outlined in section 3.3.1 (162). Stratifying children by age group (0 to 2; 3 to 5 and 6 to 12 years) and comparing colonization patterns between period 1 and period 2, there was a decrease across all age groups for *S. pneumoniae* colonization. Colonization by *S. pneumoniae* decreased among adolescents (13-18 years) from 21.4% in period 1 to 12.3% in period 2 (p=0.0015) and among adults 19 to 45 years from 10.7% to 5.8% (p=0.0002); Table 5.1.

Table 5.1 Prevalence of bacterial pathogens in children and adults in Period 1 and Period 2

Pathogen	Period	Population age group								
		0-2 years	3-5years	6-12years	13-18years	19-45years	>45years	Overall children	Overall adults	Overall
OVERALL COLONIZATION										
[1]SP	Period 1[11]	331 (82.9)	219 (80.2)	186 (60.0)	36 (21.4)	68 (10.7)	12 (5.1)	736 (74.9)	116 (11.2)	852 (42.4)
	Period 2[12]	666 (73.3)	307 (72.2)	242 (50.0)	35 (12.3)	69 (5.8)	22 (5.9)	1215 (67.0)	126 (6.8)	1341 (36.7)
	AOR[10]	**0.57 (0.41,0.76)**	**0.65(0.45,0.96)**	**0.63(0.47,0.85)**	**0.42(0.25,0.72)**	**0.51(0.36,0.73)**	1.27(0.61,2.64)	**0.60(0.50,0.72)**	**0.57(0.44,0.75)**	**0.63(0.55,0.73)**
	p-value	**0.0002**	**0.0282**	**0.0022**	**0.0015**	**0.0002**	0.5225	**<.0001**	**<.0001**	**<.0001**
PCV7 serotypes	Period 1	180(45.1)	97(35.5)	59(19.0)	9(5.7)	19(3.0)	4(1.7)	336(34.2)	32(3.1)	368(18.3)
	Period 2	213(23.5)	121(28.7)	61(12.6)	6(2.1)	13(1.1)	4(1.1)	395(21.8)	23(1.2)	418(11.4)
	AOR	**0.37(0.29,0.48)**	0.76(0.54,1.05)	**0.58(0.39,0.87)**	0.38(0.13,1.10)	**0.35(0.17,0.71)**	0.63(0.15,2.58)	**0.49(0.41,0.58)**	**0.39(0.22,0.67)**	**0.49(0.42,0.58)**
	p-value	**<0.0001**	0.0986	**0.0083**	0.0752	**0.0038**	0.5204	**<.0001**	**0.0006**	**<.0001**
Non PCV7 serotypes	Period 1	155(38.9)	125 (45.8)	132 (42.6)	28 (17.7)	51 (8.1)	8 (3.4)	412 (42.0)	87 (8.5)	499 (24.8)
	Period 2	456 (50.2)	187 (44.3)	182 (37.6)	28 (9.8)	52 (4.4)	16 (4.3)	825 (45.6)	96 (5.2)	921 (25.2)
	AOR	**1.65(1.29,2.11)**	0.95(0.69,1.31)	0.84(0.62,1.13)	**0.47(0.26,0.84)**	**0.60(0.40,0.89)**	1.59(0.67,3.75)	1.17(1.00,1.38)	**0.66(0.49,0.90)**	1.02(0.89,1.17)
	p-value	**<.0001**	0.7607	0.2468	**0.0115**	**0.0111**	0.2947	0.0515	**0.0075**	0.7627
[2]HI	Period 1	226 (56.6)	174 (63.7)	147 (47.4)	30 (17.8)	111 (17.5)	32 (13.5)	547 (55.7)	173 (16.7)	720 (35.8)
	Period 2	490 (54.0)	233(55.2)	178 (36.8)	34 (11.9)	99 (8.3)	29 (7.8)	901 (49.7)	162 (8.8)	1063(29.1)
	AOR	0.88(0.69,1.12)	**0.72(0.52,0.99)**	**0.60(0.45,0.81)**	**0.53(0.31,0.93)**	**0.44(0.33,0.59)**	**0.55(0.32,0.94)**	**0.73(0.62,0.86)**	**0.48(0.38,0.60)**	**0.66(0.58,0.75)**
	p-value	0.3088	**0.0445**	**0.0009**	**0.0257**	**<0.0001**	**0.0285**	**0.0001**	**<0.0001**	**<0.0001**
[3]SA	Period 1	34 (8.5)	27 (9.9)	60 (19.4)	56 (33.3)	213 (33.6)	55 (23.2)	121 (12.3)	324 (31.2)	445 (22.1)
	Period 2	81 (8.9)	43 (10.2)	76 (15.7)	67 (23.5)	313 (26.3)	58 (15.6)	200 (11.0)	438 (23.7)	638 (17.4)
	AOR	1.03(0.67,1.57)	1.09(0.65,1.82)	0.79(0.54,1.16)	**0.53(0.34,0.81)**	**0.70(0.56,0.86)**	**0.59(0.39,0.89)**	0.94(0.74,1.20)	**0.66(0.56,0.79)**	**0.75(0.65,0.86)**
	p-value	0.9015	0.7482	0.2333	**0.0039**	**0.0008**	**0.0127**	0.6346	**<0.0001**	**<0.0001**
SINGLE PATHOGEN COLONIZATION										
Any SP serotype only	Period 1	127 (31.8)	59 (21.6)	56 (18.0)	19 (12.0)	30 (4.7)	7 (3.0)	242 (24.6)	56 (5.4)	298 (14.8)
	Period 2	249 (27.4)	98 (23.2)	96 (19.8)	15 (5.3)	37 (3.1)	11 (3.0)	443 (24.4)	63 (3.4)	506 (13.8)
	AOR	0.81(0.62,1.05)	1.04(0.72,1.52)	1.14(0.79,1.64)	**0.39(0.19,0.81)**	0.62(0.38,1.03)	1.03(0.39,2.71)	0.96(0.80,1.15)	**0.60(0.41,0.87)**	0.88(0.74,1.03)
	p-value	0.1137	0.8235	0.4871	**0.011**	0.0628	0.9567	0.6496	**0.0066**	0.1118

PCV7 serotype only	Period 1	68 (17.0)	28 (10.3)	12 (3.9)	7 (4.4)	5 (0.8)	2 (0.8)	108 (11.0)	14 (1.4)	122 (6.1)
	Period 2	79 (8.7)	43 (10.2)	21 (4.3)	4 (1.4)	8 (0.7)	2 (0.5)	143 (7.8)	14 (0.8)	157 (4.3)
	AOR	**0.47(0.33,0.67)**	0.98(0.59,1.63)	1.17(0.56,2.45)	0.33(0.09,1.15)	0.80(0.26,2.50)	0.58(0.08,4.21)	**0.65(0.50,0.85)**	0.55(0.26,1.16)	**0.65(0.51,0.84)**
	p-value	**<0.0001**	0.9279	0.6668	0.0821	0.7063	0.5877	**0.0016**	0.1151	**0.0008**
HI only	Period 1	28 (7.0)	20 (7.3)	20 (6.5)	13 (8.2)	57 (9.0)	28 (11.8)	68 (6.9)	98 (9.5)	166 (8.3)
	Period 2	90 (10.0)	31 (7.3)	38 (7.9)	13 (4.6)	43 (3.6)	19 (5.1)	159 (8.8)	75 (4.1)	234 (6.4)
	AOR	1.42(0.90,2.22)	1.02(0.56,1.85)	1.25(0.71,2.20)	0.51(0.23,1.15)	**0.39(0.26,0.58)**	**0.40(0.21,0.74)**	1.26(0.94,1.70)	**0.40(0.29,0.55)**	**0.75(0.61,0.92)**
	p-value	0.1286	0.9569	0.4468	0.1029	**<0.0001**	**0.0033**	0.1288	**<0.0001**	**0.0061**
SA only	Period 1	2 (0.5)	4 (1.5)	23 (7.4)	43 (27.2)	154 (24.3)	46 (19.4)	29 (3.0)	243 (23.6)	272 (13.5)
	Period 2	16 (1.8)	10 (2.4)	50 (10.3)	50 (17.5)	266 (22.4)	50 (13.4)	76 (4.2)	366 (19.8)	442 (12.1)
	AOR	3.46(0.78,15.3)	1.61(0.49,5.27)	1.68(0.98,2.88)	**0.55(0.34,0.88)**	0.87(0.69,1.09)	**0.60(0.39,0.94)**	**1.85(1.17,2.91)**	**0.78(0.64,0.93)**	0.88(0.75,1.04)
	p-value	0.1014	0.4337	0.0582	**0.0132**	0.2267	**0.0266**	**0.0082**	**0.0074**	0.1429
CO-COLONIZATION										
[4]SP + HI	Period 1	176 (44.1)	138 (50.5)	101 (32.6)	9 (5.7)	14 (2.2)	0	415 (42.3)	23 (2.2)	438 (21.8)
	Period 2	360 (39.7)	180 (42.7)	124 (25.6)	10 (3.5)	17 (1.4)	6 (1.6)	664 (36.6)	33 (1.8)	697 (19.0)
	AOR	0.83(0.65,1.06)	0.74(0.54,1.02)	**0.67(0.49,0.93)**	0.50(0.19,1.36)	0.61(0.30,1.26)	-	**0.74(0.63,0.87)**	0.80(0.47,1.38)	**0.74(0.63,0.86)**
	p-value	0.1349	0.0629	**0.0161**	0.1752	0.1835	-	**0.0003**	0.4308	**0.0001**
[5]PCV7 + HI	Period 1	97 (24.3)	63 (23.1)	32 (10.3)	2 (1.3)	6 (0.9)	0	192 (19.5)	8 (0.8)	200 (10.0)
	Period 2	116 (12.8)	65 (15.4)	29 (6.0)	0	5 (0.4)	1	210 (11.6)	6 (0.3)	216 (5.9)
	AOR	**0.44(0.33,0.60)**	**0.62(0.42,0.92)**	**0.50(0.29,0.85)**	-	0.40(0.12,1.35)	-	**0.49(0.39,0.61)**	0.37(0.13,1.10)	**0.49(0.39,0.60)**
	p-value	**<0.0001**	**0.0178**	**0.0108**	-	0.1391	-	**<0.0001**	0.0742	**<0.0001**
[6]SP + SA	Period 1	10 (2.5)	7 (2.6)	11 (3.5)	5 (3.2)	19 (3.0)	5 (2.1)	28 (2.9)	29 (2.8)	57 (2.8)
	Period 2	25 (2.8)	11 (2.6)	10 (2.1)	6 (2.1)	8 (0.7)	4 (1.1)	46 (2.5)	18 (1.0)	64 (1.7)
	AOR	1.10(0.52,2.33)	1.17(0.44,3.11)	0.51(0.21,1.25)	0.57(0.17,1.95)	**0.24(0.10,0.55)**	0.57(0.15,2.18)	0.90(0.55,1.45)	**0.35(0.19,0.63)**	**0.61(0.43,0.89)**
	p-value	0.809	0.7498	0.1411	0.3729	**0.0008**	0.4106	0.6538	**0.0005**	**0.0089**
[7]PCV7 + SA	Period 1	6 (1.5)	3 (1.1)	3 (1.0)	0	5 (0.8)	2 (0.8)	12 (1.2)	7 (0.7)	19 (0.9)
	Period 2	7 (0.7)	4 (0.9)	4 (0.8)	2 (0.7)	0	0	15 (0.8)	2 (0.1)	17 (0.5)
	AOR	0.53(0.17,1.61)	1.02(0.22,4.72)	0.88(0.19,4.00)	-	-	-	0.73(0.34,1.57)	**0.17(0.03,0.82)**	**0.50(0.26,0.97)**
	p-value	0.2638	0.9832	0.8684	-	-	-	0.4154	**0.0271**	**0.0416**
[8]HI + SA	Period 1	4 (1.0)	1 (0.4)	8 (2.6)	5 (3.2)	35 (5.5)	4 (1.7)	13 (1.3)	44 (4.3)	57 (2.8)

	Period 2	8 (0.8)	4 (0.9)	4 (0.8)	7 (2.5)	32 (2.7)	3 (0.8)	16 (0.9)	42 (2.3)	58 (1.6)
	AOR	0.71(0.20,2.49)	2.64(0.29,24.3)	**0.29(0.09,0.99)**	0.76(0.23,2.48)	**0.50(0.30,0.81)**	0.51(0.11,2.33)	0.64(0.31,1.35)	**0.53(0.34,0.81)**	**0.56(0.39,0.82)**
	p-value	0.5977	0.3925	**0.0483**	0.6522	**0.0054**	0.3857	0.2456	**0.0036**	**0.0025**
[9]SP + HI + SA	Period 1	18 (4.5)	15 (5.5)	18 (5.8)	3 (1.8)	5 (0.8)	0	51 (5.2)	8 (0.7)	59 (2.9)
	Period 2	32 (3.5)	18 (4.3)	12 (2.5)	4 (1.4)	7 (0.6)	1 (0.3)	62 (3.4)	12 (0.7)	74 (2.0)
	AOR	0.77(0.42,1.41)	0.77(0.38,1.58)	**0.39(0.19,0.84)**	0.60(0.13,2.86)	0.83(0.26,2.64)	-	**0.64(0.43,0.94)**	0.83(0.33,2.04)	**0.66(0.46,0.93)**
	p-value	0.402	0.4821	**0.0154**	0.5242	0.7469	-	**0.0215**	0.6772	**0.0187**
Non-Colonized	Period 1	34 (8.5)	29 (10.6)	73 (22.6)	61 (38.6)	319 (50.4)	147 (62.0)	136 (13.8)	527 (51.3)	663 (33.0)
	Period 2	128 (14.1)	70 (16.6)	150 (31.0)	180 (63.2)	778 (65.4)	278 (74.7)	348 (19.2)	1236 (67.0)	1584 (43.2)
	AOR	**1.85(1.23,2.79)**	**1.65(1.03,2.66)**	**1.50(1.08,2.09)**	**2.98(1.97,4.51)**	**1.90(1.56,2.32)**	**1.85(1.30,2.64)**	**1.64(1.31,2.05)**	**1.98(1.69,2.32)**	**1.80(1.58,2.04)**
	p-value	**0.0033**	**0.0381**	**0.0156**	**<0.0001**	**<0.0001**	**0.0007**	**<0.0001**	**<0.0001**	**<0.0001**
Total	**Period 1**	**399**	**273**	**310**	**158**	**633**	**237**	**982**	**1028**	**2010**
	Period 2	**908**	**422**	**484**	**285**	**1188**	**372**	**1814**	**1845**	**3659**

[1]SP – *Streptococcus pneumoniae,* [2]HI – *Haemophilus influenzae,* [3]SA – *Staphylococcus aureus,* [4]SP + HI – dual carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae,* [5]PCV7 + HI - dual carriage of PCV7 serotypes and *Haemophilus influenzae,* [6]SP + SA – dual carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus,* [7]PCV7 + SA – dual carriage of PCV7 serotypes and *Staphylococcus aureus,* [8]HI + SA – dual carriage of *Haemophilus influenzae* and *Staphylococcus aureus,* [9]SP + HI + SA – triple carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus.*

[10] Number of individuals carrying a particular pathogen and percentage are given in parenthesis. We also present the adjusted odds ratios (AOR) derived from multivariate regression models that included other confounders such as the age of the subject, fuel used for cooking or heating, any child attending day care in the household, the number of people living in the same household and the number of children below the age of 5 years living in the household (significant p-values are in bold)

[11]Period-1 (2009, pre-PCV introduction), [12]Period-2 (2011-post-PCV introduction)

The prevalence of *H. influenzae* colonization decreased with increasing age in both periods, (chi square for trend p<0.001. Comparing period 1 to period 2, there was a decline in *H. influenza*e colonization in the age groups 3 to 5 years (63.7% vs. 55.2%; p=0.04) and 6-12 years (47.4% vs. 36.8%; p<0.001), but not among those 0 to2 years (56.6% vs. 54.0%; p=0.31); Table 2. Among adolescents/adults, colonization of *H. influenzae* decreased from 16.7% in period 1 to 8.8% in period 2 (p<0.0001); Table 2. This inlcuded a decrease in the prevalence in colonization among adults 19 to 45 years (17.5% vs. 8.3%; p<0.001) and among those older than 45 years (13.5% vs. 7.8%; p=0.029). A declining trend was also observed among adolescents (17.8% vs. 11.9%, p= 0.026).

The prevalence of *S. aureus* colonization tended to be higher among older children and adults than in young children in both periods; Table 5.1. In all age-groups in children there was no change in prevalence of *S. aureus* colonization between the two periods, while overall *S. aureus* colonization decreased from 31.2% to 23.7% (p<0.0001) among adults/adolescents; Table 5.1.

Co-colonization by at least two pathogens was higher in period 1 compared to period 2. A decrease in co-colonization from period 1 to period 2 was observed for *S. pneumoniae* and *H.influenzae* among children 3 to 5 years (50.5% vs. 42.7%; p=0.07) and 6 to 12 years (32.6% vs. 25.6%; p=0.016); but not among children 0 to 2 years (44.1% vs. 39.7%; p=0.14); Table 2. PCV7 serotypes and *H. influenzae* co-colonization decreased in children (19.5% vs. 11.6; p<0.0001), while *H. influenzae* and *S. aureus* co-colonization also declined in children 6 to12 years (2.6% vs. 0.8%; p=0.0483). No change was, however, observed for co-colonization between *S. aureus* and overall *S. pneumoniae* among children (2.9% vs. 2.5%; p=0.6538), nor specifically *S. aureus* and PCV7 serotype colonization (1.2% vs. 0.8%, p=0.4154); Table 5.1. Concurrent colonization by all three bacteria declined from 5.8% in

Period-1 to 2.5% in Period-2 among children 6 to 12 years (p=0.0154), but remained unchanged in the other age-groups; Table 5.1.

5.3.2 Bacterial associations in children

S. pneumoniae was positively associated with *H. influenzae* colonization in period 1 in children aged 3 to 5 years and 6 to 12 years (adjusted odds ratio [AOR] 3.49, 95% CI 1.79-6.83 and AOR 7.50, 95% CI 3.95-14.3, respectively); Table 5.2, and in all childhood age-groups in period 2 (AOR 2.05, 95% CI 1.45-2.90 in 0 to 2 years; AOR 4.26, 95% CI 2.47-7.35 in 3-5 years and AOR 4.78, 95% CI 2.93–7.78 in 6 to 12 years); Table 5.2. In addition, a negative association between pneumococcal and *S. aureus* colonization was observed only among children 6 to 12 years in period 2 (AOR 0.36, 95% CI 0.17-0.80); Table 5.2.

H. influenzae colonization was positively associated with *S. pneumoniae* colonization in both period 1 and period 2 for children 3 to 5 years (AOR 3.50, 95% CI 1.79-6.86 and AOR 4.26, 95% CI 2.46–7.37, respectively) and 6 to12 years (AOR 8.02, 95% CI 4.18–15.4 and AOR 4.74, 95% CI 2.92–7.71, respectively), but only in period 2 for children 0 to 2 years (AOR 2.01, 95% CI 1.42–2.86); Table 5.2.

S. aureus was negatively associated with both *S. pneumoniae* (AOR 0.45, 95% CI 0.21-0.97) and *H. influenzae* (AOR 0.36, 95% CI 0.16-0.80) in period 2 in children 6 to 12 years, with similar trends observed in other childhood age groups; Table 5.2. A negative association was also detected betweeen *S. aureus* and non PCV13 serotypes in children aged 6 to 12 years in period 2. (AOR 0.32, 95% CI 0.13-0.80), Table 5.3.

Table 5.2 Effects of PCV7 serotype carriage on other bacterial carriage in children (stratified by age group) and adults

Investigated risk factors for bacterial carriage in children	Carriage of *H. influenzae*		Carriage of *S. aureus*		Carriage of *S. pneumoniae*		Carriage of PCV7 serotypes	
	Period 1	**Period 2**	**Period 1**	**Period 2**	**Period 1**	**Period 2**	**Period 1**	**Period 2**
	OR[2] (95% CI)[4]	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	AOR[3] (95% CI)	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)
0-2 years								
Child carrying *S. pneumoniae* (yes/no)?	1.60(0.95,2.71) 1.53(0.86, 2.70)	**2.13(1.58, 2.88)** **2.05(1.45, 2.90)**	0.95(0.38, 2.40) 1.20(0.25, 5.81)	0.85(0.51, 1.40) 0.76(0.37, 1.55)				
Child carrying PCV7 serotypes (yes/no)?	1.15(0.77, 1.71) 1.09(0.71, 1.68)	1.34(0.98, 1.83) 1.24(0.86, 1.79)	0.96(0.47, 1.94) 1.39(0.42, 4.65)	0.93(0.54, 1.60) 0.61(0.23, 1.65)				
Child carrying *H. influenzae* (yes/no)?			1.44(0.69, 2.99) 2.42(0.40, 14.6)	0.85(0.53, 1.35) 0.66(0.25, 1.72)	1.60(0.95, 2.71) 1.56(0.88, 2.77)	**2.13(1.58, 2.88)** **2.01(1.42, 2.86)**	1.15(0.77, 1.71) 1.09(0.71, 1.69)	1.34(0.98, 1.83) 1.22(0.85, 1.76)
Child carrying *S. aureus* (yes/no)?	1.44 (0.69, 2.99) 2.53(0.43, 15.0)	0.85(0.53, 1.35) 0.81(0.31, 2.14)			0.95(0.38, 2.40) 1.36(0.27, 6.79)	0.85(0.51, 1.40) 0.81(0.39, 1.66)	0.96(0.47, 1.94) 1.39(0.42, 4.58)	0.93(0.54, 1.60) 0.62(0.23, 1.66)
Child carrying both *S. pneumoniae* and *H. influenzae* (yes/no)?			1.20(0.59, 2.43) 0.53(0.07, 3.80)	0.87(0.55, 1.40) 1.32(0.43, 4.07)				
Child carrying both PCV7 serotypes and *H. influenzae* (yes/no)?			1.00(0.45, 2.23) 0.58(0.13, 2.62)	0.98(0.51, 1.92) 2.01(0.57, 7.07)				
Child carrying both *S. pneumoniae* and *S.aureus* (yes/no)?	1.40(0.63, 3.12) 0.52(0.07, 3.70)	1.13(0.65, 1.95) 0.99(0.32, 3.10)						
Child carrying both PCV7 serotypes and *S.aureus* (yes/no)?	1.15(0.40, 3.29) 0.61(0.14, 2.70)	1.55(0.57, 4.23) 1.63(0.45, 5.85)						
Child carrying both *H. influenzae* and *S.aureus* (yes/no)?					0.92(0.30, 2.82) 0.44(0.06, 3.27)	1.48(0.67, 3.27) 1.17(0.38, 3.66)	0.84(0.35, 2.01) 0.57(0.13, 2.54)	1.25(0.61, 2.54) 1.99(0.57, 6.99)
3-5 years								
Child carrying *S. pneumoniae* (yes/no)?	**3.64(1.96, 6.76)** **3.49(1.79, 6.83)**	**4.12(2.60, 6.55)** **4.26(2.47, 7.35)**	1.09(0.39, 3.04) 0.77(0.20, 2.95)	0.75(0.38, 1.48) 0.74(0.27, 1.99)				
Child carrying PCV7 serotypes (yes/no)?	1.34(0.79, 2.26) 1.44(0.81, 2.55)	1.41(0.91, 2.17) 1.29(0.77, 2.16)	0.49(0.19, 1.25) 0.75(0.18, 3.18)	1.09(0.55, 2.16) 0.79(0.24, 2.63)				
Child carrying *H. influenzae* (yes/no)?			0.81(0.36, 1.82) 0.27(0.03, 2.71)	0.81(0.43, 1.53) 0.63(0.15, 2.60)	**3.64(1.96, 6.76)** **3.50(1.79, 6.86)**	**4.12(2.60, 6.55)** **4.26(2.46, 7.37)**	1.34(0.79, 2.26) 1.48(0.84, 2.64)	1.41(0.91, 2.17) 1.30(0.78, 2.17)
Child carrying *S. aureus*	0.81(0.36, 1.82)	0.81(0.43, 1.53)			1.09(0.39, 3.04)	0.75(0.38, 1.48)	0.49(0.19, 1.25)	1.09(0.55, 2.16)

*(*yes/no)?	0.39(0.04, 3.85)	0.66(0.16, 2.65)			0.96(0.26, 3.60)	0.76(0.28, 2.08)	0.88(0.21, 3.64)	0.72(0.22, 2.35)
Child carrying both *S. pneumoniae* and *H. influenzae* (yes/no)?			0.98(0.44, 2.18) 3.15(0.26, 39.0)	0.78(0.41, 1.48) 1.15(0.22, 5.87)				
Child carrying both PCV7 serotypes and *H. influenzae* (yes/no)?			0.36(0.11, 1.25) 0.54(0.08, 3.82)	1.26(0.58, 2.76) 2.00(0.44, 9.21)				
Child carrying both *S. pneumoniae* and *S.aureus* (yes/no)?	1.24(0.49, 3.15) 2.00(0.17, 24.2)	1.32(0.61, 2.88) 1.05(0.21, 5.32)						
Child carrying both PCV7 serotypes and *S.aureus* (yes/no)?	0.56(0.11, 2.84) 0.57(0.08, 4.17)	1.82(0.55, 6.00) 2.56(0.56, 11.7)						
Child carrying both *H. influenzae* and *S.aureus* (yes/no)?					3.90(0.50, 30.2) 2.11(0.17, 25.7)	1.71(0.57, 5.16) 1.10(0.21, 5.78)	0.40(0.11, 1.44) 0.41(0.06, 2.95)	1.78(0.74, 4.28) 2.39(0.54, 10.7)
6-12 years								
Child carrying *S. pneumoniae* (yes/no)?	**6.09(3.63, 10.2)** **7.50(3.95, 14.3)**	**6.14(4.04, 9.33)** **4.78(2.93, 7.78)**	**0.55(0.31, 0.98)** 0.59(0.25, 1.39)	**0.35(0.20, 0.59)** **0.36(0.17, 0.80)**				
Child carrying PCV7 serotypes *(*yes/no)?	**4.21(2.23, 7.98)** **4.42(1.99, 9.81)**	**2.83(1.63, 4.90)** **2.34(1.22, 4.50)**	1.56(0.80, 3.05) 1.71(0.42, 7.01)	1.21(0.60, 2.45) 1.00(0.31, 3.20)				
Child carrying *H. influenzae* (yes/no)?			0.82(0.46, 1.44) 1.23(0.43, 3.51)	**0.40(0.22, 0.72)** 0.46(0.15, 1.41)	**6.09(3.63, 10.2)** **8.02(4.18, 15.4)**	**6.14(4.04, 9.33)** **4.74(2.92, 7.71)**	**4.21(2.23, 7.98)** **4.62(2.10, 10.2)**	**2.83(1.63, 4.90)** **2.31(1.20, 4.44)**
Child carrying *S. aureus (*yes/no)?	0.82(0.46, 1.44) 1.27(0.46, 3.52)	**0.40(0.22, 0.72)** **0.45(0.21, 0.97)**			**0.55(0.31, 0.98)** 0.65(0.28, 1.54)	**0.35(0.20, 0.59)** **0.36(0.16, 0.80)**	1.56(0.80, 3.05) 1.30(0.32, 5.18)	1.21(0.60, 2.45) 1.01(0.32, 3.23)
Child carrying both *S. pneumoniae* and *H. influenzae* (yes/no)?			0.63(0.34, 1.16) 0.74(0.19, 2.90)	**0.43(0.22, 0.82)** **0.42(0.20, 0.92)**				
Child carrying both PCV7 serotypes and *H. influenzae* (yes/no)?			1.70(0.82, 3.55) 1.47(0.27, 7.91)	1.32(0.56, 3.13) 2.49(0.47, 13.3)				
Child carrying both *S. pneumoniae* and *S.aureus* (yes/no)?	1.93(0.88, 4.23) 0.74(0.19, 2.81)	2.13(0.90, 5.02) 2.02(0.46, 8.79)						
Child carrying both PCV7 serotypes and *S.aureus* (yes/no)?	**4.74(1.31, 17.2)** 2.76(0.52, 14.6)	3.07(0.89, 10.6) 2.12(0.40, 11.2)						
Child carrying both *H. influenzae* and *S.aureus*					1.55(0.65, 3.69) 0.56(0.15, 2.09)	3.10(0.99, 9.77) 2.07(0.48, 8.96)	**4.32(1.88, 9.93)** 2.17(0.42, 11.3)	**5.93(2.12, 16.6)** 2.17(0.40, 11.8)

(yes/no)?								
Children (≤ 12 years)								
Child carrying *S. pneumoniae* (yes/no)?	**3.83 (2.84, 3.16)** **3.35 (2.41, 4.65)**	**3.89 (3.16, 4.78)** **2.82 (2.16, 3.69)**	**0.54 (0.36, 0.79)** 0.68 (0.45, 1.04)	**0.50 (0.38, 0.66)** **0.59 (0.39, 0.89)**				
Child carrying *H. influenzae* (yes/no)?			0.79 (0.55, 1.15)	**0.58 (0.43, 0.78)** 0.59 (0.33, 1.04)	**3.83 (2.84, 5.16)** **3.87 (2.71, 5.54)**	**3.89 (3.16, 4.78)** **3.36 (2.45, 4.62)**	1.15 (0.77, 1.71) 1.10 (0.73, 1.66)	**1.77 (1.41, 2.21)** 1.25 (0.96, 1.64)
Child carrying *S. aureus* (yes/no)?	0.79 (0.55, 1.15)	**0.58 (0.43, 0.78)** **0.50 (0.25, 1.00)**			**0.54 (0.36, 0.79)** 0.65 (0.40, 1.04)	**0.50 (0.38, 0.66)** **0.56 (0.33, 0.94)**	0.96 (0.47, 1.94)	0.92 (0.64, 1.30)
Child carrying both *S. pneumoniae* and *H. influenzae* (yes/no)?			0.72 (0.50, 1.05)	**0.60 (0.40, 0.82)** 1.43 (0.71, 2.89)				
Child carrying both *S. pneumoniae* and *S. aureus* (yes/no)?	1.50 (0.94, 2.41) 1.13 (0.67, 1.89)	1.45 (0.98, 2.15) 1.67 (0.72, 3.89)						
Child carrying both *H. influenzae* and *S. aureus* (yes/no)?					1.44 (0.77, 2.69)	**2.02 (1.17, 3.48)** 1.99 (0.74, 5.29)	0.84 (0.35, 2.01)	**1.97 (1.22, 3.17)** **1.78 (1.01, 3.11)**
Adults (> 12 years)								
Adult carrying *S. pneumoniae* (yes/no)?	**1.70 (1.04, 2.75)** **1.71 (1.05, 2.79)**	**6.83 (4.41, 10.6)** **7.34 (4.22, 13.1)**	1.15 (0.75, 1.77)	1.07 (0.69, 1.66)				
Adult carrying *H. influenzae* (yes/no)?			0.98 (0.69, 1.41)	**1.86 (1.30, 2.65)** **1.90 (1.30, 2.77)**	**1.70 (1.04, 2.75)** 1.62 (0.99, 2.65)	**6.83 (4.41, 10.6)** **7.34 (4.05, 13.1)**	**2.33 (1.04, 5.22)**	**5.36 (2.15, 13.4)** **6.48 (4.10, 10.3)**
Adult carrying *S. aureus* (yes/no)?	0.98 (0.69, 1.41)	**1.86 (1.30, 2.65)** **2.04 (1.33, 3.12)**			1.15 (0.75, 1.77)	1.07 (0.69, 1.66)	1.13 (0.52, 2.47)	0.32 (0.07, 1.37)
Adult carrying both *S. pneumoniae* and *H. influenzae* (yes/no)?			1.01 (0.43, 2.37)	1.38 (0.67, 2.81)				
Adult carrying both *S. pneumoniae* and *S.aureus* (yes/no)?	1.46 (0.65, 3.26)	**7.63 (3.51, 16.6)** 0.82 (0.29, 2.32)						
Adult carrying both *H. influenzae* and *S.aureus* (yes/no)?					1.60 (0.73, 3.50)	**4.28 (2.14, 8.57)** 0.76 (0.31, 1.85)	2.16 (0.63, 7.37)	1.59(0.21, 12.1)

[1] For the multivariate model, we included other variables such as any sibling attending of day care, fossil fuel used for cooking or heating, age of the study participant and the number of people in the household, number of children below <5 years.

[2]OR: odds ratio; [3]AOR: adjusted OR; [4]CI: confidence interval (multivariate Generalized Estimate Equation models);

Table 5.3 Effects of non PCV7 serotype carriage on other bacterial carriage in children (stratified by age group) and adults

Investigated risk factors for bacterial carriage in children	Carriage of *H. influenzae*		Carriage of *S. aureus*		Carriage of NVT7[5]-serotypes	
	Period 1[6]	**Period 2[7]**	**Period 1**	**Period 2**	**Period 1**	**Period 2**
	OR[2] (95% CI)[4]	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	AOR[3] (95% CI)	AOR (95% CI)	AOR (95% CI)	OR (95% CI)	AOR (95% CI)	AOR (95% CI)
0-2 years						
Child carrying NVT7-serotypes (yes/no)?	1.17 (0.78,1.75) 1.18 (0.76,1.82)	**1.53 (1.17,1.99)** **1.57 (1.16,2.14)**	0.86 (0.42,1.77) 0.68 (0.19,2.42)	0.89 (0.56,1.40) 1.09 (0.54,2.19)		
Child carrying *H. influenzae* (yes/no)?					1.17 (0.78,1.75) 1.19 (0.76,1.84)	**1.53 (1.17,1.99)** **1.58 (1.16,2.15)**
Child carrying *S. aureus (*yes/no)?					0.86 (0.42,1.77) 0.75 (0.21,2.69)	0.89 (0.56,1.40) 1.15 (0.57,2.30)
Child carrying both NVT7-serotypes and *H. influenzae* (yes/no)?						
Child carrying both NVT7-serotypes and *S.aureus* (yes/no)?	1.74 (0.53,5.75) 1.13 (0.24,5.34)	0.92 (0.49,1.74) 0.65 (0.24,1.76)				
Child carrying both *H. influenzae* and *S.aureus* (yes/no)?					0.97 (0.40,2.31) 1.02 (0.21,4.88)	1.02 (0.54,1.93) 0.62 (0.23,1.67)
3-5 years						
Child carrying NVT7-serotypes (yes/no)?	**2.01 (1.21,3.33)** **1.88 (1.08,3.27)**	**2.44 (1.64,3.64)** **2.63 (1.64,4.22)**	1.71 (0.76,3.84) 0.94 (0.25,3.58)	0.75(0.39,1.42) 0.82(0.29,2.30)		
Child carrying *H. influenzae* (yes/no)?					**2.01 (1.21,3.33)** **1.83 (1.05,3.20)**	**2.44 (1.64,3.64)** **2.61 (1.63,4.20)**
Child carrying *S. aureus (*yes/no)?					1.71 (0.76,3.84) 1.00 (0.27,3.72)	0.75 (0.39,1.42) 0.97 (0.35,2.69)
Child carrying both NVT7-serotypes and *H. influenzae* (yes/no)?			1.63 (0.73,3.64) 2.86 (0.47,17.3)	0.64 (0.31,1.35) 0.81 (0.20,3.21)		
Child carrying both NVT7-serotypes and *S.aureus* (yes/no)?	1.76 (0.55,5.61) 2.10 (0.34,12.9)	1.14 (0.43,3.06) 0.55 (0.14,2.15)				
Child carrying both *H. influenzae* and *S.aureus* (yes/no)?					**3.59 (1.13,11.43)** 2.68 (0.44,16.25)	0.97(0.41,2.30) 0.59(0.15,2.34)
6-12 years						
Child carrying NVT7-serotypes (yes/no)?	**2.41 (1.52,3.82)** **2.72 (1.58,4.66)**	**3.87 (2.61,5.72)** **3.17 (2.01,5.00)**	**0.41 (0.22,0.76)** 0.53 (0.21,1.33)	**0.31(0.17,0.57)** **0.33(0.13,0.82)**		
Child carrying *H. influenzae* (yes/no)?					**2.41 (1.52,3.82)** **2.74 (1.59,4.72)**	**3.87 (2.61,5.72)** **3.13 (1.99,4.92)**

Child carrying *S. aureus (*yes/no)?					**0.41 (0.22,0.76)** 0.65 (0.26,1.61)	**0.31 (0.17,0.57)** **0.32 (0.13,0.80)**
Child carrying both NVT7-serotypes and *H. influenzae* (yes/no)?			**0.32 (0.14,0.74)** 0.49 (0.12,1.93)	**0.32 (0.14,0.73)** 1.22 (0.28,5.35)		
Child carrying both NVT7-serotypes and *S.aureus* (yes/no)?	0.86 (0.31,2.36) 0.30 (0.08,1.19)	1.74 (0.60,5.04) 1.16 (0.26,5.19)				
Child carrying both *H. influenzae* and *S.aureus* (yes/no)?					0.46 (0.19,1.12) 0.31 (0.08,1.20)	1.26 (0.46,3.44) 1.25 (0.29,5.43)
Children (≤ 12 years)						
Child carrying NVT7-serotypes (yes/no)?	**1.80 (1.40, 2.32)** **1.79 (1.34,2.37)**	**2.38 (1.98,2.87)** **2.15 (1.72,2.67)**	0.70 (0.47,1.02) 0.67 (0.36,1.23)	**0.54 (0.40,0.73)** 0.68 (0.43,1.07)		
Child carrying *H. influenzae* (yes/no)?					**1.80 (1.40,2.32)** **1.75 (1.32,2.32)**	**2.38 (1.98,2.87)** **2.12 (1.70,2.63)**
Child carrying *S. aureus (*yes/no)?					0.70 (0.47,1.02) 0.68 (0.37,1.25)	**0.54 (0.40,0.73)** 0.67 (0.43,1.05)
Child carrying both NVT7-serotypes and *H. influenzae* (yes/no)?			0.71 (0.46,1.11) 1.05 (0.46,2.40)	**0.54 (0.37,0.77)** 0.93 (0.48,1.79)		
Child carrying both NVT7-serotypes and *S. aureus* (yes/no)?	1.30 (0.71,2.39) 0.84 (0.37,1.92)	1.24 (0.77,1.98) 0.80 (0.41,1.58)				
Child carrying both *H. influenzae* and *S. aureus* (yes/no)?					1.02 (0.61,1.70) 1.01 (0.45,2.26)	1.12 (0.72,1.76) 0.92 (0.47,1.79)
Adults (> 12 years)						
Adult carrying NVT7-serotypes (yes/no)?	1.33 (0.75,2.37) 1.42 (0.70,2.90)	**6.99 (4.38,11.1)** **8.35 (4.51,15.4)**	1.15 (0.71,1.87) 1.08 (0.62,1.87)	1.25(0.78,1.99) 1.22(0.63,2.34)		
Adult carrying *H. influenzae* (yes/no)?					1.33 (0.75,2.37) 1.47 (0.72,3.00)	**6.99 (4.38,11.1)** **8.32 (4.48,5.47)**
Adult carrying *S. aureus (*yes/no)?					1.15 (0.71,1.87) 1.09 (0.63,1.90)	1.25 (0.78,1.99) 1.25 (0.65,2.40)
Adult carrying both NVT7-serotypes and *H. influenzae* (yes/no)?			0.97 (0.34,2.83) 0.83 (0.24,2.90)	1.48 (0.70,3.15) 0.75 (0.25,2.22)		
Adult carrying both NVT7-serotypes and *S.aureus* (yes/no)?	1.15 (0.43,3.07) 0.76 (0.22,2.65)	**7.32 (3.26,16. 5)** 0.65 (0.22,1.93)				
Adult carrying both *H. influenzae* and *S.aureus* (yes/no)?					1.27 (0.49,3.28) 0.71 (0.20,2.47)	**4.66 (2.26,9.61)** 0.67 (0.22,1.99)

[1] For the multivariate model, we included other variables such as any sibling attending of day care, fossil fuel used for cooking or heating, age of the study participant and the number of people in the household, number of children below <5 years.

[2]OR: odds ratio; [3]AOR: adjusted OR; [4]CI: confidence interval (multivariate Generalized Estimate Equation models); [5]NVT7-serotypes: serotypes not included in the seven-valent vaccine[6]Period 1 (2009, pre-PCV introduction), [7]Period 2 (2011, post-PCV introduction)

5.3.3 Bacterial associations in adults

S. pneumoniae colonization was positively associated with *H. influenzae* colonization in period 1 (AOR 1.71, 95% CI 1.05-2.79) and period 2 (AOR 7.34, 95% CI 4.22-13.1); Table 5.2, including a positive association between non PCV13 serotypes and *H. influenzae* colonization in period 2 (AOR 8.35, 95% CI 4.51-15.4), Table 4. *H. influenzae* colonization was also positively associated with *S. aureus* colonization in period 2 (AOR 1.90, 95% CI 1.30-2.77) but not in period 1; Table 5.2.

5.4 Discussion

To date there is a paucity of data regarding the effect of PCV7 vaccination on bacterial nasopharyngeal ecology across different age-groups from the same population. Our study showed, that there was a decrease in *S. pneumoniae* colonization among children <2 years of age who had been targeted for PCV immunization, while the prevalence of *H. influenzae* and *S. aureus* colonization remained unchanged in this age group following PCV7 introduction. Among adolescents/adults from the same population, however, there were temporal declines in *S. pneumoniae, H. influenzae* and *S. aureus* colonization following infant PCV7 immunization. The decrease in *S. pneumoniae* vaccine serotypes colonization prevalence among the adolescents/adults, (162) may have resulted from PCV vaccination of young children reducing transmission of pneumococci to these older unvaccinated age groups. This decrease in vaccine serotype colonization was larger than the increase in non-vaccine serotype colonization in children resulting in a net decrease in overall pneumococcal colonization. Non-vaccine serotypes may be less efficient colonizers as demonstrated by an increase in prevalence of colonization in PCV vaccinated but not among unvaccinated children. Also, a decrease in non-vaccine pneumococcal serotypes colonization was observed

in adults, we have previously alluded this decrease to be possibly due to increased antiretroviral therapy coverage in adults (162). This may again be a plausible explanation for the decline observed in the prevalence of *H. influenzae* and *S. aureus* colonization among adults/adolescents.

The prevalence of *H. influenzae* colonization among children in our study (55%) was higher than observed among Kenyan children aged 0 to 5 years (26%) and 5 to 9 years (24%), (169) but lower than that reported among infants in The Gambia (70% compared to 52% among infants in our study) (100). Our study, however, observed a decline in *H. influenzae* nasopharyngeal colonization among older children 3 to 12 years but not among PCV vaccinated children <2 years of age, following the implementation of the PCV immunization program. This is in contrast to the increase in prevalence of *H. influenzae* colonization which was observed among vaccinated children in The Netherlands, following a booster dose of PCV at 11 months of age after a three dose primary series schedule (108). However, an earlier study also from The Netherlands, in which children were vaccinated with a two dose primary series and a booster dose of PCV, did not demonstrate an increase in *H. influenzae* colonization at 11 months of age (104). In our population there was no change in *H. influenzae* colonization after the booster dose at 9 months. This supports data from a study in Fiji where there was no change in *H. influenzae* in vaccinated children younger than 2 years (95).

Decreases in *H. influenzae* colonization were noted in the unvaccinated age-groups in our study. Studies in neonatal rats show that when *H. influenzae* is inoculated first in the respiratory tract followed by *S. pneumoniae*, there is an increase in *H. influenzae* density, however, when the order of inoculation is reversed competition between the two species was observed (170). This observation was found to be strain specific (170). In our study we demonstrated a positive association between non-vaccine serotype and *H. influenzae* carriage

in all age-groups. This may explain the decrease in *H. influenzae* carriage in unvaccinated age-groups which may have been caused by a decrease in non-vaccine pneumococcal serotype carriage observed in these age-groups (162). Our study was, however, done within two years of PCV7 implementation, and the full effects of vaccination may not be evident and established in the population and our results may reflect short term shifts in the nasopharyngeal microbiome associated with vaccination or temporal variation in colonization prevalence.

The inhibition of *S. aureus* by hydrogen peroxide produced by the pneumococcus results in a negative relationship between *S. aureus* and *S. pneumoniae* (93, 94). Also, it is thought to be immune mediated, specifically CD4+ T-lymphocyte mediated (102) and due to a decrease in mucosal immunity (65). This is supported by the absence of this relationship among HIV-infected children (65, 97) and HIV-infected adults (102). Our study was carried out in a setting with high prevalence (23.9%) of HIV-infection among adults (162). We observed a reduction in *S. aureus* prevalence among adolescents and adults but not among children. Most of the studies that investigated the effect of PCV on *S. aureus* colonization among children have also failed to detect an increase in prevalence of *S. aureus* colonization in the PCV era (65, 95, 106, 107). An exception was a study in The Netherlands where an increase in the prevalence of *S. aureus* colonization was observed in children aged 11-12 months following 3 doses of PCV7 and at three years of age (103, 108). We did not observe any increase in *S. aureus* colonization post PCV7 booster among vaccine recipients (data not shown). In South Africa, PCV was administered with a modified 2+1 dose schedule versus the 3+1 in The Netherlands, which may impact on the time it takes to realise shifts in bacterial interactions. In the USA, however, as part of a *S. aureus* surveillance program between 2001 and 2004, decrease in *S. aureus* colonization was observed among children and adults following the introduction of a 3+1 PCV7 immunization into the national

immunization program in 2000 (171). This decrease may, however, have been a transient early effect of PCV vaccination, since nine years later the prevalence of *S. aureus* colonization among children was similar to pre-vaccination levels (107). A South African study on the long-term effects of PCV in the context of a randomised placebo trial found no differences in *S. aureus* and *H. influenzae* colonization after 5 years of vaccination between vaccinees and placebo-recipients (65).

Colonization by multiple pathogens is common in African children, in part due to host and environmental factors (100). The synergism between *S. pneumoniae* and influenza virus has been well described (172) as well as other interactions between bacteria and viruses, including respiratory syncytial virus, rhinovirus, adenoviruses, to mention but a few (173). We did not isolate respiratory viruses from our samples and are therefore unable to deduce the effect of seasonal respiratory viruses on colonization. However, co-carriage of multiple pathogens is also supported by data among adolescents/adults, where *H. influenzae* colonization was positively associated with both *S. aureus* and *S. pneumoniae.* It has been demonstrated that among PCV naïve children *S. pneumoniae* colonization, mainly by vaccine serotypes, is negatively associated with *S. aureus* carriage and positively associated with *H. influenzae* carriage (93, 94). The studies that reported on the same paradigm following pneumococcal vaccination show that such associations are probably maintained (65, 103, 104). In our study population the previously established positive association in period 1 between *S. pneumoniae* and *H. influenzae* as well as the negative association between *H. influenzae* and *S. aureus* were observed in children also in period 2.

Limitations of our study include that it was cross-sectional rather than longitudinal, which could have provided greater insight into the timing of acquisition of the different bacteria and their interactions in colonization. Also, we only sampled the nasopharynx rather than the anterior nares for *S aureus*, however, swabs from the nasopharynx have been used before for

detection of multiple respiratory pathogens (103, 104). All nasopharyngeal samples were processed by means of culture-based techniques, which are inferior to molecular detection methods for the identification of multiple carriages. Additionally, we do not have *H. influenzae* typing data to determine the serotypes associated with colonization. Moreover, we did not look for other pathogens, for example *Moraxella catarrhalis* and respiratory viruses which could have helped us to better understand the dynamics of colonization. We sampled more females than males in either study period limiting the generalizability of our study findings. Lastly, due to uncertainty over self-reported HIV status we did not stratify our analysis by HIV status among adults.

Our results on early changes in the interactions between *S. pneumoniae*, *H. influenzae* and *S. aureus* suggest that the *S. aureus* carriage in children was not affected by PCV7 immunization and that there was a decrease in colonization in the adult population. It is therefore, unlikely that disease due to *S. aureus* would increase in our population. Continued surveillance is however, needed to monitor changes with vaccination in later years.

Chapter 6: Trends in community-wide effect on invasive pneumococcal disease and associations with colonization, Soweto, South Africa following infant pneumococcal conjugate vaccination

6.1 Introduction

Pneumococcal nasopharyngeal colonization precedes pneumococcal disease, hence, serotypes identified in invasive disease isolates are also identified in the nasopharynx at the time of illness (21, 30, 31). PCV vaccinated compared to unvaccinated children have an approximately 50% reduced risk of vaccine serotype nasopharyngeal colonization acquisition (174). Since young children are the main source of transmission of pneumococci in communities (21), targeted PCV immunization of children has interrupted transmission of vaccine serotypes within the communities (73). Consequently, reduction in prevalence of vaccine serotype colonisation has also been observed among PCV unvaccinated individuals at the household and community level (9, 71, 73, 80, 84, 162).

Concurrently, a decline in the incidence of vaccine serotype IPD among PCV vaccinated and unvaccinated individuals has been observed, (7, 10, 11). In the USA, the indirect effect of childhood PCV immunization has resulted in 2.2-fold greater number of IPD cases prevented among PCV unvaccinated adults compared to that prevented among age-groups targeted for PCV vaccination (10). There is a paucity of data from low and middle income countries on the effectiveness of childhood PCV immunization in protecting against IPD among PCV unvaccinated age groups, which would be essential to fully evaluate the public health impact and cost-effectiveness of this intervention (123).

Weinberger et al. propose that ecological nasopharyngeal vaccine serotype and non-vaccine serotype colonization studies prior and following childhood PCV immunization can be used to impute the direct and indirect effectiveness of childhood PCV immunization against IPD (122). This would be pertinent to most low and middle income countries, where high-quality surveillance systems to directly monitor the impact of PCV against IPD are generally lacking.

The aim of this study was to analyse the direct and indirect effectiveness of infant PCV immunization among HIV-infected and HIV-uninfected children and their mothers in Soweto, South Africa. Furthermore, we evaluated the fit of the proposed model by Weinberger et al,(122) to determine whether temporal changes in vaccine serotype colonization relative to childhood PCV immunization was predictive of the direct and indirect effect of PCV against IPD (122).

6.2 Methods

6.2.1 Study population

A series of pneumococcal colonization studies were undertaken in mother-child pairs in Soweto (Gauteng, South Africa), which has a population of 1.4 million of whom 125,000 are under five years age (175). We enrolled HIV-infected and HIV-uninfected children >3 months to 5 years age and their mothers aged >18-45 years age. We specifically targeted enrolling mothers of the children, since women in our setting have greater contact with children than men, which possibly also contributes to them having a higher incidence of IPD than men (47).

The majority of the Soweto population seeks medical care from 23 primary health care centres and a single public-hospital (Chris Hani Baragwanath Academic Hospital; CHBAH) where approximately 90% of all hospitalizations from the community occur. The

effectiveness of infant PCV-immunization against IPD in this community was included as part of a recent national analysis on the direct and indirect effect against IPD in South Africa (112). The current analysis on IPD expands on the previous report by providing temporal changes in incidence of IPD in children and women from Soweto (112).

Details of vaccine introduction into the national immunization program were previously described, in section 2.3.

6.2.2 Pneumococcal colonization studies in Soweto (2007 to 2012)

Prior to PCV7 introduction into the national immunization program, we undertook a longitudinal cohort study to investigate the dynamics of pneumococcal transmission in HIV-uninfected mother-child pairs between January 2007 and April 2009 (pre-PCV era) as reported (158). This survey was not designed as part of this study. Since the use of longitudinal data could overestimate colonization prevalence, to transform the data into cross-sectional data, a single randomly selected visit for each mother-child pair was used. Briefly, the study showed an association of vaccine serotype acquisition between young children and their mothers (140), and that children transmitted PCV7 serotypes more often to their mothers than vice-versa (105). Subsequently, two cross-sectional colonization prevalence surveys were undertaken in the same population in 2010 (PCV7 era) and 2012 (PCV13 era) as described (176). This study showed a significant reduction of PCV7 serotype colonization in both HIV-infected and HIV-uninfected mothers and their children; and an increase of non-vaccine serotype colonization in children but not in the mothers (176).

In all the colonization studies, nasopharyngeal swabs were collected in children and nasopharyngeal and oropharyngeal swabs from the mothers. Samples were kept on ice for a maximum of 6 hours and stored at -70^{o}C until processing. The methods of sample collection, culture and serotyping are detailed in section 2.4 (162).

6.2.3 Invasive pneumococcal disease surveillance (2005 -2012)

Laboratory-based surveillance for IPD in South Africa has been ongoing since 1999 by the National Institute for Communicable Diseases (NICD) (177). Since 2003, this included an enhanced component at twenty-four sentinel hospitals, including CHBAH, in which detailed individual case-level demographic and clinical data are collected. IPD cases were defined as isolation of *S. pneumoniae* from normally sterile sites including cerebrospinal fluid (CSF), blood and joint fluid. Recurrent IPD episodes at least 21 days apart in the same individual were considered as new cases.

The standard culture method at CHBAH for blood uses the BacT/Alert microbial detection system (Organon Teknika, Durham, North Carolina, USA). Autolysed blood culture specimens, macroscopically chocolate-colored with or without pleomorphic Gram-positive cocci on microscopy, were tested with a latex agglutination kit (Wellcogen Bacterial Antigen Kit, Remel Europe Ltd, Dartford, UK). Viable pneumococcal isolates were serotyped by Quellung method using specific antisera (Statens Serum Institute, Copenhagen, Denmark). Serotype 6A was distinguished from 6C by Quellung (162).

Both IPD and colonizing pneumococcal isolates were serotyped at the Centre for Respiratory and Meningitis Diseases at NICD as described in section 2.4.

6.2.4 Statistical analysis

PCV7 serotypes were defined as any of the 7 valent vaccine serotypes (i.e. 4, 6B, 9V, 14, 18C, 19F and 23F). Furthermore, since antibody to serotype 6B confers cross-protection against serotype 6A, the latter was analysed separately (150). The additional serotypes included in PCV13 (i.e. 1, 3, 5, 7F and 19A) were categorised as PCV13 additional serotypes; whereas all non PCV13 serotypes and non-typeable strains were categorized as non-vaccine serotypes. Non viable isolates were prorated based on identified serotypes as its been shown that the distribution of serotypes among viable isolates determined by culture and among non-viable isolates determined by PCR is similar (178).

Incidence of IPD for children, 0 to 3 months, >3 months to 24 months and >24 to 60 months, 5 to 18 years of age, and adults 18 to 45 years and >45years of age were calculated using the annual number of IPD cases identified by mid-year population estimates for Soweto. The population denominators were available through Statistics South Africa [25]. The average of the IPD incidence between 2005 and 2008 represented the pre PCV rate. We then compared this pre PCV era incidence to that in 2010 (PCV7 era) and 2012 (PCV13 era) and calculated the percent change in the rate of IPD, as well as the absolute difference between the pre PCV era and PCV7 era and PCV13 era. Differences in the rates including the 95% confidence interval were used to evaluate the significance of observed changes in IPD. The Chi-square test was used to assess differences in proportions with a p-value of <0.05 considered as a statistically significant result. Since the HIV prevalence among IPD cases in years prior to 2008 could have been overestimated because of a lower proportion (70% in 2005 vs >88% in 2012) of cases having had an HIV test done, we used a previously developed methodology to account for this potential bias (112).

For colonization data we compared colonization prevalence in the different study period stratified by HIV-status. Both colonization and IPD data were imputed into a theoretical model (Model 1) proposed by Weinberger *et al*. (122) to calculate the expected IPD incidence post PCV introduction and compared model-predicted values to the observed IPD incidence changes, for age groups, >3 to 24 months, >24 to 60 months and women 18-45 years old. The model assumes that the ratio of IPD incidence to carrier prevalence for any serotype is constant, such that a change in carriage prevalence will result in a proportional change in IPD incidence.

6.3 Results

6.3.1 Temporal changes in pneumococcal colonization

Compared to the pre PCV era there was a reduction in PCV7 serotype colonization prevalence in the 2010 survey; and reduction in the additional PCV13 serotypes in the 2012 survey, Fig 6.1 and Table 6.1.

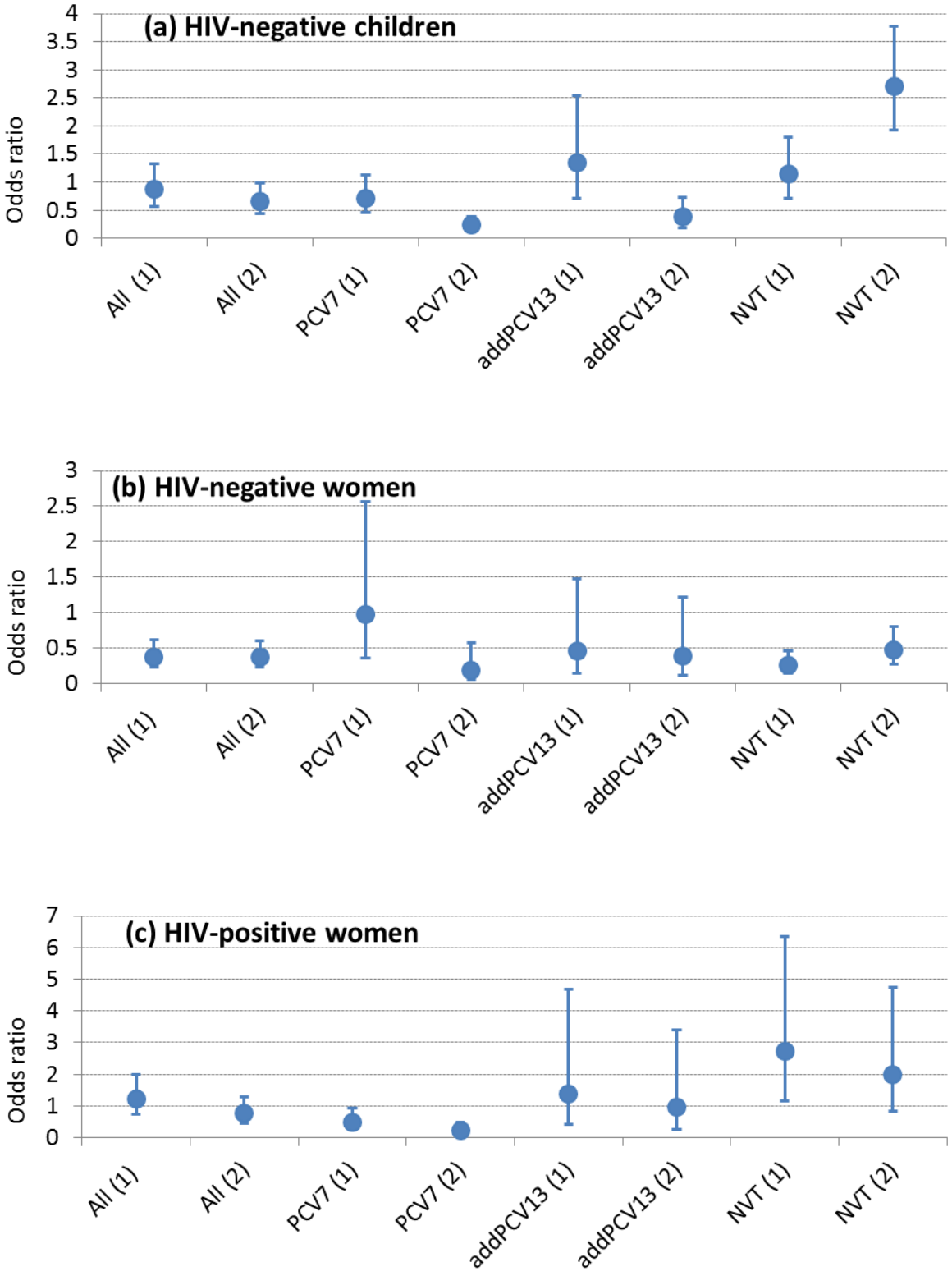


Figure 6.1 Comparison of carriage prevalence by age group between baseline year 2007-2009 and 2010 (1) or baseline year and 2012 (2). A, HIV-uninfected children >3months to 5 years, B, HIV-uninfected women 18-45 years, C, HIV-infected women 18-45 years.

Table 6.1 Comparison of pneumococcal colonization by HIV-status in children and mothers enrolled in 2007-2009 (Pre-PCV era); 2010 (PCV7- era) and 2012 (PCV13-era), in Soweto, South Africa.

Group		All		HIV-uninfected		HIV-infected	
Children >3 to 24months							
		Total	OR (95%CI)		OR (95%CI)		OR (95%CI)
Total per group	2007-2009	251		125		-	
	2010	638		423		215	
	2012	935		791		144	
All serotypes	2007-2009	158 (62.9)		83 (66.4)		-	
	2010	392 (61.4)	0.93 (0.69-1.27)	267(63.1)	0.87 (0.57-1.32)	125 (58.1)	
	2012	515 (55.1)	0.72 (0.54-0.96)	448(56.6)	0.66 (0.44-0.98)	67 (46.5)	0.63 (0.41-0.96)*
PCV7 serotypes	2007-2009	83 (33.1)		38 (30.4)		-	
	2010	154 (24.1)	0.64 (0.47-0.89)	101(23.9)	0.72 (0.46-1.12)	53 (24.7)	
	2012	96 (10.3)	0.23 (0.17-0.32)	76 (9.6)	0.24 (0.16 - 0.38)	20 (13.9)	0.49 (0.28-0.87)*
Additional-PCV13 serotypes	2007-2009	24 (9.6)		13 (10.4)		-	
	2010	76 (11.9)	1.27 (0.79-2.08)	57 (13.5)	1.34 (0.71-2.54)	19 (8.8)	
	2012	38 (4.1)	0.40 (0.24-0.68)	33 (4.2)	0.38 (0.19-0.73)	5 (3.5)	0.37 (0.14-1.02)*
Non-PCV13 serotypes	2007-2009	51 (20.3)		32 (25.6)		-	
	2010	174 (27.3)	1.47 (1.03-2.09)	119(28.1)	1.14 (0.72-1.79)	55 (25.6)	
	2012	381 (40.7)	2.70 (1.93-3.77)	339(42.9)	2.18 (1.42-3.34)	42 (29.2)	1.20 (0.75-1.92)*
Mothers							
Total per group	2007-2009	251		125		126	
	2010	1376		672		704	
	2012	1556		948		608	
All serotypes	2007-2009	50 (19.9)		28(22.4)		22 (17.5)	
	2010	209 (15.2)	0.72 (0.51-1.01)	65 (9.7)	0.37 (0.23-0.61)	144 (20.5)	1.22 (0.74-1.99)
	2012	176 (11.3)	0.51 (0.36-0.73)	92 (9.7)	0.37 (0.23-0.60)	84 (13.9)	0.76 (0.45-1.27)
PCV7 serotypes	2007-2009	18 (7.2)		5 (4.0)		13 (10.3)	
	2010	63 (4.6)	0.62 (0.36-1.07)	26 (3.9)	0.97 (0.36-2.57)	37(5.3)	0.48 (0.25-0.94)

	2012	22 (1.4)	0.19	(0.10-0.35)	7 (0.7)	0.18	(0.06-0.57)	15 (2.5)	0.22	(0.10-0.47)
Additional-PCV13 serotypes	2007-2009	7 (2.8)			4 (3.2)			3 (2.4)		
	2010	33 (2.4)	0.86	(0.37-1.96)	10(1.5)	0.46	(0.14-1.48)	23 (3.3)	1.38	(0.41-4.68)
	2012	26 (1.7)	0.59	(0.25-1.38)	12(1.3)	0.39	(0.12-1.22)	14 (2.3)	0.97	(0.27-3.41)
Non-PCV13 serotypes	2007-2009	25 (10.0)			19(15.2)			6 (4.8)		
	2010	117 (8.6)	0.84	(0.53-1.32)	29 (4.3)	0.25	(0.14-0.46)	84 (11.9)	2.71	(1.16-6.35)
	2012	128 (8.2)	0.81	(0.52-1.27)	73 (7.7)	0.47	(0.27-0.80)	55 (9.0)	1.99	(0.84-4.73)

Data on comparison of carriage prevalence in 2010 compared to 2012 has been described in Chapter 4.

*Comparison between carriage in 2010 and 2012, no data for baseline year (2007-2009)

6.3.2 Temporal changes in pneumococcal colonization among children

Compared to the pre PCV era, there was a reduction in PCV7 serotype colonization prevalence among HIV-uninfected children by 2010; and reduction in the PCV13 additional serotypes by 2012, Figures 6.1a, 6.1b, 6.1c and Table 6.1. The change was evident among children aged <3 months-24 months, among whom the overall colonization prevalence decreased from 62.9% to 55.1% (p=0.025), PCV7 serotype from 33.1% to 10.3% (p<0.001) and PCV13 additional serotypes from 9.6% to 4.1% (p=0.001) between the pre PCV era and 2012. During the same period, non-vaccine serotype colonization increased from 20.3% to 40.7% (p<0.001), Figure 6.1a and Table 6.1. Notably, the reduction in PCV7 serotype colonization was already evident within one year of PCV7 introduction (OR: 0.64; p=0.007); which declined further by 2012 (OR: 0.23; p<0.001). In contrast, colonization prevalence due to PCV13 additional serotypes between the pre PCV era and 2010 was similar (9.6% vs 11.9%; p=0.32; whilst decreasing to 4.1% one year following PCV13 introduction (OR: 0.40 p<0.001); Figure 6.1a and Table 6.1. Compared to the pre PCV era, the prevalence of non-vaccine serotype colonization increased from 20.3% to 40.7% by 2012 (OR: 2 .70; p<0.001); Figure 6.1a and Table 6.1.

Similar pre-PCV era data were unavailable for HIV-infected children, with the observed changes in colonization prevalence between 2010 and 2012 being previously described in section 4.3.2; and Table 6.1 [28].

6.3.3 Pneumococcal colonization in women aged 18-45 years

Overall, the prevalence of pneumococcal colonization in women was 19.9% in the pre PCV era, which decreased to 15.2% (p=0.06) and then 11.3% (p<0.001) by 2010 and 2012, respectively. Stratifying by HIV-infection status, overall pneumococcal prevalence declined

from 22% in the pre PCV area compared to 9.7% in 2010 (p<0.001) and 9.7 in 2012 (p<0.001) among HIV-uninfected women; whilst it remained unchanged in HIV-infected women (range: 17.5% to 13.9%); Figures 6.1b, 6.1c and Table 6.1. PCV7 serotype colonization remained unchanged between the pre PCV era and 2010 (4.0% vs 3.9%) but declined to 0.7% by 2012 (p=<0.001) among HIV-uninfected women whilst in HIV-infected women it decreased from 10.3% in the pre-PCV era to 5.3% by 2010 (p<0.001) and 2.5% by 2012 (p<0.001). PCV13 additional serotypes trended to also decrease from 3.2% in the pre PCV era to 1.5% in 2010 (p=0.181) and 1.3% by 2012 (p=0.094) in HIV-uninfected women; but remained unchanged in HIV-infected women (2.4% to 3.3% in 2010 and 2.3% in 2012) .There was also a decline in non-PCV13 serotype colonization among HIV-uninfected women between the pre PCV era (15.2%) compared to 2012 (7.7%; p=0.005), whereas this trended to being higher among HIV-infected women (4.8% in pre PCV era vs 9.0% in 2012; p=0.113); Table 6.1.

6.3.2 Invasive pneumococcal disease

We identified 4,144 IPD cases between 2005 and 2012 at CHBAH of which 2,533 (61%) had viable isolates for further characterization. The overall IPD rate decreased from 55.5 per 100,000 to 18.0 per 100,000 in 2012 among all ages; Figure 6.2A. Although the incidence of IPD was highest in children ≤2 years throughout, the magnitude of this difference compared to other age-groups narrowed over time; Figure 6.2B. HIV-infected individuals had higher IPD rates compared to HIV-uninfected, regardless of the age of the individual, a difference which persisted even in 2012 (Figures 6.3 and 6.4).

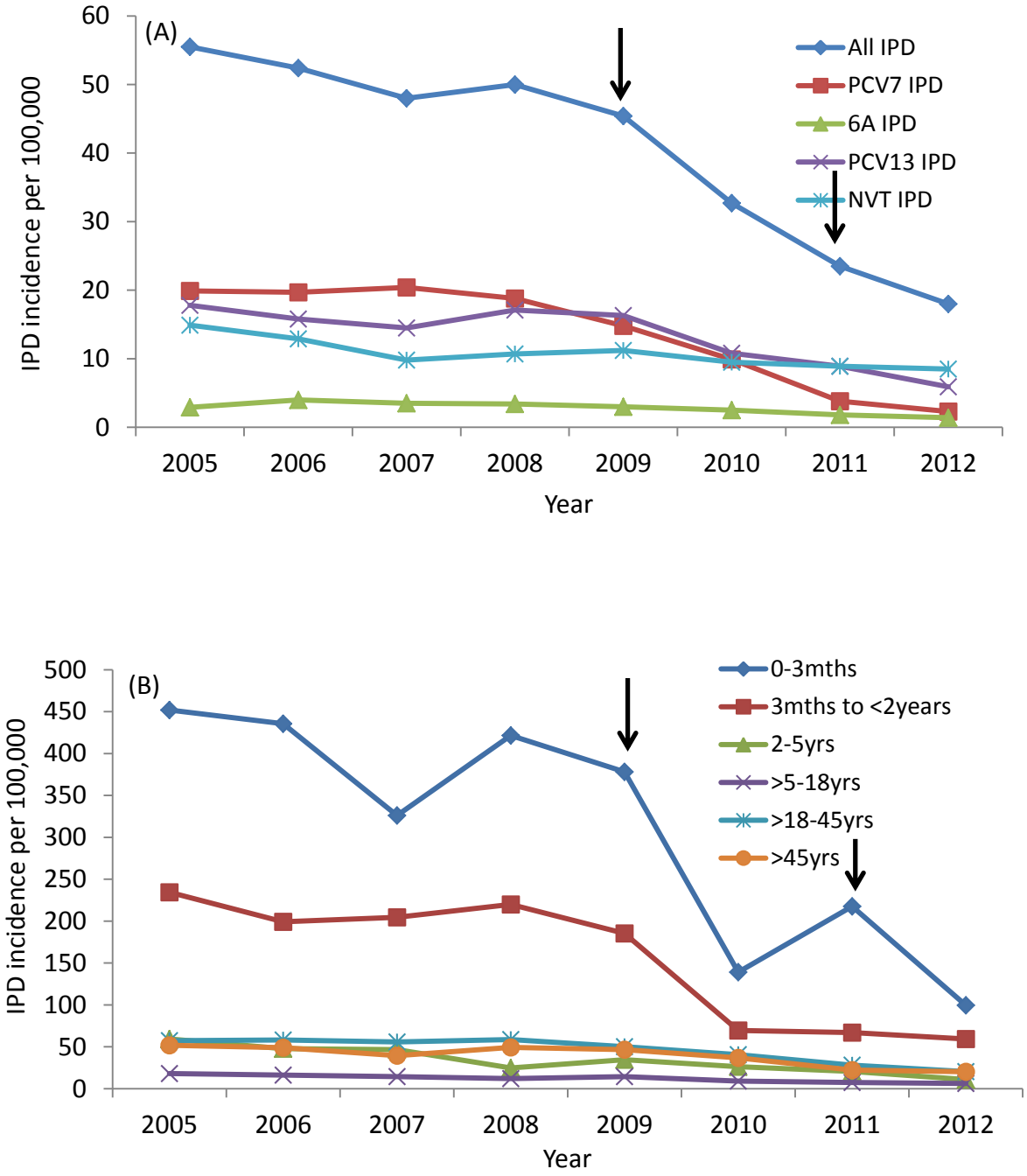
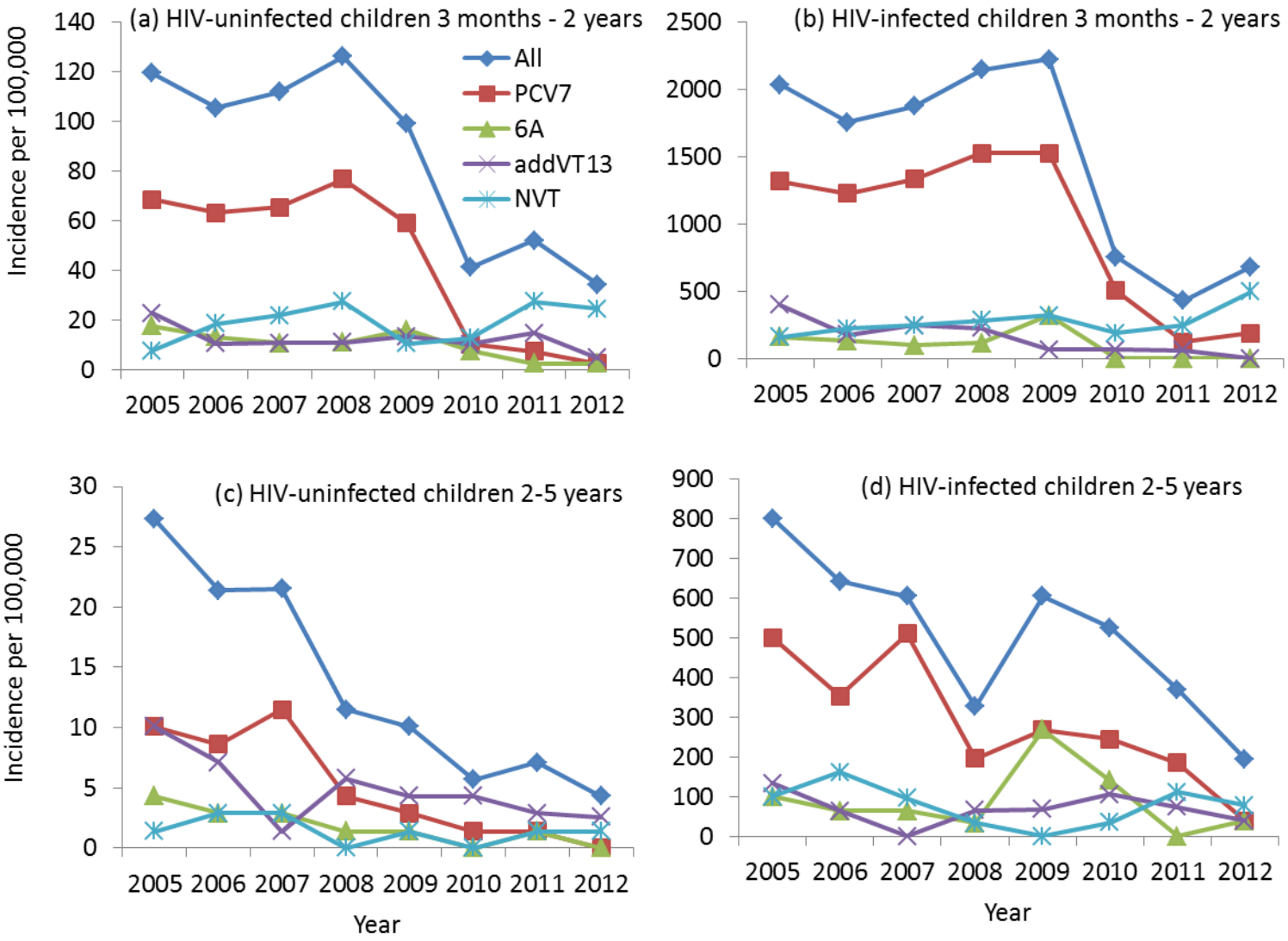


Figure 6.2: IPD trends in Soweto, South Africa, 2005-2012, A, Overall IPD by vaccine serotype, B by age group. Arrows indicate introduction of PCV7 in 2009 and PCV13 in 2011


(a) HIV-uninfected children 3 months - 2 years
Incidence per 100,000
All
PCV7
6A
addVT13
NVT
(b) HIV-infected children 3 months - 2 years
(c) HIV-uninfected children 2-5 years
(d) HIV-infected children 2-5 years
2005 2006 2007 2008 2009 2010 2011 2012
Year

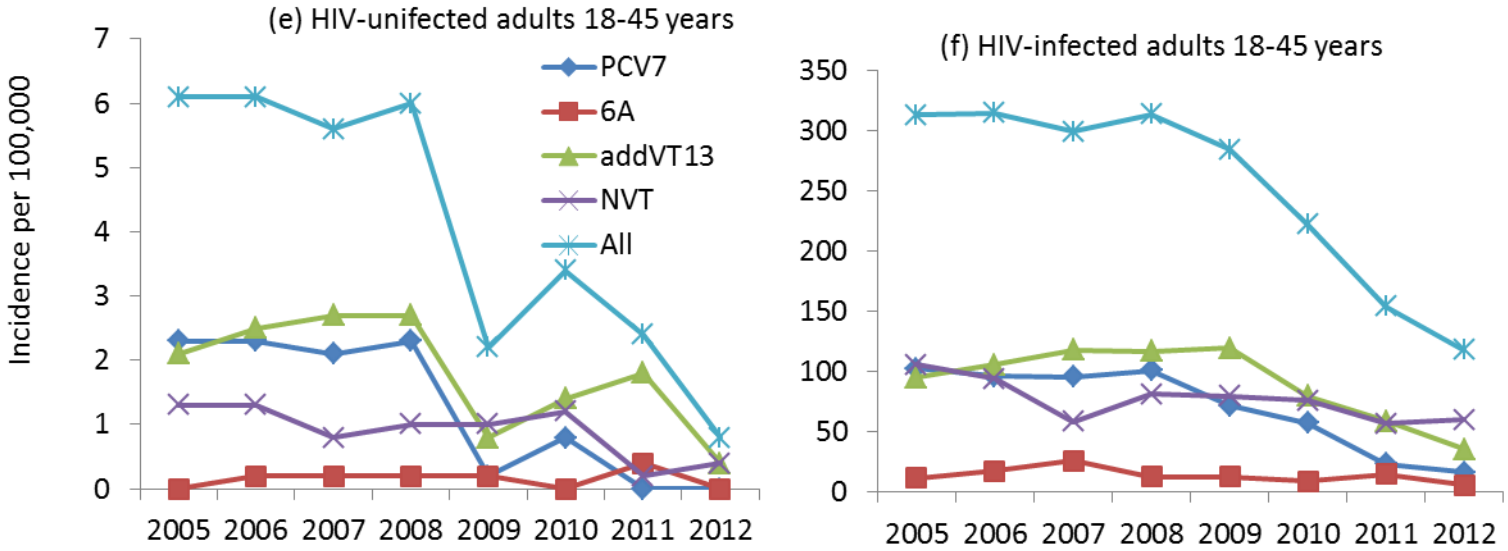
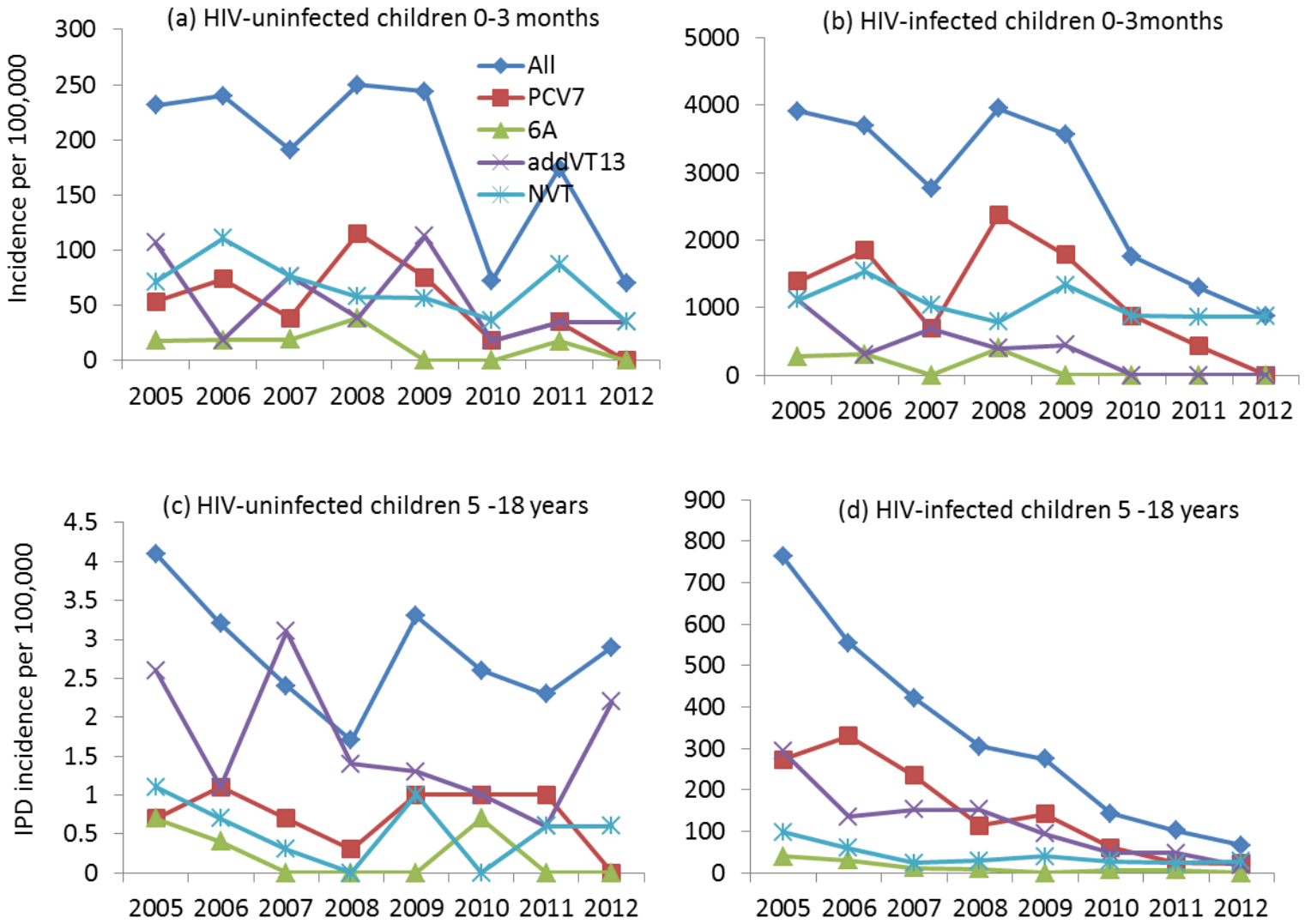


Figure 6.3 Invasive pneumococcal disease incidence in children >3 months to 5 years and adults aged >18 to 45 years by sero-group over time (2005-2012). A, HIV-uninfected children >3months to 2 years, B, HIV-infected children >3 months to 2 years, C.HIV –uninfected children >2 to 5 years, D, HIV-infected children >2 to 4 years, E, HIV-uninfected adults >18 to 45years, F. HIV-infected adults >18 to 45 years


(a) HIV-uninfected children 0-3 months
Incidence per 100,000
All
PCV7
6A
addVT13
NVT
(b) HIV-infected children 0-3months
(c) HIV-uninfected children 5 -18 years
IPD incidence per 100,000
(d) HIV-infected children 5 -18 years
2005 2006 2007 2008 2009 2010 2011 2012

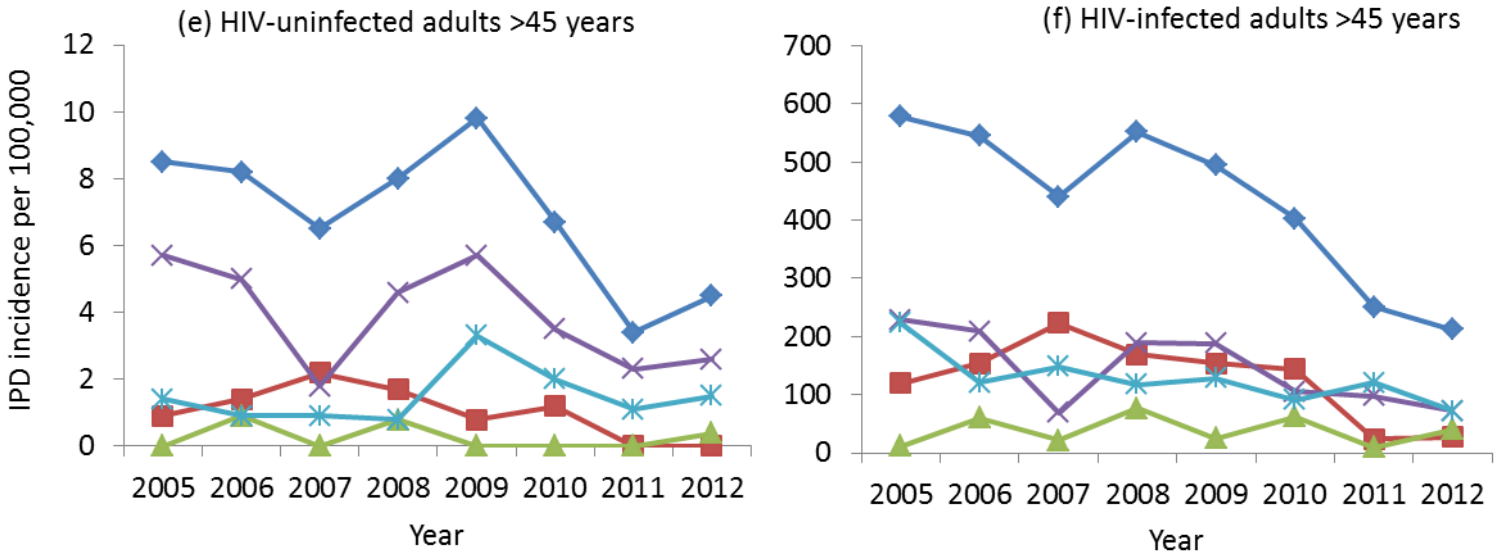


Figure 6.4 Invasive pneumococcal disease incidence in children 0 to 3 months, 5- 18 years and adults aged >45 years by sero-group over time (2005-2012). A, HIV-uninfected children 0 to 3months, B, HIV-infected children 0 to 3 months, C.HIV –uninfected children 5 to 18 years, D, HIV-infected children 5 to 18 years, E, HIV-uninfected adults >45years, F. HIV-infected adults >45 years

6.3.4 Changes in Invasive Pneumococcal Disease

In children >3-24 months of age, the annual overall incidence of IPD (per 100,000) in the pre-PCV era was 214.7, including an incidence of 137.3, 19.5 and 26.4, respectively for PCV7 serotype, serotype 6A and PCV13 additional serotypes (Table 6.2). By 2012 the overall IPD incidence in the >3-24 month age group had declined by 72.4% (95% CI; 58.5 to 82.4%). This included reductions in PCV7 serotype (-93.1%), serotype-6A (-87.9%) and PCV13 additional serotype IPD (-82.1%; $p<0.05$ for all observations); Table 6.2. No significant changes were observed in the incidence of non PCV13 serotype IPD between the pre-PCV era and 2012 (Table 6.2).

Table 6.2 Rate changes of invasive pneumococcal disease cases among children 3months to <2 years of age, and 2 to 5 years of age by year and serotype group, South Africa, 2010 and 2012 compared with baseline pre-PCV years 2005-2008.

	Number and rate (cases per 100,000)						Change in rate			
	2005-2008		2010		2012		2010 vs baseline		2012 vs baseline	
Serotypes	N	Rate[1]	n	Rate	n	Rate	Rate difference[2] (95% CI[3])	% change (95% CI)	Rate difference[2] (95% CI)	% change (95% CI)
3 months to <2 years of age										
All serotypes	341	214.7	28	69.6	25	59.3	-145.1 (-179.5 to -110.8)	-67.6 (-78.8 to -52.3)	-155.4 (-188.0 to -122.9)	-72.4 (-82.4 to -58.5)
PCV7 serotypes	218	137.3	12	29.8	4	9.5	-107.5 (-132.3 to -82.6)	-78.3 (-88.9 to -61.3)	-127.8 (-148.2 to -107.3)	-93.1 (-98.1 to -82.1)
6A	31	19.5	3	7.5	1	2.4	-12.1 (-22.9 to -1.2)	-61.8 (-92.5 to +22.4)	-17.2 (-25.4 to -8.9)	-87.9 (-99.7 to -27.1)
PCV13-additional serotypes	42	26.4	5	12.4	2	4.7	-14.0 (-27.5 to -0.05)	-53.0 (-85.5 to +18.6)	-21.7 (-32.1 to -11.4)	-82.1 (-97.9 to -31.1)
Non-PCV13 serotypes	50	31.5	8	19.8	18	42.7	-11.6 (-27.9 to +4.7)	-36.9 (-74.2 to +34.2)	+11.2 (-10.4 to +32.8)	+35.6 (-25.6 to +136.5)
HIV-uninfected										
All	174	115.8	16	41.4	14	34.5	-74.4 (-101.0 to -47.8)	-64.3 (-80.0 to -40.3)	-81.3 (-106.3 to -56.4)	-70.2 (-84.1 to -48.7)
PCV7 serotypes	103	68.6	4	10.3	1	2.5	-58.2 (-74.9 to -41.5)	-84.9 (-96.0 to -60.2)	-66.1 (-80.2 to -52.0)	-96.4 (-99.9 to -79.5)
6A	20	133.1	3	7.8	1	2.5	-5.6 (-16.1 to +5.0)	-41.7 (-88.9 to +96.5)	-10.9 (-18.4 to -3.3)	-81.5 (-99.6 to +15.7)
PCV13-additional	21	14.0	4	10.3	2	4.9	-3.6 (-15.4 to +0.8)	-26.0 (-81.5 to +119.4)	-9.1 (-18.1 to +0.02)	-64.8 (-96.0 to +44.2)

serotypes										
Non-PCV13 serotypes	28	18.6	5	12.9	10	24.6	-5.7 (-18.9 to +7.6)	-30.6 (-79.1 to +82.0)	+6.0 (-10.8 to +22.7)	+32.2 (-42.7 to +180.3)
HIV-infected										
All	167	1947.3	12	757.1	11	676.1	-1190.2 (-1710.5 to -669.9)	-61.1 (-80.3 to -30.3)	-1271.2 (-1768.1 to -774.4)	-64.9 (-82.2 to -35.6)
PCV7 serotypes	115	1341.0	8	504.7	3	184.4	-836.2 (-1263.3 to -409.1)	-62.4 (-84.1 to -23.3)	-1156.6 (-1478.4 to -834.7)	-86.1 (-97.2 to -58.4)
6A	11	128.3	0	-	0	0	-128.3 (-204.1 to -52.5)	-	-128.3 (-204.1 to -52.5)	-
PCV13-additional serotypes	23	268.2	1	63.1	0	0	-205.1 (-370.3 to -39.9)	-76.5 (-99.4 to +44.9)	-268.2 (-377.8 to -158.6)	-
Non-PCV13 serotypes	19	221.6	3	189.3	8	496.6	-322.7 (-268.5 to +203.9)	-14.6 (-83.8 to +190.2)	+270.2 (-84.8 to +625.1)	+124.1 (-15.1 to +436.2)
2-5yrs										
All serotypes	130	44.6	19	26.2	8	11.0	-18.4 (-32.5 to -4.3)	-41.2 (65.7to -4.4)	-33.6 (-44.4 to -22.8)	-75.3 (-89.6 to -49.9)
PCV7 serotypes	72	24.7	8	11.0	1	1.4	-13.7 (-23.2 to -4.1)	-55.3 (-81.4 to -7.1)	-23.3 (-29.7 to -17.0)	-94.4 (-99.9 to -67.9)
6A	16	5.5	4	5.5	1	1.4	+0.03 (-6.0 to +6.1)	+0.6 (-75.5 to +211.7)	-4.1 (-7.9 to -0.3)	-74.9 (-99.4 to +61.5)
PCV13-additional serotypes	25	8.6	6	8.2	3	4.1	-0.3 (-7.7 to +7.1)	-3.5 (-67.6 to +141.1)	-4.5 (-10.2 to +1.3)	-51.8 (-90.7 to +57.9)
Non-PCV13 serotypes	17	5.8	1	1.4	3	4.1	-4.5 (-8.3 to -0.6)	-76.3 (-99.4 to +51.1)	-1.7 (-7.1 to +3.7)	-29.2 (-86.7 to +145.0)
HIV-infected										

All serotypes	57	20.4	4	5.8	3	4.3	-14.7 (-22.4 to -6.9)	-71.9 (-92.6 to -23.9)	-16.1 (-23.3 to -9.0)	-79.0 (-95.8 to -35.5)
PCV7 serotypes	24	8.6	1	1.4	0	-	-7.2 (-11.6 to -2.7)	-83.3 (-99.6 to +2.5)	-8.6 (-12.0 to -5.2)	-
6A	8	2.9	0	-	0	-	-2.9 (-4.9 to -0.9)	-	-2.9 (-4.9 to -0.9)	-
PCV13-additional serotypes	17	6.1	3	4.3	2	2.9	-1.8 (-7.5 to +3.9)	-29.2 (-86.7 to +144.7)	-3.2 (-8.1 to +1.7)	-53.1 (-94.7 to +97.6)
Non-PCV13 serotypes	5	1.8	-	-	1	1.4	-1.8 (-3.4 to -0.2)	-	-0.4 (-0.4 to +2.9)	-20.3 (-98.3 to +612.4)
HIV-infected										
All serotypes	73	591.6	15	542.3	5	194.6	-67.3 (-365.4 to -230.7)	-11.4 (-52.8 to +55.9)	-397.0 (-615.0 to -179.0)	-67.1 (-89.6 to -19.7)
PCV7 serotypes	48	389.0	7	244.7	1	38.9	-144.3 (-356.4 to +67.7)	-37.1 (-75.0 to +39.8)	-350.1 (-484.0 to -216.2)	-90.0 (-99.8 to -41.5)
6A	8	64.8	4	139.8	1	38.9	+74.9 (-69.2 to +219.2)	+115.6 (-52.5 to +704.9)	-25.9 (-114.5 to +62.6)	-40.0 (-98.7 to +347.8)
PCV13-additional serotypes	8	64.8	3	104.9	1	38.9	+40.0 (-86.9 to +166.9)	+61.7 (-72.4 to +573.8)	-25.9 (-114.5 to +62.6)	-40.0 (-98.7 to +347.8)
Non-PCV13 serotypes	12	97.3	1	34.9	2	77.9	-62.3 (-150.2 to +25.6)	-64.1 (-99.2 to +142.9)	-19.4 (-140.5 to +101.7)	-20.0 (-91.3 to +259.6)

[1] average rate for period 2005-2008, [2] cases per 100,000 population; [3]CI, confidence interval;

In children >24-60 months age, the annual overall incidence (per 100,000) of IPD decreased by 75.3% (95%CI: 49.9% to 89.6%) from the pre-PCV era, (44.6) compared to 2012 (11.0). This included a decline in incidence of PCV7 serotype IPD from 24.7 to 11.0 by 2010 (-55.3%; 95%CI: -7.1% to -81.4%), which declined even further to 1.4 by 2012 (-94.4%; 95%CI: -67.9% to -99.9%). In contrast, IPD due to PCV13 additional and non-vaccine serotypes remained unchanged over this period.

Among HIV-uninfected children 3-24 months age, the incidence (per 100,000) of PCV7 serotype IPD compared to the pre-PCV era (68.6) decreased by 84.9% by 2010 (10.3; $p<0.001$) and by 96.4% in 2012 (2.5; $p<0.001$), Figure 6.3a and Table 6.2. Similarly, among HIV-uninfected children >24-60 months age, the incidence of PCV7 serotype IPD declined by 83.3% from 8.6 in the pre-PCV era to 1.4 by 2010 ($p=0.016$), whilst no PCV7 serotype cases were recorded in 2012 (100% reduction; $p<0.001$); Figure 6.3c and Table 2. The incidence of PCV13 additional and non-vaccine serotypes remained unchanged for both childhood age groups between the pre-PCV era up until 2012.

Among HIV-infected children >3-24 months age, the incidence of PCV7 serotype IPD declined by 62.4% from the pre-PCV7 era (1,341) compared to 2010 (504.7; $p=0.001$) and by 86.1% compared to 2012 (184.4; $p<0.001$), Figure 6.3b and Table 6.2. Furthermore, the incidence of PCV13 additional serotypes declined from 268.2 in the pre-PCV era to 63.1, by 2010 ($p=0.05$); whilst no cases were observed in 2012 (100% reduction; $p=0.009$). The incidence of non-vaccine serotype IPD remained unchanged among the >3-24 month age-group of HIV-infected between the pre-PCV era and 2012.

Among HIV-infected children >24-60 months age, the incidence of PCV7 serotype IPD trended to decline from 389.0 in the pre-PCV era to 244.7 by 2010 (-37.1%; p=0.124), and 38.9 in 2012 (-90%; p=0.061); Figure 6.3d and Table 6.2. Similar to HIV-uninfected children, the incidence of PCV13 additional serotypes and non-vaccine serotype IPD was unchanged between the pre-PCV era until 2012.

Among adults 18-45 years of age, overall serotype, PCV7 serotype, PCV13 additional serotypes and non PCV13 serotype IPD incidence (per 100,000) were 57.5, 18.6, 20.6 and 15.3, respectively in the pre-PCV era; Table 6.3. By 2010, we observed reductions in the incidence of overall serotype (-29.3%; p<0.001), PCV7 serotype (-44.1%; p<0.001) and PCV13 serotype IPD (-28.2%; p=0.002). Non-vaccine serotype IPD was unchanged in 2010 (-8.7%; p=0.23). By 2012, further declines were observed in overall IPD (-64.4%: p<0.001), PCV7 serotype (-84.9%; p<0.001), and PCV13 additional serotype IPD (-69.5%; p<0.001. Furthermore, we also observed a reduction in incidence of non PCV13 of -31.9% between the pre-PCV era (15.3) and 2012 (10.4; p=0.002).

Table 6.3 Rate changes of invasive pneumococcal disease cases among adults 18 to 45 years of age, by year and serotype group, South Africa, 2010 and 2012 compared with baseline pre-PCV years 2005-2008.

	Number and rate (cases per 100,000)						Change in rate			
	2005-2008		2010		2012		2010 vs baseline		2012 vs baseline	
Serotypes	N	Rate	n	Rate	n	Rate	Rate difference (95% CI)	% change (95% CI)	Rate difference# (95% CI)	% change (95% CI)
All serotypes	1330	57.5	242	40.6	124	20.5	-16.9 (-22.9 to -10.9)	-29.3 (-38.6 to -18.9)	-37.0 (-41.8 to -32.3)	-64.4 (-70.4 to -57.2)
PCV7 serotypes	431	18.6	62	10.4	17	2.8	-8.2 (-11.4 to -5.1)	-44.1 (-57.9 to -26.9)	-15.8 (-18.0 to -13.6)	-84.9 (-91.3 to -75.6)
6A	70	3.0	9	1.5	6	1.0	-1.5 (-2.7 to -0.3)	-50.1 (078.1 to +0.3)	-2.0 (-3.1 to -0.9)	-67.3 (-88.4 to -25.1)
PCV13-additional serotypes	476	20.6	88	14.8	38	6.3	-5.8 (-9.4 to -2.2)	-28.2 (-43.5 to -9.7)	-14.3 (-17.0 to -11.6)	-69.5 (-78.7 to -57.6)
Non-PCV13 serotypes	353	15.3	83	13.9	63	10.4	-1.3 (-4.7 to +2.1)	-8.7 (-28.9 to +16.3)	-4.9 (-7.9 to -1.8)	-31.9 (-48.7 to -10.7)
HIV-uninfected										
All serotypes	114	5.9	17	3.4	4	0.8	-2.5 (-4.5 to -0.5)	-42.1 (-67.4 to -3.1)	-5.1 (-6.5 to -3.8)	-86.5 (-96.4 to -64.6)
PCV7 serotypes	43	2.2	4	0.6	0	-	-1.4 (-2.5 to -0.03)	-63.9 (-90.6 to -0.5)	-2.2 (-2.9 to -1.6)	-
6A	3	0.2	0	-	0	-	-0.2 (-0.3 to +0.2)	-	-0.2 (-0.3 to +0.02)	-
PCV13-additional serotypes	48	2.5	7	1.4	2	0.4	-1.1 (-2.3 to +0.2)	-43.3 (-78.4 to +25.9)	-2.1 (-3.0 to -1.2)	-84.0 (-98.1 to -39.0)

Non-PCV 13 serotypes	21	1.1	6	1.2	2	0.4	+0.1 (-0.9 to +1.2)	+11.0 (-63.3 to +184.4)	-0.7 (-1.4 to +0.03)	-63.5 (-95.9 to +49.5)
HIV-infected										
All serotypes	1216	310.2	225	222.3	120	117.9	-87.9 (-121.8 to -54.0)	-28.3 (-38.1 to -17.3)	-192.3 (-219.7 to -164.9)	-62.0 (-68.8 to -54.1)
PCV7 serotypes	388	99.0	58	57.3	17	16.7	-41.7 (-59.4 to -23.9)	-42.1 (-56.9 to -23.6)	-82.3 (-94.9 to -69.6)	-83.1 (-90.3 to -72.6)
6A	67	17.1	9	8.9	6	5.9	-8.2 (-15.3 to -1.1)	-47.9 (-77.2 to +4.8)	-11.2 (-17.4 to -5.0)	-65.5 (-87.8 to -20.9)
PCV13-additional serotypes	428	109.2	81	80.0	36	35.4	-29.2 (-49.4 to -8.9)	-26.7 (-42.9 to -6.9)	-73.8 (-89.3 to -58.3)	-67.6 (-77.6 to -54.4)
Non-PCV13 serotypes	332	84.7	77	76.1	61	59.9	-8.7 (-27.9 to +10.7)	-10.2 (-30.8 to +15.4)	-24.8 (-42.3 to -7.2)	-29.2 (-47.1 to -6.8)

Among HIV-uninfected adults, the incidence (per 100,000) of overall serotype, PCV7, PCV13 additional and non PCV13 serotype IPD were 114, 43, 48 and 21, respectively in the pre-PCV era; Figure 6.3e and Table 6.3. The incidence of PCV7 serotype IPD subsequently declined by 63.9% in 2010 (p=0.016), whilst no PCV7 cases were observed in 2012 (p<0.001). Similar decline (-84.0%) were observed in incidence of PCV13-additional IPD (p=0.001); whilst non-vaccine serotypes were not significantly different (1.1 vs 0.4; p=0.076) between the pre-PCV era and 2012.

Among HIV-infected adults, the incidence (per 100,000) of overall, PCV7, PCV13 additional and non PCV13 serotype IPD were 1 216, 388, 428 and 332, respectively in the pre-PCV era; Figure 6.3f and Table 6.3. By 2010, the incidence of PCV7 serotype IPD had declined by 42.1% (p<0.001); and further by 83.1% (p<0.001) in 2012. Similarly, we observed a 26.7% (p=0.004) reduction in PCV13additional serotypes between the pre-PCV era and 2010 (109.2 vs. 80 cases); and a further decline by 2012 (-67.6%; p<0.001); Figure 6.3f and Table 6.3. A more modest decline in incidence of non-vaccine serotype IPD from 84.7 in the pre-PCV era to 59.9 in 2012 (-29.2%; p=0.005) was also observed.

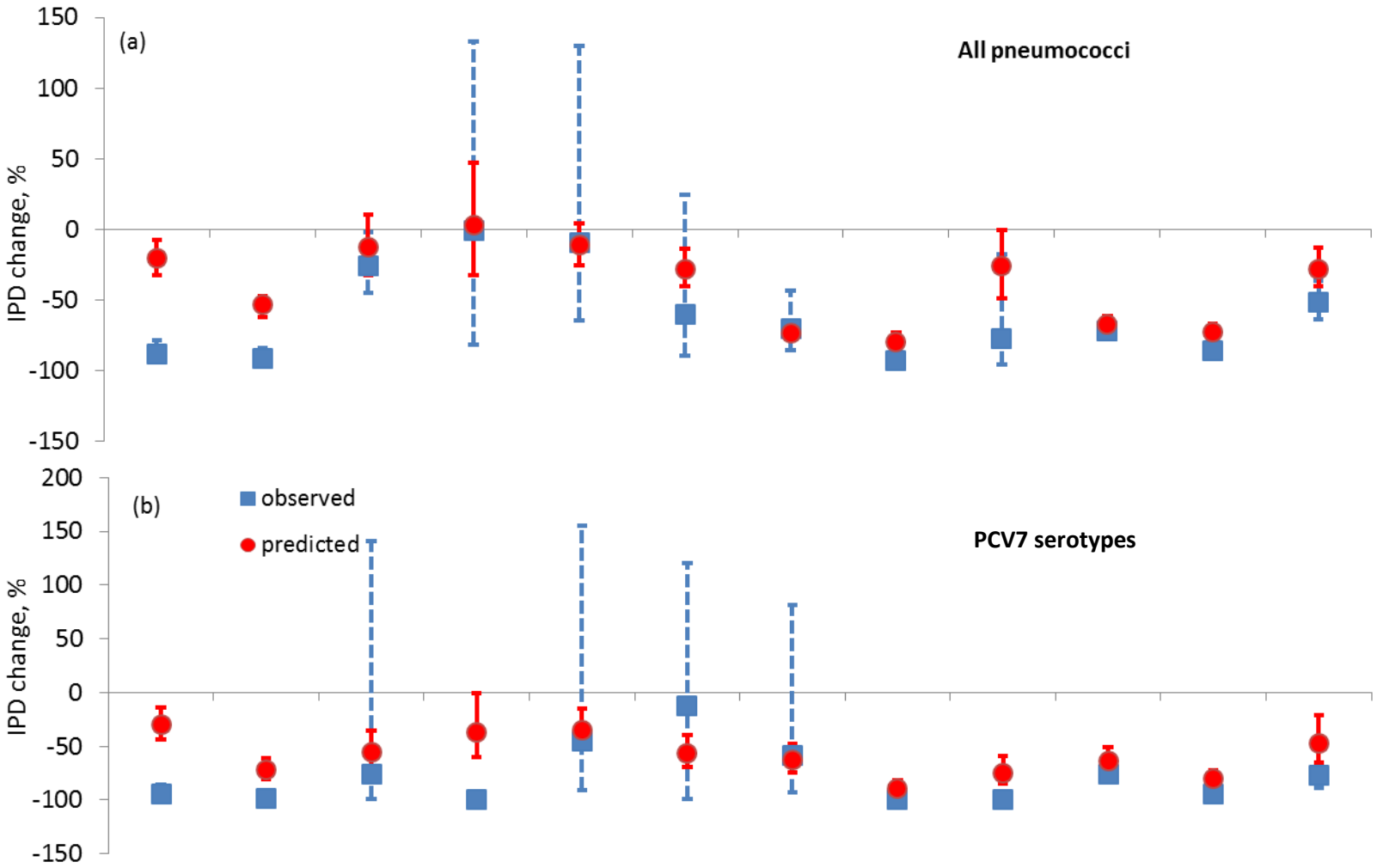
Among HIV-infected adults 18-45 years age, the incidence of overall serotype, PCV7 serotype, PCV13 additional serotypes and non PCV13 serotype IPD incidence (per 100,000) were 1216, 388, 428 and 332, respectively in the pre-PCV era; Figure 6.3f and Table 6.3. By 2010, the incidence of PCV7 serotype IPD had declined by 42.1% (p<0.001) and further by 83.1% (p<0.001). Similarly, we observed a 26.7% reduction in PCV13 additional serotypes between the pre-PCV era and 2010 (incidence 109.2 vs. 80; p=0.004); and a further decline by 2012 (-67.6%; p<0.001); Figure 6.3f and Table 3. A more modest decline in incidence of

non-vaccine serotype IPD from 84.7 in the pre-PCV era to 59.9 in 2012 (29.2%; p=0.005) was also observed.

IPD trends in women aged 18-45 years are presented in Section 6.3.3, Tables 6.4 and 6.5.

6.3.5 Evaluation of model using temporal nasopharyngeal colonization to Predict IPD incidence

Using a model proposed by Weinberger et al., we simulated the expected incidence of IPD for overall serotypes, PCV7 serotype, PCV13 additional serotype and non-vaccine serotype; based on the observed prevalence of pneumococcal colonization for these serotype groups in the pre-PCV era and then in 2010 and 2012. This was undertaken for HIV-uninfected children aged >3-24 months and for HIV-infected and HIV-uninfected women 18-45 years age in whom pre-PCV era and PCV era colonization data were available. Also, we compared for HIV-infected children aged >3-24 months, and for both HIV groups among children age >24-60 months and women 18-45 years, the predicted incidence of IPD using colonization prevalence in 2010 (PCV7 era) compared to 2012 (PCV13 era). The results of the model are shown in Figures 6.5 and 6.6 and Tables 6.4 and 6.5.


(a)
All pneumococci
IPD change, %
150
100
50
0
-50
-100
-150
(b)
observed
predicted
PCV7 serotypes
IPD change, %
200
150
100
50
0
-50
-100
-150

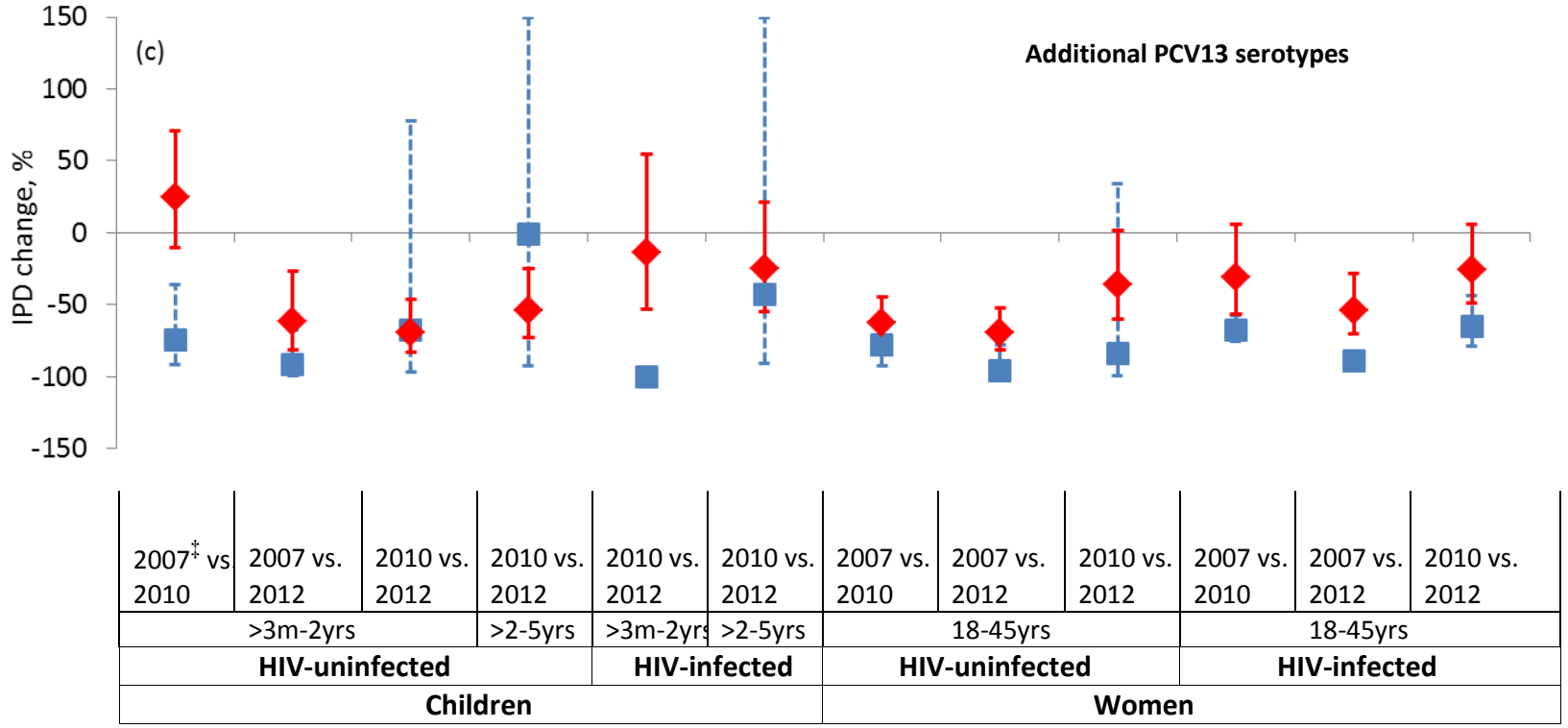
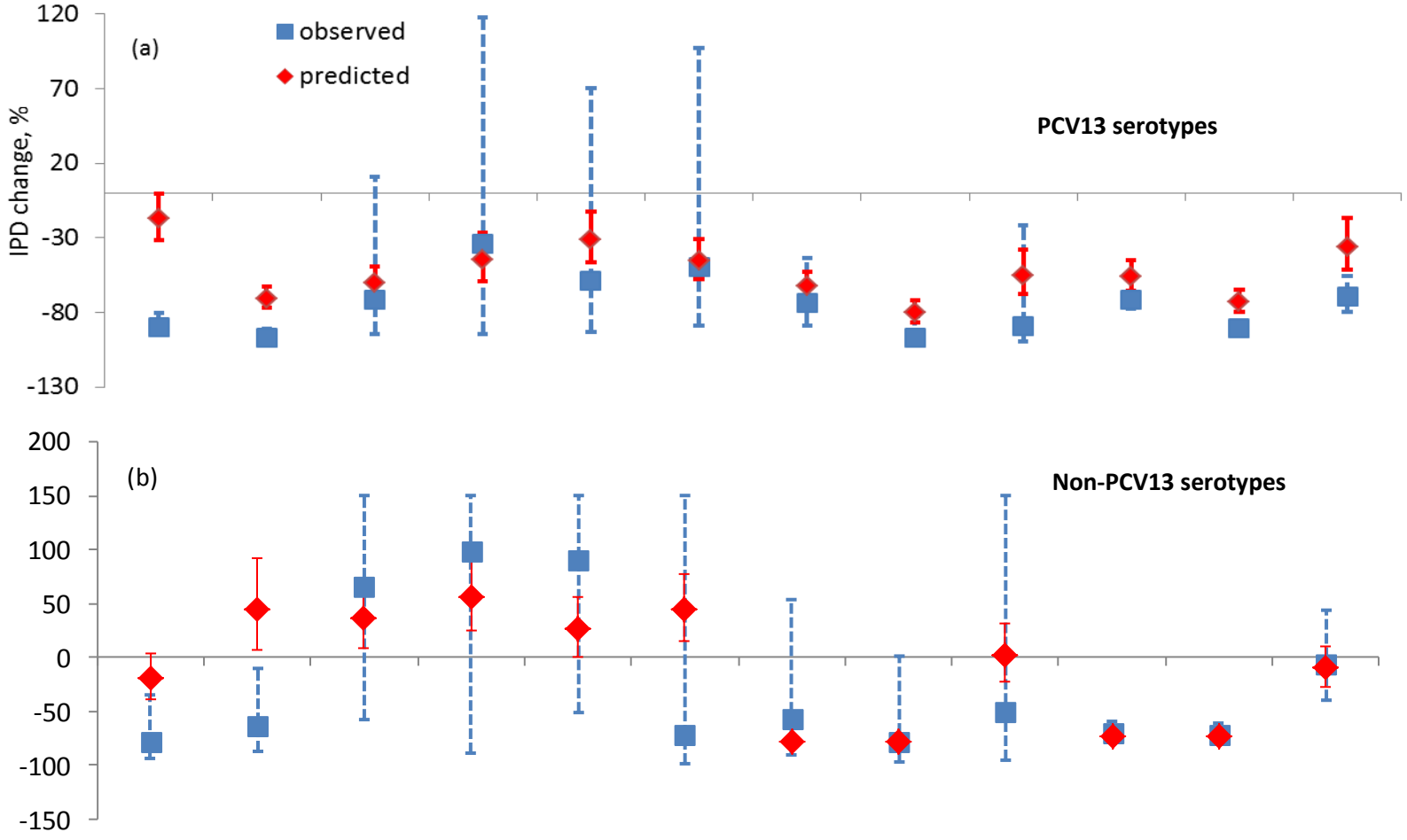


Figure 6.5 Comparison of observed and model-predicted IPD changes in children >3mths-2 years, >2-5 years old and women 18-45 years old during pre- and post-vaccination periods for all pneumococcal, PCV7 and additional PCV13 IPD



2007‡ vs. 2010	2007 vs. 2012	2010 vs. 2012	2010 vs. 2012	2010 vs. 2012	2010 vs. 2012	2007 vs. 2010	2005 vs. 2012	2010 vs. 2012	2007 vs. 2010	2007 vs. 2012	2010 vs. 2012
>3m-2yrs			>2-5yrs	>3m-2yrs	>2-5yrs	18-45yrs			18-45yrs		

HIV-uninfected	HIV-infected	HIV-uninfected	HIV-infected
Children		Women	

Figure 6.6 Comparison of observed and model-predicted IPD changes in children >3mths-2 years, >2-5 years old and women 18-45 years old during pre- and post-vaccination periods for PCV13 and non-vaccine serotype IPD

Table 6.4 Observed and model-predicted changes in overall incidence of invasive pneumococcal disease and disease due to vaccine serotypes

		All pneumococcal disease		PCV7 serotype disease		Additional PCV13 serotype disease	
Group	**Years compared**	**Observed IPD**	**Predicted IPD**	**Observed IPD**	**Predicted IPD**	**Observed IPD**	**Predicted IPD**
		% change (95% CI)	% change (95% CI)	% change (95% CI)	% change (95% CI)	% change (95% CI)	% change (95% CI)
Children							
HIV- (>3mnths-2yrs)	Pre-PCV era[1] vs 2010[2]	-87.9	-20.2	-94.5	-29.6	-74.7	-24.8
		(-93.6, -78.8)	(-32.1, -7.64)	(-98.5, -85.4)	(-43.3, -13.5)	(-91.6, -36.0)	(-10.5, +71.2)
	Pre-PCV era vs 2012[3]	-91.7	-53.5	-98.7	-72.1	-91.9	-62.2
		(-96.1, -84.2)	(-62.2, -42.7)	(-100, -92.5)	(-80.1, -61.4)	(-99.1, -67.4)	(-81.1, -27.1)
	2010 vs 2012	-26.2	-12.9	-76.2	-55.3	-68.2	--69.3
		(-44.6, -2.27)	(-32.2, 10.2)	(-99.5, 140.8)	(-69.5, -35.8)	(-96.9, +77.7)	(-82.8, -46.7)
(>2-5 years)	2010 vs 2012	-0.71	3.39	-100.0	-36.8	-0.71	-54.2
		(-81.5, 133.1)	(-32.1, 46.9)		(-60.3, -0.37)	(-92.8, +1270)	(-72.7, -25.2)
HIV+ (>3mnths-2yrs)	2010 vs 2012	-9.11	-11.4	-45.5	-35.1	-100.0	-14.0
		(-64.8, 129.5)	(-25.6, 4.31)	(-91.2, 155.4)	(-51.3, -14.7)	-	(-53.6, +54.6)
(>2-5 years)	2010 vs 2012	-60.1	-28.3	-12.3	-56.1	-43.0	-25.4
		(-89.1, 24.4)	(-40.6, -14.1)	(-98.9, 120.0)	(-68.8, -39.2)	(-90.8, +167)	(-55.3, +21.4)
Mothers (18-45yrs)							
HIV-	Pre-PCV era vs 2010	-70.1	-73.2	-58.5	-63.0	-77.7	-62.4
		(-85.3, -43.1)	(-77.8, -68.1)	(-93.1, 81.6)	(-74.2, -47.7)	(-92.5, -44.5)	(-75.1, -44.3)
	Pre-PCV era vs 2012	-93.1	-79.8	-100.0	-88.8	-96.4	-69.9
		(-98.6, -78.4)	(-84.5, -73.3)		(-93.6, -81.1)	(-99.9, -77.9)	(-81.6, -52.0)
	2010 vs 2012	-77.4	-25.8	-100.0	-74.9	-83.7	-35.7
		(-95.9, -17.9)	(-49.2, -0.16)		(-84.9, -59.2)	(-99.6, +34.2)	(-60.1, +1.50)
HIV+	Pre-PCV era vs 2010	-71.5	-66.9	-76.3	-63.4	-67.9	-31.4
		(-76.2, -66.0)	(-72.1, -61.0)	(-83.2, -67.2)	(-73.0, -51.0)	(-75.9, -57.8)	(-56.5, +5.7)
	Pre-PCV era vs 2012	-86.2	-73.0	-94.5	-80.0	-88.8	-53.7
		(-89.3, -82.6)	(-78.0, -66.4)	(-97.3, -89.9)	(-85.8, -72.3)	(-92.9, -83.0)	(-70.7, -28.6)
	2010 vs 2012	-51.7	-27.9	-76.6	-47.2	-65.1	-25.9
		(-63.6, -36.5)	(-40.5, -13.1)	(-89.1, -54.2)	(-65.1, -21.6)	(-78.8, -44.2)	(-49.0, +5.8)

n/a –there were no PCV7 serotypes detected in HIV-uninfected women during 2012

[1]Pre-PCV era, 2005 to 2008, [2]2010 as PCV7 era, [3]2012 as PCV13 era

Table 6.5 Observed and model-predicted changes in non-vaccine serotype and additional PCV13 serotypes IPD

		Non-vaccine serotype disease		PCV13 serotype disease	
Group	**Years compared**	**Observed IPD**	**Predicted IPD**	**Observed IPD**	**Predicted IPD**
		% change (95% CI)	% change (95% CI)	% change (95% CI)	% change (95% CI)
Children					
HIV- (>3mnths-2yrs)	Pre-PCV era[1] vs 2010[2]	-78.4 (-94.7, -34.5)	-19.9 (-39.5, +4.5)	-89.7 (-95.2, -80.1)	-17.2 (-31.5, -0.75)
	Pre-PCV era vs 2012[3]	-64.0 (-87.3, -9.6)	+44.9 (+7.7, +92.0)	-97.0 (-99.4, -91.1)	-70.7 (-77.1, -62.9)
	2010 vs 2012	+66.8 (-57.6, +677)	+36.4 (+9.4, +68.3)	-71.4 (-94.9, 11.1)	-60.1 (-68.9, -49.5)
(>2-5 years)	2010 vs 2012	+98.6 (-89.7, +11616)	+56.3 (+25.7, +91.8)	-33.8 (-94.5, 117.8)	-44.8 (-59.0, -26.9)
HIV+ (>3mnths-2yrs)	2010 vs 2012	+90.9 (-51.5, +789)	+26.2 (+0.4, +56.8)	-59.1 (-93.0, 70.4)	-30.9 (-46.3, -12.3)
(>2-5 years)	2010 vs 2012	-71.5 (-99.4, +188.1)	+44.4 (+15.8, +77.9)	-49.9 (-89.2, 97.1)	-45.7 (-57.9, -30.8)
Mothers (18-45yrs)					
HIV-	Pre-PCV era vs 2010	-57.0 (-90.3, +54.1)	-78.2 (-82.4, -73.4)	-73.6 (-88.9, -43.6)	-62.7 (-71.1, -52.5)
	Pre-PCV era vs 2012	-79.0 (-97.8, +1.50)	-78.8 (-83.6, -72.9)	-97.1 (-99.9, -82.5)	-80.3 (-86.6, -71.6)
	2010 vs 2012	-51.1 (-95.6, +240)	+1.7 (-22.2, +31.4)	-89.1 (-99.8, -21.6)	-55.0 (-67.7, -38.3)
HIV+	Pre-PCV era vs 2010	-70.5 (-79.3, -58.8)	-73.8 (-78.9, -67.9)	-71.9 (-77.3, -65.3)	-56.3 (-65.8, -44.8)
	Pre-PCV era vs 2012	-72.5 (-80.8, -61.2)	-72.6 (-78.2, -66.0)	-90.8 (-93.5, -87.1)	-73.1 (-79.7, -64.8)
	2010 vs 2012	-6.5 (-39.6, +44.5)	-10.2 (-27.1, +9.6)	-69.7 (-79.7, -55.6)	-35.9 (-51.3, -16.8)

[1]Pre-PCV era, 2005 to 2008; [2]2010, PCV7 era; [3]2012, PCV13 era

Among HIV-uninfected children 3-24 months age, the model underestimated the decline in overall-serotype IPD between the pre-PCV7 era and 2012 (observed- 91.7%; 95% CI:-96.1 to -84.2 vs. predicted -53.5%; 95%CI:-62.2 to -42,7), and similarly for PCV7 serotype (observed -98.7%; 95%CI:-100 to -92 vs. predicted -72.1%; 95%CI: -80.1 to -61.4). The observed decline between the pre-PCV era and 2012 in PCV13 additional serotype IPD was -91.9% (95%CI:-99.1 to -67.4), which although higher than the predicted point estimate 9-62.2%; 95%CI: --81.1 to -27.1) there was overlap of the 95% confidence(Figure 6.5a and Table 6.4). For non-vaccine serotype IPD, we observed a decrease of 64.0% (95% CI:-87.3 to -9.6) whereas the model predicted an increase of 44.9% (95%CI: 7.7 to 92.0); Figure 6.6b and Table 6.5. A similar trend was observed when comparing the pre-PCV era to 2010. The

model's predicted compared to observed changes in IPD incidence was, however, more comparable when limited to comparing changes in prevalence of colonization between 2010 and 2012 only, for all serotypes (observed:-26.2%, 95%CI: -44.6 to -2.27 vs. predicted -12.9%, 95%CI: -32.2 to 10.2), PCV7 serotypes (observed: -76.2%, 95%CI:-99.5 to 140.8 vs. predicted -55.3%, 95% CI: -69.5 to -35.8), and PCV13 additional serotypes (observed: -68.2%, 95%CI: -96.9 to +77.7 vs. predicted of -69.3%, 95% CI: -82.8 to -46.7).

In older unvaccinated HIV-uninfected children aged >24-60months, comparing the pre-PCV era to 2012 the model underestimated the decline in PCV7 serotype disease (observed: -100% vs predicted -36.8%, 95% CI: -60.3 to -0.37). The wide confidence interval for PCV13 additional and non-vaccine serotypes did not allow for further comparison of the observed and predicted changes in this in this age-group.

In HIV-infected children, in whom pre-PCV colonization data were unavailable, between 2010 and 2012 in children aged >3-24 months, the point-estimate of the observed (-9.11%, 95%CI:-64.8 to 129. 5) and predicted (11.4%, 95% CI; -25.6 to 4.31) decrease in overall IPD were similar, albeit not significant for either. Similar point estimates were also observed for PCV7 serotype IPD, observed (-45.5%, 95%CI: -91.2 to +155.4) and predicted (-35.1, 95%CI: -51.3 to -14.7). There was, however, a 100% decline (in PCV13 additional serotype IPD between 2010 and 2012, whereas the model prediction was a non-significant decline of 14.0% (95%CI: -53.6 to +54.6). The predicted point estimate for non PCV13 serotypes (+26.2%, 95% CI +0.4 to +56.8) was lower than the observed (+90.9%, 95% CI: -51.5 to +789).

Among older HIV-infected children (>24 to 60 months) the predicted point estimate for decrease in incidence of overall serotype IPD (-28.3%, 95%CI: -40.6% to -14.1%) was lower than the observed decline (-60.1%, 95%CI: -89.1% to +24.4%). There was overlap of the 95% CI between the observed (-43.0%, 95%CI: -90.8 to +167) and predicted decline in PCV13 additional serotype IPD (-25.4%, 95% CI:-55.3, to +21.4).

Among HIV-uninfected women aged 18-45 years comparing the pre-PCV era and 2012, the observed reduction in overall serotype IPD (-93.1%, 95% CI: -98.6 to -78.4) was similar to the model predicted estimate (-79.8%, 95% CI: -84.5 to -73.3). Similarly, the observed and model-predicted reductions were similar for PCV7 serotype IPD (-100% vs -88.8%, 95% CI: 93.6, -81.1), PCV13 additional serotypes (-96.4%, 95% CI: -99.9 to -77.9 vs -69.9%, 95% CI: -81.6 to -52.0), and non-vaccine serotype IPD (-79.0%, 95% CI: -97.8, +1.50 vs -78.8, 95% CI: -83.6 to -72.9). Similar synergies in the observed and predicted estimates were observed when comparing the pre-PCV era to 2010.

Similarly, among HIV-infected women when comparing the pre-PCV era to 2012, the observed estimates closely mirrored the model-predicted reductions in IPD for all serotypes (-86.2% [95% CI: -89.3 to -82.6] vs -73.0 [95% CI: -78.0 to -66.4]), PCV7 serotype (-94.5% [95% CI: -97.3 to -89.9] vs -80.0% [95% CI: -85.8 to -72.3]) and non-vaccine serotypes (-72.5% [95% CI: -80.8 to -61.2] vs -72.6% {95% CI: -78.2 to -66.0]). The model predicted estimates in reduction for PCV13 additional serotype (-31.4%, 95% CI: -56.5, +5.7) was, however, lower compared to observed reduction (-67.9%, 95% CI: -75.9 to -57.8).The model predicted and observed changes in IPD data between 2010 and 2012 were comparable, for all serotypes, PCV7 serotypes, PCV13 additional serotypes and non PCV13 serotypes.

6.4 Discussion

Our study shows that the introduction of PCV into the South African childhood national immunization program resulted in reduction of vaccine serotype IPD among all ages. These changes were mirrored by a decline in colonization of vaccine serotypes temporally associated with childhood PCV immunization, including among HIV-infected and HIV-uninfected PCV-unvaccinated older children and women as discussed in Chapter 4 (179). In this analysis, we expand on the latter observations and illustrate that serial studies on NP vaccine serotype colonization can be used to predict post-PCV changes in the incidence of IPD among children and women, particularly in age groups not targeted for vaccination.

The effectiveness of a 2+1 schedule on IPD in South Africa has been previously evaluated in a case control study in South Africa, (180) in which two or more doses of PCV7 were shown to be 74% effective in reducing IPD among HIV-uninfected, whereas no significant reduction was observed in HIV-infected children [12% (95% CI, −449% to 77%)]. Similarly three or more doses of PCV7 conferred 90% protection against vaccine serotype IPD among HIV-uninfected; whereas the reduction in HIV-infected children was not significant (-57%; 95% CI: -96 to 371) (180). We, however, observed significant decreases in IPD among HIV-infected children during the study period similar to the observation at national level (112). In this study non-vaccine serotype IPD was unchanged among HIV-infected children although national data observed a decrease that was alluded to the increase in anti-retroviral therapy usage among children (112).

Childhood PCV immunization in South Africa reduced the burden of IPD among older children and women/adults through reduced transmission of vaccine serotype pneumococci (or the commonly isolated serotypes in both carriage and IPD). In our population the decrease

in IPD among individuals 19-45 years of age, represents a significant public health impact of infant PCV vaccination as this group has the highest HIV prevalence. The reduction in vaccine serotype disease among HIV-uninfected unvaccinated groups can be attributed mainly to indirect effects, although this may also result from secular trends. However, among HIV-infected individuals in addition to the effect of PCV, increased availability of ART also resulted in a reduction in IPD including non-vaccine serotype disease (112). The indirect protection against IPD observed in our study, is similar to observations in the USA, where indirect protection was observed following implementation of the childhood PCV7 immunization program (10, 11). In the USA across all age groups between 1998/99 (pre-PCV era) and 2003 there was a 75% decline in IPD due to any serotype and 94% decrease in IPD caused by PCV7 serotypes, including among individuals greater than 65 years supporting an indirect effect on vaccination (10, 11, 110).

In children, we observed substantial replacement of non-vaccine serotype colonization, but no concomitant increase in IPD due to these non-vaccine serotypes. Similarly,in the UK despite an increase in non-vaccine serotype carriage there was a net reduction in IPD and this was attributed to smaller case carrier ratios (i.e., low invasiveness) of these serotypes (59). In the USA, the incidence of IPD due to non-vaccine serotypes , in particular 19A, increased by 58% among children aged less than 2 years and by 135% in children aged 2 to 4 years in the PCV era compared to the pre vaccine introduction period (114). This effect was manifest as early as three years post PCV introduction (114, 115). Similarly in the UK, serotype 19A increased significantly following PCV7 vaccination of infants (59). However, serotype 19A increases have also been described in unvaccinated communities (117). In our population the transitioning from PCV7 to PCV13 within a short period of time may have prevented the

emergence of increase in any of the additional serotypes (including 19A) which are included in PCV13.

An important component of our study was to determine whether serial studies on nasopharyngeal colonization, which is easier and cheaper to undertake than surveillance for IPD, could be used to impute the magnitude of changes in IPD following introduction of childhood PCV immunization. In this regard, we observed a strong association in temporal decline of colonization by vaccine serotype and IPD among age-groups that were not targeted for PCV vaccination, when comparing the pre PCV era to the PCV era. The strength of this association was, however, less evident in young children 3-24 months of age, in whom the reduction in IPD exceeded the percentage change in colonization by the vaccine serotypes. Consequently, using the serial colonization studies and pre PCV era IPD incidence, we observed that the magnitude of a model-predicted reduction in vaccine serotype IPD was similar to the observed reduction of vaccine serotype IPD for HIV-infected and HIV-uninfected adults and children outside the age group that were targeted for PCV immunization.

We found similarity in the model predicted and observed IPD reductions in vaccine serotype IPD among women 18-45 years old, regardless of HIV-status. This is despite HIV-infected adults having higher prevalence of pneumococcal, including vaccine serotype, colonization; (47, 153, 159, 160, 181) and a higher incidence of IPD (112). This reflects that the risk of IPD is inherently associated with colonization, hence among adults in whom there is generally a low-modest prevalence of pneumococcal colonization, serial surveillance of changes in colonization can be used to determine whether an indirect effect against IPD is materialising in the community. Also, among children aged 2 to 5 years in whom we did not

have pre PCV era data, the model also performed well for vaccine serotypes regardless of HIV status when comparing early and post-vaccination years. Estimates for non-vaccine serotypes were, however, less accurate for both HIV-infected and HIV-uninfected unvaccinated individuals.

The model predicted changes in vaccine serotype IPD was, however, less well aligned to the observed reductions in the age group (>3 to 24 months) who were targeted for PCV immunization. This is likely because the model does not account for the direct protection against progression to IPD which vaccinated children derive through humoral immunity induced by vaccination, (122) over and above any further reduced risk secondary to lesser community wide transmission of the vaccine serotypes. The model predicted reduction in PCV serotypes among this young age group (<3 to 24 months) was, however, somewhat closer to the observed reduction in HIV-infected than HIV-uninfected children. This could be because of the poorer immunogenicity and lesser direct protection through PCV immunization in HIV-infected children; as had been alluded to in the case control study and the previous 9 valent PCV efficacy trial in South Africa (54, 180).

Although the theoretical model predicted reductions that were similar to the observed reductions in women, regardless of HIV-status, significant changes in anti-retroviral treatment might alter the susceptibility of HIV-infected individuals to IPD and this may render the modelling results less accurate (122). However, a previous study from our setting showed no changes in the incidence of IPD in HIV-infected individuals with increase in ART coverage (154). Also, the additional serotypes included in PCV13, including 1 and 5 which account for approximately 20% of pneumococcal disease in African children, are infrequently identified in healthy individuals and have much greater invasive potential (36, 50). The

model, however, assumes equal reduction in carriage and IPD, irrespective of the serotype, and hence may be less accurate in identifying changes in disease due to these serotypes which are infrequently identified among healthy individuals. Measuring colonization at the time of IPD (16) may improve the accuracy of estimates including among vaccinated children. Also, the smaller sample size for our data compared to national estimates may have impacted on accuracy of estimates (122). Despite, these shortfalls, our analysis indicate that the introduction of PCV resulted in a marked reduction in both vaccine serotype carriage prevalence in both vaccinated and non vaccinated groups and that any significant decreases in carriage resulted in proportional declines in vaccine serotype IPD.

A limitation of our study is that we did not have carriage data for HIV-infected children prior to the introduction of PCV in the national immunization program, so some analysis was limited to HIV-uninfected children. This may, however, become less important in settings such as ours where mother to child HIV transmission rate is now <2% (128), and will continue to fall with improvement in HIV care and treatment.

In summary, our study show that PCV use in South Africa reduced vaccine serotype carriage and IPD in vaccinated children as well as in unvaccinated mothers. In the vaccinated age group, the effectiveness of the vaccine on IPD is greater than the effectiveness of the vaccine on colonization. On the other hand, changes in colonization due to the vaccine in un-vaccinated individuals can approximate IPD changes in the same population and can potentially be used in resource limited settings to predict post PCV introduction vaccine serotype changes in IPD including in HIV-infected populations. For both vaccinated and unvaccinated individuals model estimates for non-vaccine serotypes were not comparable.

Chapter 7 Integrated Discussion and Conclusion

The United Nations set a 2015 target to achieve a series of Millennium Development Goals (MDG). Many low-income countries will be unable to attain MDG Goal 4 i.e. "to reduce by two thirds, between 1990 and 2015, the under-five mortality rate"(182). Among children aged five years and less, pneumonia and diarrhoea remain leading causes of disease and death (183) and the prevention of these diseases needs to be prioritised. Most cases of severe pneumonia, including deaths, are due to bacterial infections, particularly due to *S. pneumoniae*, *H. influenzae* and *S. aureus* (183).

In the absence of PCV immunization, *S. pneumoniae* was responsible for 18.3% of severe pneumonia cases globally (17) and at least 20-37% of pneumonia associated with alveolar consolidation on CXR (53, 54). PCV immunization has the potential to reduce under-5 childhood pneumonia mortality in low-income countries, where 97% of pneumonia deaths occur (184). The higher pneumococcal colonization prevalence in low and medium income country settings mirror the disproportionate burden of pneumonia and IPD in these countries compared to high-income countries. Furthermore, childhood PCV immunization could indirectly reduce pneumococcal disease even among PCV-unvaccinated age-groups, due to immunization of young children interrupting the transmission of the vaccine serotypes in the community (185).

This thesis evaluated the effect of PCV introduction on the epidemiology of nasopharyngeal colonization in South Africa, a country with a high prevalence of HIV among adults. In addition we examined the effect of childhood PCV immunization on colonization by other potential bacterial pathogens, i.e. *S. aureus* and *H. influenzae*. Lastly, we sought to address

whether studies on changes in prevalence of serotype-specific colonization could be used to predict IPD disease trends post-vaccine introduction in HIV-infected and HIV-uninfected individuals.

We have described NP pneumococcal colonization prior and during the time after PCV introduction occurred by age-group and HIV-status in two study settings in South Africa (162, 176). This work highlights the high prevalence of pneumococcal carriage in young children and up to 12 years of age. In addition, HIV-infected adults were twice as likely to be colonized by pneumococcus, including PCV serotypes, compared to HIV-uninfected adults prior to and during the PCV era (176). These findings are consistent with other studies in Zambia and South Africa (132, 140, 181). We initially hypothesised that in the absence of adult PCV vaccination, HIV-infected adults could serve as a reservoir of vaccine serotype colonization in the population, hence possibly undermining the indirect effect of childhood PCV immunization. Our studies, however, subsequently showed that transmission of pneumococcus adults to children was minimal even in settings with a high prevalence of adult HIV-infection (186).

Following childhood PCV immunization, decline in vaccine serotype colonization prevalence were observed in age groups targeted for vaccination. Furthermore, we observed a temporal reduction in prevalence of pneumococcal colonization among unvaccinated age groups. The reduction in vaccine serotypes colonization among adults 18-45 years of age with a high prevalence of HIV-infection provides the first evidence that infant PCV immunization confers indirect protection against NP colonization among HIV-infected adults who remain at high risk (20-fold greater) of invasive pneumococcal disease even when treated with ART. However, we also observed that the prevalence of pneumococcal colonization remained

higher in HIV-infected compared to –uninfected women overall as well as for PCV13-serotypes during the PCV-era. This likely contributes to HIV-infected individuals remaining at greater risk of developing IPD, albeit at lower incidence than in the pre-PCV era. The Joint Committee on Vaccination and Immunisation in the United Kingdom has not recommended PCV vaccination for high risk groups, based on them likely benefiting from indirect protection (187). Given the high cost of PCV vaccination for governments, improvements in ART management and ensuring high levels of infant PCV vaccine coverage might be adequate to protect HIV-infected adults against vaccines-serotype IPD in settings such as South Africa.

The high nasopharyngeal pneumococcal colonization prevalence among age groups not targeted for PCV immunization may have implications for the magnitude of the indirect effects and duration of time it would take to materialize, more so where there are no catch up campaigns for older children (123). Using a unique dosing schedule of PCV, given at 6, 14 and 40 weeks of age (2+1) of age (infant PCV immunization without a catch-up campaign for older children in South Africa, was associated with reduction in NP vaccine serotype (PCV7 and PCV13 additional serotypes) colonization across all age-groups. Children younger than 2 years targeted in most PCV immunization programs are "key transmitters" (16) and it may be adequate to vaccinate only this age group. This is supported by our study findings; and similarly in a study from Kenya where a similar magnitude of indirect effect on vaccine serotype IPD was observed among older children between a setting where a catch-up campaign was initiated (76) compared to another where there was no catch-up campaign (77). Also, our studies have shown that indirect effects can materialize with PCV vaccine coverage of just over 50% in young children (<12 months) as was the case in rural South Africa. Vaccine coverage rates of 65-75% were also sufficient to induce indirect effect in both Kenya and Massachusetts (76, 188).

While an increase in non-vaccine serotype colonization was observed among PCV vaccinated children, a decrease in non-vaccine serotype colonization was observed among adults in rural South Africa and in HIV-infected women during the PCV era (162, 179). The decrease in non-vaccine serotype among these groups could be due to temporal fluctuations in circulation of these serotypes, rather than a specific effect of childhood PCV immunization. Furthermore, we hypothesise that transmission of non-vaccine serotypes from children to adults is less efficient, and there might be a lag when colonization replacement due to these non-vaccine serotypes materializes among unvaccinated age groups. Additionally, improved management of HIV-infected adults with ART over the same period when PCV immunization of young children was being rolled out, could have contributed to the decrease in non-vaccine serotypes observed in HIV-infected adults.

With the introduction of PCV into national immunization programs, there is a need to determine what other changes might occur in nasopharyngeal bacterial colonization which could indicate whether disease due to these other potential pathogens might also change. In this thesis we described the concurrent changes in *H. Influenzae* and *S. aureus* colonization following PCV7 infant immunization in a rural African community with a high HIV prevalence among adults (189).

Our study showed temporal reductions in pneumococcal and *H. influenzae* nasopharyngeal colonization among children targeted for vaccination. Additionally, *S. aureus* colonization was unchanged in vaccinated children, whilst there was a decrease in *S. aureus* among unvaccinated age-groups. In a randomised controlled trial, higher colonization prevalence of *S. aureus* and *H. influenzae* was observed in children as well as their parents following infant vaccination with PCV7 (108). An increase in *S. aureus* colonization has also been reported in vaccinated children but not among PCV unvaccinated controls following PCV7 vaccination

of infants (104). This was evident at 12 months of age, but not at an older age. Also, studies from the USA indicate that the increase in *S. aureus* following PCV immunization was only transient, with no long term increase in *S. aureus* colonization observed (171). The relationship between *S. aureus* and non-vaccine serotype colonization may therefore be different to the known negative relationship between vaccine serotype and *S. aureus* colonization. This could explain our observation in which *S. aureus* colonization was unchanged in children, despite the significant increase in non-vaccine serotype colonization. Continuous monitoring on the impact of childhood PCV immunization on colonization by these bacteria are, however, warranted to rule out any possible increase in disease due to these bacteria which have disease causing potential (190).

Pneumococcal carriage prevalence mirrors IPD incidence by age group. In our population, IPD incidence was highest in young children aged <2 years and in adults 18-45 years age (179). The high incidence in adults aged 18-45 years is due to the high prevalence of HIV-infection in this age group. Additionally, for all age-groups, HIV-infected individuals had higher rates of IPD compared to HIV-uninfected age-groups (112, 179). For children, <2 years age among HIV-uninfected children, although we did not have data on HIV-exposure status, there is emerging evidence that these children are three-fold more likely to develop IPD compared to HIV-unexposed uninfected children (191). This is particularly important as the population of HIV-infected children decreases but HIV-uninfected exposed children increase. The effectiveness of PCV in HIV-exposed uninfected children has, however, been shown to be similar compared to HIV-unexposed children (180). In our study setting of Soweto, we observed a 93% decrease in PCV7 serotype IPD in children age 3-24 months of age comparing the pre-PCV era to 2012. This compared well to the 88% decrease observed nationwide in South Africa during the same period (112). Similar reductions in vaccine

serotype IPD has also been observed in countries that adopted a 3+ 1 vaccine dosing schedule (56, 192). HIV-infected children, who are still at high risk of IPD even in the PCV–era, had lower reductions in IPD compared to HIV-uninfected children (112, 179). Similarly, among HIV-infected adults aged 18-45 years, the observed reductions in vaccine serotype IPD were smaller than those in HIV-uninfected individuals (179).

In the USA, between 1998/1999 (pre-PCV era) and 2007, a 91% reduction in PCV7 serotype IPD was described in HIV-infected adults (14). Similarly, a 67% reduction in PCV7 serotype IPD was observed among HIV-infected adults in Spain within six years of childhood PCV introduction (193). Continued reductions in IPD incidence in HIV-infected individuals is therefore expected with more years of vaccine use, in addition to improved access to anti-retroviral treatment. The demonstration of indirect effect in this age group is important and indicates the added public health benefit of PCV immunization which is specifically targeted at infants in countries where there is a substantial burden of adult HIV-infection.

While an increase in non-vaccine serotype colonization was observed in childhood age-groups targeted for PCV immunization, we did not identify an increase in IPD due to these non-vaccine serotypes (112, 179). In South Africa transitioning from PCV7 to PCV13 within two years most likely mitigated against some of this increase, in particular due to some of the additional serotypes included in PCV13 (i.e. 19A and 7F) which were associated with higher rates of IPD post PCV7 introduction in some settings. Among HIV-infected individuals, a decrease was observed in non-vaccine serotype IPD among HIV-infected adults, while this remained unchanged in HIV-uninfected adults. Improved access to ART among adults could have contributed to this decline of non-vaccine serotype IPD among the HIV-infected adults. This indicates that in addition to PCV infant immunisation, improved ART management of

HIV-infected individuals is needed to further reduce the burden of IPD in settings such as ours.

Colonization and IPD data was available pre and post vaccine introduction for mother-infant pairs in Soweto. We applied a theoretical model that has been used to predict changes in IPD based on colonization data in three settings (among American Indians, British Population and Portugal) (122). Our analyses was stratified by HIV-status, as interventions in HIV management may affect changes in risk for IPD (113). Our findings showed that the model correctly predicted changes in vaccine serotype IPD for unvaccinated mothers regardless of HIV status. However, estimates for non-vaccine serotype were not comparable. Furthermore, among childhood age groups targeted for vaccination, the observed reductions in IPD were greater than the predicted estimates. This was likely due to the direct effect of PCV vaccination in this age group preventing progression to IPD not being accounted for in the model (122). Alternatively, to improve accuracy of estimates it has been proposed to swab individuals at time of illness to allow for the detection of serotypes 1, 4, 5 and 7F which are seldom isolated from carriage in healthy children (16).

Natural fluctuations of circulating pneumococcal serotypes may account for some of the observations in our data. However, the effect of PCV introduction was evident in both colonization and IPD studies. We also sampled participants in the winter season each year to account for any seasonal variation. Also the IPD surveillance program has been ongoing for at least 15 years and provides a good baseline for evaluating the effect of infant PCV immunization. Ideally we would have liked to confirm self-reported HIV status in the

Agincourt area. However, our results even though limited to self-reporting of HIV status are comparable to those among confirmed HIV-infected adults in Soweto.

In conclusion, infant PCV-immunization with fairly modest vaccine coverage and in the absence of a catch-up campaign of older children was associated with indirect protection against vaccine serotype colonization and IPD in a high HIV-prevalent setting. Currently, there is no evidence of increases in *S. aureus* and *H. influenzae* in the nasopharynx. However, continuous monitoring is required to measure the long-term effects of infant PCV immunization on bacterial interactions in the nasopharynx at a population level. Finally, the use of serial colonization surveys has the potential to help estimate the indirect effect of childhood PCV immunization against vaccine serotype IPD in age groups not targeted for immunization, including among high risk HIV-infected adults.

List of future research

1. Continued NVT monitoring in carriage. Although NVT are less commonly associated with disease, however, these serotypes may acquire virulence factors associated with disease causing potential. As such continued monitoring of trends in colonization and IPD is essential to fully evaluate the impact of pneumococcal conjugate vaccine introduction

2. A study tailored to evaluate bacterial interactions at population level and stratified by HIV status is essential. While our study does not show significant shifts at the moment, this may change with continued vaccine pressure.

3. Measuring colonization at the time of disease maybe more accurate than colonization in healthy population. This study will evaluate the fitness of colonization data in estimating changes in IPD.

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Appendix 1: Questionnaires

Adult Questionnaire
Participants number [___][___][___][___][___][___]

Section 1:Study site			
Soweto	[___]	Agincourt	[___]
Clinic PHRU = 01 RMPRU = 02 PHC=03	[___][___]	Village	[___][___]
File number		Dwelling	[___][___][___][___]
Field worker (enter name)		Field worker (enter name)	
Visit date	[___][___][___][___] /[___][___] /[___][___]	Visit Date	[___][___][___][___] /[___][___] /[___][___]

Section 2: Demographic information				
Q1	Name (not to be entered on database)	Name : 1a Surname: 1b		
Q2	Participants number		2	[___][___][___][___][___][___]
Q3	Date of birth		3	[___][___][___][___] /[___][___] /[___][___]
Q4	Gender	Male = 01 Female=02	4	[___][___]
Q5	Occupation	Mining =01 Farming= 02 Construction=03 Other=04 Unemployed =05 Pensioner=06 School=07	5	[___][___]
Section 3: Risk factors				
Q6	Do you smoke?	Yes = 01(→Q7) No =02 (→Q9)	6	[___][___]
Q7	How many cigarettes a day?		7	[___][___]
Q8	Do you smoke indoors?	Always = 01 Sometimes = 02 Rarely = 03 Never = 04	8	[___][___]
Q9	Do you take snuff?	Yes=01(→Q10) No = 02 (Q11)	9	[___][___]
Q10	How many days a week do you take snuff?		10	[___]
Q11	Do you drink alcohol?	Yes=01(→Q12) No=02 (→Q13)	11	[___][___]
Q12	How often do you drink alcohol	Almost	12	

Completed by:_____ 181
Date: _____

			everyday=01 On weekends=02 Social occasions=03		[___][___]
Q13	Do you suffer from any chronic illnesses?		Yes= 01(→Q14) No= 02 (→Q15)	13	[___][___]
Q14	If Yes to above please specify?	Hypertension =01 Diabetes=02 Malignancy=03 Current TB=04 Other =05 More than one of the above=06		14	[___][___]
Q15	Do you know your HIV status?		Yes=01 (→Q16) No=02(→Q21)	15	[___][___]
Q16	Complete?	Positive =01 (→Q17) Negative=02 (→Q21) Refused to disclose=03(→Q21)		16	[___][___]
Q17a	Last CD4+ count			17a	[___][___][___]
17b	%CD4+			17b	[___][___].[___]
17c	State date when last CD4+ was taken			17c	[___][___][___][___] /[___][___] /[___][___]
17d	Viral load			17d	[___][___][___][___]
17c	State date when last viral load was taken			17e	[___][___][___][___] /[___][___] /[___][___]
Q18a	Are you on Co-trimoxazole (Bactrim, Septran, Triprim) prophylaxis?		Yes=01 (→Q18b) No=02(→ Q19)	18a	[___][___]
Q18b	Compliance	Most of the time (5-7days) = 01 Regular (3-4days) = 02 Poor (1-2days) = 03 Never =04		18b	[___][___]
Q18c	State date when Co- trimoxazole prophylaxis was started			18c	[___][___][___][___] /[___][___] /[___][___]
Q19	Are you on ARV's		Yes=01→Q20 No=02→Q21	19	[___][___]
Q20	Complete table below			20	

Completed by:_____ 182
Date: _____

Details of ARV therapy

	Name	Abbreviation	Date started
Q20a	Lamivudine (Epivir)	3TC	[___][___][___][___] /[___][___] /[___][___]
Q20b	Stavudine (Zerit)	d4T	[___][___][___][___] /[___][___] /[___][___]
Q20c	Efavirenz (Stocrin)	EFV	[___][___][___][___] /[___][___] /[___][___]
Q20d	Neverapine (Viramune)	NVP	[___][___][___][___] /[___][___] /[___][___]
Q20e	Lopinavir/Ritonavir (Kaletra)	LPV/r,	[___][___][___][___] /[___][___] /[___][___]
Q20f	Didanosine (Videx)	ddI,	[___][___][___][___] /[___][___] /[___][___]
Q20g	Zidovudine(Retrovir)	AZT,	[___][___][___][___] /[___][___] /[___][___]
Q20h	Abacavir(Ziagen)	ABC,	[___][___][___][___] /[___][___] /[___][___]
Q20i	Ritonavir (Norvir)	RTV	[___][___][___][___] /[___][___] /[___][___]
Q20j	Nelfinavir (Viracept)	NFV	[___][___][___][___] /[___][___] /[___][___]
Q20k	Other (specify)		[___][___][___][___] /[___][___] /[___][___]

Section 4: Current symptomatic disease

Q21	Documented current fever	Yes=01 No=02	21	[___][___]
Q22	Are you currently coughing?	Yes=01 No=02	22	[___][___]
Q23	Are you currently sneezing/having a runny nose?	Yes=01 No=02	23	[___][___]
Q24	Are you currently on TB treatment?	Yes=01 (→Q27) No=02 (→Q25)	24	[___][___]
Q25	Have you been treated for TB in the past year?	Yes= 01(→Q26) No =02	25	[___][___]

Completed by:_____ 183
Date: _____

		(→Q28))		
Q26	State date of completion of treatment		26	[___][___][___][___] /[___][___] /[___][___]
Q27	Enter codes of anti TB drugs*	[___][___], [___][___], [___][___], [___][___], [___][___]		
Q28	Are you suffering from any other illness at the moment (specify)			

***** Rifampicin ® = 01, Isoniazid (H) = 02, Pyrazinamide (Z) = 03, Ethambutol (E) = 04, Streptomycin (S) = 05, other = 06

Section 5: Examination

Q29	Record actual temperature		29	[___][___].[___]
Q30	Record if cough/runny nose/sneezing was noted during the interview	Yes = 01 No = 02	30	[___][___]
Q31	Sore throat/Pharyngitis noted on examination	Yes = 01 No = 02	31	[___][___]

Section 6: Current antibiotic treatment

Q32	Are you currently taking any antibiotics	Yes = 01 (→Q33) No = 02 (→ Q34)	32	[___][___]
Q33	Tick all that apply			

Details of current antibiotic therapy

	Name	**Code** (see below)**	**Start Date**	**Comment(eg medication seen)**
Q33a.			[___][___][___][___] /[___][___] /[___][___]	
Q33b.			[___][___][___][___] /[___][___] /[___][___]	
Q33c.			[___][___][___][___] /[___][___] /[___][___]	

Completed by:_____ 184
Date: _____

Q33d.			[___][___][___][___] /[___][___] /[___][___]	

**β –lactams = 01, Co-trimoxazole = 02, Cephalosporins = 03, Metronidazole = 04, Macrolides = 05, Tetracycline =06, Aminoglycosides = 07, Quinolones = 08, Other=09

Section 7: Previous hospitalization

Q34	Have you been hospitalized in the past 3 months*** (see codes below)	Yes=01(→Q35) No=02 end of questionnaire)	35	[___][___]

Q35	a)Duration of hospital stay	b)Diagnosis
i)	[___][___]	
ii)	[___][___]	
iii)	[___][___]	

***Less than 3 days = 01, 3-7days = 02, 7-14days = 03, Greater than 14days = 04

Child Questionnaire

Participants number [___][___][___][___][___][___]

Section 1:Study site			
Soweto	[___]	Agincourt	[___]
Clinic site PHRU = 01 RMPRU = 02 PHC = 03		Village	[___][___]
File number		Dwelling	[___][___][___][___]
Mothers ID	[___][___][___][___][___][___]		
Field worker (enter name)		Field worker (enter name)	
Visit date	[___][___][___][___] /[___][___] /[___][___]	Visit Date	[___][___][___][___] /[___][___] /[___][___]

Section 2: General information				
Q1	Name(not to be entered on database)	1aName 1b. Surname		
Q2	Study ID		2	[___][___][___][___][___][___]
Q3	Date of birth		3	[___][___][___][___] /[___][___] /[___][___]
Q4	Is child still breastfeeding	Yes = 01(→Q6) No = 02 (→Q5)	4	[___][___]
Q5	Has child ever been breastfed?	Yes = 01 (→Q6) No = 02 (→Q7)	5	[___][___]
Q6	If Yes to Q4 and Q5 state duration of breastfeeding to the nearest month?		6	[___][___]
Q7	Current weight in kilograms		7	[___][___][___].[___][___]
Q8	Current height in centimeters		8	[___][___][___]
Section 3: Daycare/School attendance				
Q9	Does this child attend daycare/regular play group/school?	Yes = 01 No = 02	9	[___][___]
Q10	If yes how many times a week		10	[___]
Section 4: Risk factors				
Q11	Do you know the HIV status of this child?	Yes = 01(→Q12) No = 02(→Q13)	11	[___][___]
Q12	If Yes specify	Positive = 01 (→Q15) Negative = 02 (→Q19)	12a	[___][___]
12b	State date when last tested		12b	[___][___][___][___] /[___][___] /[___][___]

Completed by: _____ 186

Date: _____

Q13	Was mother tested for HIV?	Yes= 01(→Q14) No= 02 (→Q19)	13	[___][___]
Q14	State result	Positive = 01 (→Q19) Negative = 02 (→ Q19) Refused to disclose = 03 (→Q19) Unknown= 04 (→Q19)	14	[___][___]
14b	State date when last tested		14b	[___][___][___][___] /[___][___] /[___][___]
Q15	a) Last CD4+ count of child b) % CD4+ c) Date when last CD4+ was taken d) Viral load e) Date when last viral load was taken		15a 15b 15c 15d 15e	[___][___][___] [___][___].[___] [___][___][___][___] /[___][___] /[___][___] [___][___][___][___] [___][___][___][___] /[___][___] /[___][___]
Q16	a) Is child on Co-trimoxazole (Bactrim, Septran, Triprim) prophylaxis?	Yes = 01(→ Q16b) No = 02 (→Q17)	16a	[___][___]
	b) Compliance (How often does child take Co- trimoxazole prophylaxis)	Most of the time (5-7days) = 01 Regularly (3-5days) = 02 Poor (1-2days) = 03 Never = 04	16b	[___][___]
	c) State start date of Co-trimoxazole prophylaxis		16c	[___][___][___][___] /[___][___] /[___][___]
Q17	Is child on ARV's	Yes=01 (→Q18) No=02 (→Q19)	Q17	[___][___]
Q18	If yes complete table			

Details of ARV therapy

	Name	Abbreviation	Date started
Q18a	Lamivudine (Epivir)	3TC	[___][___][___][___] /[___][___] /[___][___]
Q18b	Stavudine (Zerit)	d4T	[___][___][___][___] /[___][___] /[___][___]
Q18c	Efavirenz (Stocrin)	EFV	[___][___][___][___] /[___][___] /[___][___]
Q18d	Neverapine (Viramune)	NVP	

Completed by: _____

Date: _____

			[___][___][___][___] /[___][___] /[___][___]
Q18e	Lopinavir/Ritonavir (Kaletra)	LPV/r,	[___][___][___][___] /[___][___] /[___][___]
Q18f	Didanosine (Videx)	ddI,	[___][___][___][___] /[___][___] /[___][___]
Q18g	Zidovudine(Retrovir)	AZT,	[___][___][___][___] /[___][___] /[___][___]
Q18h	Abacavir(Ziagen)	ABC,	[___][___][___][___] /[___][___] /[___][___]
Q18i	Ritonavir (Norvir)	RTV	[___][___][___][___] /[___][___] /[___][___]
Q18j	Nelfinavir (Viracept)	NFV	[___][___][___][___] /[___][___] /[___][___]
Q18k	Other (specify)		[___][___][___][___] /[___][___] /[___][___]

Section 5: Current disease

Q19 19b	Documented current fever Record actual temperature	Yes =01 No =02	19 19b	[___][___] [___][___].[___]
Q20	Is child currently coughing?	Yes =01 No =02	20	[___][___]
Q21	Does child have a runny nose	Yes =01 No =02	21	[___][___]
Q22	Is child currently on TB treatment?	Yes=01 (→Q24) No=02 (→Q23)	22	[___][___]
Q23	Has the child been treated for TB in the past one year?	Yes=01 (→Q24) No=02 (→Q26)	23	[___][___]
Q24	If Yes to Q23, please state date of completion of treatment		24	[___][___][___][___] /[___][___] /[___][___]
Q25	Give codes of TB drugs if Yes to Q22 above*	[___][___], [___][___], [___][___], [___][___], [___][___]		
Q26	Is child suffering from any other medical illness at the moment (specify)			

***** Rimcure = 01, Rimatacid = 02, Streptomycin (S) = 03, Other = 04

Completed by: _____ 188
Date: _____

|Section 6: Examination

Q27	Record actual temperature		27	[___][___].[___]
Q28	Record if cough/runny nose/sneezing was noted during the interview	Yes = 01 No = 02	28	[___][___]
Q29	Sore throat/Pharyngitis noted on examination	Yes = 01 No = 02	29	[___][___]

Section 7: Current antibiotic therapy

Q30	Is child currently taking any antibiotics	Yes = 01 (→Q31) No = 02 (Q32)	30	[___][___]
Q31	If Yes list below			

Details of current antibiotic therapy

	Name	**Code** (see below)**	**Start Date**	**Comment(eg medication seen)**
Q31a.			[___][___][___][___] /[___][___] /[___][___]	
Q31b.			[___][___][___][___] /[___][___] /[___][___]	
Q31c.			[___][___][___][___] /[___][___] /[___][___]	
Q31d.			[___][___][___][___] /[___][___] /[___][___]	
Q31e.			[___][___][___][___] /[___][___] /[___][___]	

**β –lactams = 01, Co-trimoxazole = 02, Cephalosporins = 03, Metronidazole = 04, Macrolides = 05, Tetracycline =06, Aminoglycosides = 07, Quinolones = 08, Other=09

Completed by: _____ 189
Date: _____

Section8: Previous hospitalization

Q32	Have you been hospitalized in the past 3 months	Yes=01(→Q33) No=02 (→Q34)	32	[___][___]
Q33	a)Duration of hospital stay (for each admission)***		b) Diagnosis	
i)	[___][___]			
ii)	[___][___]			
iii)	[___][___]			

***Less than 3 days = 01, 3-7days = 02, 7-14days = 03, Greater than 14days = 04

Section 9: Immunisation history

Q34a) Road to health card seen Yes=01 (→Q31b) No= 02 (end of questionnaire)
[___][___]

Q34b) Immunisation history (please provide date when vaccine was administered)

Age of Child	**Vaccines needed**		**Date when vaccine given**	**Tick if not given**
At birth	BCG		[___][___][___][___] /[___][___] /[___][___]	
	Polio (0)		[___][___][___][___] /[___][___] /[___][___]	
6 weeks	Polio (1)	IPV [___] OPV [___] Not recorded [___]	[___][___][___][___] /[___][___] /[___][___]	
	DPT	aPettussis [___] wPertussis [___] Not recorded [___]	[___][___][___][___] /[___][___] /[___][___]	
	Haemophilus Influenza B		[___][___][___][___] /[___][___] /[___][___]	
	Pentaxim		[___][___][___][___] /[___][___] /[___][___]	
	Hepatitis B (1)		[___][___][___][___] /[___][___] /[___][___]	
	Pneumoccocal Conjugate Vaccine (PCV) 1		[___][___][___][___] /[___][___] /[___][___]	
	Rotavirus		[___][___][___][___] /[___][___] /[___][___]	
10 weeks	Polio	IPV [___] OPV [___]	[___][___][___][___] /[___][___] /[___][___]	

		Not recorded [___]		
	DPT	aPettussis [___] wPertussis [___] Not recorded [___]	[___][___][___][___] /[___][___] /[___][___]	
	Vaccine		**Date when vaccine was given**	**Tick if not given**
	Haemophilus Influenza B		[___][___][___][___] /[___][___] /[___][___]	
	Hepatitis B (2)		[___][___][___][___] /[___][___] /[___][___]	
14 weeks	Polio	IPV [___] OPV [___] Not recorded [___]	[___][___][___][___] /[___][___] /[___][___]	
	DPT	aPettussis [___] wPertussis [___] Not recorded [___]	[___][___][___][___] /[___][___] /[___][___]	
	Haemophilus Influenza B		[___][___][___][___] /[___][___] /[___][___]	
	Pentaxim		[___][___][___][___] /[___][___] /[___][___]	
	Hepatitis B (3)		[___][___][___][___] /[___][___] /[___][___]	
	PCV (2)		[___][___][___][___] /[___][___] /[___][___]	
	Rotavirus		[___][___][___][___] /[___][___] /[___][___]	
9 months	Measles (1)		[___][___][___][___] /[___][___] /[___][___]	
	PCV (3)		[___][___][___][___] /[___][___] /[___][___]	
18 months	Polio (4)	IPV [___] OPV [___] Not recorded [___]	[___][___][___][___] /[___][___] /[___][___]	
	DPT	aPettussis [___] wPertussis [___] Not recorded [___]	[___][___][___][___] /[___][___] /[___][___]	
	Haemophilus Influenza B		[___][___][___][___] /[___][___] /[___][___]	
	Pentaxim		[___][___][___][___] /[___][___] /[___][___]	
	Measles (2)		[___][___][___][___] /[___][___] /[___][___]	
5years	Td-1 (Tetanus and reduced strength Diphtheria)		[___][___][___][___] /[___][___] /[___][___]	
12 years	Td2		[___][___][___][___] /[___][___] /[___][___]	

Completed by: _____ 191
Date: _____

Household/ participant number [___][___][___][___][___][___]

Effect of Introduction of PCV upon nasopharyngeal ecology

Section 1:Study site			
Soweto	[___]	Agincourt	[___]
Clinic name PHRU = 01 RMPRU= 02 PHC = 03	[___][___]	Village	[___][___]
		Dwelling	[___][___][___][___]
Field worker (enter name)		Field worker (enter name)	
Visit date	[___][___][___][___] /[___][___] /[___][___]	Visit Date	[___][___][___][___] /[___][___] /[___][___]

Section 2: List of household residents, relationship to household head and study participation

Q1	Q2	Q3	Q4	Q5	Q6
Name (not to be entered in database)	Gender Male = 01 Female=02	Date of birth	Relationship * (see codes below)	Primary care giver to any of the children under 5years Yes=01 No=02	Enrolled Yes= 01 No = 02
	[___][___]	[___][___][___][___] /[___][___] /[___][___]	[___][___]	[___][___]	[___][___]
	[___][___]	[___][___][___][___] /[___][___] /[___][___]	[___][___]	[___][___]	[___][___]
	[___][___]	[___][___][___][___] /[___][___] /[___][___]	[___][___]	[___][___]	[___][___]
	[___][___]	[___][___][___][___] /[___][___] /[___][___]	[___][___]	[___][___]	[___][___]
	[___][___]	[___][___][___][___] /[___][___] /[___][___]	[___][___]	[___][___]	[___][___]
	[___][___]	[___][___][___][___] /[___][___] /[___][___]	[___][___]	[___][___]	[___][___]
	[___][___]	[___][___][___][___] /[___][___] /[___][___]	[___][___]	[___][___]	[___][___]

*01= Household head, 02=Spouse, 03=Son or daughter, 04= Sister, 05= Brother, 06=Nephew/Niece, 07=Parent, 08= Uncle/Aunt, 11= other relative 12=grandchild 13= son in law/daughter in law 14=parent in law 15=adopted/foster/step child

Section 3: General household questions

Q7	7.1 How many adults live in the household? 7.2 How many children 6- 18years live in the household? 7.3 How many children aged 5 or less live in the household? 7.4 Total number of people in the household [add 6.1 and 6.2 and 6.3]		7.1 7.2 7.3 7.4	[___][___] [___][___] [___][___] [___][___]
Q8	For any of the children aged 5 or less did they attend daycare/regular playgroup during the past month?	Yes= 01 No= 02	8	
Q9	How many people smoke in the dwelling?		9	[___][___]
Q10	How many rooms are used for sleeping in the dwelling?		10	[___][___]
Q11	What fuel is used for cooking in the dwelling?	Electricity = 01 Paraffin/gas= 02 Coal/wood = 03	11	[___][___]
Q12	What fuel is used for heating in the dwelling?	Electricity = 01 Paraffin/gas= 02 Coal/wood = 03 No heater = 04	12	[___][___]