EPIDEMIOLOGY OF *STREPTOCOCCUS PNEUMONIAE* POST-PNEUMOCOCCAL CONJUGATE VACCINE INTRODUCTION IN SOUTH AFRICA

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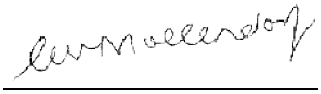
DECLARATION

I, Claire Emily von Mollendorf, declare that this thesis is my own work. It is being submitted for the degree of Doctor of 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.



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Date: 11 November 2016

ABSTRACT

BACKGROUND

Streptococcus pneumoniae is a leading cause of severe invasive bacterial infections globally; estimated to cause over 300,000 deaths in children <5 years in 2015. Pneumococcal conjugate vaccine (PCV) introduction in South Africa has been associated with changes in invasive pneumococcal disease (IPD) risk groups and emerging serotypes. Serotype 1 pneumococcal disease is highly invasive, fluctuates annually but tends to have lower mortality and antibiotic-resistance than other serotypes. Paediatric antiretroviral treatment (ART) and HIV prevention of mother-to-child transmission programme improvements in South Africa have resulted in a growing number of HIV-exposed-uninfected (HEU) children who have higher rates of infectious diseases than unexposed children. It is important to identify risk groups changes with new interventions and define IPD burden pre- and post-PCV introduction in developing countries.

OBJECTIVES

In South Africa we aimed to estimate severe pneumococcal disease burden in the pre- (2005-2008) and post-PCV era (2013) amongst HIV-infected (HI) and HIV-uninfected (HU) children <5 years of age; describe the epidemiology of serotype 1 IPD in all age groups from 2003 to 2013; describe the epidemiology of IPD in HEU children <1 year of age from 2009 to 2013 and the risk factors related to IPD in HI and HU children post-PCV introduction (2010 to 2012). All analyses included PCV introduction impact.

METHODS

A model using national laboratory-based IPD surveillance data as the baseline was used to determine the total burden of severe hospitalised pneumococcal disease and related mortality in South Africa in children aged <5 years. Adjustments were made for specimen-taking practices and care seeking differences. Vaccine probe studies were used to calculate non-bacteraemic pneumococcal pneumonia case numbers. Observed case fatality ratios were applied to estimated case numbers to determine pneumococcal death numbers.

All patients with laboratory-confirmed IPD were included in the serotype 1 analysis. We calculated incidence rates, determined factors associated with serotype 1 disease and conducted a space-time analysis using SaTScan with a Bernoulli model for comparison. Maps to visualise serotype 1 clusters were generated using ArcGIS.

Surveillance data was used to compare IPD incidence and mortality in HEU, HIV-unexposed-uninfected (HUU) and HI infants. Factors associated with HIV status were compared using a multinomial regression model and logistic regression for mortality factors. A matched case-control study nested within the surveillance programme was used to determine risk factors associated with IPD in HU and HI children aged <5 years. Data was analysed using conditional logistic regression.

RESULTS

In the pre-vaccine era (2005-2008) in South Africa, roughly 196,100 (148,000-251,000) cases of severe pneumococcal disease were estimated annually in children aged <5 years, an incidence of 3799/100,000; the rate was reduced by 67% in 2013, likely due to PCV and other interventions. In addition 8600 (7000-10220) pneumococcal-related annual deaths were estimated pre-vaccine and 3600 in 2013, a rate difference of 99/100,000 child-years.
Over an 11-year period two clusters (2003-2004 and 2008-2012) of serotype 1 infection were detected in all age groups with reductions in incidence noted in 2013. Among children aged <5 years, those with serotype 1 IPD had shorter hospital stays, fewer penicillin-nonsusceptible cases (adjusted odds ratio (aOR) 0.02, 95% confidence interval (CI) 0.01–0.05), lower HIV prevalence (aOR 0.19, 95% CI 0.12–0.31) and lower in-hospital death rates (aOR 0.38, 95% CI 0.19–0.76) than children with non-serotype 1 IPD.
The incidence of IPD was greatest in HI infants (272-654/100,000), then HEU infants (33-88/100,000) and HUU infants (18-28/100,000). Young HEU infants (37% [59/175]) were more likely to die than HUU infants (32% [51/228]; adjusted relative risk ratio, 1.76, 95% CI 1.09–2.85]). On case-control analysis a number of factors were shown to be associated with an increased risk of IPD in the post-PCV period. In HU children these factors included underlying medical conditions (aOR = 1.99, 95% CI 1.22–3.22), attending day care (aOR = 1.58, 95% CI 1.01–2.47) or having been exposed to HIV perinatally (aOR = 1.62, 95% CI 1.10–2.37), while PCV vaccination reduced the odds of IPD (aOR = 0.67, 95% CI 0.46–0.99). Predisposing factors in HI children included malnutrition (aOR = 2.68, 95% CI 1.40–5.14) and recent tuberculosis (aOR = 5.12, 95% CI 1.69–15.50), while current ART reduced the odds of IPD (aOR = 0.13, 95% CI 0.05–0.38).

CONCLUSION

Pneumococcal disease represents a major public health burden in young children in South Africa. PCV and other HIV-associated interventions resulted in a significant reduction in

both invasive disease and non-bacteraemic pneumonia. Serotype 1 IPD has distinctive clinical features with temporal decreases noted post-PCV13 introduction. With improvements in interventions to prevent and treat HIV, a resultant growing HEU infant population has been observed with an increased risk of IPD compared with HUU children. Risk factors related to socio-economic conditions and intense exposure to infection continues to be important causes of IPD in children. A full understanding of PCV impact on pneumococcal disease burden is needed to support ongoing national policy decisions on PCV use.

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers:

a) Burden of potentially vaccine-preventable pneumococcal disease in children <5 years of age in South Africa, 2005-2008 and 2013. Claire von Mollendorf, Stefano Tempia, Anne von Gottberg, Susan Meiring, Vanessa Quan, Linda de Gouveia, Charles Feldman, Jeane Cloete, Shabir Madhi, Katherine L. O'Brien, Keith P. Klugman, Cynthia G. Whitney, Cheryl Cohen
Paper with revisions submitted to PLOS One

b) Epidemiology of serotype 1 invasive pneumococcal disease in all ages in South Africa, 2003-2013. Claire von Mollendorf, Cheryl Cohen, Stefano Tempia, Susan Meiring, Linda de Gouveia, Vanessa Quan, Sarona Lengana, Alan Karstaedt, Halima Dawood, Sharona Seetharam, Ruth Lekalakala, Shabir A. Madhi, Keith P. Klugman, Anne von Gottberg, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Emerging Infectious Diseases 2016; 22(2):261-270. (http://dx.doi.org/10.3201/eid2202.150967)

c) Increased Risk for and Mortality from Invasive Pneumococcal Disease in HIV-Exposed but Uninfected Infants Aged <1 Year in South Africa, 2009–2013
Claire von Mollendorf, Anne von Gottberg, Stefano Tempia, Susan Meiring, Linda de Gouveia, Vanessa Quan, Sarona Lengana, Theunis Avenant, Nicolette du Plessis, Brian Eley, Heather Finlayson, Gary Reubenson, Mamokgethi Moshe, Katherine L. O'Brien, Keith P. Klugman, Cynthia G. Whitney, and Cheryl Cohen for GERMS-SA. Clinical Infectious Diseases 2015;60(9):1346–56. (doi: 10.1093/cid/civ059)

d) Risk Factors for Invasive Pneumococcal Disease Among Children Less Than 5 Years of Age in a High HIV Prevalence Setting, South Africa, 2010 to 2012
Claire von Mollendorf, Cheryl Cohen, Linda de Gouveia, Nireshni Naidoo, Susan Meiring, Vanessa Quan, Sonwabo Lindani, David P. Moore, Gary Reubenson, Mamokgethi Moshe, Brian Eley, Ute M. Hallbauer, Heather Finlayson, Shabir A. Madhi, Laura Conklin, Elizabeth R. Zell, Keith P. Klugman, Cynthia G. Whitney, and Anne von Gottberg, for the South African IPD Case-Control Study Group. The Pediatric Infectious Disease Journal 2015;34(1):27–34. (doi: 10.1097/INF.0000000000000484)

The publishers have given permission for reprinting of published papers. My roles in each of the publications are included in Appendix A.

CONTENTS

PREFACE

I was employed as a medical officer at the National Institute for Communicable Disease six years ago to run the invasive pneumococcal disease case-control study.
During this time I completed my Masters in Epidemiology (distance learning) through the London School of Hygiene and Tropical Medicine (2011). My thesis explored the reported increase in ceftriaxone resistance in *Streptococcus pneumoniae*.
I identified a theme for a PhD in 2013 and enrolled in the University of the Witwatersrand's School of Public Health PhD programme in April 2014. This programme has helped to grow me as a student and person.
I have been privileged to present my work at numerous local and international meetings and conferences, including the International Symposium on Pneumococci and Pneumococcal Diseases, where the forerunners in pneumococcal disease present current research and data.
None of this would have been possible without the support and mentorship of my two supervisors, Prof Cheryl Cohen and Prof Anne von Gottberg, who have pushed me to achieve my full potential.
I hope that my work will inspire others to pursue a PhD in their chosen field.

Claire von Mollendorf, 11 November 2016

ABBREVIATIONS

PCV: Pneumococcal conjugate vaccine

IPD: invasive pneumococcal disease

ART: antiretroviral treatment

HIV: human immunodeficiency virus

HEU: HIV-exposed-uninfected

HI: HIV-infected

HU: HIV-uninfected

HUU: HIV-unexposed-uninfected

CI: confidence interval

VT-IPD: vaccine type invasive pneumococcal disease

NPNM: non-pneumonia non-meningitis

ARTI: acute respiratory tract infections

CXR: chest radiograph

PPSV23: 23-valent polysaccharide vaccine

PCV7: 7-valent pneumococcal conjugate vaccine

PCV10: 10-valent pneumococcal conjugate vaccine

PCV13: 13-valent pneumococcal conjugate vaccine

CSF: cerebrospinal fluid

Hib: *Haemophilus influenzae* type b

NVT-IPD: non-vaccine type invasive pneumococcal disease

PMTCT: prevention of mother-to-child transmission

PCV9: 9-valent pneumococcal conjugate vaccine

VE: vaccine efficacy

VT: vaccine type

NVT: non-vaccine type

EPI-SA: Expanded Programme on Immunisation in South Africa

GERMS-SA: Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa

DALYs: disability-adjusted life years

QALYs: quality-adjusted life years

ST1: Serotype 1

GIS: Geographic Information System

BACKGROUND

INTRODUCTION

Streptococcus pneumoniae has long been recognised as a significant disease-causing pathogen. It was first identified as pathogenic in 1881 when two microbiologists, Louis Pasteur and George Sternberg, each individually injected human saliva into rabbits and isolated this bacterium [1]. As early as 1918 Sir William Osler referred to the pneumococcus as the "captain of the men of death" as it killed young adults in the "prime of their lives" [2]. It was finally given its current name in 1974 [3].

S. pneumoniae is a small gram-positive diplococcus that affects children and adults worldwide. At least 93 different serotypes have been described. The pneumococcus causes severe disease such as bacterial pneumonia, meningitis and sepsis, but is also a common coloniser. Colonising rates can vary between different age groups and settings: 5-10% in adults and 10-50% in children in developing countries, but up to 86% in children in some African countries [4]. Much of the virulence of *S. pneumoniae* originates from its polysaccharide capsule which can prevent phagocytosis [5]. Despite a number of therapeutic strategies, such as antibiotics and vaccines, *S. pneumoniae* continues to kill individuals of all ages till the present day. It has been treated with antibacterial drugs since the late 1930s but widespread overuse of antibiotics in recent years has led to the creation of penicillin-resistant strains of *S. pneumoniae*.

Studies from South Africa have shown an increased risk of invasive pneumococcal disease (IPD) in HIV-infected (HI) adults and children [6-11]. Following the widespread introduction of antiretrovirals in 2004, a significant reduction in IPD was noted in children [12], but not in adults [13].

BURDEN OF PNEUMOCOCCAL DISEASE

In developing countries pneumonia is a serious disease in both adults and children. Pneumonia accounts for almost one in seven deaths in young children with nearly a million deaths annually, 50% of which are in sub-Saharan Africa according to the World Health Organization [14]. In South Africa the incidence of severe pneumococcal disease in children <5 years of age was estimated as having been approximately 3000 per 100,000 and the death rate as around 100 per 100,000 population in the year 2000 [15].

Annual estimates for pneumonia in children <5 years of age in South Africa in 2010, derived from a Global Action Plan for Pneumonia and Diarrhoea model, were reported as 705,554 cases for all acute respiratory tract infections (ARTI) and 33,436 new episodes of *S. pneumoniae* pneumonia; severe disease was a proportion of this, namely 78,749 cases for all severe ARTI and 10,052 severe new episodes of *S. pneumoniae* pneumonia [16]. This manuscript reported country level estimates of the model. The estimates were modelled based on the prevalence of five main risk factors for childhood pneumonia obtained from country-based demographic health or cluster surveys. A meta-analysis assessed the size of the risk factor effect on pneumonia incidence [16]. The portion of pneumonia deaths attributable to *S. pneumoniae* was derived from the meta–analysis of pneumococcal conjugate vaccine (PCV) vaccine efficacy against chest radiograph (CXR) confirmed pneumonia [15]; it was assumed that the etiologic fraction of *S. pneumoniae* (33%) among cases was similar to that among the deaths [15].

Baseline data from a national pneumococcal surveillance programme pre-PCV introduction (2003-2008) demonstrated that IPD rates were 6-fold higher in the <1 year age group compared with children 1–4 years of age, and HI infants had a 21-fold greater risk of disease than HU infants <5 years of age [17]. Even though PCV had lower efficacy amongst HI children [18,19], these children had a higher burden of IPD than HU children resulting in a projected 18-fold greater reduction in the absolute burden of IPD in HI compared to HU children if PCV were introduced [20,21].

Bacteraemia is approximately four-fold more common in HI compared to HU children with pneumococcal pneumonia [20]. Disease among HI children is more likely to be caused by vaccine serotypes than among HU children [22] and there is also an increased prevalence of paediatric serotypes in HI adults [6]. Higher single drug [23] and multidrug [22] antibiotic resistance has been reported in children and HI individuals [6,8]. HI individuals with a lower CD4 count tend to have a higher mortality from bacteraemic pneumococcal pneumonia than individuals with a higher CD4 [24].

Three methods, some of which have been touched on above, have been proposed to estimate the burden of different pneumococcal syndromes [15]. Firstly, a proportional approach using a mortality or morbidity envelope for the relevant clinical syndrome with allocation of cases or deaths attributable to *S. pneumoniae*; secondly, an incidence-based approach using clinical disease incidence to derive cause specific cases, and then case-fatality ratios to estimate the number of deaths; or lastly "triangulation" which compares the relative occurrence of one syndrome relative to another. All three methods were used to determine

case and death numbers in a pneumococcal global burden paper [15]. To estimate the proportion of pneumonia attributable to *S. pneumoniae* the vaccine efficacy against a specific pneumonia endpoint was first divided by the overall efficacy against vaccine type invasive pneumococcal disease (VT-IPD) and then by the proportion of VT-IPD in the population where the study was conducted. An adjustment was also made for the effect of *Haemophilus influenzae* serotype b vaccination in the relevant study population. The estimated proportion of each pneumonia endpoint attributed to *S. pneumoniae* was based on a meta-analysis [25-27] of vaccine efficacy trial results [20,21,28-32]. Pneumonia deaths numbers were estimated by applying the proportion of pneumonia deaths attributable to *S. pneumoniae* to overall estimates of pneumonia deaths in children 1-59 months of age. For meningitis, an incidence-based approach was used in countries where *S. pneumoniae* incidence and case fatality rates were available. Estimates were also combined using meta-analysis results [25-27]. The burden of non-pneumonia non-meningitis (NPNM) invasive disease was based on the ratio of NPNM to meningitis cases multiplied by the estimated number of meningitis cases for each country. The number of deaths caused by NPNM was calculated by multiplying the severe NPNM cases by an appropriate case fatality rate [15]. Global pneumonia death models often split case and death rates by aetiological agent. The Rudan, et. al. paper [16] used previous determined proportionate estimates for *S. pneumoniae* and accounted for vaccine use.

Although the number of countries in the African region, who have introduced PCV since it first became available in 2000, has increased exponentially, the availability of published pre-vaccine and post-vaccine data is limited to a small number of countries. South Africa was the first African country to introduced PCV into its public National Immunisation Programme in April 2009 and is one of the few African countries to have robust pre-vaccine pneumococcal surveillance data. South African impact data will help to inform vaccine introduction into other low- and middle-income countries in terms of vaccine type, schedule, duration of protection, replacement and risk groups in the post-PCV era.

PNEUMOCOCCAL SEROTYPES AND VACCINES

Polysaccharide antigens are large molecules which interact directly with B cells and induce T-independent antigens [33]. Due to the absence of T-cell reactions polysaccharide vaccines fail to induce significant and sustained antibody responses in young children less than 18 months of age. Even in older children and adults, antibody responses are relatively short lived and booster response cannot be produced by repeated exposure. By conjugating

the polysaccharide to a protein carrier, a T-cell response can be induced [33]. T cells stimulate a more vigorous immune response and also promote a more rapid and long-lasting immunologic memory. Conjugate vaccines are immunogenic in very young children, prime for memory responses and also provide "herd immunity" [34].

In 1983 the 23-valent polysaccharide vaccine (PPSV23) was released and included serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F, covering 90% of serotypes found in pneumococcal bacteraemia. This vaccine should only be used in children older than two years of age due to poor antibody responses in younger children [35]. When healthy adults are vaccinated antibody levels persist for at least 5 years, but may be shorter in adults with underlying illnesses.

In February 2000, the heptavalent pneumococcal conjugate vaccine (PCV7; serotypes 4, 9V, 14, 19F, 23F, 18C, and 6B) was licensed in the US and this vaccine included several serotypes that were resistant to antibiotics. In addition, it was highly effective in children <2 years of age. Higher valency vaccines, such as the 10-valent PCV (PCV10), which includes additional serotypes 1, 5, 7F, and 13-valent PCV (PCV13), which includes additional serotypes 1, 3, 5, 6A, 7F, 19A, later replaced PCV7. The 10 most common pneumococcal serotypes account for about 62% of invasive disease worldwide. The serotypes in PCV7 cause the highest incidence of IPD in the first 2 years of life and then decrease over the next few years of life [36].

Serotypes differ by age (6B, 9V, 14, 19F, are most common in young children) and by disease syndrome (serogroups 6, 10 and 23 are more frequently isolated from cerebrospinal fluid (CSF) than from blood, while serotypes 1, 4, and 14 are more frequently isolated from blood) [36]. Serotypes 1 and 7F are more likely to be invasive than carried, whereas other types are mainly involved in carriage. Carriage duration varies by age and is generally longer in children [36].

OUTBREAK PNEUMOCOCCAL SEROTYPES

Most of the historical pneumococcal outbreaks in the early part of the 20th century were caused by serotypes 1, 2, and 5 [37]. A reduction in outbreaks has been noted with very few reported in the present day [38]. Reasons for the reduction in outbreaks may be related to the availability of antibiotics which has changed transmission dynamics and colonisation, improvements in socioeconomic conditions and an increase in vaccine serotypes in recent years [39]. Viral respiratory infections in defined populations have been implicated in a

predisposition to pneumococcal pneumonia outbreaks with the predominant strains carried in that population [40-43].

Pneumococcal outbreaks usually occur in closed communities. Pre-2006 a systematic review of 42 outbreaks identified serotype 14 as the most common cause [44], but most studies included patients with underlying medical conditions in hospitals or care facilities and often included asymptomatic carriers [45]. When outbreaks were limited to non-hospitalised poor young adults and a single serotype, three out of the five studies were due to serotype 1. Two serotype 1 outbreaks were in homeless shelters and one in a military training facility [46,47].

Serotype 1 has been shown to cause outbreaks in Africa [48]. The African meningitis belt is a region of sub-Saharan Africa, extending from Senegal to Ethiopia that is characterised by hyperendemic seasonal peaks of acute bacterial meningitis and sporadic epidemics [49]. It is not clear why pneumococcal meningitis in the meningitis belt is seasonal and usually presents with one predominating serotype. Even though climatic factors increase the incidence of meningitis in the meningitis belt, these factors do not explain the increase in serotype 1 above other pneumococcal serotypes. In addition other socio-economic and underlying conditions predisposing to IPD are similar between other African countries and the meningitis belt.

A study comparing opsonophagocytic activity against pneumococcal disease in Burkina Faso and the United Kingdom (UK) showed that even though functional activity was generally low in both groups, a higher serotype 1 activity was observed during childhood in Burkina Faso, with similar levels during adolescence and adulthood in both populations [50]. Despite this disease incidence in the UK was much lower. The low level of functional antibodies was consistent with infrequent carriage of serotype 1 [51-53] and reduced natural immunity compared with other serotypes. A general defect in humoral immunity amongst the Burkina Faso population was ruled out. Climatic factors have been shown to play a key role in predisposing people in the meningitis belt to meningitis [53-55]. These environmental factors were thought to increase the incidence of serotype 1 in Burkina Faso [50] even though both the UK and Burkina Faso populations had low natural immunity to serotype 1.

Serotype 1 epidemiology

Serotype 1 is commonly isolated in IPD but rarely causes asymptomatic nasopharyngeal colonization [37,56]. Serotype 1 pneumococci have been isolated from swabs taken from ill patients with pneumonia [57] or close contacts of patients with serotype 1 IPD [58].

A meta-analysis demonstrated the difference in carriage duration between serotypes; with a shorter duration in serotype 1, which has a high attack rate, compared with other pneumococcal serotypes [59].

Serotype 1 has been shown to have lower antibiotic resistance than serotypes that are more commonly carried asymptomatically (e.g. 6B and 23F) [60,61]. Prior antibiotic use may clear susceptible serotype 1 pneumococci, making it less likely to be grown on culture [48]. Serotype 1 shows substantial year-to-year variability that is more marked than other serotypes [62]. In terms of the influence of vaccination on serotype 1, although it can't be excluded, there was data that showed that serotype 1 increased prior to PCV introduction in some countries [63,64] and had no correlation in others [61], likely due to the epidemic nature of serotype 1.

Serotype 1 was found to be an important cause of disease among older children and among HU children in the pre-PCV era in South Africa [17]. Serotype 1 IPD tends to occur in patients who are younger and healthier than IPD caused by other serotypes [65]. Serotype 1 is found across all age groups [36] and the age differences in reported rates may be due to relative differences in other serotypes. Compared to other serotypes, serotype 1 has lower morbidity and mortality [65,66] and frequently causes uncommon presentations of IPD, including empyema [67] and peritonitis [68].

The serotype 1 capsule contains zwitterionic polysaccharides which have sections of opposing charges, allowing it to elicit a T-cell dependent immune response [48]. This is in contrast to the capsular polysaccharides of other serotypes that function as T-cell independent antigens. The serotype 1 capsule has been shown to be more resistant to opsonisation and complement deposition than other serotypes, except for serotype 5 [69]; as such it is thought that the serotype 1 capsule may function in a different way to other serotypes resulting in a difference in virulence. There were also differences in pneumolysin in some serotype 1 isolates, which also impacts colonisation and virulence [48]. The zwitterionic polysaccharide capsule has been found to be related to abscess formation in animal models and thus cause empyema [48].

Serotype 1 is contained in both the PCV10 and PCV13 vaccines. This thesis focusses on the effects and impact of PCV13 only as this is the vaccine that replaced PCV7 in the routine national immunisation programme in South Africa.

RISK FACTORS OTHER THAN HIV-EXPOSED UNINFECTED CHILDREN

It is important to establish risk factors for pneumococcal disease in different settings and following different interventions such as antiretroviral treatment (ART) or PCV, to establish priority groups for public health interventions. Prior to the introduction of PCV there was limited data on risk factors for IPD in South Africa. Local studies showed a significant higher risk of IPD in HIV-infected children (41-fold) with "paediatric" pneumococcal serotypes, than –uninfected children [11]. HIV-infected children in this study had high rates of malnutrition and underlying tuberculosis. Other studies from the US also showed that a higher risk of pneumococcal disease with HIV was in the pre-ART era [70]. In contrast in HIV-uninfected children with IPD in South Africa had underlying infections, like chronic liver and renal disease in the pre-vaccine era [11]. Young children (<2 years of age) and the elderly are most at risk of invasive disease caused by the pneumococcus, as are males [71]. Other risk factors include alcoholism, smoking [72], day care for children and asplenia [73].

Also in the pre-vaccine era a case-control study in children <5 years of age in The Gambia showed lack of maternal income, exposure of the child to indoor smoke from cooking or cigarette smoke, poor weight gain and serious illness in the child in the last few months as risk factors associated with pneumococcal disease [74]. Another case-control study conducted in the US prior to the introduction of PCV7 identified a number of risk factors for IPD [75]. Cases of IPD were identified from a laboratory-based surveillance programme and controls from the community. On multivariable analysis recent day care attendance was associated with an increased risk in all age groups. In infants 2-11 months old IPD was associated with a decreased likelihood of current breastfeeding, in 12-23 month olds with recent antibiotic use and in older children (24-59 months) with crowding [75].

Following the introduction of PCV7 in the US in 2000 another case-control study (2001-2004) assessed the changes in risk factors associated with IPD [76]. In children who had received at least one PCV7 dose, the strongest risk factor for cases with vaccine-type IPD was an underlying medical condition; other factors included male gender, having no health insurance and been less likely to be up-to-date for *Haemophilus influenzae* type b (Hib) vaccination. Unvaccinated cases with VT-IPD had additional risk factors similar to those seen in the pre-PCV period, namely day care attendance and black race which was not seen in vaccinated children. Risk factors for NVT-IPD included children with underlying conditions (in households with smokers), day care attendance among black children, children from low-income households, male gender, history of asthma, low birth weight or grommets. Cases were less likely than controls to be up-to-date with Hib vaccination or to live in households with children younger than 18 years.

In the post-PCV period prior to our study, there was no data exploring the changes in risk groups for pneumococcal disease in South Africa. A case-control study in South Africa in the post-PCV era and published after our risk factor paper, assessed risk factors for presumed bacterial pneumonia hospitalisations in young HIV-uninfected children [77]. This study reported black race, malnutrition with (OR 3.30, 95% CI 2.28-4.79) and without crowding (OR 6.68, 95% CI 4.74-9.42), crowding among well-nourished children (OR 2.29, 95% CI 1.89-2.78), previous pneumonia hospitalisation (OR 2.35, 95%CI 1.65 to 3.35) and smoking by the primary caregiver (OR 5.15, 95%CI 2.94 to 9.03) as risk factors.

Risk factors for antibiotic non-susceptible disease have been shown to include previous hospital admissions due to antibiotic exposure, day care attendance due to crowding and highly likelihood of transmission, HIV infection and malnutrition possibly due to previous admissions and antibiotic therapy [78]. Female sex [79], probably due to transmission from children and pneumococcal serotypes commonly found in children ('paediatric serotypes'), due to prolonged carriage [80], are also associated with higher antibiotic resistance.

HIV-EXPOSED UNINFECTED CHILDREN

Groups of particular interest in South Africa are HI and HEU (HIV-exposed-uninfected) children in view of the fact that around a third of all pregnant women test positive for HIV at antenatal clinics nationally in the country [81]. An improvement in treatment to prevent the transmission of HIV from pregnant women to their babies has resulted in an increasing number of exposed but uninfected infants. Prior to our study, HIV exposure was shown to be associated with an increased risk of lower respiratory tract infections and bacterial infections, but had not been described as a specific risk factor for IPD.

A review article by Evans, et. al. highlighted the challenges when reviewing studies including HEU children [82]. The difficulties in reviewing these studies included that earlier studies did not include HIV testing in infants, follow-up testing was often not done for HIV-uninfected mothers and infants, HIV-unexposed groups were often not included for comparison, breast feeding practices differed between groups and HIV and ART exposures were often not described. Despite these limitations mortality in young HI and HEU children <2 years of age has been shown to be higher than that of HIV-unexposed-uninfected (HUU) children. Factors such as maternal CD4 count and health status impact the outcome in the HEU group [83]. A number of factors have been proposed to contribute to the poor health and nutrition among HEU children: lack of parental care, infant feeding practices, immune

abnormalities, exposure to other infections and antiretroviral drugs [84]. HEU children have been shown to have a significantly higher all-cause outpatient consultation rate and LRTI-associated visit rate in infants <6 months of age [85]. In South Africa, HEU infants hospitalised with pneumonia tended to have higher treatment failure rates than HUU infants [86]; HEU infants were prone to higher infectious morbidity [87] and were at higher risk for more severe infections requiring hospitalisation [88]. Data from high-income countries also showed a correlation between the risk of serious bacterial infections among HEU infants and maternal CD4 values during pregnancy [89].

A small cohort of 27 HEU and 28 HUU infants from South Africa in 2009 showed a non-significant three-fold increase in hospitalisations for infections in HEU compared with HUU children in the first year of life [88]. Most infections were respiratory tract infections. The HEU infants did not have more reported infectious events or higher rates of malnutrition. No comparison could be made with regards to the role of breastfeeding as only one HEU infant was breastfed.

In South Africa a case-control study which assessed risk factors for presumed bacterial pneumonia hospitalisations in HIV-uninfected children [77] identified that maternal HIV infection was a risk factor for pneumonia in infants who were exclusively breastfed (OR 2.33, 95% CI 1.53 to 3.55). These results differed from another study in Kenya which showed a decreased risk of pneumonia in breastfed HEU infants [90]. The authors of the South African study suggested that the inconsistent association between breastfeeding and pneumonia in their study may have been due to unmeasured confounding.

A pooled analysis which included a number of HIV prevention of mother-to-child transmission (PMTCT) studies demonstrated that risk factors for death among young HEU children included having a mother with a low CD4 count or who died [83]. In Tanzania low birth-weight was also a risk factor for death among HEU infants [91], while in Zimbabwe, mortality within the first 2 years of life was significantly higher amongst HEU than HUU children [92]. Other studies also showed advanced maternal disease to be a risk factor for mortality [89,93]. Additional risk factors for death among HEU children included low birth weight, male sex, maternal death, malnutrition [92], severe maternal anaemia, single mother and low household income. Mortality was highest in HEU infants <6 months of age mainly associated with lower respiratory tract infections [92].

A cohort study from 3 African countries showed differential mortality after 12 months of follow-up amongst HI (42%), HEU (7.2%) and HUU (4.8%) infants. Infants were more likely to die if their mother experienced a severe adverse event or died; and in HI mothers

with low CD4 counts or high viral loads [94]. In Zambia a cohort of 620 HEU infants were followed up to 4 months of age. Overall infant mortality was 4.6% and those whose mothers had low CD4 cell counts (≤350 cells/mL) were almost three times more likely to die or be hospitalised after adjusting for other factors. The most common cause of infant death and hospitalisation was pneumonia and/or sepsis [95].

General immunological responses in HIV-exposed-uninfected children

HEU children have been reported to have a number of distinct immunological differences from HUU children [96]. The magnitude of cytotoxic lymphocyte activity, CD4 helper T cell responses and natural killer cells responses in HEU infants have all been found to be associated with whether the infant is infected perinatally with HIV or not [97-99]. Transplacental transfer of specific maternal antibodies, including tetanus and measles antibodies, are reduced in HEU infants compared to HUU infants [100-103]. This reduction may be related to impaired B cell function, less efficient IgG placental transfer and myeloid dendritic cells activity [104]. Absolute CD4 and naive CD8 T cells have been found to be reduced in HEU children and some studies have shown thymic function disruption in HEU infants [105,106]. These immunological changes may have a negative impact on the HEU child's response to infection and to immunisation in early life. Their immune response to *S. pneumoniae* and other encapsulated bacteria, which requires functional antibodies, may also be reduced [96].

A number of metabolic and haematological changes have been reported in HEU infants exposed to ART during PMTCT [96]. Metabolic changes are likely caused by mitochondrial toxicity which can result in mitochondrial dysfunction with neuropathy, development of lactic acidosis, cardiac growth and functional abnormalities [107-109]. Haemoglobin levels, platelets, total lymphocytes, neutrophils, CD4 and CD8 T cell counts may all be reduced [110-112]. Further studies are required to determine the extent to which ART directly alters immune responses and to assess the functional significance of the changes in haematological parameters.

Impact of breastfeeding in HIV-exposed-uninfected children

Another explanation for a higher mortality in HEU compared with HUU infants is that HIV-infected mothers chose not to breastfeed or are unable to breastfeed due to advanced HIV

disease. Breast milk contains a number of different immune-related compounds including IgA, leukocytes, lysozyme, lactoferrin, interferon-ɣ and cytokines [113]. Some compounds prevent adherence of pathogens to the upper respiratory tract and gastrointestinal tract mucosa in the infant providing passive protection against invasive infections. Breast milk may also stimulate the child's own immune system. In developing countries breastfeeding reduces mortality from acute respiratory infection and diarrhoea [114], with a 6 times higher odds of mortality reported in non-breast-fed infants <2 months of age. Even in developed countries, studies have shown a protective effect of breastfeeding against acute infections [115,116].

VACCINE EFFICACY OF PNEUMOCOCCAL CONJUGATE VACCINES IN CLINICAL TRIALS

Vaccine probe studies can be used to estimate the total burden of pneumococcal disease incidence that is preventable by PCV by calculating the difference in disease incidence between vaccinated and unvaccinated people [117]. Estimates can be calculated separately for different disease syndromes. Vaccine probe studies can gauge the contribution of the pneumococcus to different clinical syndromes and explore causality in disease pathogenesis. Vaccine probe studies can use either a randomised or non-randomised design to measure the incidence of IPD pre- or post-PCV introduction and be incorporated into the design of a vaccine efficacy study or be applied retrospectively [117].

A Cochrane review exploring PCV effect on IPD and CXR pneumonia included eleven publications from 6 randomised control trials conducted in 5 different countries (South Africa, The Gambia, the USA, Philippines and Finland) [118]. These trials included 113,044 children <2 years of age; 57,015 who received PCV and 56,029 who received a placebo or another vaccine. The evidence on PCV efficacy against IPD was considered high quality while the evidence against pneumonia was of moderate quality. Of the 5 trials that included all-cause mortality data, none had sufficient numbers to explore this outcome and only 2 trials had data on all-cause admissions. The pooled vaccine efficacy (VE) for vaccine type IPD (VT-IPD) in HU children was 80% (95% confidence interval (CI): 58-90) while the VE for all serotype IPD was 58% (95% CI: 29-75) and the all-cause mortality VE was 11% (95% CI: -1-21%). The findings for HI children were similar although point estimates were slightly lower than in HU children [118].

Vaccine efficacy of pneumococcal vaccines in developed countries

A Kaiser Permanente trial was conducted in Northern California (NCKP study) from October 1995 to April 1999 to evaluate PCV7 efficacy [28]. The final analysis included 40 fully vaccinated IPD VT-IPD cases, 39 of which were controls, translating into a VE of 97.4% (95% CI: 82.7 to 99.9%). There was an 89.1% (95% CI: 73.7 to 95.8%) reduction in all-serotype IPD in children who had received ≥1 dose of PCV7. Another trial which used a group-randomised design to recruit Native American children ≤2 years of age demonstrated a per-protocol VE of 76.8% (95% CI: –9.4-95.1) [119]. None of the non-vaccine type (NVT) results were statistical significance.

Vaccine efficacy of pneumococcal vaccines in developing countries

Two clinical randomised, placebo-controlled, double-blind vaccine efficacy trials were conducted using a 9-valent PCV (PCV9) in Africa [21,29]. This vaccine included additional serotypes 1 and 5 which were not included in the PCV7 vaccine that was ultimately licensed. In the South African VE trial, a total of 39,836 children were included, 19,922 in the PCV9 group and 19,914 in the placebo group [21]. In the per-protocol analysis, the VE was 85% (95% CI: 32-98) for HU children and 65% (95% CI: 24-86) against VT-IPD (the primary endpoint) in HI children. In the Gambian trial 17,437 children were randomised of those screened, 8718 into the vaccine group and 8719 into the placebo group [29]. The original primary endpoint was all-cause childhood mortality, but this was changed to radiologically confirmed pneumonia due to concerns that the original study endpoint would not be met. Efficacy against a secondary endpoint of the study, VT-IPD was 77% (95% CI: 51–90).

VACCINE EFFECTIVENESS OF PNEUMOCOCCAL CONJUGATE VACCINES FROM CASE-CONTROL STUDIES

Vaccine effectiveness of pneumococcal vaccines in developed countries

In the US, a matched case-control study was conducted from 2001-2004 to assess the effectiveness of PCV7 against IPD following the introduction of this vaccine in 2000 [120]. Cases were identified using the Active Bacterial Core Surveillance programme and community controls from birth registers; matching was by age and zip code. Vaccines were

administered at 2, 4, 6 and 12–15 months of age. The study showed a high vaccine effectiveness against VT-IPD with ≥1 dose of PCV7 in healthy children (96% [95% CI: 93–98]) as well as good efficacy against penicillin non-susceptible disease (76% [95%CI: 63–85]) and multidrug-resistant strains (77% [95%CI: 62–86]).

A paper from the UK using enhanced surveillance data utilised the indirect cohort method to assess the vaccine effectiveness of PCV7 against VT-IPD and individual serotypes [121]. The analysis included all first episode IPD cases aged ≥5 months from November 2006 to May 2010. In children <14 months of age, the adjusted vaccine effectiveness for 1 dose was 56% (95% CI: -7-82) and for 2 doses was 83% (95% CI: 60-93); the vaccine effectiveness for 2 doses in children ≥14 months was 93% (95% CI: 70-98). The authors concluded that their vaccine effectiveness estimates were lower than in the US study possibly due to residual confounding.

Vaccine effectiveness of pneumococcal vaccines in South Africa

In South Africa, a matched case-control study was conducted to assess the effectiveness of PCV7 against IPD [122]. Cases of IPD were identified through a national laboratory-based surveillance programme while controls were enrolled from wards or outpatient departments at the same hospital as their case. Cases and controls were also matched by age and HIV status. The study was able to show that ≥2 PCV7 doses were effective against VT-IPD amongst HU children (74% [95% CI: 25–91]), but not against HI children ≥16 weeks of age (−12% [95% CI: −449-77]). Among HIV-exposed-uninfected (HEU) children, the vaccine effectiveness of ≥2 doses was 92% (95% CI: 47–99) against VT-IPD, while that against all-serotype multidrug-resistant IPD was 96% (95% CI: 62–100) among HU children. A single dose of PCV7 given at about 6 weeks provided no protection against VT-IPD, unlike the effectiveness shown in the USA [122].

A later analysis from the same South African case-control study determined PCV13 effectiveness (Cheryl Cohen, submitted). Amongst HU children the vaccine effectiveness of ≥2 PCV13 doses against PCV13-serotype IPD was 85% (95% CI: 37-96), 92% (95% CI: 40-99) against the 6 additional PCV13 serotypes and 52% (95% CI: -12-79) against all serotype IPD. When the data from both children who received PCV7 or PCV13 was combined the vaccine effectiveness against PCV7-serotype IPD was 87% (95% CI: 38-97) in HEU children and 90% (95% CI: 53-98) in HU malnourished children. Significant effectiveness could still not be shown for ≥2 doses amongst HI children (91% [95% CI: -35-100]) against PCV13-

serotype IPD or against the 6 additional PCV-13 serotypes (82% [95% CI: -155-100]) even though the point estimate was higher than that shown in the PCV7 study.

PNEUMOCOCCAL CONJUGATE VACCINE IMPACT

When using surveillance systems to monitor the impact of PCV it is important to remember the inherent biases associated with trend data [123]. There may be natural fluctuations in serotypes and changes in specimen-taking practices following vaccine introduction [124,125]. Post-vaccine introduction less blood cultures may be taken due to clinicians expecting less disease, resulting in an inflation of the change in VT disease and a reduction in NVT disease detection. Increases in trends may also be reported [126,127]. These changes in specimen-taking practices may be more marked in outpatient departments than hospitalised children. It is important to ensure that there is stability in the pre-vaccine years used for the baseline calculation of rates and sufficient post-vaccine years to determine the actual change in disease incidence and the extent of replacement disease [123]. Incomplete case ascertainment can make the establishment of a stable baseline for subsequent comparison difficult. In ecological studies changes in disease incidence following vaccine introduction cannot always be causally attributed to vaccine introduction. PCV effectiveness and impact data is now available from more than 50 countries (http://view-hub.org/viz/). Only a few of these studies are discussed in detail below.

Pneumococcal conjugate vaccine impact on invasive pneumococcal disease in developed countries

A meta-analysis of 21 datasets from different surveillance systems showed a reduction in all IPD and VT-IPD in children <5 years of age by 1 year after introduction of PCV7 and which continued till 7 years post-introduction [128]. Most sites showed an increase in NVT-IPD in at least one post-introduction year. In adults, VT-IPD decreased significantly by the second year following PCV7 introduction and was more gradual when compared to children. Only adults aged ≥50 years showed significant increases in NVT-IPD post-PCV introduction. The impact of PCV7 on VT-IPD has shown consistent results in high-income countries, with reductions in incidence ranging from 79% to 100% [129-137]. However the overall reduction in incidence of all serotype IPD showed more variation across studies (37-80%), likely due to differences in serotype distributions [129-139].

In the UK, PCV7 was introduced in 2006 and replaced by PCV13 in 2010. Comparing IPD incidence rates from 2004-2006 to those in 2009-2010, in children <2 years of age, there was a 46% reduction in all IPD, 97% reduction in VT disease and an 81% increase in NVT disease [136]. In the elderly (≥65 years) there was a 13% reduction in all IPD, 79% reduction in VT disease and a 48% increase in NVT disease. After PCV13 introduction, IPD incidence decreased by 32% across all ages by 2013-14 [140]. The incidence of PCV7 VT disease continued to decline in all age groups and the additional six serotypes in PCV13 also showed a significant decrease of 69%. Compared with the pre-PCV7 baseline years the overall incidence of IPD in 2013-14 was reduced by 56% and the incidence of PCV7 VT disease by 97%; the incidence of non-PCV13 IPD, compared with the pre-PCV baseline, increased by 28%.

In the USA, PCV7 was introduced in 2000 and replaced by PCV13 in 2010. In 2001 in children <2 years of age, compared with 1998-1999, the incidence of PCV7 VT disease was reduced by 78%, while all vaccine-related IPD (6A, 9A, 19A) was reduced by 50% and NVT disease increased by 27%; the latter was not significant [141]. Following PCV13 introduction, time series models were used to compare what would have been seen in the absence of PCV13 with what was actually observed [142]. In children <5 years of age in 2012-2013 there was a reduction of 64% in all IPD, 93% reduction in the additional 6 PCV13 serotypes and no evidence of replacement disease. In adults ≥65 years of age, there was a 12% reduction in all IPD and a 58% reduction in the additional 6 PCV13 serotypes. There was also no significant increase in NVT disease in this age group.

Pneumococcal conjugate vaccine impact on pneumonia and hospitalisation in developed countries

A number of studies have reported the impact of PCV7 on pneumonia-associated hospitalisation. Reported reductions in all-cause pneumonia hospitalisations in children post-PCV7 introduction range from 13% to 65% after introduction of PCV-7 [139,143,144]. It was suggested that the range of reductions was possibly due to differences between countries in the common serotypes causing pneumonia, the aetiology of pneumonia and differential duration of time since PCV7 introduction [145]. In the United States it was estimated that 41,000 all-cause pneumonia hospitalisations were avoided in children <2 years of age through the introduction of PCV7, a 39% (22-52%) reduction [143]; by the end of 2004, there was a 65% (47-77%) reduction in the rate of pneumococcal pneumonia admissions in

children <2 years of age and a 21% (6-34%) reduction in all age groups. The control group of dehydration admission rates showed no change in young children.

In Canada PCV7 was first introduced as part of the national immunisation programme in 2002. A review of data from 2004 to 2010 showed an overall decrease in the incidence of all-cause and pneumococcal pneumonia [146]. This was driven primarily by decreases in incidence in children <5 years and the elderly (≥65 years). In all age groups the incidence of all-cause pneumonia declined from 361 (359-364) per 100,000 in 2004-2005 to 347 (345-349) per 100,000 in 2009-2010 and for pneumococcal pneumonia from 6.40 (6.09-6.73) per 100,000 to 5.08 (4.81-5.36) per 100,000 in the same years.

Pneumococcal conjugate vaccine impact on invasive pneumococcal disease and carriage in developing countries

Studies on the impact of PCV on IPD are currently been conducted in at least 21 developing countries and impact on nasopharyngeal carriage in 18 countries (http://view-hub.org/viz/).

The KEMRI Wellcome Trust Research Programme in Kenya launched a study of PCV10 effectiveness in the routine childhood immunisation schedule in Kenya. The study endpoints included invasive pneumococcal disease, radiologically proven pneumonia and all-cause hospital admissions pre- and post-PCV10 introduction. Routine immunisation of children <12 months of age began in January 2011. The surveillance programme showed a significant reduction in VT and all serotype IPD by 2012 (http://www.kemri-wellcome.org). In 2012 PCV10 effectiveness was estimated to be 72% (95% CI 34–88) against VT-IPD in children <5 years of age. In terms of nasopharyngeal carriage, vaccine effectiveness for VT disease was 64% (95% CI 49–74) in children <5 years of age and 66% (38–82) in individuals ≥5 years of age [147]. There was a significant increase in NVT carriage in children <5 years of age 37% (95% CI 13–65); however overall there was a slight decline in pneumococcal carriage prevalence because the magnitude of the decline in VT carriage was greater than NVT increase [147].

To investigate the impact of PCV7 on pneumococcal nasopharyngeal carriage, a cluster-randomised trial was conducted in 21 villages in The Gambia over a 5 year period (December 2003 to June 2008) [148]. PCV7 was given to children <30 months of age in all the villages, while older children and adults were randomised to receive either one dose of PCV7 (11 vaccinated villages) or meningococcal serogroup C conjugate vaccine (10 control

villages). Nasopharyngeal swabs were collected in cross-sectional surveys before vaccination and at 3 points after vaccination. A time trend analysis demonstrated a reduction in VT pneumococcal carriage prevalence in all age groups in both villages following vaccination, showing both direct and indirect vaccine effects. The herd effect was shown from 6 months after vaccination and persisted for at least the next 2 years. Only small differences were noted in NVT pneumococcal carriage prevalence between the different villages [148].

A population-based surveillance system in the Gambia compared the incidence of baseline IPD rates pre-PCV13 (May 2008-May 2010) and rates post-PCV13 (Jan 2013-Dec 2014) introduction [149]. A total of 320 cases of IPD were identified over the 7 year period. In children 2-23 months of age there was a 55% (95% CI 30-71%) reduction in the incidence of all IPD and an 82% (95% CI 44-91%) reduction in the incidence of PCV13 serotypes. Similarly in children 2-4 years of age there was a 56% (95% CI 25-75%) reduction in the incidence of all IPD and a 68% (95% CI 39-83%) reduction in the incidence of PCV13 serotypes. Reductions in other age groups were not significant, as were the increases in non-vaccine type disease.

In South Africa a national, laboratory-based surveillance programme for IPD demonstrated a reduction in the incidence of disease, when rates were compared between four pre-vaccine (baseline) years (average of 2005 through 2008) and two different post-vaccine years (2011 and 2012) [150]. In children <2 years of age (vaccinated group), the incidence rates for all pneumococcal serotypes decreased from 54.8 to 17.0 cases per 100,000 person-years (69% reduction) between these 2 periods, while PCV7 serotypes showed an 89% reduction (32.1 to 3.4 cases per 100,000 person-years). The additional 5 serotypes (excluding 6A) in PCV13 and not in PCV7 also showed a significant 57% reduction by 2012. Among HU children, PCV7 VT disease decreased by 85%, whereas disease caused by non-PCV13 serotypes increased by 33%. Similarly in HI children PCV7 VT disease decreased by 86%, although the overall rate of disease was more than 20 fold greater than in HU children, and there was no significant change in non-PCV13 serotype disease. The absolute difference in HI children between VT and NVT disease was 55%. Among adults 25 to 44 years of age, there was evidence of herd immunity with the rate of PCV7 VT disease declining by 57%, and all serotype disease by 34% [150]. This surveillance programme does not capture outpatient or non-invasive disease.

Pneumococcal conjugate vaccine impact on pneumonia and hospitalisation in developing countries

There is limited data on PCV impact on pneumonia and hospitalisations from low and middle income countries. Various studies on the impact of PCV on pneumonia are ongoing in a number of developing countries (http://view-hub.org/viz/). Post–PCV7 introduction a study from Poland reported a 65% reduction in pneumonia hospitalisations [151] and a study from Uruguay showed a 56% reduction in CXR confirmed pneumonia [152]. A later study from Uruguay showed a continued reduction in hospitalisations for non-consolidated pneumonia (46.4%) in the post-vaccine era and an overall decrease in consolidated pneumonia (27.3%) despite an slight increase in numbers in 2012 [153]. The authors investigated possible causes for this increase including changes in the diagnosis or hospitalisation of patients with suspected pneumonia, but could find none. They suspected an increase in other bacterial aetiologies. In Brazil, soon after the introduction of PCV10, an interrupted time-series analysis measured the rates of hospitalisation for pneumonia and non-respiratory causes among children in 5 cities [154]. During the post-vaccination period there was a significant difference in the reductions of pneumonia hospitalisation rates and non-respiratory hospitalisation rates in 3 of the cities.

REPLACEMENT PNEUMOCOCCAL DISEASE

With the expanded use of PCV globally it is important to estimate the scale of serotype replacement to determine the net decline in disease and the overall benefit of vaccination. The magnitude of the increase in non-vaccine serotypes varies between different settings and is depended on host and *S.pneumoniae* population level characteristics [123].

Randomised-controlled clinical trials are unable to demonstrate indirect effects or replacement disease due to the fact that only a limited number of individuals are vaccinated [155-157]. Data on the extent of replacement disease is mainly being derived from observational studies which are subject to various biases. Replacement is more marked in well-vaccinated populations [158].

Vaccination with conjugate vaccines has resulted in a change in pneumococci colonising the nasopharynx, from mainly VT serotypes to predominantly NVT serotypes with little or no net change in the bacterial carriage prevalence [21,123,159,160]. The increase in NVT carriage may partially be due to the artefact known as “unmasking” [161] in which the reduction in prevalence of VT serotypes has made it easier to detect the presence of NVT serotypes in the population. Other contributing factors may be the increase in acquisition of new serotypes [162] and a higher NVT colonisation density in vaccinated individuals [163].

NVT-IPD incidence has also increased [123]. In the USA, 10 years after PCV7 introduction, the surveillance system showed a decrease in VT disease and increase in NVT disease especially serotype 19A. The increase was most apparent among hospitalised cases. The incidence of NVT disease in children <5 years of age increased from 16·8 per 100 000 population in 1998–99 to 22·1 per 100 000 population in 2006–7 (32%); however the overall rate of IPD in the paediatric population was still significantly lower than in the pre-vaccine era [137].

The increase in NVT infections in England and Wales post-PCV7 introduction was greater than that reported in the USA [136]. This may be as most children in the UK programme were hospitalised, whereas the US surveillance system also included non-hospitalised children (68% of <5 year olds in 1998–99) [137] resulting in a substantially higher prevalence of IPD in the USA compared with England and Wales. For pneumococcal meningitis the increase in NVT cases in children <5 years in the USA and England/Wales was similar at over 70% [136,137]. The increase in NVT disease in individuals aged 65 years or older in the USA was 32% by 2006–07 [137] compared with 48% in England and Wales by 2009–10 [136]. Serotypes 17F and 19A were major causes of replacement pre-PCV13 introduction [123,136], while data regarding serotype 1 was contradictory [21,29]. In 2013-14, post-PCV13 introduction, non-PCV13 serotypes increased significantly in children <5 years and adults ≥45 years, compared with the pre-PCV13 baseline across all age groups. For children <5 years, the increase in non-PCV13 IPD compared with the pre-PCV13 baseline was most marked in 2013-14 and at this point the overall IPD incidence was higher than 2012-13 [140].

Increases in NVT disease are likely due to a number of factors; however vaccination probable provides the strongest pressure for this increase. Other contributing factors may include antibiotic use and resistance and long-term secular trends [164-166].

A meta-analysis, including 19 datasets in children <5 years, showed a significant increase in NVT-IPD rates by 2 years post-PCV introduction, which increased through 5 years, with some plateauing till year 7 [128].

There is a possibility that serotype replacement could substantially reduce the impact and benefits of vaccines. Replacement serotypes could be associated with resistance, even multidrug resistance. The amount of replacement thus far, especially with PCV13 use (which covers serotype 19A) is small in comparison to the overall reduction in disease. However there is a need for broader valency vaccines, universal vaccines and adequate surveillance [128].

JUSTIFICATION AND OBJECTIVES

There are limited data on the epidemiology of pneumococcal disease from Africa and other developing countries. As PCV is gradually introduced into developing countries, it is important to have good data regarding pneumococcal disease and the impact of the vaccine. As South Africa was the first African country to introduce PCV into its routine national immunisation programme and there is a long-standing national pneumococcal surveillance programme with good baseline data, it is a good platform to describe the changes in pneumococcal serotypes and the overall impact of PCV on disease burden. We used the IPD surveillance data to build a burden model to describe the overall burden of hospitalisations and deaths in the pre- and post-PCV period. We also calculated the proportion of disease averted as all these components are important for health policy.

Serotype 1 pneumococcal disease has been shown to have distinctive clinical and microbiological characteristics in other studies and is an important cause of disease in low and middle income countries. Although it has been shown to cause outbreaks in the African meningitis belt the epidemiology of serotype 1 is likely different in South Africa a high HIV prevalence setting. Serotype 1 is contained in the PCV13 vaccine and it is important to describe its epidemiology in the post-vaccine era. There is conflicting data regarding whether PCV13 will result in a reduction in serotype 1 disease and in view of its epidemic nature ongoing surveillance is important.

Risk factors for pneumococcal disease in South Africa are anticipated to differ somewhat from those traditionally reported from developed countries as there are high rates of HIV and malnutrition and lower socioeconomic conditions in certain communities. HIV-infected children have been shown to have an increased risk of IPD but data are limited on other risk factors for IPD in South Africa. It is important to describe the change in risk in these children the era of PCV and paediatric HIV treatment and care. In HIV-uninfected children it is important to describe the risk factors for ongoing disease in the era of PCV. The identification of risk groups guides policy makers with regards to allocation of resources and clinical management by alerting clinicians to who may present with pneumococcal disease and who to treat empirically.

With improvements in prevention of mother-to-child HIV transmission (PMTCT) programmes and high maternal antenatal HIV prevalence rates, there has been a reduction in HIV-infected children but an increasing number of HEU infants. There were no published

data exploring the risk of IPD-associated hospitalization or mortality amongst HEU children at the time of conceptualisation of this PhD.

This thesis aimed to assess the public health impact of introducing the pneumococcal conjugate vaccine into the Expanded Programme on Immunisation in South Africa (EPI-SA), including the changes in disease burden and risk factor groups in the country as well as trends in disease caused by specific important serotypes. It is hoped that the findings from this thesis will be useful in guiding future pneumococcal vaccination policies and development in South Africa and other developing countries. The thesis focused predominantly on children <5 years of age where the greatest effects of PCV were observed. For the serotype 1 analysis all age groups were included to provide a complete picture of disease clustering for this serotype.

The primary objectives for this thesis were:

1. To estimate the burden of hospitalised pneumococcal cases and in-hospital deaths caused by severe pneumococcal clinical syndromes (meningitis, bacteraemic and non-bacteraemic pneumonia, and non-pneumonia non-meningitis invasive disease), among HI and HU children <5 years in South Africa, in the pre-vaccine (2005-2008) and post-vaccine (2013) era (Paper I).
2. To describe the epidemiology of IPD due to serotype 1 in all age groups in South Africa from 2003 to 2013, including the impact of PCV (Paper I).
3. To describe the epidemiology of IPD in HEU children <1 year of age, compared with HUU and HI children in South Africa from 2009 through 2013 and include changes over this time period due to PCV and paediatric antiretroviral treatment introduction (Paper III).
4. To describe the risk factors related to IPD in HI and HU South African children eligible to receive PCV7 through the EPI-SA and the changes in risk factors associated with vaccination between 2010 and 2012 (Paper IV).

METHODS

STUDY SETTING

In 2015, South Africa had a total population of around 54,96 million people with an annual birth rate of 22,7 per 1000 and an infant mortality rate of 34,4 per 1000 live births. The HIV prevalence rate was approximately 11.2% in the general South African population in 2015 [167], as compared with 18.8% in 2008 [168]. The antenatal HIV prevalence rate remained stable at around 30% from 2004 [81], while the HIV prevalence in children <5 years of age decreased over time from 4.4% in 2006 to 3.5% by 2013 due to marked improvements in the PMTCT programme; the estimated perinatal HIV transmission rate decreased from 16.4% in 2006 to 2.4% in 2012 [169,170]. The number of HI infants identified under the age of 2 months, fell by 46% between 2008 and 2012 [170]. Access to ART has gradually improved since its introduction in the public sector in South Africa in 2004, and the overall estimated coverage in HI children requiring treatment was 63% in 2012 [171,172]. The number of women newly infected with HIV declined by 21% from 2009 to 2012 in South Africa.

PCV7 was available in the private sector and limited areas of the public health sector in South Africa from 2008. The vaccine was introduced into the EPI-SA in April 2009. It was replaced by PCV13 from May 2011. PCV is administered in the EPI as 2 primary doses (6 and 14 weeks) with a booster dose at 9 months. This differs from the schedule that was used in the vaccine efficacy trial (6, 10 and 14 weeks) conducted in South Africa with PCV9 [21].

There are a number of different estimates of vaccination coverage rates for PCV in South Africa, all of which have limitations [173]. According to WHO-UNICEF estimates, vaccination coverage for the third dose of PCV increased from 10% in 2009 to 65% in 2014, while official administrative country estimates reported 94% in 2014.

STUDY DESIGN

Data from a number of studies was utilised in order to address the objectives of this thesis. Descriptions of the study methods and statistical analyses are discussed in detail within the methods sections of each paper. A brief description of methods as well as some additional issues related to different study designs is discussed below.

Burden models

Burden of disease models usually combine multiple data sources to calculate the number of cases and deaths from defined diseases in a certain population. For our burden model we used a robust active laboratory-based surveillance programme for the baseline case numbers for meningitis, bacteraemic pneumonia and non-meningitis non-pneumonia syndromes, adjusting for differences in specimen taking practices. We calculated non-bacteraemic pneumonia cases using data from a vaccine probe study [20]. We applied observed case fatality ratios from hospitalised cases to calculate death rates. This approach differed from previous estimates as it used a bottom-up approach.

We conducted one-way sensitivity analyses adjusting one variable at a time using different assumptions. Adjusted variables included community HIV prevalence, deaths in the community, a specimen-taking practice adjustment based on IPD incidence rates from a clinical trial conducted in South Africa, different adjustment rates for HIV-infected and –uninfected children and a lower vaccine attributable reduction ratio for chest X-ray confirmed pneumonia instead of clinical pneumonia, compared to bacteraemic pneumonia. For death rates we also explored different case fatality ratios. Tornado diagrams were used to depict the sensitivity of the case and death estimates to changes in selected variables. These diagrams show the effect on the base rate by varying each input variable one at a time, while keeping all the other input variables at their initial base value. High and low values may be chosen for each input. The results are displayed as a bar graph with the variation for each variable from the base rate.

To account for uncertainty in our estimates and calculate confidence intervals we used bootstrapping. It is usually not feasible to sample repeatedly from the same population so the best estimate is to resample randomly from a sample. Every resample has the same number of observations as the original sample, so the bootstrap method models the impact of the actual sample size [174]. Bootstrapping allows measures of accuracy to be assigned to sample estimates, verifies replicability of results and allows inferences to be made regarding key parameters. The simulated samples were used to calculate an estimated population distribution.

Spatial-temporal analysis for detection of IPD clusters

Outbreaks or clusters of serotype 1 pneumococcal disease have not been previously described in South Africa despite a long standing surveillance programme, but this may have simply been due to the fact that they were missed. To determine whether any serotype 1 clusters occurred within an 11 year period (2003-2013) we conducted a space-time scan analysis using a Bernoulli model [175,176] to compare cases (serotype 1 IPD cases) to controls (non-serotype 1 IPD cases) rather than a rate (Poisson) model. The space-time analysis was implemented using SatScanTM version 9.3.1 (http://www.satscan.org/) [177]. SatScanTM is able to detect spatial, temporal or spatio-temporal disease clusters and determine whether they are random and whether they are significant or not. For Bernoulli models SaTScan uses a shifting window of varying proportions to evaluate clusters. The area of search varies in size, shape and direction. At each point SaTScan calculates the number of observed and expected observations and the likelihood function.

A Poisson-based model can be used when events in a geographical area are Poisson-distributed and assumed to be independent with a known underlying population at risk. The dependent variable is a count of the number of cases that occur over a follow-up period. One can estimate a rate ratio associated with a given predictor or exposure. A Bernoulli model is a discrete-time stochastic model that takes only two values, 0 (controls) and 1 (cases) and determines whether there is a significant difference in clustering of cases versus that of controls. The SaTScan Bernoulli model uses a likelihood ratio test of the probability of a group of patients within a potential cluster being a case versus a control [178]. SaTScan uses elliptical windows to identify potential cluster boundaries and this may not be a realistic representation of the population at risk.

We chose to use a Bernoulli as opposed to a Poisson model for a number of reasons. Firstly we felt that the comparison of cases to controls from the same geographical area and time period would minimise biases potentially introduced by temporal and geographical differences in specimen-taking practices, healthcare seeking behaviour or improvements of the surveillance system over time. Secondly the Bernoulli model is independent of the underlying population distribution. Following the introduction of PCV7 from 2009 there was a reduction in control numbers; to account for this we adjusted positively by the percent reduction from the pre-vaccine period [150]. We obtained average monthly estimated reductions in control numbers (from 2009 to 2013) assuming that there was no PCV introduction. Monthly adjustment factors were also differentiated by province as PCV7 uptake was

assumed to differ by geographical area. It was necessary to adjust controls to obtain a stable baseline by essentially removing the impact of PCV7 on serotypes in this group; this allowed for true increases in cases to be identified and not just relative case changes.

Surveillance data for monitoring trends in IPD

A number of countries have population-based surveillance programmes for monitoring infectious diseases. In South Africa, surveillance for IPD started in 1999 [179] and the programme was expanded into a more comprehensive national active laboratory-based surveillance system, called GERMS-SA (Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa) in 2003. This robust surveillance system provided us with good baseline data pre-PCV introduction to show trends post-PCV introduction [150]. Cases of IPD were considered as hospitalized individuals with *S. pneumoniae* cultured from normally sterile site specimens. The GERMS-SA programme includes over 200 microbiological laboratories ("non-enhanced" sites) which submit isolates and basic demographic information (age, gender, date of specimen collection, and body fluid source of isolate). Trained surveillance officers at 24 sentinel hospitals ("enhanced" sites) located in all nine provinces collect additional data including HIV status, discharge diagnosis and outcome.

A number of factors can influence trend data besides the exposure of interest, in this case PCV. There was an improvement in healthcare services, ART for adults and children and HIV PMTCT programmes over the same period which also would have impacted trend data and needed to be accounted for in interpretation of PCV impact data [150].

Surveillance data was used in three of the manuscripts included in this PhD. Firstly it was used for the baseline rates of the pneumococcal burden model for the different invasive clinical syndromes in both the pre- (2005-2008) and post-PCV (2013) period. In addition to the introduction of PCV, other interventions were instituted in South Africa between these two periods. We attempted to account for these other changes by using adjusted denominators stratified by HIV status, and using actual case numbers with altered HIV prevalence rates. Secondly it was used to calculate IPD incidence rates in HEU, HUU and HI infants in one year pre- (2009) and post-PCV (2013) introduction using adjusted denominators as before. Lastly we also used surveillance data in all age groups to demonstrate changes in serotype 1 pneumococcal trends over an 11 year period using pre- (2003-2008) and post-PCV (2013) time points.

Case-control study

A case-control study design was used to explore risk factors for pneumococcal disease in children; this was a secondary objective of the study, the primary being vaccine effectiveness. In our case-control study [122] which enrolled cases with IPD from 24 sentinel hospitals, there was only one group of controls. Controls were children hospitalised in wards or attending outpatient departments at the same hospital as the case. This group of controls were chosen based on convenience and the fact that we did not have mechanisms in place to enrol community controls e.g. through registers or telephone lists. We matched for age, as vaccination status is integrally linked to age; HIV status, as PCV efficacy differed in HU and HI children in the South African clinical trial; and hospital site as access to care differs between different provinces of South Africa. We explored risk factors associated with all-serotype, VT, NVT and penicillin nonsusceptible IPD. We also determined the change in risk factors associated with PCV introduction by stratifying by vaccination status.

RESULTS AND DISCUSSION

Detailed study results are presented in full within the results sections of each paper. Here we discuss some of the main issues arising from the results.

Surveillance data was used as the baseline for most of our studies. There are inherent limitations in the use of surveillance data. Our laboratory-based surveillance programme underestimates the full burden of pneumococcal disease as it only includes patients who present to healthcare facilities and have samples taken; we aimed to address this in our burden model (paper I). There is often missing data associated with the use of surveillance programmes; we assumed that data were missing at random and imputed values for serotype for trend analyses. Interventions other than vaccination, for example PMTCT and ART, impact IPD disease trends. It is often difficult to tease out the proportional contribution of all these strategies on disease reduction using surveillance data.

BURDEN OF POTENTIALLY VACCINE-PREVENTABLE PNEUMOCOCCAL DISEASE IN CHILDREN

Summary of results and comparison with other burden models

The estimates from our burden model demonstrated that approximately 196,100 (178,500-210,400) total cases of severe hospitalised pneumococcal disease occurred annually in the pre-vaccine era. In 2013, 5 years after PCV introduction, only 67,300 total cases were estimated, a rate reduction of 2528 cases per 100,000 child-years. In terms of deaths approximately 8600 (7000-10200) pneumococcal-related annual deaths were estimated in the pre-vaccine period and 3600 in 2013, a rate difference of 99 per 100,000 child-years. These changes were assumed to be due to PCV as well as HIV-related interventions.

Prior to our study, the only available burden estimates for pneumococcal disease for South Africa were derived from global pneumococcal models which included data on South Africa using a proportional mortality top down approach to calculate burden of disease.

Most national disease burden estimates for pneumococcal disease are derived from models that use country specific inputs with a pneumonia component which is calculated from randomised control trial data. Data is then aggregated in a meta-analysis at the global level. In contrast we used country-specific surveillance data on IPD as the input data for

building our burden model, a bottom up approach, and adjusted for underestimation in case ascertainment. Our model differed from the global model of O'Brien et al. in its conceptual approach and in terms of several input parameters; it allowed us to assess the degree to which the models calculated similar or different disease rates. Death rates for South Africa reported by the pre-PCV era global model [15] were 101 (72-140) per 100,000 py for total pneumococcal death rates, 9 (6-25) per 100,000 py for meningitis and 82 per 100,000 (60-90) py for pneumonia in the <5 year old age group in 2000. Rates from our model for the pre-vaccine period (2005-2008) were similar: 166 (87-261), 7 (6-8) and 139 (59-243) per 100,000 py in these respective groups, but our rates in 2013 were lower than an updated model [180] which reported 203 (164-241) per 100,000 py for total pneumococcal death rates, 19 (16-23) per 100,000 py for meningitis and 166 (133-198) per 100,000 py for pneumonia in the <5 year old age group.

As mentioned the pneumococcal global burden paper used three different methods for determining incidence and death rates for different pneumococcal syndromes [15]. Both this model and our model used an incidence based approach for meningitis. We were able to use this approach as we had actual meningitis case numbers and appropriate case fatality rates. The O'Brien model adjusted case fatality rates for access to care, while we adjusted our meningitis estimates for specimen taking practices, resulting in similar estimates. The global model used a proportional approach for pneumonia using vaccine efficacy trial results [20,21,28-32] for WHO-defined clinical pneumonia to determine the proportion of cases attributable to *S. pneumoniae* and WHO-defined CXR positive pneumonia for deaths. For pneumonia we used actual case numbers and CFRs for bacteraemic pneumonia and used vaccine efficacy trial results [20] to estimate non-bacteraemic pneumonia numbers. There are limitations in using the vaccine probe approach for allocating aetiological fractions; clinical definitions err on the side of higher sensitivity but low specificity in detecting pneumococcal pneumonia. The global model used triangulation to determine the burden of NPNM invasive disease from meningitis cases while our model used actual non-meningitis non-pneumonia cases. Lastly the global model calculated numbers of HIV-infected children by using HIV prevalence rates while our model used HIV data from the surveillance programme.

Burden of disease models usually combine multiple data sources to calculate the number of cases and deaths from defined diseases in a certain population. Disease burden may be reported as incidence rates as described in our model; however disability-adjusted life years (DALYs) or quality-adjusted life years (QALYs) measurements may also be used [181]. Death rates are used to compare mortality in different regions of the world and vital

registration systems are often used. These registration systems usually underestimate the true number of deaths [182]. In our model we used death rates observed in hospitalised cases captured through our surveillance programme.

Considerations for interpreting burden model results

Burden models are often particular to the setting in which they were calculated as they usually use locally specific data. The choice of potential covariates and structure of the model introduces uncertainty and variability into the model. It is important to account for model uncertainty otherwise standard error calculations will underestimate the overall equivocality of the results. Bayesian model averaging or stratification of data may be used to deal with the variability [183].

A pathogen-based incidence approach like our burden model usually uses data acquired from different surveillance sources, including notification or lab surveillance data for symptomatic cases, hospital data for hospitalised cases and vital registration systems for death rates. Data from these routine surveillance sources need to be adjusted when estimating disease burden to correct for underreporting (misdiagnosis) and under-ascertainment (cases who never seek health care). The multiplication factors used to adjust the data should be age-specific and disease-specific at minimum [184].

As previously mentioned, in South Africa in the post-PCV period a number of other interventions, including improvements in PMTCT and paediatric ARTs changed the profile of pneumococcal disease in HIV-infected children. Data was not available for all adjustment parameters in the post-PCV era resulting in some pre-PCV parameters been used for these calculations. This may have overestimated the contribution of HIV to pneumococcal disease in 2013 and inflated our calculation of case numbers and deaths. In 2013, the estimated number of deaths in children <5 years of age for South Africa was 44000 (35000-55000), 40% during the neonatal period and 15% due to pneumonia [185]. Our model estimated that approximately 8% (3600) of these deaths were still due to severe pneumococcal disease, mostly pneumonia. This has important implications for management guidelines for pneumonia including choice of antibiotics and taking of blood cultures. In addition continued surveillance to monitor changes in pneumococcal disease especially replacement serotypes is important.

Generalisability of results and accounting for variability

Our burden model was based on national laboratory-based surveillance data which included isolates from both urban and rural sites. It is likely that representivity differed between these sites, however our model adjusted for differences in specimen taking practices assuming that urban sites and certain provinces with more academic facilities were more likely to take specimens. This adjustment was also assumed to account for access to care and deaths prior to reaching facilities (which is likely higher in rural areas).

We considered alternative parameters and adjustment factors by conducting one-way sensitivity analyses by changing a single variable at a time. We explored changing the HIV prevalence (community versus hospitalised patients), using different specimen-taking estimates and lower vaccine attributable reduction ratios which all lowered our case estimates. Using alternative non-bacteraemic pneumonia estimates and adding community deaths increased our estimates

To account for variability and uncertainty around our estimates we used bootstrapping, a robust statistical method, to calculate confidence intervals. Bootstrapping relies on fewer assumptions than more traditional statistical approaches but is often more accurate in certain circumstances, requires smaller sample sizes and is easier to implement for complicated statistics. Bootstrapping assumes that each sample is identically and independently distributed. It draws many more sub-samples than other methods and provides less biased and more consistent results than the Jackknife method for example [186].

Remaining gaps and implications following our study

Our burden model only estimated the burden of severe pneumococcal disease in children <5 years of age in a middle income country. Pneumococcal burden estimates are still required for older children and adults as well as for non-invasive pneumococcal disease, for example otitis media, in order to build the complete burden pyramid. In addition the cost of pneumococcal disease in our setting and the cost-effectiveness of PCV in South Africa would be important to determine.

We were not able to accurately estimate the individual contribution of PCV and HIV interventions (ART and PMTCT) to the reduction in IPD observed in 2013. Based on findings from the surveillance programme which showed the reduction in IPD between 2005-2008 and 2012 [150] we made some assumptions regarding the impact of PCV. We assumed that all reductions in PCV7 serotypes (85%) in HIV-uninfected children <2 years of age were

due to PCV7, while in HIV-infected children <2 years of age the impact of PCV (55%) was assumed to be the difference between changes in the PCV7 serotype rates (86%) and NVT serotype rates (31%), the latter assumed to be due to the effect of ART.

In our study we noted differential changes in PCV13 and non-PCV13 serotypes in 2013 in HIV-infected and HIV-uninfected children by syndrome. In HIV-infected meningitis cases aged <5 years, there was a reduction in both PCV13 (90%) and non-PCV13 (22%) serotypes; while in contrast PCV13 serotypes were reduced (90%) in HIV-uninfected children, but non-PCV13 serotypes increased by 27%. A similar picture was seen with non-pneumonia non-meningitis rates. For incidence rates in bacteraemic and non-bacteraemic pneumonia cases, there was a decrease in PCV13 serotypes in HIV-infected (89%) and –uninfected (83%) children, while non-PCV13 serotypes increased in both groups (1.1 fold in HIV-infected and 1.9 fold in HIV-uninfected children). For all syndromes combined for children aged <5 years there was a 85% reduction in PCV13 serotypes in HIV-infected children with a 75% increase in non-PCV13 serotypes; in HIV-uninfected children there was an 83% reduction and a 98% increase, respectively. The increases in non-PCV13 serotypes are indicative of replacement disease which has been observed in surveillance data for children <5 years of age from the UK [187], but not in the US [188].

EPIDEMIOLOGY OF SEROTYPE 1 INVASIVE PNEUMOCOCCAL DISEASE

Summary of results and comparison with other serotype 1 studies

Serotype 1 (ST1) IPD has been described to have a number of distinct characteristics when compared to other pneumococcal serotypes. In our study we demonstrated that over 11 years of surveillance (2003-2013), the incidence of serotype 1 (ST1) IPD fluctuated significantly with two clusters of ST1 IPD from May 2003 to December 2004 and September 2008 to April 2012. ST1 is amongst the most frequently isolated IPD serotype and has been shown in other studies to fluctuate year-on-year and cause outbreaks [189,190].

When we explored factors associated with ST1 IPD, compared with all other serotypes, in children <5 years of age we found that older children (1-4 years) were more likely to have ST1 IPD than the youngest group (<1 year). In the <5 year olds, the most common serotypes prior to PCV introduction (2003-2008) were 14, 6B, 6A, 19F, 23F and 19A; while in the post-PCV era (2010-2013) in the same group, serotypes 19A, 6A, 8, 23F, 6B, 19F and 35B had the highest numbers. ST1 IPD differed by province and year

demonstrating its epidemic-prone nature. Young children with ST1 IPD were hospitalised for shorter periods, were less likely to have HIV or die or have resistant disease compared with children with non-ST1 IPD. Other studies have shown that ST1 has low mortality and rarely causes antibiotic resistance. ST1 has a short duration of carriage which allows reduced opportunity for recombination, genetic diversity and antibiotic resistance [48].

Serotype 1 was the most common serotype in older children and adults (≥5 years) in the pre-vaccine era and the post-vaccine era. These older individuals with ST1 IPD had lower rates of hospitalisation and were hospitalised for shorter periods when compared to individuals with non-ST1 IPD. HIV and resistance rates were also lower and pneumonia was more common than meningitis in ST1 IPD. ST1 tends to affect young adults without underlying conditions and frequently causes uncommon clinical manifestations of pneumococcal disease such as empyema and peritonitis [48]. When ST1 is associated with pneumonia it is usually bacteraemic pneumonia [191]. When we analysed factors associated with in-hospital deaths in patients with ST1 IPD, the extremely young (<1 year), those with underlying conditions and those with meningitis were more likely to die. In older individuals the elderly and extremely ill were also more likely to die. Our findings regarding ST1 disease, i.e. syndrome, age and underlying conditions, were similar to a study from Israel (2000-2009) [192].

Considerations for interpreting cluster results

Spatial epidemiology has been described as the "study of spatial variation in disease risk or incidence" [193] and it can be used to advise public health decision making [194]. Our spatio-temporal analysis identified two large serotype 1 clusters, which were not recognised prospectively, due to the limitations of laboratory-based surveillance data. There is a delay in the processing and serotyping of samples from laboratory-based surveillance which hinders the ability to identify community-wide clusters in real time. In addition clusters were over prolonged periods and across a number of provinces which complicated identification. A cluster is considered as a number of health events (in our study serotype 1 pneumococcal cases) situated in close proximity in space and/or time. Clusters may be detected using a number of different techniques which are based on cell counts, or on adjacent high cell counts, or on distance between events [195]. A large cluster may engulf surrounding regions which don't have an elevated risk of disease [196].

We explored clustering of other epidemic serotypes, i.e. serotypes 5 and 8, over the study period to assist in the interpretation of the two ST1 clusters. These serotypes fluctuated at low levels with small numbers. When we modelled serotype 5 changes in children <5 years of age, we ascertained that compared with 2005, which was considered the baseline, there were no significant increases in case numbers. Most years showed a decrease in numbers, especially 2013. Similar findings were found in individuals ≥5 years of age. When we modelled serotype 8 changes in children <5 years of age, compared with 2005, there were increasing trends in case numbers from 2011-2013 but none were significant. In persons ≥5 years of age, there were non-significant increases in 2006-2008 and 2012-2013.

We used a Geographic Information System (GIS) to analyse and present our serotype 1 cluster data spatially-referenced data. GIS is able to handle large volumes of data with repetitive tasks and compare spatial data from various sources and different spatial areas. However, GIS data does not always adequately represent spatial-temporal information as it usually represents static points in time [195]. We were able to demonstrate two clusters of serotype 1 by district level during May 2003 to December 2004 (Gauteng, Mpumalanga, Limpopo and North-West Provinces) and September 2008 to April 2012 (KwaZulu-Natal, Free State, Gauteng, North-West, Mpumalanga and Eastern Cape Provinces). We were only able to map ST1 IPD incidence for all age groups at district level, so minor changes in incidence and clusters at the individual healthcare facility level may have been missed.

Generalisability of results

Only patients who had relevant samples taken were identified as IPD cases and included in our surveillance programme. This means that more severe cases that potentially did not reach a health facility or died soon after admission would not be represented by the surveillance programme. Most of our enhanced sites were urban or peri-urban sites which may not be completely reflective of rural sites.

Remaining gaps and other considerations following our study

The PCV13 vaccine has been found to induce antibodies against the serotype 1 capsule in children; however in two clinical trials from The Gambia and South Africa using PCV9, protection could not be demonstrated against serotype 1 clinical disease, although case numbers were small [197,198]. In addition these two trials, which used three infant vaccine doses

only, showed that serotype 1 vaccine failures occurred after 12 months of age [199]. It was suggested that a booster dose may be necessary for protection against this serotype. As mentioned the serotype 1 capsular polysaccharide is particularly resistant to opsonisation and it is uncertain whether protein-conjugate vaccination will be as effective against serotype 1 disease as against other serotypes.

In the United Kingdom PCV13 was introduced in April 2010 in a 2+1 schedule. Annual serotype specific incidence rates showed a significant reduction in serotype 1 rates between 2008-2010 and 2013-2014 in all age groups [140]. An indirect cohort study, including data up to 3.5 years after PCV13 introduction, showed protection against serotype 1 with a vaccine effectiveness of 84% (95% CI 54-95) [200].

In South Africa a case control study demonstrated a high vaccine effectiveness point estimate against serotype 1 (89% [95% CI -82-100]) although the results were not statistically significant (Cohen 2016, submitted). In the USA no reductions in serotype 1 incidence rates were shown, but case numbers were small [142].

As ST1 is an outbreak serotype with fluctuating annual cases it is important for surveillance programmes to monitor for increasing case numbers. The main aim of monitoring for outbreaks would be to determine if there are any modifiable risk factors or public health changes which could be made to stop the spread of the outbreak. In addition it is important to determine if new serotype 1 clones are emerging as these may have different characteristics in terms of antibiotic resistance.

Following the introduction of PCV13 it is assumed that serotype 1 case numbers will decrease although previous studies showed differing results. Our study only included data on ST1 up to 2 years post-PCV13 introduction. It is important to continue to monitor trends in outbreak prone serotypes included in PCV13 to document whether there is indeed a sustained reduction in these serotypes.

INCREASED INVASIVE PNEUMOCOCCAL DISEASE RISK AND MORTALITY IN HIV-EXPOSED BUT UNINFECTED INFANTS

Comparison with other studies including HIV-exposed-uninfected children

The PMTCT programme for HIV in South Africa has improved significantly over the last decade. Mother-to-child HIV transmission rates decreased from 16.4% in 2006 to 2.4%

in 2012 [170], despite an antenatal HIV prevalence of around 30% over the same period. This resulted in an increasing population of HEU children and less HI children.
To explore the risk of disease and mortality associated with IPD in HEU children we included cross-sectional data from a surveillance programme as well as data from a nested case-control study. We were able to use the cohort data to determine incidence of HEU to HUU and HI children while the case-control data allowed us to explore risk factors for IPD associated with being HIV exposed but uninfected.

Our case-control study showed that the single risk factor which remained significant in all the subgroup analyses for IPD in HU children was been exposed to HIV. For all-serotype IPD and NVT-IPD, HEU children had a two times higher odds of IPD [OR 1.62 (95% CI=1.10–2.37) and OR 1.96 (95% CI=1.25–3.07), respectively]. The increased risk was more marked for VT-IPD [OR 3.05 (95% CI=1.10–2.37)], especially in unvaccinated children [OR 8.80 (95% CI=1.23–62.94)]. Prior to our study, HIV exposure had not been described as a specific risk factor for IPD; it had however been shown to be associated with an increased risk of lower respiratory tract infections and bacterial infections.

All-cause hospitalisation rates and complicated hospital admissions are more frequent in HEU than in HUU infants [86,88]. Some infectious diseases, including respiratory tract infections [201], are more common and often more severe among HEU than HUU children. HEU children tend to have more severe infections, for example persistent diarrhoea [202], complicated acute malaria [203] and higher treatment failure rates with pneumonia [86]. In our study we showed that HEU children were more likely to have pneumococcal pneumonia than other syndromes when compared with HUU children..

A pooled analysis of three longitudinal community studies from Uganda, Tanzania and Malawi found that both maternal HIV infection and maternal death increased the risk of childhood death [204]. In Botswana discontinuation of breastfeeding increased the risk of death in HEU compared with HUU children [205]. In our dataset we only had breastfeeding data for 17-35% of cases across all years and could therefore not make definite conclusions regarding the contribution of breastfeeding as a risk factor in our population. Not accounting for breastfeeding in our study may have resulted in confounding in our comparison of HEU, HUU and HI children as breastfeeding practices possibly differed between these groups.

We established that the incidence of IPD was three-fold higher in HEU infants <6 months of age when compared with HUU infants. It is possible that breastfeeding differed between HEU and HUU children in our study. Breastfeeding is protective against IPD and mortality which may have resulted in residual confounding in our risk factor and mortality

analyses. A number of studies could not show a significant difference in mortality between HEU and HUU children [206-209]. In our study we demonstrated that HEU infants less than 6 months of age with IPD were more likely to die than HUU infants likely due to differences in breastfeeding practices. This increased risk was not shown in the older infants.

In our study HI infants were at higher risk of malnutrition, previous hospital admissions, non-susceptible pneumococcal disease and death than HEU infants. In addition children who were malnourished and who had pneumococcal meningitis were also more likely to die. A study in Tanzania (1995-1997) enrolled 474 HU and 69 HI children and followed them up until 12 months of age. CD4 counts were measured in children every 3 months and they decreased linearly among HI children and increased linearly among HEU children. Predictors of IPD in HI and HEU children included malnutrition and advanced maternal HIV [210]. As shown in the literature, for HI infants, we found that those who had a low CD4 count (CD4 percent <30) were 2-3 times more likely to die [infants <6 months (OR 2.43, 95% CI = 1.12-5.28) and >6-<12 months (OR 2.73, 95% CI = 1.16-6.41) then those with a higher CD4 count. CD4 T-cell depletion rates in HI children are predictive of mortality and poor clinical outcome. A prospective cohort study in South Africa in 848 mother–child pairs determined that the main causes of infant morbidity were gastrointestinal and respiratory infections. The mortality rate among HI infants was eight times higher than among HU infants [93].

Considerations for interpreting surveillance data results

Surveillance data is used to monitor population health to develop and direct public health strategies, for example to target specific high risk and underserved groups. It allows large-scale between-country comparisons. Surveillance data may be limited by under-reporting and misclassification, trend changes due to logistic and operational reasons, changes in case definitions or testing strategies and multiple interventions. In our surveillance programme decreasing trends in IPD rates were demonstrated after the introduction of PCV. Some of the reductions in HI individuals were also attributed to improvements in HIV care. To account for the impact of HIV interventions in HI individuals when exploring trend data, it was assumed that reductions in non-vaccine type disease was due to HIV interventions while vaccine-type disease was mainly influenced by PCV introduction; the difference in rate reductions between VT-IPD and NVT-IPD were then assumed to be due to PCV introduction [150]. This assumption is not entirely accurate as HIV-interventions also reduce VT-IPD; it is

however difficult to tease out the differential impact of PCV and HIV-interventions on disease. To ensure that changes in laboratory testing did not influence trend rates, PCR results were excluded from reported trends. The number of hospitals and laboratories covered by the surveillance programme increased between 2005 and 2012 from 126 to 459 hospitals (and 109 to 215 laboratories); however, 94% (32,922/35,192) of cases were reported from 150 hospitals, and more than 70% of these hospitals reported cases for the full period [150].

Generalisability and implications of results

As mentioned only certain patients with IPD would have been captured by our surveillance programme. In addition only patients with clinical data were included in some analyses, which may have limited the generalisability of some of our findings to certain groups. However we used national data for incidence calculations and had large numbers of children <1 year of age in our dataset which would likely make our results applicable to other low/middle income countries with high HIV prevalence. In addition our findings were similar those from other studies [83,208].

With increasing numbers of HEU children who have a higher risk of IPD when compared to HUU children, it is important to ensure that HIV-infected pregnant women receive ART to optimise the outcome of the infant and reduce HIV transmission. It is also important recognise these children to ensure that they receive appropriate doses of PCV to reduce their risk of IPD and its negative health outcomes, including death.

CHANGING RISK FACTORS FOR INVASIVE PNEUMOCOCCAL DISEASE IN YOUNG CHILDREN IN A HIGH HIV PREVALENCE SETTING

As part of a nested case-control study we explored the risk factors predisposing children to IPD in South Africa. In HU children, besides from being HIV-exposed other risk factors in this group included having siblings <5 years old, underlying medical conditions, preceding upper respiratory tract infections and day care attendance, while been vaccinated with PCV7 was protective.

In HI children, advanced HIV disease was more common in cases than controls; being recently diagnosed with tuberculosis and malnutrition increase the odds of IPD in cases versus controls, while use of ART was protective.

Comparison with other risk factor studies

We described risk factors in HU children which were in line with conventional pneumococcal risk factors. In our study these risk factors included, day care attendance which may increase IPD risk due to crowding which aids in transmission of pneumococci and higher pneumococcal loads, mixing of children with different pneumococci and possible cessation of breastfeeding [75]; living with siblings less than 5 years of age due to high rates of pneumococcal carriage [211], underlying medical conditions [75], poor socioeconomic conditions which are often associated with crowding and different odds in different race groups and by sex. Vaccination with the conjugate pneumococcal vaccine reduced the odds of disease in HU children [120].

A number of factors increased the risk of antibiotic-resistant IPD. In HU children we found that black race, a recent hospital admission, been HIV exposed and attending day care was associated with penicillin-nonsusceptible disease. In HI children, being malnourished, recent tuberculosis or a respiratory tract infection increased the odds of disease, while ART use reduced the odds of penicillin-non-susceptible disease. Upper respiratory tract infections may increase vulnerability to bacterial infections by causing changes in the respiratory epithelium which promote bacterial invasion [212]. Previous studies and reviews [78] have showed a positive association between day care attendance and resistant disease, likely due to crowding, lack of hygiene and a higher use of antibiotics as children often have recurrent respiratory tract infections. Previous admissions and respiratory tract infections are associated with antibiotic use which in turn results in increased resistance, especially if antibiotics are used inappropriately.

A case-control study nested in the US Active Bacterial Core surveillance programme [76] explored risk factors associated with IPD in the PCV7 era and demonstrated that cases and controls differed in terms of race, gender, history of breastfeeding, smoke exposure in the home, day care attendance, caregiver education level, presence of underlying illnesses, household income and healthcare coverage. This study showed a reduction in risk among traditional risk groups following PCV introduction, while children with underlying conditions still remained at risk of IPD [76]; similarly in our study children with underlying medical conditions remained at risk of IPD.

Considerations for interpreting case-control study results

Prior to introduction of PCV into the EPI-SA, a randomised controlled trial showed good efficacy of this vaccine in a high HIV prevalence setting [21]. Post-PCV introduction into routine use, other study designs need to be used to evaluate vaccine effectiveness as placebo controlled trials are no longer ethically acceptable. One option is a case-control study design [213,214]. Case-control studies can be used for rare diseases, such as IPD, and are less costly and time-consuming then randomised trials or cohort studies. Case-control studies are however subject to bias and confounding. There are a number of strategies to minimise bias [215]: attention must be given to the choice of cases and controls, for example having a clear case definition for cases, random selection of controls, matching of cases and controls; ensuring that there are no confounding associations between detection of disease and risk factor exposure and use of statistical techniques, such as stratification or logistic regression. In addition use of "sham" outcomes and exposures can be used to determine whether bias affected the results of the study [215].

Choosing the correct control group is vital to the conduct of a case-control study and when considering differences in risk factors between cases and controls. Differences may occur if cases are compared to hospital or community controls [216,217]. In our study we included hospitalised controls or those seen at the hospital outpatient department due to ease of access. Hospitalised controls may not be representative of children in the community from which the case originated as they may have different vaccination and disease risk factor status with different IPD risk factors. For example, if controls were hospitalised with gastroenteritis many risk conditions would be elevated in these children resulting in some risk factors been potentially missed or underestimated when compared with IPD cases. However, there are advantages to using hospital controls; besides for the convenience of enrolment, their health-seeking behaviour is likely similar to hospitalised IPD cases. We had to take these differences into account when we analysed risk factors for cases with IPD.

Matching is an important strategy to control for known confounders and ensure that there are enough controls in each subgroup [218,219]. However, as in our study, some cases were excluded in the analysis when no matched controls could be found, and especially in the HI group, enrolment of eligible HI controls often took months as it was difficult to find controls who met the eligibility criteria. An enrolment algorithm was followed if sufficient controls were not identified within a prescribed period and cases were often closed with one or two controls.

Generalisability of results

The public health impact of pneumococcal vaccines as well as the risk factors associated with IPD may differ between and within countries. We analysed risk factors by province as we had national data and risk factors were not statistically different across provinces. However, enhanced sites that collected risk factor data were predominantly urban or peri-urban sites and our results may therefore not be generalisable to more rural areas of South Africa. It is likely that our results may be generalisable to other developing countries with a high HIV prevalence.

Pre-PCV introduction in the USA, age (<2 years of age) and race (black) were risk factors for pneumococcal disease. Following PCV introduction, rates in black children decreased by 83% to similar rates to those in white children. In addition there was a reduction in the proportional difference in antibiotic non-susceptible disease in young children [220]. In our study we showed a higher risk of IPD in HU children of black race, especially antibiotic resistant disease.

Another study from South Africa [88] showed that similar to our study, young infants who were HIV-exposed but uninfected were shown to have an increased risk of severe disease.

Remaining gaps and implications following our studies

Even though there were limitations in our choice of control group which may have resulted in some risk factors been missed, our risk factors were comparable to previous studies. We only had breastfeeding data for <40% of all cases and could therefore not make definite conclusions regarding its role as a risk factor in our population. In other studies, however, breastfeeding has been shown to be protective against IPD and mortality and should be advocated for to improve child health. HI infants who were malnourished were more likely to die from IPD. Improving the nutritional status of HI infants and ensuring they receive appropriate ART is a key part of management. It is important to continue to document changes in risk factors for IPD with continued use of PCV, especially higher valency vaccines, and ongoing decreases in HIV rates in children. It is important to identify risk factors in order to prioritise interventions to reduce pneumococcal disease.

CONCLUSION

This doctoral thesis has demonstrated that even in the post-PCV era, *S. pneumoniae* is still an important cause of invasive disease in young children in South Africa and represents a major public health burden and potential cost. There has been a reduction in HIV infection rates in young infants due to improvements in the PMTCT programme and an improvement in paediatric ART with an 18-fold greater reduction in the absolute burden of IPD in HI children. However there are increasing numbers of HEU children who we have shown to be more likely to be hospitalised with IPD. HEU infants <6 months of age were more likely to die from IPD than HUU children. From a public health point of view it important to continue targeting HEU children for PCV vaccination and ensure that IPD is diagnosed promptly especially in the very young to minimise mortality. In HU children, traditional risk factors such as poor socio-economic conditions and intense exposure to infection need to be addressed to reduce the burden of disease in these children.

It is important to continue with robust ongoing surveillance to show reductions or increases in different serotypes in the post-vaccine era. We demonstrated that serotype 1 IPD had marked temporal variability with two large clusters observed between 2003 and 2012 and apparent reductions within two years of PCV13 introduction; however an extended period is needed to better define this change.

Laboratory-based IPD surveillance should continue unchanged to allow for measurement against the baseline. In addition documentation of nasopharyngeal carriage at defined points in time may assist with prediction of which serotypes will become most prominent in the post-PCV13 period. Although vaccine-serotypes continue to decrease globally following PCV13 introduction, some increases in non-vaccine serotypes have been observed. There is a limit on the number of serotypes that can be conjugated in one vaccine and replacement non-PCV13 serotypes vary across different countries. New universal protein-based vaccines are therefore in development. A complete picture of the impact of PCV on pneumococcal disease burden is needed to support ongoing national policy decisions related to PCV use.

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APPENDICES

Appendix A - Role of the student

Appendix B - Ethical clearance certificate

Appendix C - Copies of manuscripts making up PhD

- Paper I
- Paper II
- Paper III
- Paper IV

Appendix D – Responses to examiners' comments

APPENDIX A: ROLE OF THE STUDENT

Roles performed by the student in the manuscripts making up the PhD

Claire Emily von Mollendorf, student number 9102000D, had the following roles in the studies and the writing of the manuscripts forming part of her thesis entitled: "Epidemiology of *Streptococcus pneumoniae* post-pneumococcal conjugate vaccine introduction in South Africa".

1. Burden of potentially vaccine-preventable pneumococcal disease in children <5 years of age in South Africa, 2005-2008 and 2013

Claire von Mollendorf, Stefano Tempia, Anne von Gottberg, Susan Meiring, Vanessa Quan, Charles Feldman, Jeane Cloete, Shabir Madhi, Katherine L. O'Brien, Keith P. Klugman, Cynthia G. Whitney, Cheryl Cohen

Paper finalised for submission and currently undergoing CDC clearance

Data derived from: (1) GERMS-SA: Laboratory-based Surveillance for Pathogens of Public Health Importance in South Africa; (2) NHLS Corporate Data Warehouse; (3) Published literature

Role of the student: General epidemiological support, data cleaning, data analysis, training of site staff and site visits for the GERMS-SA programme. Analysis of NHLS Corporate Data Warehouse data. Creation of burden model. Literature review, drafting of paper and revision and finalisation of paper.

2. Epidemiology of serotype 1 invasive pneumococcal disease in all ages in South Africa, 2003-2013

Claire von Mollendorf, Cheryl Cohen, Stefano Tempia, Susan Meiring, Linda de Gouveia, Vanessa Quan, Sarona Lengana, Alan Karstaedt, Halima Dawood, Sharona Seetharam, Ruth Lekalakala, Shabir A. Madhi, Keith P. Klugman, Anne von Gottberg, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Emerging Infectious Diseases 2015; 22(2):261-270

Data derived from: (1) GERMS-SA: Laboratory-based Surveillance for Pathogens of Public Health Importance in South Africa

Role of the student: General epidemiological support, data cleaning, data analysis, training of site staff and site visits. Literature review, drafting of paper and revision and finalisation of paper.

3. Increased Risk for and Mortality from Invasive Pneumococcal Disease in HIV-Exposed but Uninfected Infants Aged <1 Year in South Africa, 2009–2013

Claire von Mollendorf, Anne von Gottberg, Stefano Tempia, Susan Meiring, Linda de Gouveia, Vanessa Quan, Sarona Lengana, Theunis Avenant, Nicolette du Plessis, Brian Eley, Heather Finlayson, Gary Reubenson, Mamokgethi Moshe, Katherine L. O'Brien, Keith P. Klugman, Cynthia G. Whitney, and Cheryl Cohen for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Clinical Infectious Diseases 2015; 60(9):1346–56

Data derived from: (1) Case-control study to estimate effectiveness of a pneumococcal conjugate vaccine against invasive pneumococcal disease in South Africa; (2) GERMS-SA: Laboratory-based Surveillance for Pathogens of Public Health Importance in South Africa

Role of the student: Responsible for coordinating the case-control study, case report form design and changes, updating tools, updating the protocol, ethics applications, checking study case investigation forms, review and verification of vaccination histories from road-to-health cards, data cleaning, data analysis, training of site staff and site visits. Provided general epidemiological support to the GERMS-SA programme, joint site visits, training and data cleaning. Literature review, drafting of paper and revision and finalisation of paper.

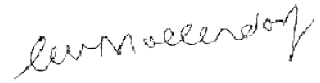
4. Risk Factors for Invasive Pneumococcal Disease Among Children Less Than 5 Years of Age in a High HIV Prevalence Setting, South Africa, 2010 to 2012

Claire von Mollendorf, Cheryl Cohen, Linda de Gouveia, Nireshni Naidoo, Susan Meiring, Vanessa Quan, Sonwabo Lindani, David P. Moore, Gary Reubenson, Mamokgethi Moshe, Brian Eley, Ute M. Hallbauer, Heather Finlayson, Shabir A. Madhi, Laura Conklin, Elizabeth R. Zell, Keith P. Klugman, Cynthia G. Whitney, and Anne von Gottberg, for the South African IPD Case-Control Study Group. The Pediatric Infectious Disease Journal 2015; 34(1):27–34

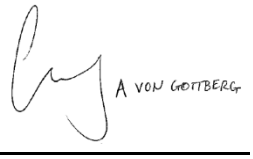
Data derived from: (1) Case-control study to estimate effectiveness of a pneumococcal conjugate vaccine against invasive pneumococcal disease in South Africa

Role of the student: Responsible for coordinating the case-control study, case report form design and changes, updating tools, updating the protocol, ethics applications, checking study case investigation forms, review and verification of vaccination histories from road-to-health cards, data cleaning, data analysis, training of site staff and site visits. Literature review, drafting of paper and revision and finalisation of paper.

All co-authors have been informed that the papers are to be used in a PhD thesis and none raised any objections regarding their use within the thesis.

Student: Claire von Mollendorf: 

Supervisor: Cheryl Cohen: 

Supervisor: Anne von Gottberg: 

APPENDIX B – ETHICAL CLEARANCE CERTIFICATE

R14/49 Dr Claire von Mollendorf et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140822

NAME: (Principal Investigator)	Dr Claire von Mollendorf et al
DEPARTMENT:	School of Public Health National Institute for Communicable Disease
PROJECT TITLE:	Epidemiology of Streptococcus Pneumoniae Post-Pneumococcal Conjugate Vaccine Introduction in South Africa
DATE CONSIDERED:	29/08/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Cheryl Cohen and Dr Anne von Gottberg
APPROVED BY:	Professor P Cleaton-Jones, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/09/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

Date 04/09/2014

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX C - COPIES OF MANUSCRIPTS MAKING UP PHD

PAPER I

Severe pneumococcal disease cases and deaths prevented by pneumococcal conjugate vaccine introduction in children <5 years of age in South Africa

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Keywords: *Streptococcus pneumoniae*, burden, children, South Africa, pneumococcal conjugate vaccine

Running title: Pneumococcal disease burden in children

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Tables: 6

Figures: 1

Supplementary material: Tables: 3; Figures: 4

Abstract

Introduction

Streptococcus pneumoniae is a leading cause of severe bacterial infections globally. A full understanding of pneumococcal conjugate vaccine (PCV) impact on pneumococcal disease burden, following its introduction in 2009 in South Africa, can support the ongoing national policy on PCV use and assist with policy decisions elsewhere.

Methods

We developed a model to estimate national burden of severe pneumococcal disease pre- (average 2005-2008) and post-PCV introduction (2013) in children aged 0-59 months in South Africa. We estimated case numbers for invasive pneumococcal disease using data from national laboratory-based surveillance, adjusted for specimen-taking practices. We estimated non-bacteraemic pneumococcal pneumonia case numbers using vaccine probe study data. To estimate pneumococcal deaths, we applied observed case fatality ratios to estimated case numbers. Estimates were stratified by HIV status to account for the impact of PCV and HIV-related interventions. We assessed how different assumptions affected estimates using a sensitivity analysis. Bootstrapping created confidence intervals.

Results

In the pre-vaccine era a total of approximately 196,100 (148,000-251,000) cases of severe hospitalised pneumococcal disease were estimated annually. In 2013, 5 years after introduction of PCV, 67,300 (49,000-95,000) cases were estimated, a rate reduction of 2528 cases per 100,000 child-years. Approximately 8600 (4500-13,500) pneumococcal-related annual deaths were estimated in the pre-vaccine period and 3600 (1000-6800) in 2013, a rate difference of 99 per 100,000 child-years.

Conclusions

A reduction in the burden of pneumococcal-associated hospitalisations and deaths, temporally associated with PCV introduction, was noted in children aged <5 years in South Africa. The impact of other interventions such as improvements in HIV care can't be excluded.

Introduction

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, meningitis, and sepsis, and was estimated to cause 335,000 (240,000-460,000) deaths in children <5 years in 2015 globally (1). In 2008, immediately before the introduction of the pneumococcal conjugate vaccine (PCV) in low income countries, the estimated number of deaths was 541,000 (376,000- 594,000) (2). In South Africa HIV-infected children, as well as HIV-exposed-uninfected children, had a higher incidence of all acute lower respiratory tract infections (LRTIs) than HIV-unexposed-uninfected children, even in the post-PCV era (3). In addition the incidence of pneumococcus-associated LRTIs still remained higher in HIV-infected than –uninfected children, although the rate ratio was not significant.

Prevention of pneumococcal disease by PCV use has been documented through effectiveness and impact data from more than 50 countries (http://view-hub.org/viz/). In South Africa, the 7-valent pneumococcal conjugate vaccine (PCV7) was introduced nationally in April 2009 and replaced by PCV13 in June 2011. National surveillance data for invasive pneumococcal disease (IPD) from South Africa showed a 69% (95% confidence interval [CI]: 65-72%) reduction in the incidence of all-serotype IPD among children aged <2 years by 2012, with contributions by PCV and HIV-associated interventions (4).

The burden of pneumococcal disease has been described in young children in the African region (5) with epidemiological studies in a number of countries including The Gambia (6, 7), Kenya (8) and South Africa (9). World Health Organization (WHO) country specific estimates of disease burden (1, 5, 10) generate summed regional and global estimates using country specific inputs for syndromic mortality (pneumonia and meningitis) along with other country specific parameters (e.g. pathogen specific case fatality ratios, HIV prevalence, population size, vaccine coverage). However, these models do not include the observed incidence of IPD. South Africa has excellent surveillance to measure IPD incidence and thus allows for an alternative model to estimate national cases and deaths from pneumococcal disease, anchoring on this observed value. The model can be updated over time to track improvements in health and be compared to other estimates.

We aimed to estimate the national burden of severe hospitalised pneumococcal disease (meningitis, bacteraemic and non-bacteraemic pneumonia, and non-pneumonia non-

meningitis invasive disease) among HIV-infected and HIV-uninfected children aged 0-59 months in South Africa in two periods: 2005-2008, before PCV was introduced, and 2013, after PCV was introduced. Estimates were based on the observed IPD incidence measures from our surveillance system, as an alternative approach to that used by the WHO.

Methods

Model overview

We developed a conceptual model to estimate the national burden of pneumococcal cases, deaths, incidence and mortality rates, in children 0-59 months in South Africa for the pre-vaccine (average 2005-2008) era and one year (2013) of the post-vaccine era (Figure 1 and supplementary figures 1a and 1b). Cases and deaths averted were calculated as the difference between these two periods, i.e. over 5 years. We used the observed cases of IPD hospitalisations from the GERMS-SA surveillance programme at both time points, stratified by disease syndrome (meningitis, pneumonia and non-pneumonia non-meningitis) and by age (<1 year of age, 1-4 years of age) as the base rate and adjusted for incomplete specimen collection based on Corporate Data Warehouse (CDW) data numbers by province among hospitalised children. Gauteng province was used as the reference province for this adjustment because of its systematic testing practices. We stratified all estimates by HIV status which has previously been documented to affect burden of disease (9). Model parameters are given in Table 1a and calculations are explained further below as well as shown in Figure 1 (and Supplementary Figures 1a and 1b). All reported case numbers were rounded to the closest 100, except when counts were less than a 100. Incidence rates were calculated from the estimated cases using denominators from Statistics South Africa (11); incidence rates were reported to the lowest case number per 100,000 population.

Estimated number of cases and incidence rates by clinical syndrome

IPD case numbers were estimated from national laboratory-based surveillance (described below) and divided into fractions attributable to meningitis, bacteraemic pneumonia and non-pneumonia non-meningitis cases on the basis of GERMS-SA clinical data. IPD case numbers were adjusted for sensitivity of blood culture among truly bacteraemic cases and CSF culture among those with pneumococcal meningitis, and incomplete specimen-taking practices among those with IPD. To estimate the number of cases of non-bacteraemic pneumococcal pneumonia, we extrapolated data from PCV probe studies in South Africa (12) by using the

PCV9 vaccine attributable reduction (VAR) ratio of clinical pneumonia to bacteraemic pneumococcal pneumonia (11:1). An additional adjustment was made for the presumed underestimation of vaccine efficacy (VE) for non-bacteraemic pneumococcal pneumonia in this study. This assumption was based on a study which showed a higher pneumococcal detection rate using urine antigen testing in non-bacteraemic pneumonia in the elderly (13). The adjustment value (1.89) was based on the ratio of the original and inflated VE from the different studies (see Supplementary Material for detailed methods).

Estimated number of deaths and mortality rates

Pneumococcal death estimates for each syndrome were calculated by multiplying case estimates (as calculated above) by observed syndrome specific case fatality ratios (CFRs) (from GERMS-SA enhanced sites) for IPD cases by age group and HIV status. For non-bacteraemic pneumococcal pneumonia we used the ratio of CFR (5:1) among all-cause community-acquired bacteraemia (28.2%) to all-cause non-bacteraemic hospital admissions (5.7%) reported in the literature from Kenya (14, 15) to calculate the non-bacteraemic pneumonia CFR. This CFR ratio was intermediate between those reported by two other trials (16, 17). Clinical trial data from The Gambia showed a death risk ratio of 4 times for end-point pneumonia (3.0%) compared to 'other infiltrates/abnormalities' pneumonia (0.8%) (16) and a CFR of 6.6% in a study comparing cases with bacteraemic pneumonia to a non-pneumonia control group (17). Mortality rates were calculated by dividing the estimated pneumococcal deaths by mid-year age-specific population denominators.

Data sources

GERMS-SA IPD surveillance programme

GERMS-SA is an active, national, laboratory-based surveillance programme for IPD and other invasive organisms. All public health sector microbiology laboratories (>200) are encouraged to submit isolates to the National Institute for Communicable Diseases (NICD) in Johannesburg. The public sector serves 84% of the South African population without private medical aid coverage (18). Some private sector laboratories also submit isolates. Of the public sector facilities, 24 sites have dedicated surveillance officers who collect clinical information on identified patients thereby defining them as enhanced sites. Laboratory-based surveillance for IPD in South Africa began in 1999 (19) and completeness of case reporting stabilised by 2005 (20). Cases of IPD were defined as illnesses in patients with *S. pneumoniae* cultured from normally sterile-body sites (e.g. cerebrospinal fluid (CSF) or

blood) or polymerase chain reaction (PCR) confirmation of culture-negative cases. Information on specimen type and age of cases is available from all sites; clinical diagnosis is reported only from cases that occurred at enhanced sites. Severe pneumococcal disease was considered as disease resulting in hospitalisation.

National Health Laboratory Service (NHLS) Corporate Data Warehouse (CDW)

The CDW is managed by the NHLS, the sole laboratory service provider for all public health facilities in South Africa. The CDW is a repository which contains archived data on all laboratory tests requested and results from public laboratories from 2003.

Additional input parameters for model

Values for input parameters of the model were derived from a number of sources. For estimates of non-bacteraemic pneumococcal pneumonia cases and adjustments for expected burden in presence of systematic blood culturing practices we used published data from a South African PCV9 vaccine clinical trial (12, 21). Published data was also used to derive the CFR for non-bacteraemic pneumococcal pneumonia (14, 15).

Population denominators

Annual age-specific population denominators used to calculate incidence and mortality rates were obtained from Statistics South Africa (11). The Thembisa model (22) was used to estimate population denominators by HIV status; these denominators accounted for the changes in mother-to-child HIV transmission rates and improvements in paediatric ARV treatment.

Statistical analysis

We calculated the percent reduction in incidence and death rates between the two periods (2005-2008 and 2013) using the following formula:

$$\% \text{ reduction} = \frac{[\text{Average incidence or death rate 2005 to 2008} - \text{Incidence or death rate 2013}]}{\text{Average incidence or death rate 2005 to 2008}}$$

Bootstrapping to create confidence intervals was used for all endpoints, to account for variability and uncertainty in detection rates, incidence rates and case-fatality rates from the surveillance data.

Human subjects review

Ethics approval was obtained for GERMS-SA surveillance (M081117) from the Human Research Ethics Committee (Medical), University of the Witwatersrand, Johannesburg, South Africa and other local hospital or provincial ethics committees, as required. Clearance for the surveillance programme was also obtained from the U.S. Centers for Disease Control (IRB 00001223).

Sensitivity analysis

A one-way sensitivity analysis was performed by changing one variable at a time to see the effect on the total number of cases and deaths (parameters in Table 1b). A Tornado diagram was fitted around the base case estimates for cases (Supplementary figure 2a) and deaths (Supplementary figure 2b) to evaluate the sensitivity of the model to changes in the assumed values of key parameters (Details in Supplementary Material).

Results

Burden of invasive pneumococcal disease in the pre-vaccine era

In the pre-vaccine era (2005-2008) an estimated national average of 196,100 (148,000-251,000) annual cases of hospitalised pneumococcal disease, an incidence of 3799 (2870-4853) per 100,000 person-years (py), occurred in children aged <5 years in South Africa (Tables 2 and 3). An average of 1100 (1000-1200) cases of pneumococcal meningitis (21 per 100,000 py); 8600 (6700-11,200) bacteraemic pneumococcal pneumonia cases (167 per 100,000 py), 181,500 (140,000-236,000) non-bacteraemic pneumococcal pneumonia cases (3515 per 100,000 py) and 4900 (3600-6100) non-pneumonia non-meningitis invasive pneumococcal disease cases (95 per 100,000 py) were estimated to occur annually in children 0-59 months of age. Based on model inputs, the overall incidence for hospitalised pneumococcal disease was higher amongst infants <1 year of age (9024 per 100,000 py) than children 1-4 years of age (2472 per 100,000 py), a relative risk of 4; incidence was also higher among HIV-infected children (41,436 per 100,000 py) than among HIV-uninfected children aged 0-59 months (1741 per 100,000 py), a relative risk of 24. Similar trend were observed in all syndromes (Table 3).

Pneumococcal related deaths and mortality rates in the pre-vaccine era

In the pre-vaccine period an average of 8600 (4500-13,500) annual pneumococcal-related deaths, translating into a mortality rate of 166 per 100,000 py, was estimated to have occurred

in children 0-59 months of age (Table 4 and 5). An average of 370 (300-400) pneumococcal meningitis deaths (7 per 100,000 py), 1400 (900-2000) bacteraemic pneumococcal pneumonia deaths (27 per 100,000 py), 5800 (2200-10,000) non-bacteraemic pneumonia deaths (112 per 100,000 py) and 1000 (200-2000) non-pneumonia non-meningitis IPD deaths (20 per 100,000 py) were estimated per year. The overall pneumococcal mortality rate, based on CFRs, was higher amongst infants (550 per 100,000 py) than children 1-4 years of age (68 per 100,000 py), a relative risk of 8, and also higher amongst HIV-infected children (1731 per 100,000 py) than amongst HIV-uninfected children aged 0-59 months (80 per 100,000 py), a relative risk of 22 (Table 5).

Impact of the pneumococcal conjugate vaccine and other interventions on the burden of disease

Based on inputted model parameters we estimated that in 2013 there were 67,300 (49,000-95,000) pneumococcal cases in children aged 0-59 months, 128,800 fewer cases than would have been expected based on the incidence of disease observed in 2005-2008 among this age group (Table 2). Since the IPD syndromic distribution observed in 2005-2008 and in 2013 drives the case and death estimates for these two periods, any reductions by syndrome are inherently a result of the differences observed by syndrome in the IPD cases. As a result of those differences, the model output had reductions in all syndromes between the pre- and the post-PCV periods. Similarly reductions were greatest in infants and HIV-infected children, the latter driven by the relative risk inputted into the model.

The overall national annual incidence of pneumococcal disease in 2013 was estimated as 1271 per 100,000 py in children 0-59 months of age, a total rate difference of 2528 per 100,000 (67% reduction) compared with the pre-PCV period (Table 3). The annual incidence of pneumococcal meningitis in 2013 was 5 per 100,000 py in children 0-59 months of age (rate difference of 16 per 100,000, 77% reduction), 57 per 100,000 py for bacteraemic pneumococcal pneumonia (rate difference of 110 per 100,000, 66% reduction), 1187 per 100,000 py for non-bacteraemic pneumococcal pneumonia (rate difference of 2328 per 100,000, 66% reduction) and 22 per 100,000 py for non-pneumonia non-meningitis pneumococcal disease (rate difference of 73 per 100,000, 76% reduction). For all syndromes incidence was highest amongst infants and HIV-infected children as is expected based on the rates observed in the IPD GERMS data.

Pneumococcal related deaths and mortality rates in 2013

In 2013 we estimated 3600 (1000-6800) annual pneumococcal deaths in children aged 0-59 months, 5000 fewer than would have been expected based on modelled pneumococcal deaths in 2005-2008 (Table 4). The overall South African annual mortality rate for pneumococcal disease in 2013 was estimated at 67 per 100,000 py in children 0-59 months of age, a rate difference of 99 per 100,000 py (59% reduction) compared with the pre-PCV years (Table 5). The mortality rate was 10 times greater in infants (237 per 100,000 py) than in children 1-4 years of age (24 per 100,000 py). The average pneumococcal meningitis mortality rate in 2013 was 2 per 100,000 py in children 0-59 months of age, a rate difference of 5 per 100,000 (76% reduction) compared with the 2005-2008 rate; 11 per 100,000 py for bacteraemic pneumococcal pneumonia (rate difference of 16 per 100,000, 58% reduction) and 49 per 100,000 py for non-bacteraemic pneumococcal pneumonia (rate difference of 63 per 100,000, 56% reduction).

Sensitivity analysis

The total numbers of pneumococcal cases and deaths estimated by the model changed depending on the values of key parameters used in the model (Table 1b); most variations resulted in lower estimates of cases and deaths (Supplementary Table 2a and 2b; Tornado diagrams, supplementary figures 2a and 2b). The inclusion of death estimates in the community did not change the numbers of cases and deaths significantly from our base model, while variations in the CFR resulted in a 16-74% increase in the estimated number of pneumococcal deaths. In the sensitivity analysis when we changed other parameters, including the VAR NBP/BPP ratio of 11:1, VAR NBP/BPP ratio of 7.6:1, the adjustment factor for systematic blood culturing and the HIV prevalence, the case numbers were reduced by 44%, 59%, 50% and 78% respectively. When the same parameters were altered in estimating pneumococcal deaths, numbers were reduced by between 33% and 49%.

Discussion

Our South African pneumococcal disease burden model has estimated that in the pre-vaccine era (2005-2008) an average of 196,100 (148,000-251,000) cases of severe pneumococcal disease were experienced per year in children 0-59 months of age. In 2013, 67,300 cases were estimated, a 2528 per 100,000 py rate difference. This 67% reduction in all serotype IPD compared with a non-PCV period was likely due to PCV introduction as well as improvements in HIV care and prevention. Other studies in the PCV13 era, which compared

reductions with the PCV7 period, showed a 64% (95% CI 59–68%) reduction in all IPD in the USA in children aged <5 years (23) and in the Gambia a 55% (95% CI 30-71%) reduction in children 2-23 months of age and 56% (95% CI 25-75%) reduction in children 2-4 years of age (24). In the UK in 2014/2015 in all age groups the overall incidence of IPD, compared to the pre-PCV7 period, declined by 47%, but an increase was noted in non-PCV13 serotypes in this period (25).

The model estimated 8600 (4500-13,500) annual pneumococcal deaths in children aged 0-59 months in the pre-vaccine era; this translated into a mortality rate of 166 per 100,000 py. In children aged 0-59 months in South Africa there was an average of 61,749 annual all causes deaths and 14,927 annual pneumonia and influenza deaths over the 2005-2008 period based on Statistics South Africa data (26); the estimated pneumococcal deaths would have made up 14% and 57% of these deaths respectively. A meta-analysis, including studies from the US, South Africa, Gambia and the Philippines, which assessed PCV efficacy on pneumonia concluded that approximately 21.2% of severe clinical pneumonia and 35.8% of CXR confirmed pneumonia was attributable to pneumococcal disease (5). In 2013 we estimated that 3600 (1000-6800) annual pneumococcal-related deaths occurred in children aged 0-59 months, a mortality rate reduction of 99 per 100,000 py. In 2013 in children <5 years there were an estimated 35,094 overall deaths and 8596 pneumonia and influenza deaths in South Africa, based on Statistics South Africa data (26); the estimated pneumococcal deaths would have contributed to 10% and 41% of these deaths respectively. A review by Izadnegahdar, et. al. in 2013 (27) proposed that even in the post-PCV13 period, *S. pneumoniae* pneumonia may still make up 44% of pneumonia deaths due to non-PCV13 serotypes. This estimate is higher than that reported in a Child Health Epidemiology Reference Group (CHERG) systematic review which estimated that in 2011 *S. pneumoniae* made up 32.7% of pneumonia deaths in the African region and globally (28).

An updated global burden model that includes a time series from 2000-2015 (1) with annual rates, calculated a total pneumococcal death rate of 203 (164-241) per 100,000 py, 19 (16-23) per 100,000 py for pneumococcal meningitis and 166 (133-198) per 100,000 py for pneumococcal pneumonia in children 1-59 months of age in 2008 for South Africa. When compared with our model which included children 0-59 months, the point estimates for the global model were slightly higher but the uncertainty ranges overlapped with our overall (166 [87-261] per 100,000 py) and pneumonia (139 [59-243] per 100,000 py) rates. Our meningitis

rates were lower than those reported in the global model (7 [6-8] per 100,000 py). In 2013, our overall (67 [19-129] per 100,000 py) and pneumonia (60 [12-124] per 100,000 py) rates overlapped with the South African estimates from the global model (70 [57-83] and 56 [45-66] per 100,000 py respectively). Our meningitis estimates were again slightly lower (2 [1-3] vs 8 [7-9] per 100,000 py) than the global model estimates. Even though the two models differed in their conceptual approach, their approach to neonatal deaths, and the inclusion of different input parameters, they provided similar death rate estimates. The global disease burden model is centred on a proportional mortality approach, taking as a given the all-pathogen deaths for meningitis and for pneumonia provided by the Maternal and Child Epidemiology Estimation (MCEE) Group. These deaths are apportioned out to pneumococcus using country specific empirical data for meningitis and PCV clinical trial data (for pneumonia). In contrast, the South Africa specific model used a bottom-up approach using IPD surveillance data as the anchor for the estimates, but it used the same PCV clinical trial data for pneumonia estimates. Lastly our model used the HIV prevalence rates for children identified with pneumococcal disease from our surveillance programme (67% in 2005-2008 and 30% in 2013) and not community prevalence rates (+/- 4% in 2008 and 2% in 2013) used by other models.

The greatest burden of pneumococcal disease in this study was contributed by pneumonia with a 96-fold higher rate than meningitis. This was similar to rates described in previous burden papers where pneumonia made up the bulk of pneumococcal disease (90-fold higher than meningitis) (5).

The pre-PCV pneumococcal incidence rates calculated by this burden model were comparable to those from a clinical trial conducted in South Africa in the pre-antiretroviral treatment, pre-vaccine era from 1998-2001. This clinical trial reported an IPD incidence of 331 per 100,000 py in young children in the placebo arm (21) which was slightly higher than this burden model rate of 283 per 100,000 py (2005-2008). The same trial demonstrated a bacteraemic pneumococcal pneumonia incidence for all children of 196 per 100,000 py (12) which was similar to our estimates of 167 per 100,000 py. The non-bacteraemic pneumococcal pneumonia rates (3515 per 100,000 py) were similar to the observed incidence of clinical lower respiratory tract infection (3565 per 100,000) rates among the placebo arm of the clinical trial (12). Our model utilised parameters from this clinical trial to calculate the

burden of non-bacteraemic from bacteraemic pneumonia, and this may have contributed to similar rates.

Stratifying data by HIV status revealed a 49% reduction in the incidence of all serotype pneumococcal disease in HIV-uninfected children, and a 67% reduction in HIV-infected children aged <5 years based on data inputted into the model. The decrease in HIV-infected children is likely due to the combined effect of PCV, antiretroviral therapy and improvements in the prevention of mother-to-child transmission of HIV programme.

Although we did not calculate costs of pneumococcal hospitalizations, there have been studies from Latin America which documented the substantial cost of pneumococcal disease and found that PCV introduction was cost-saving (29, 30). With approximately 26,000 hospitalised cases averted annually in South Africa in 2013, it is expected that PCV will have significant cost reductions for the health system despite the expense of the vaccine. A health economic study in South Africa (Cohen, personal communication) calculated the median total cost for a severe acute respiratory infection (SARI) hospitalization as ZAR 8804.25; assuming a similar cost for pneumococcal disease, ZAR 228,910,500 (US$ 19,075,875) could be saved per annum in direct hospitalization costs compared with those that would occur without the PCV program. We did not account for the impact and costs of PMTCT and ART in these calculations.

This study is subject to a number of limitations. First, the model is anchored on the GERMS-SA IPD surveillance data which is primarily drawn from public sector laboratories, so may not be representative of all sectors in South Africa; however, 84% of the population access public health care in South Africa even though not all cases detected in private laboratories are captured. Second, a number of assumptions were made to adjust for the lack of sensitivity of detecting cases, and this contributes to higher incidence rates than would be inferred from the measured cases. It is possible that some of the difference in incidence rates were true differences. Although all the assumptions were based on published literature, it is possible that some of these assumptions were not accurate. For example, using a CFR for all hospitalised cases may have overestimated the CFR for non-bacteraemic pneumonia cases and the ratio of BPP to NBP may change by serotype distribution which we did not account for in our model. In addition some estimates (e.g. vaccine probe study data) were only available for the pre-vaccine period and were assumed to be relevant to the post-PCV period.

Third, we only calculated the burden of severe pneumococcal disease and did not include pneumococcal disease that was cared for only in the outpatient setting. We were unable to include otitis media burden calculations in this model due to a lack of reliable African data. A US study showed that in children <5 years of age, acute otitis media made up 74% of pneumococcal cases (31), so this burden model is an underestimate of true pneumococcal burden. Fourth, as we used adjustment factors from a PCV-probe study in children (12) which based the diagnosis of pneumonia on clinical and CXR findings only, both of which have limitations in detection, we may have underestimated the burden of non-bacteraemic pneumonia in our calculations. We tried to account for this underestimate by including an adjustment for the increase in the known vaccine efficacy, based on more recent data, against non-bacteraemic pneumonia since these trials were conducted. Supporting evidence for this additional adjustment was seen in a recent PCV vaccine efficacy study in the elderly (13) which used a serotype-specific urinary antigen detection assay to detect vaccine-type *S. pneumoniae.* It is possible that this adjustment may have overinflated our numbers as it is not clear if the magnitude of the difference in VE for non-bacteraemic and bacteraemic pneumonia is the same for children as observed in the adult study. Fifth, we assumed similar PCV impact across all age strata among children less than 5 years and did not account for direct or indirect vaccine effects separately; we assumed that by using actual reported cases this would account for different impact rates. Lastly, there was a reduction in all-cause pneumonia deaths in South Africa over the 2005 to 2008 period (26); for the pre-PCV death rate calculations we assumed that rates were similar over this period and we may have therefore overestimated the change in pneumococcal death rates when compared with 2013.

In summary, pneumococcal disease represents a major public health burden in children <5 years of age in South Africa. Pneumococcal conjugate vaccination, in conjunction with other interventions, has resulted in a significant reduction in severe pneumococcal disease with approximately 130,000 cases and 5,000 deaths averted over a 5-year period. Although other interventions likely contribute to reductions in pneumococcal disease it is possible that PCV use may have a 'multiplier' effect of preventing other illnesses as children are generally healthier for not having had pneumonia or IPD.

Table 1a: Parameters used in base case model to estimate total number of cases, incidence and mortality rates for severe pneumococcal disease among children aged <5 years in South Africa

<table>
<tr><th>Parameter</th><th>Value used in base case model</th><th>Source of data</th></tr>
<tr><td>Number of cases of invasive pneumococcal disease for 3 clinical syndromes (meningitis, BPP, NPNM)</td><td>IPD cases detected from enhanced and non-enhanced surveillance sites</td><td>GERMS-SA surveillance programme</td></tr>
<tr><td>Adjustment factor for systematic blood culturing from South African clinical trial: for Gauteng Province only</td><td>Ratio of BPP incidence from Soweto clinical trial (1998-1999) to BPP incidence from GERMS-SA surveillance in same age group in 2005 (<2 years of age) = 23 overall, 13 in HIV-uninfected and 23 in HIV-infected children.</td><td>Madhi 2005: VE clinical trial conducted in Soweto located in the Gauteng Province</td></tr>
<tr><td>Adjustment for specimen-taking practices in provinces other than Gauteng Province

(Provincial incidences rates calculated using provincial specific cases and provincial denominators)</td><td>Provincial incidence rates were adjusted by the relative rate of blood cultures or CSF specimens collected in each province relative to Gauteng Province (baseline=1). Rate ratios differed by province and specimen type
<table>
<tr><th>2005-2008</th><th>CSF</th><th>Blood</th></tr>
<tr><td>GA</td><td>1.00</td><td>1.00</td></tr>
<tr><td>WC</td><td>1.27</td><td>1.26</td></tr>
<tr><td>KZN</td><td>2.00</td><td>6.00</td></tr>
<tr><td>NC</td><td>1.86</td><td>4.32</td></tr>
<tr><td>EC</td><td>2.81</td><td>4.58</td></tr>
<tr><td>NWP</td><td>2.48</td><td>10.51</td></tr>
<tr><td>MP</td><td>2.53</td><td>18.06</td></tr>
<tr><td>FS</td><td>2.04</td><td>2.43</td></tr>
<tr><td>LP</td><td>5.60</td><td>80.13</td></tr>
</table>
<table>
<tr><th>2013</th><th>CSF</th><th>Blood</th></tr>
<tr><td>GA</td><td>1.00</td><td>1.00</td></tr>
<tr><td>WC</td><td>0.23</td><td>0.35</td></tr>
<tr><td>KZN</td><td>2.30</td><td>35.35</td></tr>
<tr><td>NC</td><td>1.84</td><td>11.82</td></tr>
</table></td><td>NHLS Corporate Data Warehouse (CDW): Collates data on CSF and blood specimens taken nationally & submitted to NHLS laboratories</td></tr>
</table>

	EC	0.85	1.61
	NWP	1.66	15.08
	MP	2.71	40.06
	FS	0.39	10.71
	LP	5.70	82.37
Number of cases of non-bacteraemic pneumococcal pneumonia	PCV9 clinical pneumonia VAR = 410 cases/100,000 and all BPP VAR = 37 cases/100,000 = ratio 11:1 Additional adjustment for vaccine probe underestimate – VE against VT non-bacteraemic pneumonia is closer to 45% than 85% = 1.89		Madhi 2005 Bonten 2014
HIV prevalence among IPD cases; used to calculate proportion of HIV-infected and –uninfected cases.	Number of HIV-infected and –uninfected cases calculated by syndrome and year		GERMS-SA surveillance programme – HIV data available for enhanced sites
Case fatality ratio	CFR for pneumococcal bacteraemic syndromes = unadjusted pneumococcal deaths from enhanced sites/unadjusted pneumococcal cases from enhanced sites; CFR determined by age, HIV status and syndrome. CFR for non-bacteraemic pneumococcal pneumonia based on published data on difference in CFR (28.2%) between all-cause bacteraemic cases and medical non-bacteraemic cases (5.7%) in Kenya.		GERMS-SA surveillance data Ayieko 2013
Adjusted number of deaths	Adjusted number of pneumococcal deaths = CFR*Adjusted pneumococcal case numbers		GERMS-SA surveillance data
Incidence and death rates using mid-year population denominators	Incidence rates = Adjusted case numbers/population denominator Death rates = Adjusted death numbers/population denominator		Statistics South Africa data
HIV-specific denominators for incidence and death rates	Incidence and death rates by HIV status		AIDS and Demographic model developed by ASSA – was used to

		adjust Statistics South Africa denominators for HIV influence

Table 1b: Additional parameters for sensitivity analysis for severe pneumococcal disease among children aged <5 years in South Africa

<table>
<tr><th>Parameter</th><th>Value used in sensitivity model</th><th>Source of data</th></tr>
<tr><td>Number of cases of invasive pneumococcal disease for 3 clinical syndromes (meningitis, BPP, NPNM)</td><td>As for base calculations</td><td>GERMS-SA surveillance programme</td></tr>
<tr><td>Adjustment factor for systematic blood culturing from South African clinical trial: for Gauteng Province only</td><td>Ratio of IPD incidence from Soweto clinical trial (1998-1999) to IPD incidence from GERMS-SA surveillance in same age group in 2005 (<2 years of age) = 8.</td><td>Klugman 2003: VE clinical trial conducted in Soweto located in the Gauteng Province</td></tr>
<tr><td>Adjustment for specimen-taking practices in provinces other than Gauteng Province</td><td>As for base calculations</td><td>NHLS CDW</td></tr>
<tr><td>Number of cases of non-bacteraemic pneumococcal pneumonia</td><td>PCV9 Clinical pneumonia VAR = 410 cases/100,000 and all BPP VAR = 37 cases/100,000 = ratio 11:1 without additional adjustment</td><td>Madhi 2005</td></tr>
<tr><td>Number of cases of non-bacteraemic pneumococcal pneumonia</td><td>PCV9 WHO CXR confirmed VAR = 155 cases/100,000 and all BPP VAR = 37 cases/100,000 = ratio 4:1</td><td>Madhi 2005</td></tr>
<tr><td>HIV prevalence among general community used to calculate proportion of HIV-infected and –uninfected cases.</td><td>Number of HIV-infected and –uninfected cases calculated by age group and year
<table>
<tr><th>Age group</th><th>Year</th><th>HIV prevalence</th></tr>
<tr><td><2 years</td><td>2005</td><td>4.5</td></tr>
<tr><td></td><td>2006</td><td>4.3</td></tr>
<tr><td></td><td>2007</td><td>4.0</td></tr>
<tr><td></td><td>2008</td><td>3.5</td></tr>
<tr><td></td><td>2005_2008</td><td>4.1</td></tr>
<tr><td></td><td>2013</td><td>1.3</td></tr>
<tr><td>2-4</td><td>2005</td><td>5.1</td></tr>
</table></td><td>Thembisa model (Lee Johnson)</td></tr>
</table>

<table>
<tr><td></td><td><table>
<tr><td>years</td><td></td><td></td></tr>
<tr><td></td><td>2006</td><td>5.1</td></tr>
<tr><td></td><td>2007</td><td>4.9</td></tr>
<tr><td></td><td>2008</td><td>4.8</td></tr>
<tr><td></td><td>2005_2008</td><td>5.0</td></tr>
<tr><td></td><td>2013</td><td>2.5</td></tr>
</table></td><td></td></tr>
<tr><td>Case fatality ratio for all syndromes</td><td>Specific CFR by age groups and all-cause bacteraemic/all-cause non-bacteraemic syndromes<table>
<tr><th>Age group</th><th>Bacteraemic</th><th>Non-bacteraemic</th></tr>
<tr><td><1 year</td><td>34%</td><td>8%</td></tr>
<tr><td>≥1 year</td><td>23%</td><td>4.3%</td></tr>
<tr><td>All ages</td><td>28.2%</td><td>5.7%</td></tr>
</table></td><td>Berkley 2005
Ayieko 2014</td></tr>
<tr><td>Case fatality ratio of bacteraemic to non-bacteraemic pneumococcal pneumonia</td><td>Ratio of end-point pneumonia CFR (3.0%) to the CFR for ‘other infiltrates/ abnormalities’ pneumonia (0.8%) = 4:1
CFR of 6.6% in cases with bacteraemic pneumonia compared with a non-pneumonia control group</td><td>Enwere 2007

Forgie 1991</td></tr>
<tr><td>Adjusted number of deaths</td><td>Adjusted number of pneumococcal deaths = CFR*Adjusted pneumococcal case numbers – used different CFR</td><td>GERMS-SA surveillance programme</td></tr>
<tr><td>Deaths in the community</td><td>(Deaths by syndrome outside hospital/Deaths by syndrome in-hospital)*(Deaths from GERMS-SA enhanced sites) by age, syndrome and year</td><td>Vital statistics data from Statistics South Africa</td></tr>
<tr><td>Mid-year population denominators for incidence and death rates</td><td>As for base case model</td><td>Statistics South Africa data</td></tr>
<tr><td>HIV-specific denominators for incidence and death rates</td><td>As for base case model</td><td>ASSA model</td></tr>
</table>

BPP = Bacteraemic pneumococcal pneumonia; NPNM = Non-pneumonia non-meningitis; IPD = invasive pneumococcal disease; PCV9 = 9-valent pneumococcal conjugate vaccine; VAR = Vaccine-attributable reduction; VE = vaccine efficacy; CSF = cerebrospinal fluid; NHLS = National Health Laboratory Service; ASSA = Actuarial Society of South Africa; PCV13 = 13-valent PCV; CFR = case fatality ratio; NBP = Non-bacteraemic pneumococcal pneumonia
Provinces: GA = Gauteng, WC = Western Cape, KZN = KwaZulu-Natal, NC = Northern Cape, EC = Eastern Cape, NW = North West Province, MP = Mpumalanga, FS = Free State, LP = Limpopo Province

Table 2: Number of pneumococcal cases, by syndrome, in South Africa, by age and HIV status, 2005-2008 and 2013

	Total			HIV-infected			HIV-uninfected		
Syndrome /age group	**Mean case numbers 2005-2008 (95% CI)**	**Case numbers 2013 (95% CI)**	**Reduction in cases***	**Mean case numbers 2005-2008 (95% CI)**	**Case numbers 2013 (95% CI)**	**Reduction in cases***	**Mean case numbers 2005-2008 (95% CI)**	**Case numbers 2013 (95% CI)**	**Reduction in cases***
Meningitis									
<1 year	700 (680-740)	200 (180-240)	500	350 (320-390)	40 (20-60)	310	350 (320-380)	170 (130-200)	180
1-4 years	350 (300-390)	50 (30-60)	300	230 (200-250)	20 (10-30)	210	140 (120-160)	20 (10-30)	120
<5 years	1100 (1000-1200)	300 (200-320)	800	600 (540-630)	60 (50-80)	540	590 (540-630)	200 (160-220)	390
BPP									
<1 year	4100 (3000-5000)	1600 (1100-2200)	2500	2100 (1600-2700)	300 (150-550)	1800	2100 (1600-2700)	1300 (900-1800)	800
1-4 years	4500 (3500-5900)	1400 (1100-2100)	3400	2800 (2200-3700)	700 (500-1000)	2100	1700 (1300-2300)	800 (600-1200)	900
<5 years	8600 (6700-11200)	3000 (2000-4000)	5600	4900 (3800-6500)	1000 (700-1500)	3900	3800 (2800-4800)	2000 (1500-3000)	1800
NBP									
<1 year	86800 (66000-112000)	33200 (23400-45600)	53600	43600 (33000-56100)	6600 (3100-11500)	37000	43200 (32400-56500)	26600 (18300-37200)	16600
1-4 years	94700 (74000-97000)	29700 (23800-44900)	65000	59100 (45400-77300)	13900 (10100-22700)	45200	35600 (26800-48000)	15800 (12000-25000)	19800
<5 years	181500 (140000-236000)	62800 (47000-89000)	118700	102700 (79389-134106)	20400 (13800-30500)	82300	78800 (59600-101500)	42400 (32000-62000)	36400
NPNM									
<1 year	2800 (2100-3600)	600 (400-800)	2200	1400 (1100-1800)	100 (50-200)	1300	1400 (1100-1800)	500 (300-700)	900
1-4 years	2200 (1500-2600)	600 (400-800)	1600	1400 (900-1700)	300 (200-400)	1100	800 (600-1000)	300 (200-400)	500
<5 years	4900 (3600-6100)	1200 (800-1500)	3700	2700 (2000-3500)	400 (200-500)	2300	2100 (1600-2700)	800 (500-1000)	1300
Total									
<1 year	94400 (71000-120000)	35500 (24600-48300)	58900	47400 (34400-60400)	7000 (3500-12000)	40400	46900 (34700-61300)	28500 (19000-39000)	18400
1-4 years	101800 (77000-130000)	31800 (24100-47400)	70000	63500 (48100-81800)	14800 (11000-23400)	48700	38300 (28100-50700)	16900 (12400-26800)	21400
<5 years	196100 (148000-251000)	67300 (49000-95000)	128800	111000 (84000-145000)	21900 (14800-32400)	89100	85200 (63500-108000)	45400 (33000-66000)	39800

CI = confidence interval; BPP = Bacteraemic pneumococcal pneumonia; NBP = Non-bacteraemic pneumococcal pneumonia; NPNM = Non-pneumonia non-meningitis

*Reduction in cases = difference in case numbers between 2005-2008 and 2013

Table 3: Pneumococcal incidence rates in South Africa, by syndrome, age and HIV status, 2005-2008 and 2013

Syndrome and age group	Total			HIV-infected (HI)			HIV-uninfected (HU)			IRR^	
	Incidence rate* 2005-2008 (95% CI)	Incidence rate* 2013 (95% CI)	% reduction	Incidence rate* 2005-2008 (95% CI)	Incidence rate* 2013 (95% CI)	% reduction	Incidence rate* 2005-2008 (95% CI)	Incidence rate* 2013 (95% CI)	% reduction	IRR^ HI/HU 2005-8 (95%CI)	IRR^ HI/HU 2013 (95%CI)
Meningitis											
<1 year	68 (66-71)	19 (17-22)	72	868 (786-947)	285 (152-446)	67	35 (33-39)	16 (13-19)	56	25	18
1-4 years	9 (8-10)	1 (0.8-1.4)	88	102 (94-112)	15 (10-20)	85	3.5 (3-4)	0.6 (0.4-0.8)	84	26	15
<5 years	21 (20-22)	5 (4-6)	77	221 (203-233)	40 (30-50)	82	10 (9-11)	4 (3-5)	63	22	10
BPP											
<1 year	395 (300-509)	146 (102-200)	63	5043 (3785-6491)	2165 (1046-3827)	57	205 (154-266)	118 (82-165)	42	25	18
1-4 years	110 (86-142)	34 (27-51)	69	1242 (960-1620)	469 (345-745)	62	44 (33-58)	19 (14-30)	58	28	25
<5 years	167 (129-218)	57 (42-80)	66	1837 (1410-2412)	625 (439-940)	66	77 (58-97)	39 (29-57)	49	24	16
NBP											
<1 year	8298 (6316-10699)	3056 (2153-4200)	63	105906 (80010-136268)	45471 (21554-79645)	57	4296 (3234-5629)	2485 (1707-3470)	42	25	18
1-4 years	2301 (1803-2993)	705 (566-1066)	69	26085 (20062-34146)	9855 (7198-16106)	62	916 (689-1234)	389 (294-615)	58	28	25
<5 years	3515 (2710-4573)	1187 (887-1681)	66	38367 (29655-50094)	13161 (8910-19687)	66	1609 (1218-2074)	826 (623-1208)	49	24	16
NPNM											
<1 year	263 (204-342)	53 (34-77)	80	3354 (2549-4391)	784 (368-1418)	77	136 (105-177)	43 (26-63)	69	25	18
1-4 years	53 (36-64)	15 (9-20)	72	598 (405-733)	205 (117-288)	66	21 (14-27)	8 (5-11)	62	28	26
<5 years	95 (70-119)	22 (15-29)	76	1028 (756-1291)	258 (160-353)	75	45 (32-56)	15 (10-21)	66	23	17

Total											
<1 year	9024 (6743-11483)	3273 (2264-4453)	64	115171(83626-146513)	48705 (24398-80060)	58	4671 (3450-6101)	2662 (1767-3680)	43	25	18
1-4 years	2472 (1873-3185)	755 (572-1128)	69	28027 (21214-36095)	10544 (7785-16641)	62	984 (721-1302)	416 (304-659)	58	28	25
<5 years	3799 (2870-4853)	1271 (926-1795)	67	41436 (31406-54037)	14086 (9565-20904)	66	1741 (1297-2200)	884 (645-1285)	49	24	16

*Per 100,000 population; ^IRR = incidence rate ratio; IPD = invasive pneumococcal disease; BPP = Bacteraemic pneumococcal pneumonia; NBP = Non-bacteraemic pneumococcal pneumonia; NPNM = Non-pneumonia non-meningitis

Table 4: Number of pneumococcal deaths in South Africa, by syndrome, age and HIV status, 2005-2008 and 2013

	Total			HIV-infected			HIV-uninfected		
Syndrome and age group	**Mean number of deaths 2005-2008 (95% CI)**	**Number of deaths 2013 (95% CI)**	**Reduction in deaths (2005/8-2013)**	**Mean number of deaths 2005-2008 (95% CI)**	**Number of deaths 2013 (95% CI)**	**Reduction in deaths (2005/8-2013)**	**Mean number of deaths 2005-2008 (95% CI)**	**Number of deaths 2013 (95% CI)**	**Reduction in deaths (2005/8-2013)**
Meningitis									
<1 year	300 (200-400)	60 (20-110)	240	140 (90-180)	10 (3-30)	130	150 (90-200)	50 (20-90)	100
1-4 years	90 (50-130)	20 (10-40)	70	60 (10-110)	10 (4-20)	50	40 (10-60)	10 (5-20)	30
<5 years	370 (300-400)	90 (50-140)	280	200 (100-300)	20 (10-40)	180	160 (100-200)	70 (40-100)	90
BPP									
<1 year	900 (300-1600)	400 (100-900)	500	500 (100-800)	90 (20-220)	410	500 (200-800)	300 (100-700)	200
1-4 years	500 (150-900)	200 (0-700)	300	300 (0-800)	80 (0-300)	220	200 (0-500)	100 (0-400)	100
<5 years	1400 (900-2000)	600 (100-1300)	800	800 (300-1400)	200 (40-400)	600	600 (200-1000)	400 (90-900)	200
NBP									
<1 year	3900 (1200-6800)	1800 (400-3700)	2100	2000 (600-3500)	400 (80-840)	1600	1900 (600-3400)	1500 (300-3000)	400
1-4 years	2000 (0-5000)	700 (0-2900)	1300	1000 (0-3200)	400 (0-1400)	600	700 (0-2000)	400 (0-1600)	300
<5 years	5800 (2200-10000)	2600 (500-5300)	3200	3000 (1300-6000)	700 (200-1700)	2300	2700 (1000-4500)	1900 (400-3600)	800
NPNM									
<1 year	700 (100-1500)	200 (50-500)	500	300 (70-700)	40 (10-100)	260	300 (60-700)	200 (50-400)	100
1-4 years	300 (0-900)	50 (0-400)	250	200 (0-600)	20 (0-200)	180	100 (0-400)	30 (0-200)	70
<5 years	1000 (200-2000)	300 (0-600)	700	600 (100-1000)	70 (0-200)	530	500 (100-800)	200 (0-400)	300
Total									
<1 year	5800 (2500-9100)	2600 (800-4900)	3200	2900 (1200-4600)	500 (100-1000)	2400	2900 (1200-4700)	2100 (700-3900)	800
1-4 years	2800 (500-6200)	1000 (10-4000)	1800	1700 (70-4300)	500 (10-1800)	1200	1100 (40-2600)	500 (10-2200)	600
<5 years	8600 (4500-13500)	3600 (1000-6800)	5000	4600 (2200-8200)	1000 (300-2300)	3600	3900 (1700-6300)	2600 (700-4700)	1300

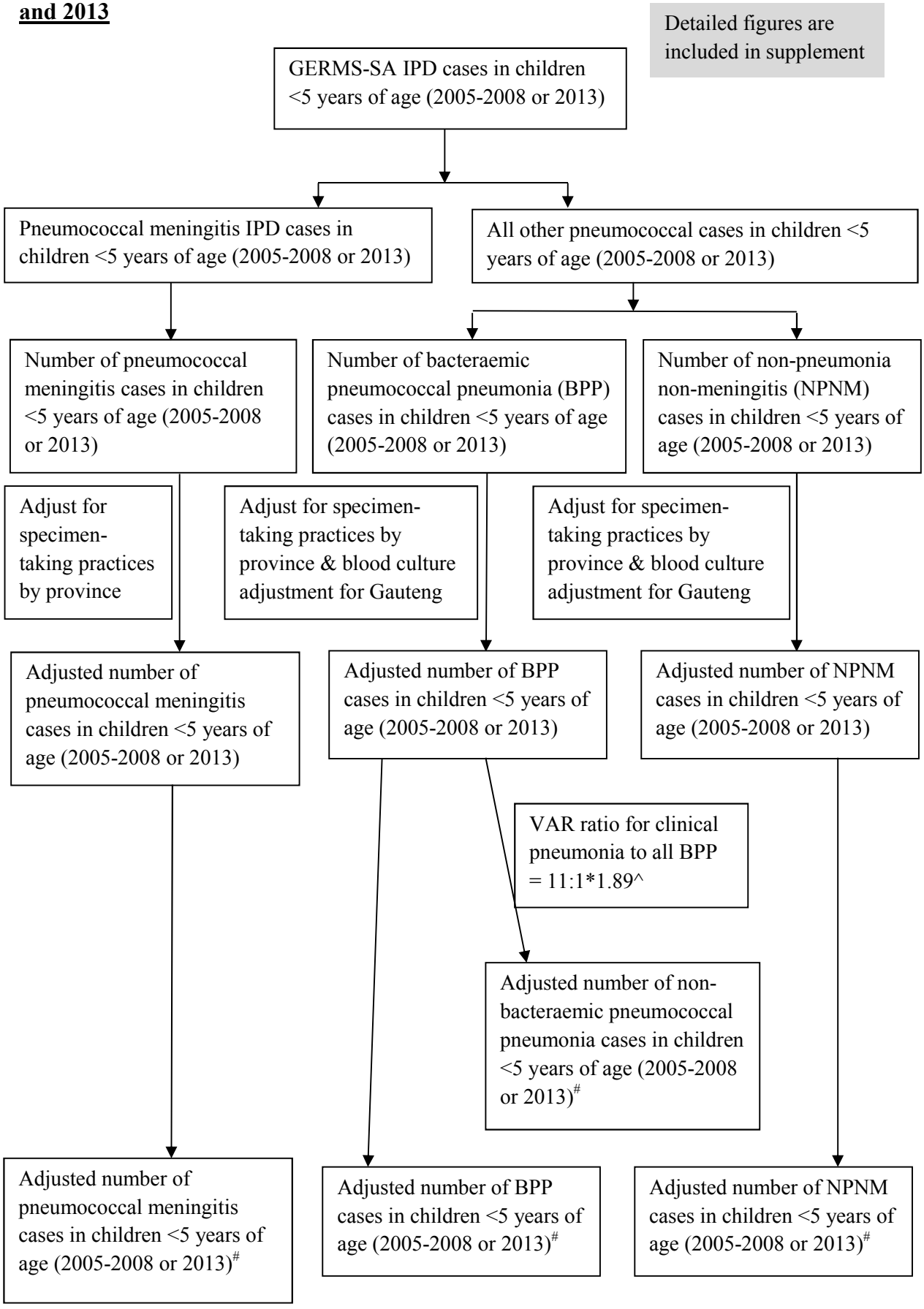
CI = confidence interval; BPP = Bacteraemic pneumococcal pneumonia; NBP = Non-bacteraemic pneumococcal pneumonia; NPNM = Non-pneumonia non-meningitis

Table 5: Pneumococcal mortality rate in South Africa, by syndrome, age and HIV status, 2005-2008 and 2013

	Total			HIV-infected (HI)			HIV-uninfected (HU)			Incidence rate ratio	
Syndrome age group	**MR* 2005-2008 (95% CI)**	**MR* 2013 (95% CI)**	**% reduction**	**MR* 2005-2008 (95% CI)**	**MR* 2013 (95% CI)**	**% reduction**	**MR* 2005-2008 (95% CI)**	**MR* 2013 (95% CI)**	**% reduction**	**IRR HI/HU 2005-2008 (95% CI)**	**IRR HI/HU 2013 (95% CI)**
Meningitis											
<1 year	26 (21-31)	6 (2-10)	78	334 (213-446)	87 (22-181)	74	14 (9-19)	5 (2-8)	65	24	17
1-4 years	2 (1-3)	0.6(0.2-1)	75	26 (6-47)	8 (3-14)	69	1 (0.2-2)	0.3 (0.1-0.5)	65	26	8
<5 years	7 (6-8)	2 (1-3)	76	73 (50-101)	15 (7-25)	79	3 (2-4)	1 (0.7-2)	63	18	15
BPP											
<1 year	88 (28-155)	40 (9-80)	54	1128 (351-1992)	601 (126-1495)	47	46 (15-83)	33 (7-66)	28	25	18
1-4 years	11 (4-21)	4 (0-16)	62	125 (0-333)	59 (0-233)	53	4 (0-12)	2 (0-9)	47	31	30
<5 years	27 (17-40)	11 (2-24)	58	279 (114-538)	109 (27-266)	61	13 (5-22)	8 (2-17)	35	21	14
NBP											
<1 year	371 (118-653)	170 (38-337)	54	4736 (1497-8444)	2526 (1707-3470)	47	192 (62-342)	138 (30-286)	28	25	18
1-4 years	46 (0-125)	18 (0-68)	62	524 (0-1421)	246 (0-985)	53	18 (0-51)	10 (0-38)	47	29	25
<5 years	112 (42-203)	49 (10-100)	56	1172 (469-2238)	458 (115-1099)	61	54 (19-92)	36 (7-71)	33	22	13
NPNM											
<1 year	65 (13-139)	21 (5-42)	68	828 (165-1748)	89 (19-225)	89	34 (6-74)	17 (4-34)	50	24	18
1-4 years	8 (0-23)	1 (0-8)	85	94 (0-255)	17 (0-120)	82	3 (0-9)	1 (0-5)	80	31	17
<5 years	20 (4-37)	5 (0-12)	74	207 (46-399)	44 (0-139)	79	10 (2-17)	4 (0-8)	58	21	11
Total											
<1 year	550 (235-871)	237 (77-454)	57	7025 (2976-11066)	1545(479-3125)	50	285 (124-464)	192 (63-363)	32	25	18
1-4 years	68 (13-150)	24 (0.3-94)	65	769 (30-1899)	330 (4-1306)	57	27 (1-67)	13 (0.2-53)	52	28	25
<5 years	166 (87-261)	67 (19-129)	59	1731 (813-3074)	626 (200-1466)	64	80 (34-128)	50 (13-91)	37	22	13

*Mortality rate (MR) per 100,000; IRR = incidence rate ratio; IPD = invasive pneumococcal disease; BPP = Bacteraemic pneumococcal pneumonia; NBP = Non-bacteraemic pneumococcal pneumonia; NPNM = Non-pneumonia non-meningitis

Figure 1: Flow diagram of the steps used to estimate the burden of invasive and non-invasive pneumococcal cases in children <5 years of age in South Africa in 2005-2008 and 2013



#For all syndromes total case numbers as well as numbers stratified by HIV status were determined

^Additional adjustment (1.89) for difference in VE estimated with use of urinary antigen

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Supplementary material

Severe pneumococcal disease cases and deaths prevented by pneumococcal conjugate vaccine introduction in children <5 years of age in South Africa

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Methods (see Figure 1a and 1b)

Model overview

Estimated pneumococcal cases, deaths and incidence rates by clinical syndrome in the pre-pneumococcal conjugate vaccine (PCV) period (2005-2008) and post-PCV period (2013)

A number of steps were followed to estimate pneumococcal case numbers, numbers of deaths and incidence rates in both the pre- and post-PCV periods:

STEP 1 (Figure 1a)

1) All GERMS-SA hospitalised IPD cases from both enhanced and non-enhanced sites from 2005 to 2008 and for 2013, were included in the baseline numbers on which the model was based because specimen type was available for all cases. An average of the case numbers for the pre-vaccine period (2005-2008) was calculated. Based on specimen type (i.e. CSF or non-CSF), IPD cases were defined as meningitis cases or non-meningitis cases. Cases numbers were determined by province and age group (<1 and 1-4 years) for each of the time periods (2005-2008 and 2013).
2) Pneumococcal meningitis cases were defined as all IPD cases with *S. pneumoniae* isolated from CSF (enhanced and non-enhanced sites) or IPD cases where the organism was isolated from blood and the clinical diagnosis was meningitis (enhanced sites). The proportion of meningitis cases diagnosed clinically, with only a positive blood culture, was determined from enhanced site cases and extrapolated to non-enhanced site cases (as for other syndromes). This extrapolation was done by province and age for each of the time periods (2005-2008 and 2013). The calculated non-enhanced site cases were combined with the diagnosed meningitis cases by province and age group (<1 year and 1-4 years).
3) Non-meningitis pneumococcal cases were defined as all the remaining IPD cases that were not meningitis cases. Among the remaining IPD cases we applied the proportions of bacteraemic pneumococcal pneumonia and non-pneumonia non-meningitis IPD cases

observed at enhanced sites in each year and by province and age group (<1 and 1-4 years) to the non-meningitis IPD cases at non-enhanced sites to calculate case numbers by syndrome. Case counts, by syndrome, from non-enhanced sites were combined with those from enhanced sites for each of the categories.

STEP 2: Figure 1b

4) Pneumococcal meningitis cases were adjusted for specimen-taking practices by province (see 5b) as certain provinces had better access to care and more cerebrospinal fluid specimens were taken in certain provinces compared with others, assuming that IPD incidence was similar across all provinces. We totalled meningitis cases and stratified by HIV (see 9) to calculate numbers of HIV-infected and –uninfected meningitis pneumococcal cases.
5) For bacteraemic pneumococcal pneumonia we adjusted for incomplete blood culturing in the Gauteng Province (see 5a) and for specimen-taking practices by province (see 5b) as certain provinces had better access to care and some provinces took more blood culture specimens for patients presenting with pneumonia. We totalled bacteraemic pneumococcal pneumonia cases and stratified by HIV (see 9) to calculate numbers of HIV-infected and –uninfected bacteraemic pneumococcal pneumonia cases.
    a. We assumed that Gauteng Province had the highest rate of specimen-taking for pneumonia but knew that not all children with pneumonia would have had a blood culture taken as part of routine practice. In contrast in a clinical trial setting all children presenting with pneumonia had a blood culture taken. To account for incomplete blood culture collection among children hospitalised with pneumonia, we adjusted the measured rate of bacteraemic pneumococcal pneumonia in 2005-2008 by a ratio (23:1) comparing the bacteraemic pneumococcal pneumonia hospitalisation incidence from a PCV clinical trial (control arm) in Soweto [1,2] to the measured bacteraemic pneumococcal pneumonia incidence in the same province (2005-2008). The same ratio was used to adjust 2013 measured case numbers.
    b. Specimen-taking practices varied across facilities and provinces. To account for differences in specimen-taking practices and the underdiagnosis of IPD, we adjusted GERMS case numbers for meningitis, bacteraemic pneumonia and non-pneumonia non-meningitis using a specimen ratio where the Gauteng province incidence was estimated to be the baseline (= 1,0) and have the most complete specimen taking after been adjusted (as in 5a), compared with the incidence rates in other provinces by year.

Data on total numbers of blood and cerebrospinal fluid specimens taken on an annual basis and submitted to public-sector laboratories was obtained from the National Health Laboratory Service Corporate Data Warehouse. These data were broken down by province, age group and year. We assumed that this adjustment would account for children who did not reach a hospital for care as this also differed between provinces.

6) To calculate the number of hospitalised non-bacteraemic pneumococcal pneumonia cases we used published data on the PCV9 attributable reduction (VAR) ratio (11:1) of clinical pneumonia to all bacteraemic pneumococcal pneumonia [2]. We used the clinical pneumonia outcome because it was found to be the most sensitive measure of pneumococcal pneumonia burden in the clinical trial (i.e. had the highest VAR). Based on a clinical trial in adults [3] which incorporated urine antigen testing for non-bacteraemic pneumonia, a higher vaccine efficacy was demonstrated against pneumonia. The ratio (1.89) of the original and inflated vaccine efficacy was used to adjust our non-bacteraemic pneumococcal pneumonia cases. The total non-bacteraemic pneumococcal pneumonia cases were stratified by HIV (see 9) to calculate numbers of HIV-infected and –uninfected non-bacteraemic pneumococcal pneumonia cases.
7) For non-pneumonia non-meningitis IPD case numbers we adjusted the observed cases from GERMS for specimen-taking practices by province (see 5b) as certain provinces had better access to care and some provinces took proportionally more blood culture specimens. We totalled the adjusted non-pneumonia non-meningitis IPD cases and stratified by HIV (see 9) to calculate numbers of HIV-infected and –uninfected non-pneumonia non-meningitis IPD cases.
8) To calculate the relative risk (RR) of IPD in HIV-infected versus HIV-uninfected cases we used the following formula:

$$RR = \frac{\text{((Number of enhanced site (ES) cases*HIV prevalence)/HIV-infected population denominator)}}{\text{((Number of ES cases – (Number of ES cases*HIV prevalence))/HIV-uninfected population denominator)}}$$

9) We stratified by HIV for all pneumococcal syndromes by estimating the number of HIV-infected cases in each group using the following formula:

$$\text{IPD HIV-infected cases} = \frac{1}{\frac{(\text{PopHIV-} + 1) * 1/\text{IPDTotal}}{(\text{RR* PopHIV+})}}$$

The number of HIV-uninfected cases was assumed to be the difference between the total number of cases and the HIV-infected cases (IPD HIV-uninfected cases = IPD Total cases – IPD HIV-infected cases).

10) We calculated the adjusted number of pneumococcal deaths for each syndrome by age group by multiplying adjusted case number estimates (as calculated above) by case fatality ratios (CFRs) observed at the GERMS-SA enhanced sites for bacteraemic cases (meningitis, bacteraemic pneumonia and non-pneumonia non-meningitis). For non-bacteraemic pneumococcal pneumonia we used CFRs from a study in Kenya [4] that observed case fatality rates among children under 5 years of age admitted to hospital for any reason with and without bacteraemia (all children had blood cultures obtained regardless of their admission diagnosis). The observed CFR for non-bacteraemic admissions was (5.7%) which was 5-fold lower than that observed for bacteraemic admissions (28.2%).
11) For all syndromes we calculated incidence and mortality rates using the adjusted case and death estimates from the model in combination with the and mid-year population estimates obtained from Statistics South Africa as denominators for different age groups (http://www.statssa.gov.za/). The Thembisa model which accounted for PMTCT and HIV treatment impact, was used for HIV specific denominators [5]. We calculated incidence rate ratios for all syndromes comparing incidence or mortality rates in HIV-infected to HIV-uninfected children (Supplementary table 1).

Supplementary table 1: Population denominators from Thembisa model for children <5 years of age in South Africa [5]

	2005	2006	2007	2008	2005_2008	2013
<1 year						
Pop Total	993446	1071503	1085777	1133330	1071014	1129271
Pop HIV+	44033	44702	41169	38857	42190	14982
Pop HIV-	949413	1026801	1044608	1094473	1028824	1114289
1-4 years						
Pop Total	3674623	3683421	3805687	3981501	3786308	4494190
Pop HIV+	212561	209114	207500	204103	208320	150315
Pop HIV-	3462062	3474307	3598187	3777398	3577989	4343875
<5 year						
Pop Total	4668070	4754925	4891464	5114831	4857322	5623461
Pop HIV+	256595	253817	248669	242960	250510	165297
Pop HIV-	4411475	4501108	4642795	4871871	4606812	5458164

Sensitivity analysis

A one-way sensitivity analysis was performed by changing one variable at a time to see the effect on the total number of cases and deaths (Tornado diagrams, supplementary figure 2a and figure 2b).

Parameters that we varied in the sensitivity analysis of cases and deaths included HIV prevalence, proportion of under 5 year old deaths that occur in the community, adjustment for the likelihood of obtaining a blood culture among hospitalised children with suspected pneumococcal disease and the ratio of bacteraemic to non-bacteraemic pneumococcal pneumonia. For estimates of pneumococcal deaths we also assessed the effect of changes in CFRs (see Table 1b for parameters).

1) For HIV prevalence we used values from the Thembisa model which reflects HIV prevalence in the community instead of the prevalence in hospitalised IPD cases used in our main analysis.
2) For the proportion of all deaths that occur in the community (which we did not include in our main analysis) we used vital statistics data from Statistics South Africa which enumerates deaths in the community and in hospitals by syndrome. The limitation is that cases that die in and out of hospital may not be directly comparable with each other in terms of severity and causation.
3) For incomplete blood culturing practices among children hospitalised with IPD, we adjusted the measured rate of bacteraemic pneumococcal pneumonia by a ratio of 8:1 which compared the IPD hospitalisation incidence from the PCV clinical trial (control arm) in Soweto [2] to the measured IPD incidence in the same province.
4) For the ratio of bacteraemic to non-bacteraemic pneumococcal pneumonia we used the same published data on the vaccine attributable reduction (VAR) ratio (11:1) of clinical pneumonia to bacteraemic pneumococcal pneumonia as in our main analysis [2] but without the additional factor (1.89) accounting for the change in vaccine efficacy [3] which was included in our main analysis.
5) A second VAR calculation using the ratio of CXR-confirmed pneumonia to bacteraemic pneumococcal pneumonia (4:1) [2] and including the additional factor (1.89) accounting for the change in vaccine efficacy [3] which was included in our main analysis (7.6:1) was conducted.
6) For the alternative calculation of non-bacteraemic pneumococcal pneumonia we calculated the number of HIV-infected and –uninfected cases separately and summed the numbers to calculate the total. The sensitivity of blood culture for diagnosing pneumococcal

pneumonia was assumed to be 3-5% in HIV-uninfected children and 18% in HIV-infected children.

7) For the CFR we used published rates for bacteraemic and non-bacteraemic disease [4] instead of the rates calculated from our surveillance programme (which were assumed to be underestimates). We used the published bacteraemic CFRs for meningitis, for bacteraemic pneumococcal pneumonia and non-pneumonia non-meningitis invasive disease, and the non-bacteraemic CFRs for non-bacteraemic pneumococcal pneumonia. The limitations with using these published rates were that they were from Kenya, the bacteraemic group included all causes of bacteraemia and all syndromes, the non-bacteraemic group included children with all medical conditions who did not have a positive blood culture and the age groups reported in this paper were different those used in our model.
8) Lastly for the CFR ratio of bacteraemic to non-bacteraemic pneumococcal pneumonia we used the death risk ratio reported by a trial from The Gambia [6]. This ratio (4:1) was considered an underestimate as it compared the CFR for end-point pneumonia (3.0%) which included the highest proportion of bacteraemic pneumonia to the CFR for 'other infiltrates /abnormalities' pneumonia (0.8%) which had the lowest proportion of positive blood cultures. The CFR for bacteraemic pneumonia was also lower than that reported in another study from The Gambia (6.6%), but the control group did not have pneumonia and no control children died [7].

Results

Sensitivity analysis

Supplementary table 2a: Sensitivity analysis for case numbers showing key variables altered in analysis, 2005-2008 and 2013

Key variables altered in analysis	**Number of cases**	
	2005-2008	**2013**
0) Base numbers	196,100	67,300
1) Community HIV prevalence	43,100	3200
2) Community deaths	196,900	68,900
3) Altered blood culturing estimates	98,100	34,400
4) NBP/BPP VAR ratio of 11:1	109,700	37,300
5) NBP/BPP VAR ratio of 7.6:1	80,300	27,200

6) Altered NBP calculations with separate HIV estimates	214,300	77,800

NBP = non-bacteraemic pneumococcal pneumonia; BPP = bacteraemic pneumococcal pneumonia

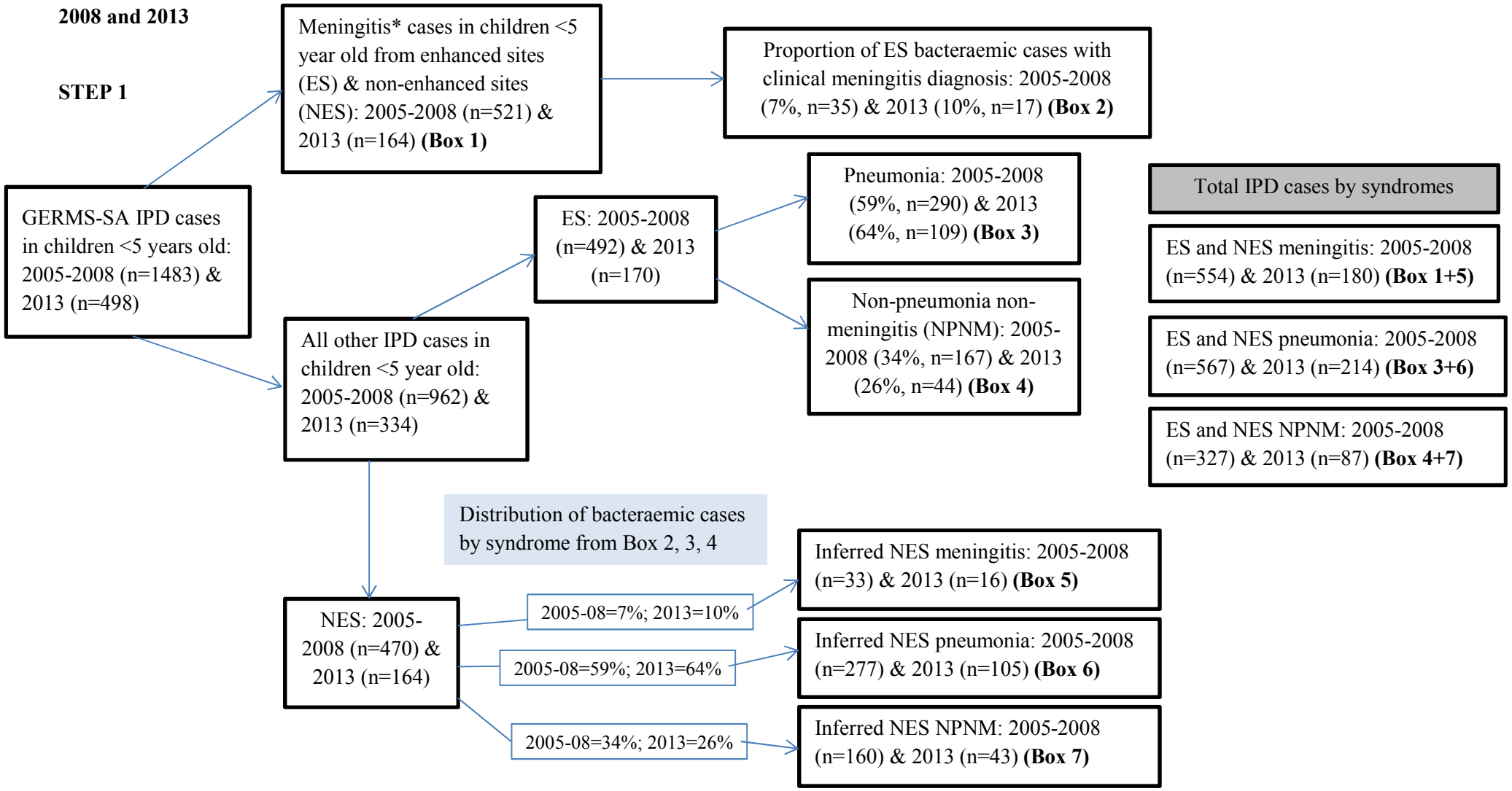
Supplementary table 2b: Sensitivity analysis for numbers of deaths showing key variables altered in analysis, 2005-2008 and 2013

Key variables altered in analysis	**Number of deaths**	
	2005-2008	**2013**
0) Base numbers	8600	3600
1) Community HIV prevalence	5300	700
2) Community deaths	8600	3700
3) Altered blood culturing estimates	4400	1800
4) NBP/BPP VAR ratio of 11:1	5800	2300
5) NBP/BPP VAR ratio of 7.6:1	4900	1900
6) Altered NBP calculations with separate HIV estimates	8700	3900
7) Separate case fatality ratios for bacteraemic and non-bacteraemic syndromes	15,000	5000
8) Adjusted case fatality ratio for BPP to NBP (4:1 ratio)	10,000	4200

NBP = non-bacteraemic pneumococcal pneumonia; BPP = bacteraemic pneumococcal pneumonia

The highest mortality rate (when new CFRs were included) in children <5 years estimated a death rate of 1492 per 100,000 py in 2005-2008 and 468 per 100,000 py in 2013.

Figure 1a: Initial step in estimating the burden of invasive and non-invasive pneumococcal cases in children aged <5 years in South Africa, 2005-2008 and 2013



*Includes cases positive for pneumococcus from CSF at ES and NES, and among ES sites, cases with pneumococcus from blood culture along with a clinical meningitis diagnosis

Figure 1b: Second step in estimating the burden of invasive and non-invasive pneumococcal cases in children <5 years in South Africa, , 2005-2008 and 2013

STEP 2

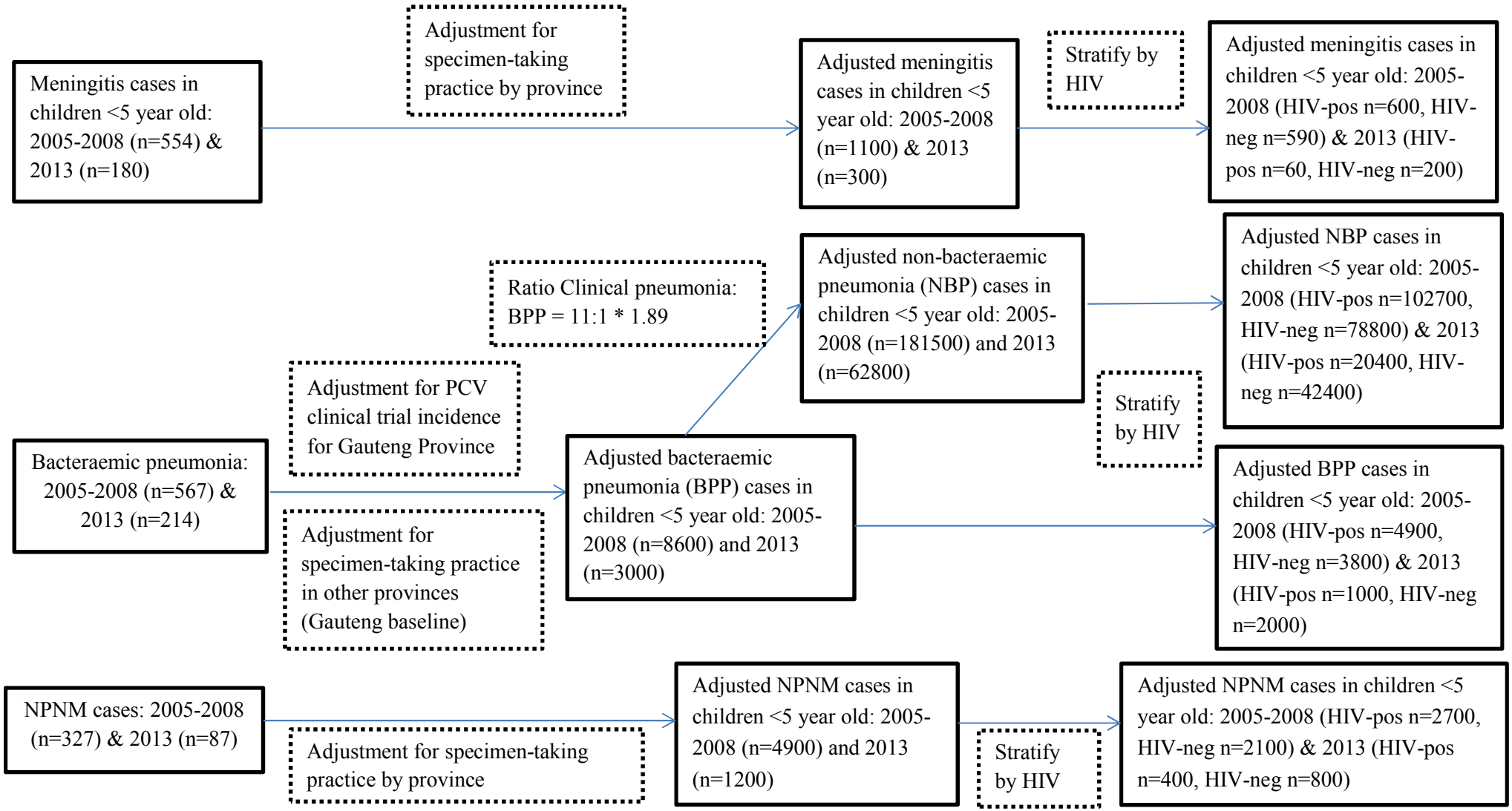


Figure 2a: Tornado sensitivity diagram representing change in pneumococcal case estimates in children <5 years of age in the pre-vaccine era, when values of key variables are modified.

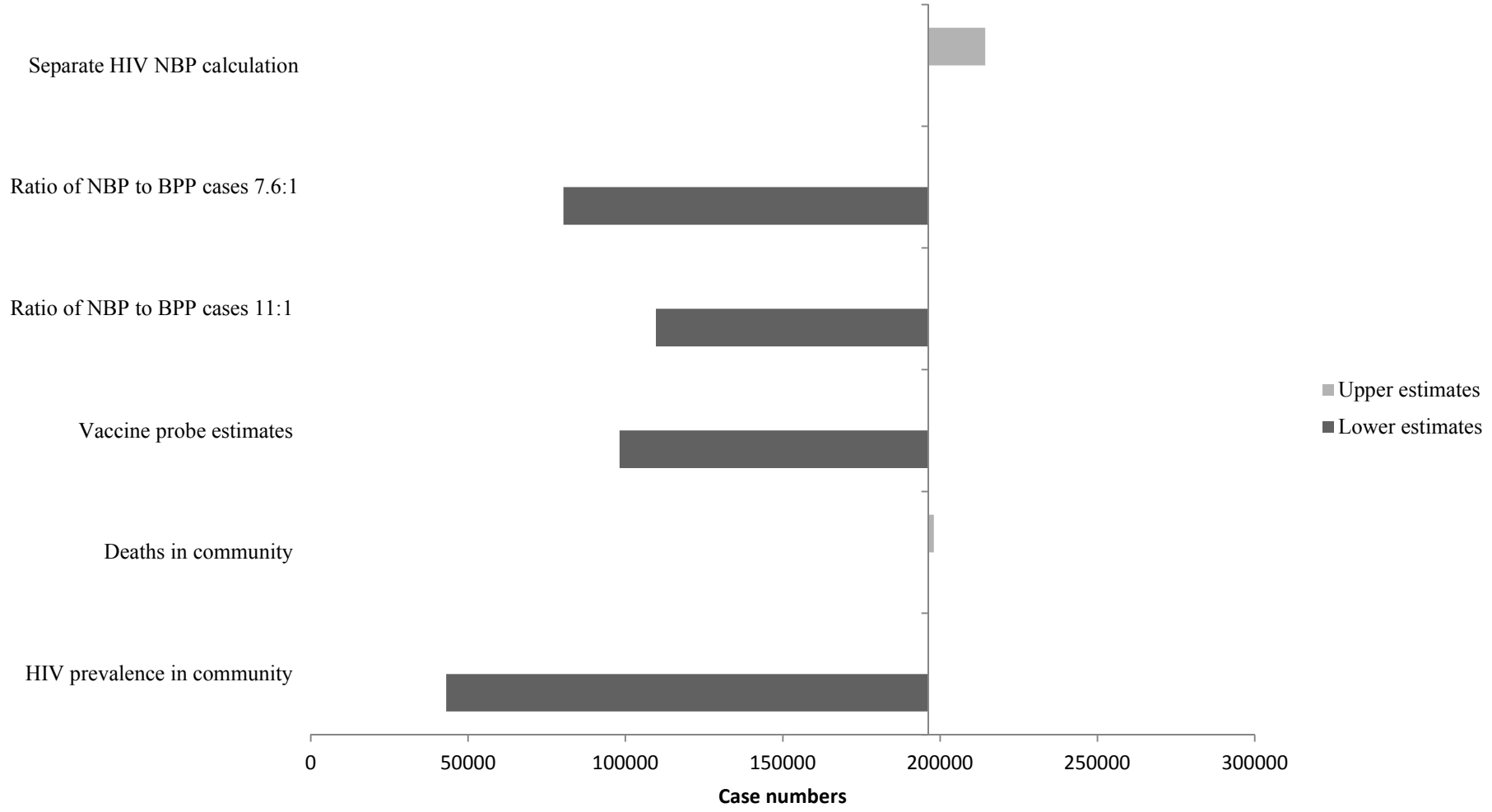
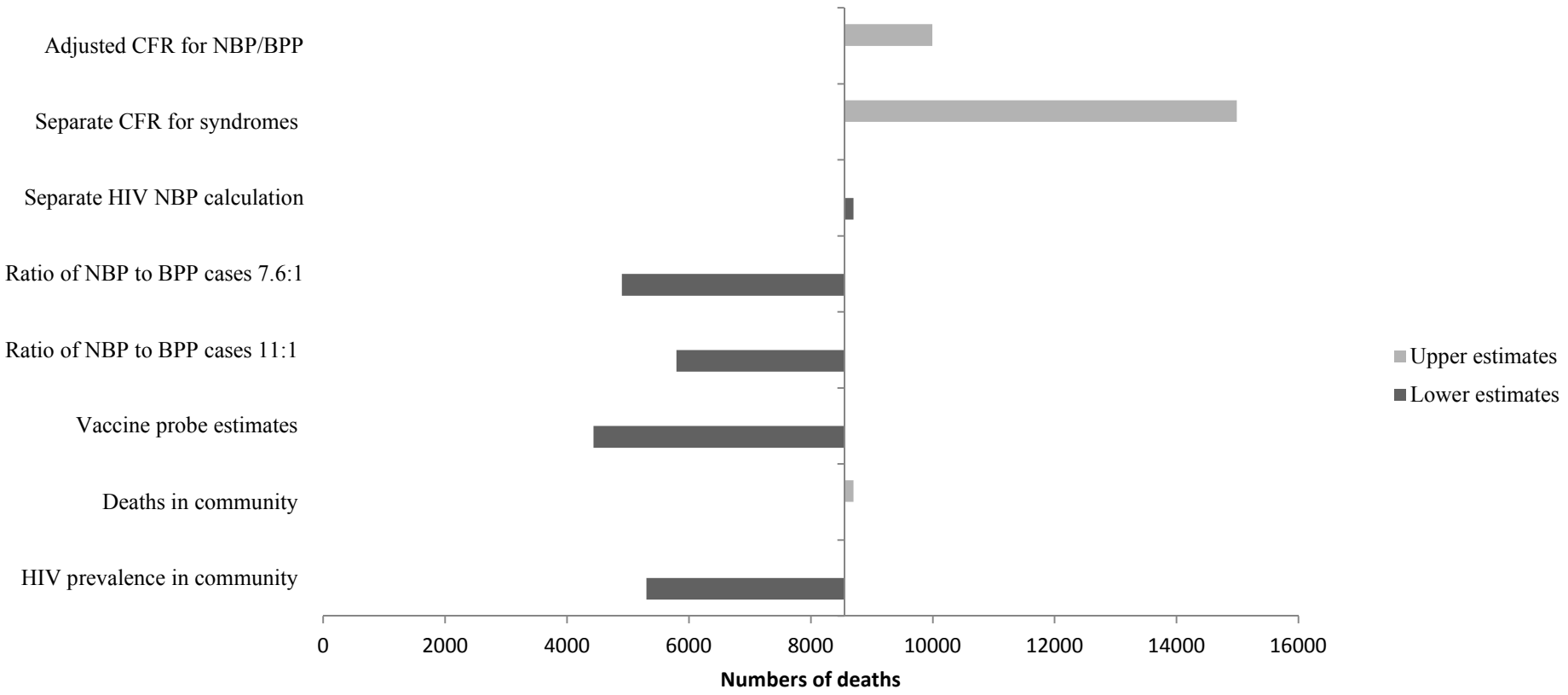


Figure 2b: Tornado sensitivity diagram representing change in pneumococcal death estimates in children <5 years of age in the pre-vaccine era, when values of key variables are modified.



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PAPER II

Epidemiology of Serotype 1 Invasive Pneumococcal Disease, South Africa, 2003–2013

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In South Africa, 7-valent pneumococcal conjugate vaccine (PCV) was introduced in April 2009 and replaced with 13-valent PCV in April 2011. We describe the epidemiology of serotype 1 *Streptococcus pneumoniae* disease during the pre- and post-PCV eras (2003–2013). Using laboratory-based invasive pneumococcal disease (IPD) surveillance, we calculated annual incidences, identified IPD clusters, and determined serotype 1–associated factors. Of 46,483 IPD cases, 4,544 (10%) were caused by serotype 1. Two clusters of serotype 1 infection were detected during 2003–2004 and 2008–2012, but incidence decreased after 2011. Among children <5 years of age, those who had non–serotype 1 IPD had shorter hospital stays, fewer cases of penicillin-nonsusceptible disease, and lower HIV prevalence and in-hospital death rates than did those with serotype 1 IPD; similar factors were noted for older patients. Serotype 1 IPD had distinctive clinical features in South Africa, and annual incidences fluctuated, with decreases noted after the introduction of PCV13.

Streptococcus pneumoniae serotype 1 is highly invasive and rarely carried asymptomatically (*1*). The incidence of serotype 1 invasive pneumococcal disease (IPD) fluctuates year to year; disease is associated with outbreaks in closed communities and hospitals and, in Africa, with communitywide meningitis outbreaks (*2*). Compared with other *S. pneumoniae* serotypes, serotype 1 tends to cause fewer cases of fatal disease, and antibiotic-resistant cases are unusual (*1*).

IPD is common in children with underlying diseases, especially HIV. A study conducted among children <18 years of age in Israel before introduction of 7-valent pneumococcal conjugate vaccine (PCV7) showed that, compared with other common serotypes, serotype 1 caused more bacteremic pneumonia and peritonitis, occurred in older children and certain ethnic groups, and affected otherwise healthy children (*3*). After PCV7 introduction, infections caused by serotypes included in the vaccine declined, but other pneumococcal serotypes (e.g., serotype 1, which was later included in 13-valent vaccine [PCV13]) became relatively more common (*4–6*); serotype 1 ranked among the top 4 serotypes infecting children <5 years of age (*7*). Although PCV7 use may have contributed to the relative increase in serotype 1 infections, some studies showed no correlation between the vaccine and serotype 1 disease incidence (*8*). Lack of correlation is likely due to the epidemic-prone nature of serotype 1 disease and annual fluctuations in disease incidence (*9*). In addition, replacement disease is mainly due to common colonizing serotypes. An indirect cohort analysis using data from the United Kingdom Health Protection Agency (now Public Health England) surveillance program could not demonstrate significant protection against serotype 1 IPD by PCV13, although the point estimate suggested protection (vaccine effectiveness 62% [95% CI –112% to 92%]) (*10*). Two trials of a 9-valent vaccine showed waning protection against serotype 1 in the absence of a booster vaccine dose in the second year of life; vaccine failures clustered in children >18 months of age (*11*,*12*).

In South Africa, PCV7 was introduced into the national immunization schedule in April 2009 as a 3-dose

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regimen for infants 6 weeks, 14 weeks, and 9 months of age; in April 2011, the vaccine was replaced with PCV13. Among children <1 year of age, reported coverage for the third dose of PCV improved from 10% in 2009 to 81% in 2012 but declined to 62% in 2013 (*13*). In 2012, after PCV13 introduction, serotype 1 IPD incidence showed a temporally associated decline in children <2 years of age (–57%, 95% CI –79% to –16%) and adults 25–44 years of age (–33%, 95% CI –46% to –17%) compared with incidence in 2005–2008 (*14*).

Information regarding *S. pneumonia*e serotype 1 epidemiology in Africa is limited. We compared serotype 1 disease epidemiology in South Africa with that of other serotypes over an 11-year period, before and after introduction of PCV7 and PCV13. We also explored whether temporal or spatial clusters of serotype 1 disease occurred during the study period.

Methods

Study Design and Setting

Persons of any age were included in the study if they were hospitalized in South Africa during 2003–2013 for laboratory-confirmed IPD and had an available *S. pneumoniae* serotype result for an isolate from a normally sterile site. Patients were identified through an active national, laboratory-based surveillance program for *S. pneumoniae*. Data were contributed by >200 hospital-based diagnostic laboratories that submitted pneumococcal isolates to the National Institute for Communicable Diseases, Johannesburg, South Africa. Most laboratories were nonenhanced sites where only isolates and accompanying laboratory report forms with patient age, sex, date and source of the specimen were submitted. However, 24 sites (primarily tertiary hospitals) implemented enhanced surveillance, in which dedicated surveillance officers collected additional clinical information on identified patients; at least 1 site was located in each South Africa province, giving national representation (*14*). Enhanced sites were chosen on the basis of convenience, interest from site investigators, and number of isolates submitted each year; thus, some differences existed between enhanced and nonenhanced sites (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/2/15-0967-Techapp1.pdf). Annual audits conducted by using a laboratory-based information system were used to identify unreported cases, which were included and used in incidence calculations.

Participants identified from enhanced and nonenhanced sites were included for determining incidence rates and cluster mapping. For the analyses of factors associated with serotype 1 pneumococcal disease and fatality, only participants from enhanced sites with detailed clinical information and known in-hospital outcomes were included.

Approval was obtained from the Human Research Ethics Committee (Medical), University of the Witwatersrand, Johannesburg (M081117), and other hospital or provincial ethics committees, as required. Informed consent was obtained for all patients.

Definitions

IPD cases were defined as disease in persons with *S. pneumoniae* detected in cultures of specimens from normally sterile sites or persons with culture-negative samples that were positive by latex agglutination and/or Gram stain microscopy or *lytA* PCR (*15*). Pneumococci were serotyped by the Quellung method (Statens Serum Institut, Copenhagen, Denmark).

Serotype 1 clusters were defined as an increase in serotype 1 IPD numbers relative to other serotype numbers in a specific geographic area and time. Cluster location was based on hospital district where cases were diagnosed; actual geographic location was considered to be the centroid of the district polygon. Other definitions are provided in the online Technical Appendix.

Incidence Estimations

We calculated annual incidence of serotype 1 disease per 100,000 population during 2003–2013 by using data for participants in defined age groups. We divided the number of age-specific, culture-positive serotype 1 IPD cases reported each year by age-specific midyear population estimates. Incidences for non–serotype 1 disease were similarly calculated. Serotype data for cases without serotype results from culture (including cases with only PCR serotype results) were imputed by age and year to obtain final incidence rates. Missing data were assumed to be random among different serotypes. Midyear population denominators were obtained from Statistics South Africa (http://www.statssa.gov.za/). To show differences in serotype incidences between prevaccine and postvaccine years, we compared an average incidence from prevaccine years (2003–2008) to 1 postvaccine year (2013). As a baseline for comparison, we included the average for years without clusters (2005–2007). CIs were calculated by using Poisson distribution for incidence rates.

Factors Associated with Serotype 1 IPD and Case-Fatality Rates

For the analyses of factors associated with serotype 1 IPD, we included only participants with culture- and PCR-positive results from enhanced sites during 2003–2013. Patients were stratified into 2 age groups (<5 and ≥5 years), and disease-associated factors in those with serotype 1 IPD were compared with those in patients with non–serotype 1 IPD by using a multivariable logistic regression model. A

second model to assess in-hospital fatalities restricted the analysis to serotype 1 IPD cases.

For both models, we assessed all variables considered significant ($p<0.2$) on univariate analysis and removed nonsignificant factors ($p\geq0.05$) by manual backward elimination. Patients with missing data for included variables were excluded. Statistical analysis was implemented by using Stata version 13.1 (StataCorp LP, College Station, TX, USA).

Spatiotemporal Analysis for Detection of Serotype 1 IPD Clusters

We conducted a space–time scan analysis to detect serotype 1 clusters by aggregating IPD cases with available serotype results from January 2003–December 2013 by month and district. To minimize potential biases introduced by temporal and geographic differences in specimen-collecting practices, healthcare-seeking behavior, or surveillance system improvements over time, we compared cases (serotype 1 IPD cases) with controls (non–serotype 1 IPD cases) from the same geographic area and time period; a Bernoulli model (*16*,*17*) was used for the comparison.

To account for control number reductions after PCV7 introduction, we adjusted (increased) observed control numbers by the percent reduction from the prevaccine period (*14*). To obtain estimated monthly numbers of controls, assuming no PCV introduction, we linearly interpolated estimated annual proportional reductions from June to June of consecutive years from 2009 through 2013. Because the percentage of reduction in the control numbers may have differed by geographic area due to locality differences in PCV7 uptake over time, we obtained monthly adjustment factors for each province. This adjustment would decrease the likelihood of detecting a cluster if, in fact, a cluster did not occur (null hypothesis).

To identify spatial clusters, we used an elliptical area of search that was allowed to vary in size, shape, and direction. Significance was assessed at $p<0.05$ over 999 replications. Space-time analysis was conducted by using SaTScan version 9.3.1 (http://www.satscan.org/); maps were generated by using ArcGIS version 9.2 (http://www.esri.com/). To calculate relative risks for districts, we divided observed number of cases by expected number of cases in each district.

Results

During 2003–2013, a total of 46,483 persons with IPD were enrolled in the study; 32,841 (71%) had viable isolates and known *S. pneumoniae* serotype, and 1,204 (3%) had serotype determination by PCR. Of the 46,483 persons, 20,564 (44%) were enrolled from enhanced sites; of these 6,211 (30%) were <5 years of age, 14,004 (68%) were ≥5 years of age, and 349 (2%) had unknown age (Figure 1). Of the 4,985 patients who died, 68% (3,365) did so within 3 days of admission. Of the 12,013 patients who recovered, 14% (1,673) were hospitalized for ≤3 days, 62% (7,427) for 4–14 days, and 24% (2,913) for >2 weeks. In the pre-PCV7 period (2003–2008), serotype 1 was the sixth most common *S. pneumoniae* serotype among children <5 years of age, but by 2013, it was eleventh. In contrast, among persons ≥5 years of age, serotype 1 was the most common serotype across all years, although case numbers decreased after PCV13 introduction.

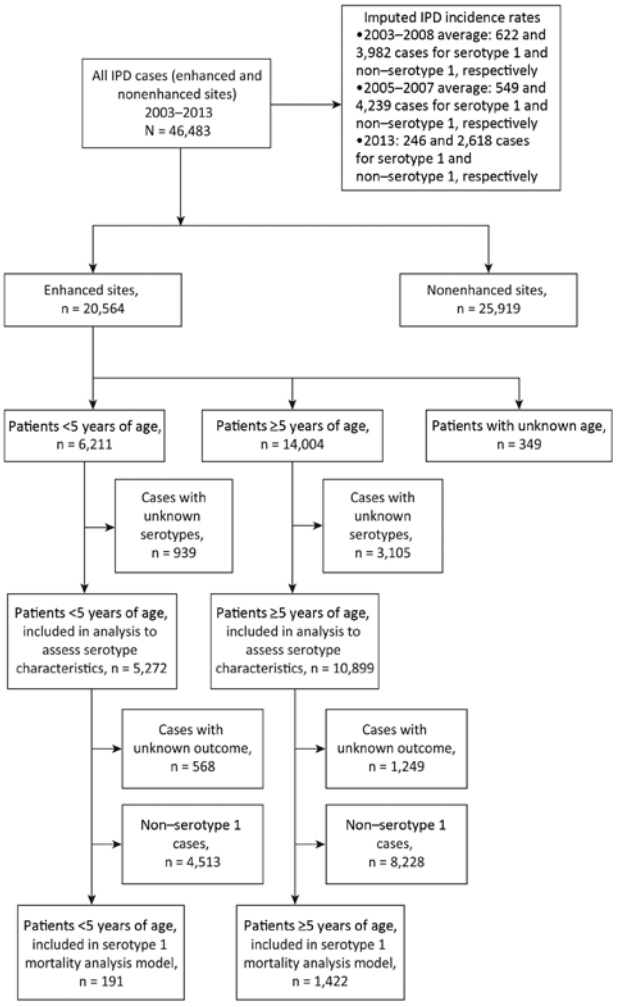

Figure 1. Selection flowchart for study of invasive *Streptococcus pneumoniae* disease (IPD) cases in South Africa, 2003–2013. Cases were reported by Group for Enteric Respiratory and Meningeal Disease Surveillance sites (GERMS-SA). Years indicate prevaccine (2003–2008), baseline (2005–2007), and postvaccine (2013) periods. Nonenhanced sites only submitted isolates and accompanying laboratory report forms, which included patient age and sex and the date and source of the specimen; enhanced sites (primarily tertiary hospitals) implemented enhanced surveillance, in which dedicated surveillance officers collected additional clinical information on identified patients.

Comparison of Enhanced and Nonenhanced Sites

Characteristics of enhanced and nonenhanced sites differed (online Technical Appendix Table 1). Compared with nonenhanced sites, enhanced sites had a higher proportion of cases among younger children, more cases from certain provinces, fewer cases in 2012–2013, more penicillin-nonsusceptible cases, more blood culture results, and fewer serotype 1 IPD cases.

Incidence of Serotype-Specific IPD in Different Age Groups

During the prevaccine era (2003–2008), serotype 1 incidence per 100,000 population was highest among persons <1 (1.8 cases), 5–9 (1.6 cases), and 25–44 (1.8 cases) years of age (Figure 2, panel A). Serotype 1 incidence did not differ significantly for 2003–2008 compared with 2005–2007, when there were no clusters. In 2013, serotype 1 incidence was highest among persons 5–9 (0.7 cases) and 25–44 (0.6 cases) years of age; reductions were significant ($p<0.001$) in all age groups except the >64-year-old age group ($p = 0.07$).

For all other serotypes during 2003–2008, the highest incidence rates per 100,000 population were among persons <1 (71.8 cases), 1–4 (13.9 cases), and 25–44 (10.1 cases) years of age (Figure 2, panel B). In 2013, the highest incidence rates were among persons <1 (27.3 cases) and >25 (>5.0 cases) years of age. Reductions in incidence among persons <5 and 25–44 years of age were significant ($p<0.001$).

The incidence of serotype 1 IPD fluctuated over the 11-year period (online Technical Appendix Figure 1). For the <5-year-old age group, incidence rates were significantly reduced in 2006 ($p = 0.01$), 2007 ($p = 0.03$), 2010 ($p = 0.006$), and 2012–2013 ($p<0.001$) compared with rates in 2005. In the ≥5-year-old age group, incidence rates were significantly higher in 2003 ($p = 0.001$) and 2004 ($p = 0.002$) compared with 2005 but lower during 2006–2008 and 2010–2013 ($p<0.001$).

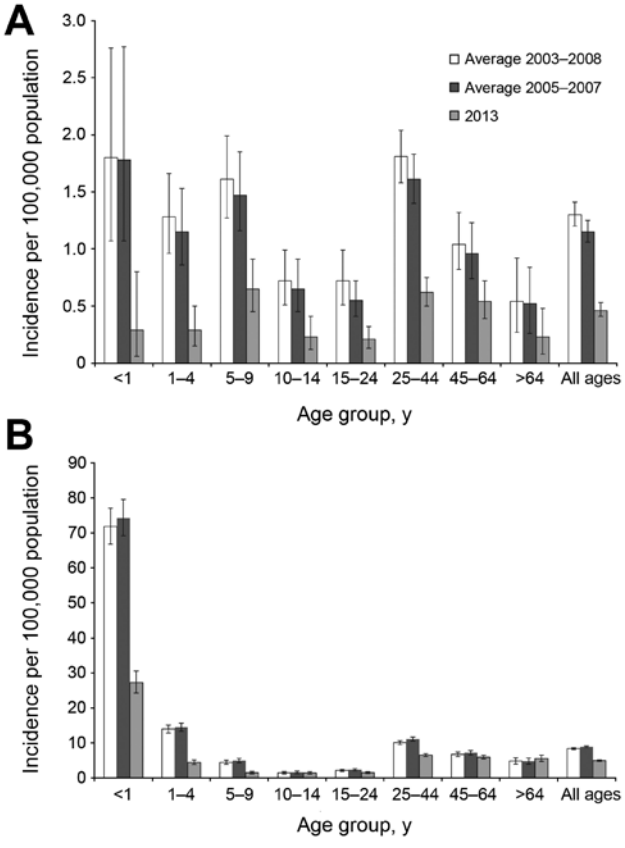

Figure 2. Incidence of serotype 1 and non–serotype 1 invasive pneumococcal disease (IPD) by age group, South Africa, 2003–2013. Years indicate prevaccine (2003–2008), baseline without clusters (2005–2007), and postvaccine (2013) periods. A) Serotype 1 IPD incidence by age group during prevaccine (no. cases = 622), baseline (no. cases = 549), and postvaccine (no. cases = 246) years. B) Non–serotype 1 IPD incidence by age group during prevaccine (no. cases = 3,982), baseline (no. cases = 4,239), and postvaccine years (no. cases = 2,618). Error bars indicate 95% CIs.

Factors Associated with Serotype 1 IPD

After adjustment for geographic location (province), year (based on prominent serotype 1 fluctuations), and clinical syndrome, we saw a difference among patients at enhanced sites who had IPD caused by serotype 1 versus other serotypes. Multivariable analysis showed a difference in disease distribution by province, year, and age among children <5 years of age; these difference were more apparent in children >3 than <1 years of age. Compared with children with non–serotype 1 IPD, those with serotype 1 disease had significantly shorter hospitalizations (≤3 days vs. 4–14 days [odds ratio (OR) 0.58, 95% CI 0.33–1.02] or ≥15 days [OR 0.44, 95% CI 0.23–0.85]) and were less likely to have HIV disease (OR 0.19, 95% CI 0.12–0.31), to die while hospitalized (OR 0.38, 95% CI 0.19–0.76), or to have penicillin-nonsusceptible disease (OR 0.02, 95 CI 0.01–0.05) (Table 1).

Among persons ≥5 years of age, serotype 1 IPD (compared with non–serotype 1 IPD) was significantly associated with province, year, and patient age: compared with persons >64 years of age, ORs (95% CIs) were 13.48 (5.53–32.82) for children 5–9 years of age; 8.02 (3.15–20.43) for children 10–14 years of age; 5.65 (2.31–13.82) for persons 15–24 years of age; 3.67 (1.53–8.76) for persons 25–44 years of age; and 2.57 (1.06–6.23) for persons 45–64 years of age (online Technical Appendix Table 2). Compared with persons with non–serotype 1 IPD, those with serotype 1 disease had significantly shorter hospitalization (≤3 days vs. 4–14 days [OR 0.86, 95% CI 0.68–1.09] and vs. ≥15 days [OR 0.64, 95% CI 0.48–0.86]) and lower rates of previous admissions (OR 0.45, 95% CI 0.35–0.57) and tuberculosis treatment (OR 0.73, 95% CI 0.57–0.95).

Table 1. Characteristics of 5,272 patients <5 years of age with invasive pneumococcal disease caused by serotype 1 or non–serotype 1 *Streptococcus pneumoniae*, South Africa, 2003–2013*

Variable	No. cases/no. total (%)		Univariate analysis†		Multivariable analysis†	
	Serotype 1	Non–serotype 1	OR (95% CI)	p value	aOR (95% CI)	p value
Age, y						
<1	63/211 (30)	2,754/5,061 (54)	Reference	<0.001	Reference	<0.001
1	35/211 (17)	1,155/5,061 (23)	1.32 (0.87–2.01)		2.36 (1.31–4.26)	
2	43/211 (20)	519/5,061 (10)	3.62 (2.43–5.40)		6.91 (3.78–12.64)	
3	37/211 (18)	355/5,061 (7)	4.56 (2.99–6.94)		12.03 (6.12–23.64)	
4	33/211 (16)	278/5,061 (5)	5.19 (3.35–8.05)		7.13 (3.60–14.13)	
Province						
Gauteng	95/211 (45)	2,067/5,061 (41)	Reference	<0.001	Reference	<0.001
Western Cape	11/211 (5)	1,158/5,061 (23)	0.21 (0.11–0.39)		0.11 (0.04–0.26)	
KwaZulu-Natal	46/211 (22)	957/5,061 (19)	1.05 (0.73–1.50)		1.04 (0.59–1.84)	
Eastern Cape	15/211 (7)	152/5,061 (3)	2.15 (1.22–3.79)		1.98 (0.74–5.28)	
Free State	25/211 (12)	383/5,061 (8)	1.42 (0.90–2.24)		1.06 (0.56–2.00)	
Mpumalanga	4/211 (2)	104/5,061 (2)	0.84 (0.30–2.32)		0.58 (0.07–4.86)	
North-West	5/211 (2)	46/5,061 (1)	2.36 (0.92–6.09)		5.65 (1.33–24.05)	
Limpopo	4/211 (2)	48/5,061 (1)	1.81 (0.64–5.13)		1.79 (0.41–7.90)	
Northern Cape	6/211 (3)	146/5,061 (3)	0.89 (0.39–2.08)		0.50 (0.15–1.64)	
Year of specimen collection						
2003	31/211 (15)	544/5,061 (11)	1.20 (0.72–1.99)	0.004	1.10 (0.49–2.49)	0.05
2004	26/211 (12)	699/5,061 (14)	0.78 (0.46–1.32)		0.58 (0.25–1.34)	
2005	32/211 (15)	672/5,061 (13)	Reference		Reference	
2006	21/211 (10)	551/5,061 (11)	0.80 (0.46–1.40)		0.77 (0.34–1.72)	
2007	15/211 (7)	547/5,061 (11)	0.58 (0.31–1.07)		0.67 (0.26–1.75)	
2008	10/211 (5)	542/5,061 (11)	0.39 (0.19–0.80)		0.40 (0.15–1.03)	
2009	23/211 (11)	494/5,061 (10)	0.98 (0.57–1.69)		1.43 (0.63–3.24)	
2010	19/211 (9)	361/5,061 (7)	1.11 (0.62–1.98)		0.82 (0.33–2.08)	
2011	19/211 (9)	240/5,061 (5)	1.66 (0.92–2.99)		1.04 (0.44–2.44)	
2012	12/211 (6)	190/5,061 (4)	1.33 (0.67–2.63)		0.49 (0.18–1.33)	
2013	3/211 (1)	221/5,061 (4)	0.29 (0.09–0.94)		0.12 (0.02–0.59)	
Medical conditions/treatment						
Length of hospital stay, d						
≤3	57/186 (31)	1,238/4,489 (28)	Reference	0.09	Reference	0.04
4–14	96/186 (52)	2,138/4,489 (48)	0.98 (0.70–1.36)		0.58 (0.33–1.02)	
≥15	33/186 (18)	1,113/4,489 (25)	0.64 (0.42–1.00)		0.44 (0.23–0.85)	
Previously hospitalized	39/164 (24)	1,676/4,110 (41)	0.45 (0.31–0.65)	<0.001		
Underlying medical condition‡	27/114 (24)	1,321/3,371 (39)	0.48 (0.31–0.75)	0.001		
Antimicrobial drug use in previous 2 mo§	10/147 (7)	742/3,549 (21)	0.28 (0.14–0.53)	<0.001		
HIV infected	43/132 (33)	2,125/3,539 (60)	0.32 (0.22–0.47)	<0.001	0.19 (0.12–0.31)	<0.001
TB treatment in previous 3 mo	11/161 (7)	570/3,928 (15)	0.43 (0.23–0.80)	0.008		
Malnourished¶	24/95 (25)	1,109/2,619 (42)	0.46 (0.29–0.74)	0.001		
Died during hospitalization	24/191 (13)	1,105/4,513 (24)	0.44 (0.29–0.68)	<0.001	0.38 (0.19–0.76)	0.006
Pneumococcal isolate characteristics						
Penicillin nonsusceptible#	4/203 (2)	2,580/4,950 (52)	0.02 (0.01–0.05)	<0.001	0.02 (0.01–0.05)	<0.001
Previous invasive pneumococcal disease**	2/211 (1)	356/5,061 (7)	0.13 (0.03–0.51)	0.004		
Clinical syndrome††						
Meningitis	59/198 (30)	1,668/4,736 (35)	Reference	0.001		
Pneumonia	124/198 (63)	2,358/4,736 (50)	1.49 (1.08–2.04)			
Bacteremia	15/198 (8)	710/4,736 (15)	0.60 (0.34–1.06)			

*All patients were reported from the enhanced Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance sites. aOR, adjusted odds ratio; OR, odds ratio; TB, tuberculosis.

†Only variables significant on univariate and multivariable analysis are shown. Variables not included are sex, race, Pitt bacteremia score, prematurity, antimicrobial drug use in previous 24 h, viable culture, and specimen type.

‡Includes asplenia or sickle cell anemia; chronic illness (i.e., chronic lung, renal, liver, cardiac disease, and diabetes); other immunocompromising conditions (i.e. including organ transplant, primary immunodeficiency, immunotherapy, and malignancy, but excluding HIV); and other risk factors (i.e., head injury with possible cerebral spinal fluid leak, neurologic disorders, burns, and chromosomal abnormalities). Excludes malnutrition.

§Use of any antimicrobial drug in 2 mo prior to admission.

¶Malnutrition was classified as a weight-for-age *z*-score of less than −2 (World Health Organization child growth standards 2009) (*18*), nutritional edema, or both.

#Considered penicillin nonsusceptible at MIC ≥0.12 μg/mL; intermediately resistant and resistant groups were combined into a nonsusceptible group.

**Invasive pneumococcal disease diagnosis >21 d before this episode.

††Clinical diagnoses were made on the basis of documented discharge diagnoses in patient medical records; clinical syndrome were separated into 3 groups: meningitis, bacteremic pneumonia, and bacteremia without focus or other diagnosis (e.g., septic arthritis, endopthalmitis, peritonitis, pericarditis).

Persons ≥5 years of age with serotype 1 disease were also significantly less likely to have HIV (OR 0.39, 95% CI 0.31–0.49) or penicillin-nonsusceptible disease (OR 0.02, 95% CI 0.01–0.04), and they were more likely than those with non–serotype 1 IPD to receive a diagnosis of pneumonia (OR 1.28, 95% CI 1.03–1.58) or bacteremia (OR 1.76, 95% CI 1.22–2.55) rather than meningitis. In-hospital death compared with recovery was not significant in the ≥5 year age group.

Factors Associated with In-Hospital Deaths among Patients with Serotype 1 IPD

We conducted multivariable analysis to explore factors associated with death in children <5 years of age with serotype 1 IPD (Table 2). Compared with 4-year-old children, those <1 year of age were more likely to die (OR 12.06, 95% CI 1.45–100.26), as were children with underlying medical conditions than those without. Odds of death were also increased among children with HIV (OR 2.82, 95% CI 1.36–5.84) or meningitis versus those with pneumonia or bacteremia. Duration of hospitalization was shorter among persons who died compared with those who recovered (<3 days vs. 4–14 days [OR 0.06, 95% CI 0.03–0.15] or ≥15 days [OR 0.02, 95% CI 0.01–0.07]).

Similar factors were associated with increased odds of death in persons ≥5 years of age with serotype 1 IPD (online Technical Appendix Table 3). In addition, death was more likely among persons who had received tuberculosis treatment in the previous 3 months (OR 1.75, 95% CI 1.25–2.45) and among severely ill persons (OR 5.26, 95% CI 3.53–7.84 for patients with a Pitt bacteremia score ≥4). No difference was seen in the odds of death by HIV status. Compared with children 5–9 years of age, persons >25 years of age had incrementally increased odds of death by age group: 25–44 years of age, OR 5.07 (95% CI 2.74–9.38); 45–64 years of age, OR 9.00 (95% CI 4.66–17.35); and >64 years of age, OR 10.13 (95% CI 4.46–23.00).

Detection of Serotype 1 IPD Clusters

Of the 46,483 IPD cases, 34,032 (73%) had available data (i.e., date of specimen collection, geographic location of patient, and serotype results) and were included in the space–time scan analysis. Of these 34,032 cases, 4,544 (13%) were caused by serotype 1 IPD. Two clusters of serotype 1 were detected. The first (713 cases) occurred during May 2003–December 2004 and affected Gauteng Province and adjacent districts of Mpumalanga, Limpopo, and North-West Provinces (Figure 3, panel A; online Technical Appendix Table 4). The second cluster (718 cases) occurred during September 2008–April 2012 and affected KwaZulu-Natal and Free State Provinces and adjacent districts of Gauteng, North-West, Mpumalanga, and Eastern Cape Provinces (Figure 3, panel B; online Technical Appendix Table 4). We also assessed clustering of disease caused by 2 other epidemic-prone serotypes (serotypes 5 and 8); neither showed significant increases in case numbers compared with numbers in 2005.

Discussion

In South Africa, serotype 1 pneumococcal disease had a number of distinct features. Children <5 years of age with serotype 1 IPD were less likely to die than were children with disease caused by other serotypes; this association between serotype 1 and death was not seen in older children and adults. Patients with serotype 1 IPD had fewer cases of penicillin-nonsusceptible disease, a lower prevalence of HIV, and less severe disease than patients with non–serotype 1 IPD. However, pneumonia and bacteremia occurred more commonly in patients with serotype 1 IPD than in patients with IPD caused by other serotypes.

Serotype 1 IPD incidence differed by geographic area and year, reflecting its epidemic potential (*1*). In older children and adults, serotype 1 was the most common serotype over the entire study period, even though numbers were lower after PCV13 introduction. Before PCV7 introduction, serotype 1 was the sixth most common serotype in children <5 years of age; by 2013, it no longer ranked in the top 10 serotypes in this age group.

IPD is common in children with underlying diseases, including HIV. Compared with infections caused by other common pneumococcal serotypes, serotype 1 IPD was associated with more bacteremic pneumonia and peritonitis, occurred in older children and specific ethnic groups, and affected otherwise healthy children (*3*).

Serotype 1 IPD has marked temporal variability (*19*) and is associated with outbreaks (*20*,*21*). In our study, we noted fluctuations in incidence rates for serotype 1 IPD, especially among young children before PCV introduction. Incidence of serotype 1 IPD decreased in all age groups after 2011, likely due to the effect of PCV13, and serotype 1 disease nearly disappeared among the youngest children by 2013, two years after PCV13 introduction (*10*). We cannot exclude that other factors (e.g., improvements in access to antiretroviral treatment and programs for the prevention of mother-to-child HIV transmission) may have contributed to this decrease (*14*,*22*). We identified 2 large clusters that were not recognized prospectively because of the difficulty in identifying communitywide clusters in real time, especially using laboratory-based surveillance.

Our findings showed differences in the geographic distribution of serotype 1 and non–serotype 1 disease. Serotype 1 has been described to occur more frequently in underprivileged populations in developing countries (*19*); in our study, differences in specimen collection practices between provinces may have

Table 2. Factors associated with death in patients <5 years of age with serotype 1 invasive pneumococcal disease, South Africa, 2003–2013*

Variable	Univariate analysis			Multivariable analysis	
	No. deaths/no. cases (%)	OR (95% CI)	p value	aOR (95% CI)	p value
Age group, y					
<1	102/355 (29)	11.49 (2.75–47.95)	<0.001	12.06 (1.45–100.26)	0.02
1	22/154 (14)	4.75 (1.08–20.88)		3.83 (0.41–35.35)	
2	11/94 (12)	3.78 (0.81–17.69)		1.30 (0.12–14.34)	
3	6/73 (8)	2.55 (0.49–13.14)		1.40 (0.12–15.82)	
4	2/59 (3)	Reference		Reference	
Province					
Gauteng	53/327 (16)	Reference	0.001		
Western Cape	15/111 (14)	0.81 (0.44–1.50)			
KwaZulu-Natal	26/111 (23)	1.58 (0.93–2.68)			
Eastern Cape	12/44 (27)	1.94 (0.94–4.01)			
Free State	11/62 (18)	1.11 (0.55–2.28)			
Mpumalanga	7/19 (37)	3.02 (1.13–8.01)			
North-West	11/23 (48)	4.74 (1.99–11.30)			
Limpopo	7/21 (33)	2.58 (1.00–6.71)			
Northern Cape	1/17 (6)	0.32 (0.04–2.49)			
Medical condition/treatment					
Length of hospital stay, d					
≤3	94/209 (45)	Reference	<0.001	Reference	<0.001
4–14	36/354 (10)	0.14 (0.09–0.21)		0.06 (0.03–0.15)	
≥15	10/160 (6)	0.08 (0.04–0.16)		0.02 (0.01–0.07)	
Pitt bacteremia score†					
0–3	102/608 (17)	Reference	<0.001		
≥4	16/28 (58)	6.61 (3.04–14.40)			
Underlying medical condition‡					
No	55/343 (16)	Reference	0.19	Reference	0.003
Yes	33/158 (21)	1.38 (0.86–2.23)		3.21 (1.49–6.91)	
Antimicrobial drug use in 24 h before admission					
No	82/504 (16)	Reference	0.05		
Yes	15/56 (26)	1.88 (1.00–3.56)			
HIV status					
HIV-uninfected	37/252 (15)	Reference	0.13	Reference	0.005
HIV-infected	52/263 (20)	1.43 (0.90–2.27)		2.82 (1.36–5.84)	
Malnourished§					
No	44/277 (16)	Reference	0.03		
Yes	43/176 (24)	1.71 (1.07–2.74)			
Clinical syndrome/specimen type					
Specimen type					
CSF	59/166 (36)	Reference	<0.001		
Blood	83/530 (16)	0.34 (0.23–0.50)			
Other	1/39 (3)	0.05 (0.01–0.36)			
Clinical syndrome¶					
Meningitis	74/209 (35)	Reference	<0.001	Reference	0.0003
Pneumonia	50/410 (12)	0.25 (0.17–0.38)		0.25 (0.11–0.54)	
Bacteremia	18/111 (16)	0.35 (0.20–0.63)		0.11 (0.03–0.42)	

*All patients were reported from the enhanced Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance sites. Only variables significant on univariate and multivariable analysis are shown. Variables not included in table are sex, year, previous hospital admission, prematurity, antimicrobial drug in previous 2 mo, and penicillin nonsusceptible invasive pneumococcal disease. aOR, adjusted odds ratio; OR, odds ratio.
†Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest and mental status. Severe disease defined as score of ≥4 points.
‡Includes asplenia or sickle cell anemia; chronic illness (i.e., chronic lung, renal, liver, cardiac disease, and diabetes); other immunocompromising conditions (i.e., organ transplant, primary immunodeficiency, immunotherapy, and malignancy, but excluding HIV); and other risk factors (i.e., head injury with possible cerebral spinal fluid leak, neurologic disorders, burns, and chromosomal abnormalities). Excludes malnutrition.
§Children with weight-for-age *z*-score of less than −2 (World Health Organization child growth standards 2009) (*18*), nutritional edema, or both.
¶Clinical diagnoses were made on the basis of documented discharge diagnoses in patient medical records, with clinical syndrome separated into 3 groups: meningitis, bacteremic pneumonia, and bacteremia without focus or other diagnosis (e.g., septic arthritis, endopthalmitis, peritonitis, pericarditis)

contributed to differences seen in disease distribution, as shown in other studies (*23*). Similar to findings by others (*24,25*), we found a difference in serotype distribution by age: serotype 1 IPD incidence was proportionally similar among older children and adults compared with that among children <1 year of age, whereas other serotypes predominated in the youngest age group and showed only a small peak in young adults. A number of factors may contribute to these age-associated differences (*25*). Compared with other serotypes, serotypes 1 and 5 are rarely carried by healthy persons; a short duration of carriage results in less opportunity for recombination events and

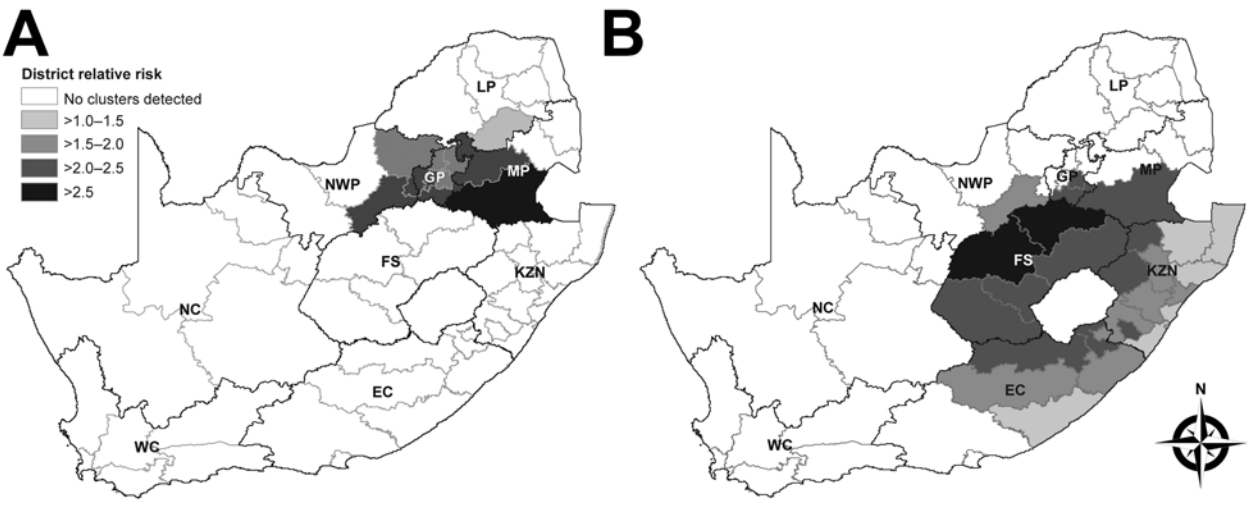

Figure 3. Serotype 1 invasive pneumococcal disease clusters by district, South Africa. A) May 2003–December 2004. B) September 2008–April 2012. Gray borders indicate district boundaries; black borders indicate provincial boundaries. Provinces: EC, Eastern Cape; FS, Free State; GP, Gauteng; KZN, KwaZulu-Natal; LP, Limpopo; MP, Mpumalanga; NC, Northern Cape; NWP, North-West; WC, Western Cape. District relative risk was calculated by dividing the observed number of cases per district by the number of cases expected by district (as determined on the basis of numbers in control groups).

less antibiotic selection pressure, resulting in reduced antibiotic nonsusceptibility in serotype 1 isolates (*26*).

Similar to findings in other studies (*3*), we found that, compared with other pneumococcal serotypes, serotype 1 caused more bacteremic pneumonia than meningitis. In addition, among HIV-uninfected children, serotype 1 IPD made up a larger proportion of disease than in HIV-infected children (*27*,*28*), suggesting that serotype 1 is more invasive and virulent, thus affecting otherwise healthy persons (*29*,*30*). Among children <5 years of age, those with serotype 1 disease were less likely to die than those with disease caused by other serotypes (*31*), and those most at risk of death were the very young (<1 year of age) and those HIV infected. In older persons, no association was found between serotype 1 disease and death when compared with other serotypes. Another analysis from the prevaccine era showed an increased risk of death among adults with serotype 1 disease compared with those with serotype 4 disease (*32*); this increased risk has been shown in few other studies (*33*).

Our study had several limitations. First, we included only patients who sought care at healthcare facilities with laboratories that submitted pneumococcal isolates to the National Institute for Communicable Diseases and who had specimens collected; patients with mild clinical pneumococcal disease treated in the community were not included. Second, we were able to map serotype 1 IPD incidence only at district level, so minor changes in incidence and clusters at the individual healthcare facility level may have been missed. Third, because of the small number of patients in the <5-year-old age group, we did not show clusters by age. We expect that reported clusters would have been similar for all ages. Fourth, we did not collect details regarding duration of symptoms before admission and thus could not assess whether intensity of symptoms when healthcare was sought affected case-fatality rates. Fifth, PCR serotype results from samples with a *lytA* cycle threshold (C_t) of ≥35 may not be accurate. We did not use PCR results in the trend analysis, and the proportion of *lytA* samples with high C_t values was low in the surveillance program (*34*), so the C_t accuracy is unlikely to have affected our results. Sixth, we used non–serotype 1 cases as our comparison group in the descriptive factor analysis; although this group changed over the study period, PCV13 serotypes (excluding serotype 1) made up >50% of this group until 2012 and 40% in 2013. Last, our study covered only a short period of observation after PCV13 introduction, making it difficult to determine whether reductions in serotype 1 IPD were due to introduction of this vaccine.

In conclusion, compared with IPD caused by other serotypes, IPD caused by serotype 1 in South Africa was characterized by shorter hospital stays, fewer cases of resistant disease, fewer in-hospital fatalities in children <5 years of age, and lower prevalence among HIV-infected persons. Serotype 1 caused disease in all age groups, although prevalence peaked in older children and young adults. Temporal reductions in serotype 1 IPD have been observed within 2 years of PCV13 introduction in South Africa; this observation must be corroborated by ongoing surveillance over an extended period of time.

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Epidemiology of Serotype 1 Invasive Pneumococcal Disease, South Africa, 2003–2013

Technical Appendix

Methods

Invasive Pneumococcal Disease Surveillance in South Africa

Invasive pneumococcal disease (IPD) surveillance began in South Africa in 1999 (*1*) and was limited to the collection of laboratory data and isolates from pneumococcal cases. The surveillance program was expanded in 2003 through GERMS-SA (Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa), a national, active, laboratory-based surveillance system. The number of hospitals and laboratories covered by the surveillance increased over time, however more than 70% of hospitals remained consistent in the program over most of the reported period (*2*).

All laboratories record basic demographic information (age, sex, date of specimen collection, and source of isolate) for all pneumococcal isolates. Enhanced surveillance with trained surveillance officers at 24 sentinel hospitals located in all nine provinces of South Africa, includes the collection of additional clinical data, for example, admission and discharge date, HIV serologic status, vaccination information and discharge diagnosis and outcome. Enhanced surveillance sites account for ≈50% of all reported pneumococcal cases nationally.

Enhanced surveillance sites were chosen based on convenience, interest from site investigators and number of isolates submitted. Larger sites with higher isolate submissions were favored, resulting in enhanced sites being mainly tertiary and some secondary (regional) hospitals. Non-enhanced sites include district, regional and tertiary public hospitals, private hospitals and clinics. The regional and tertiary hospitals however made up over 70% of isolates sent from non-enhanced sites.

To identify missed unreported cases, annual laboratory audits were conducted throughout the study period using a centralized National Health Laboratory Service Corporate Data Warehouse which consolidates cases for all public-sector laboratories. Audit cases were included in the surveillance database for incidence rate calculations. Cases were likely missed as isolates were submitted by staff working in busy routine clinical microbiological laboratories. Isolates were often delayed at the sites and submitted in batches with other surveillance organisms sent to the NICD. As *S. pneumoniae* is fastidious it was often non-viable by the time it reached the NICD.

Definitions

At enhanced sites where additional clinical information was available, underlying conditions were defined as asplenia, including sickle cell anemia; chronic illness (chronic lung, renal, liver, cardiac disease and diabetes); other immunocompromising conditions (excluding HIV), including organ transplant and malignancy; and other risk factors, including head injury with possible CSF leak, neurologic disorders, burns, chromosomal abnormalities, alcohol use and smoking. Clinical diagnoses were based on documented discharge diagnoses in patient medical records, with clinical syndrome separated into three groups: meningitis, bacteremic pneumonia, and bacteremia without focus/other. Pitt bacteremia score was calculated using 5 parameters: (1) oral temperature, (2) hypotension, (3) receipt of mechanical ventilation, (4) cardiac arrest, and (5) mental status. Severe disease was defined as a score of ≥4 points (*3*).

Serotypes were defined as serotype 1 or non-serotype 1 IPD. Penicillin non-susceptibility was categorized using 2013 Clinical and Laboratory Standards Institute breakpoints for oral penicillin V (susceptible, ≤0.06 μg/L; intermediately resistant, 0.12–1 μg/L and resistant, ≥2 μg/L) (*4*). Intermediately resistant and resistant groups were combined into a non-susceptible group for analysis. Pneumococcal disease was considered recurrent if diagnosed >21 days after a previous case in the same patient.

Other Interventions Affecting Invasive Pneumococcal Disease Trends in South Africa

Comprehensive HIV/AIDS treatment programs were implemented in South Africa in 2003 and access to treatment improved steadily with 80% coverage reported by 2012 (*5*).

Prevention of mother-to-child transmission programs also improved steadily with an associated decrease in mother-to-child HIV transmission rates from 12% in 2007 to 2.7% in 2011 (*6*) and 2.5% during 2012/2013 (*7*). This was despite a relatively constant prevalence of HIV in pregnant women of around 30% over the same period.

A manuscript describing the reduction in IPD in South Africa following the introduction of PCV (*2*) showed a 49% reduction in all serotype IPD and 85% reduction in PCV7 serotypes in HIV-uninfected children <2 years of age by 2012. In HIV-infected children PCV7 serotypes decreased by 86% and non-vaccine serotypes by 31% which showed the benefit of improvements in prevention of mother-to-child transmission of HIV, antiretroviral treatment in children and PCV7. Reductions in PCV13-serotype disease in 2009 and 2010, before the introduction of PCV13, were also most likely a result of ART. In HIV-infected children it was thought to be difficult to tease out the exact amount of reduction in pneumococcal disease due to PCV and that due to other interventions.

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Technical Appendix Table 1. Comparison of cases from GERMS-SA enhanced and non-enhanced sites for all age groups, 2003–2013

Variable	Enhanced sites n/N (%)	Non-enhanced sites n/N (%)	OR (95% CI)	p value
Age				<0.001
<1 y	3431/20,826 (16)	3470/23,397 (15)	1.21 (1.08–1.36)	
1–4 y	2899/20,826 (14)	2828/23,397 (12)	1.26 (1.12–1.41)	
5–9 y	1286/20,826 (6)	1587/23,397 (7)	0.99 (0.88–1.13)	
10–14 y	510/20,826 (2)	700/23,397 (3)	0.89 (0.77–1.04)	
15–24 y	1269/20,826 (6)	1551/23,397 (7)	1.00 (0.89–1.14)	
25–44 y	7909/20,826 (38)	9058/23,397 (39)	1.07 (0.96–1.19)	
45–64 y	2844/20,826 (14)	3371/23,397 (14)	1.04 (0.92–1.16)	
>64 y	678/20,826 (3)	832/23,397 (4)	Reference	
Sex				0.83
Female	10,686/20,984 (51)	12,510/24,516 (51)	Reference	
Male	10,298/20,984 (49)	12,006/24,516 (49)	1.00 (0.97–1.04)	
Province				<0.001
Gauteng	11,287/21,188 (53)	10,950/25,297 (43)	Reference	
Western Cape	3038/21,188 (14)	2864/25,297 (11)	1.03 (0.97–1.09)	
KwaZulu-Natal	3045/21,188 (14)	2321/25,297 (9)	1.27 (1.20–1.35)	
Eastern Cape	570/21,188 (3)	3101/25,297 (12)	0.18 (0.16–0.20)	
Free State	1365/21,188 (6)	1687/25,297 (7)	0.78 (0.73–0.85)	
Mpumalanga	726/21,188 (3)	1873/25,297 (7)	0.38 (0.34–0.41)	
North West	338/21,188 (2)	1510/25,297 (6)	0.22 (0.19–0.25)	
Limpopo	363/21,188 (2)	719/25,297 (3)	0.49 (0.43–0.56)	
Northern Cape	456/21,188 (2)	272/25,297 (1)	1.63 (1.40–1.89)	
Year				<0.001
2003	1927/21,188 (9)	1962/25,297 (8)	Reference	
2004	2297/21,188 (11)	2245/25,297 (9)	1.04 (0.96–1.13)	
2005	2488/21,188 (12)	2398/25,297 (9)	1.06 (0.97–1.15)	
2006	2202/21,188 (10)	2534/25,297 (10)	0.88 (0.81–0.96)	
2007	2148/21,188 (10)	2595/25,297 (10)	0.84 (0.77–0.92)	
2008	2051/21,188 (10)	2784/25,297 (11)	0.75 (0.69–0.82)	
2009	2039/21,188 (10)	2725/25,297 (11)	0.76 (0.70–0.83)	
2010	1918/21,188 (9)	2280/25,297 (9)	0.86 (0.78–0.93)	
2011	1615/21,188 (8)	2189/25,297 (9)	0.75 (0.69–0.82)	
2012	1305/21,188 (6)	1917/25,297 (8)	0.69 (0.63–0.76)	
2013	1198/21,188 (6)	1668/25,297 (7)	0.73 (0.66–0.81)	
Penicillin non-susceptibility				<0.001
Susceptible	10,536/16,338 (64)	10,986/16,510 (67)	Reference	
Non-susceptible	5802/16,338 (36)	5524/16,510 (33)	1.10 (1.05–1.15)	
Specimen type				<0.001
CSF	5697/21,188 (27)	11,446/25,297 (45)	Reference	
Blood culture	13,897/21,188 (66)	11,104/25,297 (44)	2.51 (2.41–2.62)	
Other specimens	1594/21,188 (8)	2747/25,297 (11)	1.17 (1.09–1.25)	
Serotype				<0.001
Non-serotype 1	19,246/21,186 (91)	22,690/25,294 (90)	Reference	
Serotype 1	1940/21,186 (9)	2604/25,294 (10)	0.88 (0.83–0.93)	

Technical Appendix Table 2. Characteristics of 10,899 patients ≥5 years of age with invasive pneumococcal disease caused by serotype 1 and non–serotype 1 *Streptococcus pneumoniae*, South Africa, 2003–2013*

	No. cases/no. total (%)		Univariate analysis†		Multivariable analysis†	
Variable	Serotype 1	Non–serotype 1	OR (95% CI)	p value	aOR (95% CI)	p value
Age group, y						
5–9	254/1,642 (15)	809/9,257 (9)	3.19 (2.29–4.45)	<0.001	13.48 (5.53–32.82)	<0.001
10–14	115/1,642 (7)	298/9,257 (3)	3.92 (2.71–5.66)		8.02 (3.15–20.43)	
15–24	201/1,642 (12)	755/9,257 (8)	2.71 (1.93–3.79)		5.65 (2.31–13.82)	
25–44	768/1,642 (47)	5,078/9,257 (55)	1.54 (1.13–2.10)		3.67 (1.53–8.76)	
45–64	257/1,642 (16)	1,839/9,257 (20)	1.42 (1.03–1.97)		2.57 (1.06–6.23)	
>64	47/1,642 (3)	478/9,257 (5)	Reference		Reference	
Black race	1452/1,576 (92)	7,854/8,889 (88)	1.54 (1.27–1.87)	<0.001		
Province						
Gauteng	951/1,642 (58)	4,804/9,257 (52)	Reference	<0.001	Reference	<0.001
Western Cape	99/1,642 (6)	1,443/9,257 (16)	0.35 (0.28–0.43)		0.24 (0.17–0.34)	
KwaZulu-Natal	228/1,642 (14)	1,469/9,257 (16)	0.78 (0.67–0.92)		0.80 (0.60–1.07)	
Eastern Cape	47/1,642 (3)	166/9,257 (2)	1.43 (1.03–1.99)		0.80 (0.39–1.63)	
Free State	130/1,642 (8)	516/9,257 (6)	1.27 (1.04–1.56)		0.89 (0.64–1.22)	
Mpumalanga	64/1,642 (4)	358/9,257 (4)	0.90 (0.69–1.19)		0.80 (0.43–1.49)	
North-West	34/1,642 (2)	148/9,257 (2)	1.16 (0.79–1.70)		2.25 (1.13–4.48)	
Limpopo	42/1,642 (3)	143/9,257 (2)	1.48 (1.04–2.11)		0.97 (0.47–2.01)	
Northern Cape	47/1,642 (3)	210/9,257 (2)	1.13 (0.82–1.56)		1.39 (0.85–2.26)	
Year of specimen collection						
2003	209/1,642 (13)	733/9,257 (8)	1.45 (1.16–1.80)	<0.001	1.17 (0.76–1.82)	0.01
2004	225/1,642 (14)	891/9,257 (10)	1.28 (1.03–1.58)		1.32 (0.87–2.00)	
2005	196/1,642 (12)	994/9,257 (11)	Reference		Reference	
2006	142/1,642 (9)	962/9,257 (10)	0.75 (0.59–0.95)		0.67 (0.42–1.09)	
2007	112/1,642 (7)	892/9,257 (10)	0.64 (0.50–0.82)		0.71 (0.44–1.14)	
2008	116/1,642 (7)	842/9,257 (9)	0.70 (0.55–0.89)		0.86 (0.56–1.32)	
2009	156/1,642 (10)	866/9,257 (9)	0.91 (0.73–1.15)		1.21 (0.80–1.84)	
2010	164/1,642 (10)	995/9,257 (11)	0.84 (0.67–1.05)		1.02 (0.66–1.57)	
2011	134/1,642 (8)	819/9,257 (9)	0.83 (0.65–1.05)		0.98 (0.63–1.51)	
2012	112/1,642 (7)	676/9,257 (7)	0.84 (0.65–1.08)		0.96 (0.62–1.48)	
2013	76/1,642 (5)	587/9,257 (6)	0.66 (0.49–0.87)		0.64 (0.40–1.04)	
Medical conditions/treatment						
Length of hospital stay, d						
≤3	481/1,443 (33)	2518/8,311 (30)	Reference	0.001	Reference	0.01
4–14	758/1,443 (53)	4289/8,311 (52)	0.93 (0.82–1.05)		0.86 (0.68–1.09)	
≥15	204/1,443 (14)	1504/8,311 (18)	0.71 (0.60–0.85)		0.64 (0.48–0.86)	
Previous hospital admission	166/1,153 (14)	2000/6,816 (29)	0.40 (0.34–0.48)	<0.001	0.45 (0.35–0.57)	<0.001
Underlying medical condition‡	310/953 (33)	2571/6,083 (42)	0.66 (0.57–0.76)	<0.001		
Antimicrobial drug use in previous 2 mo§	32/962 (3)	412/5,550 (7)	0.43 (0.30–0.62)	<0.001		
HIV infected	717/1,007 (71)	5373/6,338 (85)	0.44 (0.38–0.52)	<0.001	0.39 (0.31–0.49)	<0.001
Treated for TB in previous 3 mo	146/1,126 (13)	1373/6,659 (21)	0.57 (0.48–0.69)	<0.001	0.73 (0.57–0.95)	0.02
Died during hospitalization	461/1,422 (32)	2650/8,228 (32)	1.01 (0.90–1.14)	0.88		
Pneumococcal isolate characteristics						
Penicillin nonsusceptible¶	15/1,555 (1)	2916/8,829 (33)	0.02 (0.01–0.03)	<0.001	0.02 (0.01–0.04)	<0.001
Previous invasive pneumococcal disease**	26/1,642 (2)	396/9,257 (4)	0.36 (0.24–0.54)	<0.001	0.32 (0.16–0.63)	0.001
Clinical syndrome/specimen type						
Specimen type						
Cerebral spinal fluid	512/1,642 (31)	2626/9,257 (28)	Reference	0.05		
Blood	1025/1,642 (62)	5967/9,257 (64)	0.88 (0.78–0.99)			
Other	105/1,642 (6)	664/9,257 (7)	0.81 (0.65–1.02)			
Clinical syndrome††						
Meningitis	587/1,541 (38)	3043/8,793 (35)	Reference	0.02	Reference	0.006
Pneumonia	832/1,541 (54)	5076/8,793 (58)	0.85 (0.76–0.95)		1.28 (1.03–1.58)	
Bacteremia	122/1,541 (8)	674/8,793 (8)	0.94 (0.76–1.16)		1.76 (1.22–2.55)	

*All patients were reported from the enhanced Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance sites. aOR, adjusted odds ratio; OR, odds ratio; TB, tuberculosis
†Only variables significant on univariate and multivariable analysis are shown (exception is death during hospital admission). Variables not included in table are sex, Pitt bacteremia score, antimicrobial drug in previous 24 h, and viable culture. Prematurity and malnutrition were not included in the analysis because they were not considered relevant or actively collected for patients ≥5 years of age.
‡Includes asplenia or sickle cell anemia; chronic illness (i.e., chronic lung, renal, liver, cardiac disease, and diabetes); other immunocompromising conditions (i.e., organ transplant, primary immunodeficiency, immunotherapy, and malignancy, but excluding HIV); and other risk factors (i.e., head injury with possible cerebral spinal fluid leak, neurologic disorders, burns, chromosomal abnormalities, smoking, and alcohol use).
§Use of any antimicrobial drug in 2 mo before admission.
¶Considered penicillin nonsusceptible at MIC ≥0.12 μg/mL; intermediately resistant and resistant groups were combined into a nonsusceptible group.
**Invasive pneumococcal disease diagnosis >21 days before this episode.
††Clinical diagnoses were made on the basis of documented discharge diagnoses in patient medical records; clinical syndrome separated into 3 groups: meningitis, bacteremic pneumonia, and bacteremia without focus or other diagnosis (e.g., septic arthritis, endopthalmitis, peritonitis, pericarditis).

Technical Appendix Table 3. Factors associated with death in patients ≥5 years of age with serotype 1 invasive pneumococcal disease, South Africa, 2003–2013*

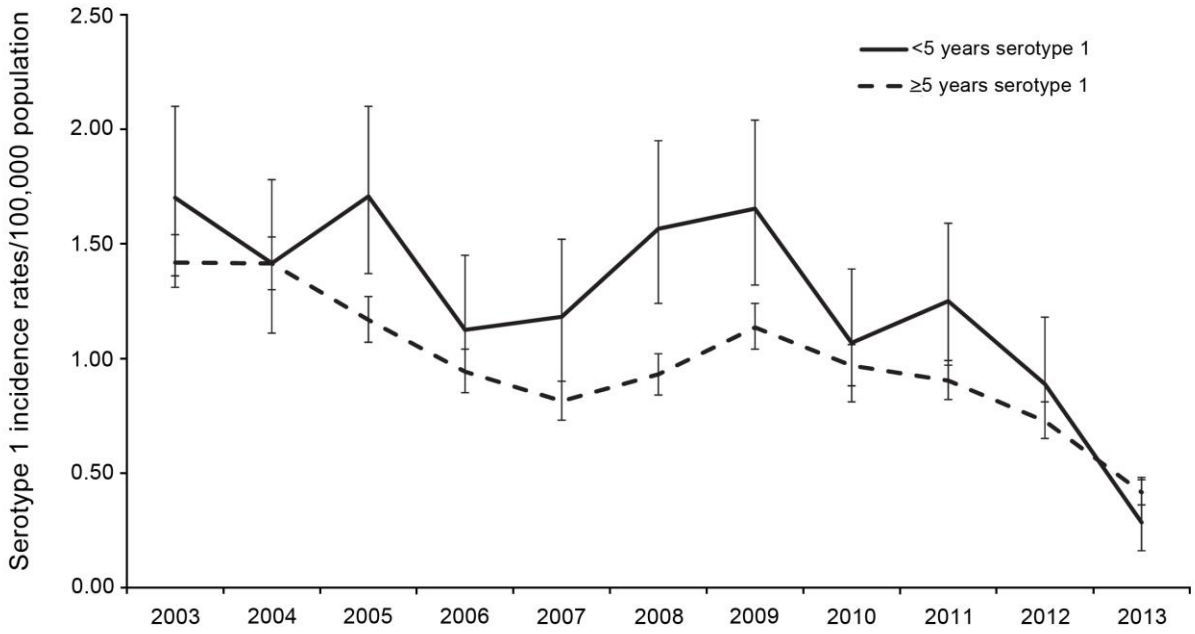
	Univariate analysis			Multivariable analysis	
Variable	No. deaths/no. cases (%)	OR (95% CI)	p value	aOR (95% CI)	p value
Demographic/socioeconomic characteristic					
Age group, y					
5–9	37/350 (11)	Reference	<0.001	Reference	<0.001
10–14	23/143 (16)	1.62 (0.92–2.84)		1.24 (0.65–4.57)	
15–24	90/362 (25)	2.80 (1.85–4.24)		3.05 (1.47–6.32)	
25–44	611/1,950 (31)	3.86 (2.71–5.50)		5.07 (2.74–9.38)	
45–64	285/686 (42)	6.01 (4.14–8.73)		9.00 (4.66–17.35)	
>64	58/133 (44)	6.54 (4.03–10.61)		10.13 (4.46–23.00)	
Race					
Nonblack	61/250 (24)	Reference	0.03		
Black	1,023/3,313 (31)	1.38 (1.02–1.86)			
Province					
Gauteng	706/2,444 (29)	Reference	<0.001		
Western Cape	54/217 (25)	0.82 (0.59–1.12)			
KwaZulu-Natal	98/329 (30)	1.04 (0.81–1.34)			
Eastern Cape	29/68 (43)	1.83 (1.12–2.98)			
Free State	59/189 (31)	1.12 (0.81–1.54)			
Mpumalanga	63/154 (41)	1.70 (1.22–2.38)			
North-West	34/70 (49)	2.32 (1.44–3.75)			
Limpopo	42/94 (45)	1.99 (1.31–3.01)			
Northern Cape	19/59 (32)	1.17 (0.67–2.03)			
Medical condition/treatment					
Length of hospital stay, d					
≥3	750/1,130 (66)	Reference	<0.001	Reference	<0.001
4–14	254/1,891 (13)	0.08 (0.07–0.09)		0.07 (0.05–0.10)	
≥15	93/577 (16)	0.10 (0.08–0.13)		0.06 (0.04–0.09)	
Pitt bacteremia score†					
0–3	744/2,920 (26)	Reference	<0.001	Reference	<0.001
≥4	258/361 (71)	7.33 (5.74–9.34)		5.26 (3.53–7.84)	
Underlying medical condition‡					
No	357/1,582 (23)	Reference	<0.001	Reference	0.004
Yes	257/827 (31)	1.55 (1.28–1.87)		1.53 (1.14–2.04)	
Antimicrobial drug use in 24 h before admission					
No	644/2,537 (25)	Reference	0.05		
Yes	32/93 (34)	1.54 (1.00–2.39)			
HIV status					
HIV uninfected	108/514 (21)	Reference	0.001		
HIV infected	610/2,165 (28)	1.47 (1.17–1.86)			
Treated for tuberculosis in previous 3 mo					
No	508/2,156 (24)	Reference	0.001	Reference	0.001
Yes	154/496 (31)	1.46 (1.18–1.81)		1.75 (1.25–2.45)	
Previous invasive pneumococcal disease§					
No	1097/3,536 (31)	Reference	<0.001		
Yes	7/88 (8)	0.19 (0.09–0.42)			
Clinical syndrome/specimen type					

Specimen type					
Cerebral spinal fluid	461/802 (57)	Reference	<0.001		
Blood	565/2,440 (23)	0.22 (0.19–0.26)			
Other	78/382 (20)	0.19 (0.14–0.25)			
Clinical syndrome¶					
Meningitis	531/982 (54)	Reference	<0.001	Reference	<0.001
Pneumonia	490/2,311 (21)	0.23 (0.19–0.27)		0.18 (0.13–0.25)	
Bacteremia	75/307 (24)	0.27 (0.21–0.37)		0.29 0.18–0.48)	

*All patients were reported from the enhanced Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance sites. Only variables significant on univariate and multivariable analysis are shown. Variables not included in table are sex, year, previous hospital admission, any antimicrobial drug used in 2 mo before admission, and penicillin-nonsusceptible invasive pneumococcal disease. Prematurity and malnutrition were not included in the analysis because they were not considered relevant or actively collected for patients ≥5 years of age. aOR, adjusted odds ratio; OR, odds ratio.
†Pitt bacteremia score calculated by using temperature, hypotension, mechanical ventilation, cardiac arrest, and mental status. Severe disease defined as score of ≥4 points.
‡Includes asplenia or sickle cell anemia; chronic illness (i.e., chronic lung, renal, liver, cardiac disease and diabetes); other immunocompromising conditions (i.e., organ transplant, primary immunodeficiency, immunotherapy, and malignancy, but excluding HIV); and other risk factors (i.e. head injury with possible cerebral spinal fluid leak, neurologic disorders, burns, and chromosomal abnormalities).
§Invasive pneumococcal disease diagnosis >21 days before this episode.
¶Clinical diagnoses were made on the basis of documented discharge diagnoses in patient medical records, with clinical syndrome separated into 3 groups: meningitis, bacteremic pneumonia, and bacteremia without focus or other diagnosis (e.g. septic arthritis, endopthalmitis, peritonitis, pericarditis).

Technical Appendix Table 4. Serotype 1 clusters, by district, in South Africa, 2003–2013

		Location			
Cluster	Period	District	Province	Relative risk	p value
1	May 2003–Dec 2004	City of Johannesburg	Gauteng	1.7	<0.001
		City of Tshwane	Gauteng		
		Ekurhuleni	Gauteng		
		Metweding	Gauteng		
		Sedibeng	Gauteng		
		West Rand	Gauteng		
		Sekhukhune Cross	Limpopo		
		Govan Mbeki	Mpumalanga		
		Nkangala	Mpumalanga		
		Bojanala	North-West		
		Southern	North-West		
2	Sep 2008–Apr 2012	Alfred Nzo	Eastern Cape	1.4	<0.001
		Amatole	Eastern Cape		
		Chris Hani	Eastern Cape		
		Ukhahlamba	Eastern Cape		
		Lejweleputswa	Free State		
		Motheo	Free State		
		Northern	Free State		
		Thabo Mofutsanyane	Free State		
		Xhariep	Free State		
		Ekurhuleni	Gauteng		
		Sedibeng	Gauteng		
		Amabuja	KwaZulu-Natal		
		Ethekwini	KwaZulu-Natal		
		iLembe	KwaZulu-Natal		
		Sisonke	KwaZulu-Natal		
		Ugu	KwaZulu-Natal		
		UMgungundlovu	KwaZulu-Natal		
		Umkhanyakude	KwaZulu-Natal		
		Umzinyathi	KwaZulu-Natal		
		Uthukela	KwaZulu-Natal		
		Uthungulu	KwaZulu-Natal		
		Zululand	KwaZulu-Natal		
		Govan Mbeki	Mpumalanga		
		Southern	North-West		



Technical Appendix Figure. Incidence rates for serotype 1 in children <5 years (n = 714) and individuals ≥5 years (n = 5167) of age, South Africa, 2003–2013. Error bars indicate CIs for incidence rates. N, imputed serotype 1 cases.

PAPER III

MAJOR ARTICLE

Increased Risk for and Mortality From Invasive Pneumococcal Disease in HIV-Exposed but Uninfected Infants Aged <1 Year in South Africa, 2009–2013

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(See the Editorial Commentary by Mofenson on pages 1357–60.)

Background. High antenatal human immunodeficiency virus (HIV) seroprevalence rates (~30%) with low perinatal HIV transmission rates (2.5%), due to HIV prevention of mother-to-child transmission program improvements in South Africa, has resulted in increasing numbers of HIV-exposed but uninfected (HEU) children. We aimed to describe the epidemiology of invasive pneumococcal disease (IPD) in HEU infants.

Methods. We conducted a cross-sectional study of infants aged <1 year with IPD enrolled in a national, laboratory-based surveillance program for incidence estimations. Incidence was reported for 2 time points, 2009 and 2013. At enhanced sites we collected additional data including HIV status and in-hospital outcome.

Results. We identified 2099 IPD cases in infants from 2009 to 2013 from all sites. In infants from enhanced sites (n = 1015), 92% had known HIV exposure status and 86% had known outcomes. IPD incidence was highest in HIV-infected infants, ranging from 272 to 654 per 100 000 population between time points (2013 and 2009), followed by HEU (33–88 per 100 000) and HIV-unexposed and uninfected (HUU) infants (18–28 per 100 000). The case-fatality rate in HEU infants (29% [74/253]) was intermediate between HUU (25% [94/377]) and HIV-infected infants (34% [81/242]). When restricted to infants <6 months of age, HEU infants (37% [59/175]) were at significantly higher risk of dying than HUU infants (32% [51/228]; adjusted relative risk ratio, 1.76 [95% confidence interval, 1.09–2.85]).

Discussion. HEU infants are at increased risk of IPD and mortality from IPD compared with HUU children, especially as young infants. HEU infants, whose numbers will likely continue to increase, should be prioritized for interventions such as pneumococcal vaccination along with HIV-infected infants and children.

Keywords. *Streptococcus pneumoniae*; HIV exposure; children; South Africa; pneumococcal conjugate vaccine.

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Programs for prevention of mother-to-child transmission (PMTCT) of human immunodeficiency virus (HIV) have expanded over the last decade in many countries. South Africa reported a decrease in mother-to-child HIV transmission rates from 12% in 2007 to 2.7% in 2011 [1] and 2.5% during 2012–2013 [2], despite a relatively constant prevalence of HIV in pregnant women of approximately 30%. This has resulted in an increasing number of HIV-exposed but uninfected (HEU) infants, especially in countries with elevated HIV prevalence such as South Africa.

All-cause hospitalization rates and complicated hospital admissions are more frequent in HEU than in HIV-unexposed and uninfected (HUU) infants [3, 4]. Some infectious diseases, including respiratory tract infections [5], are more common and often more severe among HEU than HUU children. Compared with HIV-infected children, 1 study reported similar rates of pneumonia and bacterial meningitis in HEU children, but higher rates of gastroenteritis and sepsis [6].

Some studies report higher mortality rates in HEU than HUU infants [7, 8] whereas others show no difference [9, 10]. In contrast, studies consistently report higher mortality rates in HIV-infected vs HEU or HUU infants [7]. Risk factors for mortality in HEU children include advanced maternal HIV disease [11, 12], malnutrition [8], severe pneumonia, and bacterial meningitis [6]. Mortality among HEU children peaks in younger infants (3–6 months), with death being predominantly associated with lower respiratory tract infections [8]. Within the general population, pneumococcus is estimated to cause 30%–40% of childhood community-acquired pneumonia cases [13]

There are no published data evaluating or quantifying the risk of hospitalization or mortality associated with invasive pneumococcal disease (IPD) among HEU children. We aimed to describe the epidemiology of IPD from 2009 to 2013 in South African HEU infants <1 year of age, compared to the epidemiology of IPD in similarly aged HUU and HIV-infected infants.

METHODS

Study Design and Setting

Detailed methods are available in the Supplementary Appendix. Children hospitalized from 2009 through 2013 with laboratory-confirmed IPD were prospectively identified by a national, laboratory-based, active surveillance program for *Streptococcus pneumoniae*. More than 200 routine hospital-based diagnostic laboratories (enhanced and nonenhanced hospital sites) systematically report IPD cases of all ages to the surveillance program. For the subset of cases occurring at 25 enhanced sentinel hospital sites, located in all 9 provinces, dedicated study surveillance officers collect additional clinical and demographic information.

Study Population

We included all infants <1 year of age with IPD from 2009 through 2013. For incidence calculations, infants from enhanced and nonenhanced sites were included. For analyses of factors associated with HIV exposure/infection status and mortality, only infants from enhanced sites with known HIV exposure status and in-hospital outcome were included.

Case Definitions

IPD cases were defined as *S. pneumoniae* identified from normally sterile site (eg, cerebrospinal fluid [CSF], blood, joint fluid, pleural fluid) specimens at participating sites. HUU infants were defined as infants with documented negative maternal HIV status at birth or time of illness, with or without a negative HIV enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) result for the infant. HEU infants were defined as infants who had a negative HIV PCR result with known positive maternal HIV status (verbal or documented positive result) or infants with a positive HIV ELISA result and negative HIV PCR result. HEU infants who had symptoms suggestive of HIV at the current admission were retested. HIV-infected infants were defined as infants with a positive HIV PCR result before or at time of illness.

Incidence

We calculated annual incidence of pneumococcal disease from 2009 through 2013 for infants <1 year of age, by HIV infection/exposure status, by dividing the number of laboratory-confirmed IPD cases reported each year in each category (HEU, HUU, and HIV) by the midyear population estimates for each group. Population denominators were obtained from the THEMBISA model [14]. Due to significant decreasing trends in IPD incidence rates (IRs) from 2009 through 2013, resulting from progressive pneumococcal conjugate vaccine (PCV) introduction and HIV-related interventions [15], we only presented data from 2 time points, prevaccine (2009) and postvaccine (2013) introduction.

As HIV infection/exposure status information was only available for cases identified at enhanced sites, we assumed a similar prevalence of HIV infection and exposure among cases with unknown (from nonenhanced sites) status as that found at enhanced sites. We calculated relative risk of IPD hospitalization comparing HEU children with HUU and HIV-infected children. Confidence intervals were calculated using Poisson distribution for incidence rates IRs and incidence rate ratios (IRRs).

Factors Associated With HIV Exposure Status and Death

We included infants <1 years of age with IPD from enhanced sites only, from 2009 through 2013, and developed 2 multivariable models to identify factors associated with outcome variables: (1) HIV infection/exposure status and (2) mortality.

Multinomial regression was used for comparison of factors associated with HIV infection/exposure. Multinomial regression allows modeling of outcome variables with >2 categories and relates the probability of being in category *j* to the probability of being in a baseline category. A complete set of coefficients are estimated for each of the *j* levels being compared with the baseline, and the effect of each predictor in the model is measured as relative risk ratio (RRR). HEU cases were used as the referent group and compared with HUU and HIV-infected infants so that all described differences would be related to

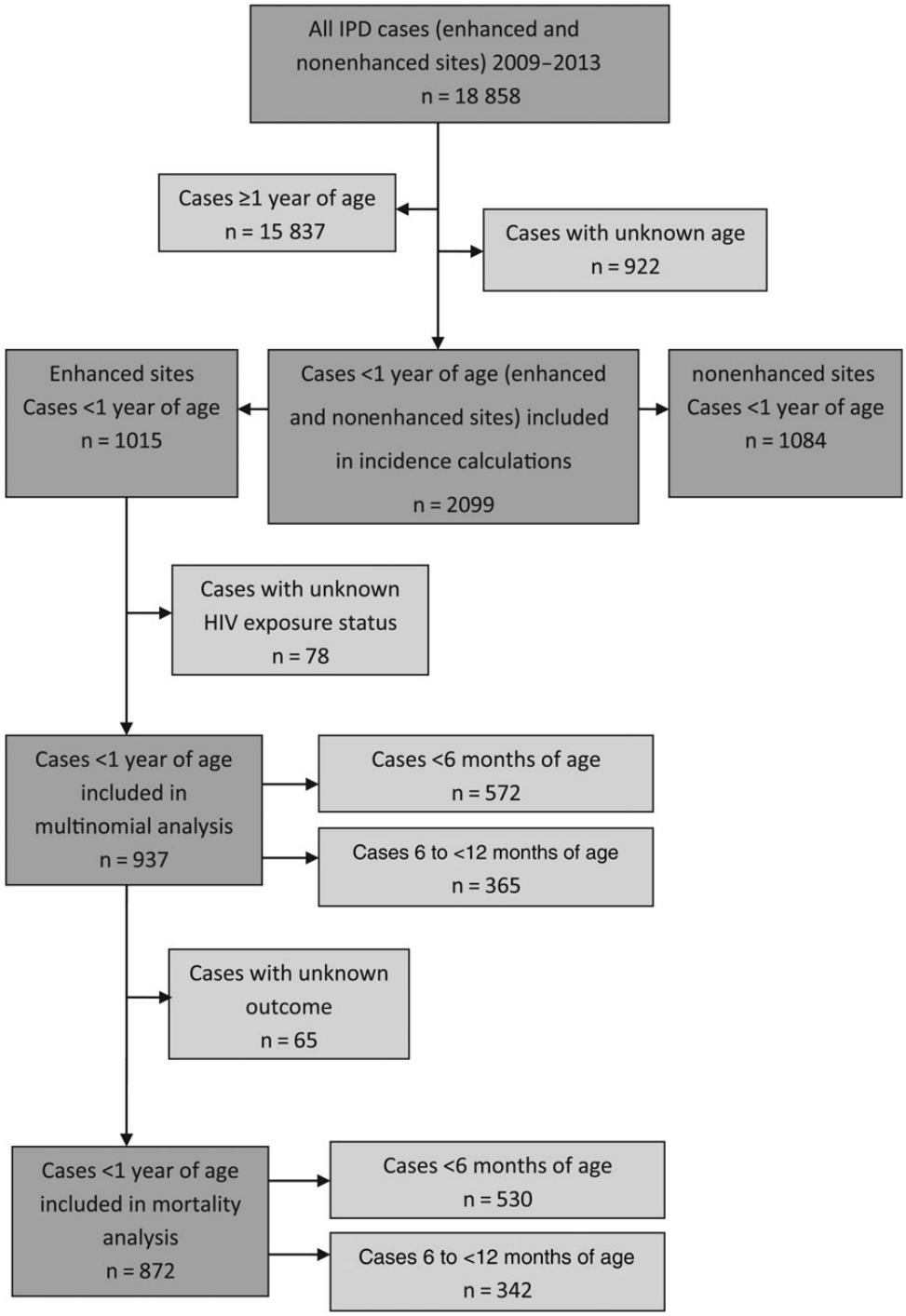

Figure 1. Patients with invasive pneumococcal disease (IPD) reported from the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa sites, 2009–2013. Abbreviation: HIV, human immunodeficiency virus.

exposed children. The model to assess factors associated with mortality used logistic regression and was presented stratified by age (<6 months and 6 to <12 months) as there was significant interaction between age and HIV infection/exposure status. Statistical analysis was implemented using Stata software, version 12 (StataCorp, College Station, Texas).

Ethics

Ethics approval was obtained for Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) surveillance (M081117) from the Human Research Ethics Committee (Medical), University of the Witwatersrand, Johannesburg, South Africa, and other local hospital or provincial ethics committees, as required.

RESULTS

We identified 2099 IPD cases in infants <1 year of age from 2009 through 2013 from all sites (Figure 1). Enhanced sites, predominantly regional and tertiary hospitals, contributed about 50% (n = 1015) of all isolates received. Nonenhanced sites included district, regional, and tertiary public hospitals, as well as private hospitals and clinics. Regional and tertiary hospitals contributed 73% (787/1084) of isolates sent from nonenhanced sites. In cases from enhanced sites, 92% (937/1015) had known HIV exposure/infection status and 86% (872/1015) had known in-hospital outcomes. Compared with nonenhanced sites, patients at enhanced sites were more likely to be diagnosed on positive blood (odds ratio [OR], 1.24; 95% confidence interval [CI], 1.04–1.48) or other specimen culture (OR, 2.36; 95% CI, 1.39–4.03), compared with CSF, as these specimens were more likely to be done at enhanced sites. Age distribution (<6 months and 6 to <12 months) did not differ (OR, 1.10; 95% CI, .92–1.31) between enhanced and nonenhanced sites (data not shown).

Breastfeeding information was only available for children enrolled in a nested case-control study. In the first 4 months of life, 33% (30/90) of HEU children, 81% (119/147) of HUU children, and 56% (43/77) of HIV-infected children were breastfed. Seventy-six percent (n = 207) of all HEU children from enhanced sites had known HIV testing dates; only 61 (29%) were tested more than a month prior to admission, and 34 (of the 61) had a known feeding status, with only 7 being breastfed.

Serotype distribution differed by known HIV status for enhanced-site patients. Across all years, isolates from HIV-infected cases were more likely to be vaccine serotypes (71% [160/225]) than isolates from HEU cases (57% [139/244]; $P < .001$), whereas prevalence was similar between isolates from HEU and HUU cases (56% [198/356]; $P = .88$). A similar proportion of cases was isolated from CSF and blood cultures in HIV-infected (33% [84/257] and 65% [167/257], respectively) and HEU cases (38% [103/273] and 61% [166/273], respectively), whereas proportions differed among HUU cases (CSF, 43% [175/407]; blood culture, 51% [207/407]; $P < .001$). The proportion of vaccine-type IPD decreased in all 3 groups between 2009 and 2013: 79% (48/61) and 30% (12/40) for HEU infants ($P < .001$); 72% (72/100) and 23% (15/64) for HUU infants ($P < .001$); and 85% (75/88) and 35% (7/20) ($P < .001$), for HIV-infected infants, respectively.

Incidence Rates

In 2009 (Table 1), IPD incidence in the <1-year age group was higher in HIV-infected compared with HUU (20-fold) and HEU infants (7-fold). HEU infants also had a 3-fold higher incidence of IPD than HUU infants. When stratified into 2 age groups, incidence was similarly highest in HIV-infected infants, intermediate in HEU infants, and lowest in HUU infants (Table 1). By 2013, although IRs had decreased due to PCV and HIV interventions, in all groups compared with 2009, relative trends in incidence by HIV exposure/infection status were

Table 1. Invasive Pneumococcal Disease Incidence Rates and Incident Rate Ratios Between Infants Aged <12 Months, <6 Months, and 6 to <12 Months, South Africa, 2009 and 2013

	Incidence Rates per 100 000 Population (95% CI)			Incidence Rate Ratio (95% CI)		
Age Group	HI	HEU	HUU	HI/HEU	HI/HUU	HEU/HUU
2009 (prevaccine)						
<6 mo	1156 (972–1364)	112 (94–132)	31 (26–37)	10.3 (8.1–13.1)	37.0 (29.0–47.2)	3.6 (2.8–4.6)
6 to <12 mo	467 (394–551)	59 (46–75)	26 (21–31)	7.9 (5.9–10.8)	18.0 (14.0–23.3)	2.3 (1.7–3.1)
<12 mo	654 (579–736)	88 (76–100)	28 (25–32)	7.5 (6.2–9.0)	23.1 (19.4–27.6)	3.1 (2.6–3.7)
2013 (postvaccine)						
<6 mo	581 (389–835)	57 (46–71)	21 (17–26)	10.1 (6.4–15.7)	27.2 (17.2–41.7)	2.7 (2.0–3.7)
6 to <12 mo	149 (92–227)	11 (6–18)	14 (11–18)	13.9 (6.7–29.5)	10.4 (6.0–17.4)	0.8 (.4–1.4)
<12 mo	272 (203–357)	33 (26–40)	18 (15–21)	8.4 (5.9–12.0)	15.0 (10.7–20.6)	1.8 (1.4–2.3)

Abbreviations: CI, confidence interval; HEU, HIV exposed but uninfected; HI, HIV infected; HIV, human immunodeficiency virus; HUU, HIV unexposed and uninfected.

similar. In 2013, among infants aged 6 to <12 months, incidence was similar between HEU and HUU cases, but case numbers were small in this age group, limiting the ability to detect relative differences in rates. Incidence rate and IRR were higher in the <6-month age group than in the 6- to <12-month age group regardless of HIV status (Table 1).

Factors Associated With HIV Exposure and Infection Status

For cases <1 year of age, with known outcomes and HIV status, the overall case-fatality ratio was high (29% [249/872]), with mortality in HEU infants (29% [74/253]) intermediate between HUU (25% [94/377]) and HIV-infected infants (34% [81/242]) ($P = .07$; Supplementary Table 1). When comparing HEU (n = 273) with HUU (n = 407) infants on multivariable analysis, HUU infants were twice as likely to be >6 months of age or to have meningitis vs pneumonia, but less likely to be of black race.

On multivariable analysis, HIV-infected infants (n = 257) (Supplementary Table 1) were more likely to be >6 months of age, to be infected with penicillin-nonsusceptible *S. pneumoniae*, to have used cotrimoxazole prophylaxis in the last month, and to have died compared with HEU children. In addition, HIV-infected infants were less likely to have underlying conditions other than HIV and malnutrition.

When we restricted the analysis to cases <6 months of age (Table 2), on multivariable analysis, HUU cases were at significantly lower risk of dying during the IPD episode, had a decreased risk of IPD caused by a penicillin-nonsusceptible strain, and had an increased risk of meningitis compared with pneumonia compared with HEU children with IPD. HIV-infected infants with IPD were more likely to have disease caused by a penicillin-nonsusceptible strain and be malnourished than HEU infants with IPD.

For cases aged 6 to <12 months (Table 3), on multivariable analysis, HUU infants were less likely to be of black race and at significantly increased risk of dying from their IPD episode compared with HEU children. HIV-infected infants were more likely to be malnourished and die than HEU infants, despite having less-severe disease at time of presentation (as assessed with Pitt bacteremia score), and fewer underlying conditions other than HIV. Among HUU cases, underlying conditions were significantly more common in infants aged 6 to <12 months (41/141 [29.1%]) than in younger infants (22/209 [10.5%]) ($P < .001$).

Factors Associated With Case Fatality

On multivariable analysis to explore factors associated with death, in infants aged <6 months (Table 4), being of black race, malnourished, or HEU or HIV-infected and having meningitis (compared with pneumonia) were associated with an increased odds of death. In infants aged 6 to <12 months (Table 5) with malnutrition (compared with no malnutrition), those with meningitis (compared with pneumonia) and HUU cases (compared with HEU cases) had increased odds of death.

DISCUSSION

In South Africa, HIV-infected pediatric numbers continue to decline due to PMTCT improvements [1]; however, numbers of HEU children remain high and are growing. We have shown that these HEU children are twice as likely to have an IPD-associated hospitalization; and that HEU children aged <6 months are less likely to survive an IPD episode than HUU children. It is important to prioritize and continue targeting these HEU children for public health interventions such as PCV vaccination.

Following PCV introduction into the national immunization program in South Africa, a significant reduction in vaccine-type disease in both HIV-infected and HIV-uninfected children was observed [15]. In our study, we similarly observed a reduction in the estimated incidence of IPD in HEU children from 2009 to 2013. Although other interventions such as improvements in maternal immune status [12] may have contributed to this, it is likely that the bulk of this reduction resulted from the introduction of PCV [15]. A case-control study from South Africa showed that PCV, when given in the routine program, was highly effective in HEU children [16]. Despite generally lower prevaccination antibody levels, HEU children respond quantitatively as well as HUU infants to routine immunization program vaccinations such as tetanus, pertussis, *Haemophilus influenzae* type b, and hepatitis B [17]. In contrast, PCV functional assays have shown that HEU children require higher antibody concentrations for effectiveness against certain pneumococcal serotypes [18].

In South Africa, antiretroviral therapy (ART) coverage in HIV-infected children increased from 2004, but by 2011, pediatric ART initiation rates still lagged behind that of adults. Nationally, the 2011–2012 coverage for children aged <18 months was reported as 54.4% with large variations between districts [14, 19]. In our study, HIV-infected children still had an elevated risk of IPD-associated hospitalization (15-fold) and IPD-related death (2-fold), compared with unexposed children. Other studies have shown that following ART introduction, although overall incidence of IPD decreased in HIV-infected children [20], the absolute risk of IPD remained approximately 20-fold greater in HIV-infected than HIV-uninfected children <2 years of age [21]. A case-control study from South Africa found that HIV exposure was associated with nearly 2 times greater odds of all serotype IPD, although the control group in this study was children hospitalized with a nonpneumonia diagnosis, a group also at increased likelihood of HIV exposure, and therefore this study likely underestimated the increased odds of IPD associated with HIV exposure [16].

In our study, a number of differences were noted between HEU, HUU, and HIV-infected infants with IPD. Malnutrition

Table 2. Univariate and Multivariate Multinomial Logistic Regression Model of Patients Aged <6 Months With Invasive Pneumococcal Disease, in Enhanced Sites, Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa, 2009–2013 (n = 572)

	HEU Cases (Reference)	HUU Cases			HIV-Infected Cases		
Characteristic	no./No. (%)	no./No. (%)	RRR (95% CI)	ARRR (95% CI)	no./No. (%)	RRR (95% CI)	ARRR (95% CI)
Demographics and socioeconomic characteristics							
Black race	172/176 (97.7)	203/239 (84.9)	0.13 (.05–.38)	0.15 (.05–.45)	129/130 (99.2)	3.00 (.33–27.16)	3.48 (.37–32.69)
Length of hospital stay							
<4 d	48/174 (27.6)	47/227 (20.7)	Reference		37/127 (29.1)	Reference	
4–14 d	90/174 (51.7)	102/227 (44.9)	1.16 (.71–1.89)		60/127 (47.2)	0.86 (.50–1.48)	
≥15 d	36/174 (20.7)	78/227 (34.4)	2.21 (1.26–3.89)		30/127 (23.6)	1.08 (.57–2.06)	
Medical conditions and treatment							
Underlying conditions[a]	21/151 (13.9)	22/209 (10.5)	0.73 (.38–1.38)		4/113 (3.5)	0.23 (.08–.68)	
Malnutrition[b]	58/168 (34.5)	81/221 (36.7)	1.10 (.72–1.67)	1.20 (.72–2.02)	80/122 (65.6)	3.61 (2.21–5.90)	3.19 (1.80–5.64)
Previous hospital admission in last 12 mo	32/172 (18.6)	42/224 (18.8)	1.01 (.61–1.68)		41/119 (34.5)	2.30 (1.34–3.94)	
In-hospital mortality	59/175 (33.7)	51/228 (22.4)	0.57 (.36–.88)	0.46 (.26–.81)	50/126 (39.7)	1.29 (.80–2.08)	1.55 (.87–2.76)
Previous IPD infection[c]	2/189 (1.1)	4/249 (1.6)	1.53 (.28–8.42)		7/134 (5.2)	5.15 (1.05–25.21)	
Cotrimoxazole prophylaxis	26/170 (15.3)	0/249 (0.0)	Not calculated		37/110 (33.6)	2.81 (1.58–4.99)	
Treated for tuberculosis	5/174 (2.9)	6/229 (2.6)	0.91 (.27–3.03)		12/116 (10.3)	3.90 (1.34–11.39)	
Pneumococcal isolate characteristics							
Penicillin nonsusceptible[d]	69/157 (43.9)	69/201 (34.3)	0.67 (.43–1.02)	0.61 (.38–.99)	64/105 (61.0)	1.99 (1.20–3.29)	1.79 (1.03–3.09)
Vaccine serotypes[e]	94/171 (55.0)	110/217 (50.7)	0.84 (.56–1.26)		77/111 (69.4)	1.86 (1.12–3.07)	
Clinical syndrome							
Pneumonia	74/183 (40.4)	77/239 (32.2)	Reference	Reference	69/131 (52.7)	Reference	Reference
Meningitis	92/183 (50.3)	129/239 (54.0)	1.35 (.88–2.04)	1.89 (1.12–3.20)	47/131 (35.8)	0.55 (.34–.89)	0.86 (.46–1.57)
Bacteremia	17/183 (9.3)	33/239 (13.8)	1.87 (.96–3.63)	1.96 (.86–4.44)	15/131 (11.5)	0.95 (.44–2.04)	1.81 (.75–4.36)
Specimen type[f]							
Blood culture	105/189 (55.6)	117/249 (47.0)	Reference		89/134 (66.4)	Reference	
Cerebrospinal fluid	81/189 (42.9)	123/249 (49.4)	1.36 (.93–2.00)		42/134 (31.3)	0.61 (.38–.98)	
Other	3/189 (1.6)	9/249 (3.6)	2.69 (.71–10.21)		3/134 (2.2)	1.18 (.23–5.99)	

Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: sex, Pitt bacteremia score, antibiotics in last 24 hours, antibiotics in last 2 months, and vaccination status.

Abbreviations: ARRR, adjusted relative risk ratio; CI, confidence interval; HEU, HIV exposed but uninfected; HIV, human immunodeficiency virus; HUU, HIV unexposed and uninfected; IPD, invasive pneumococcal disease; RRR, relative risk ratio.

[a] Asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver, or cardiac disease, and diabetes; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy, and malignancy; and other risk factors, including head injury with possible cerebrospinal fluid leak, neurological disorders, burns, and chromosomal abnormalities. Excludes malnutrition.

[b] Malnutrition was classified as weight-for-age *z* score <−2 (World Health Organization child growth standards 2009) and/or nutritional edema.

[c] Previously diagnosed with IPD >21 days prior to this episode.

[d] Penicillin-nonsusceptible minimum inhibitory concentration ≥0.12 μg/mL.

[e] Vaccine serotypes were considered as serotypes in the 13-valent pneumococcal conjugate vaccine.

[f] Elected to use clinical diagnosis rather than specimen type in multivariable model.

Table 3. Univariate and Multivariate Multinomial Logistic Regression Model in Patients Aged 6 to <12 Months With Invasive Pneumococcal Disease at Enhanced Sites, Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa, 2009–2013 (n = 365)

	HEU Cases Reference	HUU Cases			HIV-Infected Cases		
Characteristic	no./No. (%)	no./No. (%)	RRR (95% CI)	ARRR (95% CI)	no./No. (%)	RRR (95% CI)	ARRR (95% CI)
Demographics and socioeconomic characteristics							
Black race	78/81 (96.3)	132/154 (85.7)	0.23 (.07–.80)	0.15 (.03–.70)	114/117 (97.4)	1.46 (.29–7.43)	0.82 (.09–7.23)
Medical conditions and treatment							
Underlying conditions[a]	15/77 (19.5)	41/141 (29.1)	1.69 (.87–3.31)	1.99 (.90–4.37)	11/103 (10.7)	0.49 (.21–1.15)	0.29 (.09–.87)
Malnutrition[b]	31/78 (39.7)	54/147 (36.7)	0.88 (.50–1.55)	0.68 (.35–1.34)	73/109 (67.0)	3.07 (1.68–5.63)	2.36 (1.13–4.96)
Previous hospital admission in last 12 mo	19/74 (25.7)	57/148 (38.5)	1.81 (.98–3.36)		51/111 (45.9)	2.46 (1.29–4.67)	
In-hospital mortality	15/78 (19.2)	43/149 (28.9)	1.70 (.88–3.31)	3.38 (1.34–8.53)	31/116 (26.7)	1.53 (.76–3.08)	2.82 (1.02–7.78)
Pitt bacteremia score (≥4)[c]	9/76 (11.8)	21/151 (13.9)	1.20 (.52–2.77)	0.62 (.21–1.84)	5/113 (4.4)	0.34 (.11–1.07)	0.13 (.02–0.72)
Antibiotics in last 2 mo[d]	7/73 (9.6)	37/145 (25.5)	3.23 (1.36–7.66)		23/104 (22.1)	2.68 (1.08–6.63)	
Cotrimoxazole prophylaxis	8/73 (11.0)	0/158 (0.0)	Not calculated	Not calculated	50/111 (45.1)	6.66 (2.92–15.17)	11.18 (4.04–30.91)
Treated for tuberculosis	4/75 (5.3)	4/148 (2.7)	0.49 (.11–2.02)		18/112 (16.1)	3.40 (1.10–10.48)	
Pneumococcal isolate characteristics							
Penicillin nonsusceptible[e]	30/62 (48.4)	68/130 (52.3)	1.17 (.64–2.14)		74/111 (66.7)	2.13 (1.13–4.03)	
Specimen type							
Blood culture	61/84 (72.6)	90/158 (57.0)	Reference		78/123 (63.4)	Reference	
Cerebrospinal fluid	22/84 (26.2)	52/158 (32.9)	1.60 (.88–2.90)		42/123 (34.1)	1.49 (.81–2.76)	
Other	1/84 (1.2)	16/158 (10.1)	10.84 (1.40–83.92)		3/123 (2.4)	2.35 (.24–23.12)	

Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: sex, length of hospital stay, antibiotics in last 24 hours, previous IPD infection, vaccine serotypes, vaccination status, and clinical syndrome.

Abbreviations: ARRR, adjusted relative risk ratio; CI, confidence interval; HEU, HIV exposed but uninfected; HIV, human immunodeficiency virus; HUU, HIV unexposed and uninfected; IPD, invasive pneumococcal disease; RRR, relative risk ratio.

[a] Asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver, or cardiac disease, and diabetes; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy, and malignancy; and other risk factors, including head injury with possible cerebrospinal fluid leak, neurological disorders, burns, and chromosomal abnormalities. Excludes malnutrition.

[b] Malnutrition was classified as weight-for-age *z* score <−2 (World Health Organization child growth standards 2009) and/or nutritional edema.

[c] Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest, and mental status. Severe disease defined as score of ≥4 points.

[d] Any antibiotics used in 2 months prior to admission.

[e] Penicillin nonsusceptible minimum inhibitory concentration ≥0.12 μg/mL.

Table 4. Univariate and Multivariable Analysis Showing Factors Associated With Mortality in Infants Aged <6 Months With Invasive Pneumococcal Disease, South Africa, 2009–2013 (n = 530)

	Univariate Analysis[a]			Multivariable Analysis[a]	
Characteristic	CFR, no./No. (%)	OR (95% CI)	*P* Value	AOR (95% CI)	*P* Value
Demographics and socioeconomic characteristics					
Race					
Nonblack	5/44 (11.4)	Reference	.03	Reference	.02
Black	164/501 (32.7)	3.31 (1.14–9.64)		4.14 (1.22–14.04)	
Length of hospital stay					
<4 d	121/140 (86.4)	Reference	<.001		
4–14 d	35/254 (13.8)	0.02 (.01–.04)			
≥15 d	9/144 (6.3)	0.01 (.004–.02)			
Medical conditions and treatment					
Malnutrition[b]					
No	77/292 (26.4)	Reference	.27	Reference	.03
Yes	75/218 (34.4)	1.26 (.84–1.90)		1.63 (1.05–2.53)	
Pitt bacteremia score[c]					
0–3	109/440 (24.8)	Reference	<.001		
≥4	46/73 (63.0)	5.03 (2.92–8.65)			
Any antibiotics used in last 24 h[d]					
No	114/439 (26.0)	Reference	.02		
Yes	23/58 (39.7)	2.05 (1.15–3.66)			
HIV status					
HUU	51/228 (31.9)	Reference	.002	Reference	.007
HEU	59/175 (36.9)	1.77 (1.13–2.75)		1.76 (1.09–2.85)	
HIV-infected	50/126 (31.3)	2.28 (1.42–3.67)		2.25 (1.32–3.82)	
Clinical syndrome					
Pneumonia	64/223 (28.7)	Reference	.10	Reference	.009
Meningitis	90/260 (34.6)	1.47 (.98–2.22)		1.92 (1.22–3.03)	
Bacteremia	17/66 (25.8)	0.89 (.46–1.74)		0.92 (.45–1.88)	

Abbreviations: AOR, adjusted odds ratio; CFR, case-fatality rate; CI, confidence interval; HEU, HIV exposed but uninfected; HIV, human immunodeficiency virus; HUU, HIV unexposed and uninfected; OR, odds ratio.

[a] Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: age group, sex, wood fire in the home, referral, previous admission, low birth weight, underlying conditions, antibiotics in last 2 months, penicillin nonsusceptibility, previous invasive pneumococcal disease infection, cotrimoxazole prophylaxis, tuberculosis treatment, and vaccination status.

[b] Malnutrition was classified as weight-for-age *z* score <−2 (World Health Organization child growth standards 2009) and/or nutritional edema.

[c] Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest, and mental status. Severe disease defined as score of ≥4 points.

[d] Any antibiotics used in 24 hours prior to admission.

was significantly more common in HIV-infected infants, but not in HEU compared with HUU infants. This concurs with a review of studies that showed an association between HIV infection and being stunted or underweight [22]; no differences were observed in the early growth of HEU children and healthy controls [22]. Combined ART used for PMTCT has been shown to cause lower birth weight and length in some HEU infants, but this rapidly corrects over the first few months of life [23].

Other differences between the 3 IPD case groups included clinical presentation, with HUU IPD cases more likely to present with meningitis than pneumonia. HIV-infected children are less likely to be diagnosed with meningitis than other types of IPD [24], and children with meningitis have a higher mortality than children with pneumonia or bacteremia, especially if they are HIV infected [24]. Specimen-collection practices differed between different case groups, reflecting different clinical syndromes; HUU infants were less likely to have blood cultures taken than HEU and HIV-infected infants.

Racial differences, with HUU children being more likely to be of nonblack race, has been shown in other local studies [25]. Children of black race had a higher likelihood of dying with IPD, possibly reflecting poorer socioeconomic status and higher HIV infection rates. IPD in HIV-infected individuals is more often caused by antibiotic-resistant strains than IPD in HIV-

Table 5. Multivariable Analysis Showing Factors Associated With Mortality in Infants Aged 6 to <12 Months With Invasive Pneumococcal Disease, South Africa, 2009–2013 (n = 342)

	Univariate Analysis[a]			Multivariable Analysis[a]	
Characteristic	CFR, no./No. (%)	OR (95% CI)	*P* Value	AOR (95% CI)	*P* Value
Demographics and socioeconomic characteristics					
Length of hospital stay					
<4 d	67/91 (73.6)	Reference	<.001		
4–14 d	15/164 (9.2)	0.03 (.02–.07)			
≥15 d	15/99 (15.2)	0.07 (.03–.15)			
Medical conditions					
Malnutrition[b]					
No	32/177 (18.1)	Reference	.003	Reference	.001
Yes	48/158 (30.4)	2.30 (1.32–4.01)		2.58 (1.45–4.60)	
Pitt bacteremia score[c]					
0–3	66/309 (21.4)	Reference	<.001		
≥4	28/41 (68.3)	8.66 (3.94–19.05)			
HIV status					
HUU	43/149 (28.9)	Reference	.29	Reference	.06
HEU	15/78 (19.2)	0.59 (.30–1.14)		0.46 (.22–.98)	
HIV-infected	31/116 (26.7)	0.90 (.52–1.55)		0.55 (.29–1.04)	
Clinical syndrome					
Pneumonia	37/164 (22.6)	Reference	.04	Reference	.03
Meningitis	48/130 (36.9)	1.82 (1.06–3.10)		2.16 (1.19–3.92)	
Bacteremia	16/67 (23.9)	0.88 (.42–1.84)		1.03 (.48–2.24)	

Abbreviations: AOR, adjusted odds ratio; CFR, case-fatality rate; CI, confidence interval; HEU, HIV exposed but uninfected; HIV, human immunodeficiency virus; HUU, HIV unexposed and uninfected; OR, odds ratio.

[a] Only variables significant on univariate and multivariable analysis are shown. Variables not included in table: age group, sex, race, wood fire in the home, referral, previous admission, low birth weight, underlying conditions, antibiotics in last 24 hours, antibiotics in last 2 months, penicillin nonsusceptibility, previous invasive pneumococcal disease infection, cotrimoxazole prophylaxis, tuberculosis treatment, and vaccination status.

[b] Malnutrition was classified as weight-for-age *z* score <−2 (World Health Organization child growth standards 2009) and/or nutritional edema.

[c] Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest, and mental status. Severe disease defined as score of ≥4 points.

uninfected individuals [26, 27]. Antimicrobial resistance is an important adverse consequence of cotrimoxazole prophylaxis [28]; this correlated with what we found in our study.

Cohort studies suggest that mortality among children born to HIV-infected mothers is higher than that among children born to HIV-uninfected mothers [29, 30]. A pooled mortality analysis, using African data, showed a 9 times higher mortality rate in HIV-infected than HIV-uninfected children. Children with a early positive PCR result (<4 weeks of age) were more likely to die, as were those with mothers who died or who had low $CD4^+$ cell counts at delivery [31]. In our study, we observed a higher IPD-associated case fatality rate in HEU infants compared with HUU infants in the <6-month age group. The increased fatality rate among HEU children may be due to immunological differences that resolve as these children age; thus, younger HEU children may be more vulnerable to adverse clinical outcomes [32]. Other studies have also shown a higher mortality in younger HEU children [8]. In the older infants (6 to <12 months), this relationship was reversed, with HUU infants less likely to survive IPD, but there were small numbers of infants in the comparison group of HEU infants aged 6 to <12 months (n = 78). By 6 months of age, the immunologic deficit associated with HIV exposure is reduced [8], and effects of HIV exposure on adverse outcomes in this group are less marked. Last, increased case-fatality rates in older HUU infants with IPD may be due to a higher proportion of these infants having an underlying condition or, possibly, other factors leading to high mortality in HUU infants with IPD that we were not able to document. Children with underlying conditions have been extensively described to have a higher risk of IPD than healthy children [33]. HIV infection is an independent risk factor for IPD [34, 35]. This would account for the higher rate of underlying conditions in HEU and HUU infants with IPD, compared to HIV-infected infants with IPD. The difference in underlying condition rates between HEU and HUU cases was not statistically significant and no solid conclusions could be made regarding this comparison.

A number of factors are thought to contribute to differences in case-fatality rates between HEU and HUU children. Most important are different immunological deficits documented in HEU children [36–39]. Second, a clear trend has been shown between the degree of maternal immunosuppression and infant survival [40]. We did not collect details regarding maternal CD4 count or use of ART by the mother during pregnancy and could therefore not explore this association further, which was a limitation.

Our study had other limitations. As with most surveillance studies, only patients who had samples taken could be identified as an IPD case and included in the study. For the multinomial and mortality analyses, we only included IPD cases from enhanced sites with viable isolates. These enhanced-site cases were more likely to be diagnosed with positive blood cultures, which may limit the generalizability of our findings. Infection status of HEU children was decided by 1 negative PCR result in some infants, so it is possible there may have been some misclassification of HIV status. The majority of patients had PCR testing done within a month of admission and nurses were trained to request retesting in symptomatic children, which would have minimized HIV-infected children being included in the HEU group. Some data, such as cotrimoxazole prophylaxis, were ascertained on verbal report if not available in the medical records; therefore, underreporting is possible.

In conclusion, we have described a higher incidence of hospitalization for IPD in HEU children compared with HUU children, as well as a lower chance of surviving IPD in HEU children <6 months of age compared with those who are HUU. Although we did not collect maternal data, we propose that optimizing maternal immunological status for HIV-infected women during pregnancy may help to improve outcomes in HEU children. Although widespread PCV introduction has led to substantial reductions in IPD incidence in South Africa [15], some differences were observed in vaccination rates between HIV exposure groups. It is important to ensure that all HEU children receive PCV, to reduce the risk of IPD and its negative health outcomes, including death.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC), the Agency for Toxic Substances and Disease Registry, NICD/National Health Laboratory Service (NHLS), or the Global Alliance for Vaccines and Immunisation (GAVI).

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Potential conflicts of interest. C. v. M. has received honoraria from Pfizer. A. v. G. has received research funding from Pfizer. T. A. has received honoraria and conference support from Pfizer. G. R. has received honoraria and conference support from Pfizer and Sanofi. K. L. O. and K. P. K. have received research funding and honoraria from Pfizer and GlaxoSmithKline. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Increased risk and mortality of invasive pneumococcal disease in HIV-exposed-uninfected infants <1 year of age in South Africa, 2009-2013

Supplementary appendix

Table of contents

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1.2 Methods

Study design and setting

For each non-enhanced case a laboratory report form (with information on age, gender, date of specimen collection and source of specimen) and the associated pneumococcal isolate is submitted to the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa. Additional clinical and demographic information collected at enhanced sites includes admission date, HIV exposure and infection status, discharge diagnosis, vaccination status and outcome through patient interview and medical record review.

Case definitions

Laboratory testing for pneumococcus was performed as part of routine medical care. Only IPD cases diagnosed by positive culture or polymerase chain reaction (PCR), or by latex agglutination test with supporting evidence (Gram stain or PCR positive) were included.

Malnutrition was defined according to the World Health Organization (WHO) child growth standards. Malnourished infants included those with weight-for-age Z-scores less than minus two standard deviations or nutritional edema. Underlying conditions included asplenia; chronic illness, including chronic lung, renal, liver and cardiac disease; other immunocompromising conditions (excluding HIV); and other risk factors, including head injury with possible CSF leak, neurological disorders, burns and chromosomal abnormalities, but excluded malnutrition. Clinical diagnoses were based on documented discharge diagnoses in the medical records with clinical syndrome, being defined as meningitis, bacteremic pneumonia, and bacteremia without focus/other. Pitt bacteremia score was

calculated using (1) oral temperature, (2) hypotension, (3) receipt of mechanical ventilation, (4) cardiac arrest and (5) mental status. Severe disease was defined as a score of ≥4 points [1]. A case was considered to be recurrent if pneumococcal disease was diagnosed in the same patient more than 21 days after the first confirmed laboratory diagnosis of *S. pneumoniae* disease.

Cotrimoxazole prophylaxis is administered in HEU and HIV-infected children for differing time periods to prevent PCP and is not given to HUU children. We therefore included this variable *a priori* in our analysis as it could confound other associations.

Penicillin non-susceptibility was categorized using the 2010 Clinical and Laboratory Standards Institute breakpoints for oral penicillin V (susceptible, ≤0.06mg/L; intermediately resistant, 0.12-1mg/L and resistant, ≥2mg/L) [2]. The intermediately resistant and resistant groups were combined into a non-susceptible group for analysis. Vaccine-serotype (VT) IPD was defined as serotypes present in the 13-valent pneumococcal conjugate vaccine (PCV-13) (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). All other serotypes were designated non-vaccine types (NVT).

Incidence

Denominators for HIV-infected infants <1 year of age were calculated by combining denominators for new HIV infection at/before birth and new HIV infection due to breastfeeding in infants <1 year. Denominators for HEU infants were calculated from the population denominator for HIV-infected pregnant women, adjusted for live births and less HIV-infected infants <1 year of age and infants who were infected postnatally.

Factors associated with HIV exposure status and death

Multinomial regression allows modeling of outcome variables with more than 2 categories and relates the probability of being in category *j* to the probability of being in a baseline category. A complete set of coefficients are estimated for each of the *j* levels being compared with the baseline and the effect of each predictor in the model is measured as relative risk ratio (RRR).

1.3 Tables

Supplementary Table 1: Univariate and multivariate multinomial logistic regression model showing comparison of demographic, socio-economic characteristics, and underlying conditions in HIV-exposed-uninfected (HEU), HIV-unexposed-uninfected (HUU) and HIV-infected (HI) IPD cases <1 year of age, at enhanced GERMS-SA sites in South Africa, 2009-2013 (n=937)

	HEU cases Reference	HUU cases			HI cases		
	n/N (%)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)	n/N (%)	RRR[a] (95%CI)	ARRR[b] (95%CI)
Demographics and socioeconomic characteristics							
Age ≥ 6 months	84/273 (31.0)	158/407 (38.8)	1.43 (1.03-1.98)	1.82 (1.17-2.84)	123/257 (47.9)	2.07 (1.45-2.95)	2.71 (1.67-4.38)
Male Sex	147/273 (53.9)	229/407 (56.3)	1.10 (0.81-1.50)		138/256 (53.9)	1.00 (0.71-1.41)	
Black Race	250/257 (97.3)	335/393 (85.2)	0.16 (0.07-0.36)	0.13 (0.05-0.36)	243/247 (98.4)	1.70 (0.49-5.88)	1.39 (0.32-5.99)
Length of hospital stay:							
<4 days	65/251 (25.9)	85/376 (22.6)	Reference		65/244 (26.6)	Reference	
4-14 days	129/251 (51.4)	170/376 (45.2)	1.01 (0.68-1.50)		113/244 (46.3)	0.88 (0.57-1.34)	
≥15 days	57/251 (22.7)	121/376 (32.2)	1.62 (1.03-2.55)		66/244 (27.1)	1.16 (0.71-1.90)	
Medical conditions, treatment and vaccination status							

Underlying conditions[c]	36/228 (15.8)	63/350 (18.0)	1.17 (0.75-1.83)	1.31 (0.73-2.35)	15/216 (6.9)	0.40 (0.21-0.75)	0.30 (0.14-0.63)
Malnutrition[d]	89/246 (36.2)	135/368 (36.7)	1.02 (0.73-1.43)		153/231 (66.2)	3.46 (2.37-5.04)	
Previous hospital admission in last 12 months	51/246 (20.7)	99/372 (26.6)	1.39 (0.94-2.04)		92/230 (40.0)	2.55 (1.70-3.82)	
In-hospital mortality	74/253 (29.3)	94/377 (24.9)	0.80 (0.56-1.15)	1.06 (0.65-1.74)	81/242 (33.5)	1.22 (0.83-1.78)	2.03 (1.18-3.49)
Pitt bacteremia score (≥4)[e]	35/245 (14.3)	49/367 (13.4)	0.92 (0.58-1.48)		20/237 (8.4)	0.55 (0.31-0.99)	
Antibiotics in last 24 hours[f]	20/240 (8.3)	49/362 (13.5)	1.72 (1.00-2.98)		24/223 (10.8)	1.33 (0.71-2.48)	
Antibiotics in last 2 months [g]	24/235 (10.2)	69/365 (18.9)	2.05 (1.25-3.37)		45/212 (21.2)	2.39 (1.39-4.05)	
Previous IPD infection [h]	4/273 (1.5)	12/407 (3.0)	2.04 (0.65-6.40)		15/257 (5.8)	4.16 (1.36-12.73)	
Cotrimoxazole prophylaxis	34/243 (14.0)	0/407 (0.0)	Not calculated	Not calculated	87/221 (39.4)	3.99 (2.54-6.27)	4.56 (2.63-7.89)
Treated for tuberculosis	9/249 (3.6)	10/377 (2.7)	0.73 (0.29-1.81)		30/228 (13.2)	4.04 (1.87-8.71)	
Vaccination status [i]							
- 0 doses	115/238 (48.3)	134/339 (39.5)	Reference		62/173 (35.8)	Reference	
- 1 dose	64/238 (26.9)	98/339 (28.9)	1.31 (0.88-1.96)		47/173 (27.1)	1.36 (0.84-2.22)	
- 2 doses	59/238 (24.8)	107/339 (31.6)	1.56 (1.03-2.33)		64/173 (37.0)	2.01 (1.26-3.22)	
Pneumococcal isolate characteristics							

Penicillin non-susceptible [j]	99/219 (45.2)	137/331 (41.4)	0.86 (0.61-1.21)	0.74 (0.49-1.11)	138/216 (63.9)	2.14 (1.46-3.15)	1.66 (1.04-2.65)
Vaccine serotypes[k]	139/244 (57.0)	198/356 (55.6)	0.95 (0.68-1.31)		160/225 (71.1)	1.86 (1.27-2.73)	
Clinical syndrome and specimen type							
Clinical syndrome							
- Pneumonia	115/266 (43.2)	141/395 (35.7)	Reference	Reference	124/249 (49.8)	Reference	Reference
- Meningitis	118/266 (44.4)	188/395 (47.6)	1.30 (0.93-1.82)	1.61 (1.04-2.50)	94/249 (37.8)	0.74 (0.51-1.07)	0.72 (0.44-1.19)
- Bacteremia	33/266 (12.4)	66/395 (16.7)	1.63 (1.01-2.65)	1.67 (0.88-3.18)	31/249 (12.5)	0.87 (0.50-1.51)	1.05 (0.51-2.15)
Specimen type[l]							
- Blood culture	166/273 (60.8)	207/407 (50.9)	Reference		167/257 (65.0)	Reference	
- Cerebrospinal fluid	103/273 (37.7)	175/407 (43.0)	1.36 (0.99-1.87)		84/257 (32.7)	0.81 (0.57-1.16)	
- Other	4/273 (1.5)	25/407 (6.1)	5.01 (1.71-14.69)		6/257 (2.3)	1.49 (0.41-5.38)	

[a]Relative risk ratio; [b]Adjusted relative risk ratio; [c]Asplenia, including asplenia or sickle cell anemia; chronic illness, including chronic lung, renal, liver, cardiac disease and diabetes; other immunocompromising conditions (excluding HIV), including organ transplant, primary immunodeficiency, immunotherapy and malignancy; and other risk factors, including head injury with possible CSF leak, neurological disorders, burns and chromosomal abnormalities. Excludes malnutrition; [d]Malnutrition was classified as children with weight-for-age Z-score < -2 (WHO child growth standards 2009) and/or children with nutritional edema; [e]Pitt bacteremia score calculated using temperature, hypotension, mechanical ventilation, cardiac arrest and mental status. Severe disease defined as score of ≥4 points; [f]Any antibiotics used in 24 hours prior to admission; [g]Any antibiotics used in 2 months prior to admission; [h]Previously diagnosed with IPD (invasive pneumococcal disease) more than 21 days prior to this episode; [i]Vaccination status determined only for cases eligible to have received the pneumococcal conjugate vaccine;

[j]Penicillin non-susceptible MIC ≥ 0.12 µg/mL; [k]Vaccine serotypes were considered as serotypes in the 13-valent pneumococcal conjugate vaccine; [l]Elected to use clinical diagnosis rather than specimen type in multivariable model

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PAPER IV

Risk Factors for Invasive Pneumococcal Disease Among Children Less Than 5 Years of Age in a High HIV Prevalence Setting, South Africa, 2010 to 2012

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Background: Invasive pneumococcal disease (IPD) causes significant disease burden, especially in developing countries, even in the era of pneumococcal conjugate vaccine and maternal-to-child HIV transmission prevention programs. We evaluated factors that might increase IPD risk in young children in a high HIV prevalence setting.

Methods: We conducted a case–control study using IPD cases identified at 24 Group for Enteric, Respiratory and Meningeal disease Surveillance—South Africa program sites (2010–2012). At least 4 controls were matched by age, HIV status and hospital to each case. Potential risk factors were evaluated using multivariable conditional logistic regression.

Results: In total, 486 age-eligible cases were enrolled. Factors associated with IPD in HIV-uninfected children (237 cases, 928 controls) included siblings <5 years [adjusted odds ratio (aOR) = 1.68, 95% confidence interval (CI): 1.16–2.46], underlying medical conditions (aOR = 1.99, CI 1.22–3.22), preceding upper respiratory tract infection (aOR = 1.79, CI 1.19–2.69), day-care attendance (aOR = 1.58, CI 1.01–2.47), perinatal HIV exposure (aOR = 1.62, CI 1.10–2.37), household car ownership (aOR = 0.45, CI 0.25–0.83) and ≥2 7-valent pneumococcal conjugate vaccine doses (aOR = 0.67, CI 0.46–0.99). Among HIV-infected children (124 cases, 394 controls), IPD-associated factors included malnutrition (aOR = 2.68, CI 1.40–5.14), upper respiratory tract infection (aOR = 3.49, CI 1.73–7.03), tuberculosis in the last 3 months (aOR = 5.12, CI 1.69–15.50) and current antiretroviral treatment (aOR = 0.13, CI 0.05–0.38).

Conclusion: Previously identified factors related to poverty, poor health and intense exposure continue to be risk factors for IPD in children. Ensuring delivery of pneumococcal conjugate vaccine and antiretroviral treatment are important for improving disease prevention.

Key Words: pneumococcus, risk factors, HIV, HIV exposure, children, South Africa, pneumococcal conjugate vaccine

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Pneumococcal disease is an important contributor to mortality in young children in developing countries.[1] In spite of this, few studies have evaluated specific factors that might lead to severe pneumococcal infections in such settings. HIV infection, common in South Africa and several other developing countries, is one well-described factor that increases the risk of invasive pneumococcal disease (IPD) in young children.[2]

South Africa and other countries have begun aggressive programs to prevent HIV infection, and therefore pneumococcal disease and other HIV-related complications, in newborns. In South Africa, prevention of mother to child transmission and early infant diagnosis programs have been rapidly scaled up from 2008, and by 2010, treatment was being offered at more than 95% of health facilities.[3] In 2010, the percentage of HIV-exposed children who were infected was 3.5% (CI 2.9–4.1%),[3] and this dropped to 2.7% (CI 2.1–3.2%) in 2011, despite an increase in the percentage of HIV-exposed infants (31.4% to 32.2%).[4] While the numbers of HIV-exposed, uninfected (HEU) children are growing because of programs designed to prevent HIV infection in newborns, the risk of IPD in this group of children is unknown. Data from developing countries have shown an increased risk of lower respiratory tract

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infections[5] and infection-related hospitalizations in the first year of life in children born to HIV-infected mothers but who themselves are not HIV infected.[6,7]

In addition to HIV prevention programs, pneumococcal conjugate vaccines (PCVs) are now being introduced in many developing countries to prevent serious pneumococcal disease.[8] In South Africa, PCV was first introduced into the national immunization program in April 2009. Only a few studies have evaluated risk factors for ongoing disease in settings using PCV, and all are from high-income settings.[9] In addition, in studies conducted prior to PCV introduction, risk factors for IPD are well described for high-income settings[10] while data from developing countries are more limited.[11]

The main objective of this analysis was to identify factors related to IPD among HIV-infected and HIV-uninfected children eligible to receive PCV through the South African Expanded Program on Immunization, including an examination of whether HIV exposure was a risk factor for disease in HIV-uninfected children. We separately evaluated risk factors for all-serotype, vaccine-type (VT) and nonvaccine type (NVT) IPD, as well as for PCV-vaccinated and unvaccinated children.

METHODS

Study Design and Population

The study was part of a larger protocol that evaluated effectiveness of the 7-valent PCV; the methods have been previously described.[12] In brief, we used a matched case–control study design to examine IPD risk factors in South African children admitted to selected hospitals from March 2010 to November 2012. Only children aged ≥8 weeks of age who were part of the birth cohort eligible to receive PCV through the South African Expanded Program on Immunization were included in the study. The study was nested within the Group for Enteric, Respiratory and Meningeal disease Surveillance—South Africa national, laboratory-based, active surveillance program for IPD and was conducted at 24 sentinel-enhanced surveillance hospitals, each with dedicated surveillance officers who collected clinical, demographic, vaccination and outcome information for cases and controls. All data, including information on potential disease risk factors, were collected on interview and medical record review using a structured questionnaire.

IPD cases were defined as *Streptococcus pneumoniae* identified from a sterile specimen, for example, cerebrospinal fluid, blood, joint fluid and pleural fluid. VT IPD cases were defined as those caused by serotypes in the 7-valent vaccine (4, 6B, 9V, 14, 18C, 19F, 23F). Serotype 6A was excluded from the VT and NVT groups as the effect of cross-protection was unclear in this population. All other serotypes were considered to be NVT. For each case, we attempted to enroll at least 4 matched controls per case. Surveillance officers enrolled controls from children admitted to the same hospital as the case or from patients seen in the hospital casualty or outpatient departments. Controls were excluded if they were diagnosed with IPD, pneumonia or diseases with established vaccine programs, like measles and pertussis. Patients admitted with gastroenteritis were eligible as controls, although South Africa began using rotavirus vaccine in August 2009. Controls, who were at least 8 weeks of age at case specimen date, were matched by date of birth (within ±1 calendar month from date of birth of case for children ≤12 months and ±2 months for children >12 months of age), hospital and HIV status (HIV-infected or HIV-uninfected).

Malnutrition was classified as children with weight-for-age Z scores <−2 using the 2009 World Health Organization child growth standards and/or those with nutritional edema.[13] HIV status was determined by HIV enzyme-linked immunosorbent assay testing for patients ≥18 months of age and by qualitative HIV DNA polymerase chain reaction testing for children <18 months of age. HEU children were defined as children who had a negative HIV polymerase chain reaction result with a known verbal or documented positive maternal HIV result. Data were collected for a reference period (1 month prior to the specimen collection date) for each case and their matched controls.

Pneumococci were serotyped by the Quellung reaction using specific antisera (Statens Serum Institut, Copenhagen, Denmark). Specimens that were culture negative were confirmed as pneumococci using real-time *lytA* polymerase chain reaction.[14] Minimum inhibitory concentrations were determined for all isolates using broth dilution. Results were interpreted as penicillin susceptible or nonsusceptible based on the 2010 Clinical and Laboratory Standards Institute meningitis breakpoints (penicillin nonsusceptible if minimum inhibitory concentrations are ≥0.12 μg/L).[15]

Statistical Methods

Data were entered into an Access database and analyzed using STATA software (STATA version 12; Stata Corp., College Station, TX). All models were run separately for HIV-uninfected and HIV-infected children. To determine risk factors associated with IPD we ran separate multivariable models for all-serotype, VT, NVT and penicillin nonsusceptible IPD. We conducted an open-ended analysis aiming to identify all factors associated with having IPD. All factors, including socioeconomic factors, with a P value ≤0.20 in the univariate analysis were included in conditional logistic regression models for multivariable analysis. All nonsignificant factors (assessed at $P > 0.05$) were dropped from the multivariable models employing stepwise regression starting with predictors with the highest P values. We checked for collinearity and 2-way interactions in all final models. We hypothesized that factors associated with VT IPD might differ among vaccinated (children who received ≥2 PCV doses) and unvaccinated (no PCV doses) children. To assess this we contrasted the effect of each predictor using vaccination status as an interaction term for each covariate included in the model. For all multivariable models, children with missing values for certain variables were excluded from models containing these variables.

Ethical Considerations

The study was approved by the Human Research Ethics Committee, University of the Witwatersrand (M090915), the CDC Human Research and Protection Office (Protocol number 5834), the Johns Hopkins School of Public Health Institutional Review Board (Institutional Review Board number 00002484) and other provincial and institutional ethics committees involved in the study. Signed informed consent was obtained from the parents or legal guardians of the children for participation in this study.

RESULTS

A total of 817 IPD cases in children <5 years of age were screened at enhanced sites and 486 age-eligible cases were enrolled in the study from March 2010 through November 2012. Children with incomplete vaccination or HIV information (n = 24), those who refused consent (n = 2), as well as those who had received PCV-13 (n = 99) were excluded from the analysis. A total of 361 (74%) cases (124 HIV-infected and 237 HIV-uninfected) were included in the final risk factor analysis. A total of 2037 eligible age-matched children were identified as potential controls. As with cases, controls lacking vaccination information (n = 75) and those who refused consent (n = 10), were discharged from hospital before enrolment (n = 17) or had received PCV-13 (n = 613) were excluded from the analysis. In total, 1322 (65%) age-matched controls (394 HIV-infected and 928 HIV-uninfected) were included in the final risk factor analysis. Among

HIV-uninfected children, the rates of malnutrition were high in both cases (80/234, 34.2%) and controls (258/839, 30.8%), as these were mainly hospitalized children. The most common diagnoses in our control group were acute gastroenteritis and malnutrition. The proportion of controls enrolled from the outpatient department was 3.7% (34/928) for HIV-uninfected children and 50.2% (198/394) for HIV-infected children.

Factors Associated With IPD in HIV-uninfected Children

All-serotype IPD

On univariate analysis, HIV-uninfected cases differed from controls for many factors evaluated, including black race, some markers of poverty and intense exposure (Table 1). On multivariable analysis, siblings <5 years old, underlying medical conditions, preceding upper respiratory tract infections (URTIs), day-care attendance and HIV exposure were all associated with increased odds of all-serotype IPD compared with controls. Belonging to a household with a car or having received 2 or more doses of PCV-7 resulted in decreased odds for all-serotype disease (Table 1). When we restricted our analysis to the ward control group, the results did not change (data not shown).

VT and NVT IPD

The analyses in HIV-uninfected children included 64 cases of VT IPD and 128 of NVT IPD. For VT IPD, on univariate analysis only, black race, previous admission in the last 12 months and cotrimoxazole prophylaxis use were more likely in cases than controls (data not shown). On multivariable analysis, lacking a flushable toilet and HIV exposure were associated with increased odds of having VT IPD, while male sex and receiving 2 or more doses of PCV-7 resulted in decreased odds in cases compared with controls (Table 2).

On univariate analysis, when stratified by vaccination status, vaccinated cases with VT IPD were more likely to have a wood fire in the home, malnutrition and previous admission than controls, while no additional variables were identified in unvaccinated cases (data not shown). On multivariable analysis, HIV exposure was associated with VT IPD [odds ratio (OR) 3.82] in vaccinated cases, while among unvaccinated cases having an underlying condition (OR 10.11) or HIV exposure (OR 8.80) was associated with VT IPD (Table 3).

TABLE 1. Univariate and Multivariable Analysis of Risk Factors for All-serotype IPD Among HIV-uninfected Children, South Africa, 2010–2012

			HIV-uninfected Univariate Analysis*		HIV-uninfected Multivariable Analysis†	
Characteristics	Cases n/N (%)	Controls n/N (%)	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Demographics						
Black race	214/237 (90.3)	771/926 (83.3)	2.07 (1.22–3.50)	0.007		
Primary caregiver education level						
No secondary schooling	39/231 (16.9)	127/926 (13.7)	Ref.	0.03		
Some secondary schooling	133/231 (57.6)	503/926 (54.3)	0.84 (0.54–1.30)			
Completed secondary schooling	59/231 (25.5)	296/926 (32.0)	0.57 (0.35–0.93)			
Socioeconomic characteristics						
Number of siblings <5 years of age‡						
0	106/230 (46.1)	546/926 (59.0)	Ref.	0.001	Ref.	0.007
≥1	124/230 (53.9)	380/926 (41.0)	1.65 (1.22–2.24)		1.69 (1.16–2.46)	
Wood fire in home	20/234 (8.6)	48/928 (5.2)	2.60 (1.20–5.65)	0.02		
Underlying health conditions						
Underlying conditions§ excluding malnutrition	44/237 (18.6)	127/928 (13.7)	1.35 (0.91–2.00)	0.14	1.99 (1.22–3.22)	0.005
Child had URTI (in reference period¶)	110/233 (47.2)	340/928 (36.6)	1.82 (1.30–2.54)	0.001	1.79 (1.19–2.69)	0.005
Received antibiotic treatment in reference period	41/234 (17.5)	121/922 (13.1)	1.68 (1.11–2.55)	0.01		
Previous hospital admission last 12 months	66/235 (28.1)	189/928 (20.4)	1.65 (1.17–2.33)	0.005		
Day-care attendance (in reference period¶)	49/233 (21.0)	143/927 (15.4)	1.53 (1.05–2.24)	0.03	1.58 (1.01–2.47)	0.04
HIV exposure	101/229 (44.1)	271/897 (30.2)	1.92 (1.40–2.63)	<0.001	1.62 (1.10–2.37)	0.01
Household has a car	28/237 (11.8)	171/928 (18.4)	0.57 (0.36–0.90)	0.02	0.45 (0.25–0.83)	0.01
Vaccination						
Received ≥3 DTP doses for children ≥16 weeks‖	106/187 (56.7)	504/752 (67.0)	0.61 (0.41–0.90)	0.01		
Received ≥2 PCV doses for children ≥16 weeks‖	110/187 (58.8)	509/752 (67.7)	0.67 (0.46–0.97)	0.03	0.67 (0.46–0.99)	0.05

DTP indicates diphtheria–tetanus toxoids–pertussis.

*Factors not significant on univariate analysis (>0.05) not shown (male sex, living in informal residence, building material of residence, flush toilet, crowding, smoke exposure, malnutrition, low birth weight, preterm birth, had ear infection in last 12 months, household member had URTI, breastfed in reference period, diagnosed with tuberculosis in 3 months prior to reference period, received ≥3 Hep B doses for children ≥16 weeks, received influenza vaccine within last year).

†Only factors significant on multivariable analysis shown in table.

‡Siblings: there were only 42 children in the >2 sibling group so this was combined with the ≥1 group.

§Underlying conditions included sickle cell disease, chronic kidney disease, cardiac disease, immunodeficiency conditions, chronic liver disease, asthma, neuromuscular diseases, connective tissue diseases, cancer, bone marrow or organ transplant, metabolic disease, chromosomal conditions, history of head injury/head surgery, hydrocephalus with ventriculoperitoneal shunt, burns requiring hospitalization, measles in the last month, any other chronic illness.

¶Reference period is the 1 month preceding the case pneumococcal specimen collection date.

‖Only children ≥16 weeks included in the model.

TABLE 2. Factors Significantly Associated with IPD on Multivariable Analysis Among HIV-uninfected Children: Risk Factors for VT IPD

Characteristics	Cases n/N (%)	Controls n/N (%)	Multivariable Analysis*	
			OR (95% CI)	*P* Value
Male sex	25/64 (39.1)	143/255 (56.1)	0.30 (0.13–0.69)	0.005
No flush toilet in residence	28/63 (44.4)	90/255 (35.3)	3.33 (1.29–8.57)	0.01
HIV exposure	32/62 (51.6)	78/249 (31.3)	3.05 (1.30–7.14)	0.01
Received ≥2 doses of PCV (≥16 weeks of age)†	17/48 (35.4)	123/194 (63.4)	0.16 (0.07–0.40)	<0.001

*Only factors significant on multivariable analysis shown in table.
†Only children ≥16 weeks included in model.

TABLE 3. Factors Significantly Associated with IPD on Multivariable Analysis Among HIV-uninfected Children: Risk Factors for VT IPD Stratified by Vaccination Status*

	Unvaccinated (Number of Cases = 14)			Vaccinated (Number of Cases = 17)		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Underlying conditions† (excluding malnutrition)	10.11	1.20–85.33	0.03	1.13	0.27–4.67	0.87
HIV exposure	8.80	1.23–62.94	0.03	3.82	1.21–12.04	0.02

*Includes contrast statement for vaccination status.
†Underlying conditions included sickle cell disease, chronic kidney disease, cardiac disease, immunodeficiency conditions, chronic liver disease, asthma, neuromuscular diseases, connective tissue diseases, cancer, bone marrow or organ transplant, metabolic disease, chromosomal conditions, history of head injury/head surgery, hydrocephalus with ventriculoperitoneal shunt, burns requiring hospitalization, measles in the last month, any other chronic illness.

Results of the analysis of factors associated with NVT IPD were similar to those from the analysis of all-serotype IPD (Table 4). On univariate analysis only, factors that differed between HIV-uninfected cases and controls included being admitted in the last year, having received antibiotics or having a car in the household. As with all-serotype disease, on multivariable analysis, having siblings <5 years old, preceding URTI and HIV exposure were 2 times more common among cases with NVT IPD than among controls. In addition, having a wood fire in the home was also associated with NVT disease (Table 4).

Penicillin Nonsusceptible All-serotype IPD

On univariate analysis only, having a preceding URTI, or having received PCV-7 or antibiotics, was associated with penicillin-non-susceptible IPD in HIV-uninfected cases. On multivariable analysis, factors associated with penicillin nonsusceptible all-serotype IPD in HIV-uninfected cases were black race, previous hospital admission in the last year, HIV exposure and day-care attendance in the reference period (Table 5).

Factors Associated With IPD in HIV-infected Children

All-serotype IPD

Factors associated with IPD in HIV-infected cases differed from those factors that were identified as significant in HIV-uninfected cases (Table 6). On univariate analysis only, HIV-infected cases differed from controls for a number of factors, including having received antibiotics, HIV stage, CD4 count and attending HIV clinic. On multivariate analysis, being diagnosed with tuberculosis in the last 3 months, preceding URTI and the presence of malnutrition increased the odds of all-serotype disease in cases compared with controls. Using antiretroviral treatment (ART) decreased the odds of all-serotype IPD (Table 6). When we restricted our analysis to either ward or clinic control groups, the results did not change (data not shown). We also evaluated HIV stage and CD4 count as potential effect modifiers, but no statistically significant interactions were identified.

VT IPD and NVT IPD

The analysis included 46 cases of VT IPD and 49 of NVT IPD in HIV-infected children. For VT IPD, on univariate analysis only, caregiver education, previous admission in the last 12 months, URTI and antibiotic use were more likely in cases than controls (data not shown). On multivariable analysis, the factors associated with VT IPD in HIV-infected cases were similar to those for all-serotype IPD, namely having malnutrition, being diagnosed with tuberculosis in the 3 months prior to the reference period and use of ART in the reference period (Table 7).

When stratified by vaccination status, on univariate analysis, vaccinated cases with VT IPD were more likely to be malnourished, have been previously admitted, have an underlying condition and have a mother who was educated, compared with controls. On multivariable analysis only preceding URTI and use of ART were associated with HIV-infected vaccinated cases with VT IPD (n = 27) (Table 8). No risk factors were identified in the HIV-infected unvaccinated group (n = 6) on univariate or multivariable analysis, likely as a result of low numbers.

Similar factors were also associated with enhanced or reduced odds of NVT IPD in HIV-infected cases as compared with all-serotype IPD (Table 9). Having malnutrition was only significant on univariate analysis.

Penicillin Nonsusceptible, All-serotype IPD

The factors associated with IPD caused by penicillin nonsusceptible strains were the same as those identified for IPD caused by all serotypes, with similar ORs (Table 10). Use of ART was associated with lower odds of IPD with a penicillin nonsusceptible isolate [OR 0.08 (95% CI: 0.02–0.40)]. Previous antibiotic use, day-care attendance and HIV stage were not significantly associated with penicillin nonsusceptible IPD in cases on multivariable modeling (Table 10).

DISCUSSION

This study demonstrated that, in the era of programs to prevent HIV transmission to newborns and ART, HIV still plays

TABLE 4. Factors Significantly Associated with IPD on Multivariable Analysis Among HIV-uninfected Children: Risk Factors for NVT IPD

Characteristics	Cases n/N (%)	Controls n/N (%)	Multivariable Analysis* OR (95% CI)	*P* Value
Number of siblings <5 years of age†				
0	56/126 (44.4)	302/488 (61.9)	Ref.	0.002
≥1	70/126 (55.6)	186/488 (38.1)	2.01 (1.29–3.15)	
Wood fire in home	9/127 (7.1)	16/488 (3.3)	4.99 (1.31–18.96)	0.02
Had URTI (in reference period‡)	55/127 (43.3)	153/488 (31.4)	1.81 (1.10–2.96)	0.02
HIV exposure	55/124 (44.4)	141/470 (30.0)	1.96 (1.25–3.07)	0.003

*Only factors significant on multivariable analysis shown in table.
†Siblings: There were only 26 children in the >2 sibling group so this was combined with the ≥1 group.
‡Reference period is the 1 month preceding the pneumococcal specimen collection date.

TABLE 5. Factors Significantly Associated with IPD on Multivariable Analysis Among HIV-uninfected Children: Risk Factors for Penicillin-resistant, All-serotype IPD

Characteristics	Cases n/N (%)	Controls n/N (%)	Multivariable analysis* OR (95% CI)	*P* Value
Black race	92/100 (92.0)	326/402 (81.1)	3.04 (1.16–8.00)	0.02
Previous hospital admission in last 12 months	28/98 (28.6)	78/402 (19.4)	2.11 (1.14–3.88)	0.02
Day-care attendance	24/98 (24.5)	54/402 (13.4)	1.91 (1.00–3.66)	0.05
HIV exposure	44/93 (47.3)	111/388 (28.6)	2.14 (1.27–3.62)	0.004

*Only factors significant on multivariable analysis shown in table.

TABLE 6. Univariate and Multivariable Analysis of Risk Factors For All-serotype IPD Among HIV-infected Children, South Africa, 2010–2012

Characteristics	Cases n/N (%)	Controls n/N (%)	HIV-infected Univariate Analysis* OR (95% CI)	*P* Value	HIV-infected Multivariable Analysis† OR (95% CI)	*P* Value
Underlying health conditions						
Malnutrition‡ (in reference period§)	79/120 (65.8)	124/325 (38.2)	3.23 (1.95–5.34)	<0.001	2.68 (1.40–5.14)	0.003
Child had URTI (in reference period§)	72/120 (60.0)	130/385 (33.8)	3.35 (2.03–5.53)	<0.001	3.49 (1.73–7.03)	<0.001
Received antibiotic treatment in reference period§	26/120 (21.7)	44/394 (11.2)	2.08 (1.15–3.76)	0.02		
Breast-fed (in reference period§)	40/122 (32.8)	56/393 (14.3)	3.35 (1.91–5.86)	<0.001		
Received cotrimoxazole prophylaxis in last 3 months	56/121 (46.3)	232/391 (59.3)	0.62 (0.39–0.98)	0.04		
Diagnosed with tuberculosis in 3 months prior to reference period§	22/122 (18.0)	45/382 (11.8)	1.90 (1.03–3.51)	0.04	5.12 (1.69–15.50)	0.004
ART use (in reference period§)	22/113 (19.5)	119/306 (38.9)	0.34 (0.19–0.65)	0.001	0.13 (0.05–0.38)	<0.001
HIV stage						
Stage 1	10/119 (8.4)	59/372 (15.9)	Ref.	0.002		
Stage 2	6/119 (5.0)	20/372 (5.4)	1.39 (0.41–4.66)			
Stage 3	41/119 (34.5)	165/372 (44.4)	1.45 (0.64–3.31)			
Stage 4	62/119 (52.1)	128/372 (34.4)	3.28 (1.44–7.43)			
Severe immunosuppression¶	68/86 (79.1)	201/329 (61.1)	2.28 (1.23–4.22)	0.008		
Regular attendance at HIV clinic‖	23/118 (19.5)	208/383 (54.3)	0.14 (0.07–0.27)	<0.001		
Vaccination						
Received ≥3 DTP doses for children ≥16 weeks	67/109 (61.5)	264/347 (76.1)	0.50 (0.30–0.85)	0.01		

*Factors not significant on univariate analysis (>0.05) not shown (male sex, black race, living in informal residence, building material of residence, flush toilet, crowding, caregiver education level, number of siblings, smoke exposure, wood fire in the home, underlying conditions, low birth weight, preterm birth, had ear infection in last 12 months, household member had URTI, previous hospital admission in last 12 months, day-care attendance in reference period, household had electricity, received ≥3 Hep B doses for children ≥16 weeks, received ≥3 PCV doses for children ≥16 weeks, received influenza vaccine within last year).
†Only factors significant on multivariable analysis shown in table.
‡Malnutrition was classified as children with weight-for-age Z score <−2 (World Health Organization child growth standards 2009) and/or children with nutritional edema.
§Reference period is the 1 month preceding the pneumococcal specimen collection date.
¶Presence of severe immunosuppression defined as CD4% of total lymphocytes <30% for children <12 months, <25% for children 12–35 months and <20% for children 36–59 months of age.
‖Regular HIV clinic attendance defined as >2 visits in the last year.

TABLE 7. Risk Factors for IPD on Multivariable Analysis Among HIV-infected Children: Risk Factors for VT IPD

Characteristics	Cases n/N (%)	Controls n/N (%)	Multivariable Analysis* OR (95% CI)	*P* Value
Malnutrition† (in reference period‡)	34/45 (75.6)	58/131 (44.3)	4.86 (1.25–18.86)	0.02
Diagnosed with tuberculosis in 3 months prior to reference period‡	8/46 (17.4)	19/143 (13.3)	38.10 (2.68–541.76)	0.007
ART use (in reference period‡)	8/41 (19.5)	41/106 (38.7)	0.04 (0.003–0.46)	0.01

*Only factors significant on multivariable analysis shown in table.
†Malnutrition classified as children with weight-for-age Z score <–2 (World Health Organization child growth standards 2009) and/or children with nutritional edema.
‡Reference period is the 1 month preceding the pneumococcal specimen collection date.

TABLE 8. Risk Factors for IPD on Multivariable Analysis Among HIV-infected Children: Risk Factors for VT IPD Stratified by Vaccination Status*

	Unvaccinated (Number of Cases = 6)			Vaccinated (Number of Cases = 27)		
	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Child had URTI (in reference period†)	9.60	0.51–181.49	0.13	5.94	1.20–29.32	0.03
ART use (in reference period†)	<0.001	Not calculated	0.99	0.14	0.02–0.88	0.04

*Includes contrast statement for vaccination status.
†Reference period is the 1 month preceding the pneumococcal specimen collection date.

TABLE 9. Risk Factors for IPD on Multivariable Analysis Among HIV-infected Children: Risk Factors for NVT IPD

Characteristics	Cases n/N (%)	Controls n/N (%)	Multivariable Analysis* OR (95% CI)	*P* Value
Diagnosed with tuberculosis in 3 months prior to reference period†	10/49 (20.4)	13/156 (8.3)	5.00 (1.44–17.43)	0.01
Child had URTI (in reference period)†	27/49 (55.1)	50/161 (31.1)	2.86 (1.24- 6.57)	0.01
HIV clinic attendance	8/48 (16.7)	72/159 (45.3)	0.13 (0.04–0.39)	<0.001

*Only factors significant on multivariable analysis shown in table.
†Reference period is the 1 month preceding the pneumococcal specimen collection date.

TABLE 10. Risk Factors for IPD on Multivariable Analysis Among HIV-infected Children: Risk Factors for Penicillin-resistant All-serotype IPD

Characteristics	Cases n/N (%)	Controls n/N (%)	Multivariable Analysis* OR (95% CI)	P Value
Malnutrition† (in reference period‡)	49/69 (71.0)	82/205 (40.0)	2.79 (1.17–6.68)	0.02
Diagnosed with tuberculosis in 3 months prior to reference period‡	13/72 (18.1)	30/235 (12.8)	6.46 (1.30–32.0)	0.02
Child had URTI (in reference period‡)	45/72 (62.5)	86/232 (37.1)	4.32 (1.50–12.46)	0.007
ART use (in reference period‡)	12/65 (18.5)	74/178 (41.6)	0.08 (0.02–0.40)	0.002

*Only factors significant on multivariable analysis shown in table.
†Malnutrition classified as children with weight-for-age Z score <–2 (World Health Organization child growth standards 2009) and/or children with nutritional edema.
‡Reference period is the 1 month preceding the pneumococcal specimen collection date.

an important role in the risk of pneumococcal disease. Among HIV-uninfected children, HIV exposure was associated with an increased risk of IPD for all endpoints, and in HIV-infected children, risk factors for IPD were related to conditions suggesting more severe HIV disease, such as malnutrition[16] and recent tuberculosis diagnosis.[17] ART treatment, which is critical for maintaining the health of HIV-infected children, decreased the odds of IPD, likely by improving the immune system and therefore reducing the risk of opportunistic infections.[18] Unfortunately, due to the small numbers of HIV-infected children in our study, we could not show a difference in this group by degree of immunosuppression.[12]

Although the numbers of HIV-exposed children who are infected annually continue to drop due to improvements in prevention of mother to child transmission programs, more can still be done for HIV-infected and HEU children. HIV-infected children need to be identified as soon as possible, before they become ill, and be started on ART according to the current treatment guideline.[19] HIV exposure has not been previously described as a risk factor for IPD. One US study did not find a difference in IPD risk between HEU children (n = 128) and an HIV-uninfected-unexposed (n = 71) control group, but the sample size was small.[20] Neonates born to HIV-infected mothers have lower levels of pneumococcal

antibodies.[21] Compared with HIV-unexposed children, HEU children have an increased risk of severe infections requiring hospitalization,[6] with higher odds of treatment failure and worse outcome.[22] The predisposition of HEU children to infections and their worse outcome is multifactorial, with factors including environmental conditions, feeding practices and an impaired innate immune system all playing a role.[23] This risk of invasive disease in HEU children may persist despite vaccination, possibly due to subtle differences in qualitative antibody responses to PCV.[24]

In this study, factors associated with all-serotype IPD in HIV-uninfected children were comparable with traditional risk factors described from other countries with low HIV prevalence. The presence of young siblings in the household has been associated with disease risk, likely because children commonly carry pneumococci due to a lack of serotype-specific protective antibodies, with the highest incidence of carriage observed during the first 2 years of life.[25] Likewise, a strong association has been previously demonstrated between day-care attendance and pneumococcal infections due to crowding of young children with high levels of nasopharyngeal carriage.[10] Underlying medical conditions are also well documented as risk factors for IPD.[10] Vaccination with PCV[26] and improved socioeconomic status[27] are associated with a reduced risk of pneumococcal disease. In our study, having a car, reflective of a higher socioeconomic status, was associated with decreased odds, while lacking a flush toilet, reflective of a lower socioeconomic status, was associated with a higher odds of disease.

In this study, females were at increased odds for VT IPD among HIV-uninfected children. This is in contrast to other studies in children <5 years, which have found males to be at an increased risk of disease.[28] The reasons for this association in our data are unclear. There is some evidence to suggest that the ratio of male:female infection varies with serotype, but sex is unlikely to have a significant influence on the risk of IPD with a particular serotype.[28] In HIV-uninfected children, we identified previous hospital admissions as a factor associated with penicillin nonsusceptible disease; hospital admissions are often associated with antibiotic therapy, which affects the carriage rates of antibiotic-resistant strains.[10,29] Having a wood fire in the home exposes children to particulate respiratory material, which increases the risk of acute respiratory tract infections.[11] HIV-uninfected children who were of black race, possibly with lower socioeconomic status, and who attended day care had higher rates of antibiotic-resistant disease. This was possibly due to overcrowding, which facilitates respiratory transmission of pneumococci and additional increased antibiotic exposure.[29] Malnourished HIV-infected children and those treated for tuberculosis are more likely to be admitted and exposed to antibiotic treatment, which increases their risk of antibiotic-resistant disease.

Recent URTIs were found to be a risk factor for IPD in both HIV-infected and HIV-uninfected children. While the information we collected on preceding URTIs was reported from parents rather than directly measured and therefore may be inaccurate, preceding upper respiratory tract viral infections have been demonstrated to increase susceptibility to bacterial illness. Reasons for an increase in susceptibility to pneumococcal pneumonia following influenza infection[30] include local respiratory epithelium changes enhancing pneumococcal invasion in colonized individuals,[31] a decrease in mucociliary clearance and increased inflammatory responses.[32]

Studies from the United States reported a change in the factors associated with IPD following the introduction of PCV, with reductions in excess risk among traditional risk groups such as black children[9,33] and day-care attendees.[9] In contrast, children with underlying conditions[9,34] still remained at risk for IPD despite the widespread availability of PCV and the targeting of high risk groups for receipt of vaccination. In our study among HIV-uninfected unvaccinated children (n = 14), underlying conditions and HIV exposure were associated with VT disease, while among HIV-uninfected vaccinated children (n = 17), HIV exposure was a risk for VT disease.

The limitations of our study include the use of controls that were either hospitalized or presenting to hospital outpatient departments. This choice of control group was for logistical reasons as study staff members were based at hospital sites and not able to recruit directly from the cases' communities. If the observed prevalence of possible IPD risk factors were different in the control group than in the general population from which the cases came, then we may have missed some potential risk factors and overestimated others. The majority of HIV-uninfected controls were hospitalized and the most common diagnoses were acute gastroenteritis and malnutrition. This could have masked a possible role of malnutrition as a risk factor for IPD in HIV-uninfected children. HIV exposure is a risk factor for other causes of hospitalization[23] and we expected HIV exposure prevalence in our controls to be higher than in the general population. In fact, the HIV exposure prevalence among our control group (30.3%) was similar to the national reported percentage of infants exposed to HIV [32.2% (95% CI: 30.7–33.6%)] in 2011.[4] The advantage of using hospital controls is that their health-seeking behavior is likely similar to hospitalized IPD cases.

We conclude that healthcare workers should promote pneumococcal vaccination in high risk groups like HEU children at routine health visits and, if doses are missed, provide catch-up vaccination at outpatient visits or when hospitalized.

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APPENDIX D: RESPONSES TO EXAMINERS' COMMENTS:

Responses and changes in blue text

EXAMINER'S REPORT 1:

The main concerns with the PhD thesis are mainly in the following areas:

1) Justification for the study outcomes was weak in a number of places:

I have indicated below suggested areas where the justification for the studies needs to be strengthened:

a) The importance of identifying Serotype 1 outbreaks is not clear in the thesis.

Section added to justification on page 21:

There is conflicting data regarding whether PCV13 will result in a reduction in serotype 1 disease and in view of its epidemic nature ongoing surveillance is important.

Section added to page 34 and 35:

The PCV13 vaccine has been found to induce antibodies against the serotype 1 capsule in children; however in two clinical trials from The Gambia and South Africa using PCV9, protection could not be demonstrated against serotype 1 clinical disease, although case numbers were small (Madhi2007,Saaka2008). In addition these two trials, which used three infant vaccine doses only, showed that serotype 1 vaccine failures occurred after 12 months of age (Klugman 2011). It was suggested that a booster dose may be necessary for protection against this serotype. As mentioned, the serotype 1 capsular polysaccharide is particularly resistant to opsonisation and it is uncertain whether protein-conjugate vaccination will be as effective against serotype 1 disease as against other serotypes.

In the United Kingdom PCV13 was introduced in April 2010 in a 2+1 schedule. Annual serotype specific incidence rates showed a significant reduction in serotype 1 rates between 2008-2010 and 2013-2014 in all age groups (Waight 2015). An indirect cohort study, including data up to 3.5 years after PCV13 introduction, showed protection against serotype 1 with a vaccine effectiveness of 84% (95% CI 54-95) (Andrews2014).

In South Africa a case control study demonstrated a high vaccine effectiveness point estimate against serotype 1 (89% [95% CI -82-100]) although the results were not statistically significant (Cohen 2016, submitted).

As ST1 is an outbreak serotype with fluctuating annual cases it is important for surveillance programmes to monitor for increasing case numbers. The main aim of monitoring for outbreaks would be to determine if there are any modifiable risk factors or public health

changes which could be made to stop the spread of the outbreak. In addition it is important to determine if new serotype 1 clones are emerging as these may have different characteristics in terms of antibiotic resistance.

Following the introduction of PCV13 it is assumed that serotype 1 case numbers will decrease although previous studies showed differing results. Our study only included data on ST1 up to 2 years post-PCV13 introduction. It is important to continue to monitor trends in outbreak prone serotypes included in PCV13 to document whether there is indeed a sustained reduction in these serotypes.

b) The reason for identifying risk factors for pneumococcal disease.

Added to justification on page 21:

The identification of risk groups guides policy makers with regards to allocation of resources and clinical management by alerting clinicians to who may present with pneumococcal disease and who to treat empirically.

Section added to page 41:

We only had breastfeeding data for <40% of all cases and could therefore not make definite conclusions regarding its role as a risk factor in our population. In other studies, however, breastfeeding has been shown to be protective against IPD and mortality and should be advocated for to improve child health. HI infants who were malnourished were more likely to die from IPD. Improving the nutritional status of HI infants and ensuring they receive appropriate ART is a key part of management.

c) The importance of understanding scale of serotype replacement (Page 18) - not clear what the implications are.

Page 18: With the expanded use of PCV globally it is important to estimate the scale of serotype replacement to determine the net decline in disease and the overall benefit of vaccination. The magnitude of the increase in non-vaccine serotypes varies between different settings and is depended on host and *S.pneumoniae* population level characteristics.

Page 20: There is a possibility that serotype replacement could substantially reduce the impact and benefits of vaccines. Replacement serotypes could be associated with antibiotic resistance. The amount of replacement thus far, especially with PCV13 use (which covers serotype 19A) is small in comparison to the overall reduction in disease. However there is a need for broader valency vaccines, universal vaccines and adequate surveillance (Feikin 2013).

d) Justification section: needs to be strengthened to explain why it is necessary to describe the changes in serotypes or the risk factors for IPD

Information added on page 21 as highlighted above in 1a and 1b.

2) Interpretation of findings and recommendations from the work. There is generally a lack of further interpretation of findings and synthesis to determine issues arising from the work that would require in changes in clinical management, recommendations for guidelines or recommendations for further research and development in the field. I have indicated below suggested areas where the justification for synthesis of findings and further interpretation would be required:

a) Page 34 & 36, includes detail of risk factors identified in other studies- this should have been covered in the literature review- also it is more important to do the comparison rather than state the risk factors.

Results from other studies, "A number of factors have been proposed to contribute to the poor health and nutrition among HEU children: lack of parental care, infant feeding practices, immune abnormalities, exposure to other infections and antiretroviral drugs (Filteau 2009)" were moved from page 36 to the introduction (page 9).

The comparison of risk factors with our study has been highlighted on page 38: "In our study these risk factors included, day care attendance which may increase IPD risk due to crowding which aids in transmission of pneumococci and higher pneumococcal loads, mixing of children with different pneumococci and possible cessation of breastfeeding (Levine 1999); living with siblings less than 5 years of age due to high rates of pneumococcal carriage (Abdullahi 2012), underlying medical conditions (Levine 1999), poor socioeconomic conditions which are often associated with crowding and different odds in different race groups and by sex.

b) In the conclusion one can indicate what kinds of interventions are recommended based on the risk factor analysis - mention socio-economic factors as you did in the abstract

Page 41: Details regarding breastfeeding and malnutrition as risk factors are highlighted in the responses to question 1b.

Page 42: Sentence added:

In HU children, traditional risk factors such as poor socio-economic conditions and intense exposure to infection need to be addressed to reduce the burden of disease in these children.

c) What are the other conclusions- should there be a change in the vaccine, what kind of surveillance should be continued, what other questions arise from the work.

Page 42: Laboratory-based IPD surveillance should continue unchanged to allow for measurement against the baseline. In addition documentation of nasopharyngeal carriage at defined points in time may assist with prediction of which serotypes will become most prominent in the post-PCV13 period. Although vaccine-serotypes continue to decrease globally following PCV13 introduction, some increases in non-vaccine serotypes have been observed. There is a limit on the number of serotypes that can be conjugated in one vaccine and replacement non-PCV13 serotypes vary across different countries. New universal protein-based vaccines are therefore in development.

3) Scientific writing: a number of issues regarding the presentation of the studies especially in the integrative narrative were encountered, and these as well as recommendations are summarised below:

a) The use of abbreviations without expansion prior to their use. A glossary of abbreviations was not provided and is recommended. e.g. VT-IPD and NPNM on page 2.

A list of abbreviations has been added on page ix

All abbreviations were described in full the first time they were used.

b) Use of abbreviations should be consistent- in the pages 12 and 13- you refer to VE interchangeably as being vaccine efficacy and vaccine effectiveness

This has been corrected. VE now refers to vaccine efficacy and vaccine effectiveness has been written out in full.

c) The flow of the integrated narrative was at times difficult to follow.

e.g Page 1 -1st paragraph under "Burden of pneumococcal disease"- it seems it would be better to describe the problem of pneumococcal disease, as well as the reason for determining burden prior to describing all the methods for determining burden of disease.

The paragraph has been rearranged. The description of methods has been moved to the end of the section.

e.g. Page 6- paragraph on the serotype 1 capsule. It is not clear why this is mentioned and what the significance of the capsule is.

Details regarding the importance of the serotype 1 capsule have been added on page 6:
The serotype 1 capsule has been shown to be more resistant to opsonisation and complement deposition than other serotypes, except for serotype 5; as such it is thought that the serotype 1 capsule may function in a different way to other serotypes resulting in a difference in virulence. There were also differences in pneumolysin in some serotype 1 isolates, which also impacts colonisation and virulence. The zwitterionic polysaccharide capsule has been found to be related to abscess formation in animal models and thus cause empyema.

e.g. Page 7- the literature on risk factors is not clear and easy to follow. It should be clearly stated whether findings are of HIV-infected or HIV-uninfected children, in the pre- or post PCV-7 vaccine era.
Additions have been made to the risk factor section on page 7 to try and clarify which groups and periods the risk factors are relevant to:
Local studies showed a significant higher risk of IPD in HIV-infected children (41-fold) with "paediatric" pneumococcal serotypes, than –uninfected children (Madhi 2000). HIV-infected children in this study had high rates of malnutrition and underlying tuberculosis. HIV-uninfected children had underlying infections, like chronic liver and renal disease. Other studies from the US also showed that a higher risk of pneumococcal disease with HIV was in the pre-ART era. In contrast in HIV-uninfected children with IPD in South Africa had underlying infections, like chronic liver and renal disease in the pre-vaccine era.
Also in the pre-vaccine era a case-control study in children <5 years of age in The Gambia showed….

e.g. Page 34 -limitations for interpreting surveillance data- seems to be relevant to all the studies and should be maybe in the discussion section rather than directly linked to one study.
A general discussion of the limitations of surveillance data has been added on page 28:
"Surveillance data was used as the baseline for most of our studies. There are inherent limitations in the use of surveillance data. Our laboratory-based surveillance programme underestimates the full burden of pneumococcal disease as it only includes patients who present to healthcare facilities and have samples taken; we aimed to address this in our burden model (paper I). There is often missing data associated with the use of surveillance programmes; we assumed that data were missing at random and imputed values for serotype for trend analyses. Interventions other than vaccination, for example PMTCT and ART,

impact IPD disease trends. It is often difficult to tease out the proportional contribution of all these strategies on disease reduction using surveillance data."
Results and discussion were combined for each study. Even though surveillance data limitations were applicable to all the studies, specific points were abstracted from each study to highlight their specific issues. For the burden paper (paper 1) the focus was on the issues related to burden calculations, for the risk factor paper (paper 4) issues related to case control studies were discussed, while for the serotype 1 paper (paper 2) considerations related to interpretation of cluster results were covered. Issues related to surveillance results were therefore explored with regards to HIV-exposed children who were discussed in the HIV-exposure (paper 3) and risk factor paper (paper 4).

c) Inconsistency in how data are presented that hinders the flow: e.g. when describing in pneumococcal vaccines on page 4, it is not clear when the vaccine was introduced in South Africa.
The section "Pneumococcal Serotypes and Vaccines" on page 4 aimed to give a general overview of vaccines and serotypes globally. The first introduction date of PCV into South Africa is discussed on page 3 "South Africa was the first African country to introduced PCV into its public National Immunisation Programme in April 2009…"
The reference to serotype 1 in South Africa on page 4 ("Serotype 1 was found to be an important cause of disease among older children and among HU children in the pre-PCV era in South Africa") has been moved to the section discussing serotype 1 on page 6 to avoid confusion.

d) On page 26, all results should be described first and then discussion. At the end of the page, results are then represented- rather keep all results in the first paragraph and then discuss.
The main results of the thesis are presented in the four papers. The combined result-discussion section in the integrating narrative aimed to synthesise the main results by comparing them to other data. Our results are thus related to other publications throughout this section.

4) References to common terminology in the field without explanation of what this is: e.g. "meningitis belt" on page 5
Explanation has been added for meningitis belt on page 5:

The African meningitis belt is a region of sub-Saharan Africa, extending from Senegal to Ethiopia that is characterised by hyperendemic seasonal peaks of acute bacterial meningitis and sporadic epidemics.

5) Other issues identified (optional to address):

a) In the abstract, the conclusion is not aligned to what is shown in the results of the abstract and some of the aspects mentioned are not even included in the discussion of the thesis.

The abstract outlines the most important findings from the four manuscripts included in the thesis. Additions have been made to the result section to ensure that all points raised in the conclusion have been discussed in the abstract:

In the pre-vaccine era (2005-2008) in South Africa, roughly 196,100 (148,000-251,000) cases of severe pneumococcal disease were estimated annually in children aged <5 years, an incidence of 3799/100,000; the rate was reduced by 67% in 2013, likely due to PCV and other interventions. In addition 8600 (7000-10220) pneumococcal-related annual deaths were estimated pre-vaccine and 3600 in 2013, a rate difference of 99/100,000 child-years.

Over an 11-year period two clusters (2003-2004 and 2008-2012) of serotype 1 infection were detected in all age groups with reductions in incidence noted in 2013.

b) The importance of serotype 1 outbreaks is not clear in the thesis. The literature and data are adequately described but the reader is not informed on why it is important to know about outbreak serotypes.

Details regarding the importance of serotype 1 and outbreaks have been added to page 34-35 as discussed in section 1a.

c) Study design- good descriptions were given in the papers about the actual surveillance system. How many sites involved, what information is recorded, who generally is included etc. these have important implications for the interpretation of your results so should be included in page 22 under study design.

Additional details were added on page 25:

Cases of IPD were considered as hospitalized individuals with *S. pneumoniae* cultured from normally sterile site specimens. The GERMS-SA programme includes over 200 microbiological laboratories ("non-enhanced" sites) which submit isolates and basic demographic information (age, gender, date of specimen collection, and body fluid source of

isolate). Trained surveillance officers at 24 sentinel hospitals ("enhanced" sites) located in all nine provinces collect additional data including HIV status, discharge diagnosis and outcome.

d) You mention the use of bootstrapping. Some more information about when it is usually used and why it is considered more accurate could be helpful (page 29).
Additional details have been added regarding bootstrapping on pages 23 and 29:
Page 23: Every resample has the same number of observations as the original sample, so the bootstrap method models the impact of the actual sample size (Fan & Wang, 1996).
Bootstrapping allows measures of accuracy to be assigned to sample estimates, verifies replicability of results and allows inferences to be made regarding key parameters.
Page 29: To account for variability and uncertainty around our estimates we used bootstrapping, a robust statistical method, to calculate confidence intervals.
Bootstrapping assumes that each sample is identically and independently distributed. It draws many more sub-samples than other methods and provides less biased and more consistent results than the Jackknife method for example (Deng 2013).

e) Additional analyses: Paper 1 - Figures 2a and 2b on Tornado analysis- needs further explanation of what a Tornado analysis is and what it is showing.
Details regarding the use of Tornado diagrams have been added on page 24:
Tornado diagrams were used to depict the sensitivity of the case and death estimates to changes in selected variables. These diagrams show the effect on the base rate by varying each input variable one at a time, while keeping all the other input variables at their initial base value. High and low values may be chosen for each input. The results are displayed as a bar graph with the variation for each variable from the base rate.

EXAMINER'S REPORT 3:

1) Methods
a) Page 23, last paragraph: change sentence to "SatScanTM is able to detect spatial, temporal or spatio-temporal disease clusters and determine ... "
Change has been made

b) Page 24, second full paragraph: " ... to account for this we adjusted positively by the percent reduction from the pre-vaccine period". It is not clear why this was necessary for

detecting clusters of "cases" (i.e. serotype 1 disease) or how this was implemented practically.

It was necessary to adjust controls to obtain a stable baseline by essentially removing the impact of PCV7 on serotypes in this group; this allowed for true increases in cases to be identified and not just relative case changes.

Last sentence correct verb to "ensured". Sentence replaced by above.

2) Paper 1

a) Page 6, first paragraph: shouldn't this be "overestimation of vaccine efficacy for non-bacteraemic pneumococcal pneumonia" as VE is 0.45 for non-bacteremic compared to 0.85 for bacteremic pneumonia?

To estimate the number of cases of non-bacteraemic pneumococcal pneumonia, we extrapolated data from PCV probe studies in South Africa by using the PCV9 vaccine attributable reduction (VAR) ratio of clinical pneumonia to bacteraemic pneumococcal pneumonia (11:1). An additional adjustment was made for the presumed underestimation of vaccine efficacy (VE) for non-bacteraemic pneumococcal pneumonia in this study. This assumption was based on a study which showed a higher pneumococcal detection rate using urine antigen testing in non-bacteraemic pneumonia in the elderly. The adjustment value (1.89) was based on the ratio of the original and inflated VE from the different studies.

b) Page 6, second paragraph and page 7, second full paragraph: I assume the CRF for non-bacteremic hospital admissions refers to admission for any disease syndrome, not just pneumonia. Would you expect this to lead to over- or underestimates of the non-bacteremic pneumonia CFR?

A sentence has been added to the limitations section on page 13:

For example, using a CFR for all hospitalised cases may have overestimated the CFR for non-bacteraemic pneumonia cases and the ratio of BPP to NBP may change by serotype distribution which we did not account for in our model.

c) Page 9, last paragraph: add in NPNM.

Information regarding NPNM rates have been added:

…and 22 per 100,000 py for non-pneumonia non-meningitis pneumococcal disease (rate difference of 73 per 100,000, 76% reduction).

d) Page 13, second full paragraph: need to rephrase as the reductions in IPD were due to HAART as well as PCV.

A sentence highlighting the fact that we did not take other interventions into account when exploring costs was added on page 13: “We did not account for the impact and costs of PMTCT and ART in these calculations.”

e) Page 13, last paragraph: can you explain which assumptions may be inaccurate e.g. if the ratio of BPP to NBP changes by serotype distribution or HIV status.

Details were added on page 13 regarding which assumptions may be inaccurate:

For example, using a CFR for all hospitalised cases may have overestimated the CFR for non-bacteraemic pneumonia cases and the ratio of BPP to NBP may change by serotype distribution which we did not account for in our model.

f) Page 14, first paragraph: shouldn't this be "decrease in the known vaccine efficacy" since VE vs. VT NBP is 0.45 compared to 0.85 for VT BPP? See comment above re: page 6. Perhaps I have misunderstood your point

Page 14, first paragraph: "This study reported a higher efficacy ... " is unclear and needs to be rephrased.

Page 14, last paragraph: add in "in conjunction with HAART and other interventions" to emphasize importance of ART.

To address the above 3 points, changes were made to the first paragraph:

We tried to account for this underestimate by including an adjustment for the increase in the known vaccine efficacy, based on more recent data, against non-bacteraemic pneumonia since these trials were conducted. Supporting evidence for this additional adjustment was seen in a recent PCV vaccine efficacy study in the elderly which used a serotype-specific urinary antigen detection assay to detect vaccine-type *S. pneumoniae.* It is possible that this adjustment may have overinflated our numbers as it is not clear if the magnitude of the difference in VE for non-bacteraemic and bacteraemic pneumonia is the same for children as observed in the adult study.

g) Page 14: last paragraph: numbers of cases and deaths averted are "per year" and not "over a 5-year period"

The total number of cases and deaths reported were the difference between 2008 and 2013, thus over a 5-year period. A sentence to this effect was added on page 5: “Cases and deaths averted were calculated as the difference between these two periods, i.e. over 5 years.”

h) Table 1a: "Adjustment factor for systematic blood culturing from South African clinical trial": are you assuming here that "true" BPP rates did not vary between 1998 and 2008?
A sentence was included in the limitations section on page 13 (“In addition some estimates (e.g. vaccine probe study data) were only available for the pre-vaccine period and were assumed to be relevant to the post-PCV period”) to indicate that we used the clinical trial measurements for both periods which was not necessarily applicable.

"Adjustment for specimen-taking practices": couldn't these differences in blood culture rates reflect true differences in incidence?
A sentence has been added to the limitations section to reflect that there may be a true difference (page 13): “It is possible that some of the difference in incidence rates were true differences.”

Paper 2

I realize this paper is already published so my comments cannot be addressed in the manuscript, but am sharing a few thoughts nevertheless.
Page 262, incidence estimations: There is likely to be a correlation between missing serotypes and antimicrobial resistance; could your assumption of randomness bias your results?
Missing serotypes may be isolates that were not submitted by certain laboratories or non-viable isolates due to transport issues and time delays in reaching NICD. These missing isolates may on occasion be non-random, for example from certain sites during certain periods such as outbreaks with an increased number of cases.
There may even be an intrinsic feature of the organism that certain strains are more likely to autolyse than others; it has been reported that the pneumococcal capsule provides a degree of resistance to autolysis and this capacity varies between capsular serotypes (Kadioglu2008).
It may be possible therefore that the difference was not completely random.

It would be preferable to compare the period with clusters to the period without clusters rather than to the overall study period.

A comparison is made between the cluster period (2003-2008) and non-cluster period (2005-2007) in the manuscript on page 264 ("Serotype 1 incidence did not differ significantly for 2003–2008 compared with 2005–2007, when there were no clusters") in figures 2a and 2b.

Page 263, first paragraph: does the second model compare deaths (cases) to surviving patients (controls)?

The second model includes only serotype 1 cases and compares surviving to non-surviving cases.

Page 263, spatio-temporal analysis: why not look only at non-vaccine types as your control, to avoid the need to adjust for PCV use?

Replacement was observed in non-vaccine type disease which would have required adjustment. A decision was therefore made to use all other serotypes as controls.

Paper 3

Page 1347, incidence, second paragraph: is the assumption that HIV infection and exposure has similar prevalence in non-enhanced sites to that in enhanced sites reasonable? Don't the prevalence of HIV infection and risk of HIV transmission vary across sites?

There is likely some variation across sites, but as we had enhanced sites in each province it was hoped that this would account for some of the variation.

Supplemental materials, page 5: can you specify the policy for cotrimoxazole prophylaxis in HEU and HIV-infected children?

For all HEU infants start cotrimoxazole from 4-6 weeks of age and stop when PCR negative ≥6 weeks after full weaning and infant is clinically HIV negative. If infant is on formula only cotrimoxazole can stop when PCR is negative.

For HIV-infected infants, cotrimoxazole starts at 4-6 weeks, till child is >12 months. Cessation of cotrimoxazole then happens when immune reconstitution on ART occurs.