EPIDEMIOLOGY OF INVASIVE BACTERIAL INFECTIONS IN HIV-INFECTED AND HIV-UNINFECTED CHILDREN UNDER 5 YEARS OF AGE IN SOWETO, SOUTH AFRICA BETWEEN 1998-2005

Siobhan Lindsay Trenor

A research report submitted to the School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Epidemiology and Biostatistics.

Johannesburg, 2018

Candidate's declaration

I, Siobhan Lindsay Trenor, declare that this research report is my own, unaided work. It is being submitted in partial fulfilment for the degree of Master of Science in Epidemiology and Biostatistics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Siobhan Lindsay Trenor

On this 4th day of June 2018 in Soweto, Johannesburg.

To Matthew McGrath, for showing me true perseverance and courage and for inadvertently encouraging me in wanting to know more.

Presentations arising from this study

Poster presentation: *7th FIDSSA (Federation of Infectious Diseases Societies of Southern Africa) Conference*, Cape Town, 19-11 November 2017.

Oral presentation: *Wits School of Public Health Biennial Research Day*, Johannesburg, 27 November 2017 – Second prize for oral presentation.

Abstract

Introduction

Invasive bacterial infections (IBI) cause significant morbidity and mortality in infants and young children, predominantly in low and middle-income countries. There are limited data on paediatric IBI, as well as the impact of pneumococcal conjugate vaccine (PCV) on the spectrum of IBI pathogens, in African countries with a high prevalence of HIV infection. We described the epidemiology of IBIs in a cohort of children <5 years of age in Soweto, South Africa.

Objectives

To estimate the incidence of IBI in children <5 years of age that participated in the 9-valent PCV (PCV9) efficacy trial, stratified by age, PCV9 vaccination status and HIV infection status, as well as to determine which pathogens are responsible for IBI among PCV9 vaccinated and unvaccinated, and HIV-infected and –uninfected hospitalised children.

Methods

A secondary data analysis was performed using a cohort of children enrolled into the PCV9 randomised control trial (RCT) conducted in Soweto from 1998-2005. During this RCT, 39 836 children were randomised to receive either PCV9 or placebo, and surveillance was conducted by study doctors in admission wards at Chris Hani Baragwanath Academic Hospital (CHBAH) to identify hospitalised participants. Incidence of IBI was calculated using person-time (with censoring occurring on each participant's fifth birthday or date of death) and stratified by age group, sex, PCV9 vaccination status and HIV infection status. Risk factors for IBI were investigated using binomial logistic regression.

Results

A total of 395 cases of laboratory-confirmed IBI were included in the analysis. The incidence of all-cause IBI hospitalisations decreased with increasing age. PCV9 vaccination was associated with reductions in incidence of all-cause IBI hospitalisation (IRR=0.76; 95% confidence interval (CI) 0.61 to 0.93, p=0.006) which was predominantly due to the reductions in *Streptococcus pneumoniae* incidence (IRR=0.56; 95% CI 0.39 to 0.78, p<0.001). PCV9 vaccination was associated with a decrease in incidence of PCV9- and PCV9-related serotypes in HIV-infected (IRR=0.53; 95% CI, 0.20 to 0.85, p=0.005) and HIV-uninfected (IRR=0.44; 95% CI, 0.17 to 1.07, p=0.051) participants. PCV9 vaccination had no significant effect on the incidence of *Haemophilus influenzae* type b or Salmonella species infections, however, there was a trend towards higher incidence of infections due to *Staphylococcus aureus,* a trend towards decrease in incidence of *Escherichia coli* and a significant increase (IRR=3.50; 95% CI, 1.10 to 14.59, p=0.019) in Klebsiella species IBI in PCV9 vaccinated children.

Conclusion

PCV9 vaccination was effective in reducing incidence of IBI hospitalisation in HIV-infected children through significant reductions in *S. pneumoniae* incidence. HIV-infected participants and those that did not receive PCV9 were at increased risk for IBI hospitalisation. Our results show that trends in other IBI causative pathogens (specifically *Staphylococcus aureus* and Klebsiella species) should be monitored in the post-PCV era.

Acknowledgements

I would like to express my gratitude to my supervisors Dr Michelle Groome and Dr David Moore. Thank you for your time, guidance and expertise.

I would like to acknowledge the following staff members from the Respiratory and Meningeal Pathogens Research Unit for their support and for always being accessible despite their busy schedules: Alane Izu, Clare Cutland, Eleonora Mutsaerts, Marta Nunes, Nasiha Soofie, Olukemi Babalola, Sarah Downs, Shabir Madhi and Shiraz Ismail. Thank you to Michel Muteba and Linda Erasmus; classmate that were always available to advise, share reading material and review my work.

Contents

Candidate's declaration ii
Presentations arising from this study iv
Abstract v
Acknowledgements vii
Contents viii
List of figures x
List of tables xi
Nomenclature xii
1. Chapter One: Introduction 1
1.1 Background and Literature Review 1
1.1.1 Invasive Bacterial Infections 1
1.1.2 Burden of disease and disease patterns globally 3
1.1.3 Burden of disease and disease patterns in Africa 4
1.1.4 Burden of disease and disease patterns in South Africa 7
1.1.5 Pneumococcal Conjugate Vaccine 8
1.1.6 Current gaps in knowledge 10
1.2 Problem Statement 11
1.3 Objectives 11
2. Chapter Two: Materials and Methods 12
2.1 Original Study Design and Population 12
2.2 Definitions 13
2.3 Statistical Analysis 14
2.4 Ethical Approval 17
3. Chapter Three: Results 18
3.1 Description of IBI events in the cohort 18
3.2 Effect of PCV9 vaccination on IBI 26
3.2.1 HIV-uninfected participants 28
3.2.2 HIV-infected participants 28
3.2.3 Description of IBI in CSF 29
3.2.4 Description of 'other' IBI group 30
3.3 Risk factors of IBI 30
3.4 Clinical Characteristics 31
3.5 Outcomes of IBI 31
3.5.1 Severity Indicator: Death versus Survival 31
3.5.2 Severity Indicator: Prolonged Hospitalisation (>4 days versus ≤4 days) 32

3.6 Description of Klebsiella spp IBI in the cohort 34
4. Chapter Four: Discussion 35
4.1 Description of IBI in the cohort 35
4.2 The Effect of PCV9 Vaccination on IBI 38
4.2.1 HIV-uninfected participants 39
4.2.2 HIV-infected participants 39
4.2.3 Replacement disease in the cohort 40
4.3 Risk Factors of IBI 41
4.3.1 Risk factors for Coagulase-negative staphylococcus infections 41
4.4 Clinical Characteristics 42
4.5 Outcomes of IBI 43
4.6 Description of Klebsiella spp IBI in the cohort 44
4.7 Limitations 45
5. Chapter Five: Conclusions and recommendations 48
References 49
Appendices 52
Appendix 1: Human Research Ethics Committee (Medical) of the University of the Witwatersrand Approval Certificate 52
Appendix 2: Additional tables and figures 53

List of figures

Chapter 3:

Figure 3.1: Flow diagram indicating number of hospitalisation events in the cohort 19

Figure 3.2: The proportions of pathogens detected in IBI events in the cohort during hospitalisation 21

Figure 3.3: A) Incidence rates across age groups for different IBI pathogens. B) Incidence rates across age groups for *S. pneumoniae* infections (separated into PCV9 serotypes and non-PCV9 serotypes) 25

Figure 3.4: The proportions of pathogens detected in IBI events by study group (HIV and PCV9 vaccination status) 27

Appendices:

Figure S1: Pie charts showing the constitution of the group labelled as 'Other' for the purposes of the analysis. 57

Figure S2: The proportions of pathogens detected in CSF cultures during hospitalisation by HIV status and PCV9 vaccination status 61

List of tables

Chapter 3:

Table 3.1: Characteristics of the participants with IBI events included in the analysis 20
Table 3.2: Incidence of IBI hospitalisation (per 10 000 person-years - showing 95% confidence intervals) 22
Table 3.3: Risk factors associated with IBI hospitalisation 30
Table 3.4: Predictors of poor outcome (death) for participants hospitalised with IBI 32
Table 3.5: Predictors of prolonged hospitalisation (>4 days) for participants hospitalised with IBI 33
Table 3.6: Risk factors associated with invasive Klebsiella spp infection 34

Appendices:

Table S1: Contaminants that were excluded from the analysis a) Number of events per group b) Incidence per group (per 10 000 person-years) 53
Table S2: Number of IBI hospitalisations during the follow up period stratified by age group, sex, PCV9 vaccination status, HIV status and prematurity 55
Table S3: Number of invasive *S. pneumoniae* events stratified by serotype for HIV-infected and –uninfected as well as PCV9 and control groups 58
Table S4: Risk factors for coagulase-negative staphylococcus IBI 59
Table S5: Risk factors for coagulase-negative staphylococcus IBI (for children under 3 months of age) 59
Table S6: Incidence (per 10 000 person-years) of non-contaminated CSF cultures detected during hospitalisation during the follow up period 60

Nomenclature

ART	antiretroviral therapy
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	confidence interval
CoNS	coagulase-negative staphylococcus
CSF	cerebrospinal fluid
ELISA	enzyme-linked immunosorbent assay
EPI-SA	Expanded Program on Immunisation for South Africa
GAVI	Global Alliance for Vaccines and Immunisations
GBS	Group B streptococcus
Hib	*Haemophilus influenzae* type b
HIV	Human immunodeficiency virus
HIV+PCV-	HIV-infected, placebo vaccinated group
HIV+PCV+	HIV-infected, PCV9 vaccinated group
HIV-PCV-	HIV-uninfected, placebo vaccinated group
HIV-PCV+	HIV-uninfected, PCV9 vaccinated group
IBI	invasive bacterial infection
ICD	International Statistical Classification of Diseases and Related Health Problems
IPD	Invasive pneumococcal disease
IRR	incidence rate ratio
IQR	interquartile range
LMIC	low and middle-income country
OR	odds ratio
PCR	polymerase chain reaction
PCV	pneumococcal conjugate vaccine
PCV7	7-valent pneumococcal conjugate vaccine
PCV9	9-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PMTCT	prevention of mother-to-child transmission (of HIV)
PY	person years
RCT	randomised control trial

1. Chapter One: Introduction

This chapter will begin by giving a brief description and background on invasive bacterial infections (IBI) in children in terms of clinical manifestation and symptoms as well as introducing the common causative pathogens and risk factors for infection. The burden of disease and disease patterns globally will be described before looking specifically at burden of disease and disease patterns in Africa (in terms of how the epidemiology of IBI differs between low and middle-income settings compared to high income settings). The burden of disease and disease patterns in South Africa will then be described and the link between IBI and HIV infection explored. A summary on the background of PCV and its introduction into the South African immunisation program (EPI-SA) as well as current gaps in knowledge will then be given in order to provide context to this research project. The chapter will end with the main aims and objectives of this research project.

1.1 Background and Literature Review

1.1.1 Invasive Bacterial Infections

Bacterial infections cause significant morbidity and mortality in infants and young children, predominantly in low and middle-income countries (LMIC) (1). These infections manifest clinically as pneumonia, meningitis, bacteraemia, urinary tract infections, infection of the musculoskeletal system, and acute otitis media amongst other infectious syndromes. IBI are characterised by the detection of bacteria in a normally sterile site such as blood (resulting in bacteraemia), or cerebrospinal fluid (CSF, resulting in meningitis) or other body fluids to a lesser extent (2). Pneumococcus (*Streptococcus pneumoniae)* and meningococcus (*Neisseria meningitidis)* are two of the most common bacterial causes of meningitis and bacteraemia globally (2).

Many of the bacterial pathogens responsible for causing IBI are common colonisers of the nasopharynx in healthy individuals. Pneumococcus is an example of this, and is found to have very high nasopharyngeal colonisation rates in healthy children (3). Bacterial pathogens become invasive when they are able to cross the mucosal barrier and enter into the bloodstream. In some cases the

infection may cross the blood brain barrier, thereby entering the central nervous system, causing meningitis. There are several factors which increase the risk of bacterial colonisers becoming invasive. These risk factors also often increase the severity of the infection once the pathogen has become invasive, hence increasing the risk of complications and poorer outcomes. The most common risk factors for invasive pneumococcal infections in paediatric patients under the age of 5 years, are immunosuppression or underlying conditions (including HIV and diabetes) (4), child-care attendance, overcrowded households and lack of breast-feeding (5). The increased risk associated with diabetes is especially relevant for high income countries where there is a rise in non-communicable, lifestyle-related illnesses which may increase the risk of IBI (4), however, LMICs are also starting to see higher rates of non-communicable diseases which is likely to intensify the problem in these areas (6). The most common risk factors for invasive meningococcal infections include exposure to cigarette smoke and living in close contact with another infected individual (4). Studies have shown that up to half of meningitis patients have an underlying condition which predisposes them to infection, with as many as one third of these patients being immune deficient (4). Repeated meningitis episodes have been linked to anatomical defects in which there is a disruption of the blood brain barrier (4).

While an estimated 70% of bacteraemia episodes will resolve on their own and will not deteriorate clinically (7), the remaining infections will manifest clinically as pneumonia, septic arthritis, osteomyelitis, acute otitis media or sepsis (8). Sepsis in infants usually presents with fever, vomiting, skin rash, inability to feed and/or changes in breathing (7). Meningitis presents differently in different age groups (4). Generally, infants under the age of 3 months present with nonspecific signs such as lethargy, poor feeding and irritability; while older children tend to present with more typical symptoms such as neck stiffness and headache (4). Outcomes associated with meningitis are often severe, with up to 50% of meningitis survivors suffering permanent damage such as neurological deficits, cognitive impairment or hearing damage. Mortality rates attributed to meningitis are as high as 20% in high income countries, while rates are estimated to be higher in LMICs, reaching as

high as 45% in some settings (4, 9). The empiric treatment for all IBI cases is antibiotics, although regimens differ according to geographical location and clinical presentation (10).

The pathogen profiles of IBI are relatively diverse among different age groups. Pneumococcal disease incidence is highest in young children (under 2 years of age) and the elderly (over 50 years of age). Meningococcal disease incidence is highest in children (over 1 month of age) and young adults (4). Vaccination against *Haemophilus influenzae* type b (Hib), *S. pneumoniae* and meningococcus using conjugate vaccines has caused a shift in the age distribution of bacterial meningitis from being a disease that mainly affected young children and adolescents, to one that predominantly affects the elderly (4). Studies in high income countries however, have demonstrated a decrease in incidence of IPD in the elderly as well as unvaccinated children due to the reduction in bacterial carriage in vaccinated children (known as herd immunity) (11, 12).

1.1.2 Burden of disease and disease patterns globally

Global statistics indicate that approximately 6.3 million children died before the age of 5 years in 2013 (13). More than half of these deaths (51.8%) were due to infectious causes (including pneumonia, diarrhoea, sepsis and meningitis) (13). Of these deaths, 44% occur in the neonatal period (the first 28 days of life) and were exacerbated by preterm birth complications. In children aged 1-59 months the most common causes of death were injury (15%) and pneumonia (13%). The burden of paediatric deaths was disproportionately higher in LMICs compared to high income countries, with 49.6% of deaths occurring in sub-Saharan Africa and 32.1% occurring in southern Asia (13). It is estimated that nearly half of these deaths could have been prevented through vaccination, proper nutrition and increased access to health care (14).

Globally the primary causative agents for bloodstream infections are considered to be *Staphylococcus aureus*, *Escherichia coli* and coagulase-negative staphylococcus (CoNS), although it is recognised that CoNS in most cases is a contaminant and is hence usually not clinically relevant (15). Other important causative agents include Enterococcus spp*,* Klebsiella spp*, S. pneumoniae* and

Enterobacter spp (15). In children less than 1 year of age the most frequently isolated pathogen is reported to be CoNS while *S. pneumoniae* is the most frequently isolated pathogen in children aged 1-5 years. These estimates are taken from a surveillance study done between 1997 and 2002 in North America, Latin America and Europe (15). A population-based cohort study done in Switzerland between 2011 and 2015, highlighted *E. coli* (20%), *S. aureus* (15%), CoNS (11%) and *S. pneumoniae* (10%) as the leading pathogens causing bacterial sepsis in children under 17 years of age (16). Not only has the pathogen profile has been shown to vary somewhat between different age groups and geographical locations, but also between nosocomial versus community-acquired infections. Nosocomial bloodstream infections have been found to have much higher antibiotic resistance rates, especially those isolated from intensive care patients (15). In the United States it is estimated that 36 000 deaths are caused by bloodstream infections annually. It is however acknowledged, that even in this high resource setting, there could be underreporting of the burden of disease. This underreporting is mainly due to the International Statistical Classification of Diseases and Related Health Problems (ICD) coding which often records the cause of disease as the underlying source of infection (which may differ to the diagnosis of IBI) and hence these events are not considered in some reports (17).

The main causative agents for bacterial meningitis are *S. pneumoniae* and *N. meningitidis*, with *S. pneumoniae* being responsible for as many as two thirds of cases in the United States and Western Europe post introduction of PCV (4). Other important causative agents include Group B streptococcus (GBS), *E. coli*, *Haemophilus influenzae*, *Listeria monocytogenes, S. aureus* and Salmonella spp (2).

1.1.3 Burden of disease and disease patterns in Africa

The leading causes of bloodstream infections in Africa according to a meta-analysis of studies between 1984 and 2006, are reported to be *S. pneumoniae* (18.3%), *Salmonella enterica* (13.5%), *S. aureus* (9.5%) and *E. coli* (7.3%) with the majority of infections being due to Gram-negative bacteria

(18). Invasive bacterial infections in Africa and other low income settings differ in three main ways compared to high income settings. A summary of these three main differences is given below. The first is that the burden of IBI is several fold higher in LMICs than in higher income settings. Secondly, the epidemiology and aetiology of infections are different, and thirdly, the severity of outcomes due to IBI is increased in LMICs compared to higher income settings.

1.1.3.1 Burden of IBI in Africa

Overall sepsis and meningitis rates are much higher in sub-Saharan Africa and south Asia compared to the rest of the world (13). Bacterial meningitis is approximately 10 times more common in LMICs than high income areas across all age groups (4). These estimates are taken from reported infection rates however, it is widely acknowledged that this is likely to be a considerable underestimation due to lack of surveillance and monitoring, coupled with inadequate diagnostic and laboratory resources in these areas (19). Some of the most important risk factors for IBI in children under-5 years of age include: being born in a rural area, being born to poor households and being born to a mother who lacks basic education (14). These risk factors also partly explain why mortality and morbidity due to IBI are so high in African children.

1.1.3.2 Aetiology and epidemiology of IBI in Africa

Approximately 99% of infant deaths occur in LMICs. Most available data however, is generated through investigation of the 1% of deaths that occur in high income countries (20). While these data are often extrapolated to other settings, the validity of doing so is questionable due to population differences as well as differences in epidemiology of infections across different geographical locations (5). Since IBI antibiotic treatment is usually started empirically before culture confirmation of the presumed organism, it is necessary to rely on accurate knowledge of the bacterial pathogen prevalence as well as resistance patterns in the specific geographical location in order to inform the choice of antibiotic treatment (21). The variation in IBI patterns is further magnified by the high prevalence of HIV in Africa, and coinfection with malaria in the more tropical latitudes (18).

Approximately 90% of HIV-infected individuals worldwide live in LMICs, with 75% living in sub-Saharan Africa (4, 20). Data indicate that HIV-infected adults are more likely to acquire hospital-associated infections (specifically Salmonella spp and *S. aureus*) than HIV-uninfected individuals (21). Nosocomial infections and their ensuing morbidity and mortality in turn, often have a negative impact on health seeking behaviour due to poor reputation of health services in the community (19). The high rates of nosocomial infections in Africa point to issues with training and knowledge on basic infection control as well as inadequate health infrastructure, technology and thus, ultimately, to resource deficiencies (22). Another layer of complexity is that HIV-infected infants are usually exposed to co-trimoxazole prophylaxis (South African guidelines indicate that all HIV-infected and –exposed infants must receive co-trimoxazole prophylaxis from 4-6 weeks of age (23)) which could increase the risk of antibiotic resistance in this already vulnerable group (21). During the study time period, HIV treatment guidelines recommended co-trimoxazole prophylaxis for any child that tested positive for HIV on Elisa or PCR (and should be continued until CD4 count >200 cells/mm^3) (24). Antibiotic resistance would not only change patterns of disease at a population level but would also increase severity of disease at an individual level, especially in cases in which there are few alternative treatments available. The types of infections identified in African studies (specifically Gram-negative bacilli and *S. aureus*) also indicate a lack of adequate hygiene levels (especially at hospitals) and poor postnatal care (19).

1.1.3.3 Severity of IBI in Africa

The case fatality rate of bloodstream infections in Africa for all ages is estimated to be 18.1% compared to 13.0% in the United States (17, 18). There are several reasons for this, including poor healthcare, shortage of antibiotics, delays in treatment and lack of microbiological diagnostic services. A meta-analysis done on African bloodstream infections for all ages highlighted a number of cases presenting with fever, which were mistaken to be malaria cases. The misdiagnosis resulted in antibiotics being withheld which in turn resulted in increased mortality (18). The analysis also found that 5.8% of children admitted with malaria had bacterial bloodstream coinfections which

were untreated (18). Many of these settings did not have diagnostic capacities for non-malarial bloodstream infections. Another reason for poorer outcomes in African patients is the increase in prevalence of underlying conditions, especially HIV. Studies have shown increased case fatality rates of IBI in HIV-infected individuals (25). This demonstrates that not only are the patterns of disease different for HIV-infected individuals, but the outcomes of disease are more severe (4). This places a burden on systems already burdened by substantial costs involved in treating HIV-infected patients. Despite the differences between IBI in HIV-infected and HIV-uninfected individuals, the guidelines for treatment remain the same (21).

1.1.4 Burden of disease and disease patterns in South Africa

Although there are limited data on IBI in South Africa, a study done on paediatric bloodstream infections (including children aged 0-14 years), between 2008 and 2013 at a Western Cape Hospital, showed that that these infections were dominated by *Klebsiella pneumoniae* (17%), *S. aureus* (14%), *E. coli* (11%) and *S. pneumoniae* (11%) (26). *Streptococcus pneumoniae* declined from 12.5% in 2008-2010 to 8.3% in 2011-2013 ($p=0.04$), likely due to the introduction of PCV in 2009. The study found that nosocomial infection and HIV infection were significant predictors of mortality (27). As much as 47% of the analysed events were nosocomial with high rates (70%) of antibiotic resistance. *Streptococcus pneumoniae* was demonstrated to be significantly more common in HIV-infected than HIV-uninfected individuals (17.2% versus 6.7%, $p<0.001$) (27).

The burden of IBI in South Africa is closely linked to the prevalence and treatment of HIV. In 2005 it was estimated that approximately 30% of pregnant women attending public health clinics in South Africa were HIV-infected (28). The South African prevention of mother-to-child transmission programme (PMTCT) has been extremely successful, despite its late start in 2003. This has resulted in a reduction of vertical transmission rates from mother to infant from a projected 30% (in the absence of PMTCT) to 2.7% in 2011 (28). Before the rollout of antiretroviral therapy (ART) by the National Department of Health, South Africa in 2004, HIV infections were estimated to account for

just under a third of paediatric admissions at CHBAH (29, 30). HIV-infected children were treated with antibiotic prophylaxis only to prevent opportunistic infections after their first admission, as testing was done only in patients clinically suspected of having HIV infection (30). HIV prevalence decreased from 31.7% of paediatric admissions at CHBAH in 2005 to 19.3% in 2010-11 following the successful rollout of the ART program (29). The relationship between HIV with ART and IBI is demonstrated by the 50.8% (95% confidence interval (CI), 41.5% to 58.7%) reduction in invasive pneumococcal disease (IPD) in HIV-infected children between the pre- and post-ART introduction periods (31). This study was done before the introduction of PCV into the EPI-SA and there was no reduction of IPD seen among the HIV-uninfected children; hence the reduction in HIV-infected children was attributed to the introduction of ART (31).

1.1.5 Pneumococcal Conjugate Vaccine

In 1929, Avery and Goebel first described enhancing the immunogenicity of bacterial polysaccharide antigens by attaching them to a protein carrier molecule (32). The immaturity of the immune system in infants and young children makes it difficult for them to mount a protective antibody response against polysaccharide antigens. By introducing a protein carrier into these vaccines, a protective immune response can be mounted in this high risk group, providing them with some degree of immunity (32). In 1987, the Hib vaccine became the first glycoconjugate vaccine to be licensed for use in humans (32). In 1998 the first PCV efficacy study (using PCV7 – a vaccine containing cell capsule polysaccharides of 7 of the over 90 pneumococcal serotypes), conducted in the United States, was published and indicated a 100% efficacy against IPD (32). The success of these two vaccines in reducing disease has been attributed partially to the reduction in invasive disease and partially to the reduction of nasopharyngeal carriage of pathogens in vaccinated individuals and resulting in a reduction of person-to-person spread (32).

An important RCT was conducted in Soweto from 1998-2005 to determine the efficacy of the 9-valent PCV (PCV9) (33). The PCV9 included pneumococcal serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F and

23F (33). Results of this study, which randomised 39 836 children to receive either PCV9 or placebo, were crucial in the decision to introduce PCV into the EPI-SA in 2009. The study demonstrated an 83% reduction in IPD (caused by vaccine serotypes) in HIV-uninfected children and a 65% reduction in IPD (caused by vaccine serotypes) in HIV-infected children. There was a 63% decrease in IPD caused by vaccine-related serotypes (6A, 19A and 19B) in HIV-infected children. There was a significant decrease in incidence of radiologically-confirmed pneumonia as well as antibiotic resistant IPD in PCV9 recipients (33). PCV was introduced into the EPI-SA as a 7-valent formulation (PCV7) in 2009 (34). The vaccine schedule consisted of 2 primary doses at 6 and 14 weeks, with a booster dose at 9 months of age (2 + 1 regimen). PCV7 was replaced with a 13-valent formulation (PCV13) in 2011, using the same vaccination schedule (35).

A national study of South African children under 2 years of age demonstrated that 4 years after the introduction of PCV into EPI-SA, there was an 89% reduction in incidence of IPD caused by PCV7 serotypes and an 82% reduction in the incidence of penicillin non-susceptible serotypes (34). There was also a notable (50%) reduction of IPD caused by PCV7 serotypes in unvaccinated adults and a 30% reduction in children too young to have been vaccinated (34). Reductions in IPD observed in the age groups not eligible to have received PCV most likely reflect the 'indirect protection' or 'herd immunity' offered by vaccine associated reduction of pneumococcal carriage in the population (34). This is important in protecting vulnerable age groups such as neonates (who are too young to receive PCV themselves). The effectiveness of PCV13 has also been demonstrated through a case-control study in South African children which showed an 85% (95% CI, 37 to 96) effectiveness among HIV-uninfected children and a 91% (95% CI, 35 to 100) effectiveness among HIV-infected children in preventing IPD due to PCV13 serotypes (35).

Despite the reduction of burden of disease induced by the vaccination, there has been an increase in disease caused by non-vaccine serotypes and other microbes, called replacement (36). Patterns of replacement are difficult to predict due to the fact that there are over 90 pneumococcal serotypes,

only 13 of which are included in the vaccine (36). Replacement disease has also been reported for other vaccines after they had been introduced into communities. For example, in a population with high vaccine coverage of Hib, there has been reported replacement disease in which non-type b *H. influenzae* (specifically type a and non-typeable strains) have increased significantly (from 0.6 per 100 000 to 5.6 per 100 000) (37).

1.1.6 Current gaps in knowledge

The epidemiology of IBI in Africa is not well documented. Since the increased access to HIV prevention programmes, improved HIV treatment and the high coverage of PCV, the epidemiology of IBI is expected to show significant changes (26). An important aspect of current IBI studies is the change in infection patterns due to vaccination (through the replacement phenomenon as seen with PCV and Hib) as well as the rise of antibiotic resistant bacterial strains through the overuse of antibiotics. A study in Ethiopia reported that most bacterial strains isolated from blood were resistant to more than three commonly used antibiotics, with all Gram-negative bacterial isolates being resistant to ampicillin (10). This not only highlights the importance of vaccine development against bacterial pathogens, but also of the timely investigation of bacteraemia events and monitoring of antibiotic resistance patterns (10). In order to quantify the burden of IBI and to better understand the risk factors, further research on IBI aetiology and epidemiology in African children is required (26).

1.2 Problem Statement

The epidemiology of IBI is poorly documented in LMICs (26). In African countries with a high prevalence of HIV infection little is known about IBI, especially subsequent to the introduction of PCV (27). Although there are several South African studies on the effect of PCV on IPD rates (34, 38), less is known about the potential impact of PCV on the spectrum of IBI pathogens in both HIV-infected and HIV-uninfected children. Replacement disease may result in increased non-vaccine serotypes as well as the possibility of a change in incidence of other bacterial pathogens. The aim of this research was to determine whether HIV infection and PCV9 vaccination status had an effect on the epidemiology and aetiology of laboratory-confirmed IBI in children <5 years of age that participated in the PCV9 efficacy trial (1998-2005) in Soweto South Africa.

1.3 Objectives

1. To estimate the incidence of IBI in children <5 years of age that participated in the PCV9 efficacy trial in Soweto, stratified by age group (<6 months, 6-11 months, 12-17 months, 18-23 months, 24-35 months and 36-59 months), PCV9 vaccination status and HIV infection status.
2. To determine the risk factors associated with severe IBI (defined as death or prolonged hospitalisation) in the cohort.
3. To describe the seasonality of IBI in this cohort over the study time period (1998-2005).
4. To determine the pathogens responsible for IBI among PCV9 vaccinated and unvaccinated as well as HIV-infected and –uninfected hospitalised children <5 years of age.

2. Chapter Two: Materials and Methods

This chapter gives an overview of the analysis, beginning with a description of the original study on which this analysis is based. The important definitions used for the analysis are then outlined and statistical analytical methods are described in detail. The chapter ends with details of the ethical approval for the research project.

2.1 Original Study Design and Population

A RCT assessing efficacy of the PCV9 was conducted in Soweto from 1998-2005 (33). A total of 39 836 children from Soweto were randomly assigned to receive vaccination with either PCV9 or placebo at 6, 10 and 14 weeks of age. Children were eligible for inclusion in the study if they were between 28-84 days old and were unvaccinated or had received only routine Bacillus Calmette-Guérin vaccine and oral poliovirus vaccine at birth (33). Children were excluded if they had a progressive underlying neurological disorder, a history of seizures or infantile spasms or if there was a high likelihood of them moving from Soweto (33). Enrolment took place between 2 March 1998 and 30 October 2000 (33). The paediatric admission wards at CHBAH were monitored 24 hours a day and all study participants admitted for any cause were examined by a study doctor. Case report forms were completed for all admitted participants. Surveillance of paediatric admission wards continued until October 2005 (33). The study doctors were not involved in the decision to hospitalise a child, or any management decisions while hospitalised. CHBAH is a secondary and tertiary hospital that is estimated to attend to more than 90% of children in Soweto (33). Although Hib conjugate vaccine was only included into the EPI-SA in 1999, all PCV9 efficacy study participants received Hib vaccine as part of routine study procedure. EPI-SA vaccines received by study participants included diphtheria, tetanus, whole-cell pertussis, hepatitis B, and oral live trivalent poliovirus vaccine (33).

HIV testing was not done universally during this time period and hence only participants in whom HIV was clinically suspected were tested. HIV infection was confirmed by HIV DNA polymerase-chain-reaction (PCR) for children under the age of 18 months and by enzyme-linked immunosorbent

assay (ELISA) for children 18 months or older. For those children in whom HIV was clinically suspected but ELISA results were negative, a PCR was used to evaluate status (33). It was assumed that participants who were not hospitalised during the follow up period were HIV-uninfected since no HIV treatment would have been given and hence it is unlikely that an HIV-infected, untreated child would not have presented to the hospital with an opportunistic infection at some stage during this time period. Hospitalised participants without HIV results were also assumed to be HIV-uninfected since HIV was not clinically indicated and hence testing was not done in these cases (although the majority of hospitalised children were tested for HIV).

Bacteria were isolated from blood samples using an automated blood-culture system (BacT-Alert, OrganonTeknika) and from cerebrospinal fluid (CSF) using standard microbiologic procedures. Routine microbiologic methods were used to identify species (33).

2.2 Definitions

The current study comprises a secondary data analysis in the form of a retrospective cohort study. The incidence of hospitalisation for IBI was determined in the cohort of children under 5 years of age. Cases were laboratory-confirmed IBI. The definition used for IBI in the main analysis was the isolation of a bacterial organism from a non-contaminated blood culture. A new episode of infection was defined as isolation of a bacterial pathogen at least 30 days after the previous episode, or the isolation of a different pathogen within 30 days after the previous episode. An infection was considered to be a single episode if the same pathogen was found in the same or another site taken within 30 days of the original infection or during the same hospital admission period. Only admission cultures were analysed and hence infections were assumed to be community-acquired. Infections of the CSF were not included in the primary analysis but were presented separately. Blood cultures were done for most hospitalised cases whereas very few children had CSF cultures taken. The decision was hence taken to restrict the primary analysis to blood cultures in order to increase the specificity of the analysis as well as to increase the clinical relevance for treatment. Hospitalised

participants without blood cultures were assumed to be negative for IBI since the attending physician would have seen no clinical indication of bacteraemia and hence would not have requested a blood culture for these cases.

2.3 Statistical Analysis

Person-time calculations were done with censoring time occurring at each participant's fifth birthday or date of death (for participants that were reported to have died during hospital admission). Multiple events were counted per participant (given that the events were separated by at least 30 days or different pathogens were isolated within 30 days). Bacterial cultures were categorised as *S. pneumoniae*, Hib, *E. coli, S. aureus,* Klebsiella spp, Salmonella spp or other. The 'other' category included *Pseudomonas aeruginosa*, *Campylobacter* spp, *Moraxella catarrhalis*, Streptococcus spp (excluding *S. pneumoniae*), *Enterococcus faecalis*, Alcaligenes spp, *Morganella morganii*, *Acinetobacter lwoffii*, *Candida albicans*, *Shigella flexneri*, *N. meningitidis*, *Haemophilus parainfluenzae*, *Citrobacter freundii*, *Mycobacterium tuberculosis, Escherichia vulneris*, and non-typable *Haemophilus influenzae*. The following organisms were considered contaminants: Corynebacterium spp, Bacillus spp, Propionibacterium spp, Viridans streptococci, Lactobacillus spp, coagulase-negative staphylococcus (including *Staphylococcus epidermidis*), Peptostreptococcus spp, *Clostridium bifermentans*, Micrococcus spp, Streptococcus spp, other Gram-positive cocci and *Neisseria subflava*, and were excluded from the primary analysis. Contaminants were reported separately as some of these events may be considered clinically significant in certain circumstances (including HIV-infected or premature children). The numerator for incidence calculations was the number of IBI events while the denominator was person-time in years. All incidence rates are reported per 10 000 person years.

Participants were classified into four study groups (HIV-uninfected placebo recipients (HIV-PCV-), HIV-uninfected PCV9 recipients (HIV-PCV+), HIV-infected placebo recipients (HIV+PCV-) and HIV-infected PCV9 recipients (HIV+PCV+)). Incidence was stratified by age group (<6 months, 6-11

months, 12-17 months, 18-23 months, 24-35 months and 36-59 months), sex, PCV9 vaccination status, HIV infection status and the four study groups (HIV-PCV-; HIV-PCV+; HIV+PCV-; HIV+PCV+). Ninety-five percent confidence intervals for incidence rates were calculated using the Poisson distribution. Incidence rate ratios (IRR) were calculated to compare the incidence rates in different groups and p-values presented to indicate significance. The power to determine the difference in proportions between the study groups was calculated given the sample size and an alpha of 0.05. Seasonality was investigated by comparing incidence of IBI events occurring in summer (September through February) versus winter (March through August).

Risk factors for IBI hospitalisation were investigated using binomial logistic regression modelling for the outcome of IBI. The following risk factors were included: PCV9 vaccination status, sex, HIV infection status and prematurity (preterm defined as born <37 weeks of gestation, and term defined as born ≥37 weeks of gestation). Gestational age was included on the initial enrolment questionnaire for all participants; however birthweight was only collected for some hospitalised participants. Only 1515 participants (3.8%) had a recorded birthweight, whereas gestational age was recorded for almost all participants (99. 9%). Hence gestational age was used as a measure of prematurity in the logistic regression models as a risk factor for IBI. This is a limitation of the dataset as it was not possible to investigate birth weight as a risk factor for IBI. All independent variables were included in the model (rather than using stepwise modelling) since all were considered clinically important with regards to the outcome, based on existing literature (34, 39). Age was not included as an independent variable in the model since participants that were not hospitalised during the follow up time did not have a recorded age (only hospitalised participants had an age at which they were hospitalised). This had no effect on the model since age was already controlled for (between study groups) due to all participants being enrolled at the same age and followed up until 5 years of age. Odds ratios (OR) with 95% CI for the univariate and multivariate analysis were determined. Coagulase-negative staphylococcus was regarded as a contaminant for the purpose of this analysis, however literature indicates that this group (specifically *S. epidermidis*) may be pathogenic in certain

cases (40). Risk factors for CoNS infection were also investigated using binomial logistic regression modelling (as described above for IBI hospitalisations). A second analysis of CoNS infections was done with events limited only to those in participants under 3 months of age (as CoNS infections were likely to be clinically significant in these young children).

The following clinical characteristics of IBI were investigated individually using Chi-squared tests to determine if there was an association with specific IBI pathogens (*S. pneumoniae*, Hib, *E. coli, S. aureus,* Klebsiella spp, Salmonella spp or other - as defined above): fever, refusal to eat, vomiting, diarrhoea, weight-loss, night sweats, seizures and excessive crying. Univariate analysis using multinomial logistic regression was done to compare the clinical characteristics of Hib, *E. coli*, *S. aureus*, Klebsiella spp, Salmonella spp and other bacterial infections to those of *S. pneumoniae* infections. This investigation was cross-sectional (at time of hospitalisation) and included only participants hospitalised with IBI.

Severity of IBI was modelled using a logistic regression model with a binary outcome of 'survived' or 'died' as well as duration of hospitalisation (with a binary outcome of '≤4 days' or '>4 days'). Four days was chosen as the cut-off for this severity model since CHBAH uses a four day rotation which generally discharges children on or before the fourth day if possible. Children that are severely ill are generally hospitalised for longer than four days. These models included only participants hospitalised with IBI in order to compare outcomes between infections with different pathogens. The independent variables bacterial type (*S. pneumoniae*, Hib, *E. coli, S. aureus,* Klebsiella spp, Salmonella spp or other - as defined above), HIV infection status, age category (<6 months, 6-11 months, 12-17 months, 18-23 months, 24-35 months and 36-59 months), sex, PCV9 vaccination status and prematurity (as a categorical variable) were included in the models. Age was included in severity models since only participants that were hospitalised for IBI were analysed (as cross-sectional data) and hence all had age at hospitalisation.

Stata software, version 13 (StataCorp, College Station, TX) was used for all analyses and a significance level of $p<0.05$ was used for all calculations.

2.4 Ethical Approval

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical): Clearance Certificate No. M161182 (Appendix 1) on the 25th November 2016.

3. Chapter Three: Results

This chapter describes the results from the analysis starting with an overall description of IBI events in the cohort, in terms of incidence rates of the main causative pathogens and patterns of infection. The effect of PCV9 vaccination on IBI incidence rates and causative pathogens is then described, focusing on the differences between HIV-infected and –uninfected participants. The risk factors for IBI infection (including a separate description of risk factors for CoNS infection) are then described, and an overview of the main clinical characteristics and outcomes of IBI given. The chapter ends with a description of the invasive Klebsiella spp events in the cohort.

3.1 Description of IBI events in the cohort

From the 39 836 participants included in the RCT (19 922 of which received PCV9 vaccination and 19 914 of which received placebo), there were 10 516 hospitalisation events. HIV status was only confirmed for 5 307 participants (1 267 of whom were HIV-infected and 4 040 of whom were HIV-uninfected). It was assumed, as described in the methods, that those that were not tested for HIV were HIV-uninfected.

A description of the 10 516 hospitalisation events is given in Figure 3.1. The PCV9 vaccinated arm contributed to 5 180 (49.3%) of the hospital events, while the placebo vaccinated arm contributed to 5 336 (50.7%) of the hospital events. The PCV9 vaccinated arm was made up of 1 394 (26.9%) HIV-infected participants of whom 1 131 (81.1%) had blood cultures taken. Only 100 (8.8%) of these blood cultures were IBI positive. The HIV-uninfected participants made up 3 786 (73.1%) of the PCV9 vaccinated arm. A total of 2 547 (62.3%) had blood cultures taken, of which only 70 (2.8%) were IBI positive. The placebo vaccinated arm was made up of 1 561 (29.3%) HIV-infected participants of whom 1 260 (80.7%) had blood cultures taken. Only 150 (11.9%) of these blood cultures were IBI positive. The HIV-uninfected participants made up 3 775 (70.8%) of the placebo vaccinated arm. A total of 2 599 (68.9%) had blood cultures taken, of which only 75 (2.9%) were IBI positive.

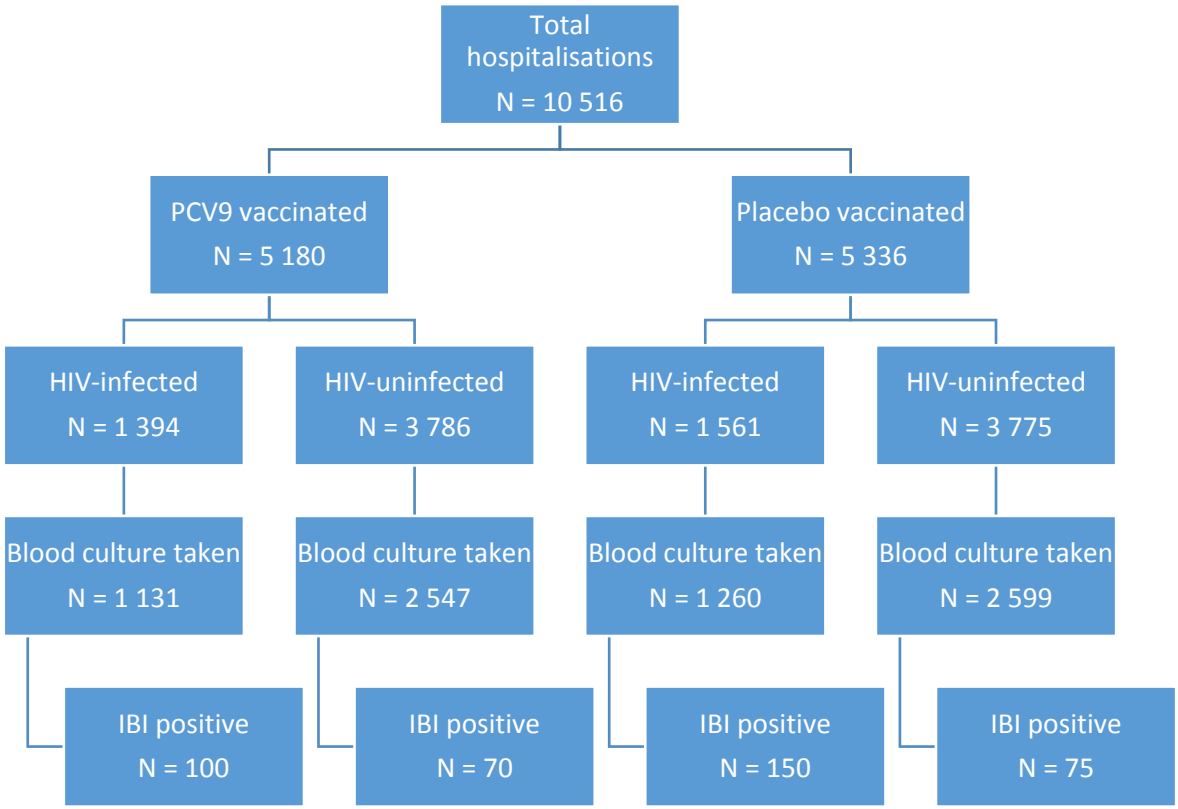


Figure 3.1: Flow diagram indicating number of hospitalisation events in the cohort

There were a total of 395 laboratory-confirmed IBI events in the total cohort over the follow up period (excluding contaminants which are described in the Appendix – Table S1). Males had 220 IBI events (99 in the PCV9 vaccinated group and 121 in the placebo group, p=0.036) while females had 175 events (71 in the PCV9 vaccinated group and 104 in the placebo group, P<0.001). The median age for hospitalisation with IBI was 9.3 months (interquartile range (IQR), 4.1-21.0), with 8.7 months (IQR, 4.5-20.3) for PCV9 vaccinated and 9.3 months (IQR, 3.5-23.3) for placebo group (p=0.497). Of the 395 events, 250 (63.3%) occurred in HIV-infected participants (100 in the PCV9 vaccinated group and 150 in the placebo group) and 145 (36.7%) were in HIV-uninfected participants (70 in the PCV9 vaccinated group and 75 in the unvaccinated group). The number of IBI events in the HIV-infected participants was very high considering that HIV-infected participants constituted only 3.2% of the total cohort (1 267 participants). Overall, there were 35 participants that had repeat IBI hospitalisations which were considered as separate events for the purposes of the analysis (15 were in the PCV9 vaccinated group and 20 in the placebo group) (Table 3.1).

Table 3.1: Characteristics of the participants with IBI events included in the analysis

Characteristic	PCV9 (N=170)	Placebo (N=225)	Total (N=395)
Sex			
Male (%)	99 (58.2%)	121 (53.8%)	220 (55.7%)
Female (%)	71 (41.8%)	104 (46.2%)	175 (44.3%)
Age at hospitalisation – median (IQR) in months	8.7 (4.5-20.3)	9.3 (3.5-23.3)	9.3 (4.1-21.0)
HIV Status			
Infected	100 (58.8%)	150 (66.7%)	250 (63.3%)
Uninfected	70 (41.2%)	75 (33.3%)	145 (36.7%)
Number of with repeat hospitalisations	15 (8.8%)	20 (8.9%)	35 (8.9%)

Figure 3.2 shows the proportions of contributing pathogens responsible for the 395 IBI events. The commonest bacterial isolate was *S. pneumoniae* which was detected in 154 events (39.0%). *E. coli* was detected in 84 events (21.3%), *S. aureus* in 29 events (7.3%), Salmonella spp in 26 events (6.6%), Hib detected in 23 events (5.8%), Klebsiella spp were detected in 18 events (4.6%) and the remaining 61 events (15.4%) were due to other pathogens (including Streptococcus spp (2. 8%), *P. aeruginosa* (2.3%), *N. meningitidis* (2.0%), *A. lwoffii* (1.5%), Campylobacter spp (1.5%), *E. faecalis* (1.5%), *C. albicans* (0.8%), Alcaligenes spp (0.5%), *M. morganii* (0.5%), *M. tuberculosis* (0.5%), *C. freundii* (0.3%), *E. vulneris* (0.3%), non-typable *H. influenzae* (0.3%), *H. parainfluenzae* (0.3%), *M. catarrhalis* (0.3%), and *S. flexneri* (0.3%)) which were grouped for the purpose of this analysis. A summary of the pathogens grouped as 'other' is presented in the Appendix (Figure S1).

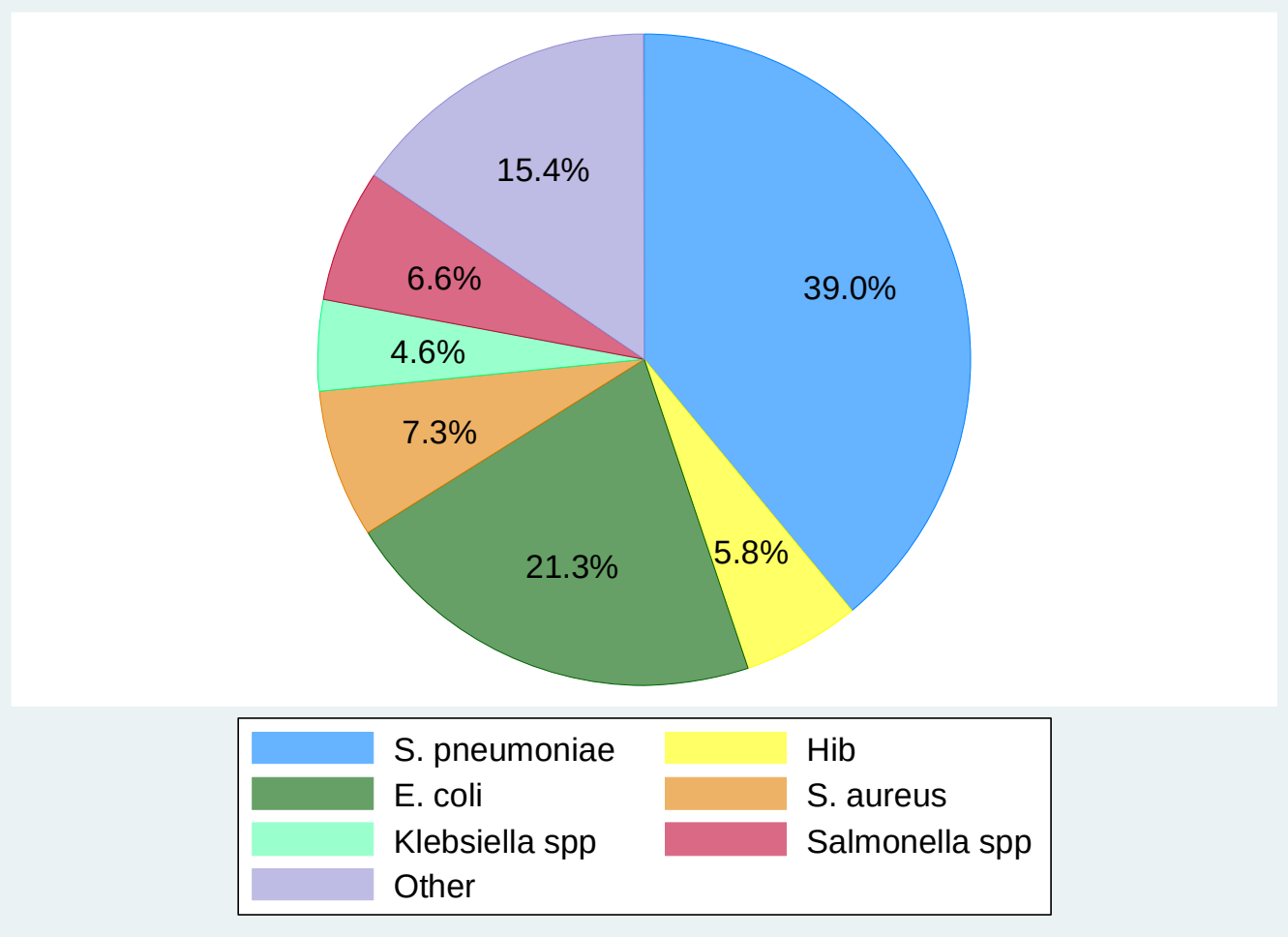


Figure 3.2: The proportions of pathogens detected in IBI events in the cohort during hospitalisation

Table 3.2 presents the incidence rates (per 10 000 person-years (PY) of follow up time) for bacterial pathogens detected as non-contaminated blood cultures during hospitalisation (number of events are presented in Table S2 – Appendix). *Streptococcus pneumoniae* had the highest incidence rate (8.01; 95% CI, 6.80 to 9.38), followed by *E. coli* (4.37; 95% CI, 3.49 to 5.41). The incidence of *S. pneumoniae* was significantly higher than *E. coli* (IRR 1.83, p<0.001). Klebsiella spp had the lowest incidence (0.94; 95% CI, 0.56 to 1.48). The incidence of *S. pneumoniae* was 8.56 (p<0.001) times higher than the incidence of Klebsiella spp.

Table 3.2: Incidence of IBI hospitalisation (per 10 000 person-years - showing 95% confidence intervals)

Characteristic	*S. pneumoniae*	PCV9 serotypes*	Non-PCV9 serotypes*	Hib	*E. coli*	*S. aureus*	Klebsiella spp	Salmonella spp	Other**	All
Overall	8.01 (6.80-9.38)	5.67 (4.66-6.84)	1.35 (0.88-1.98)	1.20 (0.76-1.80)	4.37 (3.49-5.41)	1.51 (1.01-2.17)	0.94 (0.56-1.48)	1.35 (0.88-1.98)	3.17 (2.43-4.08)	20.55 (18.57-22.68)
Age Group										
<6 months* **	28.99 (20.98-39.05)	15.51 (9.83-23.27)	6.74 (3.23-12.40)	4.05 (1.48-8.81)	22.25 (15.32-32.25)	10.11 (5.66-16.68)	6.07 (2.77-11.52)	5.39 (2.33-10.63)	18.88 (12.55-27.29)	95.75 (80.65-112.85)
6-11 months	15.18 (10.24-21.67)	9.61 (5.79-15.01)	3.04 (1.11-6.61)	4.05 (1.75-7.98)	12.65 (8.19-18.67)	2.02 (0.55-5.18)	1.52 (0.31-4.44)	2.53 (0.82-5.90)	5.56 (2.78-9.96)	43.51 (34.80-53074)
12-17 months	11.15 (6.99-16.88)	9.12 (5.40-14.41)	1.01 (0.12-3.66)	1.52 (0.31-4.44)	4.56 (2.09-8.66)	1.52 (0.31-4.44)	1.01 (0.12-3.66)	3.04 (1.12-6.62)	3.55 (1.43-7.31)	26.35 (19.68-34.55)
18-23 months	6.59 (3.51-11.27)	5.58 (2.78-9.98)	5.58 (2.78-9.98)	0.51 (0.1-2.83)	2.53 (0.82-5.92)	1.01 (0.12-3.66)	0.51 (0.01-2.82)	0.51 (0.01-2.82)	2.53 (0.82-5.92)	14.20 (9.43-20.52)
24-35 months	4.31 (2.51-6.90)	3.30 (1.76-5.64)	0.76 (0.16-2.22)	0.51 (0.6-1.83)	2.54 (1.22-4.66)	0.52 (0.01-1.41)	0.76 (0.16-2.22)	1.52 (0.56-3.31)	1.52 (0.56-3.31)	11.41 (8.33-15.27)
36-59 months	3.68 (2.46-5.29)	3.17 (2.05-4.68)	0.51 (0.14-1.30)	0.38 (0.08-1.11)	0.25 (0.03-0.92)	0.51 (0.14-1.30)	0 (0-0.47)	0 (0-0.47)	0.51 (0.14-1.30)	5.33 (3.84-7.20)
Sex										
Male	8.61 (6.86-10.67)	6.43 (4.93-8.24)	1.14 (0.57-2.04)	1.14 (0.57-2.04)	5.19 (3.85-6.84)	1.87 (1.11-2.95)	1.04 (0.50-1.91)	1.14 (0.57-2.04)	3.84 (2.70-5.29)	22.82 (19.90-26.04)
Female	7.41 (5.79-9.35)	4.91 (3.61-6.53)	1.57 (0.88-2.58)	1.25 (0.65-2.19)	3.55 (2.46-4.96)	1.15 (0.57-2.05)	0.84 (0.36-1.65)	1.57 (0.88-2.58)	2.51 (1.61-3.73)	18.27 (15.66-21.19)
IRR (p-value)	*1.16 (0.356)*	*1.31 (0.163)*	*0.73 (0.432)*	*0.91 (0.827)*	*1.46 (0.088)*	*1.63 (0.207)*	*1.24 (0.658)*	*0.73 (0.432)*	*1.53 (0.103)*	***1.25 (0.028)***
Vaccination										
PCV9	5.72 (4.31-7.45)	3.64 (2.54-5.06)	1.56 (0.87-2.57)	1.14 (0.57-2.05)	1.14 (0.57-2.05)	1.98 (1.19-3.09)	1.46 (0.80-2.44)	1.25 (0.64-2.18)	2.70 (1.77-3.96)	17.68 (15.12-20.55)
Placebo	10.31 (8.38-12.55)	7.70 (6.05-9.67)	1.15 (0.57-2.05)	1.25 (0.65-2.18)	5.31 (3.95-6.98)	1.04 (0.50-1.91)	0.42 (0.11-1.07)	1.46 (0.80-2.45)	3.64 (2.54-5.07)	23.42 (20.46-26.69)
IRR (p-value)	***0.56 (<0.001)***	***0.47 (<0.001)***	*1.36 (0.443)*	*0.92 (0.838)*	*0.65 (0.050)*	*1.90 (0.099)*	***3.50 (0.019)***	*0.86 (0.700)*	*0.74 (0.252)*	***0.76 (0.006)***
HIV Status										
Infected	250.60 (207.08-300.57)	179.31 (142.82-222.28)	43.21 (26.39-66.73)	30.24 (16.54-50.75)	116.66 (87.64-152.21)	21.60 (10.36-39.73)	21.60 (10.36-39.73)	36.73 (21.39-58.80)	62.65 (41.96-89.98)	540.09 (475.21-611.35)
Uninfected	2.03 (1.43-	1.39 (0.91-	0.32 (0.12-	0.48 (0.22-	1.60 (1.08-	1.01 (0.61-	0.43 (0.18-	0.48 (0.22-	1.71 (1.17-	7.73 (6.52-9.10)

	2.78)	2.03)	0.70)	0.91)	2.28)	1.58)	0.84)	0.91)	2.41)	
IRR (p-value)	***123.70 (<0.001)***	***129.36 (<0.001)***	***135.08 (<0.001)***	***63.04 (<0.001)***	***72.94 (<0.001)***	***21.33 (<0.001)***	***50.65 (<0.001)***	***76.54 (<0.001)***	***36.72 (<0.001)***	***69.87 (<0.001)***
Prematurity										
Term	7.65 (6.43-9.04)	5.32 (4.31-6.50)	1.33 (0.85-1.98)	1.00 (0.59-1.58)	4.05 (3.17-5.09)	1.50 (0.99-2.18)	0.72 (0.38-1.23)	1.33 (0.85-1.98)	2.88 (2.15-3.78)	19.14 (17.17-21.27)
Preterm	12.63 (7.07-20.83)	10.11 (5.22-17.65)	1.68 (0.20-6.08)	4.21 (1.37-9.83)	9.26 (4.62-16.57)	1.68 (0.20-6.08)	4.21 (1.37-9.83)	1.68 (0.20-6.08)	7.58 (3.47-14.39)	41.26 (30.53-54.55)
IRR (p-value)	*1.65 (0.080)*	*1.90 (0.051)*	*1.27 (0.698)*	***4.22 (0.014)***	***2.29 (0.020)***	*1.12 (0.808)*	***5.84 (0.004)***	*1.27 (0.698)*	***2.63 (0.016)***	***2.16 (<0.001)***
Study group										
HIV+PCV+	176.22 (125.31-240.90)	122.00 (80.40-177.50)	45.18 (21.67-83.10)	27.11 (9.95-59.01)	99.41 (62.30-150.50)	31.63 (12.72-65.17)	31.63 (12.72-65.17)	27.11 (9.95-59.00)	58.74 (31.28-100.45)	451.85 (367.64-549.57)
HIV+PCV-	318.74 (251.55-398.37)	231.81 (175.11-301.03)	41.40 (19.85-76.13)	33.12 (14.30-65.25)	132.46 (90.61-187.00)	12.42 (2.56-36.29)	12.42 (2.56-36.29)	45.53 (22.73-81.47)	66.23 (37.86-107.56)	620.93 (525.54-728.62)
IRR (p-value)	***0.55 (0.002)***	***0.53 (0.005)***	*1.09 (0.847)*	*0.82 (0.724)*	*0.75 (0.303)*	*2.55 (0.179)*	*2.55 (0.179)*	*0.60 (0.316)*	*0.89 (0.754)*	***0.73 (0.013)***
HIV-PCV+	1.70 (0.97-2.77)	0.85 (0.37-1.68)	0.53 (0.17-1.24)	0.53 (0.17-1.25)	1.17 (0.59-2.10)	1.28 (0.66-2.24)	0.75 (0.30-1.54)	0.64 (0.24-1.39)	1.38 (0.74-2.37)	7.45 (5.81-9.42)
HIV-PCV-	2.35 (1.47-3.56)	1.92 (1.14-3.04)	0.11 (0.00-0.59)	0.43 (0.12-1.09)	2.02 (1.22-3.16)	0.75 (0.30-1.54)	0.11 (0.00-0.59)	0.32 (0.07-0.93)	2.03 (1.22-3.17)	8.01 (6.30-10.04)
IRR (p-value)	*0.73 (0.332)*	*0.44 (0.051)*	*4.99 (0.126)*	*1.25 (0.757)*	*0.58 (0.147)*	*1.71 (0.266)*	***6.98 (0.039)***	*1.99 (0.346)*	*0.68 (0.293)*	*0.93 (0.667)*
Season										
Summer	7.50 (5.87-9.45)	4.90 (3.60-6.51)	1.35 (0.72-2.32)	0.83 (0.36-1.64)	4.17 (2.98-5.68)	1.35 (0.72-2.32)	0.63 (0.23-1.36)	1.15 (0.57-2.05)	2.50 (1.60-3.72)	18.13 (15.54-21.04)
Winter	8.52 (6.78-10.58)	6.44 (4.94-8.26)	1.35 (0.72-2.31)	1.56 (0.87-2.57)	4.57 (3.32-6.14)	1.66 (0.95-2.70)	1.25 (0.64-2.18)	1.56 (0.87-2.57)	3.85 (2.71-5.30)	22.97 (20.04-26.20)
IRR (p-value)	*1.14 (0.432)*	*1.32 (0.156)*	*1.00 (0.995)*	*1.87 (0.153)*	*1.10 (0.674)*	*1.23 (0.590)*	*1.99 (0.169)*	*1.36 (0.446)*	*1.54 (0.100)*	***1.27 (0.019)***

* PCV9 serotypes included: 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F as well as PCV9-related serotypes 6A, 19A and 19B; Vaccine-serotypes and non-vaccine serotypes do not add up to the total pneumococcal incidence as latex positive, culture negative *S. pneumoniae* cultures were not serotyped.

** Other bacterial infections include: Streptococcus spp *(n=11), P. aeruginosa (n=9), N. meningitidis (n=8), A. lwoffii (n=6) ,* Campylobacter *spp (n=6), E. faecalis (n=6), C. albicans (n=3),* Alcaligenes spp *(n=2), M. morganii (n=2), M. tuberculosis (n=2), C. freundii (n=1), E. vulneris (n=1),* non-typable *H. influenzae (n=1), H. parainfluenzae (n=1), M. catarrhalis (n=1), and S. flexneri (n=1)*

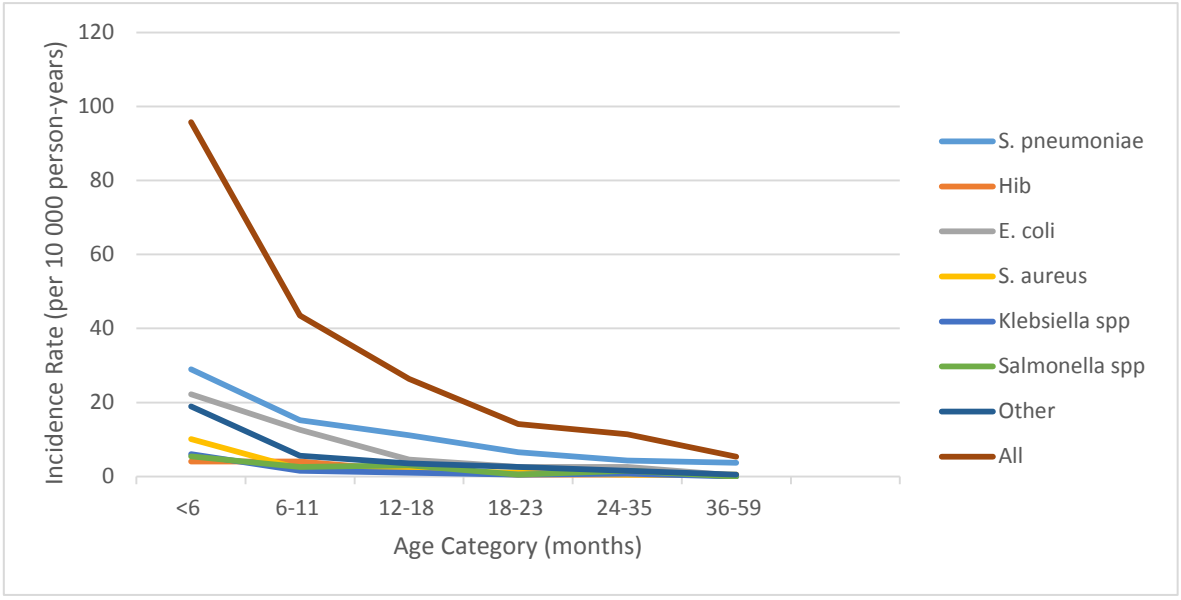
****Enrolment took place at 28-84 days old hence the <6 month group excludes children under 28 days*

Abbreviations: HIV-PCV- = HIV-uninfected placebo recipients; HIV-PCV+ = HIV-uninfected PCV9 recipients; HIV+PCV- = HIV-infected placebo recipients; HIV+PCV+ = HIV-infected PCV9 recipients; PCV9 = 9-valent pneumococcal conjugate vaccine.

There was an overall trend towards decreasing incidence of IBI with increasing age (Figure 3.3A). Participants under 6 months of age had the highest incidence of IBI as compared to other age groups. There was a sharp decline in incidence between the <6 months and 6-11 months age groups (IRR 2.20, p<0.001). Figure 3.3B shows the overall incidence of *S. pneumoniae* IBI stratified by PCV9 serotypes (including PCV9-related) and non-PCV9 serotypes. Incidence of both PCV9 serotypes (including PCV9-related) and non-PCV9 serotypes decreased with increasing age.

An analysis of the contaminants (Appendix: Table S1) revealed a high rate of contamination due to *S. epidermidis* (387.08; 95% CI, 359.76 to 415.93), which was responsible for 73.3% of total blood culture contamination. Other important contaminants in the cohort included Corynebacterium spp (which were responsible for 9.7% of total blood culture contamination with an incidence rate of 48.39; 95% CI, 39.05 to 59.28) and Bacillus spp (which were responsible for 5.3% of total blood culture contamination with an incidence rate of 26.53; 95% CI, 19.76 to 34.89). The proportion of contaminated blood cultures (for all hospitalisations in the cohort) was similar between the HIV-infected and HIV-uninfected participants (12.9% versus 12.3% respectively, p=0.467), however the incidence rates of contaminating organisms were significantly higher in HIV-infected participants (incidence rate of 678.35; 95% CI, 605.38 to 757.68) than in HIV-uninfected participants (incidence rate of 34.60; 95% CI, 31.99 to 37.37) indicated by an IRR of 19.61 (95% CI, 17.08 to 22.47, p<0.001). Vaccination with PCV9 had no significant effect on incidence of contaminants in either the HIV-infected or HIV-uninfected participants. The incidence for HIV-infected PCV9 vaccinated participants was 700.36 (95% CI, 594.45 to 819.71) while for HIV-infected control participants incidence was 658.18 (95% CI, 559.85 to 768.81) resulting in an IRR of 1.06 (95% CI, 0.85 to 1.34, p=0.582). The incidence for HIV-uninfected PCV9 vaccinated participants was 32.90 (95% CI, 29.33 to 36.78) while for HIV-uninfected control participants incidence was 36.30 (95% CI, 32.55 to 40.38) resulting in an IRR of 0.91 (95% CI, 0.77-1.06, p=0.201).

A)



B)

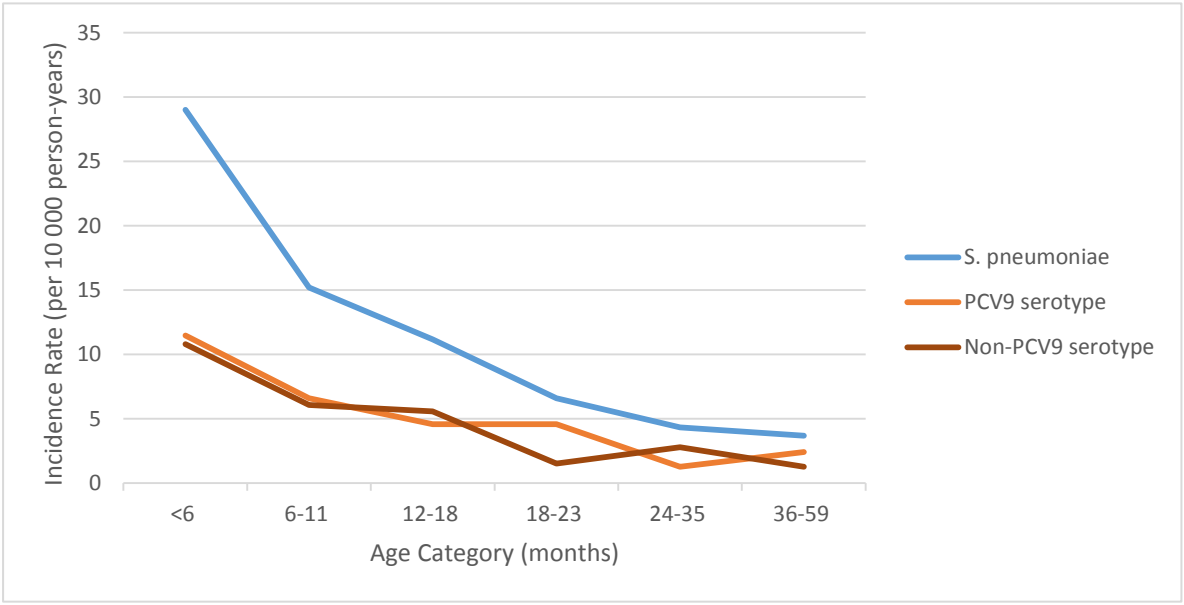


Figure 3.3: A) Incidence rates across age groups for different IBI pathogens. B) Incidence rates across age groups for *S. pneumoniae* infections (separated into PCV9 serotypes* and non-PCV9 serotypes).

* PCV9 serotypes included: 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F and PCV9-related serotypes (6A, 19A and 19B).

Note: Enrolment took place at 28-84 days old hence the <6 month group excludes children under 28 days.

Overall, males (incidence rate of 22.82; 95% CI, 19.90 to 26.04) had a higher incidence of IBI than females (incidence rate 18.27; 95% CI, 15.66 to 21.19) as indicated by a significant IRR of 1.25 (95% CI, 1.02 to 1.53, p=0.028). Stratification by bacterial pathogen indicated that this was a non-

discriminatory, cumulative effect rather than being caused by any specific pathogen (i.e. no specific pathogen affected males significantly more than females; Table 3.2). A separate analysis of CoNS infections (Appendix – Table S4) indicated that males have a 34% higher odds of CoNS infections as compared to females (p<0.001).

HIV-infected participants (incidence rate of 540.09; 95% CI, 475.21 to 611.35) had significantly higher incidence of IBI for all pathogens as compared to HIV-uninfected participants (incidence rate of 7.73; 95% CI, 6.52-9.10) with an IRR of 69.87 (95% CI, 56.71 to 86.33, p<0.001). The incidence for *S. pneumoniae* was higher in the HIV-infected (250.60; 95% CI, 207.08 to 300.57) compared to the HIV-uninfected participants (2.03; 95% CI, 1.43 to 2.78) with an IRR of 123.70 (95% CI, 85.09 to 183.47, p<0.001).

An analysis of seasonality indicated a slight increase in IBI incidence during winter. Incidence in summer was 18.13 (95% CI, 15.51 to 21.04) while incidence in winter was 22.97 (95% CI, 20.04 to 26.20) resulting in an IRR of 1.27 (95% CI, 1.03 to 1.55, p=0.019). This was a cumulative effect rather than being due to an increase in any specific pathogen during winter (changes for all individual pathogens were non-significant; Table 3.2).

3.2 Effect of PCV9 vaccination on IBI

The overall IBI incidence rate was significantly lower for PCV9 vaccinated (17.68; 95% CI, 15.12 to 20.55) versus control participants (23.42; 95% CI, 20.46 to 26.69) indicated by an IRR of 0.76 (95% CI, 0.61 to 0.93, p=0.006). Incidence of *S. pneumoniae* was 5.72 (95% CI, 4.31 to 7.45) in the PCV9 vaccinated participants as compared to 10.31 (95% CI, 8.38 to 12.55) in the control group (IRR=0.56; 95% CI, 0.39 to 0.78, p<0.001). This decrease in *S. pneumoniae* incidence in the vaccinated group was due to the significant decrease in incidence of PCV9- and PCV9-related serotypes. Incidence in PCV9 vaccinated was 3.64 (95% CI, 2.54 to 5.06) while incidence in control participants was 7.70 (95% CI, 6.05 to 9.67) resulting in an IRR of 0.47 (95% CI, 0.30 to 0.71, p<0.001). There was no significant overall change in incidence of non-PCV9 serotypes due to vaccination. Incidence in PCV9

vaccinated was 1.56 (95% CI, 0.87 to 2.57) while incidence in control group was 1.15 (95% CI, 0.57 to 2.05) resulting in an IRR of 1.36 (95% CI, 0.59 to 3.28, p=0.443). The vaccinated group showed a significant increase in incidence of Klebsiella spp. Incidence in PCV9 vaccinated was 1.46 (95% CI, 0.80 to 2.44) versus 0.42 (95% CI, 0.11 to 1.07) in the control group resulting in an IRR of 3.50 (95% CI, 1.10 to 14.59, p=0.019). There was a trend towards decrease in *E. coli* in the PCV9 vaccinated group which had an incidence of 1.14 (95% CI, 0.57 to 2.05) versus 5.31 (95% CI, 3.95 to 6.98) in the control group resulting in an IRR of 0.65 ((95% CI, 0.40 to 1.02, p=0.050); Table 3.2).

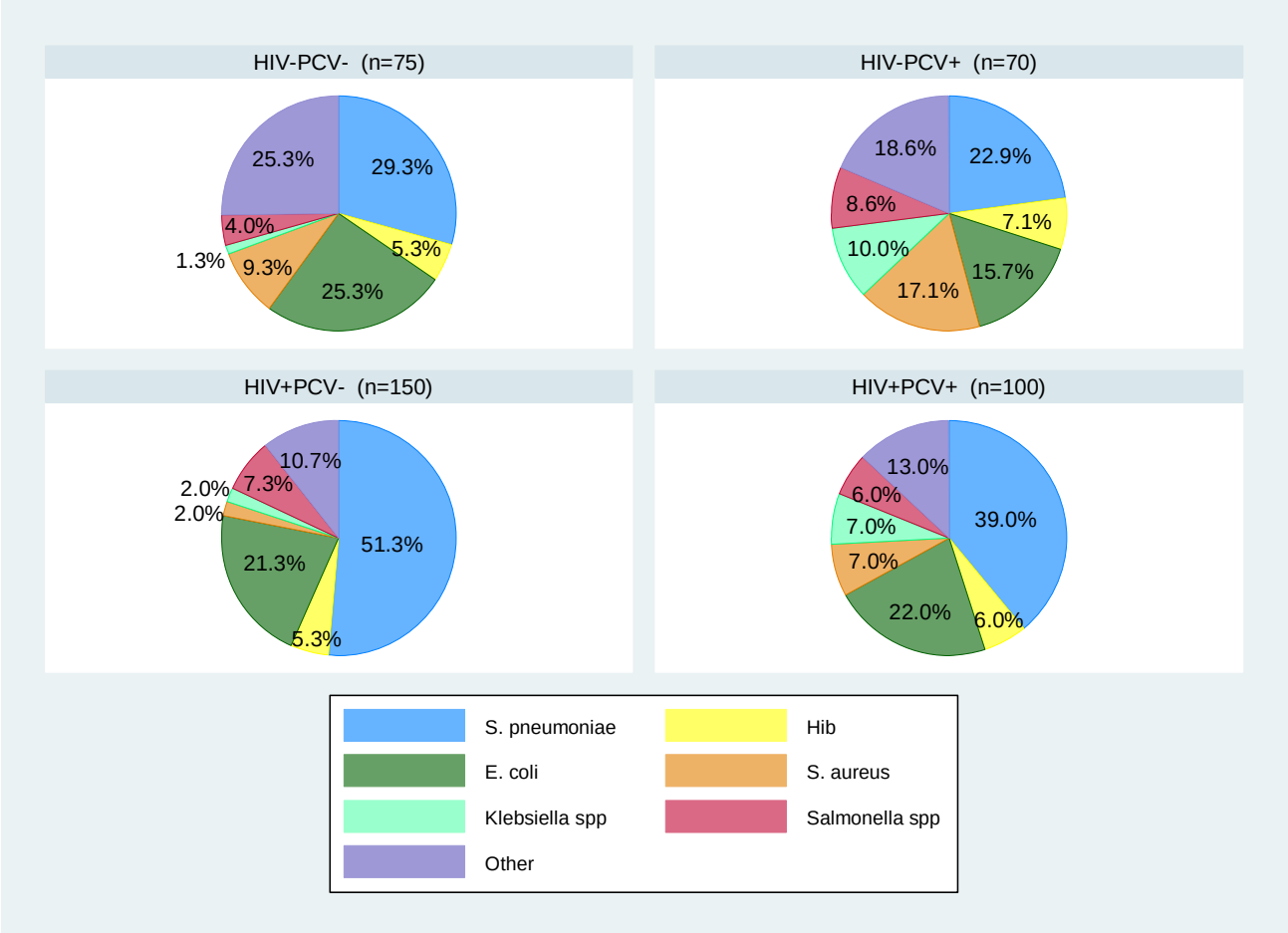


Figure 3.4: The proportions of pathogens detected in IBI events by study group (HIV and PCV9 vaccination status)

Abbreviations: Hib = *H. influenzae* type b; HIV-PCV- = HIV-uninfected placebo recipients; HIV-PCV+ = HIV-uninfected PCV9 recipients; HIV+PCV- = HIV-infected placebo recipients; HIV+PCV+ = HIV-infected PCV9 recipients.

3.2.1 HIV-uninfected participants

Streptococcus pneumoniae was the leading cause of IBI in both the HIV-infected and HIV-uninfected participants, despite vaccination (i.e. *S. pneumoniae* still had the highest incidence in the vaccinated group compared to other pathogens). Incidence rates indicate that vaccination in HIV-uninfected participants had no overall effect in reducing all-cause IBI hospitalisations, with incidence of 7.45 (95% CI, 5.81 to 9.42) in the PCV9 vaccinated and 8.01 (95% CI, 6.30 to 10.04) in the control group and a resulting IRR of 0.93 (95% CI, 0.66 to 1.31, p=0.667). There was no reduction in *S. pneumoniae* infections, with incidence of 1.70 (95% CI, 0.97 to 2.77) in the PCV9 vaccinated and 2.35 (95% CI, 1.47 to 3.56) in the control group, resulting in an IRR of 0.73 (95% CI, 0.36 to 1.45, p=0.332). There was a decrease in incidence of PCV9- and PCV9-related serotype IBI events (IRR=0.44; 95% CI, 0.17 to 1.07, p=0.051) in vaccinated (0.85; 95% CI, 0.37 to 1.68) compared to unvaccinated (1.92; 95% CI, 1.14 to 3.04) HIV-uninfected participants. There was no significant change in incidence of non-PCV9 vaccine serotype IBI events between vaccinated (0.53; 95% CI, 0.17 to 1.24) and unvaccinated (0.11; 95% CI, 0.00 to 0.59) HIV-uninfected children, indicated by an IRR of 4.99 (95% CI, 0.56 to 235.81, p=0.126). Vaccination in the HIV-uninfected participants was associated with a significant increase in Klebsiella spp IBI, with an incidence of 0.75 (95% CI, 0.30 to 1.54) in the PCV9 vaccinated and 0.11 (95% CI, 0.00 to 0.59) in the control group and a resulting significant IRR of 6.98 (95% CI, 0.90 to 314.58, p=0.039). This study was underpowered (<80.00% power) to determine a significant difference in incidence in the HIV-uninfected group between PCV9 and placebo vaccinated participants as the differences between the groups were small.

3.2.2 HIV-infected participants

Receipt of PCV9 in the HIV-infected group was associated with a decrease in *S. pneumoniae* incidence and an increase in the proportion of infections due to Klebsiella spp and Salmonella spp, however the proportions of most pathogens remained largely unchanged (Figure 3.4). Incidence rates (Table 3.2) indicated that there was a significant reduction in overall IBI in the vaccinated HIV-infected participants (incidence of 451.85; 95% CI, 367.64 to 549.57) compared to unvaccinated HIV-

infected participants (incidence of 620.93; 95% CI, 525.54 to 728.62) indicated by a significant IRR of 0.73 (95% CI, 0.56 to 0.94, p=0.013). This reduction was specifically caused by the large reduction in *S. pneumoniae* incidence (with incidence of 176.22 (95% CI, 125.31 to 240.90) in the PCV9 vaccinated and 318.74 (95% CI, 251.55 to 398.37) in the control group) indicated by a significant IRR of 0.47 (95% CI, 0.30 to 0.71, p=0.002). In particular, reduction in *S. pneumoniae* incidence was due to the reduction in PCV9- and PCV9-related serotype infections (with incidence of 122.00 (95% CI, 80.40 to 177.50) in the PCV9 vaccinated and 231.81 (95% CI, 175.11 to 301.03) in the control group) indicated by a significant IRR of 0.53 (95% CI, 0.32 to 0.85, p=0.005). There was no significant change in IBI due to non-PCV9 serotypes between vaccinated (45.18; 95% CI, 21.67 to 83.10) and placebo participants (41.40; 95% CI, 19.85 to 76.13) with an IRR of 1.09 (95% CI, 0.41 to 2.92, p=0.847). This study was well powered to determine the differences in incidence seen between the PCV9 and placebo vaccinated participants in the HIV-infected groups (89.96% power to determine the difference in *S. pneumoniae* incidence, 79.31% power to determine the difference in overall IBI incidence and 82.89% power to determine the difference in incidence of PCV9- and PCV9-related serotypes).

3.2.3 Description of IBI in CSF

In the analysis of CSF isolates observed in the study cohort, the proportions of *S. pneumoniae* were reduced in the vaccinated group compared to the placebo group for both the HIV-infected and HIV-uninfected participants (although IRRs were non-significant for both, p=0.19 and p=0.21 respectively). The proportion of CSF infections due to *S. pneumoniae* (51.6%; 95% CI, 41.5 to 61.8%) was significantly higher (p=0.026) than the proportion in blood infections (38.9%; 95% CI, 34.2 to 43.8%) (Appendix Figure S2 and Table S6).

3.2.4 Description of 'other' IBI group

A description of the 'other' group is given in the Appendix (Figure S1). This demonstrates that Gram negatives constituted the largest proportion of this group and that their proportion increased in the PCV9 vaccinated group. *Mycobacterium tuberculosis* and fungal (*C. albicans*) infections were only found in the placebo groups, although the numbers were too small to make conclusive statements regarding the significance of these findings.

3.3 Risk factors of IBI

After controlling for sex and prematurity, only receipt of PCV9 and HIV status retained significance in the model which was used to determine risk factors for IBI in the study cohort (Table 3.3). The odds of IBI hospitalisation among PCV9 vaccinated participants was 21.00% (95% CI, 2.60 to 35.40%) lower than the odds among unvaccinated participants. The odds of hospitalisation with IBI among HIV-infected participants was 25.85 (95% CI, 20.93 to 31.91) times greater than the odds for HIV-uninfected participants. Prematurity was a significant risk factor in the univariate analysis, but in the multivariate analysis it did not retain significance (p=0.629) (Table 3.3).

Table 3.3: Risk factors associated with IBI hospitalisation

	Univariate		**Multivariate ***	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Vaccination				
Control	Ref	-	Ref	-
PCV9	0.75 (0.62-0.92)	0.005	0.79 (0.65-0.97)	0.027
Sex				
Female	Ref	-	Ref	-
Male	1.22 (0.99-1.48)	0.052	1.12 (0.91-1.37)	0.278
HIV				
Uninfected	Ref	-	Ref	-
Infected	25.90 (21.07-31.95)	<0.001	25.85 (20.93-31.91)	<0.001
Prematurity				
Term	Ref	-	Ref	-
Preterm	1.77 (1.31-2.39)	<0.001	0.93 (0.68-1.27)	0.629

* Adjusted for PCV9 vaccination, sex, HIV status and prematurity.

Abbreviations: OR = odds ratio; PCV9 = 9-valent pneumococcal conjugate vaccine; Ref = referent.

A separate analysis of CoNS IBI was conducted (Appendix: Table S4) to determine which risk factors were important for infection with this pathogen. The multivariate analysis indicated that male sex (OR = 1.34; 95% CI, 1.15 to 1.55, p<0.001), HIV infection (OR = 7.07; 95% CI, 6.04 to 8.28, p<0.001) and preterm birth (OR = 2.06; 95% CI, 1.69 to 2.50, p<0.001) were risk factors for isolation of CoNS in blood cultures. When this analysis was restricted to events in participants under 3 months of age only (Appendix: Table S5), it was found that HIV (OR = 6.80; 95% CI, 4.72-9.79, p<0.001) and preterm birth (OR = 2.17; 95% CI, 1.40-3.36, p<0.001) were risk factors for isolation of CoNS in blood cultures.

3.4 Clinical Characteristics

In terms of clinical presentation of IBI infections, *E. coli* (p<0.001), *S. aureus* (p<0.001), Klebsiella spp (p<0.001) and 'other' (p<0.001) were significantly less likely to be associated with fever as compared to infections with *S. pneumoniae*. IBI caused by *S. aureus* was associated with refusal to eat (p=0.018). *Escherichia coli* infections were associated with vomiting (p=0.004). *Escherichia coli* (p=0.002) and Salmonella spp (p=0.044) were significantly associated with diarrhoea. There was no association between type of bacterial pathogen and weight-loss (p=0.170), night sweats (p=0.326), seizures (p=0.159) or excessive crying (p=0.937).

3.5 Outcomes of IBI

3.5.1 Severity Indicator: Death versus Survival

The odds of severe outcome (death) decreased with increasing age (Table 3.4). The odds of dying for HIV-infected participants were 4.60 (95% CI, 2.38 to 8.89) times the odds for HIV-uninfected participants (p<0.001). Klebsiella spp, *E. coli,* Hib and 'other' showed a trend towards increased odds of death compared to *S. pneumoniae* (although not significant in the analysis). *Staphylococcus aureus* IBI was associated with a trend towards reduced odds of death compared to *S. pneumoniae* (although not significant). There was no significant change in odds of death for PCV9 vaccinated versus placebo groups in either HIV-infected (p=0.522) or HIV-uninfected (p=0.141) participants.

History of having been born prematurely was not associated with an increased odds of death (p=0.204) (Table 3.4).

Table 3.4: Predictors of poor outcome (death) for participants hospitalised with IBI

	Univariate		**Multivariate ***	
	OR	P-value	OR	P-value
Bacteria type				
S. pneumoniae	Ref	-	Ref	-
Hib	1.52 (0.55-4.20)	0.427	1.57 (0.52-4.72)	0.423
E. coli	1.93 (1.05-3.57)	0.038	1.79 (0.91-3.51)	0.092
S. aureus	0.50 (0.14-1.76)	0.272	0.58 (0.15-2.34)	0.432
Klebsiella spp	2.16 (0.75-6.22)	0.160	2.36 (0.72-7.77)	0.156
Salmonella spp	1	-**	1	-**
Other	1.06 (0.50-2.23)	0.904	1.18 (0.52-2.70)	0.690
HIV				
Uninfected	Ref	-	Ref	-
Infected	3.17 (1.74-5.80)	<0.001	4.60 (2.38-8.89)	<0.001
Age category				
1-5 months	Ref	-	Ref	-
6-11 months	1.35 (0.75-2.44)	0.323	1.05 (0.54-2.03)	0.885
12-17 months	0.54 (0.23-1.24)	0.146	0.42 (0.17-1.04)	0.062
18-23 months	0.23 (0.05-1.00)	0.050	0.22 (0.05-1.01)	0.051
24-35 months	0.29 (0.10-0.86)	0.025	0.16 (0.05-0.51)	0.002
36-59 months	0.40 (0.15-1.09)	0.073	0.25 (0.08-0.72)	0.011
Sex				
Female	Ref	-	Ref	-
Male	0.90 (0.55-1.47)	0.677	0.82 (0.48-1.41)	0.474
Vaccination				
Placebo	Ref	-	Ref	-
PCV9	1.26 (0.77-2.05)	0.353	1.57 (0.90-2.74)	0.112
Prematurity				
Term	Ref	-	Ref	-
Preterm	0.71 (0.32-1.59)	0.410	0.57 (0.24-1.36)	0.204

* Adjusted for bacterial type, HIV infection, age category, sex, PCV9 vaccination and prematurity.

**All participants infected with *Salmonella* survived (outcome did not differ) and hence could not be modelled.

Abbreviations: Hib = *H. influenzae* type b; OR = odds ratio; PCV9 = 9-valent pneumococcal conjugate vaccine; Ref = referent.

3.5.2 Severity Indicator: Prolonged Hospitalisation (>4 days versus ≤4 days)

As shown in the multivariate analysis, HIV-infected participants had significantly higher odds of being hospitalised for longer than 4 days as compared to HIV-uninfected participants (p=0.042). There was a trend towards higher odds for longer hospital stay for participants hospitalised with *E. coli, S.*

aureus, Klebsiella spp and 'other' as compared to *S. pneumoniae* (however this was not significant) (Table 3.5). There was a trend towards higher odds for shorter hospital stay for Hib and Salmonella spp as compared to *S. pneumoniae* (although not significant). Odds of longer hospitalisations did not change for preterm versus term babies (p=0.436) (Table 3.5).

Table 3.5: Predictors of prolonged hospitalisation (>4 days) for participants hospitalised with IBI

	Univariate		**Multivariate***	
	OR	P-value	OR	P-value
Bacteria type				
S. pneumoniae	Ref	-	Ref	-
Hib	0.72 (0.30-1.72)	0.456	0.80 (0.33-2.00)	0.633
E. coli	1.31 (0.75-2.29)	0.339	1.48 (0.83-2.64)	0.188
S. aureus	0.93 (0.41-2.08)	0.858	1.09 (0.46-2.54)	0.848
Klebsiella spp	1.31 (0.47-3.68)	0.606	1.34 (0.45-3.93)	0.598
Salmonella spp	0.66 (0.28-1.51)	0.322	0.70 (0.30-1.67)	0.427
Other	1.34 (0.72-2.51)	0.353	1.53 (0.80-2.93)	0.202
HIV				
Uninfected	Ref	-	Ref	-
Infected	1.48 (0.97-2.25)	0.066	1.61 (1.02-2.56)	0.042
Age category				
1-5 months	Ref	-	Ref	-
6-11 months	0.63 (0.37-1.09)	0.101	0.63 (0.36-1.11)	0.107
12-17 months	0.92 (0.48-1.78)	0.814	0.89 (0.45-1.75)	0.734
18-23 months	1.22 (0.51-2.89)	0.652	1.34 (0.56-3.25)	0.513
24-35 months	1.16 (0.57-2.34)	0.689	1.08 (0.51-2.27)	0.845
36-59 months	1.16 (0.56-2.39)	0.697	1.08 (0.50-2.34)	0.851
Sex				
Female	Ref	-	Ref	-
Male	1.10 (0.73-1.65)	0.661	1.04 (0.68-1.58)	0.863
Vaccination				
Placebo	Ref	-	Ref	-
PCV9	1.14 (0.76-1.72)	0.532	1.13 (0.73-1.74)	0.603
Prematurity				
Term	Ref	-	Ref	-
Preterm	1.33 (0.70-2.50)	0.384	1.30 (0.67-2.53)	0.436

* Adjusted for bacterial type, HIV infection, age category, sex, PCV9 vaccination and prematurity.

Abbreviations: Hib = *H. influenzae* type b; OR = odds ratio; PCV9 = 9-valent pneumococcal conjugate vaccine; Ref = referent.

3.6 Description of Klebsiella spp IBI in the cohort

The incidence of Klebsiella spp was shown to be significantly increased in PCV9 vaccinated participants (Table 3.2). This was further investigated to determine which risk factors were associated with invasive Klebsiella spp. Table 3.6 indicates that HIV infection (OR= 15.49; 95% CI, 5.98 to 40.12, p<0.001), preterm birth (OR=2.96; 95% CI, 1.03 to 8.53, p=0.044) and PCV9 vaccination (OR=3.83; 95% CI, 1.26 to 11.65, p=0.018) are significantly associated with Klebsiella spp IBI.

Table 3.6: Risk factors associated with invasive Klebsiella spp infection

	Univariate		**Multivariate ***	
	OR	P-value	OR	P-value
Vaccination				
Control	Ref	-	Ref	-
PCV9	3.52 (1.16-10.69)	0.027	3.83 (1.26-11.65)	0.018
Sex				
Female	Ref	-	Ref	-
Male	1.20 (0.47-3.05)	0.697	1.09 (0.43-2.78)	0.850
HIV				
Uninfected	Ref	-	Ref	-
Infected	17.34 (6.84-43.96)	<0.001	15.49 (5.98-40.12)	<0.001
Prematurity				
Term	Ref	-	Ref	-
Preterm	4.75 (1.69-13.35)	0.003	2.96 (1.03-8.53)	0.044

* Adjusted for PCV9 vaccination, sex, HIV infection and prematurity.

Abbreviations: OR = odds ratio; PCV9 = 9-valent pneumococcal conjugate vaccine; Ref = referent.

The 18 invasive Klebsiella spp infections included in the analysis were spread out over a period of 4 years with 9 events occurring in 1999, 4 events occurring in 2000, 3 events occurring in 2001 and 2 in 2002. Even though there were more events in 1999 than any other year, there were no more than 2 events per month. It is thus unlikely that these infections constituted an outbreak. None of the 18 participants hospitalised with Klebsiella spp infections had recorded previous hospitalisations and hence these infections were unlikely to be nosocomial in nature. Half of the Klebsiella spp infections (n=9) occurred in participants under the age of 6 months and only one event was in a child under the age of 3 months.

4. Chapter Four: Discussion

Streptococcus pneumoniae and *E. coli* were found to be the commonest causes of IBI hospitalisations in this cohort. This chapter will start by exploring some of the possible reasons for incidence rates being higher in the current cohort than those that have been reported in other settings. HIV-infected participants had significantly higher incidence rates for all causative pathogens, however PCV9 was shown to be effective in reducing overall IBI incidence in this high risk group. Vaccination was shown to significantly reduce incidence of PCV9- and PCV9-related serotypes in HIV-infected participants with marginally significant reductions in the HIV-uninfected participants. The main risk factors for IBI hospitalisation were found to be HIV infection and non-receipt of PCV9. There was however, an increase in hospitalisations due to Klebsiella spp in the PCV9 vaccinated participants. The context of these findings in terms of existing literature is discussed in this chapter.

4.1 Description of IBI in the cohort

This analysis estimated overall IBI incidence rates of 20.55 (95% CI, 18.57 to 22.68) per 10 000 person-years, with invasive *S. pneumoniae* incidence rates of 8.01 (95% CI, 6.80 to 9.38) per 10 000 person-years in a South African cohort under-5 years of age during an important efficacy trial of PCV9 (1998 through 2005). Greenhow et al (2017) reported IBI rates of 9.70 (95% CI, 79.40 to 11.70) per 10 000 person-years and invasive *S. pneumoniae* incidence rates of 7.45 (95% CI, 5.90 to 9.30) per 10 000 person-years in the pre-PCV era in California, United States (41). These rates declined to 2.90 (95% CI, 2.04 to 3.89) and 1.00 (95% CI, 0.50 to 1.80), respectively, post-PCV introduction. Incidence rates were estimated from otherwise healthy, full-term children and as such are expected to be significantly lower than for the current study setting with a high HIV prevalence.

IBI incidence rates observed in this study confirm the large burden of infectious disease in Africa as opposed to those seen in higher income settings. The American study demonstrated a very different age distribution compared to the current study. In the American study there was a spike in invasive *S. pneumoniae* in the 12-18 month age group (pre-PCV era). In the era post-introduction of PCV this

age-related incidence spike was not appreciated, however the overall pattern of decreasing IBI incidence with increasing age (as evident in the current study) was still not seen. This likely represents differences in community-acquired infections between settings. In high income countries children are more likely to be exposed to pathogens later on when they go to nursery school or day care, whereas in a middle or low income setting children are likely to be exposed to pathogens at an earlier age (possibly as a result of the pathogen concentration effect of larger households, including lack of sanitation and communal living which would be expected to enhance carriage and transmission of organisms between family members) (5, 42). In low income settings, younger children may also be at higher risk of malnutrition compared to older children that are more readily able to fend for themselves. Middle and low income settings would also experience higher rates of intrinsic factors such as malnutrition and immunodeficiency (due to higher rates of HIV and malnutrition) which may impact on the ability to suppress overgrowth of colonising pathogens, especially in young children with developing immune systems.

Male children were found to have higher incidence rates of IBI overall as compared to female children, which corroborates findings from other studies. A study in Korea demonstrated that male children are 1.4 times as likely to be hospitalised with IBI than females of the same age (2). It has been found that males have overall higher morbidity and mortality compared to females due to their increased susceptibility to and severity of disease (43). One theory for this is that females are able to mount a stronger humoral and cellular immune response and hence have increased protection against certain pathogens (reducing carriage and increasing clearance) (43). Females have also been shown to have increased antibody titres post-vaccination with measles vaccine (among others) as compared to males (43). In the current study, this difference between the sexes was particularly illustrated in the analysis of CoNS IBI which showed that males were significantly more likely to be hospitalised with CoNS bacteraemia as compared to females. The clinical relevance of CoNS, and specifically *S. epidermidis*, infections however is debatable and is discussed in further detail below.

The incidence of IBI in HIV-infected children was nearly 70 times greater than that of HIV-uninfected children ($p<0.001$). Almost two thirds (63.3%) of the IBI events occurred in HIV-infected participants, despite HIV-infected participants constituting only 3.2% of the total cohort. This proportion is in line with previous findings in South Africa which estimate that 70.0% of IPD occurs in HIV-infected children, while the estimated HIV-infected population of children make up <5.0% of the total population (in 2008) (36). With increased access to ART and improved PMTCT, the burden of infectious disease in association with HIV infection is likely to continue to decrease. There is still however, a large population of HIV-exposed children who, despite being HIV-uninfected, is at an increased risk for being hospitalised with IBI (44).

An analysis of contamination indicated that the proportion of contaminated blood cultures (for all hospitalisations in the cohort) was similar between the HIV-infected and HIV-uninfected participants, however, the incidence of contaminants was significantly higher in HIV-infected participants compared to HIV-uninfected participants. This could be because HIV-infected children are more susceptible to infections (which may or may not be clinically relevant); alternatively, it could be a result of bias due to the fact that the proportion of HIV-infected children that were hospitalised was much higher than the proportion of HIV-uninfected children that were hospitalised and hence more blood samples were taken, which would result in more contamination events.

Escherichia coli was the second leading cause of IBI (after *S. pneumoniae*) in all study groups. This indicates that besides being one of the leading causes for diarrhoea-associated morbidity and mortality in infants and children, the role of *E. coli* in IBI should not be underestimated (45). A study from Ghana reported the proportion of bloodstream infections caused by *E. coli* to be around 8.1% (46). This estimation is lower than the current study in which 21.3% of all bloodstream infections were caused by *E. coli*. The high *E. coli* incidence highlights the need for improvements in infrastructure in terms of access to clean water and sanitation in the present community.

4.2 The Effect of PCV9 Vaccination on IBI

PCV9 vaccination significantly reduced all-cause IBI incidence rates in the cohort. This reduction was primarily due to the reduction in incidence of *S. pneumoniae*. When stratified by serotype, it was shown that the reduction in *S. pneumoniae* was a result of the significant decrease in infections due to PCV9- and PCV9-related serotypes. There was no overall change in incidence of non-PCV9 serotypes between vaccinated and placebo recipients.

Incidence rates were still relatively high for *S. pneumoniae* in the vaccinated participants (5.72; 95% CI, 4.31 to 7.45 per 10 000 person-years) as compared to reported rates from high income settings (1.0; 95% CI, 0.50 to 1.80 per 10 000 person-years) (41). Additional pneumococcal serotypes (specifically 3, 6A, 7F and 19A) were included in the PCV vaccine with the introduction of PCV13 to the EPI-SA in 2011 (47). The current analysis indicated that there was already a significant decrease in the PCV13 serotypes (IRR=0.46; 95% CI, 0.21 to 0.95, p=0.024) with PCV9 vaccination. This was largely due to the decrease in serotype 19A in the PCV9 vaccinated children. It is important to note that PCV9 was never licensed or introduced into the EPI-SA, instead, PCV7 was licensed and was introduced into in 2009 (34). PCV7 was trialled extensively in developed countries while trials in developing countries focussed mainly on PCV9. Since PCV9 and PCV7 are closely related, the efficacy studies for PCV9 were used to project the performance of PCV7 in terms of immunogenicity and safety (48). Studies suggested that lower valent formulations (such as PCV7, which contains antigens present in serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) have similar or higher efficacy as compared to higher valent formulations (such as PCV9, which contains serotypes 1 and 5 in addition to the PCV7 serotypes) for serotypes included in both vaccines (48). As such, the efficacy estimated in the PCV9 trials are hypothesised to be conservative estimates of the performance of PCV7 in reducing invasive disease (48). PCV7 does not include serotypes 1 or 5, however in the current analysis there was no significant reduction of these serotypes due to PCV9 vaccination (IRR for serotype 1 was 0.20; 95% CI, 0.01 to 1.79, p=0.125; IRR for serotype 5 was 0.00; 95% CI, 0.00 to 5.32, p=0.250). The incidence of serotypes 1 and 5 reported here, were however very low, supporting the introduction of PCV7.

Other reports have suggested that serotypes 1 and 5 constitute 15-20% of IPD in Africa, and hence vaccination with PCV7 may be less effective than vaccination with PCV9 in those settings (36).

4.2.1 HIV-uninfected participants

In the HIV-uninfected group there was no overall reduction in all-cause IBI associated with PCV9-vaccination, and *S. pneumoniae* incidence was not reduced. The incidence of PCV9- and PCV9-related serotypes was reduced by vaccination, however there was a trend towards an increase in non-PCV9 serotypes which diluted the overall effect of vaccination. It should be noted however, that the IBI incidence was very low in the HIV-uninfected placebo recipients and as such the number of events were too small to determine a significant change due to vaccination. These results are in keeping with the original analysis (which included approximately 2 years of follow up of participants as opposed to 5 years in the current analysis) which showed that PCV9 vaccination was significantly associated with a reduction in vaccine serotype IPD in HIV-uninfected children, however due to the increase in non-vaccine serotype IPD, there was no decrease in overall IPD (33).

4.2.2 HIV-infected participants

In the HIV-infected group, receipt of PCV9 was associated with a significant overall IBI reduction. This reduction was due to a decrease in incidence of *S. pneumoniae* IBI, specifically due to the significant decrease in PCV9- and PCV9-related serotypes (Appendix Table S3). This group showed reductions in incidence of IBI due to serotype 6A (IRR=0.24; 95% CI, 0.06 to 0.74, p=0.005), for which antigens are not included in the PCV9 vaccine. This protection against serotype 6A due to PCV vaccination has been documented and is due to immunological similarities with serotype 6B which allows some cross-reactivity (49). Cross-reactivity between 19A and 19B with the vaccine serotype, 19F, has also been reported. In the current study there was a decrease in incidence of 19A, however this was not significant (p=0.412). There was also no change in incidence of serotype 19B (p=0.522). Serotypes 6A, 19A and 19B were included as PCV9-related serotypes in the analysis. A reduction in non-vaccine serotypes in HIV-infected children has been reported elsewhere due to improved care for children

exposed to or infected with HIV (access to co-trimoxazole preventive therapy, ART, PMTCT) (34). This is however, not relevant to the current analysis as the randomised design of the study would ensure that treatment of HIV-infected children in the PCV9 and in the control groups were consistent. The data analysed were collected in the pre-ART era and hence ART and PMTCT were not considered as confounders in this analysis. A decrease in vaccine serotype and non-vaccine serotype IBI would however, be expected over time with improved HIV treatment (34). The original analysis also reported a significant decrease in vaccine serotype IPD as well as reductions in overall IPD in HIV-infected children as seen here (33).

4.2.3 Replacement disease in the cohort

As previously discussed, the HIV-uninfected participants showed no change in overall incidence of *S. pneumoniae* as the reduction in PCV9- and PCV9-related serotypes was countered by an increase in IBI due to non-PCV9 serotypes. This could be due to replacement disease (36). Replacement disease arises when vaccine-serotypes are eliminated or reduced due to vaccination, thereby creating a biological niche which is then open to be filled by non-vaccine serotypes. Although vaccination is one of the largest drivers of replacement disease, antibiotic use and resistance also influence which serotypes are increased (50). It is unclear why there was a trend towards serotype replacement in the HIV-uninfected participants but not in the HIV-infected participants in the current study. One possible explanation is that vaccination significantly reduced the incidence of PCV9 serotypes in HIV-infected participants however, there were still too many PCV9 serotype IBI events for replacement to occur. Another possible explanation for this observation is that the co-trimoxazole prophylaxis given to HIV-infected patients may have offered a protective advantage from colonisation with replacement serotypes.

There was a significant increase in Klebsiella spp in the HIV-uninfected, vaccinated group which may also have been a result of replacement. Klebsiella spp are reported to be mainly hospital-acquired (95% of cases) and are usually associated with intravenous infusions for more than 3 days (51).

The birth cohort in Soweto in the year 2000 was estimate to be 24 000 (approximately 2 000 babies born per month) (30). Since enrolment took place over 32 months, it is estimated that 64 000 babies were born during this period. This translates into approximately 31% of the birth cohort in Soweto being vaccinated with PCV9 during the study period. It is hence noted that approximately one third of the Soweto birth cohort was vaccinated in the RCT and therefore there would have been minimal impact on the overall bacterial population (compared to what would be expected in populations with high vaccine coverage). The replacement disease reported here is hence an indication of trend only, since the study design was not designed to detect the long-term impact of replacement and is not powered to detect replacement disease (50).

4.3 Risk Factors of IBI

The analysis indicated that non-receipt of PCV9 and HIV infection were significant risk factors for IBI hospitalisation. As previously discussed, the reduction in IBI hospitalisation in PCV9 vaccinated participants was specific to the HIV-infected group. Preterm delivery was a significant predictor of IBI hospitalisation in univariate analyses, however in multivariate analyses it was no longer significant. This could be explained by the fact that HIV infection has been shown to be a risk factor for preterm delivery (52). A study done in urban KwaZulu-Natal in a cohort with high HIV prevalence, demonstrated that the odds of preterm delivery for HIV-infected women were 4.09 (95% CI, 1.37 to 12.17, p=0.01) times the odds for HIV-uninfected women after controlling for multiple factors (52). The incidence rates reported here however, indicate that preterm infants experienced 2.16 times the number of IBI hospitalisations compared to full-term infants (p<0.001).

4.3.1 Risk factors for Coagulase-negative staphylococcus infections

Coagulase-negative staphylococcus was regarded as a contaminant for the purpose of this analysis, however literature indicates that this group of organisms (specifically *S. epidermidis*) may by pathogenic in certain clinical settings (40). The analysis of CoNS IBI demonstrated that male sex, HIV infection and preterm birth were risk factors for CoNS infections. In young infants (under 3 months

of age), in whom CoNS was likely to be a clinically significant infection, HIV infection and preterm birth were significant risk factors. Despite *S. epidermidis* formerly being thought of as a skin commensal microorganism, it is becoming clearer that this microorganism may be an important opportunistic pathogen responsible for a large number of nosocomial infections (40). One of the main sources of infection for this pathogen is from indwelling medical devices (40). Invasive *S. epidermidis* infection is difficult to treat due to high levels of antibiotic resistance, and resistance to host defences through slime production which results in persistent colonisation (40). The incidence rate of CoNS in premature children under 3 months of age in the current cohort was 12.12 (95% CI, 10.62 to 13.78) per 10 000 person years. It is likely that the infections were clinically significant in at least a proportion of these high risk children and would have required targeted antibiotic therapy. It is a limitation that no data on clinical and laboratory indicators to decide whether these were true nosocomial infections were available in this cohort.

4.4 Clinical Characteristics

Escherichia coli infections in the cohort had distinct clinical characteristics (including association with vomiting and diarrhoea, and being less likely to present with fever) as compared to *S. pneumoniae* IBI. *Escherichia coli* is associated with gastrointestinal tract infections and as such, cases are likely to present with diarrhoea and vomiting (53). Salmonella spp infections in the cohort were associated with diarrhoea. This has been well documented in literature, in which Salmonella spp cause enterocolitis which in turn leads to diarrhoea (54). The resulting enterocolitis is generally self-limiting however, it is estimated to lead to bloodstream infections in approximately 6% of cases (54). In this cohort, infections with Klebsiella spp and other IBI were less likely to present with fever as compared to *S. pneumoniae*. Infections with *S. aureus* were associated with refusal to eat and a lower prevalence of fever. Assessment of clinical characteristics associated with IBI was not a specific objective of the study and, as such, a detailed discussion of clinical characteristics has not been included.

4.5 Outcomes of IBI

Although clinical characteristics differ somewhat between pathogens, the type of bacterial pathogen does not have much of an effect on severity of outcome (in terms of length of hospital stay as well as mortality). A study of IBI in Korean children under the age of 5 years (1996-2005) has shown *N. meningitidis* to have the highest case fatality rate (15.4%) although the incidence of this pathogen was very low (2). The current study also had very few cases of *N. meningitidis* (n=8), however all 8 cases were reported to have survived the infection and hence the case fatality rate was much lower than expected from literature. The Korean study reported *S. pneumoniae* (12.4%) and GBS (10.4%) as having the most severe clinical outcomes (in terms of fatality rates) while *H. influenzae* (no fatalities), *S. aureus* (3.0%) and Salmonella spp (1.4%) were reported to be the least severe (2). Since GBS infection most commonly occurs in the neonatal period and this analysis only included surveillance of children beyond the neonatal period, very few GBS infections were noted (5). The Active Bacterial Core surveillance study based in the United States (1998-1999) reported a different case fatality pattern (although this study was population based and looked at patients of all ages). They found *H. influenzae* (13.9%) to have the highest case fatality rate followed by *N. meningitidis* (13.7%) (5). The current analysis did not find a significant difference in severity of outcome between bacterial pathogens.

There are other risk factors that increased severity of disease. These included age (poorer outcomes expected with younger age) and HIV infection. This is in line with findings from other studies which have reported IBI infections to show decreasing case fatality rates with increase age (with infants <3 months of age experiencing the highest case fatality rates in children under 5 years of age) (2). A study in Switzerland showed that sepsis episodes in healthy children beyond the neonatal period were significantly less likely to result in death compared to episodes in neonates as well as children with comorbidities (p-value<0.001) (16). Receipt of PCV9, sex and prematurity did not have an effect on the severity of the outcome.

4.6 Description of Klebsiella spp IBI in the cohort

This analysis found that risk factors for invasive infections due to Klebsiella spp included HIV infection and PCV9 vaccination. The Klebsiella spp events in this cohort were unlikely to constitute an outbreak since the events were spread out over the follow up period and were found to be community-acquired (since infected children had no previous record of being hospitalised). Although other studies have indicated that Klebsiella spp are significant bloodstream pathogens in South African children (51), the link between increased incidence in PCV-vaccinated children has not been documented. This requires further monitoring.

4.7 Limitations

Person-time calculations were done with censoring occurring at each participant's fifth birthday or date of death (for participants that were reported to have died during hospital admission). Since active surveillance was not done on all enrolled participants, it was assumed that participants survived until their fifth birthday unless a death was reported. Censoring would have incorrectly occurred at age 5 for participants that died after being discharged, refused health treatment or died without being admitted to CHBAH. The limitation of this passive surveillance system is that incidence may be underestimated due to the inflation of the denominator. There may also be an underestimation of incidence in the youngest age category as participants were eligible for enrolment up to 12 weeks of age (despite recommendation of first vaccination at 6 weeks), so the surveillance may have missed events for children that were enrolled late.

Not all participants in the Soweto PCV9 efficacy trial had known HIV status as HIV testing was not done routinely at the time. During the study, HIV testing was only done on study participants admitted to CHBAH in whom HIV was clinically suspected, hence participants that were not hospitalised were assumed to be HIV-uninfected (34). Since only 13.3% of the cohort had confirmed HIV status recorded, this assumption may have led to an under-estimation of the prevalence of HIV infection in the PCV9 trial cohort. The under-estimation may have been associated with an inflation of IBI incidence rates amongst HIV-infected participants in this analysis. However, as participants were followed up until 5 years of age, we would expect that any participant that was HIV-infected would have presented to the hospital at some point during this five-year period and been tested. Children that are slow progressors however, might not have had clinical signs of HIV disease in the first 5 years of life and as such would have been incorrectly classified as HIV-uninfected. In addition, using our assumption, the HIV prevalence in the cohort was 3.2%, which was very similar to HIV prevalence estimates obtained using the projections of the Actuarial Society of South Africa's 2008 AIDS and Demographic model for this time period (3.0%) (55). Another limitation was that HIV status of the mothers was unknown and hence HIV-exposure status could not be analysed for the cohort.

In the post-ART and PMTCT era, with significantly fewer HIV-infected children, there are a substantial number of HIV-exposed, -uninfected children who are themselves at risk for infectious disease-related morbidity and mortality (56). This raised susceptibility among HIV-exposed, -uninfected children is only expected to last for 6-12 months after birth. Hence analysis of HIV-exposure in the current cohort would have been beneficial for extrapolation of results and trends to the current setting.

The date on which blood cultures were taken was not recorded, although it was assumed that these cultures were done on the date of admission (for suspected sepsis cases). Since only admission cultures were analysed, infections were assumed to be community-acquired. However, potential nosocomial infections may have been included in the analysis in instances in which a child was readmitted with a different pathogen within 30 days of a previous hospitalisation episode. Potential nosocomial infections were hence not actively excluded. As participants were enrolled at approximately 6 weeks of age, pathogens responsible for infections in the neonatal period were not assessed. Since very few participants had recorded birthweight, gestational age as a measure of prematurity was used in the logistic regression models as a risk factor for IBI. Birthweight would have been a more accurate measurement as it is more objectively measured. The measure of gestational age is subject to different interpretation as well as differing methods used for ascertainment. Furthermore, as most mothers in Soweto book at antenatal clinics at or beyond 20 weeks of gestation, gestational age estimates are unlikely to have been accurate (57, 58).

The indirect effect or herd immunity is an important factor contributing to the success of PCV vaccination programs. Specifically, PCV has been shown to reduce pneumococcal nasopharyngeal carriage and invasive disease in the population in both vaccinated and unvaccinated individuals (50). Since this study was a RCT in which only a proportion of the population was vaccinated (approximately 31% of the birth cohort during the time period), the indirect protection of vaccination through reduction of nasopharyngeal carriage could not be fully appreciated. The

incidence rates reported for vaccinated participants are hence likely to be an overestimation of the incidence rates of a population with high vaccine coverage.

Since this is a historical cohort, it is likely that the IBI incidence has decreased since data collection. The introduction of PCV in 2009, and ART and PMTCT in 2004 are all likely to have contributed substantially to the reduction of IBI in the study setting since the study time period and hence the incidence rates presented here are likely to be overestimates of those expected today. The main purpose of this analysis, however, was to show how incidence rates may have changed for IBI pathogens due to PCV vaccination and that PCV vaccine in the community decreases the overall incidence of IBI.

There have been reports in the literature of PCV vaccination resulting in a reduction in antibiotic-resistant IPD (as important antibiotic-resistant serotypes are contained in the PCV vaccine) (34). This analysis did not interrogate antibiotic resistance in the cohort and it is thus recommended that the antibiotic susceptibility patterns of positive blood cultures (for *S. pneumoniae* and other bacterial pathogens) in this cohort be analysed for further information.

5. Chapter Five: Conclusions and recommendations

This analysis shows that the most common IBI pathogens in a cohort of children under-5 years of age in Soweto from 1998 to 2005 were *S. pneumoniae*, *E. coli* and *S. aureus*. The incidence for all IBI pathogens decreased as the cohort aged, with *S. pneumoniae* remaining the leading pathogen in all age groups. HIV-infected participants had higher odds of IBI hospitalisation for all invasive pathogens, while PCV9 vaccination significantly reduced the odds of IBI hospitalisation which was directly due to the decrease in *S. pneumoniae* incidence in vaccinated children.

PCV9 vaccination in this cohort resulted in a significant reduction in PCV9- and PCV9-related serotypes in both the HIV-infected and HIV-uninfected children (although this reduction in the HIV-uninfected group was not large enough to result in an overall *S. pneumoniae* reduction and a larger sample size would have been required to determine a significant reduction). PCV9 vaccination was associated with an increase in Klebsiella spp IBI. We recommend that this increase should be carefully monitored. Although this analysis did not demonstrate a significant change in IBI attributable to *S. aureus*, it did show a trend towards increase in incidence in the vaccinated participants. The reciprocal association between *S. pneumoniae* and *S. aureus* resulting in an increase in *S. aureus* due to PCV vaccination has been reported elsewhere in the literature (59). We hence recommend that the *S. aureus* incidence rates also be carefully monitored in the era of widespread access to PCV. Continued monitoring of IBI incidence rates in HIV-exposed children is important as the HIV prevalence among pregnant women is still high in the South African setting.

References

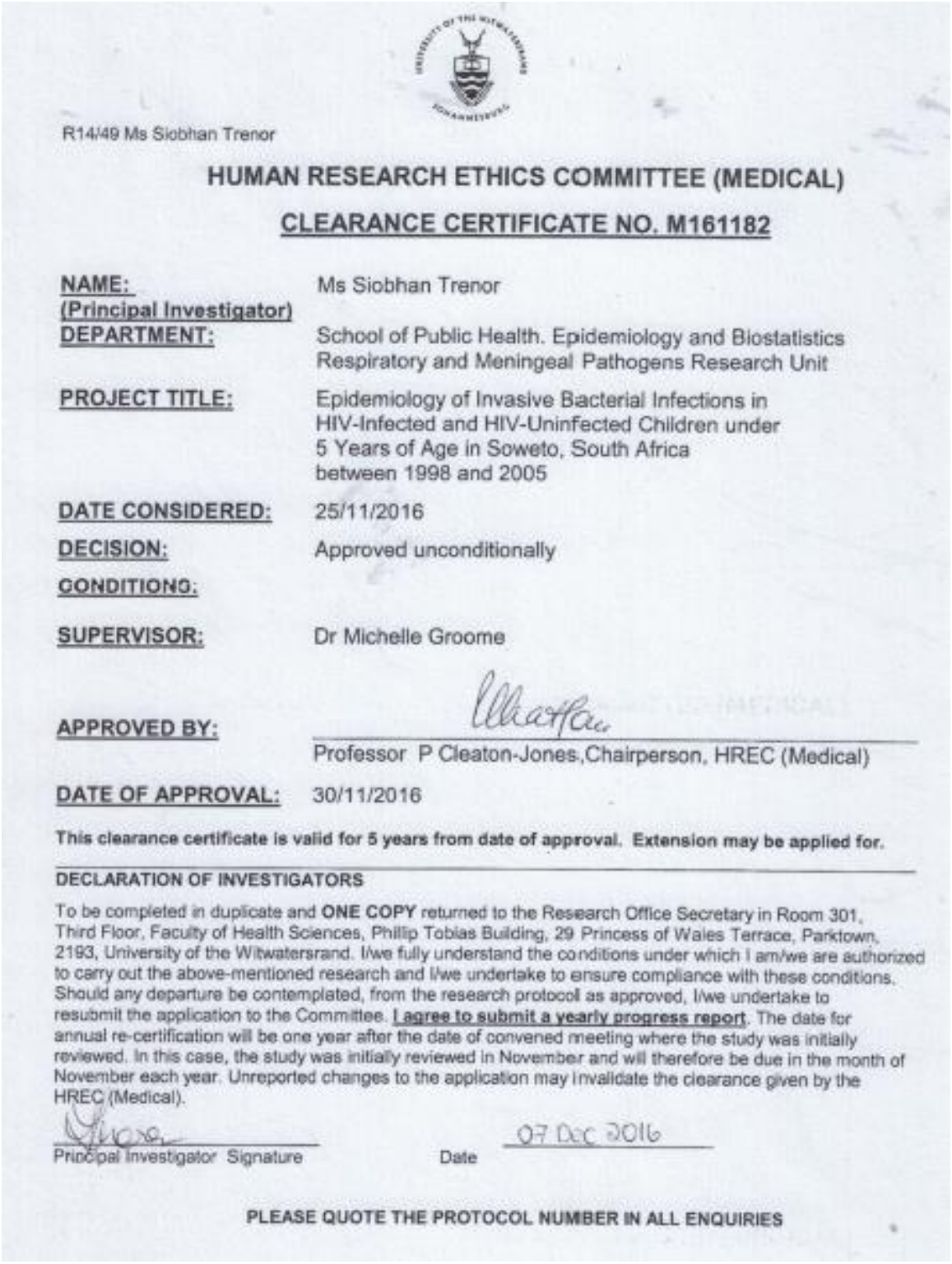
1. Enwere G, Biney E, Cheung Y, Zaman MA, Okoko B, Oluwalana C, et al. Epidemiologic and clinical characteristics of community-acquired invasive bacterial infections in children aged 2-29 months in the Gambia. Pediatr Infect Dis J. 2006;25(8):700-5.
2. Lee J, Cho HK, Kim K, Kim CH, Kim DS, Kim KN, et al. Etiology of invasive bacterial infections in immunocompetent children in Korea (1996-2005): A retrospective multicenter study. J Korean Med Sci. 2011;26(2):174-83.
3. Bogaert D, van Belkum A, Sluijter M, Luijendijk A, de Groot R, Rumke HC, et al. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. Lancet. 2004;363(9424):1871-2.
4. Adriani KS, Brouwer MC, van de Beek D. Risk factors for community-acquired bacterial meningitis in adults. Neth J Med. 2015;73(2):53-60.
5. Schuchat A, Hilger T, Zell E, Farley MM, Reingold R, Harrison L, et al. Active bacterial core surveillance of the emerging infections program network. Emerg Infect Diseases. 2001;7(1):92-9.
6. Miranda JJ, Kinra S, Casas JP, Davey-Smith G, Ebrahim S. Non-communicable disease in low- and middle-income countries: context, determinants and health policy. Trop Med Int Health. 2008;13(10):1225-34.
7. Bulloch B, Craig WR, Klassen TP. The use of antibiotics to prevent serious sequelae in children at risk for occult bacteremia: a meta-analysis. Acad Emerg Med. 1997;4(7):679-83.
8. Bennett NJ. Bacteremia Medscape 2017 [cited 2017 26 November 2017]. Available from: https://emedicine.medscape.com/article/961169-overview.
9. Wall EC, Cartwright K, Scarborough M, Ajdukiewicz KM, Goodson P, Mwambene J, et al. High mortality amongst adolescents and adults with bacterial meningitis in Sub-Saharan Africa: an analysis of 715 cases from Malawi PLoS One. 2013;8(7):e69783.
10. Zenebe T, Kannan S, Yilma D, Beyene G. Invasive bacterial pathogens and their antibiotic susceptibility patterns in Jimma University Specialized Hospital, Jimma, Southwest Ethiopia. Ethiop J Health Sci. 2011;21(1):1-8.
11. Dagan R. Use of pneumococcal conjugate vaccine to decrease rates of bacterial meningitis. Clin Infect Dis. 2008;46(11):1673-6.
12. Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, et al. Sustained reducations in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis. 2010;201(1):32-41.
13. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385:430-40.
14. World Health Organisation. Children: reducing mortality. 2016 [04 October 2017]. Available from: http://www.who.int/mediacentre/factsheets/fs178/en/.
15. Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). Diagn Microbiol Infect Dis. 2004;50(1):59-69.
16. Agyeman PKA, Schlapbach LJ, Giannoni E, Stocker M, Posfay-Barbe KM, Heininger U, et al. Epidemiology of blood culture-proven bacterial sepsis in children in Switzerland: a population-based cohort study. Lancet Child Adolesc Health. 2017;1:124-33.
17. Goto M, Al-Hasan MN. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect. 2013;19(2):501-9.
18. Reddy EA, Shaw AV, Crump JA. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis. 2010;10(6):417-32.
19. Zaidi AKM, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. Lancet. 2005;365:1175-88.

20. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? Lancet. 2005;365:891-900.
21. Phe T, Vlieghe E, Reid T, Harries AD, Lim K, Thai S, et al. Does HIV status affect the aetiology, bacterial resistance patterns and recommended empiric antibiotic treatment in adult patients with bloodstream infection in Cambodia? Trop Med Int Health. 2013;18(4):485-94.
22. Macharashvili N, Kourbatova E, Butsashvili M, Tsertsvadze T, McNutt L, Leonard MK. Etiology of neonatal bloodstream infections in Tbilisi, Republic of Georgia. Int J Infect Dis. 2009;13(4):499-505.
23. National Department of Health. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children adolescents and adults. Pretoria 2015.
24. National Department of Health. National antiretroviral treatment guidelines (HIV/AIDS policy guideline). Jacana; 2004.
25. Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, JA C. Global burden of invasive nontyphoidal Salmonella disease, 2010. Emerg Infect Diseases. 2015;21(6).
26. Dramowski A, Cotton MF, Rabie H, Whitelaw A. Trends in paediatric bloodstream infections at a South African referral hospital. BMC Pediatrics. 2015;15(33).
27. Zar HJ, Barnett W, Stadler A, Gardner-Lubbe S, Myer L, Nicol MP. Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: A nested case-control study of the Drakenstein Child Health Study. Lancet. 2016;4:463-72.
28. Burton R, Giddy J, Stinson K. Prevention of mother-to-child transmission in South Africa: an ever-changing landscape. Obstet Med. 2015;8(1):5-12.
29. Meyers TM, Dramowski A, Schneider H, Gardiner N, Kuhn L, Moore D. Changes in paediatric HIV-related hospital admissions and mortality in Soweto, South Africa 1996-2011: light at the end of the tunnel? J Acquir Immune Defic Syndr Hum Retrovirol. 2012;60(5):503-10.
30. Meyers TM, Pettifor JM, Gray GE, Crewe-Brown H, Galpin JS. Pediatric admissions with Human Immunodeficiency Virus infection at a regional hospital in Soweto, South Africa. J Trop Pediatr Afr Child Health. 2000;46:224-30.
31. Nunes MC, von Gottberg A, de Gouveia L, Cohen C, Moore DP, Klugman KP, et al. The impact of antiretroviral treatment on the burden of invasive pneumococcal disease in South African children: a time series analysis. AIDS. 2011;25(4):453-62.
32. Goldblatt D. Conjugate Vaccines. Clin Exp Immunol. 2000;119(1):1-3.
33. Klugman KP, Madhi SA, Huebner RE, Kohberger R, Mbelle N, Pierce N. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. N Engl J Med. 2003;346:1341-8.
34. von Gottberg A, de Gouveia L, Tempia S, Quan V, Meiring S, von Mollendorf C, et al. Effects of vaccination on invasive pneumococcal disease in South Africa. N Engl J Med. 2014;371:1889-99.
35. Cohen C, von Mollendorf C, de Gouveia L, Lengana S, Meiring S, Quan V, et al. Effectiveness of the 13-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South African children: a case-control study. Lancet Glob Health. 2017;5(3):359-69.
36. Madhi SA. Introduction of the pneumococcal conjugate vaccine into the South African public immunisation programme: dawn of a new era? S Afr J Epidemiolo Infect. 2008;23(4):5-9.
37. Eton V, Schroeter A, Kelly L, Kirlew M, Tsang RSW, Ulanova M. Epidemiology of invasive pneumococcal and *Haemophilus influenzae* diseases in Northwestern Ontario, Canada, 2010-2015. Int J Infect Dis. 2017;65:27-33.
38. Cohen C, von Mollendorf C, de Gouveia L, Naidoo N, Meiring S, Quan V, et al. Effectiveness of 7-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in HIV-infected and -uninfected children in South Africa: a matched case-control study. Clin Infect Dis. 2014;59(6):808-18.
39. Aly H, Herson V, Duncan A, Herr J, Bender J, Patel K, et al. Is bloodstream infection preventable among premature infants? A tale of two cities. Pediatrics. 2005;115(6):1513-8.

40. Otto M. *Staphylococcus epidermidis* - the 'accidental' pathogen. Nat Rev Microbiol. 2009;7(8):555-67.
41. Greenhow TL, Hung Y, Herz A. Bacteremia in children 3 to 36 months old after introduction of conjugated pneumococcal vaccines. Pediatrics. 2017;139(4).
42. McNally L, Jeena PM, Gajee K, Sturm AW, Tomkins AM, Coovadia HM, et al. Lack of association between the nasopharyngeal carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in HIV-1-infected South African children. J Infect Dis. 2006;194(3):385-90.
43. Muenchhoff M, Goulder PJR. Sex differences in pediatric infectious diseases. J Infect Dis. 2014;209(3):S120-S6.
44. Jallow S, Madhi SA. Pneumococcal conjugate vaccine in HIV-infected and HIV-exposed, uninfected children. Expert Rev Vaccines. 2017;16(5):453-65.
45. Bourgeois AL, Wierzba TF, Walker RI. Status of vaccine research and development for enterotoxigenic *Escherichia coli*. Vaccine. 2016;34(26):2880-6.
46. Obeng-Nkrumah N, Labi A, Addison NO, Labi JEM, Awuah-Mensah G. Trends in paediatric and adult bloodstream infections at a Ghanaian referral hospital: a retrospective study. Ann Clin Microbiol Antimicrob. 2016;15(49).
47. Cohen R, Levy C. 13-valent pneumococcal conjugate vaccine in Africa. Lancet. 2017;5(3):244-5.
48. Reingold R, Cutts F, Kamau T, Levine O, O'Brien K, Ignacio Santos Preciado J, et al. Detailed review paper on pneumoccoal conjugate vaccine. WHO Strategic Advisory Group of Experts (SAGE) on Immunization SAGE pneumococcal conjugate vaccine working group 2006.
49. Hausdorff WP, Hoet B, Schuerman L. Do pneumococcal conjugate vaccines provide any cross-protection against serotype 19A? BMC Pediatr. 2010;10(4).
50. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease following pneumococcal vaccination: A discussion of the evidence. Lancet Infect Dis. 2011;378(9807):1962-73.
51. Buys H, Muloiwa R, Bamford C, Eley. B. Klebsiella pneumoniae bloodstream infections at a South African children's hospital 2006-2011, a cross-sectional study. BMC Infect Dis. 2016;16(570).
52. Naidoo M, Sartorius B, Tshimanga-Tshikala G. Maternal HIV infection and preterm delivery outcomes at an urban district hospital in KwaZulu-Natal in 2011. S Afr J Infect Dis. 2014;31(1).
53. Puentes SS, Dunstan M. *Escherichia coli* complications in pediatric critical care. Crit Care Nurs Clin North Am. 2017;30(1):149-56.
54. Kariuki S, Gordon MA, Feasey N, Parry CM. Antimicrobial resistance and management of invasive Salmonella disease. Vaccine. 2015;33(3):21-9.
55. Actuarial Society of South Africa (ASSA). AIDS and demographic model 2008. http://aids.actuarialsociety.org.za2008.
56. Filteau S. The HIV-exposed, uninfected African child. Trop Med Int Health. 2009;14(3):276-87.
57. Ramoba MA. Pregnancy outcome in primigravid women above the age of 30 years in Soweto (MMed thesis). Johannesburg: University of the Witwatersrand; 2015.
58. Papageorghious AT, Kemp B, Stones W, Ohuma EO, Kennedy SH, Purwar M, et al. Ultrasound-based gestational-age estimation in late pregnancy. Ultrasound Obstet Gynecol. 2016;48(6):716-26.
59. Reiss-Mandel A, Regev-Yochay G. *Staphylococcus aureus* and *Streptococcus pneumoniae* interaction and response to pneumococcal vaccination: Myth or reality? Hum Vaccin Immunother. 2016;12(2):351-7.

Appendices

Appendix 1: Human Research Ethics Committee (Medical) of the University of the Witwatersrand Approval Certificate



R14/49 Ms Siobhan Trenor

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M161182

NAME: (Principal Investigator)	Ms Siobhan Trenor
DEPARTMENT:	School of Public Health. Epidemiology and Biostatistics Respiratory and Meningeal Pathogens Research Unit
PROJECT TITLE:	Epidemiology of Invasive Bacterial Infections in HIV-Infected and HIV-Uninfected Children under 5 Years of Age in Soweto, South Africa between 1998 and 2005
DATE CONSIDERED:	25/11/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Michelle Groome
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/11/2016

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 Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in November and will therefore be due in the month of November each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 07 Dec 2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2: Additional tables and figures

Table S1: Contaminants that were excluded from the analysis a) Number of events per group b) Incidence per group (per 10 000 person-years)

a)

Bacteria	**Number of Events per Group**				
	HIV-PCV-	HIV-PCV+	HIV+PCV-	HIV+PCV+	Events (% of total events)
Staphylococcus epidermidis	257/744 (34.5%)	237/744 (31.9%)	121/744 (16.3%)	129/744 (17.3%)	744 (77.3%)
Corynebacterium spp	36/93 (38.7%)	34/93 (36.6%)	15/93 (16.1%)	8/93 (8.6%)	93 (9.7%)
Bacillus spp	25/51 (49.0%)	14/51 (27.5%)	8/51 (15.7%)	4/51 (7.8%)	51 (5.3%)
Propionibacterium spp	6/15 (40.0%)	5/15 (33.3%)	1/15 (6.7%)	3/15 (20.0%)	15 (1.6%)
Viridans streptococci	10/40 (25.0%)	14/40 (35.0%)	7/40 (17.5%)	9/40 (22.5%)	40 (4.2%)
Lactobacillus spp	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	1 (0.1%)
Coagulase-negative staphylococcus (other than *S. epidermidis*)	1/5 (20.0%)	1/5 (20.0%)	3/5 (60.0%)	0/5 (0.0%)	5 (0.5%)
Peptostreptococcus *spp*	0/1 (0.0%)	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	1 (0.1%)
Clostridium bifermentans	1/1 (100.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1 (0.1%)
Micrococcus spp	4/7 (57.1%)	2/7 (28.6%)	1/7 (14.3%)	0/7 (0.0%)	7 (0.7%)
Streptococcus spp	0/2 (0.0%)	0/2 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	2 (0.2%)
Other Gram-positive cocci	0/2 (0.0%)	1/2 (50.0%)	1/2 (50.0%)	0/2 (0.0%)	2 (0.2%)
Neisseria subflava	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100.0%)	1 (0.1%)
Total	340/963 (35.3%)	309/963 (32.1%)	159/963 (16.5%)	155/963 (16.1%)	963 (100%)

b)

Bacteria	Incidence per group (per 10 000 person years) – 95% CI				
	HIV-PCV-	HIV-PCV+	HIV+PCV-	HIV+PCV+	Total
Staphylococcus epidermidis	27.44 (24.19-31.01)	25.23 (22.12-28.66)	500.88 (415.62-598.49)	582.88 (486.64-692.58)	387.08 (359.76-415.93)
***Corynebacterium* spp**	3.84 (2.69-5.32)	3.62 (2.51-5.06)	62.09 (34.5-102.41)	36.15 (15.61-71.23)	48.39 (39.05-59.28)
Bacillus spp	2.67 (1.73-3.94)	1.49 (0.81-2.50)	33.12 (14.30-65.25)	18.07 (4.92-46.28)	26.53 (19.76-34.89)
Propionibacterium spp	0.64 (0.24-1.39)	0.53 (0.17-1.24)	4.14 (0.10-23.06)	13.56 (2.80-36.61)	7.80 (4.37-12.87)
Viridans streptococci	1.07 (0.51-1.96)	1.49 (0.81-2.50)	28.98 (11.65-59.70)	40.67 (18.60-77.20)	20.81 (14.87-28.34)
***Lactobacillus* spp**	0.00 (0-0.39)	0.00 (0.00-0.39)	4.14 (0.10-23.06)	0.00 (0.00-16.67)	0.52 (0.1-2.90)
Coagulase-negative staphylococcus (other than *S. epidermidis*)	0.11 (0.00-0.59)	0.11 (0.00-0.59)	12.42 (2.56-36.29)	0.00 (0.00-16.67)	2.60 (0.84-6.07)
Peptostreptococcus *spp*	0.00 (0-.00-0.39)	0.11 (0.00-0.59)	0.00 (0.00-15.27)	0.00 (0.00-16.67)	0.52 (0.13-2.90)
Clostridium bifermentans	0.11 (0.00-0.59)	0.00 (0.00-0.39)	0.00 (0.00-15.27)	0.00 (0.00-16.67)	0.52 (0.01-2.90)
Micrococcus spp	0.43 (0.12-1.09)	0.21 (0.03-0.77)	4.14 (0.10-23.06)	0.00 (0.00-16.67)	3.64 (1.46-7.50)
Streptococcus spp	0.00 (0.00-0.39)	0.00 (0.00-0.39)	4.14 (0.10-23.06)	14.52 (0.11-25.18)	1.04 (0.13-3.76)
Other Gram-positive cocci	0.00 (0.00-0.39)	0.11 (0.00-0.59)	4.14 (0.10-23.06)	0.00 (0.00-16.67)	1.04 (0.13-3.76)
Neisseria subflava	0.00 (0.00-0.39)	0.00 (0.00-0.59)	0.00 (0.00-15.27)	4.52 (0.11-25.18)	0.52 (0.13-2.90)
Total	36.30 (32.55-40.38)	32.90 (29.33-36.78)	658.18 (559.85-768.81)	700.36 (594.45-819.71)	501.02 (469.87-533.69)

Abbreviations: HIV-PCV- = HIV-uninfected placebo recipients; HIV-PCV+ = HIV-uninfected PCV9 recipients; HIV+PCV- = HIV-infected placebo recipients; HIV+PCV+ = HIV-infected PCV9 recipients; PCV9 = 9-valent pneumococcal conjugate vaccine.

Table S2: Number of IBI hospitalisations during the follow up period stratified by age group, sex, PCV9 vaccination status, HIV status and prematurity

Characteristic	*S. pneumoniae*	PCV9 serotypes*	Non-PCV9 serotypes*	Hib	*E. coli*	*S. aureus*	Klebsiella spp	Salmonella spp	Other**	All
Overall	154	109	26	23	84	29	18	26	61	395
Age Group										
<6 months* **	43	23	10	6	33	15	9	8	28	142
6-11 months	30	19	6	8	25	4	3	5	11	86
12-17 months	22	18	2	3	9	3	2	6	7	52
18-23 months	13	11	11	1	5	2	1	1	5	28
24-35 months	17	13	3	2	10	1	3	6	6	45
36-59 months	29	25	4	3	2	4	0	0	4	42
Sex										
Male	83	62	11	11	50	18	10	11	37	220
Female	71	47	15	12	34	11	8	15	24	175
Vaccination status										
PCV9	55	35	15	11	33	19	14	12	26	170
Placebo	99	74	11	12	51	10	4	14	35	225
HIV Status										
Infected	116	83	20	14	54	10	10	17	29	250
Uninfected	38	26	6	9	30	19	8	9	32	145
Prematurity										
Term	138	96	24	18	73	27	13	24	52	345
Preterm	15	12	2	5	11	2	5	2	9	49
Season										
Summer	72	47	13	8	40	13	6	11	24	174
Winter	82	62	13	15	44	16	12	15	37	221
Study group										
HIV+PCV+	39	27	10	6	22	7	7	6	13	100
HIV+PCV-	77	56	10	8	32	3	3	11	16	150
HIV-PCV+	16	8	5	5	11	12	7	6	13	70
HIV-PCV-	22	18	1	4	19	7	1	3	19	75

* PCV9 serotypes included: 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F; vaccine-related serotypes included: 6A, 19A and 19B (33). Vaccine-serotypes and non-vaccine serotypes do not add up to the total pneumococcal events as latex positive, culture negative *S. pneumoniae* cultures were not serotyped

** Other bacterial infections include: Streptococcus spp (n=11), *P. aeruginosa* (n=9), *N. meningitidis* (n=8), *A. lwoffii* (n=6), Campylobacter spp (n=6), *E. faecalis* (n=6), *C. albicans* (n=3), Alcaligenes spp (n=2), *M. morganii* (n=2), *M. tuberculosis* (n=2), *C. freundii* (n=1), *E. vulneris* (n=1), non-typable *H. influenzae* (n=1), *H. parainfluenzae* (n=1), *M. catarrhalis* (n=1), and *S. flexneri* (n=1)

****Enrolment took place at 28-84 days old hence the <6 month group excludes children under 28 days*

Abbreviations: HIV-PCV- = HIV-uninfected placebo recipients; HIV-PCV+ = HIV-uninfected PCV9 recipients; HIV+PCV- = HIV-infected placebo recipients; HIV+PCV+ = HIV-infected PCV9 recipients; PCV9 = 9-valent pneumococcal conjugate vaccine.

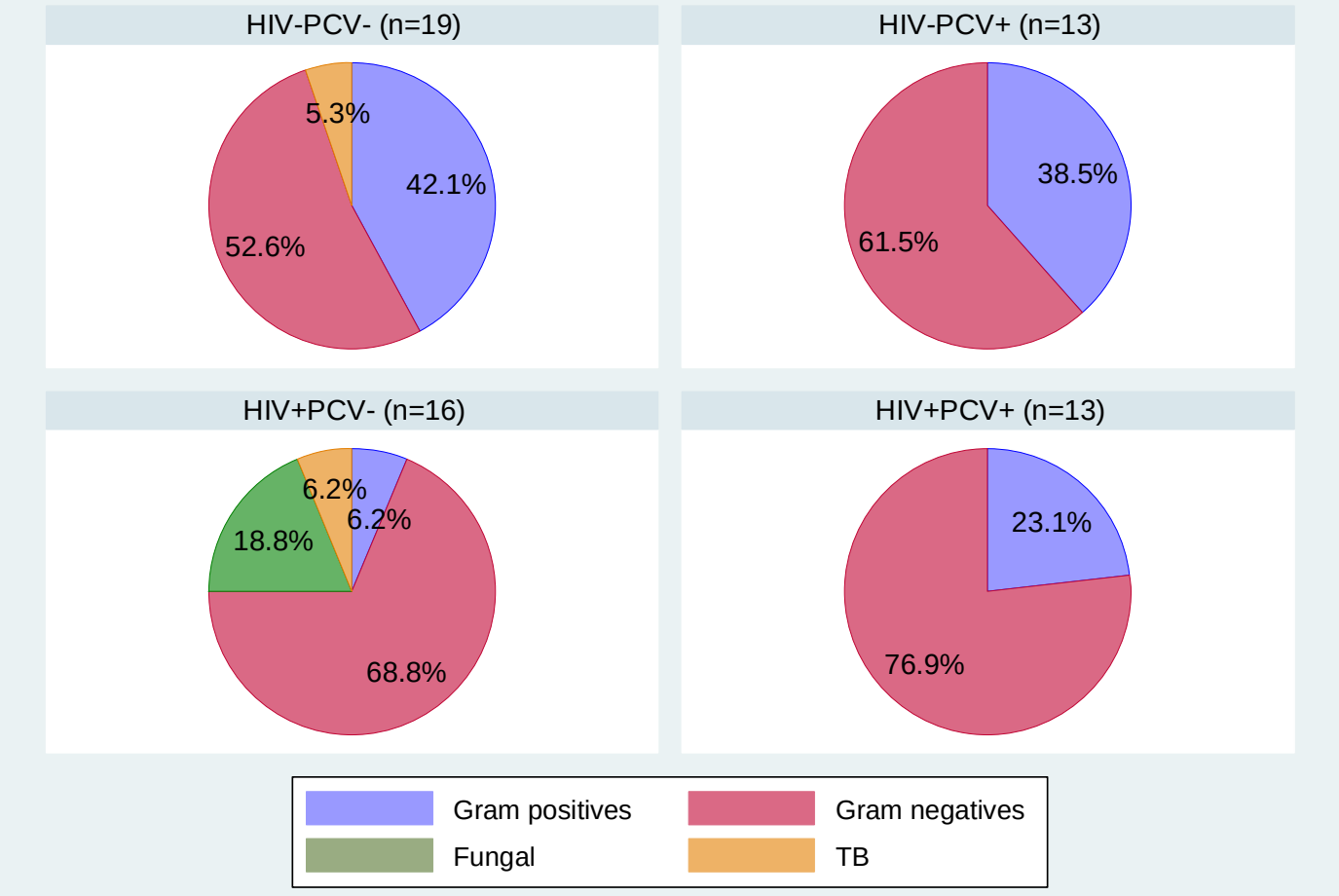


Figure S1: Pie charts showing the constitution of the group labelled as 'Other' for the purposes of the analysis.

'Other' was split into Gram positives (Streptococcus spp, *E. faecalis*), Gram negatives (*P. aeruginosa*, Campylobacter spp, *M. catarrhalis*, Alcaligenes spp, *M. morganii*, *A. lwoffii*, *S. flexneri*, *N. meningitidis*, *H. parainfluenzae*, *C. freundii*, *E. vulneris*, *H. influenzae*), fungal (*C. albicans*) and *M. tuberculosis*.

Abbreviations: HIV-PCV- = HIV-uninfected placebo recipients; HIV-PCV+ = HIV-uninfected PCV9 recipients; HIV+PCV- = HIV-infected placebo recipients; HIV+PCV+ = HIV-infected PCV9 recipients; TB = *M. tuberculosis*.

Table S3: Number of invasive *S. pneumoniae* events stratified by serotype for HIV-infected and –uninfected as well as PCV9 and control groups

Serotype	HIV-Infected			HIV-Uninfected			Total
	PCV	Placebo	Total	PCV	Placebo	Total	
1*	0	1	1	1	4	5	6
3	1	0	1	0	1	1	2
4*	0	1	1	1	2	3	4
5*	0	1	1	0	1	1	2
6A**	4	18	22	1	0	1	23
6B*	3	10	13	1	2	3	16
8	0	0	0	2	0	2	2
9V*	3	2	5	0	3	3	8
10A	1	0	1	0	0	0	1
12F	0	1	1	0	0	0	1
13	0	1	1	0	0	0	1
14*	3	8	11	0	2	2	13
15A	1	4	5	0	0	0	5
15B	1	0	1	1	0	1	2
15C	1	1	2	0	0	0	2
15F	1	0	1	0	0	0	1
16F	1	0	1	0	0	0	1
17F	1	0	1	0	0	0	1
18C*	1	0	1	1	1	2	3
19A**	3	6	9	3	1	4	13
19B**	0	1	1	0	1	1	2
19F*	4	3	7	0	1	1	8
23A	0	1	1	0	0	0	1
23F*	6	5	11	0	1	1	12
29	1	0	1	0	0	0	1
34	0	0	0	1	0	1	1
38	1	0	1	0	0	0	1
99	0	1	1	0	0	0	1
PCV9- and PCV-related serotypes	27	56	83	8	18	26	109
Non-vaccine serotypes	10	10	20	5	1	6	26
TOTAL	37	66	103	13	19	32	135

* PCV9 serotypes included: 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F as well as **PCV9-related serotypes 6A, 19A and 19B.

Abbreviations: PCV9 = 9-valent pneumococcal conjugate vaccine.

Table S4: Risk factors for coagulase-negative staphylococcus IBI

	Univariate		**Multivariate***	
	OR	P-value	OR	P-value
Vaccination				
Control	Ref	-	Ref	-
PCV	0.95 (0.82-1.10))	0.481	1.00 (0.86-1.14)	0.890
Sex				
Female	Ref	-	Ref	-
Male	1.39 (1.20-1.61)	<0.001	1.34 (1.15-1.55)	<0.001
HIV				
Uninfected	Ref	-	Ref	-
Infected	7.74 (6.63-9.03)	<0.001	7.07 (6.04-8.28)	<0.001
Prematurity				
Term	Ref	-	Ref	-
Preterm	2.78 (2.30-3.36)	<0.001	2.06 (1.69-2.50)	<0.001

* Adjusted for PCV9 vaccination, sex, HIV infection status and prematurity.

Abbreviations: OR = odds ratio; PCV9 = 9-valent pneumococcal conjugate vaccine; Ref = referent.

Table S5: Risk factors for coagulase-negative staphylococcus IBI (for children under 3 months of age)

	Univariate		**Multivariate***	
	OR	p-value	OR	p-value
Vaccination				
Control	Ref	-	Ref	-
PCV9	1.00 (0.72-1.41)	0.980	1.05 (0.75-1.47)	0.791
Sex				
Female	Ref	-	Ref	-
Male	1.38 (1.00-1.95)	0.065	1.32 (0.94-1.87)	0.112
HIV				
Uninfected	Ref	-	Ref	-
Infected	7.57 (5.30-10.82)	<0.001	6.80 (4.72-9.79)	<0.001
Prematurity				
Term	Ref	-	Ref	-
Preterm	2.99 (1.94-4.59)	<0.001	2.17 (1.40-3.36)	<0.001

*Adjusted for PCV9 vaccination, sex, HIV infection status and prematurity.

Abbreviations: OR = odds ratio; PCV9 = 9-valent pneumococcal conjugate vaccine; Ref = referent

Table S6: Incidence (per 10 000 person-years) of non-contaminated CSF cultures detected during hospitalisation during the follow up period

Bacteria	Number of events	Percentage (%)	Incidence (10 000 PYs) – 95% CI
S. pneumoniae	48	51.6	24.97 (18.41-33.11)
Hib	7	7.5	3.64 (1.46-7.50)
E. coli	7	7.5	3.64 (1.46-7.50)
S. aureus	1	1.1	0.52 (0.01-2.90)
Klebsiella spp	2	2.2	1.04 (0.13-3.76)
Salmonella spp	1	1.1	0.52 (0.01-2.90)
Other *	27	29.0	14.05 (9.26-20.44)
Total	93	100	48.39 (39.05-59.28)

* Other = *Neisseria meningitidis* (n=8); Streptococcus spp (excluding *S. pneumoniae*) (n=4); *M. tuberculosis* (n=2); *P. aeruginosa* (n=2); *A. lwoffii* (n=1); Campylobacter spp (n=1); *S. flexneri* (n=1); not specified (n=8)

Abbreviations: Hib = *H. influenzae* type b; PY = person years.

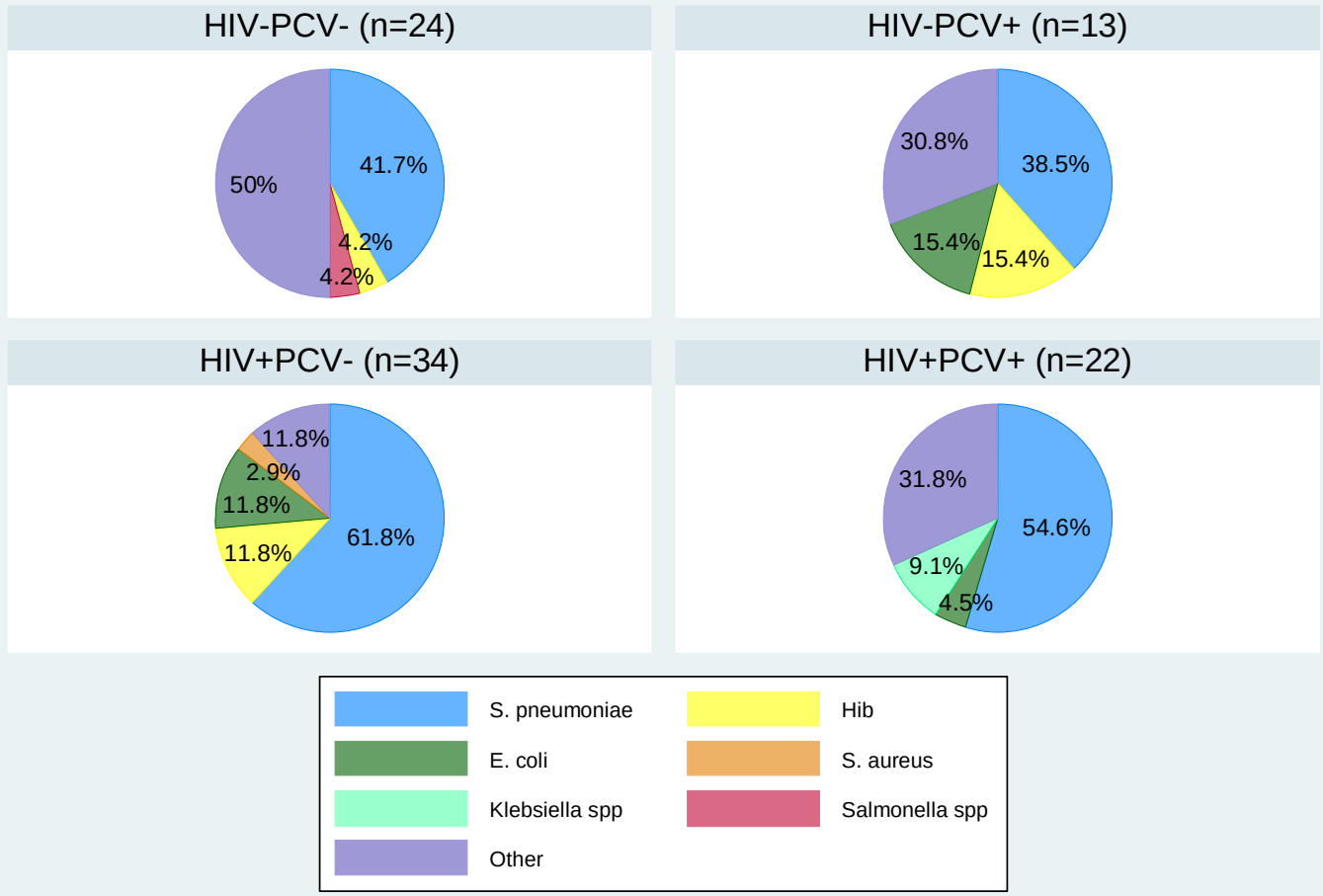


Figure S2: The proportions of pathogens detected in CSF cultures during hospitalisation by HIV status and PCV9 vaccination status

Other bacterial infections include: *N. meningitidis* (8.6%); Streptococcus spp (excluding *S. pneumoniae*) (4.3%); *M. tuberculosis* (2.2%); *P. aeruginosa* (2.2%); *A. lwoffii* (1.1%); Campylobacter spp (1.1%); *S. flexneri* (1.1%); not specified (8.6%)

Abbreviations: Hib = *H. influenzae* type b; HIV-PCV- = HIV-uninfected placebo recipients; HIV-PCV+ = HIV-uninfected PCV9 recipients; HIV+PCV- = HIV-infected placebo recipients; HIV+PCV+ = HIV-infected PCV9 recipients.