Effect of Pneumococcal Conjugate Vaccine on Invasive Pneumococcal Disease in Children Hospitalized at Chris Hani Baragwanath Hospital over a 13 Year Period

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A research report submitted to the School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Medicine in the field of Microbiology

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Declaration

I, Ifeanyi Eme, declare that this research report is entirely my own work. It is intended for submission to the University of the Witwatersrand, Johannesburg, South Africa, for the award of Master of Science in medicine degree. It has never been submitted either in part or fully to this or any other university for examination or award of any academic qualification.

Signature: 

Date: 29/05/2024

Place: Johannesburg, South Africa.

Dedication

To God Almighty and to my family.

Abstract

Background:

Globally, under-5 mortality rates remain high in Africa, particularly the sub–Saharan region, where roughly 700,000 child mortalities are caused by *S. pneumoniae* infections. The purpose of this research project was to evaluate effectiveness of the pneumococcal conjugate vaccine (PCV) on the prevalence of invasive pneumococcal disease (IPD) in hospitalized children at the Chris Hani Baragwanath Academic Hospital (CHBAH) over a 13-year period, before and after the advent of the PCV, and its subsequent incorporation into the South African Expanded Program on Immunization (EPI).

Methods:

We carried out a retrospective review of demographic, laboratory, and clinical information of *S. pneumoniae* cultured on blood and/or cerebrospinal fluid in children below the age of 14 that were hospitalized at CHBAH between January 2006 and December 2018. The investigation was stratified into pre-PCV (2006–2008), transitional-PCV (2009–2011) and post-PCV era (2012–2018).

Results:

Of 867 children hospitalised for IPD over the study period, 409 (47.2%) cases occurred in pre-PCV era, 231 (26.6%) during transitional-PCV, and 227 (26.2%) in the post-PCV era. The IPD incidence (per 100,000 persons) of IPD reduced from 41.1 in 2006 to 4.6 in 2018 ($p<0.001$). In infants <2 years, there was 79.3% reduction in IPD incidence when comparing the pre-PCV (130.9 per 100,000 persons) and post-PCV period (27.1 per 100,000 persons). We also noted a decrease in the proportion of IPD cases in both the HIV-infected and uninfected persons, comparing pre-PCV to post-PCV period.

The most prevalent vaccine serotypes were 14 (16.9%), 1 (14.0%), 6A (11.4%), 19F (11.0%), 23F (10.8%) and 6B (10.0%), whereas the predominant non-vaccine serotypes included; 15B/C (9.8%), 12F (9.3%), 16F (7.5%), NEG38 (7.5%) 35B (5.1%), and 7C (4.2%).

Using the minimum inhibitory concentration (MIC), PCV introduction resulted in increased susceptibility to penicillin and cefotaxime. For the zone sizes using the Kirby Bauer disc diffusion method, vancomycin resistance was not seen in any of the isolates. All the other

antibiotics showed increase in susceptibility over the period, apart from Erythromycin which initially tended to be relatively stable, but however showed increase in susceptibility across the eras as time goes on.

C**onclusion:**

The rate of IPD among children hospitalized at CHBAH has decreased markedly following the incorporation of the PCV into South African EPI programme. The study indicates a need for continued surveillance to monitor the change in resistance patterns and emergence in non-vaccine serotypes causing disease, as this is crucial for mapping out strategic intervention policies in South Africa.

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Table of contents

Abbreviations

BHI	Brain heart infusion broth
CDW	Corporate Data Warehouse
CHBAH	Chris Hani Baragwanath Academic Hospital
CLSI	Clinical and Laboratory Standards Institute
CPS	Capsular polysaccharide
CSF	Cerebrospinal fluid
EPI	Extended Program on Immunization
GERMS-SA	Group for Enteric, Respiratory and Meningeal disease, Surveillance in South Africa
HAART	Highly active antiretroviral therapy
HEU	HIV-exposed but uninfected
HIV	Human Immunodeficiency Virus
HPCV	Heptavalent pneumococcal polysaccharide conjugate vaccine
HREC	Human Research Ethics Committee
HUU	HIV-unexposed and uninfected
ICU	Intensive care unit
IPD	Invasive Pneumococcal Disease
MIC	Minimum Inhibitory Concentration
NHLS	National Health Laboratory Services
NICD	National Institute for Communicable Diseases
PBP	Penicillin-binding proteins
PCV	Pneumococcal Conjugate Vaccine
PCV7	7-valent Pneumococcal Conjugate Vaccine
PCV13	13-valent Pneumococcal Conjugate Vaccine
PNSP	Penicillin non-susceptible pneumococci
PRP	Penicillin-resistance pneumococci
PPCV	Pneumococcal polysaccharide-protein conjugate vaccine
RMPRU	Respiratory and Meningeal Pathogens Research Unit
SANAS	South African National Accreditation System
VGS	Viridans group streptococci
WHO	World Health Organization

List of Figures

List of Tables

Chapter 1

1.1 Background and Literature Review

1.1.1 Burden of Invasive Pneumococcal Disease

Pneumococcal bacteremia, pneumonia and meningitis have high rates of childhood morbidity and mortality worldwide, particularly in children below the age of 5, with most mortality in Africa [1-4]. An estimated 5.8 million children less than 5 years demise every year [5], and about 700,000 -1,000,000 million deaths attributable to *S. pneumoniae* infections worldwide [6]. Most deaths occur in low-income countries, and the most vulnerable groups being children below two years and immunodeficient individuals [7]. The occurrence of invasive pneumococcal disease (IPD) ranges from >5 per 100,000 populations in higher income states to 416 per 100,000 populations in low-income states [8]. Case fatality rates (CFR) being from 12.2% in high-income states to 80% in low-income states [8].

A global review on burden of ailment by *S. pneumoniae* before widespread administration of the pneumococcal conjugate vaccine (PCV), approximately 14.5 million episodes of serious pneumococcal disease befell children below the age of 5. The infections resulted in an estimated 826 000 demises in children younger than 5 years of age, and 91,000 occurred in HIV-infected [9].

Before PCV implementation in South Africa the, roughly 107,600 (95% confidence interval [CI] 83,000–140,000) episode of severe hospitalised pneumococcal ailment were calculated to have affected children aged 0–59 months, yearly. IPD incidence was shown to be more in HIV-positive children (29,159 per 100,000 person-years) than among HIV-negative children (891 per 100,000 person-years) [10]. In addition, high prevalence of HIV-infection, increases malnutrition rates, overcrowded living conditions, and increased antibiotics resistance rates, further contributes to the burden of disease; and hence the need to prevent pneumococcal disease [11, 12]. The implementation of vaccination has been shown to be effective in preventing pneumococcal infections in both children and adults [13].

Prior to the year 2000, there was the 23-valent purified capsular polysaccharide vaccine (PPV-23), that exhibited poor ability in eliciting an immune response in children below the ages of 2 and immunocompromised individuals [14]. The PP23 primarily induces T-cell-independent B-cell immune response which does not elicit memory responses in young children [15].

However, infant vaccination with PCV has proven successful in preventing pneumococcal ailments. PCV directly reduced the impact, mortality and any abnormality resulting from pneumococcal disease in children [5]. It has indirectly brought about a decline in deaths amongst individuals aged between 18-64 years [16, 17], and elderly greater than 65 years of age [18]. This is presumably due to the herd immunity created as a result of vaccination of the under two years old, and this thereby reduced the likelihood of infection for adults who lack immunity against the pneumococcal disease.

1.1.2 Microbiology of *S. pneumoniae*

As a microorganism, *S. pneumoniae*, belong to the genus; Streptococcus, the family; Streptococcaceae, the order; Lactobacillales, the phylum; Firmicutes. It is a Gram-positive organism. It is a facultative anaerobe and displays alpha-hemolysis on blood agar plate[19]. *S.pneumoniae* colonizes the nasopharyngeal tract - this is a reservoir for transmission and commonly precedes pneumococcal disease [20].

1.1.3 Morphology

S. pneumoniae, is usually found in pairs (diplococci), is non-spore forming and non-motile [19, 21]. *S. pneumoniae* colonies sometimes appears glassy or shiny colonies, and at time mucoid. Also there are the characteristic draughtsman colonies(colonies with compressed centres) exhibited by *S pneumoniae* on blood agar plates [22]. The draughtsman or carrom coin appearance is as a result of autolysis of bacteria within the flattened pneumococcal colonies, most visible when incubation is prolonged [23].

The mucoid colonies on solid medium is due to repeated disaccharide residues(Glc-GlcA), in capsular polysaccharide of some *S.pneumoniae* isolates(type 37 and type 3).This serves as protection for the organism from complement-mediated opsonophagocytosis during infection [24]. And it is also implicated with causing pneumococcal invasive diseases [25].

Pneumococcal colony variants exist, and are described according to their diameter, as small colonies, medium colonies or large colonies, and mucoid, non-mucoid or shiny considering their growth on blood agar [26]. Some morphologic appearances of *S. pneumoniae* variants are demonstrated in Figure 1.1 to 1.3.

Figure 1.1: Colonial appearance of *S. pneumoniae*, showing colonies surrounded by zone of alpha-hemolysis.

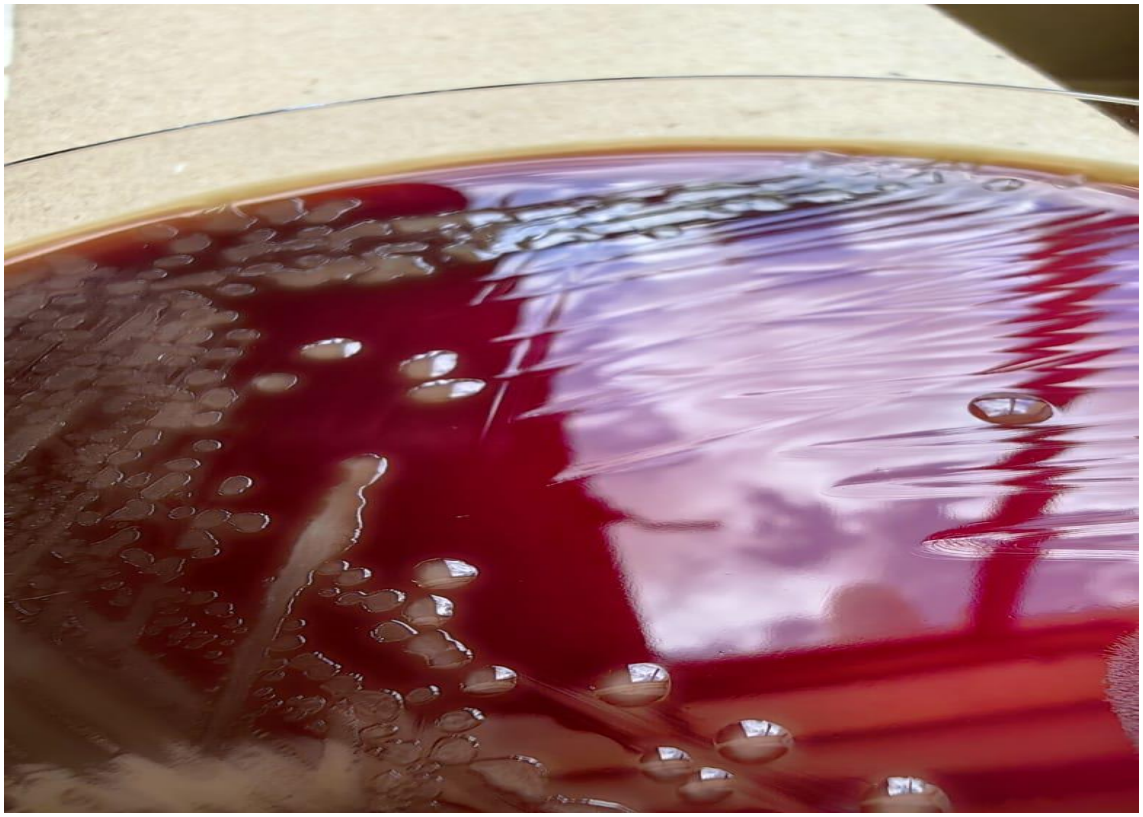

Figure 1.2: Colonial appearance of *S. pneumoniae,* showing mucoid colonies.

Figure 1.3: Colonial appearance of *S. pneumoniae* showing colonies with compressed centres.

1.1.4 Cell structure

S. pneumoniae cell wall structure is very important in maintaining the shape of the organism. It plays a major role in determining the growth and cell division, as well as how it interacts with its host. [27]. The cell structure consists of teichoic acid, covalently attached to peptidoglycan component, covered and shielded by a polysaccharide capsule.The peptidoglycan itself made up of chain-like glycans that binds with peptides assuming a net-like appearance [27-29]. In most serotypes, the thick capsulated polysaccharide that is attached with the peptidoglycan confers protection from the innate immune system of the host [29]**.**

1.1.5 Pathogenesis and virulence factors

The genomic sequence of *S. pneumoniae* appears as a circular, closed, DNA structure that is made up of base pairs (two nitrogen-containing bases). Depending on the strain, the DNA could contain from 2.0 up to 2.1 million base pairs [30]. *S. pneumoniae* virulence is enhanced by a set of about 154 genes, and there are 176 genes that regulate the non-invasive strain [30]. Pneumococcus is a normal flora of the upper respiratory tract; however, it can cause disease when the host immunity is compromised. Apart from the cell wall, other important virulence factors include: Invasins, (pneumolysin, various adhesins,). [16]. Furthermore, the ability of the pneumococcus to make capsular polysaccharide (CPS) is paramount for disease causing. The CPS biosynthesis proteins CpsB, CpsC and CpsD, regulate CPS production via tyrosine phosphorylation of CpsD. This CPS production and regulation enables the pneumococcus to become invasive, thus cause IPD [31] .The CPS in addition to being essential for pneumococcal virulence, distinctively identifies each of the more than 90 pneumococcal serotypes. The capsule works to protect essential component, like adhesins that are necessary in initiating colonization. It is worthy to note that considerably low amount of CPS appears to be enough to initiate colonization. [31, 32]. It is still unclear how the S pneumonia migrate into the bloodstream, from nasopharynx via the lungs. The assumption is that pneumococci are inhaled into the lungs and subsequently attached themselves to the bronchio-epithelial cells during colonization [33]. Afterwards, the cytokine-induced cell activation begins, then comes inflammation and the subsequent invasion of the bronchio-epithelial cells by the pneumococci. In mice, it has been shown that pneumococci reside inside these cells and migrate around the lung tissues [34]. The *S. pneumoniae* in due course penetrate into the circulatory system and may be cultured or isolated 12 hours after infection [34, 35].

S. pneumoniae serotypes

In excess of ninety different pneumococcal serotypes were recognized. They are distinguished based on variations in the polysaccharide antigen on the bacterial cell wall, an important virulence component. The World Health Organization (WHO), reported that, over 70 percent of all IPD is caused by approximately 20 serotypes [2, 36]. In a systematic review prior to widespread PCV, serotypes 1, 5, 6A, 6B, 14, 19F, 23F,18C,19A were responsible for ≥70 percent of IPD globally among children less than the age of 5 [37]. Another systematic review from India reported serotypes 14, 1, 19F, 6B, 5, 6A, 9V and 23F as the highest IPD causing serotypes in children <5years [38].

The use of PCV in the fight against IPD has altered the IPD serotypes in children below the age of 5. The alterations in the distribution of these serotypes are also attributable to capsular modification, mass vaccination with PCV, socioeconomic status, and frequent changes in the use of antimicrobial in the community. [39-41].Sourcing and acquiring information on local serotype distribution, as well as the changes as time goes on is important for productive IPD prevention implementations [39]. Von Gottberg et al. noted a substantial decrease in PCV-7 serotypes causing IPD in 2011 compared to the pre-PCV period (2005-2008) among children below the age of 2 [42].

1.1.6 Immunology

S. pneumoniae has been categorized serologically, on the account of the chemically and immunologically distinct polysaccharide capsules that protect the organism from being phagocytosed [43, 44]. It has been revealed that nasopharynx colonization by the pneumococcus prompts antibody reaction to protein and capsular antigens in mice and humans, and further activates Th17 CD4+ cellular immune responses [45]. These are protective responses, indicating that colonization is an initial event preceding disease [45]. Several episodes in such colonization with dissimilar IPD strains take place in infants up to the age of 2 [46-48], with a rough estimate of between 27 to 65 percent prevalence in nasopharyngeal carriage in infants. However, colonization decrease with age, as the prevalence falls to less than 10 percent in adults [49-51].The naturally acquired adaptive immunity to *S. pneumoniae* differs with age, as incidence of IPD follows a U-shaped distribution with incidence rates peaking at

infants and the elderly [52]. The protection offered against pneumococcal infection hinge on opsonisation of the organism by antibodies in a process known as phagocytosis [53].

Individuals living with acquired or genetic disorder in antibodies production or the people living with impaired acquired immune system as a result of stem cell transplantation or HIV are more susceptible to pneumococcal infections [54-56]. Furthermore, those individual having defects in the complement system, which is part of the defence against invading cells have a high rate of IPD [45, 57].

1.1.7 Clinical manifestations of pneumococcal infections

IPD episode is the isolation of *S. pneumoniae* from a sterile site, like the cerebrospinal fluid (CSF), blood, joint fluid and the pleural fluid [20].Pneumococcus is the major cause of community acquired pneumonia and meningitis in infants and adults [58, 59]. The overall burden of severe pneumococcal invasive disease were reported to be higher in HIV-positive in comparison to HIV-negative children [60, 61].

1.1.8 Some common risk factors for IPD

Pneumococcal infections mostly affect young children and the elderly. However, a number of factors have also been found to be major contributors in IPD incidences, such as socio-economic status and some underlying medical conditions. [62]. Incidence of pneumococcal disease in vulnerable infants in Europe and North America showed highest risk in children with haematological cancers or immunosuppression [14]. Immune deficient patients remain at risk of the IPD despite receiving the PCV [63]. This includes children with antibody deficiency, asplenia, defects in T-cell signaling and complement deficiency [63].

The complement system comprises several fluid-phase and membrane-associated proteins. During physiological conditions, the activation of the fluid-phase is tightly maintained, and complement activation occurs particularly on surfaces recognized as “non-self”. This activation on “non-self” is to avoid or minimize damage to the surrounding host cells. The membrane complement component helps to moderate complement activation on the host cells, and speed up uptake of foreign antigens or microbes that are “tagged” with complement fragments [64]. The fluid phase can be activated via the classical, lectin, or alternative pathway. When there are deficiencies of components of the classical pathway, it leads to the development

of autoimmune disorders and also predispose individuals to recurrent respiratory infections, especially those caused by encapsulated organisms, like *S. pneumoniae* [64].

Sickle cell disease (SCD), is another risk factor which increases vulnerability to pneumococcal disease. SCD is a genetic disorder in which hemoglobin is structurally abnormal, resulting in the episodic formation of sickle-shaped red blood cells (RBCs), causing a wide range of clinical manifestations [65]. Sickling can be triggered by environmental factors such as cold, hypoxia, low pH, dehydration of RBC, as well as adhesion molecules and cytokines associated with infections [65].Individuals living with SCD who have impaired splenic function are particularly vulnerable to infections caused by *S. pneumoniae* [65].
People living with SCD are 30 times to 600 times more vulnerable to pneumococcal disease than people of similar race and age without SCD, and IPD plays a major role in the loss of life in infants with SCD [66].

Furthermore, people with some underlying medical conditions such as organ transplantation on immunosuppressive therapy, HIV, SCD, asplenia, lymphoma and nephrotic syndrome are a ten to three hundred fold more likely to be affected with IPD in comparison to healthy people [67].

However, Halasa et al, demonstrates that the incidence of IPD in individuals with SCD dramatically decreased after the introduction of PCV into the routine childhood immunization schedule. They recorded a 93% decline in IPD incidence among children with SCD aged <5 years [66].

1.1.9 IPD in children with human immunodeficiency virus (HIV)

Globally, by 2015, there were roughly 37 million individuals living with HIV/AIDS ,and the larger part of the aforementioned number are resident in Africa, particularly, sub-Saharan Africa [68],of which 10 percent were infants below the age of 15 [69]. South Africa, has roughly 17 percent of the total number of HIV positive people worldwide, with an estimate of over 5.26 million HIV positive people [70]. The high antenatal HIV seroprevalence rates (~30%), coupled improved prevention of mother-to-child transmission, yielded increased number of HIV-exposed but uninfected (HEU) children [71]. The incidence of IPD among HIV-positive infants is 20–40 times more, in comparison to HIV-negative infants in South

Africa. In addition, HIV-exposed infants have a 2.7-fold greater risk of IPD when compared to HIV-unexposed [72].

The Highly active antiretroviral treatment (HAART) programs have greatly contributed in reductions in the overall impact of IPD in industrialized countries [73]. Estimated 2-3 folds reductions in the risk of pneumococcal disease were noted among HIV-positive adults in industrialized countries where HAART programs were properly administered, yet the burden remained 35-times more than the general population [53, 74, 75].

In South Africa, HAART was introduced in the public sector in 2004, as the prevalence rate of HIV infection in general population was estimated to be about 10 to 12 percent [60].Overall amount of coverage with HAART in adults that needed therapy increased from 10 percent in 2004 to 51 percent in 2008,albeit with regional age-group differences. [60]. For the HIV positive children, the coverage of HAART gradually increased from below 20 percent in 2003-2004 to 74 percent in 2007-2008 [60]. The Up-scaling of the HAART in HIV-positive infants resulted in 50.8 percent reduction in pneumococcal disease hospitalization, as well as 65.2 percent reduction in IPD-associated death rates [60].

1.1.10 Laboratory diagnosis

No one "gold standard" method for isolating and identifying of *S. pneumoniae* [76]. Laboratory identification of *S. pneumoniae* has been attained using one or several assays. These include; Gram stain , colonial arrangement, hemolysis on blood agar plate, pyrrolidonyl arylamidase reactivity, optochin susceptibility, solubility in deoxycholate (bile solubility), reaction with specific antisera, carbohydrate utilization, miniaturized manual systems, such as the API 20 Strep system (bioMerieux Vitek, Inc., Hazelwood, Mo.),and automated Gram Positive Identification (GPI) Card (bioMerieux Vitek, Inc.) and DNA probes (Gen-Probe, San Diego, Calif.) [76].

Previously, *S. pneumoniae* identification was depended on its bile solubility, as well as optochin susceptibility of this pathogen [77, 78]. Due to laborious and time-consuming technical issues, the bile solubility test has fallen away, leaving optochin test as the main technique for *S. pneumoniae* identification. Optochin (ethylhydrocuprein hydrochloride), a derivative of quinine, was originally proposed as a possible drug for treatment of pneumonia. However, the use of optochin has only been for laboratory diagnosis since 1955, due to its high

toxicity for humans [77]. A research study reported that a filter paper moistened with optochin positioned on a horse-blood agar previously inoculated with *S. pneumoniae* produced an inhibition zone [79]. The first optochin resistant pneumococci strain was recorded by Kontiainen and Sivonen in 1987 [80], and in the next 3 decades a rise in the number of optochin-resistant *S. pneumoniae* stains have been recorded. In contrast to what occurs with optochin-resistant pneumococci, the description of optochin-susceptible viridans group streptococci (VGS) is extremely limited [81-84]. Optochin susceptibility has been estimated to be 90 to 100 percent sensitive and a specificity of 99 to 100% [85, 86]. On the other hand, bile solubility was related with a sensitivity of ≥98 percent, as well as a specificity of 100 percent [87, 88]. As a standard, a zone size of at least 5 mm around the optochin disc identifies the isolate as *S. pneumoniae*, whereas the VGS will grow up to the optochin disc [23].

1.1.11 Antimicrobial resistance patterns of invasive pneumococcal isolates

S.pneumoniae had remained largely susceptible to all categories of antibiotics until 1977, when an outbreak of penicillin-resistance pneumococci (PRP) was noted in two major cities in South African [89, 90]. Thereafter, an overwhelming rise in infections as a result of penicillin non-susceptible pneumococci (PNSP) has been revealed worldwide [91]. A study from France, on invasive strains of the pneumococci from children below the age of 16, showed an increase in prevalence (45.2 percent) in penicillin-non-susceptible pneumococci [92]. In another report from South African, in children less than the age of 5 , the prevalence of penicillin resistance was 56% between 2006-2008 [93], wheras ceftriaxone resistance was 15% [94]. In Nigeria, 28.0 percent and 55.3 percent had full and intermediate resistance to penicillin, respectively, and 96.2% were fully resistant to trimethoprim–sulphamethoxazole [91].

However, globally, in the PCV era, significant declines in the number of *S.pneumoniae* showing resistance to penicillin (7·3%, 5·3–9·4), third-generation cephalosporins (4·5%, 0·3–8·7), sulfamethoxazole–trimethoprim (16·0%, 11·0–21·2), tetracycline (2·0%, 0·3–3·7) and macrolides (3·6%, 0·7–6·6) have been reported [95].

As the genetic content is altered, the pneumococcus is able to challenge clinical interventions, acquiring antibiotic resistance as well as switching capsular serotype to evade vaccines [96]. The observable traits expressed in penicillin resistance occurs due to gene alterations in penicillin-binding proteins [97]. Although initially called penicillin-resistant pneumococci

(PRP), they seem to obtained genetic traits that encoded non susceptibility to penicillin, as well as other most used antimicrobials. The main process of resistance involves the alterations in the genome genes encoding penicillin-binding proteins. In support, a study stated that mutations involving the target enzymes for β-lactam class antibiotics, which is the penicillin-binding proteins (PBPs), was recognized as the main resistance technique of the penicillin resistant pneumococci [98]. Like penicillin, the mechanism of resistance for cefotaxime which is a β-lactam drug, is the alteration of the penicillin-binding proteins (PBPs) [99].

There is also high proportion of *S. pneumoniae* that are resistance to macrolides [100], ranging between 20 to 40 percent. The mechanism of resistance includes: modification of the antibiotic, ribosomal target site mutations, and antibiotic transport modifications [97]. A report by Cherazard et al. noted that 22% of pneumococcal isolates are resistant to clindamycin. Like macrolide, clindamycin resistance is associated with target site modifications [101]. Fluoroquinolones resistance is low and induced by accumulated alterations in the bacteria gene, raised efflux, or acquiring the plasmid-encoded genes. The primary technique of *S. pneumoniae* resistance for tetracyclines is moderated by two genes that are responsible for the protection of ribosome. The trimethoprim-sulfamethoxazole (TMP-SMX) resistance is roughly 35 percent and is secondary to alterations in the bacterial genetic make-up [97].

1.1.12 Treatment of IPD

Swift intervention, as regards early treatment of pneumococcal disease will reduce the rate at which people suffer or die from the disease [100]. Amoxicillin is recommended as the choice treatment for infants older than 2 months [100]. In individuals with underlying conditions or resistance, cephalosporins (ceftriaxone, cefotaxime) or moxifloxacin may be advised. In severely sick patients, aminoglycosides, clavulanic acid and macrolides can also be added. Macrolides are not recommended as a monotherapy in in South Africa, because of its high resistance to *S. pneumoniae* [100]. A third-generation cephalosporin (cefotaxime or ceftriaxone) is recommended for pneumococcal meningitis. On the other hand, moxifloxacin may be combined with vancomycin or combined with a third-generation cephalosporin [102]. In South Africa, the empiric management of *S. pneumoniae* community-acquired pneumonia and meningitis (based on the MIC susceptibility profile) of *S. pneumoniae* is described in table 1.1 and 1.2. Common agents for the treatment of *S pneumoniae* infections, according to the clinical and laboratory standards institutes (CLSI) breakpoints are listed in table 1.3.

Table 1.1: Empirical antimicrobial therapy for paediatric pneumonia [103].

Age	**Outpatient**	**Hospitalised**
0 - 2 months.	Recommend hospitalise all children less than 2 months of age	1. Ampicillin /penicillin iv + aminoglycoside iv or 2. Ceftriaxone/cefotaxime iv
3 months - 5 years	1. Amoxicillin po high dose	1. Ampicillin iv/amoxicillin po high dose or 2. Cefuroxime iv/amoxicillin-clavulanic acid po or iv 3. Cefotaxime/ceftriaxone iv Add: cloxacillin if suspect Staphylococcus aureus
5 years onwards	1. Amoxicillin po high dose or 2. Macrolide po (erythromycin/clarithromycin/ azithromycin) – if suspect *Mycoplasma pneumoniae* or Chlamydia spp.	1. Ampicillin iv/amoxicillin po high dose or 2. Cefuroxime iv/amoxicillin-clavulanic acid po or iv 3. Cefotaxime/ceftriaxone iv Add: cloxacillin if suspect Staphylococcus aureus Add: macrolide if suspect *Mycoplasma pneumoniae* or *Chlamydia* spp.

po = oral, **iv** = intravenous. Notes: The first antibiotic is the antibiotic of choice. Other antibiotics represent second- and third-line choices respectively.

Table 1.2: Recommendations for choice and duration of directed therapy for *S pneumoniae* meningitis [93].

Pathogen	**Antimicrobial**	**Alternative**	**Duration (in days)**
S. pneumoniae (penicillin MIC $\leq$ 0.06 μg/ml)	Benzyl penicillin	Ceftriaxone	10-14
S. pneumoniae (penicillin MIC > 0.06 μg/ml)	Ceftriaxone	Vancomycin plus ceftriaxone	10-14
S. pneumoniae (ceftriaxone MIC $\geq$ 1 μg/ml)	Vancomycin plus ceftriaxone	Moxifloxacin plus rifampicin	10-14

Table 1.3: Interpretive categories and zone diameter breakpoints (nearest whole mm), and interpretive categories and MIC breakpoints(µg/ml) for common agents used to treat *S. pneumoniae* infections. Source: Clinical and Laboratory Standards Institute, 2017.

Antimicrobial agent	**Disc content**	**Interpretive categories and Zone Diameter Breakpoints. Nearest whole mm**			**Interpretive categories and MIC Breakpoints µg/ml**		
		S	**I**	**R**	**S**	**I**	**R**
PENICILLINS							
Penicillin	1 **µg** Oxacillin	≥20	-	-	-	-	-
Penicillin parenteral (non-meningitis)	-	-	-	-	≤2	4	≥8
Penicillin parenteral (meningitis)	-	-	-	-	≤0.06	-	≥0.12
Penicillin (oral penicillin v)	-	-	-	-	≤0.06	0.12-1	≥2
Amoxicillin(non-meningitis)	-	-	-	-	≤2	4	≥8
Amoxicillin-clavulanate(non-meningitis)	-	-	-	-	≤2/1	4/2	≥8/4
CEPHEMS(PARENTERAL)							
Cefepime (meningitis)	-	-	-	-	≤0.5	1	≥2
Cefepime (non-meningitis	-	-	-	-	≤1	2	≥4
Cefotaxime (meningitis)	-	-	-	-	≤0.5	1	≥2
Ceftriaxone (meningitis)	-	-	-	-	≤0.5	1	≥2
Cefotaxime (non meningitis)	-	-	-		≤1	2	≥4
Ceftriaxone (non-meningitis)	-	-	-		≤1	2	≥4
Ceftaroline (non-meningitis)	30µg	≥26	-	-	≤0.5	-	-
Cefuroxime(parenteral)	-	-	-		≤0.5	1	≥2
CEPHEMS(ORAL)							
Cefuroxime(oral)	-	-	-		≤1	2	≥4
Cefaclor	-	-	-		≤1	2	≥4
Cefdinir	-	-	-		≤0.5	1	≥2
Cefpodoxime	-	-	-		≤0.5	1	≥2
Cefproxil	-	-	-		≤2	4	≥8
Loracarbef	-	-	-		≤2	4	≥8

Antimicrobial agent	Disc content	Interpretive categories and Zone Diameter Breakpoints. Nearest whole mm			Interpretive categories and MIC Breakpoints µg/ml		
		S	I	R	S	I	R
CARBAPENEMS							
Meropenem	-	-	-		≤0.25	0.5	≥1
Ertapenem	-	-	-		≤1	2	≥4
Imipenem	-	-	-		≤0.12	0.25- 0.5	≥1
Doripenem	-	-	-		≤1	-	-
GLYCOPEPTIDES							
Vancomycin	30µg	≥17	-		≤1	-	-
MACROLIDES							
Erythromycin	15µg	≥21	16-20	≤15	≤0.25	0.5	≥1
Azithromycin	15µg	≥18	14-17	≤13	≤0.5	1	≥2
Clarithromycin	15µg	≥21	17-20	≤16	≤0.25	0.5	≥1
Dirithromycin	15µg	≥18	14-17	≤13	≤0.5	1	≥2
TETRACYCLINES							
Tetracycline	30µg	≥28	25-27	≤24	≤1	2	≥4
Doxycycline	30µg	≥28	25-27	≤24	≤0.25	0.5	≥1
FLUOROQUINOLONES							
Gemifloxacin	5µg	≥23	20-22	≤19	≤0.12	0.25	≥0.5
Levofloxacin	5µg	≥17	14-16	≤13	≤2	4	≥8
Moxifloxacin	5µg	≥18	15-17	≤14	≤1	2	≥4
Gatifloxacin	5µg	≥21	18-20	≤17	≤1	2	≥4
Ofloxacin	5µg	≥16	13-15	≤12	≤2	4	≥8
Sparfloxacin	5µg	≥19	16-18	≤15	≤0.5	1	≥2
FOLATE PATHWAY ANTAGONISTS							
Trimethoprim-sulfamethoxazole	1.25/23.75µg	≥19	16-18	≤15	≤0.5/9	1/192/38	≥4/76
PHENICOL							
Chloramphenicol	30µg	≥21	-	≤20	≤4	-	≥8

Antimicrobial agent	Disc content	Interpretive categories and Zone Diameter Breakpoints. Nearest whole mm			Interpretive categories and MIC Breakpoints µg/ml		
		S	I	R	S	I	R
ANSAMYCINS							
Rifampin	5µg	≥19	17-18	≤16	≤1	2	≥4
LINCOSAMIDES							
Clindamycin	2µg	≥19	16-18	≤15	≤0.25	0.5	≥1
STREPTOGRAMINS							
Quinupristin-dalfopristin	15µg	≥19	16-18	≤15	≤1	2	≥4
OXAZOLIDINONES							
Linezolid	30µg	≥21	-	-	≤2	-	-
PLEUROMUTILINS							
Lefamulin	20µg	≥19	-	-	≤0.5	-	-

1.1.13 Impact of PCV on IPD

The advent of PCV, as a preventative intervention against IPD was initiated in the USA, in 2000, and was recommended as part of immunization programs in developing countries by the WHO in March 2007 [36]. South Africa introduced the PCV7 (4,6b,9V,14,18C,19F and 23F). It was incorporated into the National Expanded Programme for Immunization (EPI) from April 2009, and it that targeted almost 70 percent of all IPD causing serotypes in South Africa. This was replaced by the PCV13 (additional serotypes: 1,3,5,6A,7F,19A) PCV in May 2011 [2, 10, 104, 105]. Vaccine coverage for the third dose of PCV was 10 percent, 64 percent and 89 percent in 2009, 2010 and 2011, respectively. The PCV7 and PCV13 are highly efficient in preventing and curbing the menace of IPD caused by vaccine serotypes [106]. Comparatively, PCV13 was reported to be more effective than the earlier generation PCV7 [107].

The success of the PCV 7 introduction into the South African EPI, and its subsequent replacement with the PCV13 was noted by analyzing data from a nationwide, laboratory-based IPD surveillance program [2, 42]. Evidently, Von Gottberg et al, demonstrated that the PCV 13 introduction was to address the shortfall due to replacement with non–PCV7-type serotypes. They revealed that the incidence of IPD caused by PCV13 serotypes, that were previously not included in PCV7, had lowered significantly among children as a result of declines in serotype 19A, as well as Serotype 1,contained in PCV13 [42].

According to WHO PCVs scheme should be given in infants using a 2primary(p)+1 or 3p+0 schedule, with the primary doses of each schedule administered before 6 months and the booster dose of the 2p+1 administered at nine months of age or at a later time. The Intervals between doses are generally at least 8 weeks apart for the 2 primary doses in the 2p+1 plan and at least four weeks apart for the 3p+0 plan [5, 108]. In South Africa, the PCV scheme involves 2 primary doses at six and fourteen weeks and a third (booster) dose at nine months of age[109]. The rate of PCV coverage in South Africa for the 3 doses was approximately 10 percent in 2009, and it uniformly rose to 64 percent by 2010 and 89 percent by 2011 [110].

The PCV reduced IPD in by 74% in HIV- uninfected children after at least two PCV doses in infants less or equal to 16 weeks of age and 90% following 3 doses in children greater or equal to 40 weeks of age [111]. With PCV introduction in the USA, IPD in children declined from

about 24.3 episodes per 100,000 persons in 1998 to 17.3 episodes per 100,000 persons in 2001 [112].

A 16-year surveillance study conducted in England and Wales, from 2000 to 2016, observed no change in pneumococcal meningitis incidence with the PCV7, due to replacement with non–PCV7-type pneumococcal serotypes, but a 48% reduction in with PCV13 [107].

A report from South Africa, noted that PCV7 reduced IPD among infants less than 2 years of age by 69%, from 54.8 cases per 100,000 person-years in 2005 to 17.0 cases per 100,000 in 2012 [42]. Declines in the rates of IPD was also observed in infants, 2 to 4 years of age (−60%; 95% CI, −67 to −51), and in children those 5 to 9 years old (−44%; 95% CI, −54 to −33) [42]. Vaccine failure is rare and has been reported as 0.19 per 100000 [106].

1.2 Justification for the study

South Africa have high prevalence of HIV-infections. In addition, there are other factors such as high rates of malnutrition, overcrowded living conditions and increase in antibiotic resistance. All these contribute to the burden of pneumococcal disease. Soweto being a low-income township of the City of Johannesburg in Gauteng, South Africa, the findings from this study setting will give a true reflection of the implications of the afore mentioned risk factors on the disease burden, and will help influence decision making in strategic intervention policies in South Africa, and beyond.

There have been numerous studies evaluating the efficacy of the PCV in reducing the burden of pneumococcal disease in children. Owing to the fact that there are differences in IPD serotypes, as regards virulence, antibiotic susceptibility, as well as in its invasiveness, it is paramount to monitor the changes on new serotypes, virulence factors and antibiotic resistance, in order to advise suitable prevention and therapeutic interventions.

We therefore undertook a 13-year trend analysis of the fruitfulness of PCV on pneumococcal disease in hospitalized children in a tertiary-level academic hospital in a low-income peri-urban community with a population of roughly 1,272 million people [113].

This study also aimed to contribute to the knowledge required to advise effective antimicrobial stewardship, and also evaluate the impact that scaling up the HAART program in South Africa had on the prevalence of IPD in HIV-infected children.

1.3 Study aim and objectives

1.3.1 Aim

The aim of this study was to assess the successfulness of PCV on IPD in hospitalized children at the CHBAH over a 13-year period, before and after the incorporation of the PCV in the South African EPI Programme.

1.3.2 Objectives

1. To determine trends in incidence of IPD at the CHBAH before- and after-PCV eras.
2. To determine serotype distribution prevalence of pneumococcal disease isolates in the pre-PCV and post-PCV eras.
3. To determine the antimicrobial susceptibility patterns of pneumococcal isolates in the pre-PCV and post-PCV eras.
4. To describe the MIC levels of penicillin and cefotaxime over the study period.
5. To describe clinical characteristics of children hospitalized with IPD over the study period.
6. To compare the incidence of IPD in HIV-positive to HIV-negative children.
7. To assess the impact that scaling-up of the HAART program in South Africa had on the incidence of IPD in HIV-positive children.
8. To determine predictors (clinical and laboratory) of mortality in children with IPD.

Chapter 2 - Methods

2.1 Study design

This was a retrospective descriptive research study between January 2006 and December 2018 of children under 14 years old hospitalized with IPD at the CHBAH.

2.2 Study population

CHBAH is a 3,400 bed (408 pediatric beds) tertiary-level hospital, in Soweto, a suburb south of Johannesburg, South Africa. This academic establishment serves 128,000 infants below the age of 5 [114]. CHBAH is the major tertiary level referral hospital serving Soweto and surrounding areas.

Children hospitalized at the CHBAH with clinical signs of pneumonia, meningitis, or sepsis, are treated empirically with broad-spectrum antibiotics. Blood and cerebrospinal fluid(CSF) samples are sent to the NHLS laboratory for processing, which includes culture and susceptibility testing. Children hospitalized with pneumonia are usually treated with oral amoxicillin, ampicillin and gentamicin, or amoxicillin-clavulanate depending on the severity and presence of comorbidities (including HIV). In contrast, children with signs of meningitis are treated with ceftriaxone/cefotaxime. Duration of treatment will depend on type of microorganism and clinical response to therapy.

2.3 Study method

Demographic, laboratory, and clinical data were made available from the NHLS/NICD laboratory information system (Corporate Data Warehouse), GERMS-SA surveillance database, as well as the paediatrics discharge summary database of CHBAH (permissions were obtained). All cases of blood/CSF positive *S. pneumoniae* isolates of children below 14 years were identified from the laboratory databases, then merged with the clinical discharge summary database. The data was cleaned, and duplicates were removed. We regarded an isolate to be a duplicate when the same organism is cultured within 14 days from the initial episode, which is in keeping with the CDC criteria of repeat infection [115].

2.4 Inclusion Criteria

All *S. pneumoniae* isolated from blood (BC) and/or cerebrospinal fluid (CSF) in children below 14 years, hospitalized at CHBAH from January 2006 to December 2018.

2.5 Sample size

No sample-size calculation was undertaken, as this was a retrospective study.

2.6 Laboratory methods

Children who had signs suggestive of systemic infections in the paediatrics wards had blood aseptically inoculated into paediatric blood culture bottles (BacT/ALERT PF bottle, Biomerieux Inc., Durham, NC, USA). Bottles were then loaded into an automated blood incubating machine (BacT/ALERT system, BioMerieux, Marcyl'Etoile, France). Specimens were processed at the local laboratory which is accredited by the South African National Accreditation System (SANAS). Positive blood cultures and cerebrospinal fluid samples were processed according to the standard operative procedure [116, 117]. A positive pneumococcal culture was confirmed by the use of colony morphology as well as demonstration of susceptibility to optochin disc. Colonies obtained are subjected to further identification and sensitivity testing, according to the Kirby Bauer disc diffusion method and/or the automated system, Microscan, Siemens, USA and interpreted as per the clinical and laboratory standards Institute (CLSI, 2006 -2018) criteria [118]. The diffusion sensitivity was supported by E-test dilution method. The following Oxoid discs were used in the disc diffusion method: 30µg chloramphenicol(C), 5µg rifampicin(R), 15µg erythromycin(E), 10 µg clindamycin (DA), 30 µg tetracycline (TE), 25 µg sulphamethoxazole/trimethoprim (SXT), 30 µg cefuroxime(CXM), 30µg cefotaxime(CTX) and 30µg ceftriaxone(CRO). CLSI criteria were used to interpret inhibitory zone diameters and MICs. The relevant E-test dilution of penicillin and cefotaxime/ceftriaxone MICs for blood culture vary from that of CSF culture, and were reported as per CLSI criteria [118, 119]. Serotyping was performed at the NICD of the NHLS.

2.7 Data variables

The following variables were analyzed from the databases:

- age
- gender
- previous admissions
- history of chronic conditions
- weight for age
- HIV status
- CD4 lymphocyte count and viral load
- diagnosis (sepsis or meningitis)
- markers of illness severity
- sequelae of disease
- serotype
- antimicrobial susceptibility
- MIC levels of penicillin and cefotaxime
- duration of hospitalization
- outcome (discharged or died in hospital)

2.8 Statistical analysis

The data warehouses were searched for *S. pneumoniae* isolated from blood and/or CSF in children under 14 years who were hospitalised at the CHBAH from January 2006 to December 2018. The three data sources were merged, and 955 cases with a documented diagnosis of IPD were identified. Duplicate cases, defined as admission records within 14 days for the same individual, were investigated, and 88 records after the first admission record were identified and excluded from the study to generate unique cases and comprehensive standardised records. There were different levels of missingness for each measured variable. We pre-processed each variable used in this study after assessing their distributions; these include addressing inconsistent records, exclusion of cases not in the inclusion criteria, recoding outlying records as missing, generating a new variable from existing variables, and binning records using an established category from the literature. We only performed a complete case analysis based on the outcome of interest for a specific objective considered in the study and where a complete case is required in the investigation.

The analysis was divided into three PCV eras, according to the implementation period in South Africa, i.e. pre-PCV era (2006–2008), and the transitional-PCV era (2009–2011) and the post-PCV era (2012–2018). A year-specific disease incidence (point prevalence) was estimated using the amount of IPD admission episodes recorded for every study year as numerator. The denominator depended on the mid-year population amount of children below 14 years who are vulnerable to contracting IPD for regions D and G in Johannesburg. The mid-year population estimates were retrieved from the Gauteng Department of Health and Social Development as per the Statistics South Africa report.

Disease prevalence was estimated for each study year and stratified by age over the three PCV eras. This was displayed graphically and supported by quantitative values to highlight the trend of IPD within the study location. The incidence risk ratio and the percentage reduction in incidence were computed to compare the group-specific incidence for the pre-PCV, as well as the post-PCV eras. IPD incidence was calculated using the formula defined below

$$P = \frac{All\ new\ and\ pre{-}existing\ cases\ of\ IPD \text{ during a given time period}}{Divided\ by\ Population\ \ of\ children\ during\ the\ same\ time\ period} \quad x\ 100{,}000$$

Afterwards, the incidence ratio was estimated using $PR = p1/p2$, where $p1$ and $p2$ are the incidence of, e.g. HIV-infected patients in the pre-PCV and the post-PCV eras. The formula below was used to estimate 95% confidence interval (CI) of the PR

$$Ln(\widehat{PR}) \pm z\sqrt{\frac{(n_1 - x_1)/x_1}{n_1} + \frac{(n_2 - x_2)/x_2}{n_1}}, \qquad (1)$$

where x_1 and x_2, are the observed IPD cases for, e.g. HIV-infected patients in each era and n_1 and n_2 are the total number of observed IPD cases in each era (including uninfected patients). The standard normal distribution is denoted by *z*.

The trend in the HIV infection rate and the percentage of children on HAART treatment in South Africa was assessed to determine the effect that scaling up the HAART program in South

Africa had on the prevalence of IPD in HIV-positive children. National estimates of HAART coverage among children were extracted and pooled from two sources [120, 121].

We conducted a descriptive analysis of serotypes distribution prevalence, as well as the antimicrobial susceptibility patterns of IPD isolates across the eras. For the serotype distribution prevalence, we conducted a two-sample proportion test to determine if the difference observed in serotype proportion between the pre-PCV and post-PCV eras is significant at a 95% confidence interval. The Z-test of equality of two proportions is equivalent to the chi-square test when comparing the difference in population proportions between only two groups. The formula for a two-sample Z-test is defined as

$$Z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\hat{p}(1-\hat{p})\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}, \qquad (2)$$

where p_1 and p_2 are the serotype proportions observed in the pre-PCV and post-PCV, n_1 and n_2 are the total number of serotypes reported in pre and post-PCV, and $\hat{p}$ is the total proportion of successes in the two eras, such as $\hat{p} = (k1 + k2)/(n1 + n2)$. The distributions of the observed cases of serotypes across the two eras were assessed for the validity of the assumptions of the Z-test. This test was computed using the R software, and a p-value of <0.05 was considered a significant difference in the observed proportions. In addition, the descriptive analysis of minimum inhibitory concentration levels of penicillin and cefotaxime over the study years stratified by the three eras was conducted.

Further, we used the chi-square test of association to determine if there is a relationship between the characteristics of children hospitalized with IPD and the eras. We used the univariate logistic regression analysis to estimate the odds ratio (OR) and 95% confidence interval (CI) for risk factors of IPD-associated death during hospitalization. The logistic regression formula is defined by

$$\mathrm{P} = \frac{e^{\beta_0} + e^{\beta_1}}{1 + e^{\beta_0} + e^{\beta_1}}, \qquad (3)$$

Where P is the probability of death during hospitalisation, and the regression coefficients are denoted as β_s.

2.9 Ethics approval

Ethics approval was obtained from the Human Research Ethics committee (HREC), University of the Witwatersrand, prior to initiation of the study. Ethics Clearance Certificate No: M201057

Chapter 3 - Results

3.1 The clinical characteristics of the children hospitalized for IPD

Overall, 867 children were hospitalized for IPD over the study period. The clinical characteristics of these children are described in table 3.1. There were 409 cases of IPD during pre-PCV period, 231cases in transition-PCV period, and 227 cases in post-PCV period. There were less IPD cases amongst HIV-infected children in post-PCV era, in comparison to the pre-PCV era (p=0.033). There were no differences in age (p=0.825), gender (p=0.541), CD4+ count (p=0.875) and viral load (p=0.640). Although mortality rates were lower in the pre-PCV period, a large proportion of outcomes were missing.

Table 3.1**:** The clinical characteristics of children hospitalized with invasive pneumococcal disease over the study period.

	Variables	**pre-PCV** (N=409)	**transition** (N=231)	**post-PCV** (N=227)	**Overall** (N=867)	**p-value**
Age Group	<2 years	216 (52.8%)	113 (48.9%)	136 (59.9%)	465 (53.6%)	0.825
	2-4 years	56 (13.7%)	34 (14.7%)	26 (11.5%)	116 (13.4%)	
	5-13 years	81 (19.8%)	42 (18.2%)	43 (18.9%)	166 (19.1%)	
	Missing	56 (13.7%)	42 (18.2%)	22 (9.7%)	120 (13.8%)	
Gender	Female	186 (45.5%)	99 (42.9%)	88 (38.8%)	373 (43.0%)	0.541
	Male	222 (54.3%)	132 (57.1%)	139 (61.2%)	493 (56.9%)	
	Missing	1 (0.2%)	0 (0%)	0 (0%)	1 (0.1%)	
HIV Status	Negative	120 (29.3%)	97 (42.0%)	141 (62.1%)	358 (41.3%)	0.033
	Positive	185 (45.2%)	100 (43.3%)	55 (24.2%)	340 (39.2%)	
	Missing	104 (25.4%)	34 (14.7%)	31 (13.7%)	169 (19.5%)	
CD4+ Count	≤200	44 (10.8%)	27 (11.7%)	14 (6.2%)	85 (9.8%)	0.875
	201-500	53 (13.0%)	28 (12.1%)	16 (7.0%)	97 (11.2%)	
	>500	57 (13.9%)	30 (13.0%)	15 (6.6%)	102 (11.8%)	
	Missing	255 (62.3%)	146 (63.2%)	182 (80.2%)	583 (67.2%)	
Viral load	<400	15 (3.7%)	7 (3.0%)	1 (0.4%)	23 (2.7%)	0.640
	400-10000	12 (2.9%)	9 (3.9%)	3 (1.3%)	24 (2.8%)	
	>10000	115 (28.2%)	56 (24.2%)	28 (12.3%)	199 (23.0%)	
	Missing	267 (65.3%)	159 (68.8%)	195 (85.9%)	621 (71.6%)	
Specimen type	Blood	237 (57.9%)	122 (52.8%)	141 (62.1%)	500 (57.7%)	0.472
	CSF	50 (12.2%)	32 (13.9%)	38 (16.7%)	120 (13.8%)	
	Missing	122 (29.8%)	77 (33.3%)	48 (21.1%)	247 (28.5%)	
Previous Admission	No	211 (51.6%)	121 (52.4%)	139 (61.2%)	471 (54.3%)	0.887
	Yes	106 (25.9%)	64 (27.7%)	48 (21.1%)	218 (25.1%)	
	Missing	92 (22.5%)	46 (19.9%)	40 (17.6%)	178 (20.5%)	
Outcome	Dead	25 (6.1%)	8 (3.5%)	49 (21.6%)	82 (9.5%)	0.043
	Alive	199 (48.7%)	152 (65.8%)	161 (70.9%)	512 (59.1%)	
	Missing	185 (45.2%)	71 (30.7%)	17 (7.5%)	273 (31.5%)	

3.2 Trends in IPD incidence

Figure 3.1 and table 3.2 show the decline in the incidence (population point prevalence) of IPD from the pre-PCV era to the post-PCV era. The IPD incidence significantly reduced from 41.1 per 100,000 persons in 2006 to 4.6 per 100,000 persons in 2018.

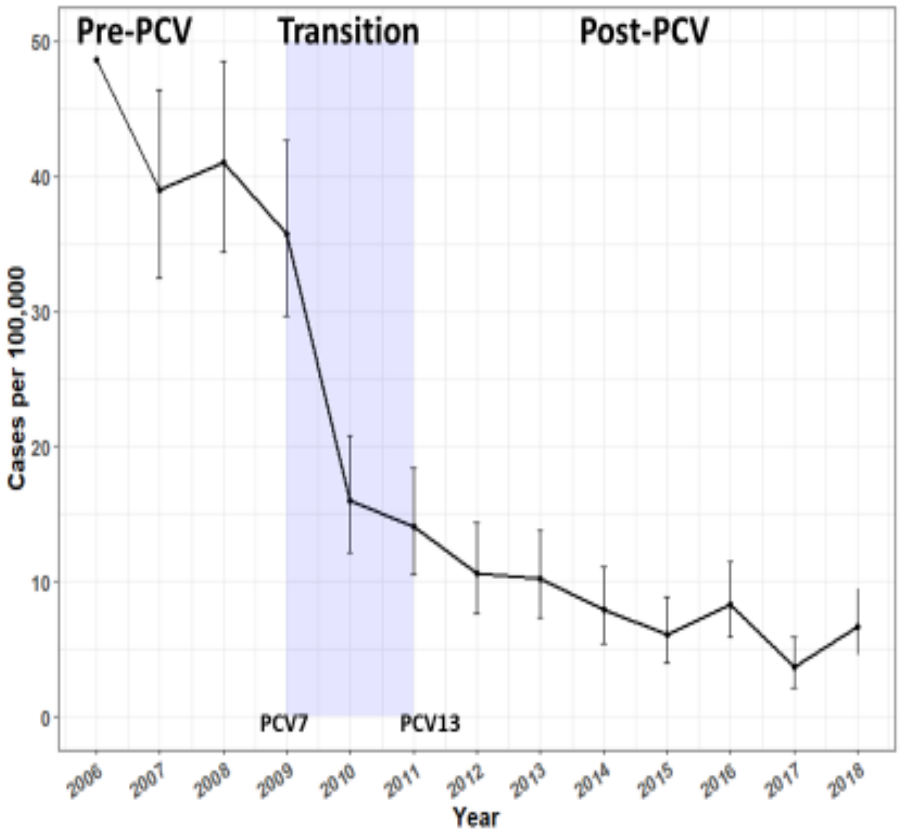

Figure 3.1: The overall incidence of invasive pneumococcal disease from 2006 through 2018.
Footnote: The period from 2006 through 2008 constitutes the pre-PCV period. The period from 2012 through 2018 represent the post-PCV era. The transition period is between 2009 and 2011 during which the PCVs were introduced. The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in 2009, and the 13-valent pneumococcal conjugate vaccine (PCV13) was introduced in 2011.

Table 3.2: Trend in IPD incidence (per 100,000)

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
N	150	124	135	122	57	52	41	41	33	26	37	17	32
LCI	48.6	39.0	41.0	35.7	16.0	14.0	10.6	10.2	7.9	6.0	8.3	3.7	6.7
P	41.1	32.4	34.4	29.7	12.1	10.5	7.6	7.3	5.5	3.9	5.9	2.2	4.6
UCI	57.0	46.5	48.5	42.7	20.8	18.4	14.4	13.8	11.1	8.8	11.5	5.9	9.4

Abbreviations: N=total number of cases, LCI=lower comfidence interval, P=point prevalence (incidence), UCI=upper comfidence interval.

3.3 Incidence of IPD stratified by age

The incidence of IPD was stratified by three age groups: <2 years, 2 to <5 years, and 5 to <14 years (Figure 3.2). We observed decline in incidence among all age categories, particularly in children less than 2 years of age. The sharp decline between 2009 and 2010 among the <2 year age group coincided with PCV7 introduction in the South African EPI in 2009.

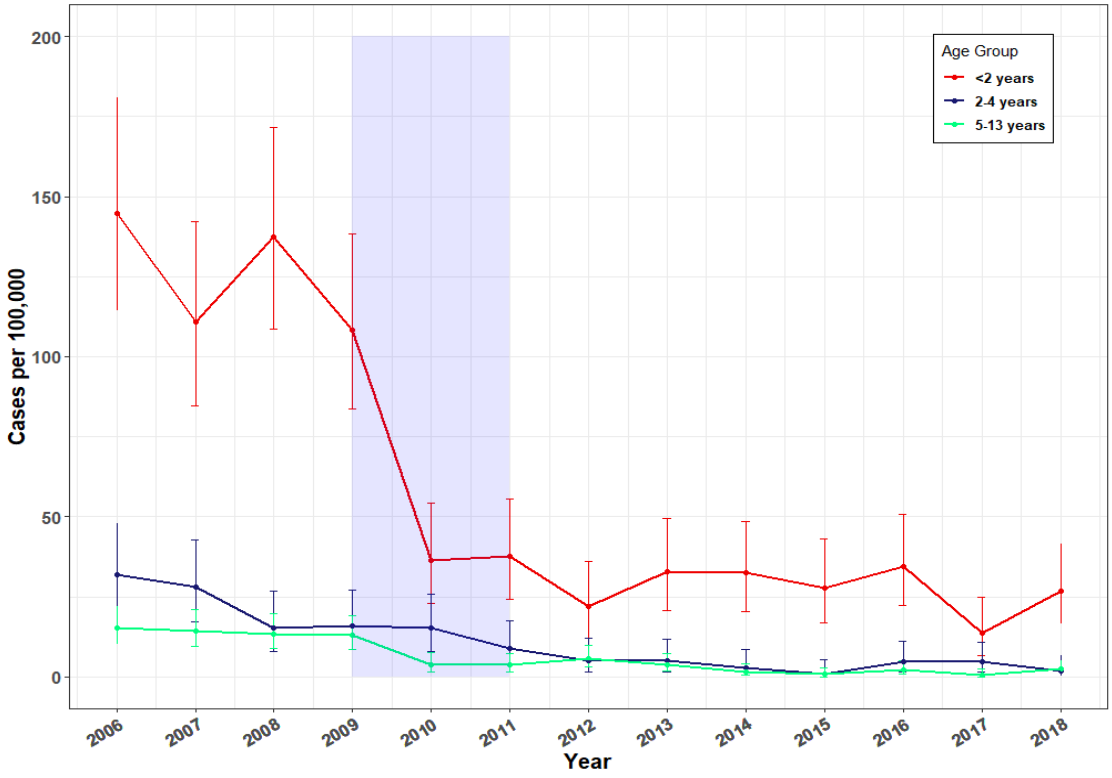

Figure 3.2: Incidence of IPD stratified by age

There were significance differences ($p<0.001$) comparing the IPD prevalence in the pre PCV and post PCV among all the age categories (Table 3.3). In children <2 years of age, there was a 79.3% reduction in IPD incidence when comparing the pre PCV (130.9 per 100,000 persons) and post PCV (27.1 per 100,000 persons). Among the 2 to <5years and 5 to <14years, there was 85.5% and 83.2% respectively (Table 3.3).

Table 3.3: Incidence ratio of IPD across the eras of PCV

	Pre-PCV N (95% CI)	**Transition N (95% CI)**	**Post-PCV N (95% CI)**	**Prevalence Ratio**	**p-value**	**%reduction**
	409	**231**	**227**			
Overall	42.8 (38.7-47.1)	21.6 (18.9.24.6)	7.5 (6.5-8.6)	5.7 (5.6-5.8)	<0.001	82.4 (82.3-82.6)
Age	**216**	**113**	**136**			
<2 years	130.9(114.0-149.5)	59.5(49.1-71.5)	27.1(22.7-32.0)	4.8 (4.7-5.0)	<0.001	79.3(74.5-84.1)
	56	**34**	**26**			
2-<5 years	24.9(18.8-32.3)	13.2(9.1-18.4)	3.6(2.3-5.2)	6.9 (6.5-7.3)	<0.001	85.5(85.1-86.0)
	81	**42**	**43**			
5-<14 years	14.3(11.4-17.8)	6.8(4.9-9.2)	2.4(1.7-3.2)	6.0 (5.6-6.3)	<0.001	83.2(82.9-83.5)

3.4 Hospitalization prevalence of IPD stratified by HIV status

The proportion of IPD was estimated for each study year by HIV status (Figure 3.3 and Table 3.4). The graph shows a decline in slope of HIV-infected from pre PCV to post PCV era. The prevalence of IPD amongst HIV-infected children declined from 67% in 2006 to 19.2% in 2018.

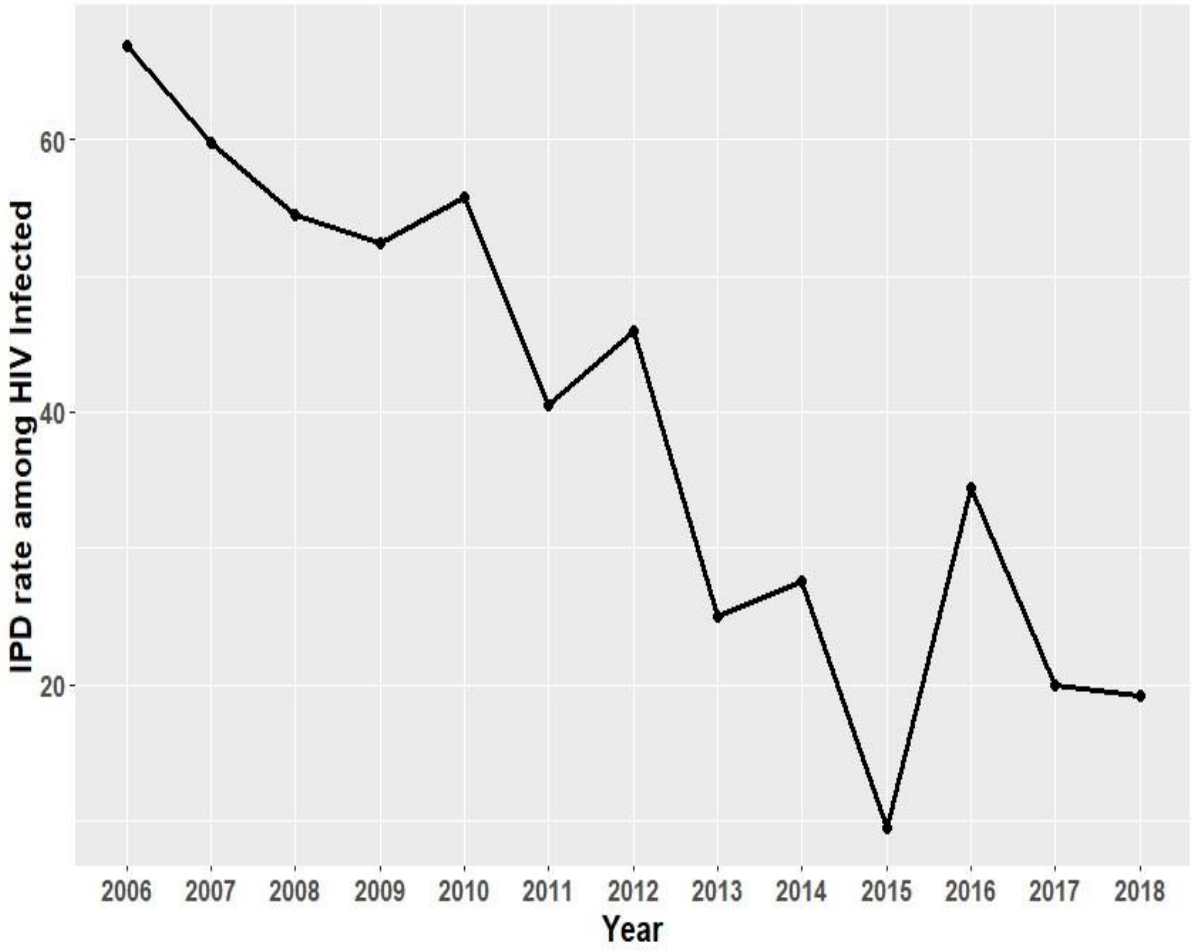

Figure 3.3: Trend in the IPD hospitalization prevalence in HIV-infected children

Table 3.4: The proportion of children with IPD stratified by HIV status

YEAR	HIV STATUS	n (%)
2006	HIV-Uninfected	37 (33.0)
	HIV-Infected	75 (67.0)
2007	HIV-Uninfected	37 (40.2)
	HIV-Infected	55 (59.8)
2008	HIV-Uninfected	46 (45.5)
	HIV-Infected	55 (54.5)
2009	HIV-Uninfected	49 (47.6)
	HIV-Infected	54 (52.4)
2010	HIV-Uninfected	23 (44.2)
	HIV-Infected	29 (55.8)
2011	HIV-Uninfected	25 (59.5)
	HIV-Infected	17 (40.5)
2012	HIV-Uninfected	20 (54.1)
	HIV-Infected	17 (45.9)
2013	HIV-Uninfected	27 (75.0)
	HIV-Infected	9 (25.0)
2014	HIV-Uninfected	21 (72.4)
	HIV-Infected	8 (27.6)
2015	HIV-Uninfected	19 (90.5)
	HIV-Infected	2 (9.5)
2016	HIV-Uninfected	21 (65.6)
	HIV-Infected	11 (34.4)
2017	HIV-Uninfected	12 (80.0)
	HIV-Infected	3 (20.0)
2018	HIV-Uninfected	21 (80.8)
	HIV-Infected	5 (19.2)

3.5 Impact of the HAART program on IPD incidence

Figure 3.4 shows the prevalence of IPD amongst HIV infected children under 14 years of age and percentage HAART coverage over the study period [120, 121]. There were significant reductions in the prevalence of IPD amongst HIV infected children with increasing HAART coverage (Figure 3.4).

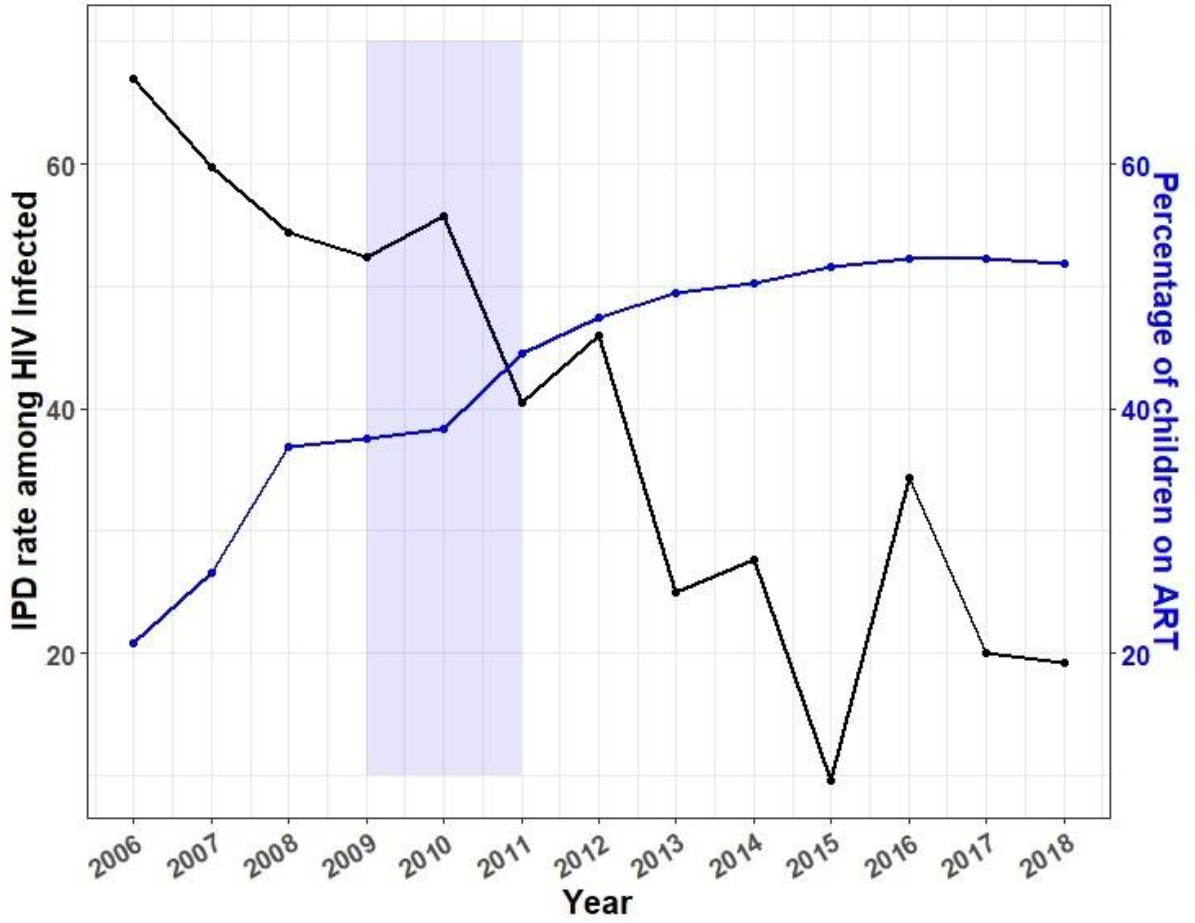

Figure 3.4**:** The impact of scaling-up of the HAART program on the prevalence of invasive pneumococcal disease in HIV-infected children in South Africa

3.6 Serotype distribution of IPD isolates

The serotype distribution of IPD isolates are illustrated in tables 3.5 and 3.6. The prevalence of the most common vaccine serotypes was 14(16.9%), 1(14.0%), 6A (11.4%), 19F (11.0%), 23F (10.8%) and 6B (10.0%) (Table 3.5). The non-vaccine serotypes that occurred the most frequently were 15B/C (9.8%), 12F (9.3%), 16F (7.5%), NEG38(7.5%) 35B (5.1%), and 7C (4.2%) (Table 3.6).

When comparing the pre- and post-PCV eras, there were significant differences for serotype 3 (p=0.002), serotype 5 (p=0.014), serotype 9V (p=0.020), and serotype 14(p=0.023) (Table 3.7).

Table 3.5. The pneumococcal vaccine serotype distribution prevalence of invasive pneumococcal isolates in the pre- and post-PCV era

SSEROTYPE	Pre-PCV			Transition			Post-PCV							
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Overall
	N=94 (%)	N=84 (%)	N=93 (%)	N=79 (%)	N=33 (%)	N=22 (%)	N=16 (%)	N=9 (%)	N=8 (%)	N=3 (%)	N=7 (%)	N=2 (%)	N=5 (%)	N=455 (%)
1	12 (12.8%)	11 (13.1%)	14 (15.1%)	10 (12.7%)	5 (15.2%)	3 (13.6%)	6 (37.5%)	1 (11.1%)	1 (12.5%)	0 (0%)	1 (14.3%)	0 (0%)	0 (0%)	64 (14.1%)
3	2 (2.1%)	2 (2.4%)	1 (1.1%)	1 (1.3%)	3 (9.1%)	1 (4.5%)	2 (12.5%)	1 (11.1%)	1 (12.5%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	15 (3.3%)
4	6 (6.4%)	6 (7.1%)	4 (4.3%)	4 (5.1%)	0 (0%)	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (14.3%)	0 (0%)	0 (0%)	22 (4.8%)
5	0 (0%)	0 (0%)	1 (1.1%)	2 (2.5%)	1 (3.0%)	0 (0%)	1 (6.3%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (1.3%)
6A	14 (14.9%)	9 (10.7%)	7 (7.5%)	12 (15.2%)	5 (15.2%)	1 (4.5%)	1 (6.3%)	1 (11.1%)	1 (12.5%)	0 (0%)	1 (14.3%)	0 (0%)	0 (0%)	52 (11.4%)
6B	11 (11.7%)	10 (11.9%)	9 (9.7%)	7 (8.9%)	7 (21.2%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	1 (14.3%)	0 (0%)	0 (0%)	46 (10.1%)
7F	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (9.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.4%)
9V	0 (0%)	3 (3.6%)	5 (5.4%)	2 (2.5%)	0 (0%)	1 (4.5%)	2 (12.5%)	1 (11.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (40.0%)	16 (3.5%)
14	16 (17.0%)	15 (17.9%)	21 (22.6%)	16 (20.3%)	1 (3.0%)	5 (22.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (28.6%)	0 (0%)	1 (20.0%)	77 (16.9%)
18C	4 (4.3%)	7 (8.3%)	2 (2.2%)	1 (1.3%)	3 (9.1%)	1 (4.5%)	0 (0%)	1 (11.1%)	0 (0%)	0 (0%)	1 (14.3%)	0 (0%)	1 (20.0%)	21 (4.6%)
19A	5 (5.3%)	6 (7.1%)	9 (9.7%)	4 (5.1%)	3 (9.1%)	5 (22.7%)	1 (6.3%)	0 (0%)	2 (25.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	35 (7.7%)
19F	13 (13.8%)	8 (9.5%)	13 (14.0%)	6 (7.6%)	1 (3.0%)	0 (0%)	2 (12.5%)	1 (11.1%)	1 (12.5%)	2 (66.7%)	0 (0%)	2 (100%)	1 (20.0%)	50 (11.0%)
23F	11 (11.7%)	7 (8.3%)	7 (7.5%)	14 (17.7%)	4 (12.1%)	2 (9.1%)	1 (6.3%)	2 (22.2%)	1 (12.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	49 (10.8%)

Table 3.6: The non-vaccine pneumococcal serotype and the frequency of occurrence

SSEROTYPE	Pre-PCV			Transition			Post-PCV							
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Overall
	N=24 (%)	N=16 (%)	(N=16) (%)	N=15 (%)	N=11 (%)	N=18 (%)	N=16 (%)	N=27 (%)	N=19 (%)	N=15 (%)	N=19 (%)	N=5 (%)	N=13 (%)	N=214 (%)
10F	1 (4.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
12F	2 (8.3%)	0 (0%)	3 (18.8%)	3 (20.0%)	1 (9.1%)	3 (16.7%)	1 (6.3%)	2 (7.4%)	1 (5.3%)	3 (20.0%)	1 (5.3%)	0 (0%)	0 (0%)	20 (9.3%)
13	2 (8.3%)	1 (6.3%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (2.3%)
15A	1 (4.2%)	0 (0%)	1 (6.3%)	0 (0%)	1 (9.1%)	1 (5.6%)	1 (6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (2.3%)
15B/C	1 (4.2%)	4 (25.0%)	1 (6.3%)	2 (13.3%)	1 (9.1%)	2 (11.1%)	4 (25.0%)	3 (11.1%)	0 (0%)	0 (0%)	2 (10.5%)	1 (20.0%)	0 (0%)	21 (9.8%)
16F	4 (16.7%)	0 (0%)	2 (12.5%)	1 (6.7%)	0 (0%)	1 (5.6%)	2 (12.5%)	2 (7.4%)	2 (10.5%)	1 (6.7%)	1 (5.3%)	0 (0%)	0 (0%)	16 (7.5%)
17F	2 (8.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.7%)	2 (10.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (2.3%)
18A	1 (4.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
18F	2 (8.3%)	1 (6.3%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (1.9%)

Table 3.6 cntd. The non-vaccine pneumococcal serotype and the frequency of occurrence

	Pre-PCV			Transition			Post-PCV							
33B	1 (4.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
33D	1 (4.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
33F	2 (8.3%)	1 (6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.7%)	1 (5.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5 (2.3%)
7C	1 (4.2%)	3 (18.8%)	0 (0%)	0 (0%)	1 (9.1%)	2 (11.1%)	1 (6.3%	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (4.2%)
8	3 (12.5)	3 (18.8%)	3 (18.8%)	2 (13.%	1 (9.1%)	4 (22.2%)	0 (0%)	2 (7.4%)	3 (15.8%)	7 (46.7%)	4 (21.1%)	1 (20.0%)	4 (30.8%)	37 (17.3%)
10B	0 (0%)	1 (6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
19F/6A	0 (0%)	1 (6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
23A	0 (0%)	1 (6.3%)	2 (12.5%)	0 (0%)	0 (0%)	0 (0%)	1 (6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (1.9%)
19B	0 (0%)	0 (0%)	1 (6.3%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (1.4%)
34	0 (0%)	0 (0%)	1 (6.3%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	1 (3.7%)	1 (5.3%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	5 (2.3%)

Table 3.6 cntd. The non-vaccine pneumococcal serotype and the frequency of occurrence

	Pre-PCV			Transition			Post-PCV							
35F	0 (0%)	0 (0%)	1 (6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
42	0 (0%)	0 (0%)	1 (6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
11A	0 (0%)	0 (0%)	0 (0%)	1 (6.7%	0 (0%)	0 (0%)	0 (0%)	2 (7.4%	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (1.4%)
22F	0 (0%)	0 (0%)	0 (0%)	1 (6.7%	0 (0%)	0 (0%)	1 (6.3%	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.9%)
9N	0 (0%)	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0%)	0 (0%)	2 (0.9%)
10A	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (9.1%)	0 (0%)	0 (0%)	1 (3.7%	0 (0%)	1 (6.7%)	1 (5.3%)	0 (0%)	0 (0%)	4 (1.9%)
35B	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (27.3%)	1 (5.6%)	1 (6.3%	2 (7.4%)	4 (21.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (5.1%)
6A/B	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (9.1%)	0 (0%)	0 (0%)	2 (7.4%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0%)	0 (0%)	4 (1.9%)
NEG42	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (9.1%)	0 (0%)	1 (6.3%	1 (3.7%	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (1.4%)
15ABCF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (11.1%)	0 (0%)	2 (7.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (1.9%)
18ABC	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)

Table 3.6 cntd. The non-vaccine pneumococcal serotype and the frequency of occurrence

	Pre-PCV			Transition			Post-PCV							
23B	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0%)	1 (7.7%)	3 (1.4%)
10AB	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6.3%	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
18B	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 6.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
21	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 6.3%	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
12ABF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (7.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.9%)
19BF	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (7.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (0.9%)
NEG38	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (15.8%)	1 (6.7%)	4 (21.1%	2 (40.0%	6 (46.2%	16 (7.5%)
20	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
12A/B/F/44/46	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (10.5%)	0 (0%)	0 (0%)	2 (0.9%)
6C	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0%)	0 (0%)	1 (0.5%)
27	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (20.0%)	0 (0%)	1 (0.5%)
INS	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	1 (0.5%)
POOL G	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (7.7%)	1 (0.5%)

Table 3.7: The p-values of the vaccine serotypes comparing the pre PCV and post PCV eras.

Serotype	**pre-PCV**	**post-PCV**	***p*-value**
1	0.18	0.14	0.420
3	0.10	0.02	0.002
4	0.02	0.06	0.257
5	0.04	0.00	0.014
6A	0.08	0.11	0.517
6B	0.04	0.11	0.125
7F	0.00	0.00	NA
9V	0.10	0.03	0.020
14	0.06	0.19	0.023
18C	0.06	0.05	0.720
19A	0.06	0.07	0.728
19F	0.18	0.13	0.298
23F	0.08	0.09	0.781

NA=Not applicable

3.7 Antimicrobial resistance patterns of IPD isolates

The antimicrobial resistance patterns of IPD isolates in the pre-PCV and post-PCV periods are summarized in table 3.8, albeit a large proportion of missing information. All but 2 isolates were susceptible to moxifloxacin. Linezolid showed susceptibility to all, but one isolate. For Rifampicin, all isolates recorded post PCV were susceptible, while the few resistant isolates were identified in pre PCV and transition periods [1 (0.8%) in 2007, 2 (1.5%) in 2008, 4 (3.3%) in 2009, and 1 (0.8%) in 2010].

Table 3.9 summarizes the MIC of penicillin and cefotaxime using the MIC breakpoints according to the CLSI guidelines. For penicillin MIC, a total of 319 (36.8%) were susceptible, 33(3.8%) were resistant and 261 (30.1%) were intermediate. For cefotaxime, 583(67.2%) were susceptible, 26 (3.0%) were intermediate, while 4 (0.5%) were resistant.

Table 3.8: The disc susceptibility patterns of the invasive pneumococcal isolates.

Antibiotics	Pre-PCV			Transition			Post-PCV						
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
	N=150 (%)	N=124 (%)	N=135 (%)	N=122 (%)	N=57 (%)	N=52 (%)	N=41 (%)	N=41 (%)	N=33 (%)	N=26 (%)	N=37 (%)	N=17 (%)	N=32 (%)
CLINDAMYCIN													
Susceptible	5 (3.3%)	2 (1.6%)	31 (23.0%)	48 (39.3%)	30 (52.6%)	26 (50.0%)	25 (61.0%)	15 (36.6%)	22 (66.7%)	16 (61.5%)	14 (37.8%)	5 (29.4%)	16 (50.0%)
Resistant	5 (3.3%)	4 (3.2%)	14 (10.4%)	17 (13.9%)	7 (12.3%)	3 (5.8%)	1 (2.4%)	0 (0%)	0 (0%)	1 (3.8%)	1 (2.7%)	0 (0%)	1 (3.1%)
Missing	140 (93.3%)	118 (95.2%)	90 (66.7%)	57 (46.7%)	20 (35.1%)	23 (44.2%)	15 (36.6%)	26 (63.4%)	11 (33.3%)	9 (34.6%)	22 (59.5%)	12 (70.6%)	15 (46.9%)
ERYTH_AZITH													
Susceptible	21 (14.0%)	13 (10.5%)	29 (21.5%)	43 (35.2%)	30 (52.6%)	24 (46.2%)	23 (56.1%)	14 (34.1%)	21 (63.6%)	17 (65.4%)	12 (32.4%)	5 (29.4%)	16 (50.0%)
Resistant	11 (7.3%)	8 (6.5%)	18 (13.3%)	21 (17.2%)	7 (12.3%)	5 (9.6%)	3 (7.3%)	1 (2.4%)	1 (3.0%)	1 (3.8%)	3 (8.1%)	0 (0%)	1 (3.1%)
Missing	118 (78.7%)	103 (83.1%)	88 (65.2%)	58 (47.5%)	20 (35.1%)	23 (44.2%)	15 (36.6%)	26 (63.4%)	11 (33.3%)	8 (30.8%)	22 (59.5%)	12 (70.6%)	15 (46.9%)
LEVOFLOXACIN													
Susceptible	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Missing	150 (100%)	124 (100%)	135 (100%)	122 (100%)	57 (100%)	52 (100%)	41 (100%)	41 (100%)	32 (97.0%)	26 (100%)	37 (100%)	17 (100%)	32 (100%)
LINEZOLID													
Susceptible	2 (1.3%)	0 (0%)	44 (32.6%)	65 (53.3%)	36 (63.2%)	29 (55.8%)	26 (63.4%)	15 (36.6%)	21 (63.6%)	14 (53.8%)	14 (37.8%)	4 (23.5%)	17 (53.1%)
Resistant	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Missing	148 (98.7%)	124 (100%)	91 (67.4%)	57 (46.7%)	21 (36.8%)	23 (44.2%)	15 (36.6%)	26 (63.4%)	12 (36.4%)	11 (42.3%)	23 (62.2%)	13 (76.5%)	15 (46.9%)

Table 3.8 cntd. The disc susceptibility patterns of the invasive pneumococcal isolates.

Antibiotics	Pre-PCV			Transition			Post-PCV						
MOXIFLOXACIN													
Susceptible	2 (1.3%)	1 (0.8%)	41 (30.4%)	65 (53.3%)	37 (64.9%)	30 (57.7%)	26 (63.4%)	15 (36.6%)	19 (57.6%)	16 (61.5%)	14 (37.8%)	5 (29.4%)	16 (50.0%)
Resistant	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.0%)	1 (3.8%)	0 (0%)	0 (0%)	0 (0%)
Missing	148 (98.7%)	123 (99.2%)	94 (69.6%)	57 (46.7%)	20 (35.1%)	22 (42.3%)	15 (36.6%)	26 (63.4%)	13 (39.4%)	9 (34.6%)	23 (62.2%)	12 (70.6%)	16 (50.0%)
RIFAMPICIN													
Susceptible	2 (1.3%)	0 (0%)	37 (27.4%)	60 (49.2%)	36 (63.2%)	30 (57.7%)	26 (63.4%)	15 (36.6%)	22 (66.7%)	18 (69.2%)	15 (40.5%)	4 (23.5%)	13 (40.6%)
Resistant	0 (0%)	1 (0.8%)	2 (1.5%)	4 (3.3%)	1 (1.8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Missing	148 (98.7%)	123 (99.2%)	96 (71.1%)	58 (47.5%)	20 (35.1%)	22 (42.3%)	15 (36.6%)	26 (63.4%)	11 (33.3%)	8 (30.8%)	22 (59.5%)	13 (76.5%)	19 (59.4%)
TRIME_SULF													
Susceptible	3 (2.0%)	4 (3.2%)	16 (11.9%)	25 (20.5%)	10 (17.5%)	13 (25.0%)	10 (24.4%)	12 (29.3%)	10 (30.3%)	12 (46.2%)	6 (16.2%)	3 (17.6%)	8 (25.0%)
Resistant	13 (8.7%)	11 (8.9%)	30 (22.2%)	40 (32.8%)	27 (47.4%)	17 (32.7%)	15 (36.6%)	3 (7.3%)	12 (36.4%)	5 (19.2%)	8 (21.6%)	2 (11.8%)	8 (25.0%)
Missing	134 (89.3%)	109 (87.9%)	89 (65.9%)	57 (46.7%)	20 (35.1%)	22 (42.3%)	16 (39.0%)	26 (63.4%)	11 (33.3%)	9 (34.6%)	23 (62.2%)	12 (70.6%)	16 (50.0%)

Abbreviations for table 3.9: TRIME SULF-Trimethoprim sulfamethoxazole, ERYTH AZITH-Erythromycin- azithromycin.

Table 3.9. The minimum inhibitory concentration levels of penicillin and cefotaxime over the study period.

	Pre-PCV			Transition			Post-PVC							
	2006	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**Overall**
Penicillin	**N=150 (%)**	**N=124 (%)**	**N=135 (%)**	**N=122 (%)**	**N=57 (%)**	**N=52 (%)**	**N=41 (%)**	**N=41 (%)**	**N=33 (%)**	**N=26 (%)**	**N=37 (%)**	**N=17 (%)**	**N=32 (%)**	**N=867 (%)**
Intermediate	53 (35.3%)	58 (46.8%)	56 (41.5%)	39 (32.0%)	18 (31.6%)	11 (21.2%)	5 (12.2%)	4 (9.8%)	6 (18.2%)	1 (3.8%)	6 (16.2%)	1 (5.9%)	3 (9.4%)	261 (30.1%)
Sensitive	65 (43.3%)	40 (32.3%)	53 (39.3%)	38 (31.1%)	17 (29.8%)	22 (42.3%)	21 (51.2%)	20 (48.8%)	13 (39.4%)	14 (53.8%)	8 (21.6%)	3 (17.6%)	5 (15.6%)	319 (36.8%)
Resistant	0 (0%)	2 (1.6%)	0 (0%)	17 (13.9%)	3 (5.3%)	3 (5.8%)	2 (4.9%)	1 (2.4%)	2 (6.1%)	0 (0%)	2 (5.4%)	1 (5.9%)	0 (0%)	33 (3.8%)
Missing	32 (21.3%)	24 (19.4%)	26 (19.3%)	28 (23.0%)	19 (33.3%)	16 (30.8%)	13 (31.7%)	16 (39.0%)	12 (36.4%)	11 (42.3%)	21 (56.8%)	12 (70.6%)	24 (75.0%)	254 (29.3%)
Cefotaxime	**(N=150)**	**(N=124)**	**(N=135)**	**(N=122)**	**(N=57)**	**(N=52)**	**(N=41)**	**(N=41)**	**(N=33)**	**(N=26)**	**(N=37)**	**(N=17)**	**(N=32)**	**(N=867)**
Intermediate	0 (0%)	3 (2.4%)	0 (0%)	10 (8.2%)	2 (3.5%)	3 (5.8%)	1 (2.4%)	1 (2.4%)	2 (6.1%)	0 (0%)	3 (8.1%)	1 (5.9%)	0 (0%)	26 (3.0%)
Sensitive	118 (78.7%)	97 (78.2%)	109 (80.7%)	82 (67.2%)	35 (61.4%)	35 (61.4%)	26 (63.4%)	24 (58.5%)	19 (57.6%)	15 (57.7%)	13 (35.1%)	4 (23.5%)	8 (25.0%)	583 (67.2%)
Resistant	0 (0%)	0 (0%)	0 (0%)	2 (1.6%)	1 (1.8%)	0 (0%)	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (0.5%)
Missing	32 (21.3%)	24 (19.4%)	26 (19.3%)	28 (23.0%)	19 (33.3%)	16 (30.8%)	13 (31.7%)	16 (39.0%)	12 (36.4%)	11 (42.3%)	21 (56.8%)	12 (70.6%)	24 (75.0%)	254 (29.3%)

3.8 Predictors of mortality in children with IPD

In the univariate analysis, there was an association between mortality and age, specimen type and PCV period (Table 3.10). In a multivariable analysis, the adjusted odds of death were 3.38-fold (95%CI: 1.41-8.07; p=0.006) for children with pneumococcal meningitis, and 3.44-fold (95%CI: 1.31-9.05; p=0.012) for children in the post-PCV era (Table 3.10).

Table 3.10: Predictors of mortality in children that demised from IPD

	Variable	Overall n (%)	Dead n (%)	Alive n (%)	OR (95% CI)	p-value	aOR (95%CI)	p-value
Age Group	<2 years	314 (52.9%)	62 (75.6%)	252 (49.2%)	1			
	2-<5 years	83 (14.0%)	6 (7.3%)	77 (15.0%)	0.32 (0.13-0.76)	0.010	0.46 (0.13-1.78)	0.278
	5-<14 years	109 (18.4%)	10 (12.2%)	99 (19.3%)	0.41 (0.20-0.83)	0.014	0.33 (0.08-1.03)	0.055
	Missing	88 (14.8%)	4 (4.9%)	84 (16.4%)				
Gender	Female	252 (42.4%)	34 (41.5%)	218 (42.6%)	1			
	Male	341 (57.4%)	48 (58.5%)	293 (57.2%)	1.05 (0.65-1.69)	0.839	0.95 (0.43-2.01)	0.846
	Missing	1 (0.2%)	0 (0%)	1 (0.2%)				
CD4 Count*	<=200	53 (8.9%)	6 (7.3%)	47 (9.2%)	1			
	>500	67 (11.3%)	1 (1.2%)	66 (12.9%)	0.12 (0.01-1.02)	0.052		
	201-500	64 (10.8%)	6 (7.3%)	58 (11.3%)	0.81 (0.25-2.68)	0.730		
	Missing	410 (69.0%)	69 (84.1%)	341 (66.6%)				
Viral Load*	<400	11 (1.9%)	1 (1.2%)	10 (2.0%)	1			
	>10000	127 (21.4%)	5 (6.1%)	122 (23.8%)	0.41 (0.04-3.86)	0.436		
	400-10000	15 (2.5%)	1 (1.2%)	14 (2.7%)	0.71 (0.04-12.83)	0.819		
	Missing	441 (74.2%)	75 (91.5%)	366 (71.5%)				
HIV Status	Negative	265 (44.6%)	38 (46.3%)	227 (44.3%)	1			
	Positive	230 (38.7%)	21 (25.6%)	209 (40.8%)	0.60 (0.34-1.60)	0.077	1.06 (0.30-1.52)	0.895
	Missing	99 (16.7%)	23 (28.0%)	76 (14.8%)				
Specimen Type	Blood	340 (57.2%)	34 (41.5%)	306 (59.8%)	1			
	CSF	82 (13.8%)	23 (28.0%)	59 (11.5%)	3.51 (1.93-6.38)	<0.001	3.38 (1.41-8.07)	**0.006**
	Missing	172 (29.0%)	25 (30.5%)	147 (28.7%)				
Previous Admission	No	324 (54.5%)	49 (59.8%)	275 (53.7%)	1			
	Yes	137 (23.1%)	15 (18.3%)	122 (23.8%)	0.69 (0.37-1.28)	0.238	1.36 (1.60-3.15)	0.480
	Missing	133 (22.4%)	18 (22.0%)	115 (22.5%)				
PCV Eras	Pre-PCV	224 (37.7%)	25 (30.5%)	199 (38.9%)	1			
	Transition	160 (26.9%)	8 (9.8%)	152 (29.7%)	0.42 (0.18-0.95)	0.038	0.47 (0.11-1.92)	0.291
	Post-PCV	210 (35.4%)	49 (59.8%)	161 (31.4%)	2.42 (1.43-4.09)	<0.001	3.44 (1.31-9.05)	**0.012**

*CD4 and Viral load not included in multivariate model because of missing values

Chapter 4 - Discussion

Following the introduction of the PCV in South Africa, there has been notable decline in the prevalence of pneumococcal disease in all age groups and HIV-infected children, thus greatly reduced the burden of the disease [42, 122]. Surveillance for changes in serotypes, virulence factors and antibiotic resistance, is however necessary to advise appropriate medical interventions. These observations are shown in this study, which extends over a long period of time, and includes years before and after the introduction of the PCV in the peri-urban population of Soweto.

4.1 Trends in incidence of IPD and serotype distribution

We report a declining trend in the incidence of IPD at CHBAH over the periods of study from 41.1 episodes per 100,000 populations in 2006 to 4.6 episode per 100,000 populations in 2018. There was an 82.4% (95%CI: 82.3-82.6) reduction in the incidence of IPD when comparing the pre- to post-PCV era. Our findings are consistent with changes in the national South African IPD incidence – the incidence (per 100 000) declined by 79% reduction in infants below the age of 5, from 30 in 2005 to 6 in 2017 [10]. Our incidence in 2018 is however lower than other developing countries where the incidence has been reported between 5 and 416 per 100,000 [8].

The incidence declined for all age categories, with the steepest decline in infants below the age of 2. There was 79.3% total reduction in IPD when comparing the pre PCV (130.9 per 100,000 persons) versus post PCV (27.1 per 100,000 persons) era. Similarly, there was a 69% (95% CI: 65-72) reduction in IPD in infants less than 2 years nationally with increasing PCV coverage [42]. There were also 85.5% and 83.2% reduction in IPD among the 2 to <5years and 5 to <14years, respectively.

Over the entire study period, the most prevalent serotypes were 14 (16.9%), 1 (14.0%), 6A (11.4%), 19F (11.0%), 23F (10.8%) and 6B (10.0%). There was a significant decline in vaccine serotypes 3 (p=0.002), 5 (p=0.014), 9V (p=0.020), and serotype 14 (p=0.023) comparing the pre and post PCV era. Comparing our findings with other studies, the commonest disease causing serotypes in France were 18C, 14, 6B, 19F, 19A, 9V, 23F, 1, 7F, 9A, 38 [92]. Similar

findings to this study was reported in India; serotypes 14, 1, 19F, 6B, 5, 6A, 9V and 23F were the most frequent causing invasive disease in infants <5years of age [38].

Non-vaccine serotypes that occurred most frequently in our study was 15B/C (9.8%), 12F (9.3%), 16F (7.5%), NEG38 (7.5%) 35B (5.1%), and 7C (4.2%). This is in contrast to the study from the Netherlands and Turkey where non vaccine serotypes 6C, 23B and 11A were most common in children [39, 123].

4.2 The effect of HIV and ART on IPD trends

Comparing the eras, graphically, we observed a descending slope of IPD amongst HIV-infected children from pre PCV to post PCV era. This is in agreement with findings from other studies and highlights that HIV-infected children are more vulnerable to IPD than the uninfected individuals, and that PCV introduction have proven successful at preventing IPD in HIV-positive children [60, 61, 124].

We further evaluated the results of the scaling-up of the HAART program in South Africa on the prevalence of IPD in HIV-positive children. National estimates of HAART coverage among children were extracted and pooled, from two sources [120, 121]. Graphically, we see a decline in the pre PCV era as HAART coverage increases – this may suggest an association between HAART coverage and the burden IPD, but we cannot discount other factors that may also have influenced the burden of disease (such as less overcrowding or improved nutrition). Similarly, a study from Spain reported that the prevalence of pneumococcal bacteraemia dropped from 24.1 cases per 1000 patient-years in the pre-HAART period to 8.2 cases per 1000 patient-years in the HAART period [73]. In our setting, Nunes et al, reported that HAART was successful in reducing IPD in HIV-positive adults by 85.5 percent [53].

4.3 Antimicrobial resistance patterns in children with IPD

We noted a large proportion of missing information. In particular, all data for Ceftriaxone, Ceftriaxone and penicillin for both CNS and non CNS infections were missing up till PCV transition period. Presumably because surveillance antibiotic susceptibility for them had not started.

Vancomycin resistance was not seen in any of the isolates. Apart from Erythromycin which tended to be relatively stable, but, however showed increase in susceptibility with time across

the eras, all the other antibiotics show an increase in susceptibility over the period. This is likely because of a decrease antibiotic pressure since the introduction of PCV into South African EPI Programme. In addition, it could also be the fact that pneumococcal vaccine serotypes contained in the PCVs are more resistant to antibiotics, but there has been increased susceptibility to antibiotics due to their decline, and it is consistent with findings from other countries [38, 125].

Using the MIC breakpoints of penicillin and ceftriaxone according to the CLSI guidelines, for penicillin, we noted 36.8% susceptibility, 3.8% resistance and 30.1% intermediate susceptibility. As for ceftriaxone, 67.2% were susceptible, while 3.0% and 0.5% were intermediate and resistant respectively. Our study findings are in support of a global systematic review that noted significant declines in the number of *S. pneumoniae* showing resistance to penicillin (7.3%, 95%CI: 5.3–9.4) and third-generation cephalosporins (4.5%, 95%CI: 0.3–8.7) [125].

4.4 Clinical characteristics and predictors of mortality

We found no difference in the clinical characteristics between the pre-PCV and post-PCV periods except for less HIV-infected cases and an increased mortality rate in the post PCV period. The decline in HIV infection is likely from successes of the prevention of mother to child transmission Programme in South Africa over the study period. The findings of increased mortality need to be interpreted with caution as 45% of children had missing outcomes in the pre PCV period.

Lastly, we determined predictors of mortality in children hospitalized with IPD at CHBAH. Children had an increased odds of death if they presented with pneumococcal meningitis – these findings were consistent with the literature [42, 126]. Children also had an increased odds of death if they presented in the post PCV era. We were unable to ascertain whether these children were unvaccinated or presented with more virulent non-vaccine serotypes causing disease in the PCV era.

4.5 Limitations

This study had several limitations. Our dataset was exclusively hospital-based cases and our findings may likely underestimate the burden of disease in the population. Secondly, as this was a retrospective study of available datasets, there were many missing variables. Thirdly, we were unable to ascertain the inoculum amount of blood culture and the method used during sampling, therefore cannot properly place the rates of contaminations and culture yields. Lastly, the absence of vaccination history from our data set is a further limitation of this study.

Chapter 5 - Conclusion

5.1 Conclusions and Recommendations

We report that PCV introduction in the South African EPI had resulted in minimizing the burden of IPD in hospitalized children at the CHBAH, which is consistent with national and global findings. These findings were consistent across all age groups and in HIV-infected children. Furthermore, we show declines in vaccine serotypes. Importantly, PCV has resulted in less antibiotic pressure and increase in susceptibility to various antibiotics over time.

We recommend that a pneumococcal serotype and antimicrobial surveillance continue in the post-vaccine period to monitor any change in resistance patterns and emergence in non-vaccine serotypes causing disease. This is particularly important as South African EPI transitions from PCV13 to PCV 10 in 2024.

Chapter 6 - References

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Appendices

Appendix 1: Study variables

Study number: M201057

Number	Variable	Data Source
1	Age	CDW-NHLS GERMS-SA CHBAH-PEADS
2	Gender	CDW-NHLS GERMS-SA CHBAH-PEADS
3	Height	CHBAH-PEADS
4	Weight	CHBAH-PEADS
5	CD4 Count	CHBAH-PEADS
6	Viral load	CHBAH-PEADS
7	HIV Status	CHBAH-PEADS
8	Specimen type	CDW-NHLS
9	Previous Admin	CHBAH-PEADS
10	Outcome	CHBAH-PEADS
11	Isolates	CDW-NHLS GERMS-SA
12	Antimicrobial profile	CDW-NHLS GERMS-SA
13	Serotype	GERMS-SA

Appendix 2: Approval of Title by Postgraduate Research Office of Faculty of Health Sciences

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Dear Mr Ifeanyi Erne Private
Bag 3 Wits, 2050 Fax:
027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

21 June 2021
Person No:
1637492
PAG

Mr I Eme
15 Caro Street
Robertsham
2091
South Africa

Master of Science in Medicine: Approval of Title

We have pleasure in advising that your proposal entitled Effect of pneumococcal conjugate vaccine on invasive pneumococcal disease in children hospitalised at Chris Hani Baragwanath Hospital over a 15 year period. has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix 3: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Mr I Eme

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M201057

NAME: (Principal Investigator)	Mr I Eme
DEPARTMENT:	School of Patholgy Department of Clinical Microbiology and Infectious Diseases Medical School University
PROJECT TITLE:	*Effect of pneumococcal conjugate vaccine on invasive pneumococcal disease in children hospitalized at Chris Hani Baragwanath Hospital over a 15 year period*
DATE CONSIDERED:	2020/10/30
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professors Z Dangor, SA Madhi & Dr J Wadula
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/11/16

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

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 submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in October and therefore reports and re-certification will be due in the month of **October** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator — Date: 17/11/2021

Appendix 4: Approval for laboratory data access from NHLS

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

01 December 2021

Applicant: Ifeanyi Eme
Institution: NHLS
Department: Medical Microbiology
Email: ifeanyi.eme@nhls.ac.za
Tel: 011 489 8730 **Cell:** 073 430 6727

CC: Jeanette Wadula
Senior Pathologist

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"EFFECT OF PNEUMOCOCCAL CONJUGATE VACCINE ON INVASIVE PNEUMOCOCCAL DISEASE IN CHILDREN HOSPITALISED AT CHRIS HANI BARAGWANATH HOSPITAL OVER A 15 YEAR PERIOD" Ref No: PR2010554** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix 5: Permission letter to access data from GERMS-SA dataset

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

DIVISION OF PUBLIC HEALTH SURVEILLANCE & RESPONSE
1 Modderfontein Road, Sandringham, 2192
Tel: +27 (0)11 386-6400

Fax: +27 (0)11 386 6221
12 December 2018

Prof Clement Penny
Chairperson
Wits HREC (Medical)
University of the Witwatersrand

Dear Prof Penny

MSC.MED protocol for IFEANYI EME (MRA05)

As Principal Investigator of the GERMS-SA surveillance programme, I hereby give permission for Dr Eme to have access to the GERMS-SA dataset for invasive *Streptococcus pneumoniae* in children <14 years of age from January 2004 to December 2018 for secondary data analysis for completion of his MMED.

He will be granted access to the following variables that are required for his analysis for his masters entitled: IMPACT OF THE PNEUMOCOCCAL CONJUGATE VACCINE (PCV) ON THE BURDEN OF INVASIVE PNEUMOCOCCAL DISEASE (IPD) IN CHILDREN HOSPITALISED AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL: A 15 YEAR TREND ANALYSIS.
UniqueID, date of birth, age category, admission date, specimen collection date, diagnosis, outcome, specimen type, serotype, antimicrobial susceptibility testing, discharge date, predisposing conditions, HIV status, first –line antibiotics given.
The data will be available to him on presentation of the ethics clearance certificate from Wits HREC (Medical).

Yours sincerely

Dr Vanessa Quan
Principal Investigator GERMS-SA surveillance programme
Division of Public Health Surveillance and Response
011 386 6012 vanessaq@nicd.ac.za

Appendix 6: Clearance certificate from Medical Advisory Committee, CHBAH

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE
CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 28th September 2021

TITLE OF PROJECT:

Effect of Pneumococcal Conjugate Vaccine on invasive Pneumococcal Disease in Children Hospitalised at the Chris Hani Baragwanath Academic Hospital over 15 year Period.

UNIVERSITY: Witwatersrand

Principal Investigator: Mr I Eme
Department: NHLS

Supervisor : Prof S Madhi / Prof Z Dangor / Dr J Wadula

Permission Hedd Department (where research conducted): Yes

NHRD No. 202109 036

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of Witwatersrand.
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

ittee Approval.

Approved/Not Approved
Hospital Management
Date: 29/09/2021

Recommended
(On behalf of the MAC)
Date: 28/09/2021

(On alf of the MAC)