HAEMOPHILUS INFLUENZAE BACTERAEMIA IN ADULTS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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0300130P

A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Internal Medicine

Johannesburg 2018

DECLARATION

I, Hajira Ismail declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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ABSTRACT

Introduction

Haemophilus influenzae type b (Hib) was the major cause of meningitis in young children worldwide before the conjugate Hib vaccine became available in the late 1980s as part of national immunization programs. However, Hib vaccination does not confer protection against other serotypes of *H. influenzae* (Hi). The Hib vaccine was first introduced in The Gambia in 1997 and was introduced in South Africa as part of the National Expanded Program on Immunization (EPI) in 1999. Between 2000 and 2004 rates of Hib disease decreased significantly in <1 year old and <5 year old children, indicating the impact of the Hib vaccine introduced in 1999. Comparison of pre- and post-vaccination burden of disease data in adults is not yet available in Sub-Saharan Africa, including South Africa. In 2008, infants that were vaccinated from birth (i.e. in 1999) and who may have become infected with Hib are currently still considered as paediatric patients. This signifies that there is a large gap in data regarding the incidence as well as clinical and microbiological characteristics of Hi disease in adults. A literature survey has revealed that there are few studies that describe and highlight the profile of Hi infection in adults and no recent studies of adult bacteraemic disease in South Africa have been published. Therefore the aim of this study was to describe the clinical and microbiological characteristics of all adult cases with Hi bacteraemia at Chris Hani Baragwanath Academic Hospital (CHBAH) between January 2008 and December 2014.

Study Design and Methods

A single centre retrospective cohort study at CHBAH in Soweto was performed on patients ≥18 years of age who had a positive blood culture for Hi, from 2008-2014. Patients were identified from the 'Group for Enteric, Respiratory and Meningeal Disease Surveillance' in South Africa (GERMS-SA) database.

Results and Discussion

A total of 45 cases were identified. There were 6-9 cases annually until 2014 when there was only one. The median age was 40 years (IQR=20) and 23/45 (51%) were female.

The foci of infection comprised pneumonia (33), bacteraemia without a focus (7), septic arthritis (3), meningitis (2), empyema (1), vasculitis (1) and cellulitis (1). A total of 35 (77%) cases had pre-disposing diseases of whom 33 (of 34 tested) were HIV-infected. Eleven (25%) had pre-existing diseases other than HIV that included chronic lung disease (4), diabetes mellitus (2), cancer (1), COPD (1), chronic kidney disease (1), diabetes with cancer (1), diabetes with chronic kidney disease and congestive cardiac failure (1). Twenty one cases had active TB. Sixteen (36%) patients died (12 early <2 days from admission). Seven isolates were resistant to ampicillin, but none to amoxycillin-clavulanate. A total of 23/43 (53%) cases were non-typeable Hi, 10/43 (23%) Hib and 10/43 (23%) were of the non-Hib strains. No adults with Hib were identified after 2010.

Conclusions

H. influenza bacteraemia was an uncommon infection which disproportionately affected the HIV-infected and had a high mortality. There was a steady small number of 6-9 cases annually until a sudden marked decrease in 2014 with no Hib cases found in the last 4 years. It would be important to know if this decrease has been sustained. In addition, there was limited bacterial resistance and non-vaccine serotypes predominated.

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ACRONYMS

AIDS – Acquired immunodeficiency syndrome

ART – Antiretroviral therapy

CRF – Case report form

CDW – Central Data Warehouse

CSF – Cerebrospinal fluid

EPI – Expanded Programme on Immunization

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

Hib – *Haemophilus influenzae* type b

HIV – Human immunodeficiency virus

NHLS – National Health Laboratory Service

NICD – National Institute for Communicable Diseases

NTHi – Non-typeable Haemophilus influenzae

1. INTRODUCTION

1.1. Overview of the *Haemophilus influenzae* Bacterium

Haemophilus influenzae (*H. influenzae*) is a pleomorphic, non-spore-forming, fastidious, facultative anaerobic gram-negative coccobacillus bacterium that requires two erythrocyte growth factors viz. X (hemin) and V (nicotinamide-adenine-dinucleotide) that are released following lysis of red blood cells. *H. influenzae* causes *H. influenzae* disease (Murphy, 2010) but despite its name, it does not cause influenza (MacNeil et al., 2011; Moxon, 1986). Studies have shown that the bacterium can spread through the air via respiratory droplets. However, the ability to spread is limited, although having close contact with infected individuals may lead to pandemics (Ulanova and Tsang, 2009; CDC, 2016; 2015). The incubation period of *H. influenzae* disease is not certain, but could be as short as a few days (Erwin and Smith, 2007).

Nasopharyngeal colonization of encapsulated *H. influenzae* Type b is uncommon and occurred in approximately 2-5% of children in the pre-vaccine era and even less after widespread vaccination. A larger bacterial load or the presence of a concomitant viral infection can potentiate the infection. The colonizing bacteria invade the mucosa and enter the bloodstream. The presence of antibodies, complement, and phagocytes determines the clearance of the bacteremia. When the bacterial concentration exceeds a critical level, it can disseminate to various sites, including meninges, subcutaneous tissue, joints, pleura, pericardia, and the lungs.

The spread of bacteria by direct extension to the eustachian tubes causes otitis media. Spread to the sinuses leads to sinusitis. Spread down the respiratory tract results in bronchitis and pneumonia. Eustachian tube dysfunction, antecedent viral upper respiratory tract infection (URTI), foreign bodies, and mucosal irritants, including smoking, can promote infection in patients with underlying chronic obstructive pulmonary disease (COPD) or cystic fibrosis (CF). Persons at high risk for invasive *H. influenzae* disease include adults who are >65 years of age and those with asplenia, sickle cell disease, complement deficiencies, Hodgkin's disease, congenital or acquired hypogammaglobulinemia, and T-cell immunodeficiency states such as HIV infection (Takala et al., 1990; Sarangi et al., 2000; Dworkin et al., 2007).

1.2. Description of *H. influenzae* Strains

Pfeiffer (1892) was the first to isolate *H. influenzae* as a potential cause for influenzae but was later clarified by Pittman (1931) who identified the six encapsulated types of *H. influenzae* based on the presence of capsular polysaccharides with encapsulated strains known to be more invasive. Encapsulated strains are reactive with typing antisera, whereas unencapsulated strains are nonreactive and are thus referred to as non-typeable *H. influenzae* (NTHi).

The capsule is the major virulence factor and antigen of *H. influenzae* since it prevents complement-mediated bacteriolysis in the absence of opsonizing antibody (Agrawal and Murphy, 2011; Wenger and Ward, 2004; MacPherson et al., 1946; Leidy et al., 1960). Based on the antigenic properties, six serotypes of encapsulated *H. influenzae* have been

identified and designated a through f. Among the strains, serotype b or *H. influenzae* type b (Hib) causes the majority of invasive *H. influenzae* disease in individuals that have not been vaccinated by the conjugate Hib vaccine. In addition, NTHi frequently colonizes the nasopharyngeal mucosa as well as the lower respiratory tract in up to 80% of children and adults and, in some instances, the conjunctivae and genital tract. This is considered crucial for the development of natural immunity against the pathogen, simultaneously with exposure to cross-reactive environmental antigens (Murphy, 2010; Moxon, 1986).

NTHi is also known to colonize the sputum of adults with chronic bronchitis. The presence of a bacterial pathogen in the sputum of adults with COPD does not establish the etiology of exacerbations because the same pathogens colonize COPD patient airways during clinically stable periods. However, several studies have supported the conclusion that bacteria cause exacerbations of COPD and that NTHi is the most common bacterial cause of exacerbations of COPD (Agrawal and Murphy, 2011). These pathogens release highly active antigens that induce enhanced airway inflammation, which is a characteristic feature of COPD (Faden et al., 1995; Abdullahi et al., 2008; Oh et al., 2008; Hall and Lyman, 2006; de Carvalho et al., 2011). Keefer and Rammelkamp (1942) confirmed that *H. influenzae* was a cause of pneumonitis in adults from a study that described a 30-year-old man with lobar pneumonia with *H. influenzae* isolated from blood and sputum.

In addition, this study noted that the majority of invasive *H. influenzae* disease was caused by the encapsulated species of Hib, with its polyribosyl ribitol phosphate (PRP) capsule. It accounts for more than 95% of invasive *H influenzae* diseases in children <5 years of

age and 50% of invasive diseases in adults, including bacteremia, meningitis, cellulitis, epiglottitis, septic arthritis, pneumonia, and empyema. Less-common invasive Hib infections include endophthalmitis, urinary tract infection, abscesses, cervical adenitis, glossitis, osteomyelitis, and endocarditis. In South Africa and abroad, the number of NTHi cases has been increasing as shown in **Figures 1 and 2** most probably in response to the Hib conjugate vaccine introduction (Wenger and Ward, 2004; Atkinson et al., 2009; Chang et al., 2010; von Gottberg et al., 2006; 2012; Dajani, 1979; Bisgard et al., 1998; Roberts et al., 2011; Levine et al., 1997).

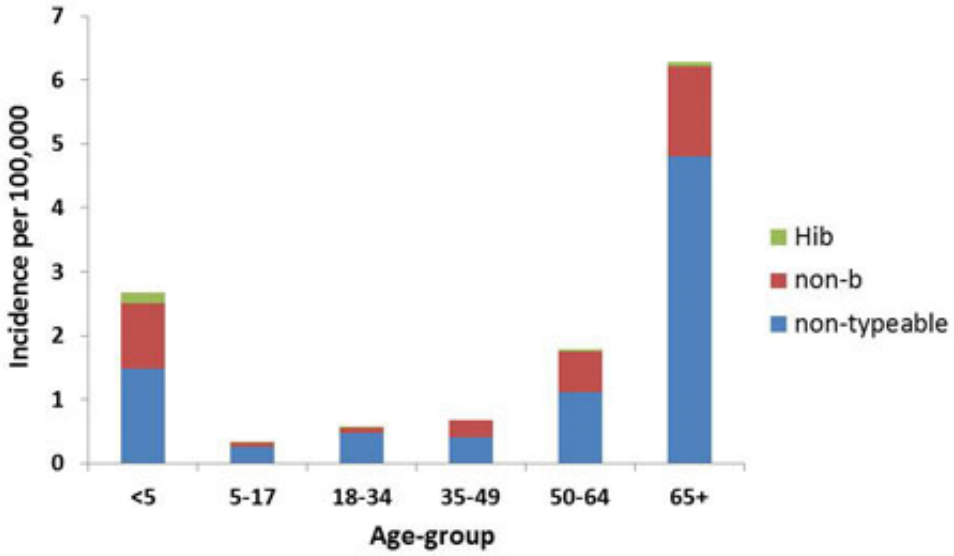


Figure 1: Bar graph showing the estimated incidence of *H. influenzae* in the USA by age-group and serotype from 2001-2012. *(Source: National Center for Immunization and Respiratory Diseases, Division of Bacterial Diseases, CDC; http://www.cdc.gov/hi-disease/about/types-infection.html).*

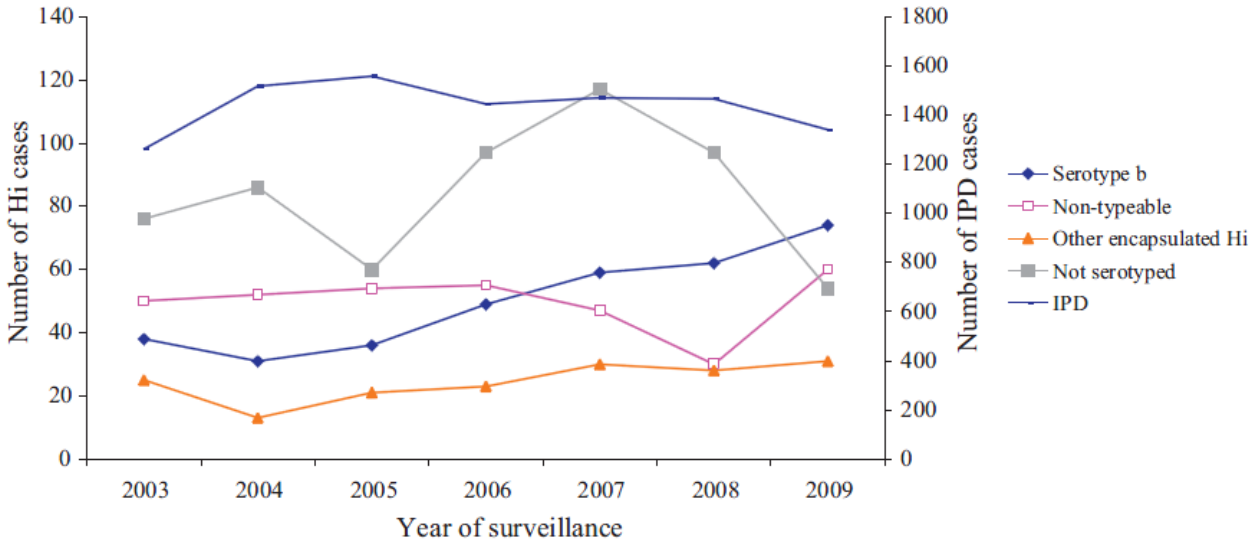


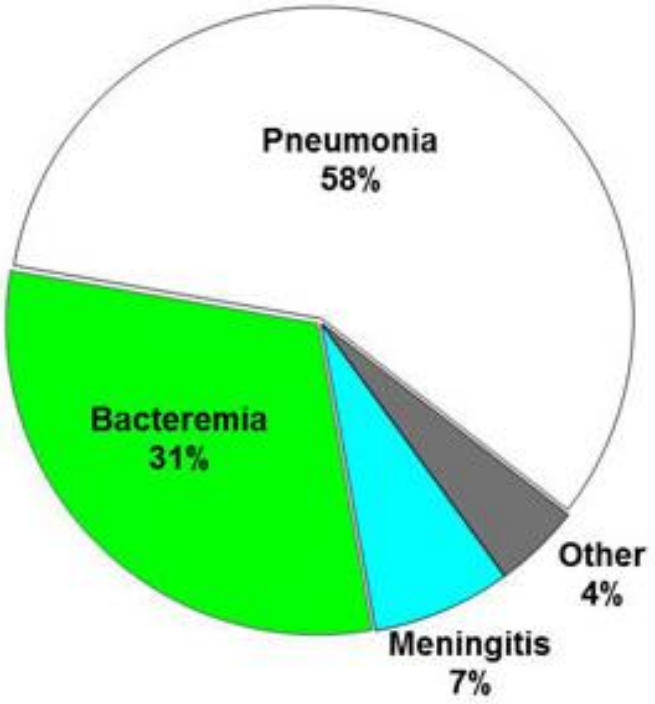
Figure 2: Profiles showing the number of reported cases of invasive *H. influenzae* disease in children <5 years (n=1455), by serotype and year in South Africa from 2003–2009. Invasive pneumococcal disease (IPD) documented for children <5 years is depicted for the same time period. Hib= serotype b; NTHi= non-typeable; other encapsulated Hi= serotypes a, c–f *(Source: von Gottberg et al, 2012).*

The other encapsulated strains of *H. influenzae* occasionally cause invasive disease such as *H influenzae* type a (Hia) which has been known to cause invasive disease such as meningitis and which is clinically indistinguishable from that caused by Hib. When *H. influenzae* invades the spinal fluid or blood (or other sterile sites) it is referred to as 'invasive disease' which is usually severe and can be fatal. It includes clinical diseases such as meningitis, bacteremia or sepsis, epiglottitis, pneumonia, septic arthritis and cellulitis (Bachiller et al., 2000).

The most frequently described childhood *H. influenzae* infection includes meningitis, epiglottitis, otitis and arthritis (Rubach et al., 2011) **(Figure 3)**. Studies have shown that NTHi strains can also cause invasive *H. influenzae* disease. However, they are known to

cause the bulk of respiratory infections, otitis media (mainly in infants and young children) as well as pneumonia and chronic bronchitis in geriatrics (Stupka et al., 2009).

In South Africa, all childhood diseases for which vaccines are available are scheduled within the routine National Expanded Program of Immunization (EPI). The vaccine is administered at 6, 10, 14 and 72 weeks as part of the EPI. Invasive *H. influenzae* is a notifiable disease. This assists with monitoring the number of cases still occurring and enables health authorities to control any outbreaks with mass immunization. This is particularly important for any case of suspected invasive *H. influenzae*. However, diseases such as bronchitis, sinusitis, and otitis media are non-invasive and are therefore not considered as notifiable diseases. Invasive Hib disease affects mostly infants and young children who have not been vaccinated and manifests as an array of acute pyogenic infections that are often accompanied by bacteraemia (CDC, Epidemiology and Prevention of Vaccine-Preventable Diseases, Hib, 2009).



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1.3. Diagnosis of *H. influenzae* Bacteraemia

A provisional diagnosis of bacteraemia is usually prompted by a history and clinical findings. A confirmed diagnosis requires bacterial cultures of blood. For meningitis, CSF culture is the gold standard (Levine et al., 1997). The detection of capsular antigens in serum, CSF or concentrated urine using immune-electrophoresis, latex agglutination or enzyme linked immune-sorbent (ELISA) assay may be diagnostic and can be found in 90% of culture proved cases of invasive *H. influenzae* meningitis.

The clinical microbiology interpretation of results still depends on the quality of the specimens received for analysis. The volume of blood obtained for each blood culture

request is the most important variable in recovering bacteria from patients with bacteraemia and therefore the impact of correct specimen management on patient care is crucial as it determines patient outcomes and influences therapeutic decisions. The diagnosis of bacteraemia is one of the most vital functions of clinical microbiology laboratories. For most etiologic agents of bacteraemia (including the recovery of fastidious *H. influenzae*), conventional blood culture methods provide results within 48 hours and incubation for >5 days is seldom required when modern automated continuous-monitoring blood culture systems and media are used [1, 2].

1.4. Treatment of *H. influenzae* Bacteraemia

Without treatment, *H. influenzae* infection can be fatal. Ampicillin or amoxicillin over a period of 10 days is often satisfactory for less severe *H. influenzae* infections. In South Africa an amoxicillin-clavulanic acid combination is selected for treating the majority of adult cases with pneumonia. Most cases of *H. influenzae* bacteraemia require hospitalization. Parenteral 3rd generation cephalosporins (mainly cefotaxime or ceftriaxone) are used as initial therapy when meningitis is known or suspected. The site of infection as well as the clinical response determines the duration of antibiotic therapy (Chang et al., 2010; Roberts et al., 2011). When *H. influenzae* causes a non-invasive infection, oral antibiotics may be given to treat and prevent complications (Robbins et al., 1973; Murdock et al., 1959; Brumfitt et al., 1957; Holdaway and Durk, 1967).

Expanded-spectrum cephalosporins have become standard therapy for meningitis due to *H. influenzae* since the emergence of chloramphenicol-resistant and β-lactamase-

producing *H. influenzae* strains. β-lactamases have a detrimental effect in that they protect the *H. influenzae* bacteria and interfere with the therapy of infection with penicillins, resulting in a sheltering effect. Amoxy-clav is suitable for non-CSF infections. The rate of isolation of β-lactamase-producing *H. influenzae* strains has steadily increased over the last decades and has not decreased since the introduction of the Hib conjugate vaccine. The rates of isolation of β-lactamase-producing strains vary worldwide and are higher for NTHi strains. Antibiotic therapy has changed to cefotaxime or ceftriaxone combined with meropenem for meningitis patients in regions with β-lactamase-Negative Ampicillin-Resistant (BLNAR) *H. influenzae*.

1.5. The *H. influenzae* Type b Vaccine Era

In the USA and other countries, invasive *H. influenzae* disease was recognized as a nationally notifiable disease in 1991. Since then several *H. influenzae* disease surveillance studies have been completed over a few decades to, 1) monitor the effectiveness of global Hib immunization programs, 2) assess the progress made toward eliminating Hib disease, 3) define the frequency and epidemiology of invasive *H. influenzae* disease as well as non-Hib disease, 4) detect the emergence of non-type b *H. influenzae* disease and 5) determine appropriate verification and validation criteria for serotyping. Hib was the major cause of meningitis in young children before the availability of the conjugate Hib vaccine in the 1980s as part of national immunization programs in various countries around the world (Bisgard et al., 1998) **(Figures 4 and 5).** The Hib vaccine does not protect individuals against other serotypes (a, c-f) of *H. influenzae*. The Hib vaccine was first introduced in The Gambia in 1997 and was introduced in South

Africa as part of the National Expanded Program on Immunization (EPI) in 1999 with an estimated coverage of 92% by 2004 (Howie et al. 2013; von Gottberg et al., 2006; 2012). Between the years 2000 and 2004 the rates of invasive Hib disease decreased significantly, by 65% and 71% in <1-year-old and <5 year old children, respectively (Visser and Hoosen, 2014). This clearly demonstrated the impact of the Hib vaccine introduced in South Africa during 1999. Each vaccine consists of a carrier protein covalently conjugated to the polyribosylribitol phosphate (PRP) of the outermost layer of the microorganism. The process of conjugation changes the polysaccharide from a T-cell-independent to a T-cell-dependent antigen and significantly improves immunogenicity in young children.

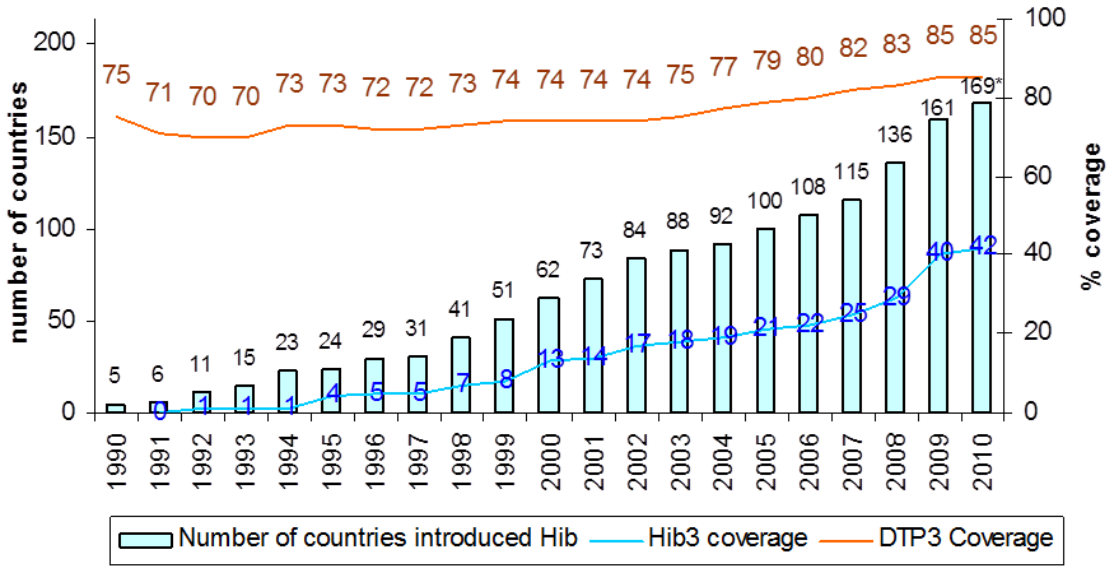


Figure 4: Number of countries that introduced the Hib vaccine and global infant DTP3 and Hib3 coverage from 1990-2010 *(Source: WHI/IVB Database & WHO/UNICEF coverage estimates 1980-2010, July 2011. *For all years, country totals include partial and full introductions, and for 2010, figure includes 3 countries that had partial introduction).*

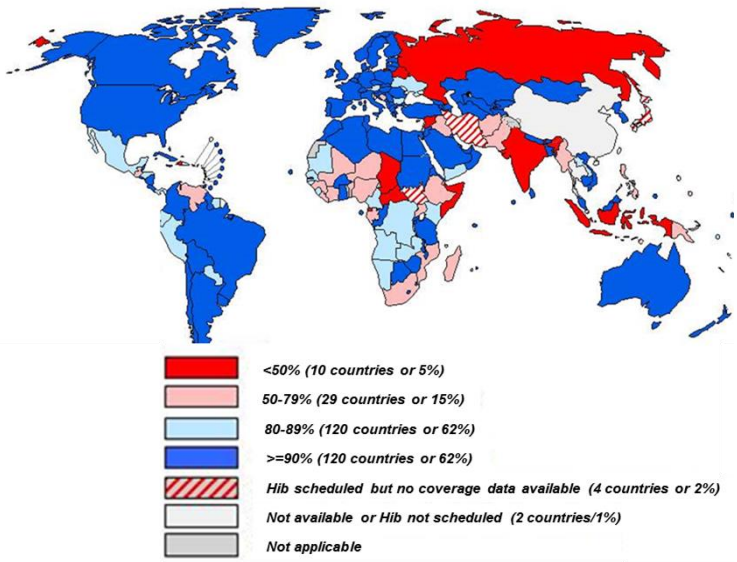


Figure 5: Immunization coverage with third dose of Hib containing vaccines in infants during 2014. *(Source: WHO/UNICEF coverage estimates 2014 revision, 24 July 2015. 194 WHO member states).*

During the year 2008-2014 (which are the years for this study), infants that were vaccinated from birth (i.e. in 1999) and who may have become infected with invasive Hib are currently still considered as paediatric patients. The first vaccinated adult cohort will only be available in 2017. There is a gap in data regarding the incidence as well as clinical and microbiological characteristics of invasive *H. influenzae* disease in adults (who were non-vaccinated at the time) who are being infected with *H. influenzae* **(Figure 6).**

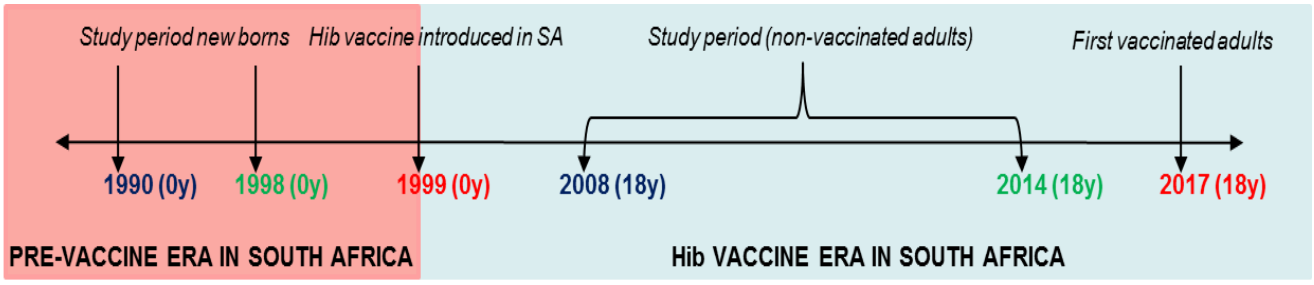


Figure 6: Timeline describing the pre- and post-Hib vaccination era in South Africa.

In the pre-Hib vaccine era, the colonization rate of Hib in infants and children was 3-5%. The rate is close to zero in countries that employ widespread Hib vaccination. The reduction of circulating strains in the population results in a herd effect, contributing importantly to the efficacy of the vaccine. Despite the immunogenicity and high efficacy of Hib conjugate vaccines in infants, a small number of Hib infections occur in vaccinated children. Children who experience Hib disease despite vaccination appear to have a defect in immunological priming, resulting in lower-avidity antibodies to Hib capsular polysaccharide. Since the introduction of vaccination, the number of cases of Hib disease in non-immunized individuals in industrialized nations has decreased by >90%, with reductions of >95% reported for meningitis as shown in **Table 1.** Reductions of 50-75% have been seen even in countries where vaccine uptake has been only moderate. In South Africa, National laboratory-based surveillance data established within the first five years of introduction of the conjugate Hib vaccine in the routine child immunization schedule between July 1999 and June 2004 revealed a decrease in Hib disease among South African children following conjugate vaccine introduction and identified cases of non-typeable disease associated with HIV infection. The number of reported Hib cases among children <1 year of age decreased by 65%, from 55 cases in 1999-2000 to 19 cases in 2003-2004 (von Gottberg et al., 2006).

Table 1: Incidence of meningitis caused by Hib in children aged 0-5 years in selected areas of the world before and after introduction of the Hib conjugate vaccine.

Geographic area (yr of comparison)	No. of cases/100,000 population	
	Prevaccination	Postvaccination
United States (1987 vs 1995)	54	<1
Canada (1985 vs 1994)	~44	<1
Brazil (1988–1996 vs 1997)	22	10
Chile (1995 vs 1998)	40	<2
Uruguay (1992-1993 vs 1995)	17–22	1
Scandinavia (1970s vs 1995)	31	<1
Austria (1991 vs 1993–1996)	11	<1
Netherlands (1970s vs 1993-1994)	22–40	0.3
Spain (1993–1995 vs 1997)	14	~0
Switzerland (1976–1990 vs 1991–1993)	25	8
United Kingdom (1991-1992 vs 1993-1994)	15	0.6
Israel (1989–1992 vs 1995)	18	<1
Australia (1991-1992 vs 1993-1994)	21	6
The Gambia (1990–1993 vs 2002)	60	0
Kenya (2000-2001 vs 2004-2005)	66	7.6
Malawi (1997–2002 vs 2005)	20–40	0
Uganda (2001 vs 2003–2006)	42	<3

Host defenses include the activation of the alternative and classical complement pathways and antibodies to the PRP capsule. The antibody to the Hib capsule plays the primary role in conferring immunity. Newborns have a low risk of infection, likely because of acquired maternal antibodies. When these transplacental antibodies to the PRP antigen wane, infants are at high risk of developing invasive *H. influenzae* disease, and their immune responses are low even after the disease. Therefore, they are at high risk of repeat infections since prior episodes of *H. influenzae* do not confer immunity. By age 5 years, most children have naturally acquired antibodies. The Hib conjugate vaccine induces protection by inducing antibodies against the PRP capsule. However, the Hib conjugate vaccine does not provide protection against NTHi strains. Since the widespread use of the Hib conjugate vaccine, NTHi has become a more prominent pathogen.

1.6. Literature Survey

A survey of the literature has revealed that there are not many studies that have described and highlighted the profile of *H. influenzae* infection particularly in adults. A summary is provided in **Table 2** that reflects the clinically significant data describing and highlighting the profile of *H. influenzae* infection in adults and children. The majority of these studies were undertaken in the USA and have either been prospective or retrospective nationwide surveillance-type studies of invasive *H. influenzae* mainly in children with a few studies reporting data on adult cases. Furthermore, a large proportion of these studies have reported that NTHi was the major serotype for the isolates obtained from either a blood or CSF sample as a source. Case mortality rates ranged from 1-59% with many studies reporting several predisposing illnesses for bacteraemia.

Table 2: Summary of the epidemiologically significant data describing and highlighting the profile of *H. influenzae* infection

Source Country	Study Details	Number of Cases / Incidence	Predisposing Illness	Type of Disease	Mortality	Serotype of Isolates	Specimen Source	References
STUDIES OF ADULTS ONLY								
USA	Invasive Hi in adults ≥65 years reported to Illinois Department of Public Health (Springfield) from 1996-2004	770 cases 1.1-3.9 /10^5	None reported	80.5% bacteremia, 13.6% meningitis, 5.8% other	12.7%	14.9% Hib, 30.8% non-Hib (27.3% Hie, 59.6% Hif), 54.2% NTHi	Blood, CSF	Dworkin et al., [14]
UK	Retrospective study on Hi in adults of 5 NHS regions from 1990-1995	446 cases 0.73/10^5	CLD (64), malignancies (42), CVD (39)	65% pneumonia with bacteraemia 18% bacteraemia, , 9% meningitis	59%	35% Hib, 2% Hie, 7% Hif, 57% NTHi	Blood, CSF	Saranji et al., [15]
Finland	A prospective nationwide surveillance of invasive Hib of adults in Finland from 1985-1988	31 cases 22/10^5	71% occurred in patients with severe underlying conditions	Septicemia (13), pneumonia (7) epiglottitis (6), meningitis (3), arthritis (2)	26%	Hib	Blood, CSF	Takala et al.
USA	Hib with and at risk for AIDS at a municipal hospital in Bronx (NY) from 1983-1991	27 cases	Hib with AIDS (10), AIDS (7), IV drug abuse (9), CLD (10)	Bacteraemia (10), pneumonia (9)	Not reported	15 Hib, 2 Hic, 2 Hif, 1 Hia, 9 NTHi	Sputum, blood	Casadevall et al.,
USA	Prospective surveillance of Hi at hospital and referral bacteriology labs in Atlanta from 1988-1990	194 cases	CLD (4.9/10^5), HIV (41/10^5), malignancy	70% pneumonia, obstetric infection, epiglottitis, tracheobronchitis, meningitis (1)	Not reported	50% Hib, 47.5% NTHi, 2.5% Hif	Blood, CSF, joint, pleural fluid	Farley et al.,
USA	Identification of adult Hi pneumonia at Beth Israel Medical Center in NYC with risk factors for AIDS from 1984-1986	413 cases	AIDS risk factors (34), IV drug abuse (23), homosexuals (6), AIDS (9), developed AIDS (4), ARC (13), P. carinii pneumonia (4), KS (4)	100% pneumonia, 53% chest pain, 47% dyspnea,	2.94%	1 Hib, 3 NTHi	Blood (4), sputum	Schlam et al.,
USA	Invasive Hi disease in adults >65, 2011	299 cases	COPD (36%), CAD (34.6%), CHF (32.8%), DM (28.4%)	81% bacteramic pneumonia, 13% bacteraemia, 4% meningitis	19%	78% NTHi	Blood, CSF	Blain et al.
USA	Hi pneumonia in adults at the Ben Taub General, Jefferson Davis and Houston Veterans Administration Hospitals from 1967-1977	41 cases	None reported	Bacteraemia (27), haemophilus bacteremia (23) with peumonia.	11%	82% Hib, 1% Hie, 2% NTHi	Blood, pleural fluid, tracheal aspirate, sputum	Wallace et al.,
USA	Review of 3 hospitals of the University of Colorado Medical Center in >20 year olds with coexistence of pneumonia from 1970-1974	24 cases	54% CLD, treated TB (1), bronchiectasis (1), 29% RTI, 50% alcoholism, LD (4), Laennec's cirrhosis (3), biliary obstruction (1), DM (1), Parkinson's (1), Bruton's hypo-γ-globulinemia (1), sarcoidosis (1), SCC (1), fever (6), afebrile (2), leucopenia (3)	75% bronchopneumonia, pleural disease, empyema, 35% pneumonia	33%	Not reported	Blood, pleural fluid, sputum	Levin et al.,
Taiwan	Retrospective survey of invasive Hi in adults in a teaching hospital from 1999-2002	15 cases	Not reported	Pneumonia (5), empyema (2), PID (2), peritonitis (2), periorbital cellulitis with abscess (2), endophthalmitis (1), primary bacteremia (1)	Not reported	Hib (2), NTHi (8)		Wang et al.,

USA	Population-based study of the epidemiology and outcomes of invasive Hiin Utah adults from 1998-2008	121 cases with $0.66/10^5$	DM (24), immunodeficiency (15), CLD (14), cancer (11), asthma (3), COPD (3), CHF (3), LD (3), CKD (3), iv drug abuse (1), pneumonia (46)	83% bacteraemia, 12.4%, meningitis, 4.6% other sterile sites	22% (66% in >65 yr olds)	43% NTHi, 9% Hib, 49% non-Hib, 25% Hif, 15% Hia	Sterile site, blood, CSF, joint, pleural, or pericardial fluid	Rubach et al.,
STUDIES OF ADULTS AND CHILDREN								
India	Prospective surveillance of acute Hi at 6 academic referral Indian Hospitals from 1993-1997	121 cases (18 adults)	Crohn's disease (1), hydrocephalus (1), SCC (1)	23.1% pneumonia, 23.1% abscess, 15.4% meningitis, 15.4% septicemia and fever, 15.4% other , 1% peritonitis	11%	97.5% Hib, Hia (1), Hid (1), Hie (1)	Blood, CSF	IBIS Study Group
Canada	Invasive Hi isolated at University of Manitoba Health Sciences Centreand Children's Hospital in Winnipeg, Manitoba from 2000-2004	53 cases (17 adults)	Not reported	Not reported	Not reported	Hia (26), NTHi (20), Hib (3), Hic (1), Hid (10, Hif (1)	Blood, CSF	Tsang et al.
USA	A review of Hi data from Alaska and Northern Canadaat CDC's Arctic Investigations Program from 2000-2005	138 cases	Not reported	33% meningitis, 29% pneumonia, 12% septic arthritis	Hia 5-15%	33% NTHi, 48% Hia, 31% Hib, 14% Hif5% Hid, 2% Hic, 1%Hie	Blood , CSF	Bruce et al.
USA	Surveillance for invasive Hi disease through Active Bacterial Core sites from 1989-2008	4838 cases (3339 adults)	Not reported	53.3 pneumonia, 34.7% bacteremia, 7.6% meningitis	15.3%	Hia, Hie, Hif	Blood, CSF, joint, pleural fluid	MacNeil et al.,
Argentina	Hi at 90 sites of National Lab Network for Meningitis and Acute Bacterial Respiratory Infection from 2005-2010	313 cases (34 adults)	Not reported	40.3% pneumonia, 30% meningitis, 26.5% bacteremia	Not reported	63.3% NTHi, 20.1% Hib, 11.2% Hia, 4.8% Hif, 2.6% other	Sterile sites	Efron et al.,
Sweden	Hi isolates in 3 Swedish regions with a population of ± 3 million from 1997-2009	410 cases	68% immunodeficient Hif, 30 cancers, 4 COPD, 3 CKD, 2 DM, 1 CVD, 1 organ transplant, 2 chemotherapy	70% pneumonia, meningitis, epiglottitis, soft tissue infections, cholangitis, sepsis (48%), septic shock (59%), immunodeficiency (37%)	8%	NTHi (191), Hib (29), Hif (44), Hie (1), non-Hib (3)	Blood and CSF	Resman et al.,
Australia Canada Denmark	Surveillance for Hi bacteremia in 7 regions of the International Bacteraemia Surveillance Collaborative from 2000-2008	406 cases $1.31/10^5$	25% cancer, 24% CLD, 12% DM, 10% CHF, 8% MI, 7% LD, 7% CVD, 6% CKD, 5% PUD, 4% PVD, 3% arthritis, 3% dementia, 1% HIV	43% RTI, 38% no focus of infection, 6% polymicrobial	20%	6% Hib, 17% Hiac-f, 75% NTHi	Blood	Laupland et al.,
Canada	Invasive Hi disease in British Columbia with strain biology and population at risk after >15 yrs of Hib vaccine from 2008-2009	98 cases	Not reported	Not reported	Not reported	75% Hib (>21 yrs), 80% Hia (2 adults >50 yrs), 83% NTHi (>20 yrs), Hif (only in adults)	Blood, CSF, CSF and blood, sterile sites	Shuel et al.,
Canada	Epidemiology of invasive Hi infection in Ontario in provincial Public Health Labs from 1989-2007	1455 cases	Not reported	Sepsis	Not reported	70.3% NTHi, 13.6% Hif, 62.3% NTHi, 20.4% Hib, 11.6% Hif, 3.2% Hie, 2.1% Hia, 0.2% Hic, 0.2% Hid	Blood, CSF, sterile sites	Adam et al.,
USA	Population- and lab surveillance for Hi for the Active Bacterial Core surveillance of the CDC's EIP Network from 1999-2008	4839 cases (4011 adults)	21.9% COPD, 19.4% DM, 4.2% HIV, 18.8% ASCVD	56.9% pneumonia, 29.7% bacteraemia, 5% meningitis	17%	60.5% NTHi, 25.1% typeable, 14.4% unknown	Blood, CSF sterile site	Livorsi et al.
Europe	Surveillance of invasive Hi disease by the EU IBIS a total of 28 countries from 1996–2006	10081 cases (7211 adults)	Not reported	30% bacteremia, 17.4% meningitis, 18.4% pneumonia, 18.4% other, 16.2% unknown	8.1%	28.1% Hib, 7.8% non-Hib, 44.3% NTHi	Blood, CSF sterile site	Ladhani et al.

Slovenia	National surveillance of Hi from Slovenian labs at National Institute of Public Health from 1993–2008	292 cases	Not reported	Not reported		Hia (2), Hib (16), Hic (2), Hid (5), Hif (3), NTHi (80)	Normally sterile sites	Kastrin et al.
STUDIES OF CHILDREN ONLY								
South Africa	National, laboratory-based surveillance for Hi from 2003-2009	2562 cases	49% HIV infected.	60% meningitis, 26% pneumonia, 5% sepsis without focus, 2% arthritis, cellulitis (2), epiglottitis (1), osteomyelitis (1)	16%	Hib (349), Hia (50), Hif (80), other encapsulated (41), NTHi (348)	Blood, CSF	Van Gottberg et al.
USA	Population-based data on the incidence of Hib among Navajo and White Mountain Apache children <2 years from 1992-2000	19 cases	No underlying immunodeficiency	bacteremia/sepsis (10), meningitis (7), pneumonia (5), cellulitis (2), and arthritis (2)	1 child died of meningitis	Not reported	Blood , CSF, joint and pleural fluid	Millar et al. 1
USA	Hi among children aged <18 years living in Utah from 998–2008	91 cases	Not reported	61% bacteremia, 50% meningitis	1%	28% Hia, Hib, Hic (2), Hid (1), Hif (3)	Blood, CSF, sterile sites	Bender et al.
USA	Population-based surveillance data for Hia of Navajo and WMA children aged 15 years from 1988–2003	378 cases	Not reported	50% meningitis, 27.6% pneumonia, 17.1% other, 9.2% bacteremia without focus	2.6%	34.2% Hia,	Blood, CSF, joint fluid	Millar et al. 2
Germany	Cases of invasive Hi in children <10 years collected from a hospital-based surveillancesystem (Hospital-ESPED) and a lab-based surveillance system (Lab-ESPED) from 1998–2005.	192 cases	Prematurity (12), immunodeficiency (5), Down syndrome (1), CF (3), pneumonia (1), gangliosidosis (1), RSV infection (1), cardial malformation (1)	Meningitis (78), pneumonia (22), epiglottitis (6), septic arthritis (2), osteomyelitis (1), other foci (17), septicaemia without focus (24)	6%	Hib (64, NTHi (93)	Blood , CSF	Kalies et al.

Footnotes:

AIDS: Acquired Immune Deficiency Syndrome; ARC: Aids Related Complex; ASCVD: Atherosclerotic Cardio Vascular Disease; CAD: Coronary Artery Disease; CF: Cystic Fibrosis; CHF: Congestive Heart Failure; LD: Liver Disease (chronic); CKD: Chronic Kidney Disease; CLD: Chronic Lung Disease; COPD: Chronic Obstructive Pulmonary Disease; CSF: Cerebrospinal Fluid; CVD: Cerebrovascular Disease; DM: Diabetes Mellitus; HIV: Human Immunodeficiency Virus; KS: Karposi's Sarcoma, MI: Myocardial Infarction; PUD: Peptic Ulcer Disease, PVD: Peripheral Vascular Disease; RSV: Respiratory Syncytial Virus; RTI: Respiratory Tract Infection; SCC: Squamous Cell Carcinoma; TB: Tuberculosis

To our knowledge there has been no study undertaken in the vaccine era to describe the disease profile of adults in Southern Africa with *H. influenzae* bacteraemia. Therefore, this study retrospectively reviewed cases of *H. influenzae* bacteraemia from January 2008 to December 2014 to describe the demographics, predisposing diseases, clinical features, microbiological serotypes and outcome in adults at a large public sector hospital in South Africa i.e. the Chris Hani Baragwanath Academic Hospital (CHBAH) (Soweto, Johannesburg, South Africa).

1.7. Study Aim

The aim of this study was to describe the clinical and microbiological characteristics of adult patients with *H. influenzae* bacteraemia at the Chris Hani Baragwanath Academic Hospital (CHBAH) from January 2008 to December 2014.

1.8. Objectives

To achieve the aim of this study the following objectives were identified:

1.8.1. Determine the number of patients with *H. influenzae* bacteraemia per year from January 2008 to December 2014.

1.8.2. Assess the clinical characteristics of adult patients with *H. influenzae* bacteraemia with respect to demographics, predisposing diseases, and type of infection.

1.8.3. Analyze the microbiological characteristics of *H. influenzae* in terms of the capsular types (serotypes), and β-lactamase production (susceptibility to ampicillin).

1.8.4. Evaluate the in-hospital mortality of patients with *H. influenzae* bacteraemia.

2. STUDY DESIGN AND METHODS

A single center retrospective study based on record review was undertaken at the Chris Hani Baragwanath Academic Hospital (CHBAH) which is a 2700-bed public sector hospital based in Soweto (Johannesburg, South Africa).

National laboratory-based surveillance for *H. influenzae* is conducted by the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). GERMS-SA is a national public and private sector network of clinical microbiology laboratories in South Africa that participate in surveillance programs for bacterial and fungal pathogens of public health importance. National surveillance for invasive *H. influenzae* started in 1999 (Huebner et al., 1999) and was enhanced by the collection of clinical data from 2003 at 25 sites across the country, including CHBAH. GERMS-SA is an initiative of the National Institute for Communicable Diseases (NICD) which is a division of the National Health Laboratory Services (NHLS).

Case Report Forms (CRF) are used by microbiology laboratories to submit clinical isolates/specimens to GERMS-SA according to specific case definitions for confirmation

and further characterization, and by surveillance officers to submit clinical and outcome data.

Adult cases with *H. influenzae* bacteraemia were identified from the GERMS-SA database and their data was supplied. Hospital records for these patients were obtained from the hospital archives as a source of data together with the GERMS-SA data.

2.1. Patient Population

Patients ≥18 years of age with a positive blood culture for *H. influenzae* from the 1st January 2008 until 31st December 2014 were included in the study. These patients were identified from the GERMS-SA database.

2.2. Inclusion Criteria

Inclusion criteria were all male and female patients ≥18 years of age who had a positive blood culture for *H. influenzae* from January 2008 to December 2014.

2.3. Exclusion Criteria

Patients with sterile site cultures positive for *H. influenzae* from cerebrospinal, ascitic or pleural fluid were excluded if there was no co-existing *H. influenzae* bacteraemia.

2.4. Microbiological Methods

The NHLS at CHBAH undertakes culturing of blood samples that are requested by clinicians. Briefly, venous blood is collected via a sterile syringe from an adult patient

suspected of having a bacteraemia. Clinicians are instructed to inoculate 5mL of venous blood into each of Bactec™ Plus Aerobic/F and Bactec™ Plus Anaerobic/F bottles (Becton Dickinson, Sparks, MD). Once a signal is detected the Time To Detection (TTD) is recorded followed by Gram staining and inoculating two non-selective agar plates, a blood agar and a Chocolate Agar Plate (CAP), as well as a selective and differential plate, MacConkey agar, for each signal positive bottle. These are then incubated aerobically at 35°C for 18-24 hours and observed for growth. Anaerobic bottles receive an additional Brucella blood agar plate that is incubated anaerobically for 7 days **(Figure 7)**.

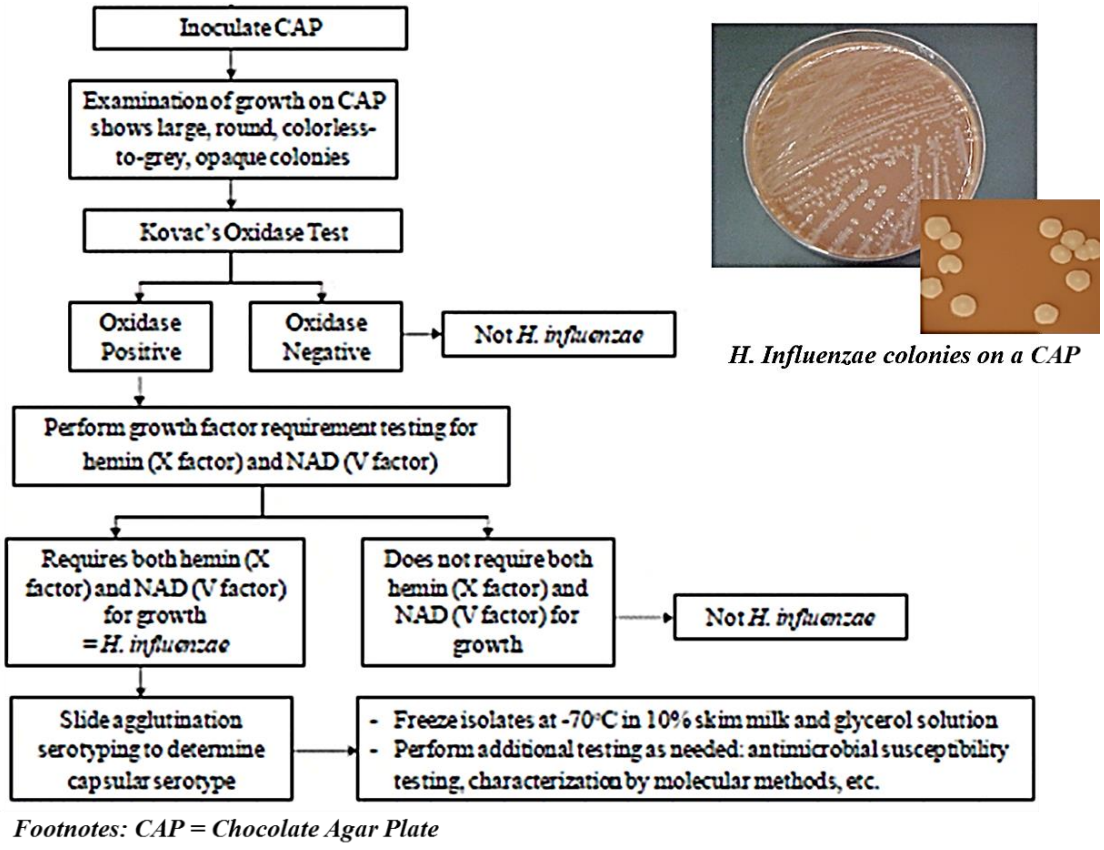


Figure 7: Flow-chart showing the algorithm used for Flow chart for identification and characterization of a *H. influenzae* bacteraemia isolate.

If the NHLS identifies *H. influenzae* they send the isolate on a Dorset slope to the NICD for confirmation of diagnosis, serotyping and assessment for β-lactamase production.

2.5. Patient Data

The GERMS-SA CRF includes basic demographic data, clinical as well as laboratory details: such as date of birth, age, gender, province, clinical diagnosis, outcome, specimen type and date of specimen collection. The following information was then extracted from the patient hospital records and GERMS-SA data and transferred to a data sheet (Appendix 1): 1) age, 2) gender, 3) co-morbid diseases, 4) polymicrobial bacteraemia, 5) clinical syndrome, 6) hospital department (i.e. medicine, psychiatry, obstetrics and gynaecology and surgery), 7) outcome, 8) capsular serotype, 9) white cell count and 10) β-lactamase production/antibiotic susceptibility. After examining the hospital clinical records, the outcomes included those patients who were discharged alive, or who may have died in hospital within 30 days of a positive blood culture.

2.6. Explanation of Study Variables

2.6.1. Age

This was calculated from the date of birth to the date of specimen collection. Age was obtained from the medical records. The age groups that were used were chosen because Statistics SA (Statistics SA, 2009) uses those age bands: 18-24 years; 25-44 years; 45-64 years and ≥ 65 years.

2.6.2. Year of infection

This was the year the specimen was collected. This variable was grouped according to the specific year from 2008 to 2014.

2.6.3. Gender

This was obtained from the medical records and was grouped as either male or female.

2.6.4. Clinical syndrome

This information was obtained from the medical records. Clinical syndrome was categorized as follows:

2.6.4.1. *Meningitis: H. influenzae* identified in CSF and/or with clinical features suggestive of meningitis.

2.6.4.2. *Pneumonia:* A diagnosis of pneumonia entered in the medical record or *H. influenzae* isolated from blood and pleural fluid during the hospital admission.

2.6.4.3. *Bacteremia without focus:* When *H. influenzae* was isolated from blood and no other localized clinical syndrome was described.

2.6.4.4. *Septic arthritis: H. influenzae* identified from joint fluid with clinical features suggestive of septic arthritis.

2.7. Predisposing Conditions

A predisposing condition is a factor that increases a person's susceptibility to develop *H. influenzae* infection. This information was obtained from the medical records. The

following predisposing conditions were included based on findings from previous studies and included chronic conditions such as chronic obstructive pulmonary disease (COPD), and post-TB bronchiectasis as chronic lung disease (CLD), chronic kidney disease (CKD), congestive heart failure (CHF), diabetes mellitus (DM), chronic liver disease, cancer, immunosuppressive therapy (steroids or chemotherapy), connective tissue disorders, HIV infection (including CD4 count and whether on ART), and current smoker (Resman et al., 2011; Laupland et al., 2010; Farley et al., 1992; Kalies et al., 2009; Pedersen et al., 2010).

2.8. In-Hospital Outcome

The outcome of *H. influenzae* cases during their hospital stay. This information was obtained from the medical records. The variable was grouped as follows: 1) discharged, 2) absconded or refused hospital treatment and 3) died.

2.9. Presence of Active TB

This information was obtained from the medical records.

2.10. Reliability and Validity of GERMS-SA Records and Data

The data used in this study was obtained from the GERMS-SA database and hospital records. GERMS-SA employed standard operational procedures to ensure that practices were standardized amongst all laboratories and surveillance officers. Their staff is exposed to ongoing training and the completing of a standardized CRF contributed to ensuring reliability of the data obtained from GERMS-SA. CRFs were checked by medical

officers at the NICD to ensure accuracy of the data captured by GERMS-SA and audits were also conducted to ensure complete case finding.

2.11. Data Analysis

The extracted data was transferred to a Microsoft Excel spreadsheet for further analysis. Data was analyzed using descriptive statistics such as computation of the mean, median, mode, range, standard deviation and coefficient of variation as well as a chi-squared (χ^2) test for measures of association between the nominal data obtained. The degrees of freedom were computed from the number of variants minus 1 with the relative standard set at $p<0.05$. The continuous data was analyzed by a single sample student's t-test at a significance level of 0.05 with n-1 degrees of freedom.

2.12. Ethics Approval

Permissions were obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and CHBAH Medical Advisory Committee to undertake the study (Appendix 2). Permissions were also obtained from the Internal Medicine Department of the University of the Witwatersrand, from the NHLS for the use of laboratory data and GERMS-SA for the provision of the database for the January 2008 to December 2014 culture-confirmed cohort of H. *influenzae*. There was no need for informed consent because this was a retrospective study.

2.13. Confidentiality and Access Issues

Confidentiality was ensured throughout the study by assigning a study number to each case with the names kept separately and secured. Data was captured on a MS Excel database which was on a secure server and password protected.

3. RESULTS

3.1. Determination of the Number of Cases Infected with *H. influenzae* per year from January 2008 to December 2014

A total of 225 adult and paediatric cases had *H. influenzae* isolated from a sterile site at CHBAH from 1 January 2008 to 31 December 2014. These patients were identified in the GERMS-SA database. Of these patients, 70/225 (31%) were adults (≥18 years), 149/225 (66%) were children and 6/225 (3%) cases had no age due to missing data. From the 70 isolates from adults which were sent to GERMS-SA from CHBAH, there were 45 (64%) cases of bacteraemia which comprised the study population, **(Figure 8).** There were 6-9 patients per year, except for 2014 when there was only 1. Thirty-nine (87%) patients had their hospital record retrieved from the CHBAH archives. Information for the other 6 were obtained exclusively from the GERMS-SA database and there were no polymicrobial bacteremia's.

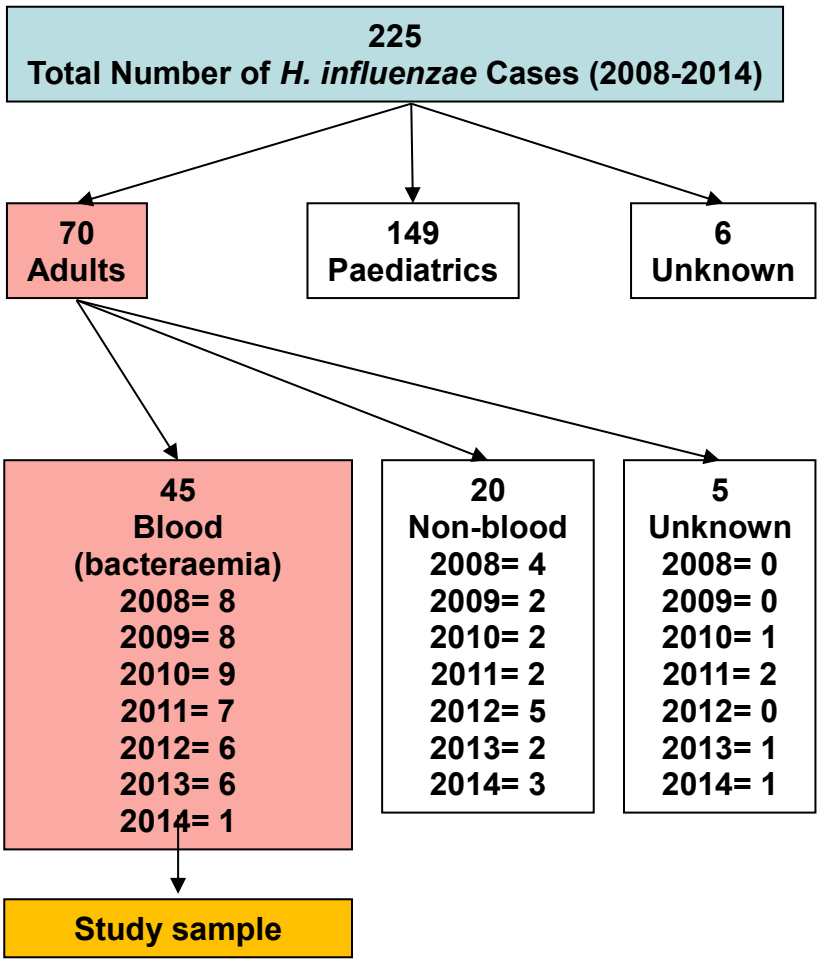
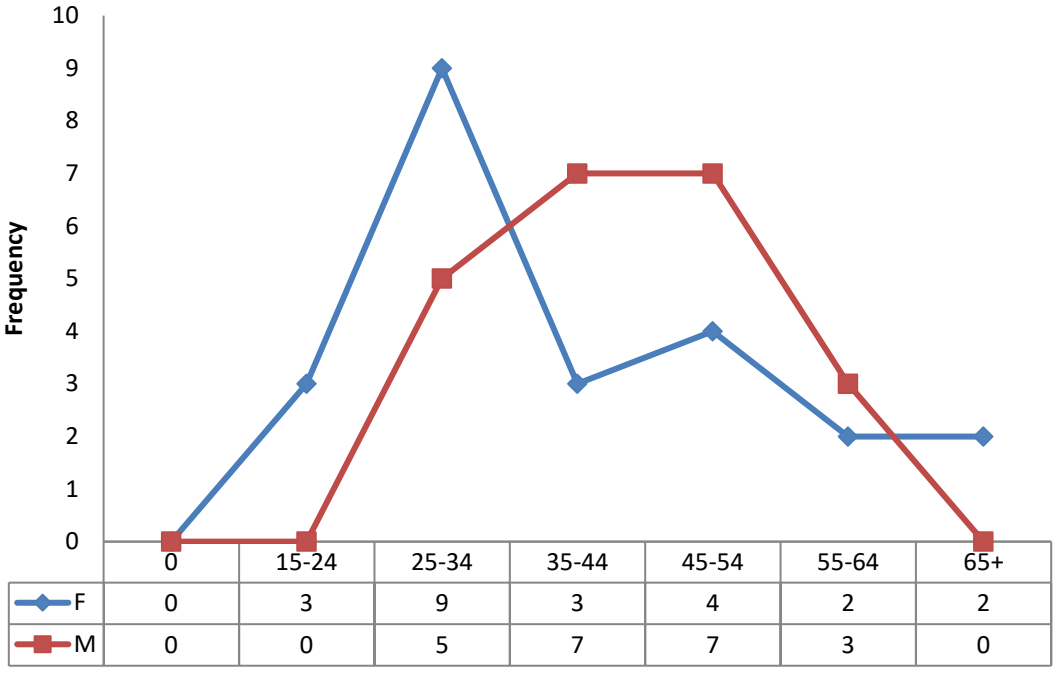


Figure 8: Schematic outlining the study patient population and culture site of isolates at the CHBAH from 1 January 2008 to 31 December 2014.

3.2. Patient Demographics

Forty-five patients were studied, of whom 23 (51%) were female and 22 (49%) were male **(Figure 9).** The median age was 40 years (IQR=20-60).



	0	15-24	25-34	35-44	45-54	55-64	65+
F	0	3	9	3	4	2	2
M	0	0	5	7	7	3	0

Figure 9: Frequency of *H. influenzae* infection of the 45 patients with bacteraemia by age band and gender at the CHBAH from 1 January 2008 to 31 December 2014.

3.3. Identification and Assessment of the Clinical Characteristics of Adult Patients with *H. influenzae* Bacteraemia

3.3.1. Site of infection

Thirty-four (75.55%) patients presented with pneumonia of whom 1 had empyema. Seven (15.55%) had bacteraemia without a focus, 3 (6.66%) had septic arthritis, 2 (4.44%) had meningitis, and 1 (2.22%) presented with cellulitis. Two patients had more than 1 clinical presentation. Thirteen/14 aged 25-34 years had pneumonia **(Figure 10).** Of the 34 patients with pneumonia, 21 (61.8%) had active TB. A total of 12 patients stayed <2 days in hospital.

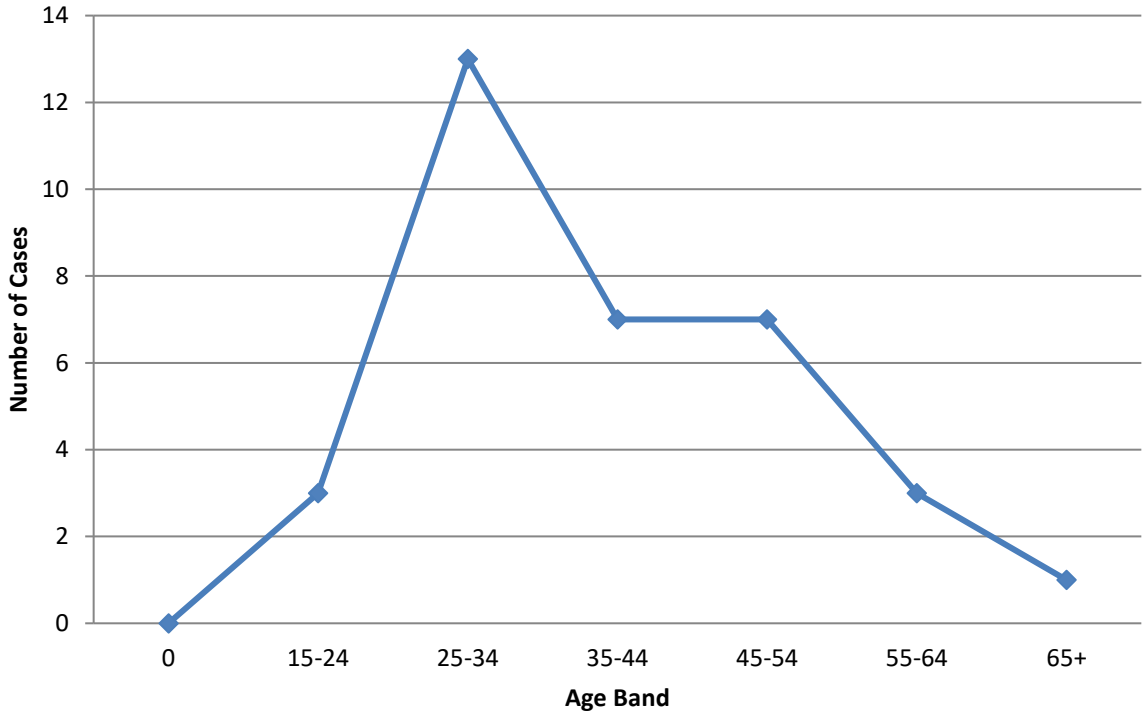


Figure 10: The number of cases of bacteraemic *H. influenzae* pneumonia by age band at CHBAH from January 2008 to December 2014.

3.3.2. Predisposing diseases

A total of 35/45 (77.8%) patients had predisposing disease including HIV infection (1 had no information on whether there was a predisposing disease). Of those, 11/45 (24%) had pre-existing diseases other than HIV. Pre-existing diseases included chronic lung disease (4), diabetes mellitus (4), cancer (2), chronic kidney disease (2), COPD (1) and CCF (1). Two patients had more than 1 predisposing disease. All 5 patients with chronic lung disease or COPD presented with pneumonia. Among the patients with diabetes mellitus, 3 had bacteraemia without a focus and 1 had a septic arthritis.

Of the 45 cases, 7 were recorded as smokers, 21 were non-smokers, and 17 had no documentation. Among the smokers 6 presented with pneumonia and 1 with empyema.

HIV results were retrieved for 34/45 (75%) of the *H. influenzae* cases of whom 33/34 (97%) were HIV infected and 1/34 (3%) was HIV-uninfected. The 33 cases that were HIV positive had a median age of 40 years **(Figure 11**). None of the 10 cases noted to have no predisposing disease were tested for HIV infection. *H. influenzae* infection in previously healthy adults should prompt HIV as an underlying disease.

The mean CD4 count was 152.23 cells/mm^3 and the median was 80 cells/mm^3 (range = 3 - 1458 cells/mm^3) and 22/33 (67%) had a CD4 count of <200cells/mm^3. Ten patients were on ART. None had a viral load recorded.

The average white cell count for the HIV positive patients was calculated at 11.72 with a median value of 11.27 and mode 17.5 (range = 1.20 - 40.98).

Pneumonia was the most common diagnosis among the HIV positive patients and the 1 HIV negative patient was an 89-year-old female who was also diagnosed with pneumonia. After pneumonia, bacteraemia without a focus (4), meningitis (2), septic arthritis (1), empyema (1) and 1 case of pneumonia with septic arthritis followed. Of the 11/34 cases with an unknown HIV status, pneumonia was also the most common diagnosis (6/11) (54.5%) followed by bacteraemia without a focus (2), pneumonia with smoke inhalation (1) and septic arthritis with cellulitis (1).

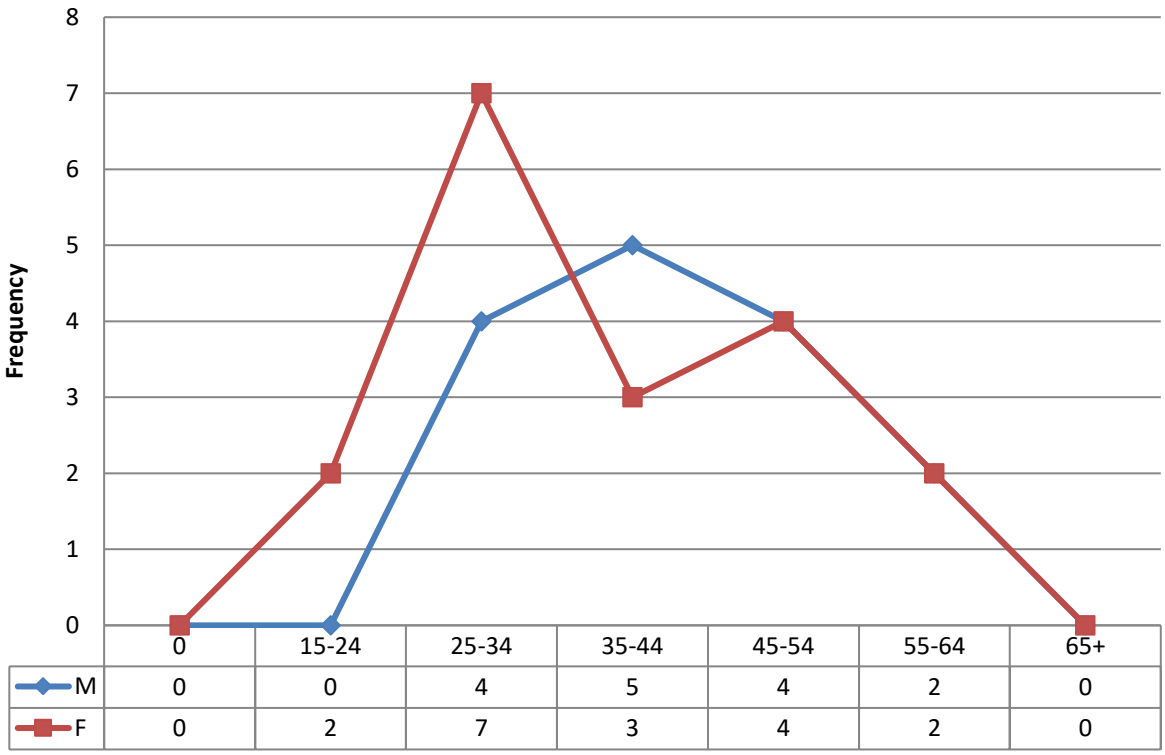


Figure 11: Frequency of HIV positive patients with *H. influenzae* infection by age band and gender at the CHBAH from 1 January 2008 to 31 December 2014.

3.3.3. *H. influenzae* serotypes

Specimens with viable isolates were available for serotyping for 43/45 (96%) of the reported cases. Of the 43 cases 19/43 (44%) were typeable and 24/43 (56%) were non-typeable *H. influenzae* (NTHi) **(Table 3).** Of the 19 typeable cases, 10/19 (52%) were identified as serotype b (Hib), 4/19 (21%) as serotype f (Hif), 2/19 (11%) as serotype a (Hia), 2/19 (11%) as serotype e (Hie) and 1/19 (5%) as serotype d (Hid). **(Figure 12).** Interestingly, there were no cases of Hib found after the year 2010. A total of 12/26 (46%) NTHi cases were identified between 2008-2010 and thereafter 12/19 (63%) cases occurred from 2011-2014. This suggests similar numbers of NTHi cases but an overall

reduction in the proportion of typeable *H. influenza* in the latter period, mainly due to the absence of Hib.

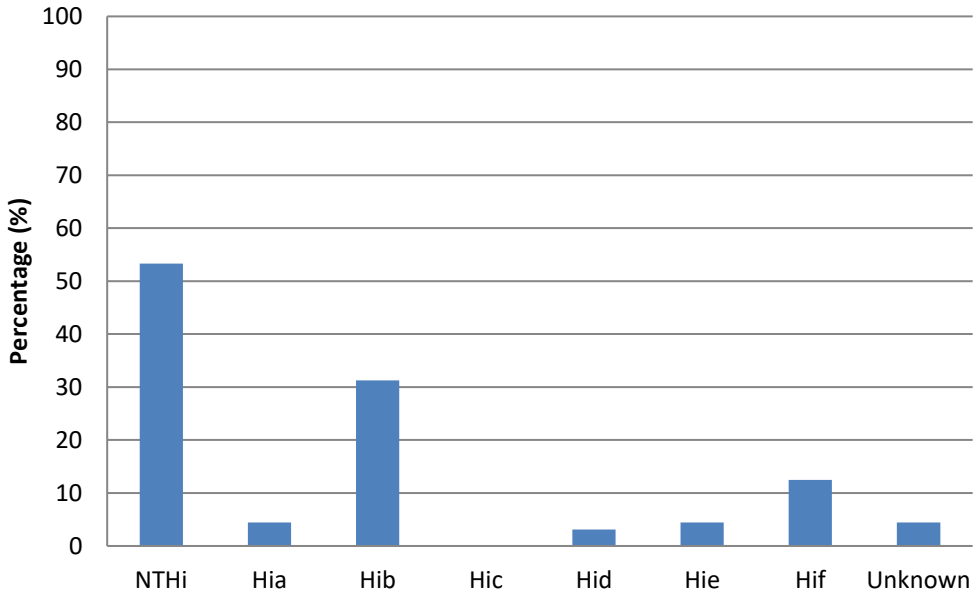


Figure 12: Serotypes of reported cases of *H. influenzae* bacteraemia in adults >18 years at CHBAH between January 2008 to December 2014.

Table 3: Reported cases of *H. influenzae* serotypes in adults >18 years of age at the CHBAH between January 2008 to December 2014.

H. influenzae n (%)	2008	2009	2010	2011	2012	2013	2014	Total
Hib	5 (63)	3 (38)	2 (20)	0 (0)	0 (0)	0 (0)	0 (0)	10
Other typeable	1 (13)	0 (0)	3 (30)	2 (33)	1 (17)	2 (33)	0 (0)	9
NTHi	2 (25)	5 (63)	5 (50)	3 (50)	5 (83)	3 (50)	1 (100)	24
No isolate available	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)	1 (17)	0 (0)	2
Total	8	8	10	6	6	6	1	45

H_0=reject. There was no association between the year and H. influenza strain (p>0.05).

Table 4 provides a summary of the serotypes of *H. influenza* by clinical presentation. Pneumonia presented in 16/24 (66%) of NTHi cases, which is similar to 12/19 (63%) among typeable cases.

Table 4: Serotypes of *H. influenzae* by clinical presentation.

Clinical presentation	Hia	Hib	Hid	Hie	Hif	NTHi	Unknown	Total
Pneumonia	1	7	1	2	3	17	2	**33**
Empyema	0	0	0	0	0	1	0	**1**
Septic arthritis	1	2	0	0	0	0	0	**3**
Sepsis syndrome	0	1	0	0	0	4	0	**5**
Meningitis	0	0	0	0	1	1	0	**2**
Unknown	0	0	0	0	0	1	0	**1**
Total	**2**	**10**	**1**	**2**	**4**	**24**	**2**	**45**

H_0=reject. The type of H. influenzae strain was independent of the clinical presentation (p>0.05).

3.4. Evaluation of the Mortality

3.4.1. Death by diagnosis

Sixteen/45 (36%) patients died of whom 12/16 (75%) died in <2 days after admission (early death). Seven/11 (63.63%) of those with pneumonia, as well as those with bacteraemia without a focus (2), septic arthritis/cellulitis (1), meningitis (1) and empyema (1) who died, did so early (i.e. <2 days after admission) **(Table 5).**

Table 5: Diagnosis of patients and case fatality rates by clinical presentation.

Clinical Diagnosis	Total No. of Cases	No. Deaths in <2 days	No. of Deaths in >2 days	No. Alive After 7 days	Case Fatality Rate at 7 days
Pneumonia	34	7	4	23	35.48
Bacteraemia without focus	6	2	0	4	33.33
Septic arthritis/cellulitis	1	0	0	1	0.00
Septic arthritis	1	1	0	0	100.00
Meningitis	2	1	0	1	50.00
Empyema	1	1	0	0	100.00
Total	**45**	**12**	**4**	**29**	**36.36**

3.4.2. Death in those with Pneumonia

Of the 33 cases that were HIV positive 25/33 (76%) were diagnosed with pneumonia and among the episodes with a known outcome, 5/25 (20%) were fatal. A total of 22/33 (73%) cases that were HIV positive were not on ART and of these 7/22 (32%) died. Mortality was 7/22 (32%) for those not on ART and 3/10 (30%) for those on ART.

3.4.3. Death by pre-existing disease

A total of 35/45 (77.8%) patients had pre-existing disease and of these 11/35 (31.42%) had died **(Table 6).**

Table 6: Effect of pre-existing diseases on outcome.

Pre-existing Disease	No. Surviving	No. of Deaths	Unknown	Total
Absent	0	1	0	**1**
Present	24	11	0	**35**
Unknown	0	0	9	**9**
Total	**24**	**12**	**9**	**45**

H_0=accept. The number of deaths/survival was dependent on the presence or absence of pre-existing disease ($p<0.05$)

3.4.4. Death by serotype

Death by serotype was as follows: NTHi strain 10/24 (43%), 4/10 (40%) Hib, 1/2 Hia, 1/2 Hie and 1/4 Hif. Clinical presentations in those who died were similar for infections caused by capsulated and non-encapsulated *H. influenzae* strains. Of the 16/45 (36%) of cases that had died, 10/16 (59%) were identified as NTHi, followed by 4/16 (23%) Hib, 1/16 (6%) Hia, 1/16 (6%) Hie and 1/16 (6%) Hif strains. Pneumonia presented in 33/45 (73%) cases and of these 16/33 (51%) were of the NTHi strain with 7/16 (41.1%) fatal cases.

3.5. Antibiotic susceptibility

Table 7 provides the susceptibility of *H. influenzae* isolates (n=35) to 6 antimicrobials. Of 35 isolates with susceptibility testing, 7 (20%) were resistant to ampicillin and 17 (49%) were resistant to cotrimoxazole. There was no data on beta-lactamases. All were susceptible to amoxicillin-clavulanate.

Table 7: Susceptibility of isolates of *H. influenzae* to 6 antimicrobials

Antimicrobial	No. of Isolates Susceptible	No. of Isolates Resistant	Unknown	% Susceptible (N=35)
Ampicillin	28	7	10	62.2
Amoxy-Clav	35	0	10	77.7
Carbapenem	35	0	10	77.7
Chloramphenicol	29	5	11	64.4
Cotrimoxazole	18	17	10	40.0
Ciprofloxacin	35	0	10	77.7

4. DISCUSSION

4.1. Patient Selection and Study Design

This study at CHBAH reported adult *H. influenzae* bacteraemic infections between January 2008 and December 2014 and encompassed a period after introduction of the conjugate Hib vaccine into the immunization schedule in South Africa. In this study, we identified patients retrospectively from the GERMS-SA database. Thereafter patient records were collected from CHBAH archives. The reporting to GERMS-SA by the CHBAH laboratory remained constant throughout the study period.

4.2. Demographics and Frequency

From January 2008 through December 2014, a total of 45 cases of *H. influenzae* bacteraemia in adults >18 years of age were hospitalized. Reasons for exclusion of cases between 2008 and 2014 included adults aged >18 years where *H. influenzae* were isolated from other sites and not a bacteraemia. Examples of these include pleural fluid, joint aspirates, cerebrospinal fluid but had no concurrent bacteraemia. The numbers were

stable from 2008-2013. It is interesting that there was but one case in 2014, but we could not confirm this drop in numbers subsequently as the study had ended.

The frequency of infection was higher in females than in males in the younger age band of 25-34 years. This may possibly have been due to females having more interaction with younger children who are the primary carriers of Hib strains if not immunized. Previous non-South African studies have reported that most cases of *H. influenzae* infection occurred in the older age group (>65 years) (Schlam et al., 1986; Wang et al., 2008; Casadevall et al., 1992; Smith et al., 1998; Askenasy et al., 1995). However, in our study (even though the numbers were small), the median age was much younger at 40 years. This is suggestive that the higher burden of HIV in the younger age bands was the reason. The immunosuppressive state caused by HIV may have resulted in a higher risk of *H. influenzae* infection. According to statistical data released by Stats SA (2009), the highest burden of HIV infection in South Africa is predominantly in the younger age groups.

4.3. Clinical Presentation

In keeping with previous studies, the most common clinical presentation of *H. influenzae* was pneumonia (Levine et al., 1997; Wallace et al., 1978; Quintilani et al., 1971). The diagnosis of pneumonia was made by clinicians eliciting the signs and symptoms that were suggestive of pneumonia or chest x-ray findings. For those patients that were started on TB treatment it may be possible that they had been simultaneously presenting with *H. influenzae* pneumonia. This finding points to having those patients at CHBAH with proven *H. influenzae* infection to also be tested for active TB. However, pulmonary TB

cannot be fully excluded as a cause of the lung findings in those patients with dual infection. Unfortunately, further clinical details of pneumonia such as a CURB-65 score (i.e. the CURB criteria, which is a clinical prediction rule that has been validated for predicting mortality in community-acquired pneumonia) could not be ascertained due to missing information from the medical records. If the CURB-65 score was available it would have been ideal to risk stratify the outcomes of the confirmed cases for *H. influenzae* pneumonia. Most cases that presented with pneumonia at the CHBAH were of the NTHi strain. This was reported previously to be associated with respiratory infections (Van Eldere, 2014).

A total of 4 cases had co-morbid diabetes mellitus. Interestingly, of these 3/4 cases presented with sepsis syndrome and 1/4 with septic arthritis. The impact of diabetes on the pathogenesis of sepsis has been studied previously by Koh et al., (2012). The main reason for which diabetes predisposes to *H. influenzae* infection may be due to the abnormalities of the host response attributed to the effect of hyperglycaemia. There is also evidence for defects in humoral immunity, and this may play a role. Meningitis may have been underrepresented by the 2 patients as we excluded patients with non-bacteraemic meningitis. As in other studies most patients had a predisposing disease (Rubach et al., 2011; Levin et al., 1997; Blain et al., 2011; Schlam et al., 1986 and Saranji et al. 2000) and possibly the rate may have been higher if the 10 patients without a predisposing disease had been tested for HIV infection.

4.4. *H. influenzae* Strains

When the Hib conjugate vaccine was introduced, various surveillance studies reported on the decrease of invasive Hib infection (Gessner and Adegbola, 2008). This resulted in a waning interest toward the study of *H. influenzae* disease with the subsequent reduction in the number of peer-reviewed publications (NTHi-Watch, 2013). Therefore, NTHi did not garner the same high profile as Hib, although NTHi is a common cause of respiratory tract infections in children and adults.

The frequency of Hib infection in the adult population of this study at CHBAH was low. The conjugated Hib vaccine was only introduced into the childhood immunization schedule during 1999 in South Africa. This meant that the adult cohort in this study (2008-2014) was not vaccinated (Figure 5). Several studies have confirmed the decline in the frequency and intensity of Hib infection in children after administering the Hib vaccine (Takala et al., 1991; Livorsi et al., 2012; MacNeil et al., 2011; Farlet et al., 1992; Murphy et al., 1993; Barbour et al., 1993, 1995; Sarangi et al., 2000). Consequently, the reduction in the number of cases of Hib infections among adults in this study can possibly be attributed to a reduction in exposure to children with Hib through a herd effect since most children would have been vaccinated as part of the childhood immunization schedule in South Africa.

This study revealed a reduction in the proportion of Hib strain with relatively stable NTHi infections in adults between the period 2008 to 2014 at the CHBAH. This is consistent with other studies that have also reported similar increases in the number of NTHi

infection especially in the elderly, immunocompromised and those that may have chronic lung disease (van Eldere et al., 2014; Murphy et al., 1987). The increase in the number of NTHi infections will have to be carefully monitored to determine new trends and the changing face of *H. influenzae* bacteraemia at CHBAH and possibly elsewhere in South Africa. Almost all the isolates (43) *H. influenzae* were typed over the study period (2008-2014) and very few infections were caused by Hi strains other than Hib (viz. Hie, Hia and Hif).

In this study, *H. influenzae* infection of adult cases at CHBAH between 2008 and 2014 was studied and a low number of Hib infections (in non-vaccinated adults) were identified. There was also a large and unexplained fall in the number of Hib cases with a proportional rise in the number of NTHi infections. There were a high number of deaths in adults of all age bands regardless of the infecting *H. influenzae* strain. Furthermore, the proportional rise in the number of NTHi infections over Hib at CHBAH over the study period warrants further investigation in a larger geographic area and the need to maintain enhanced surveillance.

A vaccine for NTHi infection is not yet available and the role of the new 10-valent pneumococcal NTHi protein D conjugate vaccine (PHiD-CV) is important to consider (MacLennan, 2016). A Phase III, open, single-center, partially randomized controlled study was conducted in South Africa between February 2009 and June 2012 with only HIV-unexposed-uninfected (HUU) children randomized to assess different PHiD-CV vaccination schedules. The study showed that the PHiD-CV was immunogenic and well-

tolerated in HIV+, HIV-exposed-uninfected (HEU) and HUU children, and has the potential to provide substantial benefit irrespective of HIV infection status (Madhi et al., 2017).

4.5. *H. influenzae* and HIV Infection

In the current study, results revealed that patients were considerably younger with more than 60% of pneumonia cases occurring in patients between 45-54 years of age. Results also revealed a low median for the CD4 count with two thirds of cases under 200 cells/mm^3. A total of 30% of patients were on ART but there was no detail on the duration of treatment and viral load. The mortality appeared to be the same irrespective of the use of ART or not. The numbers were too small for statistical significance to correlate the mortality data to the CD4 counts of the subjects.

Most of these patients with *H. influenzae* pneumonia were also HIV positive. Several studies have also reported higher number of cases of *H. influenzae* pneumonia occurring in young adults who are HIV positive (Polsky et al. 1986; Witt et al., 1987; White et al., 1985). HIV patients are known to have a deficiency in cellular immunity as well as an impaired humoral response that constantly secretes antibodies but unable to respond to new antigenic stimuli (Lane 1983; 1985; Pahwa et al., 1983; Ammann et al., 1984; Katz et al., 1986). The humoral immune response is a major defense mechanism that protects against the development of *H. influenzae* infection (Mandell et al., 1985).

Pneumonia caused by *H. influenzae* in the developed world is known to occur mostly in elderly patients with a mean age of 50-67 years and who may have also had other chronic pulmonary infections. Very few cases of *H. influenzae* pneumonia have been reported in young adults (Hirschmann and Everett, 1979; Musher et al., 1983; Levine et al., 1977; Wallace and Musher, 1978; MacFarlane, 1987).

Musher and co-workers, (1983a and b) demonstrated that patients with *H. influenzae* pneumonia had reduced antibody-mediated opsonizing activity against *H. influenzae* compared with patients that were only colonized. In addition, *H. influenzae* infection has also been reported in patients with common variable immunodeficiency and multiple myeloma by Hirschmann and Everett, (1979). Consequently, this indicates that a deficiency in the humoral response of HIV positive patients predisposes them to the development of severe *H. influenzae* infection. It may also be possible that HIV positive young adults who are also intravenous drug abusers are at increased risk for developing *H. influenzae* infection but there was no documented evidence of any patients in this series being intravenous drug users (Simon et al.1980; Everett et al., 1977; Wallace et al., 1978; Berk et al., 1982; Tillotson and Lerner, 1968).

The clinical presentation of *H. influenzae* in young adults observed in this study was similar to that reported previously in elderly patients (Granato et al., 1983; Wallace et al., 1981).

Furthermore, due to the high number of patients identified as HIV positive in this study, the possibility of a previously unrecognized HIV risk factor should be investigated in any young adult presenting with *H. influenzae*. Concerns have been raised that the Hib vaccine may increase HIV viral replication, although in another study, the Hib vaccine had no effect on HIV viral replication regardless of HIV clinical or immunological stage.

4.6. Antibiotic susceptibility

Although there was some resistance to ampicillin, amoxy-clav should have been effective against *H. influenzae.* The emergence of drug resistance is a serious challenge for the management of invasive *H. influenzae* which emphasizes the fundamental role of laboratory-based surveillance for antimicrobial resistance. The continued usefulness of antimicrobial agents will depend on the rational use of antimicrobial agents. The high rate of resistance to cotrimoxazole means that cotrimoxazole prophylaxis for HIV infected patients with low CD4 counts would not have protected those patients with resistance against invasive Hi disease. We did not have data on patients receiving prophylaxis with cotrimoxazole prior to hospitalization.

Since all tested isolates were susceptible to amoxicillin-clavulanate, we conclude that this antimicrobial agent is still a good option for the empiric treatment of community-acquired pneumonia in adult patients who may be at risk for *H. influenzae.* In addition, amoxicillin-clavulanate is used as the initial treatment for pneumonia at CHBAH.

4.7. Deaths

The overall rate of mortality from admission was relatively high (36%) across all age groups. The majority of deaths occurred early within 2 days and most likely were due to *H. influenzae*. This mortality rate is comparable to other retrospective studies of the epidemiology and outcomes of invasive *H. influenzae* in adults available from the literature. The prevalence of pre-existing disease did not lead to a higher number of deaths. As compared to other studies in the literature on adults, pneumococcal bacteraemia/pneumonia was the most common type of disease. If our study numbers were larger the risk factors for mortality could have been considered.

4.8. Study Limitations

Laboratory surveillance only captures laboratory-confirmed cases. Not all patients at CHBAH have blood cultures sent for microbiological analysis upon admission. Some patients may have been on antibiotic therapy at the time of blood cultures taken, which may have been negative as a result. This study therefore, did not capture *H. influenzae* cases that were seen at CHBAH but had no specimen that was taken. This study also does not include cases with non-invasive pneumonia. The number of cases of *H. influenzae* in this study does not reflect the true burden of disease in South Africa as it is a single institution study in Soweto. Due to the ecological nature of surveillance data, any changes in clinical practices and laboratory techniques at CHBAH from 2008-2014 could influence the results observed.

The numbers for non-type b encapsulated *H. influenzae* were small, especially by age band. It was therefore not feasible to identify whether specific predisposing conditions such as malnutrition, other chronic conditions and/or central nervous system conditions may be predictors of *H. influenzae* disease. Predisposing conditions could have been underestimated if a predisposing condition existed but was undiagnosed or if a patient was treated for a predisposing condition but was not properly documented in the medical records at CHBAH. Missing patient files and poor record keeping posed a challenge but the date obtained from GERMS-SA served as a back-up.

4.9. Strengths

The surveillance system remained stable over the years 2008-2014. The case definition was standardized and remained the same throughout the study period. Laboratory techniques used for serotyping also did not change over the study period. The surveillance was active and this ensured almost complete case ascertainment, through regular audits and site visits.

5. CONCLUSIONS

There was a steady small number of 6-9 cases annually until a sudden marked decrease of H. *influenzae* cases in 2014. It would be important to know if this decrease has been sustained. Pneumonia was the most common clinical presentation followed by bacteraemia without a focus. Meningitis may have been under-represented as the study did not include CSF as a specimen source. Nearly 80% of cases had a predisposing

disease, most commonly HIV infection. Only 2 cases had other predisposing diseases without HIV, less than what was found in comparator studies. Almost half of cases had concomitant TB and HIV infection is a common risk factor for both. It could be worth exploring the possibility of a specific association in future. One fifth of cases were resistant to ampicillin but none to amoxy-clav. This has implications where ampicillin is used as empirical treatment. Non-typeable Hi predominated as in other countries in the vaccine era. However, there was not an actual increase in the numbers and an absence of Hib was noted in the last 4 years of study.

South Africa faces a double challenge: Children with underlying HIV and protein-energy malnutrition are at higher risk to develop invasive *H. influenzae* disease but they are also more likely to be less protected by the Hib vaccine due to a lesser immune response compared to immunocompetent individuals. Thus they pose a risk to adults around HIV positive parents. Ongoing monitoring of *H. influenzae* disease in South Africa is therefore essential.

The epidemiology of *H. influenzae* disease in many countries has shifted in the post-vaccination era. NTHi now causes the majority of invasive *H influenzae* disease in all age groups. Although limited data is available on temporal and geographic variability in incidence of NTHi, use of these surveillance indicators is encouraged.

6. RECOMMENDATIONS

This study has provided some baseline data on H. influenzae infection at one site and can be expanded to more GERMS-SA surveillance sites. Surveillance for serotypes and antibiotic susceptibility should continue as well as studies on the Hib vaccine in adults with predisposing diseases. HIV-infected people are at a higher risk to develop invasive *H. influenzae* disease than their HIV uninfected counterparts. Early HIV testing is important to establish one's HIV status so that eligible people can be started on ARVs. An improvement in immune function due to ARVs may protect HIV-infected people against invasive *H. influenzae*, especially NTHi disease. A study on the impact of cotrimoxazole prophylaxis to reduce morbidity and mortality in adults with H. *influenzae* bacteraemia may also be considered.

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APPENDIX 1: DATA SHEET

HAEMOPHILUS INFLUENZAE BACTERAEMIA AT CHBAH (FILL IN OR CIRCLE)

STUDY NO:

PATIENT DEMOGRAPHICS

Age: *Gender:* M or F
Ward Dept: Medical Surgery O&G Other: …….....
DO Admission: ……
DO Discharge: ……
DO Death: ……

MICROBIOLOGICAL CHARACTERISTICS

True bacteraemia Contamination Polymicrobial Unknown significance
If polymicrobial provide list the microorganism:
……
Serotype: Typeable Non-typeable
If typeable stipulate capsular type:
……..

DIAGNOSIS

State the diagnosis:
……..
Organ system: RESP UGT GIT CNS Bone Joint Skin Other:
……..
Further relevant details of source of infection if required:
……..…….

CLINICAL AND LABORATORY CHARACTERISTICS

Co-morbid Disease: Diabetes; Chronic Lung Disease; Malignancies; Renal Disease; Immunosupression; Other
H. influenza Disease Acquisition: Community acquired; Nosocomial (hospital <90 days); Nursing home
Clinical Exam: Temp: ..... BP: ..... RR: .....
Lab Features: FBC: ..... U&E: ..... LFT: ……. Hb: ……. WCC: …….
Na: ……. Pl: ……. Urea: ……. Cr: …….

HIV PROFILE

Status: Positive Negative Unknown
CD4 count: ……..
On ARVs: Yes No

TB PROFILE

Previous TB: Yes No
Current/Active TB: Yes No
If yes state whether empiric or microbiologically confirmed: ….……

OUTCOME

Discharged Alive Died <30 days of admission

Comments:

APPENDIX 2: CLEARANCE LETTER – GERMS SA

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

DIVISION OF PUBLIC HEALTH SURVEILLANCE AND RESPONSE
National Institute for Communicable Diseases
1 Modderfontein Road, Sandringham, 2131
Tel: +27 (0)11 386 6234/6592 Fax: +27 (0)11 386 6221

30 May 2016

To whom it may concern:

I, Dr Vanessa Quan, head of GERMS-SA: Laboratory-based Surveillance for Pathogens of Public Health Importance in South Africa (WHREC M140159), hereby grant Dr Hajira Ismail (Student number: 0300130P) permission to use the GERMS-SA database for her secondary analysis required for fulfilment of the research component for the Master of Medicine (MMed) in the speciality of Internal Medicine (MFOSIMED60).

Dr Vanessa Quan
GERMS Programme Head
011 386 6012

APPENDIX 3: ETHICS CLEARANCE CERTIFICATE

R14/49 Dr Hajira Ismail

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160401

NAME: (Principal Investigator)	Dr Hajira Ismail
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Haemophilus Influenzae Bacteraemia in Adults at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	06/05/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Alan Karstaedt
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/06/2016

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 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised 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 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 review in April and will therefore be due in the month of April each year.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES