TRENDS AND FACTORS ASSOCIATED WITH INVASIVE NON-TYPEABLE AND SEROTYPE B *HAEMOPHILUS INFLUENZAE* DISEASE IN PERSONS OF ALL AGES IN SOUTH AFRICA FROM 2003-2009

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Declaration

I declare that this research report that I submit in partial fulfilment of the degree, Masters in Science in Epidemiology and Biostatistics to the School of Public Health at the University of Witwatersrand, Johannesburg is my own independent work. This research report has not been submitted previously to any institution for examination or degree purposes.

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Dr Melony Fortuin-de Smidt

April 2013

Dedication

I dedicate this work to my husband, my daughter and my son. Without the support of my husband, Theo this research report would not have been possible. I want to thank him for his unrelenting support and understanding and for looking after our children so that I can work on my research report.

Abstract

Introduction

Haemophilus influenzae type b disease (Hib) was a major cause of childhood disease but has nearly been eliminated in developed countries after the introduction of Hib conjugate vaccine. An increase in non-typeable *H. influenzae* (NTHi) disease was observed in some countries post-Hib vaccination which prompted concerns about possible serotype replacement. Hib disease in children <5 years of age decreased by a minimum estimate of 71% in South Africa after the Hib vaccine introduction in 1999. The epidemiology of Hib disease among persons >5 year olds and NTHi disease in all ages has not been described in South Africa before. The aim of this study is therefore to describe the trends and factors associated with invasive Hib and NTHi disease in South Africa, 2003-2009 among persons of all ages.

Materials and Methods

A secondary data analysis was conducted using national active laboratory-based surveillance data from January 2003 through December 2009. Surveillance was enhanced at 25 sites across the country where detailed clinical information was collected through an interview and medical record review. At non-enhanced surveillance sites only basic demographic data were collected. Overall, age-specific and serotype-specific (Hib, NTHi and non-b encapsulated *H. influenza*e) incidence rates were calculated for all invasive *H. influenzae* cases. Data from enhanced and non-enhanced sites were used to calculate incidence rates. Multivariable analysis, restricted to cases presenting to enhanced surveillance sites, was done to compare characteristics of NTHi cases to Hib cases. Chi-square test for linear trend was used for the trend analysis.

Results

A total of 2563 *H. influenzae* cases in all ages were identified from 2003 through 2009. NTHi accounted for the greatest proportion at 49% (1246/2563), followed by Hib at 33% (860/2563)

and non-b encapsulated at 18% (457/2563). Cases of *H. influenzae* from enhanced surveillance sites accounted for 50% (1278/2563) of all cases.

Hib among persons <5 years old increased from 10 cases per 1 000 000 in 2005 to 21 cases per 1 000 000 in 2008 ($p < 0.001$). Hib incidence among persons ≥5 years old increased from 0.5 cases per 1 000 000 in 2003 to 1.1 cases per 1 000 000 in 2009 ($p = 0.002$). NTHi incidence remained stable among individuals of all ages.

A greater proportion of *H. influenzae* cases were isolated from blood cultures at enhanced surveillance sites compared to non-enhanced surveillance sites, 67% (850/1278) and 58% (747/1278) respectively. The proportion of cases reported from enhanced surveillance sites and non-enhanced surveillance sites also differed by province.

Factors associated with NTHi disease compared to Hib disease, after adjusting for other factors, were clinical syndrome (lower respiratory tract infection: OR 8.31, 95% confidence interval (CI) 4.12 – 16.78; bacteremia: OR 9.24, 95% CI 3.79 – 22; compared to meningitis), predisposing conditions (OR 3.00, 95% CI 1.68 – 5.35, compared to no predisposing condition), nosocomial infection (OR 5.67, 95 %CI 1.71 – 18.82, compared to no nosocomial infection). NTHi cases were less likely to be <5 years old (OR 0.29, 95% CI 0.12 – 0.76, compared to 25-44 year age group).

Discussion

The increase in Hib disease among children <5 year old could be due to waning immunity as the primary schedule did not include a booster dose. The increase in Hib incidence observed in the ≥5 year age group could be because Hib disease increased in the <5 year olds and transmission is still ongoing. A booster dose of Hib vaccine at 18 months of age was introduced in 2010 in South Africa and ongoing surveillance is essential to monitor trends in Hib disease.

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List of abbreviations

AIDS – Acquired immunodeficiency syndrome

ALA - γ-aminolevulinic acid

ARVs – Antiretroviral therapy

CRF – Case report form

CDW – Central Data Warehouse

CNS – Central nervous system

CSF – Cerebrospinal fluid

CLSI – Clinical and Laboratory Standards Institute

95% CI – 95% Confidence interval

DTaP-Hib – Diphtheria toxoid, tetanus toxoid, acellular pertussis antigen and *Haemophilus influenzae* type b conjugate combination vaccine

DTwP-Hib – Diphtheria toxoid, tetanus toxoid, whole cell pertussis antigen and *Haemophilus influenzae* type b conjugate combination vaccine (CombActHIB, Aventis Pasteur)

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

LRTI – Lower respiratory tract infection

NHLS – National Health Laboratory Service

NICD – National Institute for Communicable Diseases

NTHi – Non-typeable *Haemophilus influenzae*

OR – Odds ratio

PCR – Polymerase chain reaction

PMTCT – Prevention-of-mother-to-child transmission

PEM – Protein-energy malnutrition

PRP – Polyribosyl ribitol phosphate

1. Introduction

1.1 Background

1.1.1 Description of *Haemophilus influenzae*, the organism

H. influenzae is a small, Gram-negative coccobacillus. Humans are the only reservoir. It is subdivided into encapsulated and non-encapsulated strains based on the presence or absence of the polysaccharide capsule. There are 6 serotypes (a-f) that can be distinguished based on antigenically distinct polysaccharide capsules. *H. influenzae* type b (Hib) is the most virulent serotype and is responsible for invasive disease, mostly among children <5 years old, which includes meningtitis, epiglottitis, bacteremia, septic arthritis and bacteremic pneumonia (1). Strains that lack a polysaccharide capsule are referred to as non-typeable *H. influenzae* (NTHi). NTHi are genetically distinct from encapsulated *H. influenzae* strains (2). NTHi strains cause mucosal infections such as otitis media, conjunctivitis and sinusitis, as well as pneumonia, especially in adults with underlying conditions (1, 3). The other non-b encapsulated serotypes (a,c,d,e and f) are rare compared to Hib and NTHi.

1.1.2 Hib disease in the prevaccine era

Hib disease was the leading cause of bacterial meningitis among children <5 years of age in the prevaccine era and was responsible for a case-fatality ratio of 2-5% and left 15-30% of survivors with neurological sequelae such as deafness (4). The global incidence of Hib disease among children <5 years old (including meningitis, bacteremic pneumonia, bacteremia and non-bacteremic pneumonia) and Hib death rates in the prevaccine era were estimated at 1342/100 000 (uncertainty range (UR) 1210 - 2180) and 60/100 000 (UR 40 - 85), respectively (5). The incidence of invasive Hib disease among children <5 years before vaccine introduction in Israel, Australia and the United States were 34/100 000 (1988-1990), 59/100 000 (1985-1988) (6) and up

to 131/100 000 (1976-1984) respectively (7). The incidence of Hib disease in developed countries is still less than an estimated 370/100 000 (including non-bacteremic cases) for Hib disease incidence observed among <5 year olds in developing countries (8). The Hib incidence in South Africa among children <5 years of age was 47/100 000 (excluding pneumonia) and 136/100 000 (including pneumonia) (9). The differences in the burden of Hib disease documented between countries and regions could be due to among others, reporting of invasive disease with and without pneumonia, specimen-taking practices, overcrowding, nutritional status of children, genetic differences, HIV prevalence (10), or regional differences in surveillance methodology (7).

Hib was responsible for more than 80% of invasive *H. influenzae* disease cases in developing countries such as South Africa (children <10 years) and Bangladesh (children <5 years) (9, 11) and in developed countries such as England, Wales and Australia (children <5 years) prior to the introduction of the Hib conjugate vaccine in these countries (12-13). The high burden of Hib disease is preventable through the use of the Hib conjugate vaccine.

1.1.3 Hib disease in the post-vaccine era

The Hib conjugate vaccine was introduced in various developed countries from the late 1980s to the early 1990s (8). There has been an overwhelming decrease in the Hib disease burden among children <5 years old after the introduction of the Hib conjugate vaccine. Most countries in Europe, North America and Asia showed a decline of more than 90% (Scandinavia - 98%, Germany – 97%, United Kingdom – 97%, Canada – 97%, United States – 98% and Israel – 97%) in Hib disease incidence. Australia however, showed a 73% decline in Hib disease incidence (8). The difference seen in the magnitude of the Hib disease decline in different world regions could be due to the usage of different formulations of the Hib conjugate vaccine. PRP-T (polyribosyl ribitol phosphate linked to tetanus toxoid protein carrier) is more immunogenic compared to PRP-OM (PRP linked to the outer membrane complex of *N. meningitidis*) and PRP-D (PRP linked to

diphtheria toxoid protein carrier), which is the least immunogenic (1). Other reasons for the difference in magnitude of Hib disease decline could be due to different coverage rates in the different countries and the homogeneity of the population. Australia, which only showed a 73% decline, has an indigenous population, the Aborigines, in which the incidence of Hib disease is higher than in the non-Aboriginal population (8).

Introduction of the Hib conjugate vaccine in Africa occurred later. The first country in Africa to implement the Hib conjugate vaccine as part of their Expanded Programme on Immunization (EPI) was The Gambia in May 1997. A 100% decline in Hib meningitis was seen among children <5 years old after 5 years of vaccine introduction (14). The Hib vaccine was introduced in South Africa in 1999 as part of the EPI. Hib disease among children <5 years decreased by 71% over a 5-year period after vaccine introduction (15). A decrease of 71% was modest compared to other countries, partly explained by the *H. influenzae* surveillance programme in South Africa starting at the same time as the vaccine introduction. The surveillance programme was improving over the study period after Hib vaccine introduction, which was evident in the more than two-fold rise in non-typeables and other non-b encapsulated serotypes, which was not covered by the Hib vaccine. The observed decrease in Hib disease was therefore an underestimation.

Although the Hib vaccine caused a dramatic reduction in Hib disease worldwide, there was concern over other non-type b serotypes and NTHi disease emerging and causing replacement disease (16-17). This concern prompted the focus to shift from Hib disease in children <5 years to the epidemiology of other non-type b serotypes and NTHi in all age groups.

1.2 Literature Review

In the prevaccine era Hib disease was responsible for causing the majority of invasive *H. influenzae* disease, mostly among children <5 years of age, compared to other encapsulated non-

b serotypes and non-typeable strains (8). Globally Hib caused an estimated 8.13 million cases of severe disease and 371 000 deaths among children <5 years in 2000 (5) The introduction of the Hib conjugate vaccine has led to a decline in Hib disease of >90% in most developed countries (8). However, a strain-specific vaccine can affect the epidemiology of Hib in all ages and create an opportunity for other serotypes to emerge (18).

H. influenzae carriage is necessary before invasive disease can occur (19). Carriage is defined as the establishment of a viable bacterial population in the pharyngeal mucosa of humans and normally does not lead to any symptoms (20). Carriage rates are age-dependent. The bacteria are acquired during the first year of life and reach a maximum during the pre-school years (3-5 years) and gradually decline thereafter to increase again in adults ≥65 years of age. Various other factors such as number of siblings, whether a child is attending day-care, socio-economic status and in adults, the presence of chronic respiratory disease can influence carriage rates (21). Carriage rates of a pathogen are important because it can in part explain the epidemiology of that disease even though the relationship between carriage and the risk of disease is complex (20).

The Hib conjugate vaccine, unlike the polysaccharide vaccine, can affect nasopharyngeal carriage of Hib. The exact mechanism by which carriage is reduced is not known but speculations are that increased concentration of serum anti-PRP antibodies leads to the passive transudation of IgG antibodies to mucosal surfaces (20). A decrease in nasopharyngeal carriage of Hib was noted among United Kingdom preschool children (median age of 3.6 years) after the introduction of the Hib conjugate vaccine (PRP-T), from 6.7% in unvaccinated children in 1991 to 1.3% in fully vaccinated children in 1995 (22). A reduction in oropharyngeal carriage rates were also observed among healthy 3-year-old children in Finland, 4 years after the Hib conjugate vaccine (PRP-D) was introduced from 3.5% in unvaccinated children to 0% in vaccinated children (23). Hib carriage rates, before the introduction of the Hib conjugate vaccine, was 4.7% among healthy children in Cape Town, South Africa (24) but carriage rates post-Hib vaccination have not been evaluated.

Differences in carriage rates observed in abovementioned studies could be due to differences in sampling of the population, specimen-taking practices and laboratory techniques.

The Hib conjugate vaccine not only reduces nasopharyngeal carriage, therefore protecting the vaccinated individual against disease, but also reduces the spread of Hib to other contacts (25). The Hib conjugate vaccine protects the unvaccinated individual (older child or adult) from acquiring the pathogen. This protection is called herd or indirect effect and increases the benefit obtained from the vaccine. The indirect effect of the Hib conjugate vaccine was reported in England and Wales after the vaccine was introduced in 1992 (26). Incidence rates of invasive Hib disease among adults ≥15 years declined from 0.17/100 000 (95% CI 0.14 - 0.22) in 1992 to 0.03/100 000 (95% CI 0.02 - 0.05) in 1998. The decline in invasive Hib incidence among adults was mirrored by a decline in the invasive Hib incidence among children <5 years old over the same time period. An active population-based surveillance programme showed a decline in Hib meningitis cases in the United States from 1988 to 1991 among children too young to be vaccinated after the introduction of the PRP-D vaccine in 1988, as well as in Canada from 1991 to 1994 after the introduction of the PRP-T vaccine in 1992 (27). No herd effect was observed in a study from the Netherlands (28). The Hib vaccine was introduced in 1993 in the Netherlands. A decline in the number of Hib meningitis cases was noted amongst a cohort of children eligible to have received the Hib vaccine from 1993 to 1996. No decline in the number of Hib meningitis cases was seen among children too old to be vaccinated (>3 years old) over the same time period. However, the number of cases was low over the entire study period (<10 cases) among the >3 year age group, which make the interpretation of changes difficult.

Various factors, such as the type of vaccine, number of doses, timing of the doses and the population characteristics, influence the impact of the conjugate vaccine on carriage. Effects on carriage may alter the duration of protection derived from the vaccine (29). Despite the introduction of the Hib conjugate vaccine in 1992 in England and Wales, an increase (0.65

cases/100 000 in 1998 to 3.90 cases/100 000 in 2003) in invasive Hib disease was noted after the initial decline (26). Various reasons were proposed for the increase in Hib disease from 1999 in all ages in England and Wales (30). The initial Hib catch-up vaccine campaign that vaccinated most children after 12 months of age expired. The catch-up vaccine campaign played a major role in indirect protection of unvaccinated individuals. Other reasons were a decrease in vaccine effectiveness over time due to the lack of a booster dose (schedule given at 2 months, 3 months and 4 months), the use of a less immunogenic DTaP-Hib combination vaccine preparation and due to a decrease in natural immunity especially among adults because of lower Hib carriage rates among younger children after vaccination. However, the incidence of invasive Hib disease following vaccine introduction, still remained well below prevaccine levels (22.91 cases per 100 000 in 1991 compared to 3.90 cases per 100 000 in 2003). Invasive Hib disease levels decreased again after a booster dose was added to the child vaccination schedule and a more immunogenic DTwP-Hib preparation was implemented (24, 31-35).

The nasopharynx can be colonized by a single strain of *H. influenzae,* for a short or a prolonged period, or by different strains sequentially (21). The colonization rates of NTHi are known to be higher among children and adults compared to Hib carriage rates. A NTHi carriage rate of 22% have been found among children <5 years old (33) and 36.5% among adults >16 years old (31). Even though NTHi are more frequently found in the nasopharynx of healthy people compared to Hib, NTHi is less virulent and is responsible for less invasive disease than Hib (3). The carriage rates of NTHi did not change significantly after the introduction of the Hib vaccine (33). However, invasive NTHi disease has become more important in the post-Hib vaccine era, as attention shifts from declining or disappearing Hib disease to the remaining *H. influenzae* disease due to NTHi or non-b encapsulated strains.

In the post-vaccine era, NTHi accounts for most of the remaining invasive *H. influenzae* cases seen. The NTHi proportion in all ages varies between studies from 44.3% (17) to 73.1% (36). The

high proportion of NTHi cases is in contrast to the prevaccine era when NTHi accounted for less than 20% of all invasive cases (9, 12, 37). NTHi incidence in all ages increased in United States (38) and Sweden (39) from 0.06/100 000 (1996) to 0.43/100 000 (2004) and 0.36/100 000 (1997) to 1.22/100 000 (2007) respectively. Other studies showed no increase in the incidence of NTHi in all ages (17, 40). Differences between studies could be due to differences in surveillance systems used (passive vs active), and whether studies only looked at bacteremia (40) or all invasive disease.

NTHi has become the major strain of *H. influenzae* in the postvaccine era in all age groups, especially among children <5 years of age (22, 24). Therefore, factors associated with NTHi disease should be further investigated. Few reports on underlying conditions in cases with NTHi disease were found. Studies that reported on underlying conditions looked at cases with invasive *H. influenzae* or cases with Hib disease (41-42). The proportion of people that presented with underlying conditions among adults with invasive *H. influenzae* (>18 years old) was as high as 43/47 (92%) in a study conducted in the Atlanta Metropolitan area, United States from 1988 to 1990 (41). Another study done in the United Kingdom from 1990 to 1995 showed that 220/350 (63%) of adults (≥ 16 years old) with invasive Hib disease had underlying conditions (43). The underlying conditions reported were chronic lung disease (29%), malignancy (19%), heart disease (18%), neurological conditions (6%), renal disease (6%), rheumatological disease (5%), liver disease (4%), diabetes mellitus (4%), splenectomy (3%), neurosurgical operation (3%) and other (4%)(43). A Finnish study conducted from 1985 to 1987 reported that alcoholism and pregnancy were important underlying conditions among persons ≥16 years old with Hib disease but this study was small (n= 31) (42). It is important to identify *H. influenzae* cases with underlying conditions because of their high risk of death. The 6-month death rate was 82% (180/220) in persons ≥16 years with underlying conditions compared to 21% (27/130) in those without underlying conditions (43).

HIV is an important underlying condition, especially in the South African setting. Very few studies reported on HIV as an underlying condition. The HIV prevalence in the study populations varied from <1% (38) to 8.8% in a study from Madrid (44). The incidence of invasive *H. influenzae* (only includes bacteremia, and specific serotypes not mentioned) was 35 times greater and up to 100 times greater among adult men, aged 20-49 years with HIV (regardless of HIV stage) and with an acquired immunodeficiency syndrome (AIDS) defining illness respectively, than among all adults in the general population within the same age band (45). An increased risk of invasive Hib disease (bacteremic pneumonia) was also seen among children aged 2-24 months who were HIV-infected compared to children HIV-uninfected (RR 21.4; 95% CI 9.4-48.4) (46). A Spanish study (1986 to 1994) in adults reported that *H. influenzae* bacteremic pneumonia occurs at a younger age in HIV-infected individuals (median age 30 years) compared to HIV-uninfected individuals (median age 59 years) (47). The majority (67%) of the HIV-infected cases were intravenous drug users. There was no difference in mortality between HIV-infected and HIV-uninfected patients (18-39 years old) with invasive *H. influenzae* (48). A recent study from the United States found that HIV was not a predictor of in-hospital mortality in both adults and children with invasive *H. influenzae* disease (49). A poor prognosis in HIV-infected individuals with invasive *H. influenzae* disease has been associated with nosocomial infection and other severe underlying conditions (47). Evidence of HIV infection as a risk factor for NTHi disease was limited.

The epidemiology of Hib disease among children <5 years of age has been described in South Africa in the early post-Hib vaccination era (1999-2003) (15) and the late post vaccination era (2003-2009) (50). However, the epidemiology of Hib disease among persons aged ≥5 years and of other non-type b serotypes and NTHi among persons of all ages remains unclear in South Africa. Also South Africa has a different age distribution and HIV prevalence compared to developed countries. HIV prevalence among 15-49 year olds in 2009 in South Africa was 17% (51) compared to 0.2% in Central and Western Europe and 0.5 % in North America (52), where most of the studies focusing on post-Hib vaccine trends were conducted. The huge burden of HIV in South

Africa will impact on the epidemiology of invasive *H. influenzae* disease. The life expectancy at birth in South Africa is 53.5 years in males and 57.2 years in females, 31% of the population is <15 years of age and only 5% of the population is older than 65 years of age (51).

Hib disease surveillance started at the same time as the introduction of the Hib vaccine in 1999. The surveillance system was still improving in the subsequent years. Therefore this study will focus on trends from 2003, when the surveillance system became more stable. This study will also focus on the era prior to the Hib booster introduction in 2010.

Hib disease is a vaccine-preventable disease, whereas NTHi disease is not. Known factors associated with NTHi disease compared to Hib disease can assist the clinician in identifying possible vaccine failures when serotype data are not yet available. Identifying high risk groups of Hib and NTHi disease, in our South African setting, is important in order to inform management of patients as well as to devise prevention strategies.

1.3 Study objectives

1.3.1 General Objective/Aim

To describe trends in invasive *H. influenzae* disease in South Africa from 2003-2009 and to determine factors associated with invasive Hib and NTHi disease in persons of all ages

1.3.2 Specific Objectives

1.3.2.1 To determine the trends and incidence of invasive *H. influenzae* disease by serotype, age group and year in South Africa from 2003-2009

1.3.2.2 To describe the seasonality of invasive *H. influenzae* disease by serotype (Hib and NTHi) and age group (<5 and ≥5 years) in South Africa from 2003-2009

1.3.2.3 To describe the characteristics of persons of all ages (<5 years and ≥5 years old) with invasive NTHi and Hib disease, in South Africa from 2003-2009

1.3.2.4 To determine the factors associated with invasive NTHi disease as compared to Hib disease among persons of all ages in South Africa from 2003-2009

2. Methodology

In this chapter the methods of the study will be described. This study was a secondary data analysis. The method section will therefore be divided into 1) Methodology of the primary study and 2) Methodology of the secondary study.

2.1 Methodology of primary study

National laboratory-based surveillance for *H. influenzae* was conducted under the auspices of the Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA). Almost all laboratories (approximately 200) across the country send isolates to a central reference laboratory at the Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases (NICD) (branch of the National Health Laboratory Service (NHLS)) in Johannesburg.

The laboratories that participated in the surveillance include all laboratories from the public health sector (NHLS), private sector (e.g. Ampath, Lancet and Pathcare), military and mining sector. National surveillance for invasive *H. influenzae* started in 1999 (53) and was enhanced in 2003 at 25 sites across the country (See Appendix A for list of enhanced surveillance sites). Convenience sampling was used to select enhanced surveillance sites. Enhanced surveillance sites were mostly academic centres and there was at least one enhanced site in every province. At these enhanced sites surveillance officers, who are registered nursing professionals, did active case finding of laboratory-confirmed cases and collected detailed epidemiological data on cases. At non-enhanced surveillance sites only limited demographic data were collected.

All participating laboratories were encouraged to send case report forms (CRFs) and isolates through regular site visits and telephone contact. Audits were conducted from 2003 for all provinces, except Kwa-Zulu Natal province, military and private laboratories, to ensure complete

case ascertainment. The surveillance was therefore active because it did not just rely on the laboratories sending the isolates.

2.1.1 Case definition

A case of *H. influenzae* was defined as isolation or identification of the organism from a normally sterile site specimen through a:

- positive culture or
- a negative culture but confirmed through a polymerase chain reaction (PCR) or
- a positive latex agglutination test which was confirmed through a Gram-stain and/or a PCR

2.1.2 Exclusion criteria

The following patients were excluded:

- Patients that were not permanent residents of South Africa - defined as people who had not been living in South Africa for the last month. The reason for this criterion was to exclude patients that were infected by *H. influenzae* in another country.

2.1.3 Data collection tools and information sources

All laboratories did routine culturing of various sterile body fluids. If the laboratories identified *H. influenzae* they were encouraged to send the isolate on a Dorset slope to the NICD together with a standardized laboratory CRF. The laboratory CRF included basic demographic data as well as laboratory details: such as date of birth, age, gender, province, clinical diagnosis, outcome, specimen type and date of specimen collection (See Appendix B for laboratory CRF).

At enhanced sites, surveillance officers were informed of a case by the microbiology laboratory as soon as it was identified. The surveillance officer located the patient in the hospital and obtained informed consent. The surveillance officer completed a standardized CRF through an interview and medical record review. The clinical CRF captured basic demographic information as well as detailed clinical information (See Appendix C for clinical CRF). If the patient had died or was

discharged the surveillance officer completed the standardized CRF by using the medical records or by conducting a telephonic interview with the patient or relative (See Appendix D1 and D2 for flow diagrams of non-enhanced and enhanced surveillance sites).

2.1.4 Laboratory techniques

Diagnostic laboratories across the country did the initial identification of the organism and then send the isolate to the NICD using a Dorset slope as the transport medium. If no growth was observed, the laboratories were still encouraged to send the clinical specimen to the NICD for further processing.

At the NICD viable isolates received were identified with the γ-aminolevulinic acid (ALA) - porphyrin test reaction and API® NH (bioMérieux® SA, Marcy-l'Etoile, France). Serotyping was done through the slide agglutination test using agglutination sera for types a-f (Murex Biotech, Dartford, Kent, England). All serotyping results were then confirmed by PCR (15). The confirmatory PCR determined the serotype by using capsule type-specific genes which avoided misclassification of serotypes compared to using slide agglutination alone (54-55). Serotypes for non-viable isolates or culture-negative specimens were identified through PCR from 2007 onwards.

Ampicillin susceptibility testing was done by using the disc diffusion test. Zone sizes were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (56). Depending on the zone size, isolates were further tested using the Epsilon Test (Etest®), to determine the minimum inhibitory concentration (MIC) in µg/ml.

2.2. Methodology of secondary study

2.2.1 Study design

Three different study designs were employed to answer the 4 objectives.

- Objective 1 and 2 (1.3.2.1 and 1.3.2.2): Retrospective cohort analysis

- Objective 3 (1.3.2.3): Descriptive cross-sectional study design
- Objective 4 (1.3.2.4): Analytical cross-sectional study design

2.2.2 Study population

The study population differed according to the objective.

- Objective 1 and 2: For the calculation of the incidence rates and seasonality, the study population consisted of the population of South Africa from January 2003 to December 2009
- Objective 3 and 4: The study population consisted of all persons who presented to an enhanced surveillance site, who had invasive Hib or NTHi identified from a normally sterile site and for whom serotype information was available.

2.2.3 Sample size

A secondary data analysis was performed therefore the available sample size was fixed. This study did not have a main exposure variable; therefore a specific variable was not used to calculate the sample size. The sample size was calculated to determine what difference in prevalence rates of any factor among persons with invasive Hib compared to persons with NTHi with a 0.05 confidence level and power of 80% and 90%, this study could pick up, given the available sample size. The values are depicted in the table below:

Table 2.1 Sample size calculations with different levels of power and prevalence differences between a case with Hib and NTHi disease assuming a confidence level (α) of 0.05.

Power	Confidence level	Prevalence of factor in persons with Hib disease	Prevalence of factor in persons with NTHI disease	Sample size for each group (1:1 ratio)
80%	0.05	45%	50%	1600
90%	0.05	45%	50%	2100
80%	0.05	40%	50%	400
90%	0.05	40%	50%	530
80%	0.05	30%	50%	100
90%	0.05	40%	50%	130
80%	0.05	20%	50%	45
80%	0.05	40%	50%	55
80%	0.05	10%	50%	20
90%	0.05	10%	50%	30

A sample size of 288 Hib cases and 395 NTHi cases would be able to identify an association with a prevalence difference of a factor in Hib cases compared to NTHi cases of 10%-20% at 80% power and a confidence level of 0.05.

2.2.4 Imputation

For individuals with missing serotype data (48%), data on serotype was imputed assuming that the serotype distribution by age group, year and specimen type was similar to cases with available serotype. Imputation was only done for trend analysis (objective 1).

2.2.5 Inclusion criteria

Inclusion criteria differed according to the specific study objective. The following persons were included:

- Objective 1
    - Persons of all ages
    - Persons that presented to enhanced and non-enhanced surveillance sites
    - Persons with serotype data available (Hib, NTHi and non-b encapsulated) from viable and non-viable isolates or
    - Persons without serotype information available (serotype was imputed see 2.2.4)
- Objective 2
    - Persons of all ages
    - Persons that presented to enhanced and non-enhanced surveillance sites
    - Persons with Hib and NTHi serotype information available from viable and non-viable isolates
- Objective 3 and 4
    - Persons of all ages
    - Persons that presented to an enhanced surveillance site

- Persons with serotype (Hib and NTHi) information available (from viable and non-viable isolates)

2.2.6 Exclusion criteria

- Objective 2
    - Persons without serotype information
    - Persons with non-b encapsulated *H. influenzae* disease
- Objective 3 and 4
    - Persons that presented to non-enhanced surveillance sites
    - Persons without serotype information
    - Persons with non-b encapsulated *H. influenzae* disease

2.2.7 Measurement of variables

2.2.7.1 Outcome variable (Objective 1)

The outcome was the number of cases of Hib, NTHi and non-b encapsulated strains, which includes serotype a, c, d, e and f. Serotyping was done as discussed in the laboratory technique section (2.1.4). Serotype information was obtained from the reference laboratory report.

- Hib: Patient with invasive disease caused by *H. influenzae* encapsulated strain type b
- NTHi: Patient with invasive disease caused by *H. influenzae* strain that lack a capsule and is not typeable.
- Non-b encapsulated: Patient with invasive disease caused by an encapsulated *H influenzae* serotypes other than type b (a, c, d, e and f)

2.2.7.2 Outcome variable (Objective 2)

The outcome was the total number of Hib cases and NTHi cases for a specific month (January to December) for the years from 2003 to 2009. Non-b encapsulated strains were excluded due to small numbers.

- Hib: Patient with invasive disease caused by *H. influenzae* encapsulated strain type b
- NTHi: A patient with invasive disease caused by *H. influenzae* strain that lack a capsule and is not typeable

2.2.7.3 Outcome variable (Objective 3 and 4)

The outcome was binary, NTHi compared to Hib disease in persons of all ages**.** Serotype information was obtained from the reference laboratory report. Non-b encapsulated serotypes were excluded due to small numbers.

2.2.7.3 Explanatory variables

- **Age:** This was calculated from the date of birth to the date of specimen collection. Age was self reported and where possible verified through the identity document. Age was treated as a categorical variable because age was not normally distributed for invasive Hib and NTHi disease. In addition, effect modification of certain risk factors was evaluated by using age groups. The age groups that were used were chosen because Statistics SA (51) used the same age bands, HIV as a risk factor could be evaluated and to avoid age groups that are too small:
    - <5 years
    - 5 – 24 years
    - 25 - 44 years
    - 45 - 64 years
    - ≥ 65 years

- **Year of infection:** This was the year the specimen was collected. Data were obtained from the laboratory results and medical records. This variable was grouped according to the specific year from 2003 to 2009.

- **Gender:** This was self reported or obtained from the medical records. It was grouped as follows:
    - Male
    - Female

- **Race:** This was self reported or obtained from the medical records. It was grouped as follows:
    - Black
    - Other: White, Asian and Coloured were included in this category (due to small numbers)

- **Province:** This was the province in which the health facility was located where the specimen was taken. This variable was grouped according to the 9 official provinces in South Africa. Limpopo and Mpumalanga provinces were combined for the analytical analysis due to small numbers. North West province was excluded from analytical analysis due to zero number of Hib and NTHi cases reported from enhanced sites from 2003-2009.

- **Clinical syndrome** (17): This information was obtained from the patient and/or medical records or clinician. Clinical syndrome was categorized as follows:
    - Meningitis: *H. influenzae* identified from cerebrospinal fluid (CSF) or *H. influenzae* identified from a blood culture and with clinical features suggestive of meningitis.
    - Lower respiratory tract infection (LRTI): *H. influenzae* identified from a blood culture or pleural fluid and with clinical features of pneumonia

- Bacteremia without focus: *H. influenzae* identified from a blood culture with no distinctive clinical syndrome
- Septic arthritis: *H. influenzae* identified from joint fluid with clinical features suggestive of septic arthritis

- **Predisposing conditions** (excluding HIV): A predisposing condition or factor that increases a person's susceptibility to develop *H. influenzae* infection. This information was obtained from the patient, medical records and the clinician. The following predisposing conditions were included based on findings from previous studies (39-41, 57-58): chronic conditions such as chronic lung, renal, liver, cardiac diseases and diabetes mellitus, immunosuppressive conditions such as malignancies, immunosuppressive therapy (steroids and chemotherapy), burns, aplastic anaemia, asplenia and primary immunodeficiency, central nervous system conditions such as cerebrovascular incident and head injury, other conditions such as pregnancy, prematurity, alcohol dependency, chromosomal abnormalities and connective tissue disorders, current smoker and protein-energy malnutrition. HIV was evaluated separately. A case with a predisposing condition could still be HIV-infected or not. Due to small numbers predisposing conditions were grouped as follows:
  - Absent
  - Present

- **HIV status:** This information was based upon standard-of-care HIV testing initiated by the attending physician or surveillance officer. HIV-uninfected status was confirmed by a laboratory test which differed according to the age of the patient. An HIV enzyme-linked immunosorbent assay test (ELISA) was done, which was confirmed by another ELISA on a second specimen in patients ≥18 months of age. A qualitative PCR test was done on children

<18 months of age (59). Data on HIV exposure was not verified and therefore could not be evaluated. This variable was binary:

- HIV-infected
- HIV-uninfected

- **In-hospital outcome:** The outcome of invasive Hib or NTHi cases during their hospital stay. This information was obtained from the medical records. The variable was grouped as follows:
  - Recovered (Discharged/ Absconded/ Refused hospital treatment)
  - Died: Died while in hospital from any cause

  Cases that absconded or refused hospital treatment were not followed up to determine the outcome and were classified as recovered.

- **Antibiotic use 24 hours prior to specimen collection date:** The use of any antibiotics (excluding cotrimoxazole prophylaxis) within 24hrs before the specimen was collected. This information was gathered through self reporting and/or from the medical records (60). The variable was grouped as follows:
  - Yes
  - No

- **Antibiotic use 2 months prior to specimen collection date:** The use of any antibiotics (excluding cotrimoxazole prophylaxis) within 2 months before the specimen was collected**.** This was gathered through self reporting and/or from the medical records (60). The variable was grouped as follows:
  - Yes
  - No

- **Cotrimoxazole prohylaxis:** The usage of cotrimoxazole for the purpose of preventing *Pneumocystis jirovecii* pneumonia in HIV-infected individuals and HIV-exposed children. This information was self reported and/or obtained from the medical records. The variable was grouped as follows:
    - Yes
    - No

- **Duration of hospital stay:** The number of days the patient stayed in the hospital calculated from the date of admission to date of discharge or death. The median number of hospital days was calculated for patients with invasive Hib disease and patients with invasive NTHi disease.

- **Nosocomial infection:** A positive specimen for invasive Hib or NTHi disease taken ≥2 days after date of hospital admission (60). The categories were as follows:
    - Yes
    - No

- **Ampicillin susceptibility:** Information on ampicillin susceptibility was obtained from the reference laboratory report. The breakpoints were defined according to the CLSI (56). The disc susceptibility testing parameters for ampicillin, using a disc content of 10µg were classified as susceptible if the disc zone measures ≥22mm and non-susceptible if the disc zone measures ≤18mm. The Etest breakpoints for ampicillin susceptibility was ≤1µg/ml and for ampicillin non-susceptibility ≥2µg/ml.
    - **Susceptible**
    - **Non-susceptibl**e (included intermediate and resistant)

- **Severity of illness:** The Pitt bacteremia score was used as a measure of the severity of illness. It was used previously in studies of pneumococcal bacteremia (61-62). The Pitt bacteremia score was calculated on the day of specimen collection and the parameters used are shown in table 2.2.

Table 2.2 Parameters for the calculation of the Pitt bacteremia score

i) Oral Temperature	**ii) Hypotension**	**iii) Mechanical ventilation**	**iv) Cardiac arrest**	**v) Mental status**
2 points - temperature of ≤35ºC or ≥40ºC 1 point - temperature of 35.1ºC-36.0ºC 0 point - temperature of 36.1ºC-38.9ºC	2 points – Systolic blood pressure less than 90 mmHg	2 points – if mechanical ventilation was received	4 points - if patient went into cardiac arrest	4 points – comatose 2 points – stuporous 1 point – disorientated 0 point - alert

Acute severe illness was defined as a Pitt bacteremia score >4 and the categories were:

- Acute severe illness (score >4)
- No acute severe illness (score ≤4)

2.3 Reliability and validity

Standard operational procedures were available to ensure that practices were standardized amongst all laboratories and surveillance officers. Ongoing training of staff and the completing of a standardized CRF contributed to ensuring reliability of the data. CRFs were checked by medical officers at the NICD to ensure accuracy of data captured. Audits were also conducted to ensure complete case finding.

2.4 Data Analysis Plans

2.4.1 Data management and cleaning

Laboratory and clinical CRFs were captured onto a Microsoft Access database. Data capturing was done by laboratory and data clerks at the NICD. Data were extracted from MS Access to MS Excel and Stata 11.0 for data analysis.

Cleaning of data amongst others consisted of checking for outliers through box plots and frequency tables. Logical checks were done. Missing data were quantified and were excluded from the analysis. A check was done to determine whether cases with missing serotype data differ from cases without serotype data with regards to exposure variables.

2.4.2 Descriptive data analysis

2.4.2.1 Incidence rates

- Overall incidence rates were calculated as the number of invasive *H. influenzae* cases per year (2003 to 2009) as the numerator and the mid-year population estimates of South Africa for that specific year as the denominator. Mid-year population estimates was obtained from Statistics SA for each year (51). All incidence rates were expressed as cases per 1 000 000 population.
- Age-specific incidence rates were calculated as the number of invasive *H. influenzae* cases in a specific age group by year (2003 to 2009) as the numerator and the mid-year population estimate of South Africa for that specific age group and specific year as the denominator (51).
- Serotype-specific incidence rates: *H. influenzae* can be divided into 2 groups: (i) Serotypeable of which Hib is the most important compared to the other non-b encapsulated serotypes (a,c,d,e, and f) and (ii) Non-serotypeable (strains that lack a capsule). Invasive Hib, NTHi and the non-b encapsulated serotypes were described in the descriptive analysis in order to explain the distribution of all serotypes in the population. The non-b encapsulated strains

were excluded from the analytical analysis. Serotype-specific incidence rates were calculated as the number of Hib cases, number of NTHi cases and number of non-b encapsulated *H. influenzae* cases (all the non-b encapsulated serotypes were grouped together) for a specific year as the numerator and the mid-year population estimate of South Africa for that specific year (2003-2009) as the denominator.

Trends of incidence rates from 2003-2009 were assessed using the chi-square test for linear trend. A p-value of <0.05 was considered a statistically significant trend. A linearity test was conducted on significant trends to assess whether the trend was linear. The percentage change was reported if the trend was significant but non-linear. The 95% confidence intervals (CI) around the incidence rates were calculated using a Poisson distribution (63).

2.4.2.2 Seasonal trends

The sum of the number of Hib and NTHi cases for a specific month, January to December, for all the years from 2003-2009 combined were calculated. The total number of Hib and NTHi cases per month for all the years (2003-2009) combined were further evaluated in persons <5 years and ≥5 years, to determine whether seasonal trends differ by age. The seasonal trends were described visually and no statistical test was performed.

2.4.2.3 Comparing characteristics of invasive H. influenzae cases reported to enhanced and non-enhanced sites

Data from cases presenting only to enhanced sites were used in the analytical objective because detailed clinical information was collected at these sites compared to only limited demographic data collected at non-enhanced surveillance sites. In order to explore possible bias which could be introduced if there are differences in characteristics of cases reporting to enhanced and non-enhanced sites, the differences between invasive *H. influenzae* cases presenting to enhanced and non-enhanced sites with regards to characteristics for which data were available were evaluated.

All the characteristics were categorical variables (age group, gender, province, serotype, clinical syndrome, year and ampicillin susceptibility). The variables were presented with frequencies and percentages. The chi-square test or the Fisher's exact test was used to compare proportions of categorical variables between the enhanced and non-enhanced sites. A p-value of <0.05 was considered statistically significant.

2.4.3 Factors associated with invasive Hib or NTHi disease

2.4.3.1 Descriptive analysis

The outcome was people who had invasive NTHi disease compared to invasive Hib disease in persons of all ages, <5 years and ≥5 years old. The analysis was stratified by <5 years old and ≥5 old because of the well-described differences in epidemiology of NTHi and Hib disease in these 2 age groups (38, 63-64). Firstly the explanatory variables were compared between the two outcome categories. The explanatory categorical variables (age group, gender, race, province, year of infection, clinical syndrome, predisposing conditions, HIV status, outcome, antibiotic use 24 hrs prior to specimen collection date, antibiotic use 2 months prior to specimen collection date, cotrimoxazole prophylaxis, severity of illness, nosocomial infection and ampicillin susceptibility) were presented with frequencies and percentages. Duration of hospital stay was a discrete variable and its distribution was evaluated through a histogram and box-and-whiskers plot. A Mann Whitney U-test was used to compare the median duration of hospital stay between cases with invasive Hib and NTHi disease in all ages because it was non-normally distributed. A chi-square test or Fisher's exact test was used to compare the categorical variables between invasive Hib and NTHi disease in all ages. A p-value of <0.05 was considered statistically significant.

2.4.3.2 Analytical analysis

Logistic regression was used for the univariate and multivariable analysis. A univariate analysis was performed to assess the association of each explanatory variable with the outcome (NTHi

disease compared to Hib disease). The 25-44 year old age group was chosen as the reference group to better display the higher risk of disease in the <5 year age group. Only variables with a p-value <0.2 were selected from the univariate analysis to be evaluated in the multivariable model. The multivariable model was built by using a manual forward selection technique. The variables were included in the multivariable model in descending order of clinical significance. The log-likelihood test was used to assess the significance of factors in explaining the odds of NTHi disease compared to Hib disease. A log-likelihood p-value of <0.05 was used for inclusion of a variable in the final model. The model's fit was tested using a Hosmer-Lemeshow goodness of fit test. Two-way interactions were evaluated.

2.5. Ethical Consideration

2.5.1 Ethical permission

Ethical permission was obtained since the inception of the surveillance programme in 1999. Ethics recertification was done on a yearly basis. Ethical permission was therefore obtained for the entire study period from 2003 to 2009. The most recent ethics recertification was obtained for GERMS-SA on 26 November 2010 (protocol number M081117). Informed consent, assent and access issues are covered under this ethics. Separate ethical permission was obtained for the secondary data analysis. The protocol was submitted to the Human Research Ethics Committee (Medical) (HREC), University of Witwatersrand, Johannesburg. Permission was obtained on 30 September 2011 (protocol number M110968) (See Appendix E for ethics clearance certificate).

2.5.2 Confidentiality and access issues

Confidentiality was ensured through conducting of the interview in a private area, sending confidential CRFs in a sealed envelope to the NICD and storing CRFs in a secure lockable space. Data was captured on a MS Access database which was on a secure server and password protected.

Patient identifiers were kept on the CRF as well as on the database because *H. influenzae* type b is a notifiable disease, to ensure that the laboratory and clinical CRFs can be matched for the same patient and that no duplication occurred and to facilitate audits by matching the GERMS-SA database to the Central Data Warehouse (CDW).

2.5.3 Risks and Benefits

Risks to the patient were minimal but potential loss of confidentiality could occur. There was no individual benefit to the patient for participating in this study and no compensation was given.

3. Results

The results will be structured according to the specific objectives of this study. Additional results, that were done to explore bias, will also be reflected.

3.1 Trends and incidence

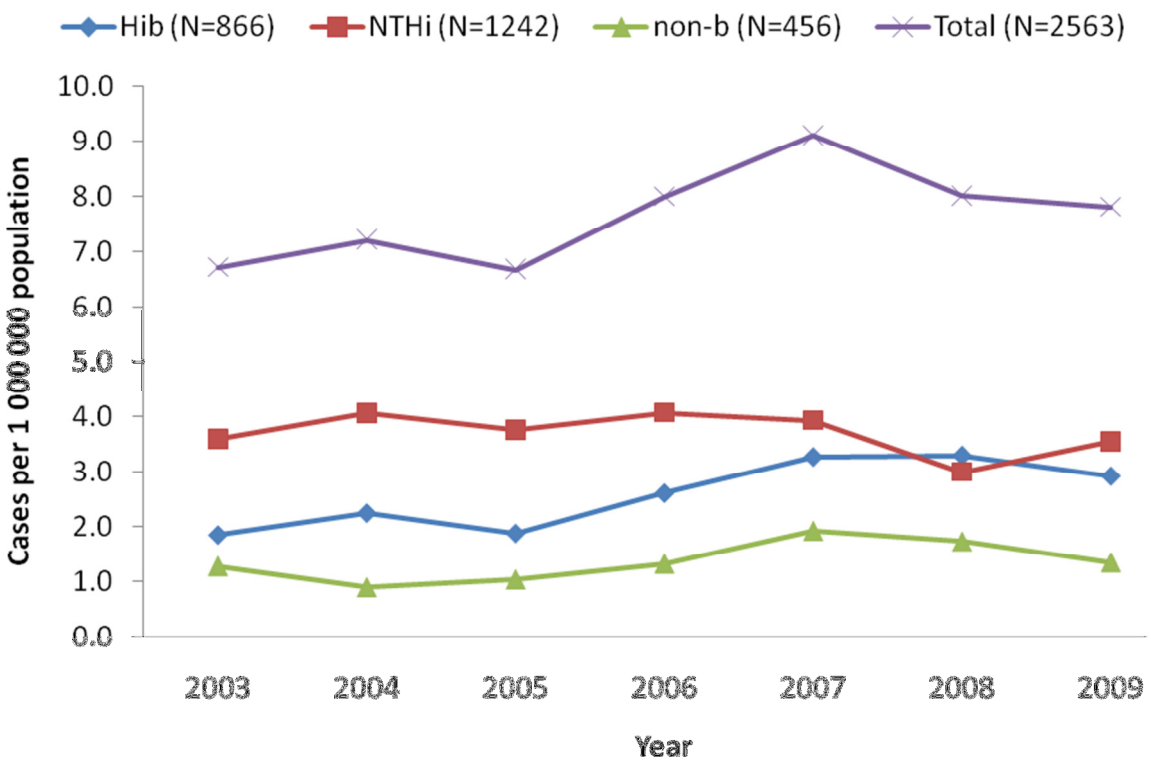
The population under surveillance was 46 429 823 in 2003, 48 253 727 in 2004, 46 888 200 in 2005, 47 390 900 in 2006, 47 850 700 in 2007, 48 687 000 in 2008 and 49 320 500 in 2009. During 2003-2009, 2563 cases of *H. influenzae* were identified through the GERMS-SA surveillance network. Fifty-six per cent (1441/2563) of all isolates or specimens were serotyped (Table 3.1). Isolates that were not serotyped include the following cases: audits, missing, contaminated and non-viable. The majority of reported cases were culture-positive (1384/2563, 54%). A small proportion was identified through PCR (210/2563, 8%) and latex agglutination test (8/2563, 0.3%) See Appendix F for a more detailed breakdown of reported cases.

Table 3.1 Number of *Haemophilus influenzae* cases serotyped (viable and non-viable) and not serotyped by year for all ages, 2003-2009

Cases	2003 N (%)	2004 N (%)	2005 N (%)	2006 N (%)	2007 N (%)	2008 N (%)	2009 N (%)	Total N (%)
Serotyped (Viable)	179 (57%)	175 (50%)	193 (62%)	202 (53%)	211 (48%)	192 (49%)	232 (60%)	**1384 (54%)**
Serotyped (Non-viable)	0	0	0	1 (0.3%)	10 (2%)	14 (4%)	32 (8%)	**57 (2%)**
Not Serotyped	133 (43%)	173 (50%)	120 (38%)	176 (46%)	215 (49%)	184 (47%)	121 (31%)	**1122 (44%)**
Total	312	348	313	379	436	390	385	**2563**

During 2003-2009, 76% of all cases were reported to the surveillance network, while 24% were identified during audits and added to the database retrospectively. NTHi disease accounted for 49% (1246/2563), Hib 33% (860/2563) and non-b encapsulated serotypes 18% (457/2563) of all cases over the 7-year period. Fifty-seven percent (1455/2563) of *H. influenzae* cases identified

were <5 years old and 43% (1108/2563) were ≥5 years old. The annual *H. influenzae* incidence in all ages increased by 16% from 2003 to 2009 (Figure 3.1). The annual Hib incidence in all ages increased from 2 cases per 1 000 000 (95% CI 1.5 - 2.3) in 2003 to 3 cases per 1 000 000 (95% CI 2.5 - 3.5) in 2009 ($p < 0.001$). The annual non-b incidence in all ages increased by 8% from 2003 to 2009. NTHi incidence in all ages remained stable for the duration of the study period.

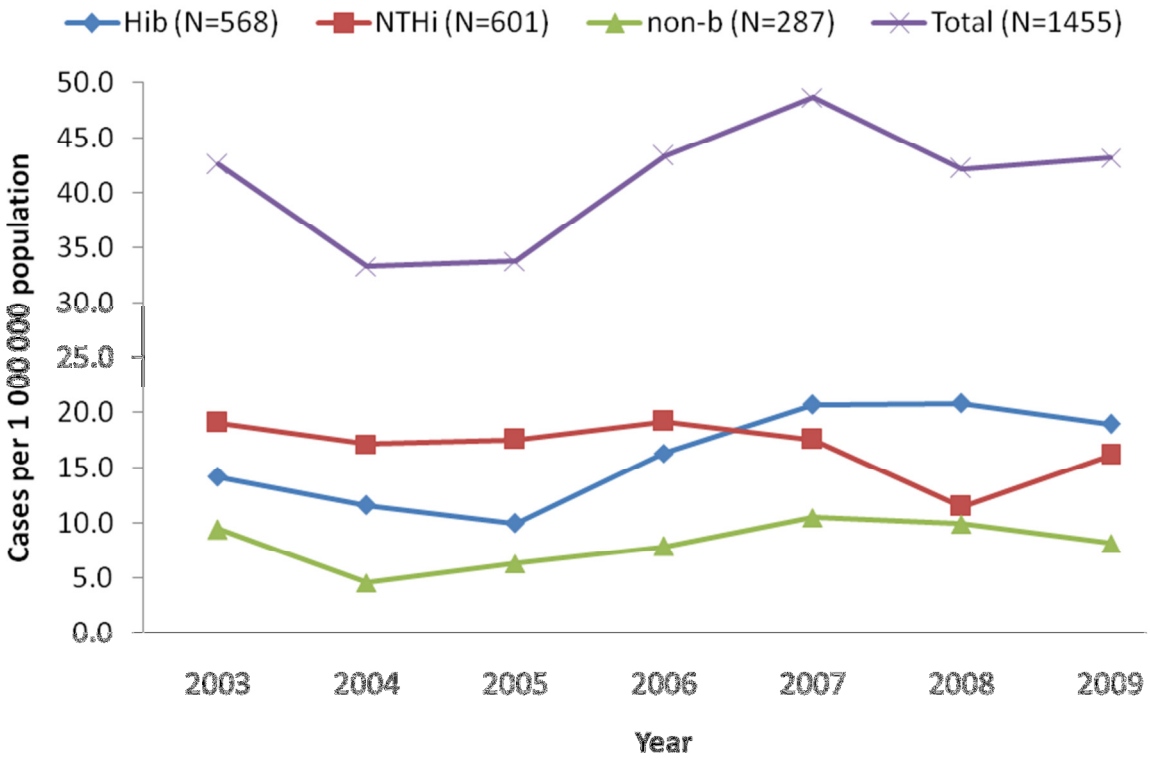


Hib – H. influenzae type b; NTHi – non-typeable H. influenzae; non-b – Encapsulated H. influenzae serotypes a,c,d,e and f.

Figure 3.1 Invasive *Haemophilus influenzae* incidence in all ages by serotype and year, 2003-2009, South Africa. Serotype-specific incidence rates were calculated assuming that cases with missing serotype data had the same serotype distribution as cases with serotype data available (Number of serotypes (%) missing per year were 133 (43%), 173 (50%), 120 (38%), 176 (46%), 215 (49%) 184 (47%) 121 (31%) respectively).

Figure 3.2 shows the overall annual *H. influenzae* incidence in <5 year olds by serotype and year, 2003-2009. No significant linear trends were observed for *H. influenzae*, Hib, NTHi and non-b

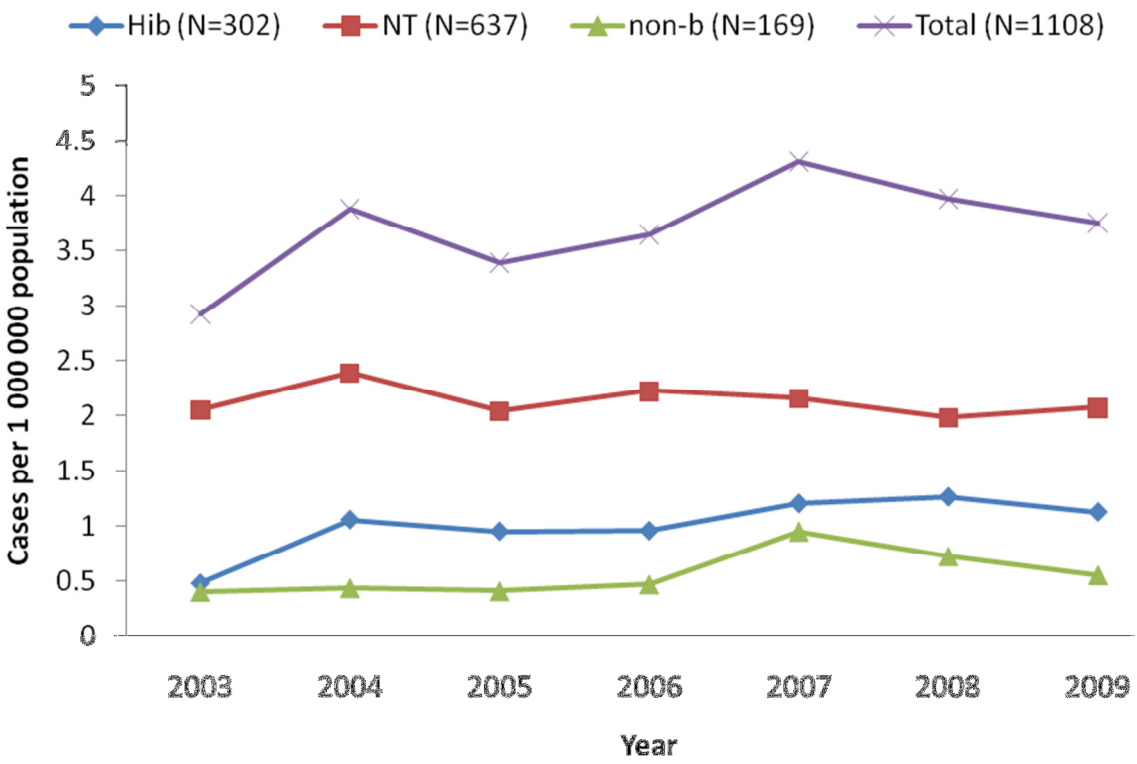
incidence from 2003-2009. However, Hib incidence in children <5 years increased from 2005-2008 from 10 cases per 1 000 000 (95% CI 7 –13) to 21 cases per 1 000 000 (95% CI 17 – 25) (p < 0.001).



Hib – H. influenzae type b; NTHi – non-typeable H. influenzae; non-b – Encapsulated H. influenzae serotypes a,c,d,e and f.

Figure 3.2 *Haemophilus influenzae* incidence by serotype and year in <5 year olds, 2003-2009, South Africa. Serotype-specific incidence rates were calculated assuming that cases with missing serotype data had the same serotype distribution as cases with serotype data available (Number of serotypes (%) missing per year were 76 (40%), 86 (47%), 60 (35%), 97 (43%), 117 (46%), 97 (45%), 54 (25%), respectively).

Figure 3.3 shows the overall annual *H. influenzae* incidence in ≥5 year olds by serotype and year, 2003-2009. The overall annual *H. influenzae* incidence in the ≥5 year olds increased from 2.9 cases per 1 000 000 (95% CI 2.4 – 3.5) in 2003 to 3.8 cases per 1 000 000 (95% CI 3.2 – 4.4) in 2009 (p = 0.019). Similarly the Hib incidence in ≥5 year olds showed an increase from 0.5 cases per 1 000 000 (95% CI 0.3 – 0.7) in 2003 to 1.1 cases per 1 000 000 (95% CI 0.8 – 1.5) in 2009 (p = 0.002). The annual NTHi incidence showed no significant linear trend from 2003-2009.



Hib – H. influenzae type b; NTHi – non-typeable H. influenzae; non-b – Encapsulated H. influenzae serotypes a,c,d,e and f.

Figure 3.3 *Haemophilus influenzae* incidence by serotype and year in ≥5 year olds, 2003-2009, South Africa. Serotype-specific incidence rates were calculated assuming that cases with missing serotype data had the same serotype distribution as cases with serotype data available (Number of serotypes (%) missing per year were 57 (46%), 87 (52%), 60 (42%), 79 (51%), 98 (53%), 87 (50%), 67 (40%), respectively).

Figure 3.4 presents the annual Hib incidence by age group and year in ≥5 year olds from 2003-2009. The only age group with Hib disease that showed a significant change was the ≥65 year age group, from 3.5 cases per 1 000 000 (95% CI 1.49 – 6.8) in 2003 to 0.9 cases per 1 000 000 (95% CI 0.10 -3.00) in 2009 (p = 0.008) (Figure 3.4). Even though not significant, a 34% decrease was seen in NTHi incidence in the ≥65 year age group from 2003-2009 (Figure 3.5). There were no significant linear trends in the annual age –specific incidence rates for non-b disease in the ≥5 year olds from 2003-2009 (figure 3.6).

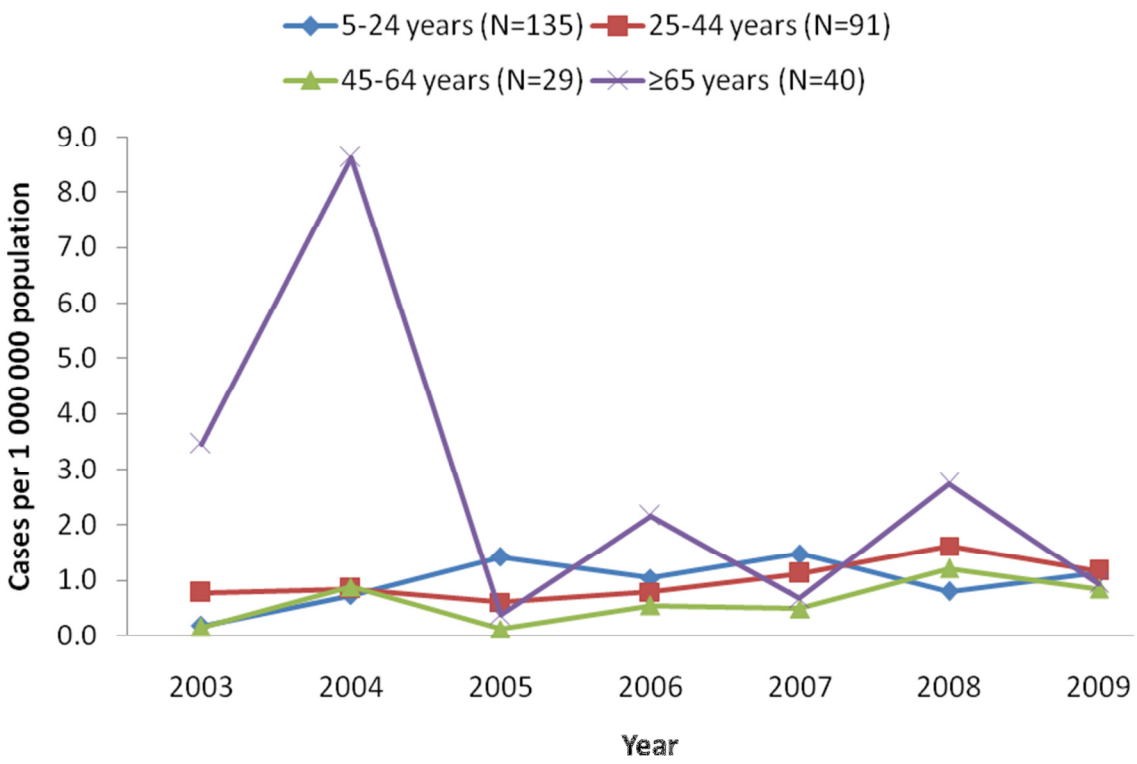


Figure 3.4 *Haemophilus influenzae* type b incidence by age group and year in ≥5 year olds, 2003-2009, South Africa.

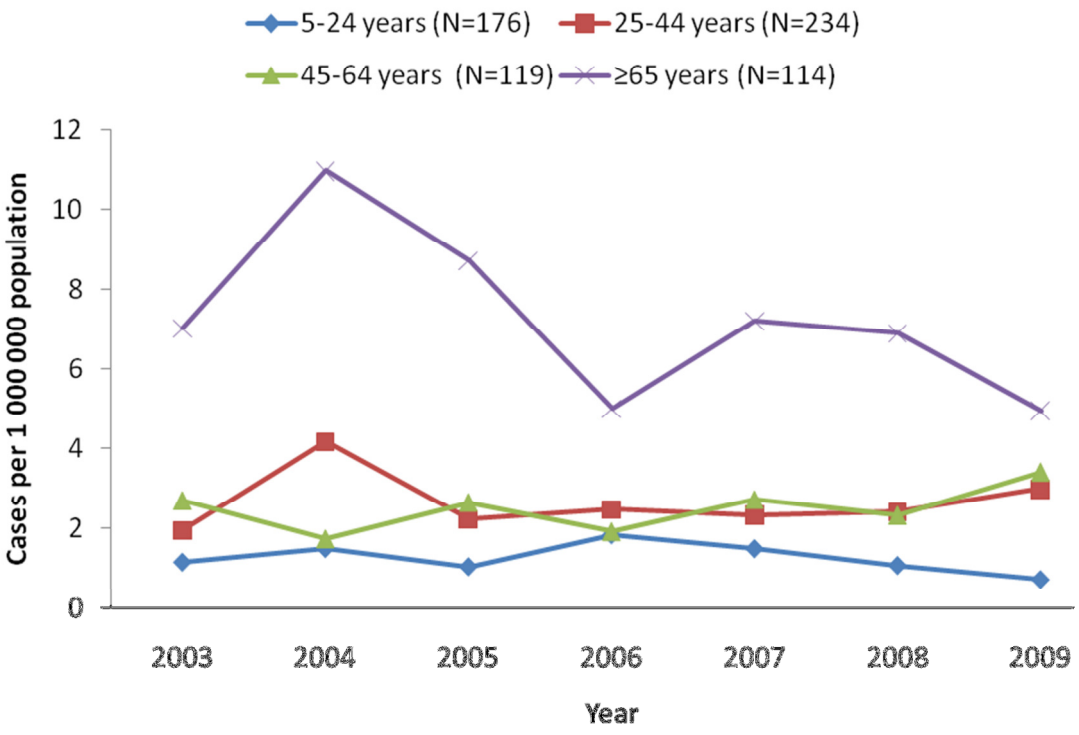


Figure 3.5 Non-typeable *Haemophilus influenzae* incidence by age group and year in ≥5 year olds, 2003-2009, South Africa

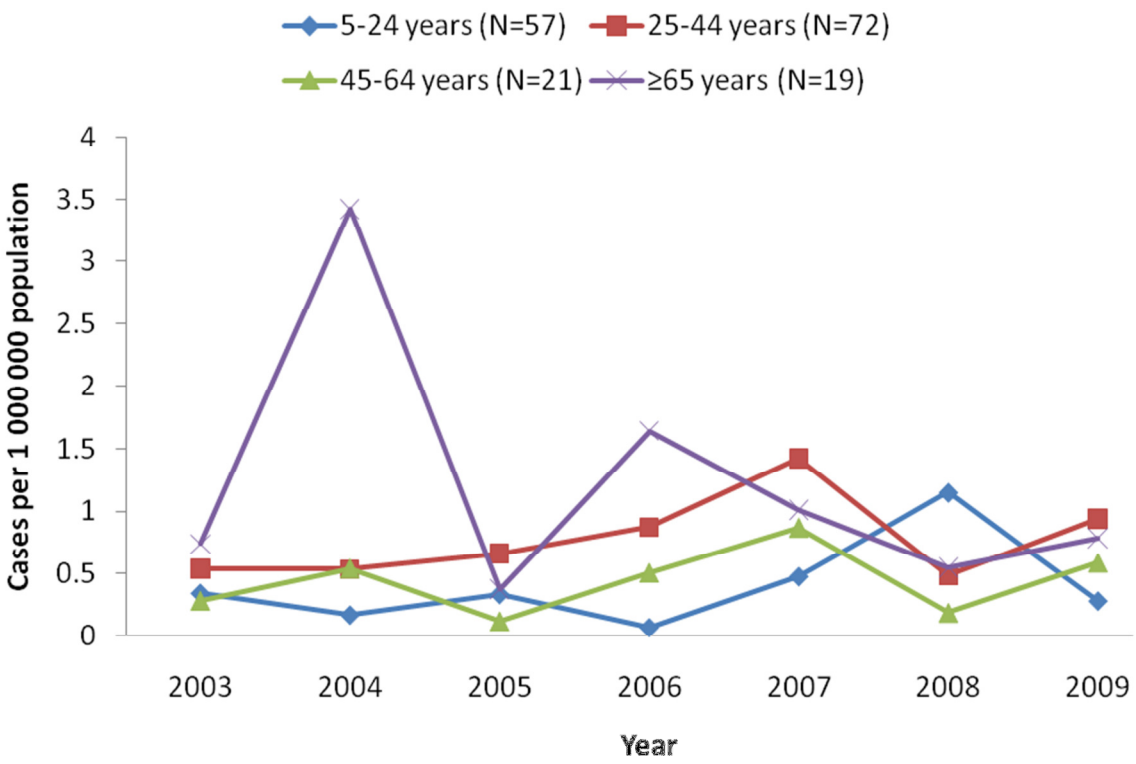
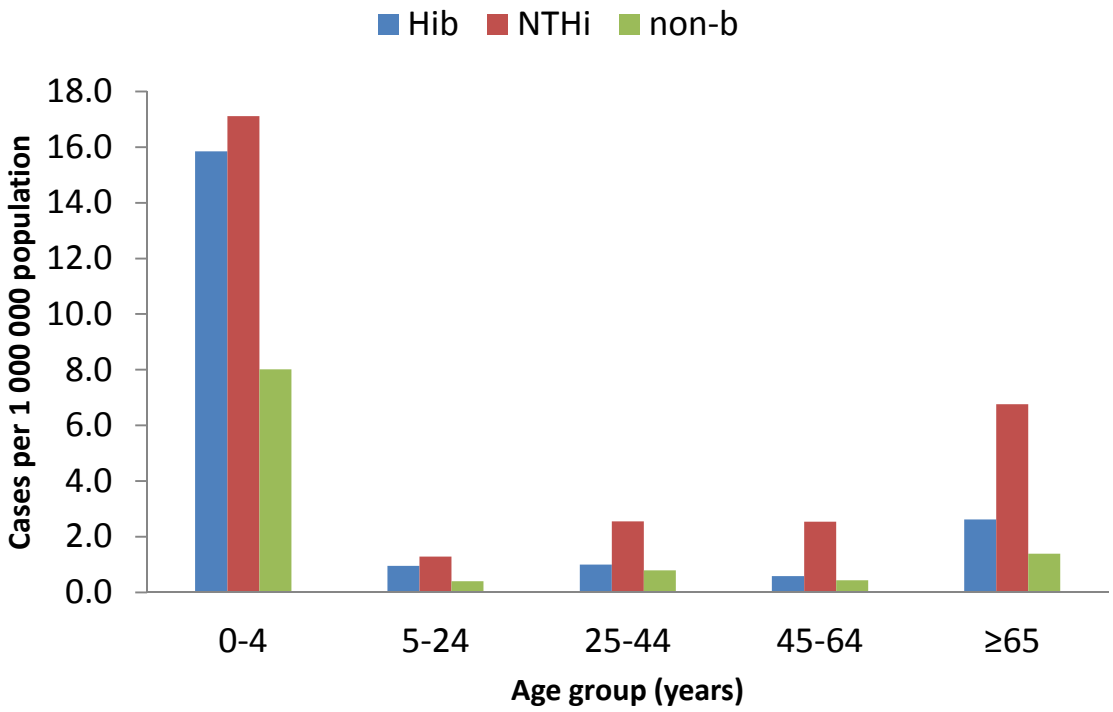


Figure 3.6 Non-b encapsulated *Haemophilus influenzae* incidence by age group and year in ≥5 year olds, 2003-2009, South Africa.

The greatest average annual (2003-2009) incidence of *H. influenzae* disease was in the 0-4 year age group, where Hib disease was as common as NTHi disease at 15.8 cases per 1 000 000 and 17.1 cases per 1 000 000, respectively (Figure 3.7). NTHi disease was the predominant serotype in the ≥65 year age group at an average annual incidence (2003-2009) of 6.8 cases per 1 000 000 compared to 2.6 cases per 1 000 000 for Hib and 1.4 cases per 1 000 000 for non-b disease. Hib disease, even though less common than in the 0-4 year age group, still occurred in the older age groups. A third peak of high average annual incidence (2003-2009) was observed in the 25-44 year age group for NTHi and non-b disease at 2.6 cases per 1 000 000 and 0.8 cases per 1 000 000 respectively.



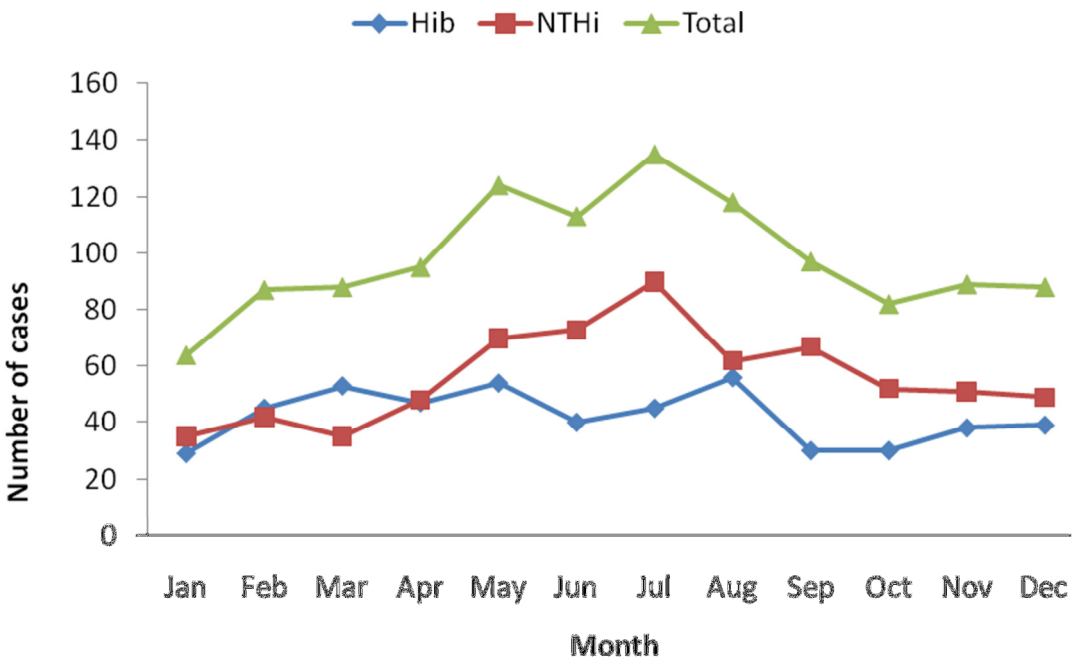
Hib – H. influenzae type b; NTHi – non-typeable H. influenzae; non-b – Encapsulated H. influenzae serotypes a,c,d,e and f.

Figure 3.7 Average annual *Haemophilus influenzae* incidence by age group and serotype from 2003-2009, South Africa. Serotype-specific incidence rates were calculated assuming that cases with missing serotype data had the same serotype distribution as cases with serotype data available (Number of serotypes (%) missing per age group were 587 (40%), 163 (44%), 200 (50%), 77 (46%), 95 (55%) respectively).

3.2 Seasonal trends

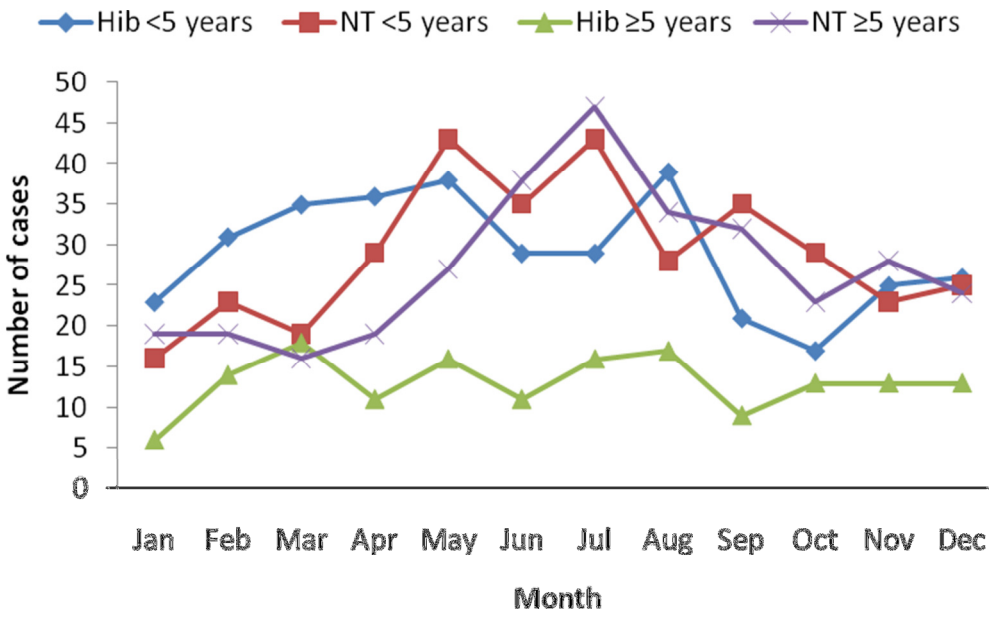
A seasonal trend was observed in cases with NTHi disease, with an increase in the total number of cases from May to September compared to the rest of the year (Figure 3.8). There was no seasonal peak observed in cases with Hib disease, with the total number of cases fluctuating between 29 and 56 from January to December.

Figure 3.9 showed the total number of Hib and NTHi cases in the <5 year and ≥5 year age group. Seasonal peaks were still observed in cases with NTHi disease regardless of age, while no distinct peaks were observed in cases with Hib disease regardless of age.



Hib – H. influenzae type b; NTHi – non-typeable H. influenzae

Figure 3.8 Total number of *H. influenzae* cases by serotype and month in all ages, 2003-2009, South Africa



Hib – H. influenzae type b; NTHi – non-typeable H. influenzae;

Figure 3.9 Total number of *H. influenzae* cases by serotype and age and by month, 2003-2009, South Africa

3.3 Characteristics of cases presenting to enhanced and non-enhanced sites

Table 3.2 Characteristics of invasive *Haemophilus influenzae* cases in persons that present to enhanced and non-enhanced surveillance sites from 2003-2009

Characteristics	Category	Enhanced site n /N (%)	Non-enhanced site n /N (%)	p-value*
Age (years)	0-4	734/1278 (57%)	721/1285 (56%)	0.938
	5-24	184/1278 (14%)	184/1285 (14%)	
	25-44	196/1278 (15%)	202/1285 (16%)	
	45-64	82/1278 (6%)	87/1285 (7%)	
	≥ 65	82/1278 (6%)	91/1285 (7%)	
Gender	Male	627/1243 (50%)	615/1235 (50%)	0.748
Province	Eastern Cape	28/1278 (2%)	199/1285 (15%)	<0.001
	Free State	79/1278 (6%)	64/1285 (5%)	
	Gauteng	586/1278 (46%)	645/1285 (50%)	
	Kwa-Zulu Natal	213/1278 (17%)	78/1285 (6%)	
	Limpopo	6/1278 (0.5%)	10/1285 (1%)	
	Mpumalanga	25/1278 (2%)	88/1285(7%)	
	Northern Cape	24/1278 (2%)	87/1285(1%)	
	North West	8/1278 (1%)	31/1285 (2%)	
	Western Cape	309/1278 (24%)	163/1285 (13%)	
Year	2003	177/1278 (14%)	135/1285 (11%)	0.015
	2004	161/1278 (13%)	187/1285 (15%)	
	2005	168/1278 (13%)	145/1285 (11%)	
	2006	197/1278 (15%)	182/1285 (14%)	
	2007	221/1278 (17%)	215/1285 (17%)	
	2008	179/1278 (14%)	211/1285 (16%)	
	2009	175/1278 (14%)	210/1285 (16%)	
Specimen	Cerobrospinal fluid	285/1278 (22%)	352/1285 (27%)	<0.001
	Blood culture	850/1278 (67%)	747/1285 (58%)	
	Joint fluid	23/1278 (2%)	10/1285 (1%)	
	Pleural fluid	79/1278 (6%)	148/1258 (12%)	
	Another**	41/1278 (3%)	28/1258 (2%)	
Ampicillin Susceptibility	Susceptible	674/803 (84%)	481/578 (83%)	0.722
Serotype	Hib†	288/683 (42%)	218/497 (44%)	0.561
	NTHi††	395/683 (58%)	279/497 (56%)	

Column percentages might not add up to 100% due to rounding off
Missing data are excluded
* p value derived from Chi^2 test
** Specimen "Another" category include specimen types such as pus from a normally sterile site (bone and brain), tissue (endometrial, lung and brain), peritoneal fluid, pericardial fluid, vitreous fluid
† Hib – *Haemophilus influenzae* type b
†† NTHi – Non-typeable *H. influenzae*

There were no significant difference with regards to age, gender, ampicillin susceptibility and the proportion of NTHi and Hib cases that presented to enhanced and non-enhanced sites (Table 3.2). However, a greater proportion of *H. influenzae* cases were derived from enhanced surveillance sites compared to non-enhanced surveillance sites in Kwa-Zulu Natal (17% vs. 6% respectively)

and Western Cape (24% vs. 13% respectively) provinces. Non-enhanced sites in Eastern Cape and Mpumalanga provinces reported 7.5 times and 3.5 times more *H. influenzae* cases compared to enhanced sites ($p < 0.001$), respectively. There was a significant difference ($p < 0.001$) in the proportion of specimen types reported from enhanced and non-enhanced sites. A greater proportion of *H. influenzae* cases were isolated from blood cultures at enhanced sites (67%) compared to non-enhanced sites (58%). A greater proportion of *H. influenzae* cases were derived from cerebrospinal fluid and pleural fluid at non-enhanced sites compared to enhanced sites, 27% vs. 22% and 12% vs. 6% respectively. The proportion of *H. influenzae* cases obtained from enhanced and non-enhanced sites differed significantly by year ($p = 0.015$).

3.4 Comparing cases with NTHi disease to Hib disease

3.4.1 Descriptive analysis

During 2003-2009, 50% (1278/2563) of *H. influenzae* cases presented to enhance surveillance sites of which 835 (65%) had a serotype determined. No significant difference were noted in age, predisposing conditions, HIV status, cotrimoxazole prophylaxis, in-hospital outcome, antibiotic use 2 months prior to specimen collection date and nosocomial infection between cases with known and unknown serotypes. However, cases with unknown serotype were less likely to have severe disease (Pitt bacteremia score >4) ($p < 0.001$), to come from Gauteng and Freestate ($p = 0.003$) and to have meningitis ($p < 0.001$).

Hib and NTHi cases accounted for 82% (683/835) of all cases serotyped, of which 42% (288/683) were Hib cases and 58% (395/683) were NTHi cases. Hib cases had the following age distribution: 0-4, 5-24, 25-44, 45-64 and ≥65 year age groups accounted for 69% (198/288), 18% (52/288), 7% (21/288), 3% 10/288) and 2% (7/288) respectively. NTHi cases had the following age distribution: 0-4, 5-24, 25-44, 45-64 and ≥65 year age groups accounted for 50% (198/395), 12% (49/395), 19% (75/395), 10% (39/395) and 9% (34/395) respectively. Hib and NTHi cases had a similar gender distribution, regardless of age category (Tables 3.3 and 3.4). The race distribution between <5

years old Hib and NTHi cases were similar (p = 0.258) (Table 3.3). A higher proportion of Hib cases ≥5 years were of black race compared to NTHi cases, 94% (81/86) and 84% (153/183) (p = 0.016), respectively (Table 3.4). The majority of Hib and NTHi cases in all ages reported were from Gauteng province (42%, 120/288 and 52%, 207/395 respectively), followed by Western Cape province (23%, 65/288 and 20%, 80/395 respectively) and Kwa-Zulu Natal province (19%, 56/288 and 17%, 68/395 respectively).

Fifty-three per cent (153/288) of all Hib cases were diagnosed with meningitis of which 74% (113/153) were among <5 year old children. Compared to this only 10% (39/392) of all NTHi cases were diagnosed with meningitis of which 38% (15/39) were <5 years old. LRTI was the most common diagnosis of all NTHi cases with 59% (232/392) compared to Hib cases with 33% (94/288) and remained the most common diagnosis regardless of age group (<5 and ≥5 years old) (Tables 3.3 and 3.4).

In all ages, the proportion of Hib and NTHi cases with a predisposing condition (excluding HIV) was 27% (51/192) and 60% (118/198) respectively. Predisposing conditions were more common in cases with NTHi disease compared to Hib disease in both <5 year olds and ≥5 year olds (Tables 3.3 and 3.4). Protein-energy malnutrition was the most common predisposing condition for both Hib (19/40, 48%) and NTHi (38/59, 64%) cases in the <5 year age group followed by chronic conditions for Hib (7/40, 18%) and NTHi cases (14/59, 24%). In persons ≥5 years, chronic conditions were the most frequent predisposing condition in Hib cases (5/11, 45 %) whereas immunosuppressive conditions were the most frequent predisposing condition for NTHi cases (14/59, 24%). If HIV infection was added as a predisposing condition, no difference was found between the proportion of ≥5 year old Hib and NTHi cases with a predisposing condition, 83% (59/71) vs 90% (117/130) respectively (p = 0.156) (Figure 3.10). The <5 year old age group however, showed a significant difference in the proportion of Hib and NTHi cases with a predisposing condition, 55% (92/166) vs 83% (119/144) respectively (p < 0.001).

The proportion of HIV-infected individuals were higher amongst older individuals (≥5 years) with Hib disease (89%) compared to children <5 years old (51%) ($p = 0.020$) with Hib disease. The proportion of NTHi cases with ampicillin non-susceptibilty were double in cases <5 years old (19%) compared to NTHi case that were ≥5 years (9%) ($p = 0.019$) (Analysis not shown)

Table 3.3 Characteristics of persons <5 years old with invasive *Haemophilus influenzae* type b and non-typeable *Haemophilus influenzae* infections presenting to enhanced sites from 2003-2009

Factor	Category	Hib* n/N (%)	NTHi** n/N (%)	p value***
Year of infection	2003	19/198 (10%)	38/198 (19%)	0.001
	2004	19/198 (10%)	34/198 (17%)	
	2005	22/198 (11%)	27/198 (14%)	
	2006	26/198 (13%)	27/198 (14%)	
	2007	38/198 (19%)	24/198 (12%)	
	2008	31/198 (16%)	14/198 (7%)	
	2009	43 /198(22%)	34/198 (17%)	
Gender	Male	97/198 (49%)	91/197 (46%)	0.578
Race	Black	173/196 (88%)	177/193 (92%)	0.258
Province	Eastern Cape	4/198 (2%)	4/198 (2%)	0.123
	Free State	15/198 (8%)	17/198 (9%)	
	Gauteng	74/198 (37%)	93/198 (47%)	
	Kwa-Zulu Natal	39/198 (20%)	40/198 (20%)	
	Limpopo	3/198 (2%)	0/198 (0%)	
	Mpumalanga	3/198 (2%)	1/198 (1%)	
	Northern Cape	6/198 (3%)	1/198 (1%)	
	North West	0/198 (0%)	0/198 (0%)	
	Western Cape	54/198 (27%)	42/198 (21%)	
Clinical	Meningitis	113/198 (57%)	15/198 (8%)	<0.001
Syndrome	LRTI‡	60/198 (30%)	128/198 (65%)	
	Bacteremia	17/198 (9%)	52/198 (26%)	
	Other†	8/198 (4%)	3/198 (2%)	
Predisposing condition (excluding HIV)	Yes	40/139 (29%)	59/102 (58%)	<0.001
HIV status	HIV infected	65/128 (51%)	94/133 (71%)	0.001
Outcome	Died	23/186 (12%)	53/175 (30%)	<0.001
Antibiotic use 24 hrs prior to specimen collection	Yes	9/155 (6%)	5/132 (6%)	0.492
Antibiotic use 2 months prior to specimen collection	Yes	17/142 (12%)	29/124 (23%)	0.014
Nosocomial[#] infection	Yes	8/185 (4%)	19/174 (11%)	0.018
Cotrimoxazole prophylaxis	Yes	19/155 (12%)	37/134 (28%)	0.001
Hospital duration in days Median (range)		10 (6-16)	9 (5-17)	0.561
Acute severe illness[##]	Yes	33/198 (17%)	68/198 (37%)	<0.001
Ampicillin susceptibility	Not susceptible	40/190 (21%)	37/193 (19%)	0.646

Column percentages does not always add up to 100% due to rounding off.

* Hib – *H. influenzae* type b

**NTHi – non-typeable *H. influenzae*

***p value derived from Chi^2 or Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables

‡LRTI – Lower respiratory tract infections

†Clinical syndrome "other" category contains the following: septic arthritis, peritonitis, cellulitis, osteomyelitis, pericarditis

#Nosocomial infection is defined as a positive specimen for *H. influenzae* taken ≥2 days after admission date

##Acute severe illness is defined as a Pitt bacteremia score of >4

Table 3.4 Characteristics of persons ≥5 years old with invasive *Haemophilus influenzae* type b and non-typeable *Haemophilus influenzae* infections presenting to enhanced sites from 2003-2009

Factor	Category	Hib* n/N (%)	NTHi** n/N (%)	p value***
Year of infection	2003	6/90 (7%)	29/197 (15%)	0.016
	2004	5/90 (6%)	37/197 (19%)	
	2005	15/90 (17%)	31/197 (16%)	
	2006	16/90 (18%)	25/197 (13%)	
	2007	16/90 (18%)	22/197 (11%)	
	2008	16/90 (18%)	24/197 (12%)	
	2009	16 /90(18%)	29/197 (15%)	
Gender	Male	43/88 (49%)	82/188 (44%)	0.414
Race	Black	81/86 (94%)	153/183 (84%)	0.016
Province	Eastern Cape	1/90 (1%)	1/197 (1%)	0.010
	Free State	5/90 (6%)	9/197 (5%)	
	Gauteng	46/90 (51%)	114/197 (58%)	
	Kwa-Zulu Natal	17/90 (19%)	28/197 (14%)	
	Limpopo	0/90 (0%)	0/197 (0%)	
	Mpumalanga	4/90 (4%)	7/197 (4%)	
	Northern Cape	6/90 (7%)	0/197 (0%)	
	North West	0/90 (0%)	0/197(0%)	
	Western Cape	11/12 (12%)	38/197 (19%)	
Clinical Syndrome	Meningitis	40/90 (44%)	24/194 (12%)	<0.001
	LRTI‡	34/90 (38%)	104/194 (54%)	
	Bacteremia	11/90 (12%)	58/194 (30%)	
	Other†	5/90 (6%)	8/194 (4%)	
Predisposing condition (excluding HIV)	Yes	11/53 (21%)	59/96 (61%)	<0.001
HIV status	HIV infected	55/62 (89%)	73/94 (78%)	0.078
Outcome	Died	13/79 (16%)	58/157 (37%)	0.001
Antibiotic use 24 hrs prior to specimen collection	Yes	3/54 (6%)	6/121 (5%)	0.869
Antibiotic use 2 months prior to specimen collection	Yes	4/46 (9%)	11/92 (12%)	0.562
Nosocomial[#] infection	Yes	1/82 (1%)	18/159 (11%)	0.006
Cotrimoxazole prophylaxis	Yes	11/58 (19%)	25/100 (25%)	0.383
Hospital duration in days Median (range)		9 (4-13)	8 (3-13)	0.233
Acute severe illness[##]	Yes	19/90 (21%)	77/197 (39%)	0.003
Ampicillin susceptibility	Not susceptible	16/83 (19%)	18/190 (9%)	0.024

Column percentages does not always add up to 100% due to rounding off.

* Hib – *H. influenzae* type b

**NTHi – non-typeable *H. influenzae*

***p value derived from Chi^2 or Fisher's exact test for categorical variables and Mann-Whitney U test for continuous variables

‡LRTI – Lower respiratory tract infections

†Clinical syndrome "other" category contains the following: septic arthritis, peritonitis, cellulitis, osteomyelitis, pericarditis

#Nosocomial infection is defined as a positive specimen for *H. influenzae* taken ≥2 days after admission date

##Acute severe illness is defined as a Pitt bacteremia score of >4

A greater proportion of NTHi cases compared to Hib cases in all ages had an acute severe illness (Pitt bacteremia score >4), 37% (145/395) vs 18% (52/288) respectively, ($p < 0.001$), a nosocomial infection, 11% (37/333) vs 3%, (9/267) respectively, ($p < 0.001$), and died while in hospital 33% (111/332) vs 14% (36/265) respectively, ($p < 0.001$). Abovementioned associations remained significant regardless of age (<5 year and ≥5 year age groups) (Tables 3.3 and 3.4).

Ampicillin non-susceptibility was more common in Hib cases compared to NTHi cases in all ages, 21% (56/273) vs 14% (55/383) respectively, ($p = 0.038$). Stratified by age group, ampicillin non-susceptibility remained more common in Hib cases (19%, 16/83) compared to NTHi cases (9%, 18/190) in the ≥5 year age group ($p = 0.024$) (Table 3.4), but in the <5 year age group no difference was observed ($p = 0.646$) (Table 3.3)

There was no significant difference between NTHi and Hib cases with regards to hospital duration and antibiotic use 24 hours before specimen collection date (Tables 3.3 and 3.4). A significant difference was found in antibiotic use 2 months prior to specimen collection date between Hib and NTHi cases in all ages, 11% (21/188) vs 19% (40/216) respectively, ($p = 0.040$) and <5 year olds, 12% (17/142) vs 23% (29/124) respectively, ($p = 0.014$) (Table 3.3), but not in the ≥5 year age group ($p = 0.562$) (Table and 3.4).

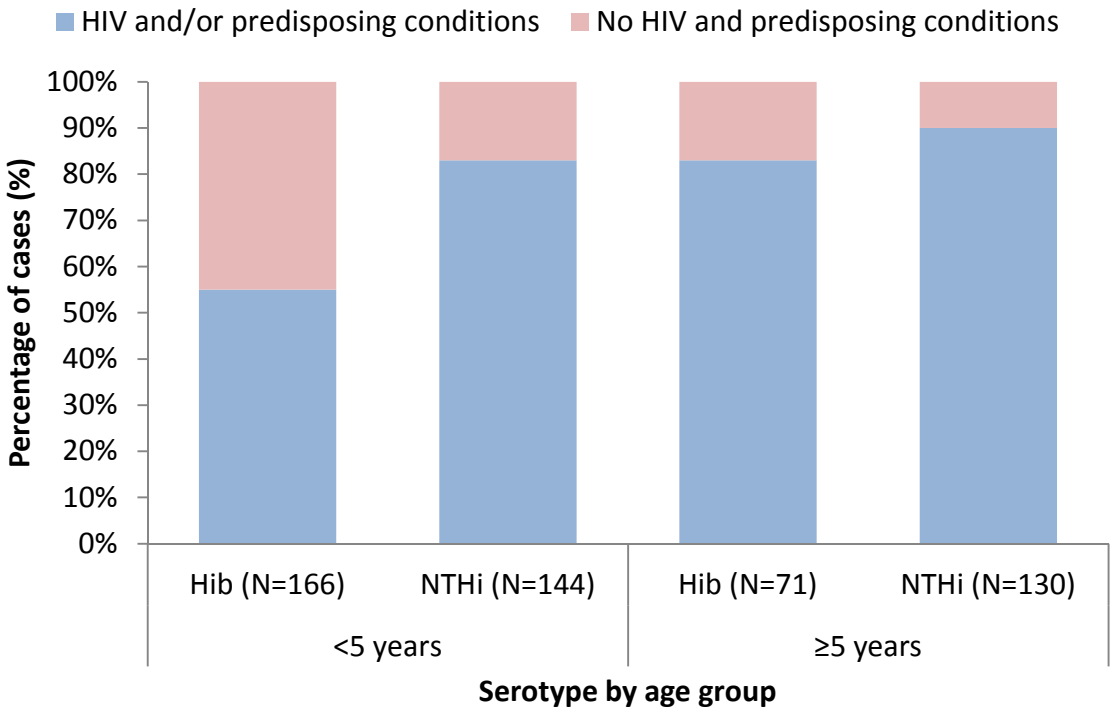


Figure 3.10 Percentage of *Haemophilus influenzae* type b and non-typeable *Haemophilus influenzae* cases by predisposing conditions and/or HIV (Human immunodeficiency virus) and age group, 2003-2009, South Africa

In all ages, NTHi cases had the highest proportion (74%, 167/227) of HIV-infected cases compared to Hib cases (63%, 120/190) (p = 0.022). A greater proportion of NTHi cases <5 years were HIV-infected compared to Hib cases, 71%, 94/133 vs 51%, 65/128 respectively (p = 0.001) (Table 3.3; Figure 3.11). In the 5-24 year age group Hib cases had a greater proportion of HIV-infected compared to NTHi cases, 94%, 34/36 vs 65% 13/20 respectively (p = 0.004) (Figure 3.11). The proportion of HIV-infected among cases with Hib and NTHi disease in the 25-44 year age group, was similar at 87% (13/15) and 84% (43/51) respectively (p = 0.823).

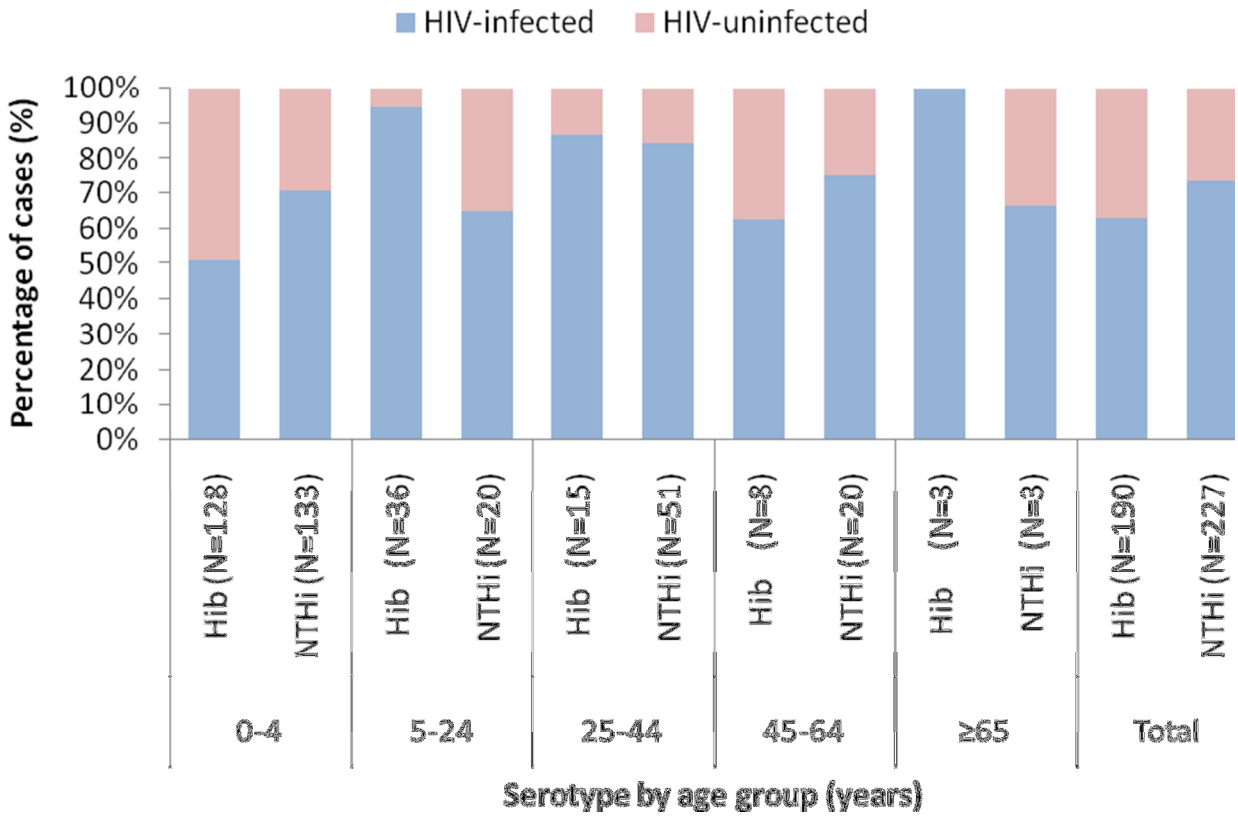


Figure 3.11 Percentage of *Haemophilus influenzae* type b and non-typeable *Haemophilus influenza*e cases by HIV status and age group, 2003-2009, South Africa

3.4.2 Predictors of NTHi disease compared to Hib disease

Factors associated with NTHi disease compared to Hib disease on univariate and multivariable analysis are shown in Table 3.5. After adjusting for other factors, a person with NTHi were more likely to have a clinical syndrome other than meningitis (respiratory tract infection OR 8.31, 95% CI 4.12-16.78, bacteremia OR 9.24, 95% CI 3.79-22.54), to have predisposing conditions other than HIV (OR 3.00, 95% CI 1.68-5.35) compared to no predisposing conditions, to die while in hospital (OR 2.33, 95% CI 1.002-5.460) compared to recovered, to have received cotrimoxazole prophylaxis (OR 2.41, 95% CI 1.10-5.32) compared to no cotrimoxazole prophylaxis received and to have a nosocomial infection OR 5.67, 95% CI 1.71-18.82) compared to no nosocomial infection. A person with NTHi was less likely to be <5 year old (OR 0.29, 95% CI 0.12-0.67) compared to being 25-44 years of age. No significant interactions were found.

Table 3.5 Univariate and multivariable analysis of factors associated with invasive non-typeable *Haemophilus influenzae* disease compared to *Haemophilus influenzae* type b disease in persons of all ages, 2003-2009, South Africa

Factor	Category	Hib n/N (%)	NTHi n/N (%)	Univariate analysis OR (95% CI)	p value	Multivariable analysis OR (95% CI)	p value
Age (years)	0-4	198/288 (69%)	198/395 (50%)	0.28 (0.17 - 0.47)	<0.001	0.29 (0.12 – 0.67)	<0.001
	5-24	52/288 (18%)	49/395 (12%)	0.26 (0.14 – 0.49)		0.24 (0.08 – 0.70)	
	25-44	21/288 (7%)	75/395 (19%)	1		1	
	45-64	10/288 (3%)	39/395 (10%)	1.09 (0.47 -2.55)		0.53 (0.16 – 1.82)	
	≥ 65	7/350 (2%)	34/395 (9%)	1.36 (0.53-3.50)		0.51 (0.05 – 5.42)	
Year of infection	2003	25/288 (9%)	67/395 (17%)	1	<0.001		
	2004	24/288 (8%)	71/395 (18%)	1.10 (0.58 - 2.12)			
	2005	37/288 (13%)	58/395 (15%)	0.58 (0.32 - 1.08)			
	2006	42/288 (15%)	52/395 (13%)	0.46 (0.25 - 0.85)			
	2007	54/288 (19%)	46/395 (12%)	0.32 (0.17 - 0.58)			
	2008	47/288 (16%)	38/395 (10%)	0.30 (0.16 - 0.57			
	2009	59 /288(20%)	63/395 (16%)	0.40 (0.22 - 0.71)			
Gender	Female	146/286 (51%)	212/385 (55%)	1			
	Male	140/286 (49%)	173/385 (45%)	0.85 (0.63 - 1.16)	0.303		
Race	Black	254/282 (90%)	330/376 (88%)	1			
	Other†	28/282 (10%)	46/376 (12%)	1.26 (0.77 – 2.08)	0.355		
Province	Gauteng	120/288 (42%)	207/395 (52%)	1	<0.001		
	Eastern Cape	5/288 (2%)	5/395 (1%)	0.58 (0.16 – 2.04)			
	Free State	20/288 (7%)	26/395 (7%)	0.75 (0.40 – 1.41)			
	Kwa-Zulu Natal	56/288 (19%)	68/395 (17%)	0.70 (0.46 – 1.07)			
	Limpopo and Mpumalanga	10/288 (3%)	8/395 (2%)	0.46 (0.18 – 1.21)			
	Northern Cape	12/288 (4%)	1/395 (0.25%)	0.05 (0.02 -0.38)			
	Western Cape	65/288 (23%)	80/395 (20%)	0.71 (0.48 – 1.06)			
Clinical Syndrome	Meningitis	153/288 (53%)	39/392 (10%)	1	<0.001	1	<0.001
	LRTI	94/288 (33%)	232/392 (59%)	9.68 (6.33 – 14.82)		8.31 (4.12 – 16.78)	
	Bacteremia	28/288 (10%)	110/392 (28%)	15.41(8.95 – 26.55)		9.24 (3.79 – 22.54)	
	Other††	13/288 (5%)	11/392 (3%)	3.32 (1.38 – 7.98)		5.15 (1.29– 20.52)	

Predisposing conditions (excluding HIV)	Absent	141/192 (73%)	80/198 (40%)	1		1	<0.001
	Present	51/192 (27%)	118/198 (60%)	4.08 (2.66 – 6.26)	<0.001	3.00 (1.68 – 5.35)	
HIV status	HIV uninfected	70/190 (37%)	60/227 (26%)	1			
	HIV infected	120/190 (63%)	167/227 (74%)	1.62 (1.07 – 2.46)	0.023		
Outcome	Recovered	229/265 (86%)	221/332 (67%)	1		1	0.049
	Died	36/265 (14%)	111/332 (33%)	3.19 (2.10- 4.86)	<0.001	2.33 (1.002 – 5.460)	
Antibiotic use 24 hrs prior to specimen collection	No	197/209 (94%)	242/253 (95%)	1			
	Yes	12/209 (6%)	11/253 (5%)	0.75 (0.32 - 1.73)	0.494		
Antibiotic use 2 months prior to specimen collection	No	167/188 (89%)	176/216 (81%)	1			
	Yes	21/188 (11%)	40/216 (19%)	1.81 (1.02 - 3.19)	0.041		
Nosocomial infection#	No	258/267 (97%)	296/333 (89%)	1		1	0.002
	Yes	9/267 (3%)	37/333 (11%)	3.58 (1.70- 7.57)	0.001	5.67 (1.71 – 18.82)	
Cotrimoxazole prophylaxis	No	183/213 (86%)	172/234 (73%)	1		1	0.025
	Yes	30/213 (14%)	62/234 (27%)	2.20 (1.36 – 3.56)	0.001	2.41 (1.10 – 5.32)	
Hospital duration Median (range)		10 (6 – 15)	9 (4 – 16)	1.01 (1.00- 1.02)	0.230		
Acute severe illness##	No	236/288 (82%)	250/395 (63%)	1	<0.001		
	Yes	52/288 (18%)	145/395 (37%)	2.63 (1.83 – 3.79)			
Ampicillin susceptibility	Non-susceptible	56/273 (21%)	55/383 (14%)	1	0.039		
	Susceptible	217/273 (79%)	328/383 (86%)	1.54 (1.02 – 2.32)			

OR – Odds ratio; CI Confidence interval

† Race "other" category contains the following: white, coloured, indian

††Clinical syndrome "other" category contains the following: septic arthritis, peritonitis, cellulitis, osteomyelitis, pericarditis

#Nosocomial infection is defined as a positive specimen for *H. influenzae* taken ≥2 days after admission date

##Acute severe illness is defined as a Pitt bacteremica score of >4

4. Discussion

Part of the aim of this study was to describe trends in invasive *H. influenzae* disease from 2003-2009, the post-Hib vaccine era. A prominent finding was the increase in Hib incidence not only among the <5 year age group but also among the ≥5 year age group. Possible reasons for the Hib increase will be explored further in this chapter. The other part of the aim of this study was to look at factors associated with NTHi disease compared to Hib disease. NTHi cases were more likely to have predisposing conditions, severe disease and to die compared to Hib cases. This chapter will elaborate further on why these differences between NTHi and Hib disease were found.

4.1 Trends

South Africa introduced the *H. influenzae* type b protein-conjugate vaccine in 1999 as part of the EPI. The vaccine was given at 6, 10 and 14 weeks without a booster dose. South Africa observed a decrease in Hib incidence among children <5 years, 1 year after introduction of the Hib vaccine (15). However, the decrease in Hib incidence was not sustained.

An increase in Hib incidence among children <5 years in South Africa was previously reported by von Gottberg et al., from 0.7 per 100 000 in 2003 to 1.3 per 100 000 in 2009 (50). The Hib incidence in children <5 years old, in the current study, showed an increase from 2005 to 2008 followed by a stabilization of the Hib incidence until 2009. However, this increase in Hib incidence was still substantially less than the Hib incidence in the pre-Hib vaccine era of 47/100 000 children <5 years old. In addition, the baseline Hib incidence in children <5 years was likely much higher than depicted in surveillance data (15) because the Hib surveillance in South Africa started in the same year as the Hib vaccine introduction. The increase in Hib incidence observed in this study is therefore small in comparison to the dramatic decline of Hib incidence post-vaccination and is more than 2000-fold less than pre-Hib vaccine levels. Cases with missing serotype data were

excluded from the von Gottberg, et al (2012) study. The proportion of cases with missing serotype data differed significantly by year, from 2003-2009, and specimen and was controlled for in the current study, which could explain the difference in trends observed between the 2 studies.

The increase in Hib disease incidence among <5 year olds in South Africa from 2005-2008 occurred 6 years after the Hib vaccine was introduced. One of the factors that might influence the duration of protection derived from a vaccine is coverage. As seen in the United States, the booster dose was deferred for 18 months due to vaccine shortage. No change was observed in the national Hib incidence among <5 year olds because of high coverage (93%) before the vaccine shortage (65).

A catch-up campaign is another factor that can impact the duration of protection derived from a vaccine. A catch-up campaign can sustain the level of protective immunity derived from a vaccine schedule without a booster dose as seen in England and Wales (66). These 2 countries experienced resurgence in Hib disease 8 years after vaccine introduction. In comparison, South Africa did not have a catch-up campaign and as a result protective immunity could have waned relatively faster.

The timing of the booster dose and its relation to duration of protection is another factor to consider. The Netherlands observed an increase in the number of Hib cases (meningitis, epiglottitis and osteomyelitis) 9 years after the introduction of the Hib vaccine in 1993 (67). The increase in Hib disease was seen despite a booster dose at 11 months of age.

Population characteristics can also play a role in Hib vaccine failures. Certain populations have an inherent higher risk of Hib disease at an earlier age such as in the Alaskan Natives (68). A resurgence of Hib disease was noted among children <5 years of age after a change in the vaccine, from PRP-OMP in 1992 to a PRP linked to a non-toxic variant of diphtheria toxin CRM197 (HbOC) in 1996.

Investigation of Hib vaccine failures that occurred in South Africa from 2003-2009 (50) showed that they were more likely to be ≥18 months of age, hinting at waning immunity as the cause of vaccine failures. HIV is another important factor to consider, especially in South Africa, even though Hib vaccine failures occurred among both HIV-infected and HIV-uninfected children (50). A primary immunization schedule produces protective antibody levels in vaccinated individuals but will wane over time if not boosted (29). The magnitude of the antibody response mounted is affected by the HIV status of an individual amongst other factors. HIV-infected children, not on antiretroviral therapy (ARVs), showed a decreased antibody response after receiving 3 doses of the Hib vaccine compared to HIV-uninfected children (69). HIV-infected individuals have therefore an estimated 35-fold higher risk of Hib vaccine failure compared to HIV-uninfected children (69).

Low carriage levels among children <5 years does not necessarily result in low or no transmission. The state of Minnesota saw an increase in Hib cases during a booster deferral period but a subsequent carriage study showed a 0% carriage rate among children <5 years old (70). This increase in Hib cases emphasized that transmission can still occur even with low carriage levels in children <5 years because school-age children can still be the reservoir for ongoing transmission as seen in England and Wales (34). Also unvaccinated and immunosuppressed children still remain vulnerable to invasive Hib disease.

The period of stabilization (2008-2009) in Hib incidence among children <5 years old in South Africa occurred before the Hib booster introduction. Therefore, another reason must account for this levelling off in Hib incidence. As discussed before HIV-infected children are vulnerable to invasive Hib disease even after receiving 3 doses of Hib vaccine (46, 69). A stabilization of HIV prevalence among children 2-14 years of age (3.5% in 2005 to 3.0% in 2008) (71) and improved access to ARVs for children <5 years old (2.1% in 2002 to 36.9% in 2007-2008) (72) could perhaps explain the plateau in Hib incidence.

Most countries in Africa have introduced the Hib conjugate vaccine, except for Somalia (GAVI approved) and South Sudan (still to apply for GAVI funding) (73). The Gambia was the first in 1998 and Burkino Faso the latest in 2006. Kenya, Malawi, Rwanda and Uganda introduced the Hib conjugate vaccine in 2002, Burundi and Zambia in 2004, Benin, Mali and Senegal in 2005. Malawi, a country with high HIV prevalence (15% in pregnant women) showed a decrease in annual Hib meningitis incidence from 20-40 cases per 100 000 in the pre-vaccine era to almost zero in 2007, 2 years after the Hib vaccine introduction (74). Mali, one of the world's six least developed nations, had a 83% decline in invasive Hib disease from a baseline of 175 cases per 100 000 (2002-2005) to 30 cases per 100 000 2 years after the vaccine introduction (75). Senegal showed a decrease in the average annual Hib meningitis incidence from 22-47 cases per 100 000 in 2003-2005 to 1.4 cases per 100 000 in 2007 (76). An active surveillance programme in Uganda showed a decline in the number of Hib meningitis cases presenting to St Mary's Hospital in Lacor from 42 in 2001 to ≤3 cases in 2003-2006 (77). The surveillance done in the majority of abovementioned countries was restricted to sentinel sites and Hib meningitis (74, 76-77). A decrease in Hib disease was reported on the background of a primary schedule at 6, 10 and 14 weeks and no booster dose. Whether this decrease in Hib disease was sustained is not known, due to a lack of further evidence from these countries.

The United Kingdom noted, similarly to South Africa, an increase in Hib incidence among persons ≥5 years after the increase of Hib incidence was observed among persons <5 years (30). In adults, Hib immunity is maintained through natural boosting (29). The Hib vaccine decrease carriage rates (78). As a result, natural boosting in adults will occur less. Hib disease in children re-emerged and unvaccinated individuals became vulnerable to Hib disease which could explain the increase in Hib disease among persons ≥5 years.

In our data, NTHi incidence in all ages remained stable from 2003 to 2009. Similarly studies from Iceland (all ages) from 1983 to 2008 (79), Germany (children) from 1998 to 2005 (57) and England

and Wales (children <15 years) from 1994 to 2008 (66) did not show a significant increase in NTHi incidence. In contrast, an increase in NTHi incidence in all ages was reported in studies from United States (38) and Sweden (39). This increase in NTHi disease could be due to an increase in reporting of cases, improvement in serotype techniques and an aging population rather than a true increase in disease (Table 4.1).

The risk of *H. influenzae* increases with every decade after 65 years of age (80). An increase in longevity in developed countries could explain the increase seen in NTHi disease in persons ≥65 years (63). In contrast our study found, even though not significant, a 34% decrease in NTHi incidence in the ≥65 year age group over the study period. The Hib incidence in the ≥65 year age group also decreased. This decrease may be unrelated to the serotype and indicates a more general cause. More specifically, a decrease in case ascertainment in this age group due to decrease access to health care facilities and/or decrease in blood culture taking practises may account for this trend. Hib, NTHi and non-b incidence peaked in the ≥65 year age group in 2004. The reason for this high peak can be related firstly, to a higher number of overall cases in the ≥65 year age group reported in 2004 compared to other years (26 (2003), 43 (2004), 22 (2005), 21 (2006), 22 (2007), 23 (2008) and 16 (2009). Secondly, the mid-year population denominator for 2004 for the ≥65 year age group was lower at 1 869 865 compared to over 2 200 000 for all the other years. This discrepancy in mid-year population denominator could not be due to a change in methodology by Statistics SA, since the cohort component approach was used for all the years except 2003. Even if this peak in incidence in 2004 is an outlier, a decreasing trend would have still been observed comparing both Hib and NTHi incidence in 2003 to 2009. In addition, only 5% of the national population are ≥65 years old (51) because the life expectancy at birth in South Africa is estimated at 53.5 years for males and 57.2 years for females (females accounted for 51% of cases with Hib disease and 55% of cases with NTHi disease in our study). The current study showed that the highest incidence of Hib disease still occurred among the <5 year old group but it is still less than in the pre-Hib vaccine era (47 per 100 000). Hib disease was also noted in the

older age groups. The highest incidence of NTHi disease also occurred among persons <5 years old. NTHi and non-b disease showed a W-shaped curve with a second peak in the ≥65 year age group and a third peak in the 25-44 year age group. However, other studies done in developed countries, showed a U-shaped curve for NTHi and non-b incidence (17, 40, 63, 81). The highest HIV prevalence in South Africa occurs in the 15-49 year age group; in 2009 it was 16.4% compared to 10.5% in the whole population (51). HIV-infected patients have a defective humoral immune response, which puts them at risk for bacterial infection such as invasive *H. influenzae* (82). We found that NTHi disease was still responsible for causing the greatest proportion of disease in ≥65 year olds. Similar findings were reported by other studies (66, 79).

Table 4.1: NTHi incidence in studies conducted at the time of and after Hib conjugate vaccine introduction

Author	Population	Study period	NTHi incidence	Hib vaccine introduction	Comments
Dworkin et al. 2007	Illinois, Unites States, all ages	1996 -2004	NTHi incidence in all ages increased from 0.06/100 000 to 0.43/100 000	1998	Passive surveillance. The increase observed could be real or due to increase reporting and an aging population.
Resman, et al. 2010	Swedish population of all ages	1997 -2009	NTHi incidence in all ages increased from 0.36/100 000 to 1.22/100 000	1992-1993	Retrospective study. Increase in specimen-taking practises could account for increase seen.
Ladhani, et al. 2010	14 European countries, all ages	1996-2006	No significant change in NTHi incidence, 0.22/100 000 to 0.28/100 000	1990s	Large prospective laboratory surveillance (10 081 cases)

Laupland, et al. 2010	Australia, Canada, Denmark and all ages	2000-2008	No signficant change in NTHi incidence, 1.00/100 00 to 0.75/100 000	Australia-1993 Canada-1992 Denmark-1993	Active surveillance. Only looked at bacteremia.

Our study revealed that NTHi disease had a seasonal pattern, with a peak during the colder months. No clear seasonal pattern was observed for Hib disease. This is in contrast to a Saudi study done in children <5 years old, looking at Hib meningitis, reported an increase number of cases during autumn (83), while a Swedish study looking at Hib in all ages showed an unimodal pattern with the highest number of cases occurring during winter (84)(Table 4.2). Another report showed that Hib had a bimodal pattern with peaks in autumn and spring but the reason is not well understood (4). Two studies, one from Australia, Denmark and Canada that only looked at bacteremia (40) and one from Illinois, United States (38), reported that the number of *H. influenzae* cases in all ages were more during the winter months compared to the summer months. Both these studies did not report on seasonality trends in <5 year olds or looked at whether seasonality patterns differ between NTHi disease and Hib disease. After the introduction of a vaccine, seasonal peaks are less pronounced or can occur later (85), as may be the case for Hib during our study period. NTHi was not affected by the Hib vaccine and still showed a seasonal pattern.

Table 4.2: Seasonal pattern of *H. influenzae* disease in the post-Hib vaccine era

Author	Population	Study period	Seasonal pattern	Hib vaccine introduction
Al-Mazrou, et al.	Saudi children <5 years old with meningitis	1995-1999	Increased number of **Hib** cases in autumn (September to November)	2000
Farhoudi, et al.	Swedish population, all ages, all invasive disease	1997-2003	Increased number of **Hib** cases in winter (November to January)	1992-1993
Laupland, et al.	Australia, Canada Denmark and all ages with bacteremia	2000-2008	Increased number of ***H. influenzae*** cases in winter	Australia-1993 Canada-1992 Denmark-1993
Dworkin, et al.	Illinois, United States, all ages, all invasive disease	1996-2004	Increased number of ***H. influenzae*** cases in winter	1998

4.2 Comparing NTHi disease to Hib disease

In our study, NTHi disease accounted for the greatest proportion (47%) of all invasive *H. influenzae* disease compared to 34% of Hib disease. The proportion of Hib disease was substantially more compared to other studies done in the post-vaccine era. In Canada, Denmark and Australia the proportion of NTHi disease was 75% and Hib only contributed 6% to all invasive *H. influenzae* disease (40). In Italy, Giufre et al. showed similar results, NTHi accounted for 73% and Hib 10% of all invasive *H. influenzae* disease (36). A study conducted in Illinois, United States found that NTHi disease contributed 54.2% and Hib 15% of all invasive *H. influenzae* disease (38). All 3 studies occurred with the background of a successful Hib conjugate vaccine immunization program, which lead to a sustained decrease in Hib incidence. However, a study done in England and Wales during a period of resurgence in Hib disease found that Hib disease accounted for 49% and NTHi disease 45% of all *H. influenzae* disease (66). Ladhani et al. found a higher proportion of

Hib cases in their study because they only included persons <15 years of age and the increase that was observed in Hib disease (0.26/100 000 in 1998 to 1.8/100 000 in 2002, 592% increased) was 5-fold more than what was observed in our study (10/1 000 000 in 2005 to 21/1 000 000 in 2008, 110% increased) among <5 year olds.

Our study revealed that more than two-thirds (69%) of Hib disease and half of all NTHi disease occurred among <5 year old children. In contrast, other studies from developed countries showed that both Hib and NTHi occur more commonly in older age groups. A study from Canada showed that 75% of Hib disease and 83% of NTHi disease occurred in >20 year olds (86). A study conducted in England and Wales just after the Hib vaccine was introduced in 1992, reported that Hib occurred more frequently in 30 year olds and 65 year olds and contributed it to increase contact with children in these two age groups (26). We found that the elderly accounted for the least number of NTHi cases (9%). In contrast, other reports revealed that NTHi disease was more frequently found in the ≥65 year old age group (17, 26, 36). The low proportion of NTHi disease seen in the elderly in our study could be due to a lower case ascertainment in this group due to lower health seeking behaviour, less specimens being done or because there are proportionately less people in this age group in South Africa compare to the younger age groups.

Similarly to our findings, other reports found a higher proportion of NTHi cases (approximately 60%) have predisposing conditions compared to Hib cases (27%) in both children and adults (36, 57-58, 79). A study from the Netherlands (81) reported that NTHi cases ≥55 years of age had the highest prevalence of predisposing conditions compared to children <5 years, which is in contrast to our findings when HIV was excluded. We found that protein-energy malnutrition (marasmus and kwashiorkor) was the most common predisposing condition, excluding HIV, in all ages. The most common predisposing conditions found in other reports were chronic lung disease, malignancy and cardiovascular disease (36, 41, 43). All the studies cited above are from

developed countries and none mentioned protein-energy malnutrition as a predisposing condition.

Protein-energy malnutrition is estimated at 5.3 cases per 100 000 children <5 years old in South Africa (87). Protein-energy malnutrition increases the risk of bacterial infections such as *H. influenzae* (88-89), by causing a secondary immunodeficiency. In addition, the underdevelopment of the thymus and peripheral lymphoid tissue (spleen and lymph nodes) causes a long lasting immune dysfunction. Children with protein-energy malnutrition develop more severe *H. influenzae* disease and have an increased mortality compared to children without malnutrition with *H. influenzae* (89). HIV is more common among older people (15-49 years old) compared to among children <5 years old in South Africa (51). Therefore, when HIV was included as a predisposing condition, persons ≥5 years old had a greater proportion of predisposing conditions compared to persons <5 years old.

Our study found a greater proportion of NTHi cases had an acute severe illness (Pitt bacteremia score of >4) compared with Hib cases. NTHi is known to cause infection in elderly people or persons with underlying conditions (3). We also found that NTHi cases were older, had more underlying conditions and also had a higher proportion of HIV-infection compared to Hib cases. These factors could have contributed to NTHi cases presenting with more severe disease on admission which resulted in a higher Pitt bacteremia score. The Pitt bacteremia score does not take into account underlying factors. The severity of NTHi disease in this study is not a reflection of a more virulent organism but rather due to underlying factors that exacerbated the presentation of NTHi cases.

Our study found that NTHi disease had a higher in-hospital mortality compared to Hib disease. A study conducted in the United States from 1999 to 2008 found that increase in age in NTHi cases (>18 years) is a risk factor for in-hospital mortality but not so for Hib cases (49). We also found that a greater proportion of NTHi cases ≥5 years died while in hospital compared to NTHi cases <5

years old. NTHi cases had more severe disease (Pitt bacteremia score of >4) compared to Hib cases (37% vs 18% respectively). An association may exist between acute severe illness and in-hospital mortality in NTHi cases but was not tested in our study. Several reports, conducted in developed countries, found a lower proportion of deaths amongst NTHi and Hib cases (57-58, 79, 81) than our study. The high HIV prevalence in our setting could contribute to a higher proportion of Hib and NTHi cases dying compared to abovementioned studies. Protein-energy malnutrition, a common predisposing condition in our study, also increases the risk of mortality by making an individual more susceptible to severe bacterial infection (90).

We found that nosocomial infection was more common in cases with NTHi disease (11%) compared to Hib cases (3%) ($p < 0.001$). More than half of NTHi cases had a predisposing condition which means that they were more likely to have been admitted to hospital for another reason than a Hib case. Nosocomial infection has been shown to be more common in patients that did not receive any prior antibiotics (91). We showed that only 5% of NTHi cases received antibiotics 24 hours before specimen collection.

There are various mechanisms of ampicillin resistance. The most important mechanism is plasmid-mediated TEM-1 type beta lactamase (92-93). Capsulated strains were found to be more resistant to ampicillin (44). The reason for this higher resistance in capsulated strains has a genetic basis, the carriage of large plasmids. Our study found that ampicillin non-susceptibility was more common in Hib cases (21%) than in NTHi cases (14%). A study from Madrid showed similar results, ampicillin susceptibility in Hib cases 45.8% vs 16.4% in NTHi cases, $p = 0.004$ (44). In contrast, a study from Canada found no ampicillin non-susceptible Hib isolates but found 20% of NTHi isolates to be ampicillin non-susceptible (86), while in Italy all the ampicillin non-susceptible isolates were from NTHi cases (36, 91). Differences in susceptibility testing techniques, the source of infection (nosocomial vs community acquired), age distribution and antibiotic treatment guidelines could account for the differences seen between studies.

Hib ampicillin non-susceptibility in our study was double that of a pre-Hib vaccine study from South Africa which showed a Hib ampicillin non-susceptibility of 10.9% (94). A second study from South Africa showed an increase in Hib ampicillin susceptibility from 16% in 1999-2000 to 31% in 2003-2004 (15). A decrease in Hib ampicillin non-susceptibility could be the reason why the current study found a lower Hib ampicillin non-susceptibility from 2003-2009 compared to 2003-2004 from the previous study. The suggested first-line antibiotic treatment for pneumonia has not changed dramatically in South Africa from 2003 to 2009 (95-96) but the prescription of antibiotics might have. Ampicillin is still recommended as the first line treatment in uncomplicated pneumonia in both children and adults. However, clinicians might opt for a clavulanic acid containing agent instead because of previously high rates of ampicillin resistance.

4.3 Predictors of NTHi disease compared to Hib disease

Age is an important risk factor for *H. influenzae* disease. In the prevaccine era Hib disease occurred almost exclusively (85%) in children <5 years of age (8). NTHi occurs more frequently in very young children and in the elderly with predisposing conditions (81, 97). Our study reiterated that young age is still an independent risk factor for Hib disease. The factor most strongly associated with NTHi disease compared to Hib disease was bacteremia (OR 9.24, 95% CI 3.79-22.54) and LRTI (OR 8.31, 95% CI 4.12-16.78). A study conducted only on adults (≥18 years) in Utah, United States from 1998-2008, after the Hib vaccine introduction, showed that meningitis was 6 times (OR 6.1, 95% CI 1.3-29) more likely to occur in persons with Hib disease than in persons with NTHi disease (64). Clinical syndrome is therefore a good predictor of whether a person has NTHi disease compared to Hib disease regardless of age. Another independent predictor of NTHi disease compared to Hib disease was having a predisposing condition (excluding HIV). Various reports have shown that predisposing conditions are more common in persons with NTHi disease than persons with Hib disease (57-58, 98). Nosocomial infection was 5 times more likely to occur in persons with NTHi disease compared to Hib disease despite controlling for other factors such as predisposing conditions, HIV status, age and acute severe illness. Mortality was

also an independent predictor of NTHi disease compared to Hib disease. Our findings suggested that HIV status is not an independent predictor of NTHi disease compared to Hib disease, probable because HIV-infected people are at increased risk to develop both NTHi and Hib disease.

4.4 Limitations

Laboratory surveillance only captures laboratory-confirmed cases. This study therefore, does not capture invasive *H*. *influenzae* cases that did not seek medical attention or died before seeking medical attention, cases that were seen at hospital but had no specimen taken. This study also does not include cases with non-invasive pneumonia. Audits were not done on private laboratories and underreporting could have occurred. The incidence of invasive H. *influenzae* in this study is an underestimate of the true burden of disease in South Africa.

Due to the ecological nature of surveillance data, any changes in clinical practices and laboratory techniques at referral hospitals could influence the trends observed.

People not resident in South Africa for the last month were excluded from the analysis. This information was only available from enhanced surveillance sites. *H. influenzae* cases that acquired the disease outside of South Africa could therefore have been included in the study from non-enhanced sites.

Patients that absconded or refused hospital treatment were assumed to be alive. No follow-up was done to assess their outcome. The numbers were very small and it was unlikely to have affected the proportion of patients that have died in hospital.

A high proportion of isolates were not serotyped and therefore extrapolation of missing serotypes was done. We made certain assumptions when we extrapolated the missing serotype data. Missing serotype data was likely to differ by age, specimen and year, therefore we controlled for it. If there was another factor that influenced missing serotype data, which was not controlled for, than our findings may have been biased.

The numbers for non-b encapsulated H. *influenzae* were very small, especially by age category. Numbers in other subgroups were also small and categories needed to be combined to increase the power of the study. We were therefore unable to look at whether specific predisposing conditions such as protein-energy malnutrition, chronic conditions, immunosuppressive conditions and central nervous system conditions were predictors of NTHi disease compared to Hib disease. Zero cases were reported from enhanced surveillance sites in North West province, which is a reflection of blood culture taking practises and not the true burden of disease in this province.

Predisposing conditions could have been underestimated if: a predisposing condition existed but was undiagnosed, if a patient was treated for a predisposing condition but they did not know the name of the condition or they were not truthful in disclosing the extent of the predisposing condition i.e. alcohol dependency.

Forty-six per cent (586/1278) of HIV status information was missing. Missing HIV status could be because the patient had died, refused testing, was unable to consent to testing or was referred to another centre for testing. If these patients with unknown status were more likely to be HIV-infected, the proportion of HIV-infected cases would have been underestimated. Also if adult patients with NTHi disease were less likely to be tested compared to children with Hib disease, the prevalence of HIV would have been underestimated in adults with NTHi disease.

4.5 Strengths

The surveillance system remained fairly stable over the years. The case definition was standardised and remained the same throughout the study period. Laboratory techniques used for serotyping also did not change over the study period. The surveillance is active and this ensures almost complete case ascertainment, through regular audits and site visits. Interviews done at enhanced surveillance sites made it possible to obtain rich clinical information.

4.6 Generalizability

The risk factor analysis only included *H. influenzae* cases from enhanced surveillance sites. Non-enhanced surveillance sites collected limited demographic data. We were therefore unable to compare all the factors included in our risk factor analysis between enhanced surveillance and non-enhanced surveillance sites. As a result, we cannot comment on the generalizability of some of the factors associated with NTHi disease compared to Hib disease. Enhanced surveillance sites were representative of non-enhanced sites and therefore potentially the entire country with regards to the age distribution, gender distribution, ampicillin susceptibility and the proportion of NTHi and Hib cases reported to the reference laboratory. However, the proportion of cases reported from enhanced surveillance sites and non-enhanced surveillance sites differed by province because in certain provinces such as North West and Limpopo, the capacity (number of patients seen, number of specimens taken and processed) of the enhanced site was less compared to all the non-enhanced sites combined in that province. In contrast in Gauteng and the Western Cape the capacity of the enhanced sites exceeded that of all the non-enhanced sites combined. The proportion of cases reported by year differed significantly between enhanced surveillance sites compared to non-enhanced surveillance sites, due to more enhanced surveillance sites being added over the years. Despite these differences between enhanced and non-enhanced surveillance sites, the representativeness of our results is not compromised. Specimen-taking practises also differed by enhanced surveillance site compared to non-enhanced surveillance site. The spectrum of bacteremia would be more real in the enhanced surveillance sites compared to non-enhanced sites where less blood cultures are being taken.

Countries that have newly implemented or are planning to implement the Hib vaccine with or without a booster dose will find these study findings relevant. Countries with similar HIV prevalence and age distribution as South Africa might see the same trends in *H. influenzae* disease. In addition, these findings will also assist clinicians in identifying high risk groups.

5. Recommendations and Conclusion

5.1 Recommendations

A booster dose for Hib was introduced in 2010 as part of the EPI. Ongoing surveillance is therefore necessary to monitor Hib disease as well as NTHi and non-b encapsulated trends.

Carriage studies might be useful in explaining the increase seen in Hib incidence among all ages. Studies to investigate the immune response mounted in both HIV-uninfected and HIV-infected (on ARVs and not on ARVs) children, especially now that a booster dose has been introduced, will also be helpful to determine the period of protection derived from the vaccine.

The incidence of NTHi disease, in our study, was lower than Hib disease in the prevaccine era. Nevertheless, NTHi disease caused more severe disease and deaths than Hib disease. A NTHi vaccine might be able to prevent NTHi disease or decrease morbidity and mortality in high risk groups, the elderly and those with predisposing conditions. The burden of NTHi disease in developed countries has prompted the development of a NTHi vaccine, of which research is still ongoing. The much greater genetic diversity of NTHi strains compared to Hib could pose a challenge in finding the most appropriate antigenic composition for the vaccine (99). An alternative would be the use of the 10-valent pneumococcal vaccine (PCV10) which is conjugated to protein D. Protein D is found on the surface of all *H. influenzae,* including NTHi strains. Various randomized trials found an increase in the protein D antibodies of more than 15-fold in infants after receiving PCV10 (100). However, whether this increase in protein D antibodies relates to clinical protection still remains to be seen, especially for NTHi disease which shows a high degree of antigenic heterogeneity.

Protein-energy malnutrition is a multi-factorial challenge. There are numerous contributing factors that need to be addressed. Immediate factors such as the promotion of breastfeeding, supplemental programmes (Vitamin A and iron), food-aid programmes, food fortification

programmes and management of diarrhoea as well as maternal education and poverty alleviation needs to be addressed (101). Fewer children will become HIV-infected with a well functioning prevention-of-mother-to-child transmission (PMTCT) programme. However, protein-energy malnutrition will still remain a risk factor for NTHi and Hib disease. Severe protein-energy malnutrition causes long-lasting immune dysfunction (88). Therefore, early diagnosis, adequate management and proper follow-up are needed to decrease prevalence of protein-energy malnutrition.

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 ones 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 which has a poorer outcome. There are conflicting views whether the Hib vaccine should be given to HIV-infected adults. Concerns were raised that the Hib vaccine increase HIV viral replication (102), while in another study (103), the Hib vaccine had no effect on HIV viral replication regardless of HIV clinical or immunological stage .

NTHi disease occurred in cases without predisposing conditions as well. It would be interesting to see whether a different biotype was responsible for disease in seemingly healthy individuals compared to individuals with predisposing conditions or whether a more virulent strain such as INT1 were responsible for NTHi disease (104).

5.3 Conclusion

Although an increase was observed in Hib disease, in all ages, in South Africa, the incidence in children <5 year olds is still substantially less compared to the pre-Hib vaccine era. NTHi incidence in all ages remains stable. Persons 25-44 years old has the third highest incidence, after persons <5 year old and ≥65 year old, of invasive *H. influenza*e disease, which is related to the high HIV prevalence in this group. South Africa faces a double challenge: persons 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. Ongoing monitoring of *H. influenzae* disease in South Africa is therefore essential.

6. Appendices

Appendix A: Enhanced surveillance sites, 2009

Hospital	Province
Nelson Mandela Academic Hospital & Mthatha Provincial Hospital	Eastern Cape
Pelonomi Hospital & Universitas Hospital	Free State
Chris Hani Baragwanath Hospital	Gauteng
Charlotte Maxeke Johannesburg Academic Hospital	Gauteng
GaRankuwa Hospital	Gauteng
Steve Biko (Pretoria) Academic Hospital & Tshwane Provincial Hospital	Gauteng
King Edward VIII Hospital	Kwa-Zulu Natal
Addington Hospital	Kwa-Zulu Natal
RK Khan Hospital	Kwa-Zulu Natal
Greys' Hospital & Edendale Hospital	Kwa-Zulu Natal
Polokwane-Mankweng Hospital Complex	Limpopo
Rob Ferreira Hospital & Themba Hospital	Mpumalanga
Kimberley Hospital	Northern Cape
Job Shimankane Tabane Provincial Hospital	North West
Red Cross Hospital, Groote Schuur Hospital & Victoria Hospital	Western Cape
Tygerberg Hospital and Karl Bremer Hospital	Western Cape

Appendix B: Laboratory case report form (for sterile isolates)

GERMS SOUTH AFRICA

Laboratory Case Report Form

LABORATORY/ HOSPITAL

Lab Specimen No: ☐☐☐☐☐☐☐☐☐☐

Hospital Name: _____ Laboratory Name: _____

Lab Contact Person: _____ Lab Telephone No: _____

PATIENT

Surname: _____ First Name/s: _____

Sex: Male: ☐ Female: ☐ Date of Birth: d d m m y y y y

Ward: _____ Age: _____

Age Unit: Days: ☐ Months: ☐ Years: ☐

Hospital Number: ☐☐☐☐☐☐☐☐☐☐ Admission Date: d d m m y y y y

Province (Residence): _____ Town (Residence): _____

DIAGNOSIS AND OUTCOME

Clinical Diagnosis: Meningitis: ☐ LRTI: ☐ Bacteraemia or fungaemia: ☐

Dysentery: ☐ Diarrhoea: ☐ Other: ☐ Specify: _____

Outcome: Recovered: ☐ Died: ☐ Unknown: ☐

SPECIMEN

Collection Date: d d m m y y y y

Gram Stain: _____ India Ink: Pos: ☐ Neg: ☐ Not done: ☐

Latex Antigen: -*Cryptococcus*: Pos: ☐ Neg: ☐ Not done: ☐

-Bacterial: Pos: ☐ Specify: _____ Neg: ☐ Not done: ☐

Was the organism cultured? Yes: ☐ No: ☐

ID: *Shigella* species: ☐ *Salmonella* Typhi: ☐ Non-typhoidal *Salmonella* species: ☐

. *Cryptococcus* species: ☐ *Klebsiella* species: ☐ *Staphylococcus aureus*: ☐

. *Haemophilus* species: ☐ *Neisseria meningitidis*: ☐ *Streptococcus pneumoniae*: ☐

Specimen type: Blood culture: ☐ CSF: ☐ Other site: ☐ Specify: _____

Was >1 organism type isolated from the specimen? Yes: ☐ No: ☐

If yes, specify organism/s: _____

PREVIOUS ISOLATES

Were any of the above organisms isolated from the patient previously? Yes: ☐ No: ☐

If yes, specify organism/s: _____

Date of Previous Isolate: d d m m y y y y Lab Specimen No: ☐☐☐☐☐☐☐☐☐☐

Send this form (with lab report) and the corresponding isolate to the NICD: Address: Receiving Office, 1 Modderfontein Road, Sandringham, 2131; Queries: Tel- 011 386 6234 Fax- 011 386 6077

Appendix C: Clinical case report form, 2009

GERMS-SA: National Laboratory-based Surveillance for Enteric, Respiratory and Meningeal Bacterial and Fungal Diseases in South Africa

Clinical Case Report Form

National Microbiology Surveillance Unit (NMSU)
TEL: 011 386 6234 OR 011 555 0353 FAX: 011 386 6077

Surveillance officer name: Signature: Date:

Sources of data: Patient/Guardian ☐ Clinician ☐ Medical records ☐ No record found ☐ Refused participation ☐

Lab Specimen No: Laboratory Name:

Hospital Name: Hospital Number: Ward: Adult Ward ☐ Paed Ward ☐

Gender: M ☐ F ☐ Unk ☐ Race: Asian ☐ Black ☐ Coloured ☐ White ☐ Unk ☐

Date of Birth: D D M M Y Y Y Y DOB Unk ☐ Age: Unit: Days ☐ Months ☐ Years ☐ Age Unk ☐

Patient Surname: Patient First Names:

Address: Town/City: Province:

Tel no: (H) (W) (C) (Neighbour)

Has patient stayed in SA for the last month: Yes ☐ No ☐ Unk ☐ If no, which country has patient come from:

ID No. Unk ☐ ARV No. Unk ☐

Was patient referred from a hospital or chronic care facility: Yes ☐ No ☐ Unk ☐ If yes, specify:

Date of admission to acute hospital: D D M M Y Y Y Y Unk ☐

Was patient transferred to a step down hospital: Yes ☐ No ☐ Unk ☐ Date of transfer: D D M M Y Y Y Y

If yes, name of step down hospital:

Final outcome of patient: Discharged ☐ Died ☐ RHT/ Absconded ☐ Unk ☐ Outcome date: D D M M Y Y Y Y

If discharged, patient discharged to: Home ☐ TB Hosp/Chronic care facility ☐ Other ☐ Specify: Unk ☐

Discharge diagnosis:

Meningitis ☐ LRTI ☐ Dysentery ☐ Diarrhoea ☐ Fungaemia/Bacteraemia without focus ☐ Other ☐ Specify:

Organism isolated: *Cryptococcus* sp. ☐ Date of specimen collection: D D M M Y Y Y Y

Haemophilus sp. ☐ *N. meningitidis* ☐ *Shigella* sp. ☐ Site of specimen collection: CSF ☐ Blood ☐

S. pneumoniae ☐ *P. jirovecii* ☐ *Salmonella* sp. ☐ Joint Fluid ☐ Other ☐ Specify

Severity of illness (on the day the positive specimen was taken):

Temp: °C Unk ☐ BP: / Unk ☐ Mechanical Ventilation: Yes ☐ No ☐ Unk ☐ Cardiac Arrest: Yes ☐ No ☐ Unk ☐

GCS: /15 Unk ☐ Mental Status: Alert ☐ Disorientated ☐ Stuporous ☐ Comatosed ☐ Unk ☐

Previous admissions in the last 12 months: Yes ☐ No ☐ Unk ☐ Number of admissions:

Cotrimoxazole prophylaxis and TB treatment (from the last 3 months and current)

Cotrimoxazole prophylaxis:	Dosage:		Date initiated:
Yes ☐ No ☐ Unk ☐			Compliant in last month: Yes ☐ No ☐ Unk ☐
TB Treatment: Drugs:	1.	3.	Date initiated:
Yes ☐ No ☐ Unk ☐	2.	4.	Date stopped:

GERMS-SA: National Laboratory-based Surveillance for Enteric, Respiratory and Meningeal Bacterial and Fungal Diseases in South Africa

Clinical Case Report Form

National Microbiology Surveillance Unit (NMSU)
TEL: 011 386 6234 OR 011 555 0353 FAX: 011 386 6077

Laboratory Specimen Number: | | | | | | | | | | |

Immunocompromising conditions:

Alcohol dependency ☐	Chronic renal failure ☐	Heart failure ☐	Kwashiokor/ Marasmus ☐	Valvular heart disease ☐	
Asthma ☐	Current smoker ☐	History of head injury/head surgery ☐	Nephrotic syndrome ☐	Malignancy ☐	Specify:_____
Burns ☐	Coronary Artery Disease ☐	Hydrocephalus with VP shunt ☐	Sickle cell anaemia ☐	Organ transplant ☐	Specify:_____
CVA/Stroke ☐	Diabetes mellitus ☐	Immunoglobulin deficiency ☐	Splenectomy/ asplenia ☐	Other ☐	Specify:_____
Cirrhosis/ liver failure ☐	Emphysema/COPD ☐	Immunosuppressive rx (steroid,chemo) ☐	Systemic Lupus Erythematosus (SLE) ☐	None ☐	Unknown ☐

HIV status prior to this admission: Pos ☐ Neg ☐ Unk ☐ HIV related counseling offered by SO: Yes ☐ No ☐

HIV status at this admission: Pos ☐ Neg ☐ Unk ☐ HIV test performed by SO: Yes ☐ No ☐

For children <18 months: HIV PCR Done: Yes ☐ No ☐ Unk ☐ If HIV unknown, is there clinical suspicion of HIV: Yes ☐ No ☐ Unk ☐

If HIV unknown, why was patient not tested: Patient died ☐ Patient not seen ☐ No guardian ☐ Patient confused/ comatose ☐

Refused consent ☐ Reason for refusal: _____ Unk ☐

CD4 count closest to specimen collection date: Absolute: Unk ☐ Percentage: % Unk ☐ Date taken: D D M M Y Y Y Y

Viral load closest to specimen collection date: <400 ☐ 400-10 000 ☐ >10 000 ☐ Unk ☐ Date taken: D D M M Y Y Y Y

Clinical markers of HIV: Diarrhoea >10days ☐ Oral candidiasis ☐ Suspected PCP ☐ None ☐

Kaposis sarcoma ☐ Tuberculosis ☐ HIV wasting ☐ Unk ☐

Any antiretroviral use: Yes ☐ No ☐ Unk ☐ If yes: Current ☐ Previous ☐ Perinatal ☐ Unk ☐

If HIV positive and no ARV use, has the patient been referred to an ARV clinic: Yes ☐ No ☐ Died ☐ Unk ☐

PLEASE COMPLETE RELEVANT SECTIONS FOR SPECIFIED ORGANISMS

***Haemophilus* spp., *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp., *Shigella* spp. ONLY**

Number of children, <18 years, living with patient: None ☐ Number ☐ Place of safety ☐ Unk ☐

Have any of these children been hospitalised in the last 3 months: Yes ☐ No ☐ Unk ☐

Antibiotic use prior to this specimen collection date:

ABX in 24hr before specimen: Yes ☐ No ☐ Unk ☐ Date initiated: D D M M Y Y Y Y

Name of antibiotic: 1. 2. 3. 4.

Other ABX in last 2 months: Yes ☐ No ☐ Unk ☐ In last 30 days: Yes ☐ No ☐ Unk ☐

Name of antibiotic: 1. 2. In last 30 to 60 days: Yes ☐ No ☐ Unk ☐

Antibiotic use in hospital during this admission (excluding TB therapy)

Weight: ☐☐.☐ kg Unk ☐ Antimicrobial therapy unknown: ☐ Antimicrobial therapy not prescribed: ☐

Name of antimicrobial	Dose	Route	Date initiated	Total doses given/no. of days
1.			D D M M Y Y Y Y	
2.			D D M M Y Y Y Y	
3.			D D M M Y Y Y Y	
4.			D D M M Y Y Y Y	
5.			D D M M Y Y Y Y	

GERMS-SA: National Laboratory-based Surveillance for Enteric, Respiratory and Meningeal Bacterial and Fungal Diseases in South Africa

Clinical Case Report Form

National Microbiology Surveillance Unit (NMSU)
TEL: 011 386 6234 OR 011 555 0353 FAX: 011 386 6077

Laboratory Specimen Number:

Haemophilus spp. and _S. pneumoniae_ ONLY

Vaccination status for ***Haemophilus influenzae***:
If <15 years old, did patient receive *Haemophilus influenzae* type b (Hib) vaccine? Yes ☐ No ☐ Unk ☐

Dose	Dose given?	Date given
6 weeks	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
10 weeks	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
14 weeks	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
18 month booster (Pentaxim)	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
Catch up/ Other	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
Catch up/ Other	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
Catch up/ Other	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y

Vaccination status for ***S. pneumoniae***:
If <15 years old, did patient receive conjugate vaccine for *S. pneumoniae*? Yes ☐ No ☐ Unk ☐

Dose	Dose given?	Date given
6 weeks	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
10 weeks	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
14 weeks	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
Catch up/ Other	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y
Catch up/ Other	Yes ☐ No ☐ Unk ☐	D D M M Y Y Y Y

Has the patient (all ages) received the 23 valent polysaccharide pneumococcal vaccine? Yes ☐ No ☐ Unk ☐

If yes give date most recently given D D M M Y Y Y Y

Source of vaccine status information:
RTHC ☐ Verbal report from caregiver ☐ Drs notes ☐ Directly from clinic ☐ Other ☐ Specify:

Cryptococcus spp. ONLY

Antifungals prior to this admission: Weight ☐☐☐.☐ kg Unk ☐

Fluconazole Yes ☐ No ☐ Unk ☐ If yes, date initiated D D M M Y Y Y Y Dose Daily ☐ BD ☐

Amphotericin B Yes ☐ No ☐ Unk ☐ If yes, date initiated D D M M Y Y Y Y Dose

Is this the first episode of cryptococcosis? Yes ☐ No ☐ Unk ☐

Management during this admission:

	Dose	Frequency	Date initiated	Total number of doses/ number of days
Fluconazole		Daily ☐ BD ☐	D D M M Y Y Y Y	
Amphotericin B		Daily ☐ BD ☐	D D M M Y Y Y Y	
Rifampicin		Daily ☐	D D M M Y Y Y Y	

Antifungal therapy unknown ☐ Antifungal therapy not prescribed ☐

Was opening intracranial pressure documented at time of first LP? Yes ☐ No ☐ Unk ☐
If yes, what was the recorded opening pressure: cm H_20 Unk ☐

On discharge, was patient given fluconazole: Yes ☐ No ☐ Unk ☐ Died ☐ Discharge dose Daily ☐ BD ☐

Pneumocystis jirovecii ONLY

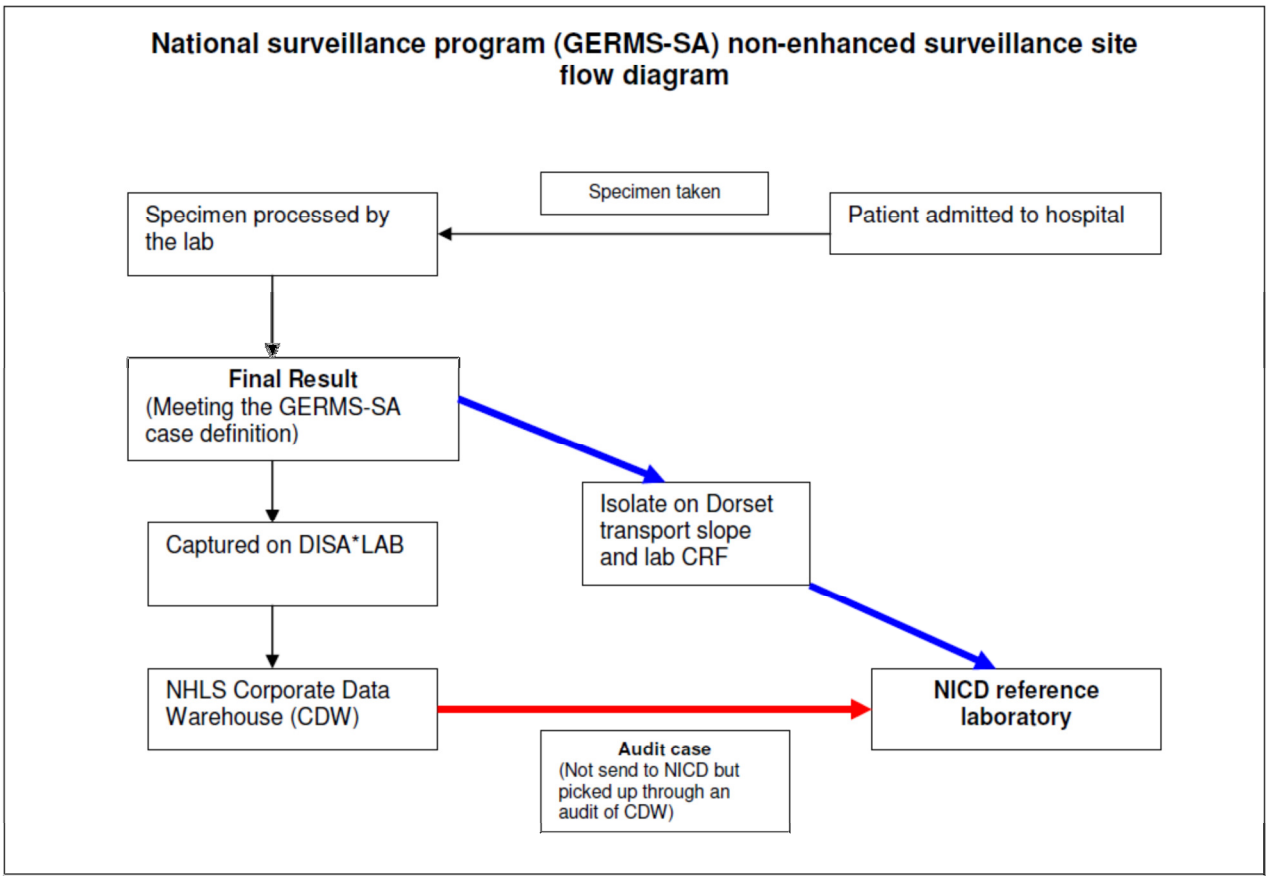
PCP treatment during this admission: Weight ☐☐☐.☐ kg Unk ☐

	Dose	Route	Date initiated	Total number of doses/ number of days
Cotrimoxazole			D D M M Y Y Y Y	
Dapsone			D D M M Y Y Y Y	
Other			D D M M Y Y Y Y	
Prednisone			D D M M Y Y Y Y	
Hydrocortisone			D D M M Y Y Y Y	

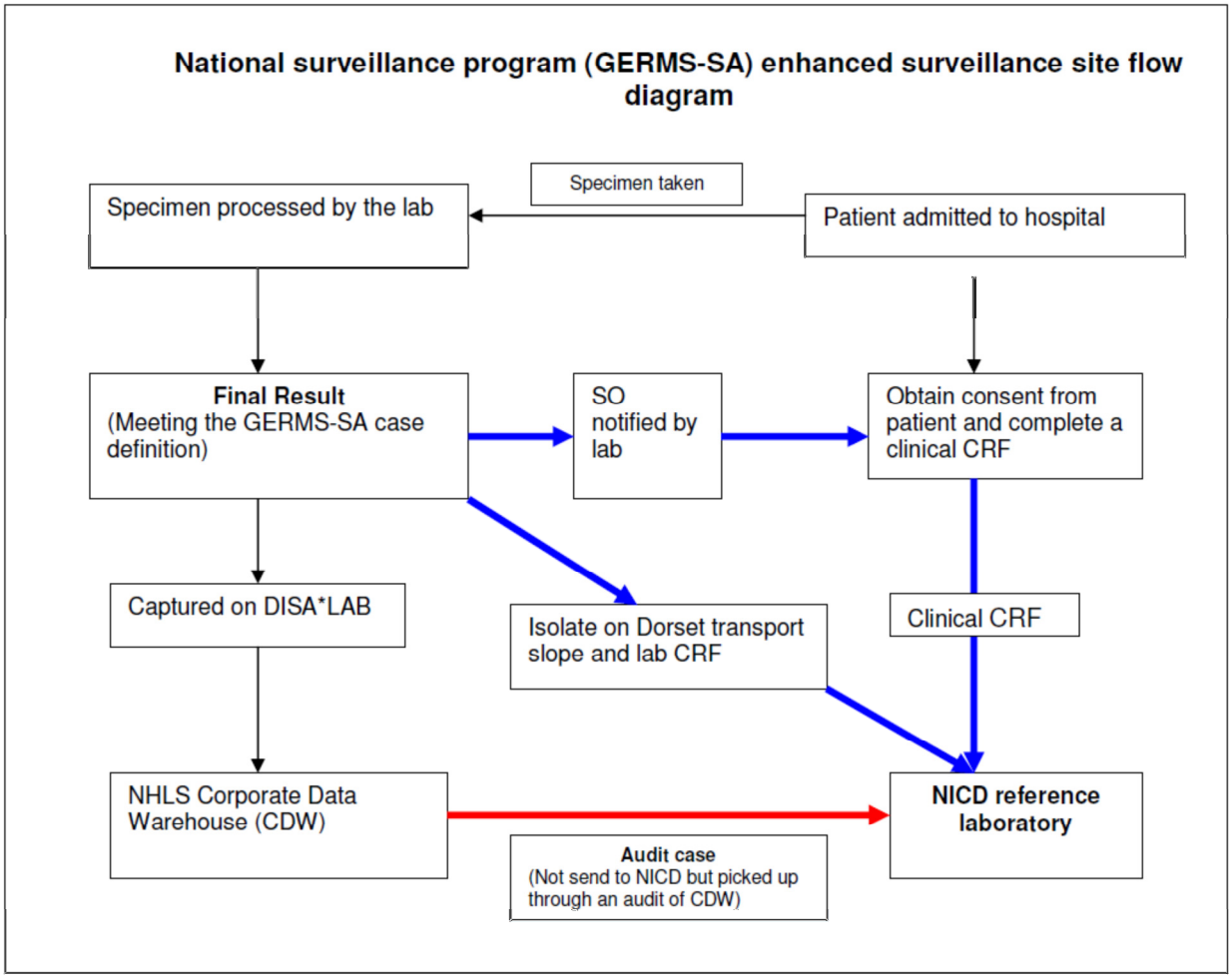
PCP therapy unknown ☐ PCP therapy not prescribed ☐

On discharge was patient given cotrimoxazole: Yes ☐ No ☐ Unk ☐ Died ☐ Discharge dose/ number of days:

Appendix D1: Flow diagram for non-enhanced surveillance sites



Appendix D2: Flow diagram of enhanced surveillance sites



Appendix E: Ethics clearance certificate

[redacted] OF THE WITWATERSRAND, JOHANNESBURG

[redacted]

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
[redacted] Dr Melony Fortuin-de Smidt.

[redacted]

[redacted]

[redacted]

[redacted]

South Africa from 2003-2010

INVESTIGATORS Dr Melony Fortuin-de Smidt.

DEPARTMENT School of Public Health

[redacted]

[redacted] Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon [redacted]

DATE 30/09/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc Supervisor : Dr Anne van Gottberg

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress report.
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix F: Number of *Haemophilus influenzae* cases reported (viable, non-viable, latex contaminated and PCR) and not reported (audits) by year for all ages, 2003-2009

	2003 **N (%)**	**2004** **N (%)**	**2005** **N (%)**	**2006** **N (%)**	**2007** **N (%)**	**2008** **N (%)**	**2009** **N (%)**	**Total** **N (%)**
Viable (cultured)	179 (57%)	175 (50%)	193 (62%)	202 (53%)	211 (48%)	192 (59%)	232 (60%)	**1384 (54%)**
PCR (non-viable)	0	0	0	0	95 (22%)	72 (18%)	43 (11%)	**210 (8%)**
Non-viable	36 (12%)	50 (14%)	47 (15%)	59 (16%)	9 (2%)	5 (1%)	1 (0.3%)	**207 (8%)**
Latex	5 (1.6%)	2 (0.6%)	0	0	0	1 (0.3%)	0	**8 (0.3%)**
Contaminated	1 (0.3%)	0	2 (1%)	6 (2%)	1 (0.2%)	1 (0.3%)	2 (0.3%)	**13 (0.5%)**
Audits	79 (25%)	116 (33%)	68 (22%)	98 (26%)	103 (24%)	111 (28%)	94 (24%)	**609 (24%)**
Total	**312**	**348**	**313**	**379**	**436**	**390**	**385**	**2563**

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