Studies on bacterial respiratory pathogens causing bacteraemia and meningitis in South Africa

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of

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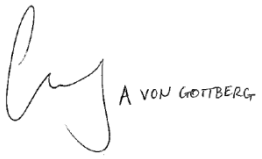
Declaration

I, Anna von Gottberg, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature: 

26th day of July 2013

Publications directly contributing to this PhD and role of the student in these studies

I wrote the initial surveillance protocol in 2002 and was principal investigator for the grant awarded from September 2002 to September 2006 that funded the surveillance enhancements that became GERMS-SA (Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa). I organised the start-up meeting in 2002 and started hiring the first members (medical officers, surveillance officers and data clerks) of the team that would become the management core to run the surveillance network. Together with this team we set up the surveillance and data methodologies that became the system allowing the collection of data and isolates used in the manuscripts contributing to this PhD. Early in 2006, the management of the broader surveillance network was taken over by a newly hired pathologist, while I continued to have primary responsibility for the surveillance, including data management and isolate processing, of the three pathogens that form part of this PhD: *Haemophilus influenzae, Neisseria meningitidis* and *Streptococcus pneumoniae*.

1. von Gottberg A, de Gouveia L, Madhi SA, du Plessis M, Quan V, Soma K, Huebner R, Flannery B, Schuchat A, Klugman K. Impact of conjugate *Haemophilus influenzae* type b (Hib) vaccine introduction in South Africa. Bull World Health Organ 2006;84:811-18.
2. von Gottberg A, Cohen C, Whitelaw A, Chhagan M, Flannery B, Cohen AL, de Gouveia L, du Plessis M, Madhi SA, Klugman KP. Invasive disease due to *Haemophilus influenzae* serotype b ten years after routine vaccination, South Africa, 2003-2009. Vaccine 2012;30:565-71.

For the above two studies I set up and guided the laboratory processing and data entry and cleaning procedures. I performed the database extraction and observational and univariate analysis with input

from my co-authors. I wrote the first draft of both papers and edited all subsequent drafts, and was both first and corresponding author for subsequent manuscript submissions to the journals.

3. von Gottberg A, du Plessis M, Cohen C, Prentice E, Schrag S, de Gouveia L, Coulson G, de Jong G, Klugman K. Emergence of endemic serogroup W135 meningococcal disease associated with a high mortality rate in South Africa. Clin Infect Dis 2008;46:377-86.

I detected the changing epidemiology and lead the epidemiological and laboratory aspects of the outbreak investigation. I performed the database extraction and observational and univariate analysis with guidance from my co-authors. For the multivariable analysis I was assisted by an epidemiologist. I wrote the first draft of the manuscript and edited all subsequent drafts, and was both first and corresponding author for the subsequent manuscript submission to the journal.

4. von Gottberg A, Klugman KP, Cohen C, Wolter N, de Gouveia L, du Plessis M, Mpembe R, Quan V, Whitelaw A, Hoffmann R, Govender N, Meiring S, Smith AM, Schrag S. Emergence of levofloxacin-non-susceptible *Streptococcus pneumoniae* and treatment for multidrug-resistant tuberculosis in children in South Africa: a cohort observational surveillance study. Lancet 2008;371:1108-13.

I lead the epidemiological and laboratory aspects of the outbreak investigation including the carriage study. I performed the database extraction and observational and univariate analysis with input from my co-authors. For the multivariable analysis I was assisted by an epidemiologist. I wrote the first draft of the manuscript and edited all subsequent drafts, and was both first and corresponding author for the subsequent manuscript submission to the journal.

5. von Gottberg A, Cohen C, de Gouveia L Meiring S, Quan V, Whitelaw A, Crowther-Gibson P, Madhi SA, Whitney CG, Klugman KP, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Epidemiology of invasive pneumococcal

disease in the pre-conjugate vaccine era: South Africa, 2003-2008 (Submitted November 2012 to Vaccine).

I set up and guided the laboratory processing and data entry and cleaning procedures. I performed the database extraction and observational and univariate analysis with input from my co-authors. For the multivariable analysis I was assisted by an epidemiologist. I wrote the first draft of the manuscript and edited all subsequent drafts, and was both first and corresponding author for the subsequent manuscript submission to the journal.

Abstract

Introduction

Analysis of surveillance data on bacterial respiratory pathogens most commonly causing bacteraemia and meningitis may be useful to measure the impact of vaccination, monitor antimicrobial resistance emergence and document changes in disease epidemiology.

Materials and methods

Active, laboratory-based, national surveillance for invasive *Haemophilus influenzae*, meningococcal and pneumococcal disease in South Africa was conducted. Isolates, cultured from normally sterile sites, were submitted for phenotypic and genotypic characterisation. Trends are described and univariate and multivariable models were used to assess differences among groups.

Results

Following the introduction of *H. influenzae* serotype b conjugate vaccine (HibCV) in 1999, the number of Hib cases reported for infants <1 year decreased by 65%, from 55 cases in 1999-2000 to 19 cases in 2003-2004. Despite high HibCV coverage, rates of Hib disease in children <5 years then increased from 0.7 per 100,000 population in 2003 to 1.3/100,000 in 2009. Among 263 Hib episodes, 135 (51%) were classified as vaccine failures and 53% of these occurred among children who were not HIV infected.

An investigation of meningococcal disease in Gauteng, revealed rates of disease which increased from 0.8/100,000 in 2000 to 4.0/100,000 in 2005; the percentage due to serogroup W135 increased during this time from 7% (4/54) of cases to 75% (221/295). Overall case-fatality ratios doubled from 11% in 2003 to 22% in 2005. Our investigations revealed that the expansion of the Hajj clone explained the emergence of serogroup W135 during this time, as 95% of W135 isolates (285/301) were identified as one clone by pulsed-field gel electrophoresis and seven representative strains belonged to the ST-11/ET-37 complex.

Among invasive pneumococcal disease (IPD) cases, 12 levofloxacin-non-susceptible pneumococci were identified in children <15 years, and were found to be associated with a history of tuberculosis (TB) treatment and nosocomial IPD in two treatment centres for multidrug-resistant TB (MDR TB). From 2003 through 2008, prior to pneumococcal conjugate vaccine (PCV) introduction, among IPD cases in children <5 years, 58% (3849/6668), 65% (4314/6668), and 85% (5669/6668) of cases and 61% (455/751), 64% (482/751), 82% (616/751) of deaths were due to serotypes included in 7-valent PCV (PCV-7), PCV-10 and PCV-13, respectively. PCV-13 had significantly higher coverage for isolates from blood culture than for isolates from cerebrospinal fluid: 3882/4531 (86%) vs. 1670/2009 (83%), p=0.009, but only differed by 3%. An analysis of risk factors revealed the relative risk of IPD was 21-fold (95% CI, 19–24) and 34-fold (29–41) greater in HIV-infected compared to HIV-uninfected children in the <1 year and 1–4-year-old age groups, respectively.

Discussion and conclusions

After initial reductions in Hib disease, vaccine failures, occurring in both HIV-infected and -uninfected children, comprised half of the rise in Hib disease detected 10 years after national introduction of Hib vaccine, given as three doses without a booster. These data contributed to the decision to add a booster dose of Hib vaccine in South Africa in 2009.

Continued surveillance of meningococcal serogroup W135 revealed evidence that this serogroup had become endemic in Gauteng causing more severe disease than the previous predominant serogroup A strain.

Paediatric fluoroquinolone use for MDR TB led to the emergence and nosocomial spread of levofloxacin-non-susceptible pneumococci. Existing pneumococcal vaccine formulations have the potential to prevent most cases and deaths from IPD among HIV-infected and -uninfected children in South Africa. Surveillance of pneumococcal meningitis may provide representative data for monitoring the impact of PCV.

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In addition, I would like to acknowledge GERMS-SA and my friends and colleagues at the NICD and CDC, Atlanta.

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List of abbreviations

EPI	Expanded Programme on Immunization
CFR	case-fatality ratio
CLSI	Clinical and Laboratory Standards Institute (formerly the National Committee on Clinical Laboratory Standards (NCCLS))
CSF	cerebrospinal fluid
DTwP	diphtheria toxoid, tetanus toxoid and whole cell pertussis antigen
HibCV	*Haemophilus influenza*e serotype b conjugate vaccine
HIV	human immunodeficiency virus
IPD	invasive pneumococcal disease
LNSSP	levofloxacin-non-susceptible *Streptococcus pneumoniae*
MDR	multidrug resistant
MIC	minimum inhibitory concentration
MIC50	minimum inhibitory concentration required to inhibit the growth of 50% of organisms
MLST	multilocus sequence typing
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
PCV	pneumococcal conjugate vaccine
PCV-7	7-valent pneumococcal conjugate vaccine
PCV-10	10-valent pneumococcal conjugate vaccine
PCV-13	13-valent pneumococcal conjugate vaccine
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
TB	tuberculosis
UK	United Kingdom
US	United States of America
ST	sequence type
StatsSA	Statistics South Africa
WHO	World Health Organization

Chapter 1 Introduction

Public health surveillance is defined as "the ongoing, systematic collection, analysis, and interpretation of health data, essential to the planning, implementation and evaluation of public health practice, closely integrated with the dissemination of these data to those who need to know and linked to prevention and control". This definition has recently been reviewed and considered to be relevant and valid in the 21st century.[1] Purposes of public health surveillance include assessing public health status of a population, defining public health priorities, evaluating programmes or interventions and doing research. The following studies will demonstrate the utility of surveillance in South Africa for a small group of encapsulated bacteria that cause vaccine-preventable bacteraemia and meningitis.

1.1 *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae*

The bacteria *H. influenzae*, *N. meningitidis* and *S. pneumoniae*, have several common characteristics that allow them to be discussed together, but they also have distinctive features that highlight differences. These three bacteria were, before vaccine introduction, the commonest causes of acute bacterial meningitis globally and in South Africa, although their relative contributions differed by time, place and person.[2] They are almost exclusively human pathogens and encapsulated forms (possessing a polysaccharide capsule) are the most important cause of severe disease. The polysaccharide capsules of *H. influenzae*, *N. meningitidis* and *S. pneumoniae* allow for their characterisation into serogroups or serotypes: 6 serotypes for *H. influenzae* (a to f), 12 serogroups for the meningococcus (A, B, C, X, Y, Z, E, W-135, H, I, K and L) and more than 90 serotypes for the pneumococcus. Disease is limited to only a few of these serogroups or serotypes: *H. influenzae* serotype b (Hib) is the predominant encapsulated strain

causing invasive disease; for pneumococcus, six to 11 serotypes account for ≥70% of invasive pneumococcal disease (IPD);[3] and only serogroups A, B, C, W-135 and Y commonly cause invasive meningococcal disease.[4] Common to all three pathogens is that current vaccines effective in disease prevention in children are predominantly based on the polysaccharide capsule conjugated to proteins, and only prevent disease due to serotypes (or in the case of meningococci, serogroups) contained in the vaccine. Only the Hib vaccine is consistently monovalent *i.e*. all Hib vaccines currently available are designed to prevent Hib disease only.

While all three bacteria are important causes of meningitis and pneumonia, there are differences in the common clinical presentations caused by each pathogen. Although the pneumococcus is a common cause of acute bacterial meningitis, by far the greatest burden of pneumococcal disease is pneumonia. In 2000, approximately 95% of the total burden of IPD in children <5 years was estimated to be pneumonia, and more than half of fatal pneumococcal pneumonia was estimated to occur in Africa (estimated 406,000 deaths in Africa (uncertainty range 297,000 to 441,000)).[5] Similarly, estimates for Hib calculated that approximately 95% of all invasive Hib disease in children <5 years was pneumonia.[6] The often cited higher proportions of invasive disease that are meningitis due to the latter two pathogens may be ascribed to regional differences in blood culturing practices.[2] Meningococcal disease is estimated to include the meninges in approximately 50% of patients, while meningococcal sepsis (abrupt onset of fever, rash and hypotension) occurs in 5% to 20% of patients.[7] Pneumonia occurs in approximately 5% to 15% of patients with invasive meningococcal disease. All three pathogens have been associated with increased disease risk among human immunodeficiency virus (HIV)-infected individuals.[8-10]

Disease severity also differs by pathogen and syndrome. Hib pneumonia in children <5 years is estimated to have a case-fatality ratio (CFR) of 4% (uncertainty range; 2% to 7%), while Hib meningitis is considerably more severe (43%; 23% to 55%).[6] Pneumococcal pneumonia CFR was in a similar range (5%; 4% to 9%) to Hib pneumonia, but global estimates of pneumococcal meningitis CFR was higher (59%; 27% to 80%).[5] Overall CFR for invasive meningococcal disease ranges from 9% to 12%, but this can increase to up to 40% among patients with meningococcal sepsis.[7;11]

1.2 Surveillance systems: some definitions

Passive surveillance relies on reporting from a healthcare provider or a laboratory without any active intervention.[12] In contrast, in active surveillance, reports from these healthcare providers or laboratories are actively sought and attempts are made to estimate the completeness of reporting. Population-based surveillance is surveillance trying to capture all cases according to a standard definition (including person, place and time) among an entire or a representative sample of a predefined population, making it possible to estimate incidence among that population. The case definition may be laboratory based (if the laboratory test is widely used and both sensitive and specific), however some diseases may be more suited to a clinical or syndromic case definition. Syndromic surveillance, with a standard clinical case definition, may include laboratory testing of a subset of patients if the funds required for this are available.

The intensive resources required for active, population-based surveillance are often not available and therefore public health agencies or countries may rely on an alternate strategy, sentinel surveillance. Sentinel surveillance systems involve a defined and limited number of reporting sites (e.g., laboratories or hospitals) that are a subset of a larger population, ideally chosen so that reports from these limited

sites will be representative of most, if not all, other sites and as a result the information from the sentinel sites can be generalised to the whole population.

1.3 Surveillance for *H. influenzae*, *N. meningitidis* and *S. pneumoniae* disease

In order for surveillance of disease due to these pathogens to optimally document burden of disease, all cases in a defined population need to be identified. This would require all patients to have equal access to hospital care, standard case definitions for clinical diagnostic procedures e.g. standard criteria for taking blood , cerebrospinal fluid (CSF) or other cultures; or for performing chest radiography; and if laboratory-based case definitions, appropriate handling of specimens and quality processing of specimens in laboratories that then report all cases. Many of these criteria are not fulfilled in surveillance systems, and so cases identified must be considered a minimum estimate, especially in low- or middle-income countries.[2] However, even if the burden of disease is underestimated, if practices over time remain constant, trends from these surveillance systems may be valid.

The surveillance case definitions for these bacterial pathogens are often laboratory based, requiring the identification of the organism in the laboratory. This significantly underestimates disease not likely to be confirmed in the laboratory e.g. pneumonia.[5;6] A strength of laboratory-based surveillance, however, is the specificity of the case definition, especially if limited to ascertainment of invasive disease *i.e.* identification of the bacteria from normally sterile-site specimens.[13] In addition, having the organism to hand allows for further characterisation, which is especially important with increasing antimicrobial resistance[14;15] and limited strain-specific coverage by vaccines.[3;16;17] Case ascertainment for meningitis may be more standardised over time in countries routinely performing lumbar punctures,[18] however in

other countries additional efforts need to be made to ensure standard lumbar puncturing practices.[19] Blood culture sampling may be especially sensitive to changes over time[18;20;21] and geographic differences.[22] Laboratory procedures require ongoing evaluation for accuracy,[23] as well as assessment of selection for reference testing.[24] Advances in molecular identification of *H. influenzae*, *N. meningitidis* and *S. pneumoniae* have resulted in improved case ascertainment in both developed and developing countries.[25;26]

Although active, population-based surveillance with a diagnostic test that is widely and readily available and has a high degree of sensitivity and specificity, may be the gold standard ,[12] sentinel surveillance has some utility for both antimicrobial resistance monitoring[27] and vaccine effectiveness.[28] In one evaluation, emerging antimicrobial resistance among pneumococci was less likely to be documented by sentinel site surveillance, but important trends over time were detected.[27]

1.4 Surveillance for *H. influenzae*, *N. meningitidis* and *S. pneumoniae* disease to monitor antimicrobial resistance

Dissemination of antimicrobial resistance data from surveillance systems may have multiple purposes. For example, creating an awareness among clinicians of increasing resistance may guide more prudent prescribing habits.[29] Geographical differences in antimicrobial resistance may also allow for region-specific interventions, while ongoing surveillance can assist in monitoring trends over time or emerging resistance. The data can be used for regional and national guidelines recommending the empiric management of certain clinical syndromes.

H. influenzae resistance was first described in the 1970s.[30] Ongoing detection of resistant isolates in Africa has challenged the empiric therapy for meningitis of penicillin and chloramphenicol still being used in some countries.[31;32] This has also been highlighted by the detection of chloramphenicol-resistant meningococci.[33] Not only is it important to monitor antimicrobials used for empiric therapy, but also those antimicrobials used to eradicate nasopharyngeal carriage of these pathogens need to be monitored for emerging resistance.[34;35]

During the 1940s, pneumococci were universally susceptible to penicillin, however in 1967, in Australia, intermediate penicillin resistance was identified for the first time and subsequently high-level resistance was detected in South Africa.[36] In 1978 in South Africa, the first multidrug resistant (MDR) pneumococci were described,[37] setting the scene for a national surveillance programme for antimicrobial-resistant pneumococci.[38] More recently, the active, population-based surveillance system has documented increasing MDR pneumococci and estimated the effects of the pneumococcal conjugate vaccines (PCV) in preventing disease due to these isolates.[39]

1.5 Surveillance for *H. influenzae*, *N. meningitidis* and *S. pneumoniae* disease and changing epidemiology without specific interventions

Serotype epidemiology has changed significantly over time globally. An analysis of pneumococcal serotypes causing disease in the US demonstrated the substantial reduction of the so-called epidemic serotypes (serotypes 1,2,3, and 5) over the last century.[22] Changes in antibiotic use, socioeconomic conditions, underlying immunocompromise of a changing population and specimen-taking practices were all proposed as possible reasons for these changes. Similarly, the more outbreak prone of the meningococcal serogroups, serogroup A, has also disappeared from industrialised countries since World

War II.[11] Good quality surveillance has also identified a substantial burden of pneumococcal meningitis masked by the meningococcal outbreaks in the African meningitis belt.[40;41]

1.6 Effect of vaccines and the surveillance for *H. influenzae*, *N. meningitidis* and *S. pneumoniae* disease

Children <2 years of age do not respond well to vaccines containing capsular polysaccharide-only antigens, however when polysaccharide antigens are conjugated to carrier proteins they are recognised as T-cell-dependent antigens and induce good antibody responses and immunological memory in infants.[42] Polysaccharide-only vaccines are still used to prevent disease due to meningococcus and pneumococcus, as older children and adults continue to be at risk of disease due to these pathogens. Newer meningococcal and pneumococcal polysaccharide-protein conjugate vaccines have however made it possible to prevent disease in infants. Hib polysaccharide-only vaccine is no longer available (infants are the predominant age group at risk of Hib disease), and only polysaccharide-protein conjugate Hib vaccines (HibCV) are available worldwide. The first commercially viable HibCV, were licensed initially in 1987 in the US for 18 months of age and from 1991 from 2 months of age.[43] In November 1999, the United Kingdom (UK) introduced the first monovalent serogroup C conjugate meningococcal vaccine after increases in the incidence of serogroup C disease.[44] It was followed by the first formulation of the PCV licensed and used routinely in the US in 2000.[45]

Conjugate vaccines induce mucosal immunity, preventing the new acquisition of vaccine-serotype strains in the nasopharynx.[42] This effect on carriage reduces the number of children who are carriers of Hib, pneumococcus or meningococcus, and decreases the chance of transmission to other at-risk individuals.[46;47] This indirect effect may, however, be more limited among individuals with co-morbid

diseases, including HIV infection.[48] As indirect effects are driven by the individuals most likely to carry the organism, targeting these age groups may maximise the indirect effects.[49]

1.6.1 Surveillance prior to vaccine introduction

Surveillance becomes essential to document the prevalence of diseases before planning an introduction in the infant immunisation programme. The global disease burden projects tried to make regional data available to help policy makers in considering the benefits of the conjugate vaccines.[5;6] Taking it one step further, global serotype distribution estimates were generated to guide future pneumococcal vaccine formulations.[3] In a similar way, careful review of meningitis surveillance data was needed to plan a monovalent serogroup A meningococcal vaccine introduction in the African meningitis belt.[16] Surveillance of meningococcal disease in England and Wales documented increasing serogroup C disease linked to the emergence of a hypervirulent strain, sequence type (ST) 11, and this set the scene for the introduction of a monovalent serogroup C vaccine.[50]

Often surveillance for vaccine-preventable diseases is initiated to allow for baseline data to monitor the effectiveness of the vaccines after introduction. Although surveillance is recommended for the introduction of these vaccines, it is not stipulated as a prerequisite.[51;52]

1.6.2 Surveillance used to measure effectiveness after vaccine introduction

HibCV was first licensed in 1987 in the US. The incidence of Hib disease in childhood has been dramatically reduced in countries using HibCV.[53-58] Serogroup C meningococcal disease has been controlled in those countries who have introduced either the monovalent (serogroup C) or quadrivalent

(serogroups A, C, Y and W-135) conjugate vaccines.[59] In addition, early surveillance data from Burkina Faso have demonstrated substantial reductions of serogroup A disease in the age groups targeted for vaccination (1 to 29 years), as well as reductions in disease in those too young or too old to have been vaccinated, one year after the introduction of the monovalent serogroup A vaccine.[60] PCV was introduced in the infant immunisation programme in the US in 2000. Substantial reductions of all IPD were documented within a year of introduction among children targeted for vaccination (69% reduction from 188 cases per 100,000 children <2 years in 1998-1999, to 59 cases per 100,000 population in 2001).[45] Reductions in disease have also been documented among individuals not vaccinated, demonstrating the potential for this vaccine to prevent disease by a herd or indirect effect.[61] PCV has also demonstrated effectiveness in preventing hospitalisations due to pneumonia in children,[62;63] and in the reduction of antimicrobial-resistant IPD.[64;65]

1.7 Aim of this study

The aim of this study was to initiate an active, laboratory-based, population-based surveillance programme in South Africa to monitor the epidemiology of bacterial causes of bacteraemia and meningitis in all ages, specifically due to the vaccine-preventable, encapsulated, bacterial pathogens of *H. influenzae, N. meningitidis* and *S. pneumoniae*. Mindful of the definition of public health surveillance, data were collected, analysed and interpreted to monitor antimicrobial resistance in *S. pneumoniae*, the changing disease epidemiology of *N. meningitidis*, and to contribute to the planning, implementation and evaluation of two new infant vaccines for the prevention of disease due to *H. influenzae* serotype b and *S pneumoniae* respectively.

Chapter 2 Materials and Methods

2.1 Case definitions

A national, laboratory-based surveillance system for invasive *H. influenzae*, *N. meningitidis* and *S. pneumoniae* was introduced in South Africa in 1999.[66] All laboratories throughout South Africa performing clinical microbiology diagnostic tests are requested to send reports of laboratory-confirmed disease together with isolates to a central laboratory in Johannesburg. Basic demographic details of the patients, such as age and gender, and date of specimen, and source of isolate are collected.

In 2003, the system was enhanced. Frequent communications and provincial visits were initiated to increase case reporting. For cases occurring at sentinel hospitals located in each of the nine provinces, we collected expanded clinical and demographic information including admission date, HIV serological status, history of tuberculosis (TB) treatment in the last three months, discharge diagnosis and outcome.

Cases were defined as patients with at least one of the three bacteria identified in normally sterile site specimens (*e.g.*, CSF, blood, joint fluid). Repeat isolates from the same patient were excluded if occurring within 21 days of the initial positive culture. Nosocomial infection was defined as specimen collection date ≥2 days after date of admission to hospital.

2.2 Laboratory processing

Isolates were received on plates or transport media from submitting laboratories. Identification of bacteria followed standard procedures.[67] Reference testing for each pathogen was performed as specified in the following chapters.

2.3 Data collection and cleaning

At enhanced sites surveillance officers complete the clinical report form (Appendix A). Data were entered in a centralised, common database for all three pathogens. Routine data checks were performed, including ensuring that individuals were not recorded more than once within 21 days. Data quality checks also comprised logical consistency tests and confirmation of outliers.

2.4 Analysis and statistical methods

Databases were extracted and analysed as specified in the following chapters.

2.5 Ethics

The national surveillance study protocols were approved by the relevant institutional ethics committees. Protocol number M021042 (GERMS-SA surveillance -principal investigator: Anne von Gottberg) was approved on 25/10/2002 by the Human Research Ethics Committee (Medical), University of Witwatersrand, Johannesburg (Appendix B) and recertified on 30/01/2004. The GERMS-SA protocol was updated and resubmitted (principal investigator: Nelesh Govender) in 2008, protocol number M081117 and approved on 28/11/2008; and has been reviewed annually since then. Only study participants or their guardians interviewed for additional clinical data at enhanced surveillance sites provided written informed consent. The carriage study described in Chapter 6 (protocol number M060634) was approved on 30/06/2006 (Appendix B). All carriage study participants or their guardians gave written informed consent.

Chapter 3 Impact of conjugate *Haemophilus influenzae* type b (Hib) vaccine introduction in South Africa

3.1 Introduction

The introduction of conjugate vaccines for the prevention of Hib disease (HibCV) in children has substantially reduced burden of disease in developed countries[68-70] and in developing countries where it has been introduced.[57;71-73] However, due to the high cost of the HibCV, they are used in only a small number of developing countries. HibCV are highly effective against invasive disease and may prevent up to 25% of radiographically-confirmed pneumonia,[74-76] although the organism remains under-recognised as a cause of severe disease and death in developing countries.[77] These vaccines have reduced effectiveness among HIV-infected children.[78;79] The vaccine-preventable burden of Hib disease, however, is likely to be greater among the HIV-infected than among uninfected children due to much higher rates of Hib disease.[80] Evaluations of the impact of HibCV introduction in populations with a high burden of HIV infection are needed.

South Africa was the first country in Africa to self-finance the incorporation of HibCV into the routine childhood immunisation programme since July, 1999. Population-based studies in South Africa had previously demonstrated rates of invasive Hib disease of 170 per 100 000 infants less than 1 year of age.[78;81] National laboratory-based surveillance for invasive Hib disease was established concurrently with HibCV introduction to document the impact of routine vaccination on Hib disease.[66] We analysed data from this national laboratory-based surveillance system for the first five years to document

changes in the number of reported cases of laboratory-confirmed *H. influenzae* disease among children <5 years of age in South Africa.

3.2 Materials and Methods

In June 1999, national laboratory-based surveillance was established for invasive *H. influenzae* and *S. pneumoniae* disease, defined as isolation from normally sterile body fluids, among South Africans of all ages.[66] All clinical laboratories in the country were requested to report cases of invasive *H. influenzae* infection and send isolates to a central reference laboratory at the National Institute for Communicable Diseases (NICD) (division of the National Health Laboratory Service (NHLS)) in Johannesburg. Multiple isolates from the same disease episode were excluded. Clinical laboratories routinely culture specimens of blood and CSF for bacterial isolation, although prior to this time, no nationwide system existed for reporting cases or collecting isolates. The number of clinical laboratories reporting cases has increased during each year of the study period, from 80 laboratories during July 1999 through June 2000 to 88, 91 and 103 in the three subsequent 12-month periods. During this period no deterioration in laboratory standards was noted. The surveillance system was enhanced in 2003 by placing additional surveillance staff in approximately 15 hospitals in seven of nine provinces, leading to an increase in the number of laboratories reporting (from 103 in July 2002–June 2003 to 126 in July 2003-June 2004). Since 2003, cases at sentinel sites throughout the country have been reviewed for outcome, HIV status (based on serology in all ages; in children less than 18 months based on serology with clinical features and/or positive polymerase chain reaction (PCR) results) and vaccination history. Laboratories were encouraged to report all cases of laboratory-confirmed disease, even if there were no isolates available for shipment. Regional laboratory audits performed yearly identified 54 laboratory-confirmed cases of *H. influenzae* in all ages during the study period (24, 5, 12, 9, and 4 cases per 12-month interval) that had not been reported to the NICD, suggesting that approximately 70% of all laboratory-confirmed *H.*

influenzae infections were reported. Cases identified by audit were added to the surveillance database. Since 1999, laboratory-confirmed cases of Hib are legally notifiable by clinicians to the national Department of Health.

A case of *H. influenzae* meningitis was defined as an individual with CSF from which *H. influenzae* was cultured. Cases of bacteraemia were defined as individuals with other clinical syndromes and growth of *H. influenzae* from blood cultures. Identification of isolates was confirmed with the gamma-aminolevulinic acid (ALA)-porphyrin test reaction and API® NH (bioMérieux® sa, Marcy-l'Etoile, France).[67] Slide agglutination for serotyping was performed using agglutinating sera for types a-f (Murex Biotech Ltd., Dartford, Kent, UK). All isolates had serotyping results confirmed with PCR.[82] Cases with no isolate available for serotyping were excluded from further analysis. Susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[83] For isolates nonsusceptible (intermediately resistant and resistant) to any antibiotic, minimum inhibitory concentrations (MIC) were determined by E-test (AB Biodisk, Solna, Sweden). Nitrocefin was used to test for beta-lactamase production. Multiple drug-resistant isolates were defined as isolates nonsusceptible to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole.

The mean age of children <5 years of age for each 12-month period was compared using the nonparametric Kruskal-Wallis test. Percentage decreases were calculated by comparing number of reported cases in July 1999-June 2000 with cases reported in July 2003-June 2004. Rates of reported cases of invasive Hib disease were calculated for 12-month periods from July 1 through June 30 of the following year. Numerators were the number of viable *H. influenzae* isolates confirmed as serotype b at the reference laboratory during the period. Denominators were mid-year population estimates obtained from Statistics South Africa (StatsSA). HibCV coverage data were obtained from the Department of

Health[84] and Health Systems Trust.[85] HIV seroprevalence estimates were obtained from antenatal clinic surveys.[86;87] Trends in the proportion of antimicrobial resistant isolates over the five July-June periods were analysed using the χ^2-test for trend. Data were managed and analysed using Epi Info software, version 6.04d.[88]

3.3 Results

In July, 1999, South Africa introduced HibCV as part of a combination product (CombActHIB®, Aventis Pasteur) containing diphtheria toxoid, tetanus toxoid and whole cell pertussis antigen (DTwP) among all children receiving their first dose of diphtheria-tetanus-pertussis vaccine. The vaccine preparation required reconstitution of dried Hib conjugate powder with DTwP as the diluent. The recommended schedule was a dose at six, 10 and 14 weeks with no booster. There was no catch-up schedule for vaccinating children who had already received their first dose of DTwP. Sporadic shortages of combination vaccine were experienced from 1999 through 2002. Prior to nationwide introduction, HibCV had been introduced beginning in March, 1998, as part of a PCV trial in Soweto (urban black community with 120,000 children <5 years of age in 1995), Gauteng, affecting a total of 19,267 children.[78] Population estimates for children <5 years of age in 2002 nationally were 4,455,000, while in Gauteng and the Western Cape, these estimates were 737,600 and 409,600 respectively.

From July 1999 through June 2004, 920 cases of invasive *H. influenzae* disease in persons of all ages were reported to the surveillance system, and the age of case-patient was provided for 847 (92%). In all, 712 (77%) of 920 isolates were recovered for serotyping and antimicrobial susceptibility testing. As the number of surveillance audits increased through the period, we identified an increasing number of laboratory-confirmed cases retrospectively for which no isolates were available. The percent of cases with isolates therefore decreased from a high of 84% in the 1999-2000 period to 74% in 2003-2004, with

a significant downward trend over the five-year period (p=0.02). Of 712 viable isolates, 300 (42%) were identified as serotype b (109, 61, 43, 44 and 43 cases for the five July-June periods respectively), 104 (15%) were other capsular types and 308 (43%) were unencapsulated *H. influenzae*.

Among case-patients of known age, 218 (78%) of 279 Hib isolates and 225 (61%) of 370 other *H. influenzae* isolates were from children <5 years of age (Table 3.1). Of the Hib cases aged 5 years and older, 16 (6%) occurred in children 5 to 9 years, 5 (2%) in 10 to 14 year olds, and 40 (14%) in adults aged 15 or older. Reported cases of invasive disease among children <5 years of age caused by Hib decreased substantially during the five-year period, while those caused by *H. influenzae* other than Hib and *S. pneumoniae* increased up to more than two-fold (Table 3.1). Among children <5 years with invasive Hib disease, 60% (130 cases) occurred in children <1 year (Table 3.2), 13% (29) in patients aged 12 to 17 months and 27% (59) in children 18 months of age or older. During the period, there was no significant change in the median age (9 months) of Hib cases among children <5 years (p=0.162). Just over half of these cases (113, 52%) were positive cultures from CSF specimens (with or without other specimens), while 104 were from blood cultures, and one from a pleural fluid specimen. No cases of epiglottitis were reported.

Table 3.1: Reported cases of invasive disease caused by *Haemophilus influenzae* and *Streptococcus pneumoniae* in South African children <5 years old, by 12-month period

Disease	Years of surveillance					
	1999/2000	2000/2001	2001/2002	2002/2003	2003/2004	% change[a]
			n (%)			
Haemophilus influenzae						
Type b	89 (65)	43 (46)	27 (30)	33 (31)	26 (17)	-71
Other typeable[b]	8 (6)	6 (6)	11 (12)	13 (12)	25 (16)	213
Non-typeable	18 (13)	19 (20)	32 (35)	35 (33)	58 (37)	217
No isolate available	22 (16)	26 (28)	21 (23)	25 (24)	46 (30)	Not applicable
All	137	94	91	106	155	12
Streptococcus pneumoniae (all serotypes)	453	691	788	733	1218	169

[a] Comparing 1999/2000 to 2003/2004.

[b] Includes serotypes a (n=10), c (n=6), d (n=5), e (n=3) and f (n=39).

Table 3.2: Reported *Haemophilus influenzae* serotype b (Hib) disease among children <5 years old, by age group and 12-month period

Age group	Years of surveillance				
	1999/2000	2000/2001	2001/2002	2002/2003	2003/2004
Reported Hib cases [a] (all age groups)	89	43	27	33	26
<6 weeks	2	2	1	2	3
6 weeks -<1 year	53	24	14	13	16
>14 weeks [b] - <1 year	43	20	13	10	14
1 - <2 years	21	8	6	4	5
2 - <3 years	9	9	4	4	0
3 - <4 years	2	0	2	5	2
4 - <5 years	2	0	0	5	0

[a] Excludes 21 cases reported without exact age specified: 4, 6, 6, 2 and 3 by 12-month period.

[b] Vaccination schedule for 3 doses of Hib conjugate vaccine: 6, 10, and 14 weeks of age

Our analyses revealed that Hib cases decreased by 65% in children <1 year of age, from 55 cases in 1999-2000 to 19 cases in 2003-4 (Table 3.3). In Gauteng, Hib cases fell from 20 to 10, and in the Western Cape, cases dropped from 12 to 2 cases, during the same time period. In Gauteng and Western Cape, rates of Hib disease as reported to the surveillance system among children <1 year decreased 57% (13.1 cases per 100 000 in 1999-2000 vs. 5.7 per 100 000 in 2003-2004; 95% confidence interval (CI) 8%-80%, p=0.04), and 85% (14.6 vs. 2.2 cases per 100 000; 95% CI 32%-97%, p=0.010) respectively (Table 3.3).

Decreases of invasive disease were also recorded nationally for children 1 to 4 years of age (Table 3.3), in whom disease decreased 79%, from 34 to 7 cases. Decreases in this age group were also evident in the two provinces with the most disease reported: from 13 to 5 cases in Gauteng and from 16 to 1 case in Western Cape. In the two provinces we analysed separately, antenatal HIV seroprevalence rates among pregnant women in 2000 were 29% in Gauteng and 9% in Western Cape, increasing to 33% and 15% in 2004 respectively (Table 3.3).

Table 3.3: Number and rates of reported cases of invasive *Haemophilus influenzae* serotype b disease in children <5 years of age in Gauteng and Western Cape provinces, and South Africa as a whole, by 12-month period

Region and age group	Years of surveillance					
	1999/2000	2000/2001	2001/2002	2002/2003	2003/2004	Total
South Africa						
<1 year	55	26	15	15	19	130
Cases/100,000	6.2	2.9	1.7	1.6	2	
1-4 years	34	17	12	18	7	88
Cases/100,000	0.9	0.5	0.3	0.5	0.2	
HIV seroprevalence rates (%) in antenatal clinic attendees	24.5	24.8	26.5	27.9	29.5	
Number of laboratories reporting nationally	80	88	91	103	126	
Gauteng Province						
<1 year	20	11	5	8	10	54
Cases/100,000	13.1	7.0	3.1	4.7	5.7	

1-4 years	13	9	6	11	5	44
Cases/100,000	2.4	1.6	1.1	1.9	0.9	
HIV seroprevalence rates (%) in antenatal clinic attendees	29.4	29.8	31.6	29.6	33.1	
Western Cape Province						
<1 year	12	6	5	2	2	27
Cases/100,000	14.6	7.1	5.8	2.3	2.2	
1-4 years	16	4	5	3	1	29
Cases/100,000	5.1	1.3	1.6	0.9	0.3	
HIV seroprevalence rates (%) in antenatal clinic attendees	8.7	8.6	12.4	13.1	15.4	

We found that all ampicillin-resistant Hib isolates produced β-lactamase. Although the numbers of Hib cases decreased during the final surveillance year, isolates of Hib were more likely to be ampicillin resistant (8 (31%), of 26 cases in 2003-4, vs. 14 (16%) of 89 isolates in 1999-2000; p=0.036, χ^2-test for trend) (Figure 3.1). In addition, even though the absolute number of cases decreased over the years, the percentage of multiple drug-resistant isolates (nonsusceptible to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole) increased: 2%, 7%, 15%, 17% and 19% for each 12-month period respectively (p=0.001, χ^2-test for trend).

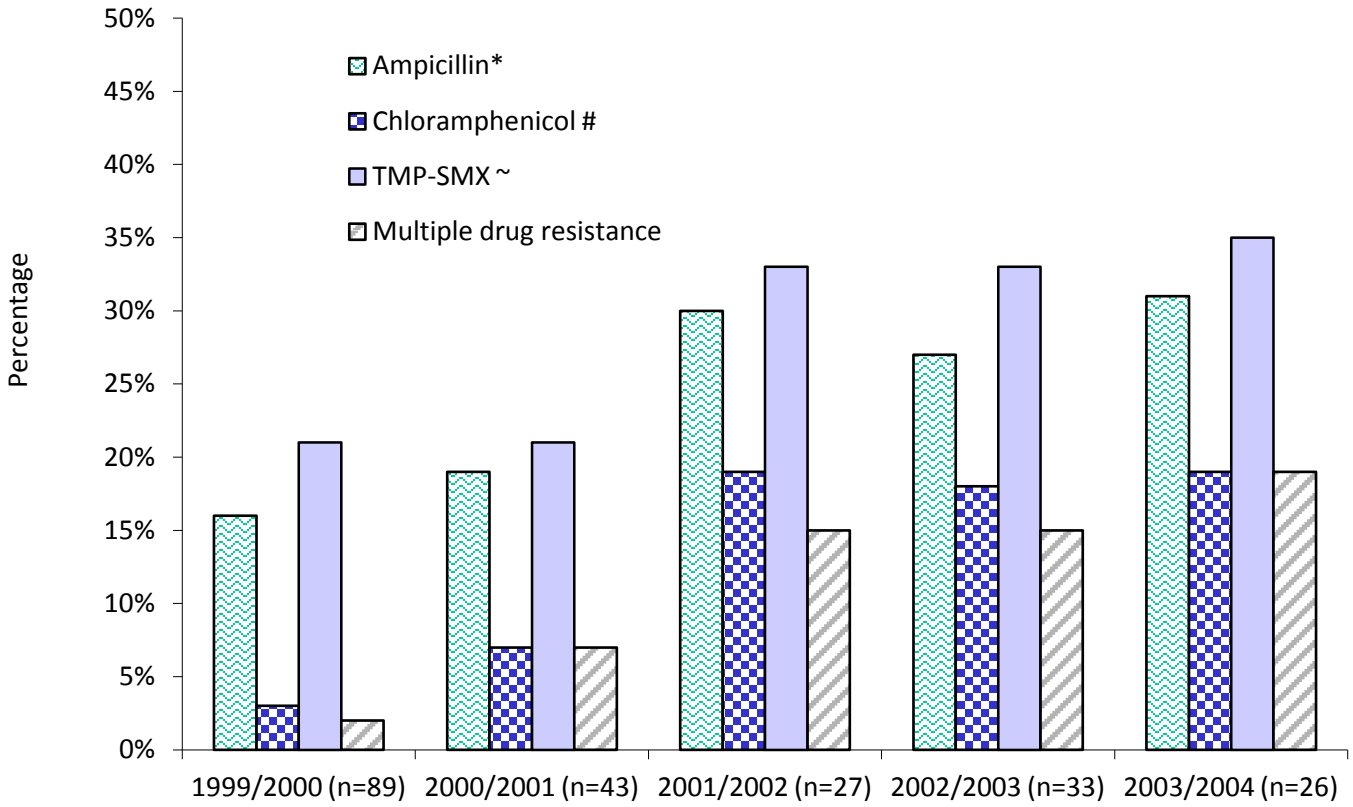

Figure 3.1 Percentage of non-susceptible isolates causing *Haemophilus influenzae* serotype b disease in children <5 years old, by category for each of the 12-month periods, South Africa

* χ^2-test for trend, p=0.04

p=0.001

~ p=0.06

Before the introduction of HibCV, a national survey of vaccination coverage in 1998 estimated that 72% of infants had received 3 doses of DTwP by one year of age. Annual coverage figures based on routine clinic reports of vaccinations estimated that 64% of South African children were fully immunised (three doses of DTwP and Hib) by one year of age in 2000, 72% in 2001 and 68% in 2002. Provincial estimates for Gauteng are lower for 2002 at 61%, but similar to the national estimates for the other 2 years; while in the Western Cape the figures are 79%, 73% and 56%, respectively.

During 18 months of enhanced surveillance from January 2003 through June 2004, 212 *H. influenzae* cases were reported nationally in children <5 years (44/154 (28%) of these confirmed cases of Hib), with 114 (54%) at sentinel hospitals (18/82 Hib (22%), 46 non-typeable, 18 other than serotype b cases, and 32 cases with no viable isolates). Vaccination status was available for 15 Hib case-patients: four were too young to be immunised, one five-month-old child was unimmunised, and 10 had received at least one dose of HibCV. A nine-month-old HIV-uninfected child who had received a third dose of the HibCV four months before presenting with Hib meningitis, subsequently died. HIV status was documented for 15 case-patients: 11 (73%) were HIV-infected, including five of eight case-patients <1 year of age. Among four case-patients who had documented receipt of one or more HibCV dose and also had known HIV-status, three (75%) were HIV-infected. Clinical outcome was reported for 29 case-patients since January 2003, five (17%) of whom died.

We observed that nationally over the five years, the median age for non-typeable disease (n=162) and disease due to serotypes other than serotype b (n=63) in children <5 years of age did not change (median age 10 months for both groups). During enhanced surveillance, children with non-typeable disease were more likely to be HIV-positive (32 of 34, 94%) than were children with Hib disease (10 of

14, 71%), p=0.051. All children who had serotypes other than b with known HIV-serostatus (12 of 12) were HIV positive.

3.4 Discussion

In South Africa, national laboratory-based surveillance for invasive Hib disease demonstrated a significant decrease in cases of invasive disease in the years following introduction of HibCV into the routine childhood immunisation schedule. While surveillance data do not provide an accurate estimate of the true burden of Hib disease due to limitations of the reporting system, the demonstration of HibCV impact at a national level is important given the decision to provide this relatively expensive vaccine for all South African children. These findings are especially important given the high prevalence of HIV in the country. Calculated cost benefits of administering HibCV to a 1992 Cape Town birth cohort (n=46,537) were calculated at between US$ 0.8 million and US$ 1.2 million.[89]

Although the laboratory reporting system in South Africa may underestimate the incidence of Hib disease in children <5 years, we believe the decrease in Hib disease we detected is a minimum estimate of the full impact of HibCV on disease in South Africa. Before introduction of HibCV, population-based studies conducted in Soweto (Gauteng) in 1997/1998 and Cape Town (Western Cape) in 1991/1992 identified annual incidence rates of invasive Hib disease of 170 cases per 100,000 children <1 year of age.[78;81] These rates are much higher than those detected by our surveillance in these two provinces in July 1999-June 2000: 13.1 and 14.6 cases per 100,000 children <1 year. In Gauteng province, HibCV had been administered to Soweto children since 1998, and nationally our surveillance system was established at the time of HibCV introduction, so initial rates of Hib disease likely reflect some impact of immunisation. In addition, the pre-immunisation studies were performed at academic hospitals where specimens are routinely collected for diagnostic purposes and laboratories are well-equipped. We have

seen improvements in case ascertainment throughout the country, reflected in the increase in isolates of non-type b *H. influenzae* and *S. pneumoniae*. For this reason, data from the national surveillance are likely to underestimate the impact of HibCV and the 65% reduction in cases among children <1 year of age during the study period should be interpreted as a minimum estimate.[70;90]

In South Africa, national estimates suggest that 8% of 1.1 million children born in 2002 were infected with HIV at birth or through breastfeeding during the first year of life.[91] In the two provinces analysed separately, reduction of Hib disease was lower in Gauteng, where HIV seroprevalence rates are more than twice those in Western Cape. HIV infection was very common among Hib cases. More data are needed to determine the relative importance of increased susceptibility to Hib disease and lower HibCV effectiveness among HIV-infected children to the persistent low rates of Hib disease in South Africa.[78;79] Antiretroviral therapy reduces mortality and progression to AIDS in infants,[92] and studies of implementation in Africa are showing promising results.[92;93] Use of antiretrovirals in pediatric care will become more common in South Africa,[94] and monitoring of Hib disease may influence decisions to include additional doses of Hib and other vaccines for such children.[95]

We observed an increase in reported cases of non-Hib disease possibly due to improved quality of surveillance, which is supported by the increase in reported pneumococcal disease over the same time period. These diseases may however also be increasing due to increases in the number of persons living with HIV infection. Data from a small number of cases from sentinel sites showed HIV infection to be more common among paediatric cases of *H. influenzae* due to non-typeable strains, than among cases of Hib. Another explanation may be replacement disease: although it has not been significant in other parts of the world[96;97] HIV-coinfection may contribute to other strains filling the niche previously

occupied by Hib. Our data do not at present allow us to distinguish the relative contributions of these factors to the observed increase.

The reported coverage with three doses of HibCV in South Africa was less than described in developed countries,[90;98] although several limitations with regard to both numerator data and population denominators hamper accurate coverage estimates.[84;85] We were unable to obtain sufficient data to determine the true incidence of HibCV failures among our case patients. Failure after complete vaccination at 14 weeks or older was recorded for only one child. Obtaining better data on vaccination histories will be necessary to determine the role of HibCV failure in infants presenting with Hib disease.[99-101] South Africa uses an accelerated schedule for HibCV at six, 10 and 14 weeks without a booster dose,[66] which might increase disease in older children due to waning immunity.[99;102] In the last year of surveillance (2003-2004) however, only 7 of 26 cases for whom age was recorded occurred after the age at which a booster may be given (children older than 12 months).

The use of national surveillance data has several important limitations. The true burden of potentially preventable Hib disease may be significantly higher than that observed by culture-confirmed disease alone.[74-77] Calculated rates of Hib disease include only cases for which viable isolates could be confirmed as type b, and for which the age of the case-patient was known. We did not adjust for the increase in the number of reporting laboratories, or the impact of enhancements made to the surveillance system in 2003, both of which likely increased the number of Hib cases identified. Low numbers of cases from many provinces may be due to several factors, including accessibility to healthcare in rural areas, reduced numbers of blood cultures being taken per patients admitted,[103] smaller rural laboratories not having resources for diagnosis unless as part of studies[104] and underreporting of diagnosed cases by the laboratory to the surveillance unit. Although laboratory-confirmed Hib cases are notifiable to the

Department of Health in South Africa, notification for this disease is uncommon; no cases were identified through the national reporting system during the 5-year period.

Documenting the reduction of disease burden through a national laboratory-based surveillance system, especially in the face of an HIV epidemic that affects a large number of newborns, supports the maintenance of HibCV in the infant immunisation programme. Increasing antibiotic resistance among Hib isolates in our population[15;80] was an additional justification for HibCV introduction. In the future, better information about underlying conditions and HibCV status together with a carefully planned case-control study will assist decision makers and public health experts in understanding possible reasons for ongoing transmission in our children. This may be especially important with the increasing survival of young children with HIV infections due to the recent introduction of comprehensive care including antiretroviral therapy.

3.5 Conclusions

Data from a newly established national laboratory-based surveillance system showed a decrease in Hib disease burden among South African children following HibCV introduction and identified cases of non-typeable disease associated with HIV infection.

Chapter 4 Invasive disease due to *Haemophlius influenzae* serotype b ten years after routine vaccination, South Africa, 2003-2009

4.1 Introduction

In Africa, HibCV has been used since 1997 in The Gambia resulting in the near elimination of invasive Hib disease.[57] Since 2000, an increasing number of African countries have introduced HibCV with support from the Global Alliance for Vaccines and Immunisation (GAVI).[105] To date, all African countries that have introduced routine HibCV have adopted a three-dose primary immunisation schedule without a booster dose, as recommended by the World Health Organization (WHO).[52] While the three-dose schedule without a booster has been highly effective in the short term for prevention of Hib disease in African countries,[55-58] it is unclear whether a booster dose will be needed to sustain reductions in Hib disease over time. In April 2009, South Africa's Expanded Programme on Immunization (EPI) programme replaced DTwP-HibCV with a pentavalent vaccine containing diphtheria and tetanus toxoids, acellular pertussis, Hib-tetanus toxoid and inactivated polio (DTaP-Hib-IPV), given at six, 10 and 14 weeks with a booster dose at 18 months of age.

Prior to the introduction of HibCV in South Africa, population-based studies demonstrated high rates of disease in young children, with eight-fold increased risk of invasive disease among HIV-infected children.[78;81] HibCV is recommended for HIV-infected children,[52] although vaccine effectiveness among HIV-infected children not receiving antiretroviral therapy (estimated at 44% to 55%) is lower than among uninfected children (91% to 97%).[78;79] Immunologic studies have demonstrated low levels of

protective antibody in antiretroviral naïve HIV-infected children following a three-dose primary immunisation schedule.[106] This response can be boosted with an additional dose in the second year of life, suggesting benefit of a booster dose for HIV-infected children. HIV prevalence in children <5 years in South Africa in 2009 was estimated to be 4%.[107]

HibCV was introduced in South Africa in 1999. In the same year, national laboratory-based surveillance for invasive *H. influenzae* disease was established.[66] From 1999 to 2004, this surveillance demonstrated a 65% reduction in invasive Hib disease among South African children <1 year.[108] Since 2005, surveillance identified annual increases in the number of invasive Hib disease cases among children who had completed the primary infant immunisation schedule. To investigate possible explanations for this increase, we reviewed cases from 2003 through 2009, prior to the introduction of a booster dose of HibCV in the routine immunisation schedule.

4.2 Materials and Methods

4.2.1 Hib vaccination in South Africa and coverage

In July 1999, HibCV was introduced into South Africa's EPI as a three-dose series recommended at six, 10 and 14 weeks of age, without a booster dose. HibCV (consisting of polyribosylribitol phosphate (PRP) covalently bound to tetanus toxoid (PRP-T)) was administered with DTwP as part of a combination product (CombActHIB®, Sanofi Aventis).[108] HibCV was administered to children who had not yet received their first dose of DTwP, with no catch-up immunisation schedule.

We obtained vaccine coverage data (2004-2008) from the Department of Health (personal communication D Kibuka, EPI, National Department of Health). Vaccination coverage was estimated

using the administrative method by dividing the number of doses administered by the estimated target population for vaccination. Previous reports estimated coverage with three doses of DTwP-HibCV at 70% among one-year olds in 2002.[108] WHO country estimates based on surveys and projections estimated coverage for three doses of DTwP-HibCV at 69% for South Africa in 2009.[109]

4.2.2 National, active, laboratory-based surveillance for *H. influenzae* disease

Since 1999, private, public, military and mining laboratories throughout the country have reported cases of laboratory-confirmed invasive *H. influenzae* disease in children and adults to the national surveillance system.[66] Available isolates were sent to a national reference laboratory for confirmation and serotyping. Cases of invasive disease were defined as isolation of *H. influenzae* from normally sterile fluids including CSF, blood and joint fluid, or a positive latex agglutination test with one additional confirmatory test (Gram stain or PCR assay[110;111]). *H. influenzae* meningitis was defined as identification of the organism in CSF or a discharge diagnosis of meningitis in a patient with bacteraemia. Pneumonia was defined as a clinical diagnosis of lower respiratory tract infection and isolation of the bacterium from blood and/or pleural fluid specimens. Non-serotype b *H. influenzae* disease included all cases confirmed as serotype a, c, d, e, and f or non-typeable. Repeat isolates from the same patient were excluded if occurring within 21 days of the initial positive culture.

Laboratory staff notified laboratory-confirmed cases of invasive *H. influenzae* disease to a national surveillance system using standard forms including case patient's age, sex, date of specimen collection and source of isolate. Since 2003, the surveillance system became an active surveillance system including enhanced surveillance conducted at sentinel sites in each of nine provinces by dedicated

surveillance officers who frequently visited laboratories to ensure reporting of identified cases. Annual audits to verify completeness of reporting were conducted for all public-sector laboratories in eight provinces. Isolates were not available for cases identified by audit. During the seven-year period, surveillance audits suggested that more than 74% of invasive *H. influenzae* disease cases had been reported to the surveillance system. There were no methodologic changes to the surveillance programme over the study period.

Additional information collected at all sites for all Hib cases among children <5 years includes admission date, HIV infection, underlying medical conditions, vaccination history, clinical diagnosis and outcome. Dates of vaccination are recorded by hospital staff from Road-to-Health vaccination cards. When vaccination cards were not available (approximately 15% of case patients age-eligible to be vaccinated), a parent or guardian was asked if the child had received infant vaccinations and if so, the approximate ages at vaccination. HIV testing was requested by admitting physicians when clinically indicated according to standard practice.[112]

Vaccine failure was defined as invasive Hib disease in a child at least four months of age who was fully vaccinated with the last dose at least 14 days before the collection of the specimen yielding Hib. A child was considered fully vaccinated if he or she had received three doses of HibCV in the first year of life. Case patients who had not completed their primary immunisation schedule were considered partially vaccinated. Children of any age who had received no HibCV were considered as unvaccinated.

4.2.3 Laboratory methods

Laboratories throughout South Africa sent *H. influenzae* isolates to the national reference laboratory for confirmation with the γ-aminolaevulinic acid (ALA)-porphyrin test or API® NH (bioMérieux® sa, Marcy-

l'Etoile, France) and serotyping by slide agglutination using antisera for types a–f (Murex Biotech Ltd., Dartford, Kent, UK). Serotyping results for all encapsulated isolates were confirmed by conventional PCR (2003-2008)[82] or real-time PCR (2009) assays.[111] Presumed non-typeable *H. influenzae* isolates were subcultured from long-term storage at -70°C on chocolate agar and were screened by PCR using previously published methods with some modifications based on in-house validation.[111;113] Multiplex real-time PCR detected the presence of *Iga1* (to confirm *H. influenzae* identification), *BexA* (encapsulation) and serotype b-specific genes. Two additional multiplex real-time PCR assays (to detect serotypes a, c, d and e, f respectively) were carried out on isolates that were *BexA* positive but negative for serotype b. B-mutant strains (strains that have lost the ability to produce a serotype b capsule)[82;114] (n=6) were classified as serotype b for this analysis. Remaining non-typeable isolates were positive for Iga1 and negative for *BexA* and serotype b-specific genes. Beginning in 2007, PCR of transport media was used to obtain serotype information for nonviable cultures sent to the reference laboratory (yielding 5 positive reactions for Hib in 2007, 4 in 2008 and 7 in 2009).[111] These cases were excluded in analyses of trends in Hib detection rates.

4.2.4 Statistical analysis

The χ^2-test was used for comparison of categorical variables and the χ^2-test for trend was used to assess linear trends over time. Age groups were defined in months: <4 (i.e., too young to be eligible for all 3 doses of Hib), 4 to 11, 12 to 17 (i.e., not eligible for the new booster at 18 months), 18 to 36, and 37 to 59. All other variables were dichotomised (yes/no), excluding missing data. Two-sided p values of <0.05 were considered significant. In univariate analyses, we used all available case information.

Age-specific detection rates were calculated from national, laboratory-based surveillance as previously described,[108] by dividing the number of confirmed Hib cases among children <5 years of age by mid-year

population estimates obtained from Statistics South Africa.[115] According to these estimates, there were 5,051,159 children <5 years in 2003 and 5,068,886 in 2009. We managed and analysed data with Epi Info software, version 6.04d[88] and Stata Version 9 (StataCorp Limited).

To compare the observed number of vaccine failures with an expected number given assumptions about vaccine efficacy and coverage, we estimated the number of vaccine failures that would be expected from 2007 through 2009 in the population <5 years in Soweto, South Africa (which has had the longest running continuous surveillance for invasive Hib infections[78]). We assumed 95% vaccine coverage in a population of 124,000 children <5 years[115] with 5.3% HIV prevalence.[107] We used estimates of 91% effectiveness of three doses of HibCV for HIV-uninfected children and 55% effectiveness for HIV-infected children.[79] For the annual risk of invasive Hib disease among non-immune children who completed the three-dose primary immunisation series, we used pre-HibCV incidence rates of 54 cases per 100,000 children <5 years per year[81] and assumed a six-fold increased risk among HIV-infected children (i.e., 324 cases per 100,000 HIV-infected children <5 years per year).[78]

4.3 Results

4.3.1 Hib vaccination coverage in South Africa

Estimated coverage with three doses of HibCV among one-year old children in South Africa rose from 71% in 2004 to 97%, 99%, 100% and 100% during 2005-2008, respectively. The lowest estimated coverage for this 3-dose regimen estimated for any of the 9 provinces of South Africa for 2004-2008 was 64% in 2004.

4.3.2 National, active, laboratory-based surveillance for *H. influenzae* disease

From January 2003 through December 2009, a total of 2562 cases of invasive *H. influenzae* disease were reported to the national surveillance system. Among 2439 cases for which patient age was reported; 1455 (60%) occurred in children <5 years of age. Specimens or isolates were available for serotyping for 868 (60%) of the reported cases among children <5 years; of these 349 were identified as serotype b, 50 as serotype a, 80 as serotype f, 41 as other encapsulated serotypes, and 348 as unencapsulated (non-typeable) *H. influenzae*. Of 349 cases of invasive Hib disease in children <5 years, 333 (95%) viable isolates were confirmed as Hib at the national reference laboratory. Hib cases in this age group increased during the surveillance period (Figure 4.1). From 2003 to 2009, the detection rate of invasive Hib disease increased from 0.7 (95% confidence interval (CI) 0.5-1.0) to 1.3 (95% CI 1.0-1.7) cases per 100,000 children <5 years ($p<0.001$). Numbers of non-typeable and other encapsulated *H. influenzae* showed no significant increase during the period (Figure 4.1). For comparison, 10,056 cases of invasive disease due to *S. pneumoniae* in patients <5 years were reported to the same surveillance system during the period, ranging from 1261 to 1558 per year, with no significant increase during the period (Figure 4.1).

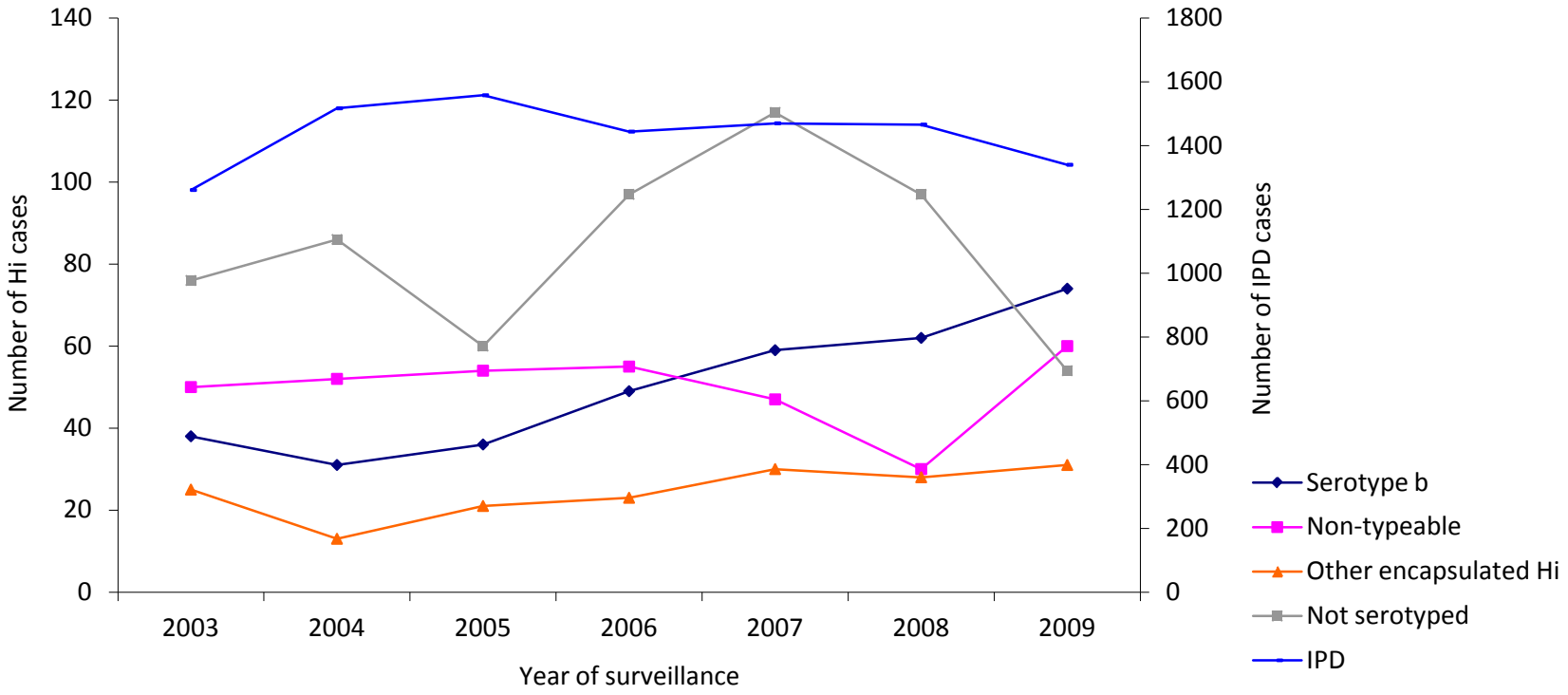

Figure 4.1 Number of reported cases of invasive *Haemophilus influenzae* (Hi) disease in children <5 years (n=1455), by serotype and year, South Africa, 2003-2009. Invasive pneumococcal disease (IPD) documented for children <5 years is depicted for the same time period.

Serotype b = *H. influenzae* serotype b; Non-typeable = non-encapsulated *H. influenzae*; other encapsulated Hi = *H. influenzae* serotypes a, c, d, e, and f

Among reported Hib case patients <5 years, 114 (33%) were 18 months of age or older and 48% were female. Syndromes included meningitis (n=211 (60%) of 349), pneumonia (90, 26%), sepsis without focus (17, 5%), arthritis (6, 2%), cellulitis (n=2), epiglottitis (n=1) and osteomyelitis (n=1), while diagnosis was not reported for 21 cases. HIV results were retrieved for 55% (191/349) of the children with Hib disease of whom 49% (93/191) were HIV infected. Hib pneumonia was relatively more common in HIV-infected children: among 191 case patients with known HIV status, 39 (42%) of 93 HIV-infected children versus 22 (22%) of 98 HIV-uninfected children had bacteraemic Hib pneumonia (p<0.004). Among 308 episodes with known outcome, 16% were fatal. CFR was 19% among 195 Hib meningitis cases versus 11% among 85 episodes of Hib pneumonia (p=0.07). CFR was 17% among 90 HIV-infected children with invasive Hib disease, 8% in 95 HIV-uninfected children and 13% in 123 children of unknown HIV status (p=0.02).

4.3.3 Vaccine failures

Determination of vaccination status improved from the first two years (31/69, 45% cases with vaccination history) to the period 2005 to 2009, when the majority of cases had vaccination status determined with no significant change (29/36, 81% in 2005 to 62/74, 84% in 2009, p=1) (Figure 4.2). Of the 349 children <5 years with Hib disease, vaccination status was determined for 263 (75%) case patients: 52 (20%) were unvaccinated and 73 (28%) were partially vaccinated. Among the 263 case patients for whom vaccination status was obtained, 138 (52%) were fully vaccinated (Figure 4.3). Of 135 invasive Hib episodes in children aged ≥4 months and vaccinated with three doses of vaccine, 74 (55%) were in children ≥18 months of age. Three children with three doses documented on vaccination records had invasive Hib disease at 3 months of age, with date of specimen collection more than 14 days after the last vaccine dose. Vaccine doses were documented on vaccination cards for 85% of children classified as having a vaccine failure. The percentage of invasive Hib disease episodes classified as

vaccine failures increased from 43% during 2003-2006 to 58% in 2007-2009 (p=0.02). Vaccine failures occurred in all race groups, were reported from all nine provinces, from the public and private sector, and no institutional clusters were identified.

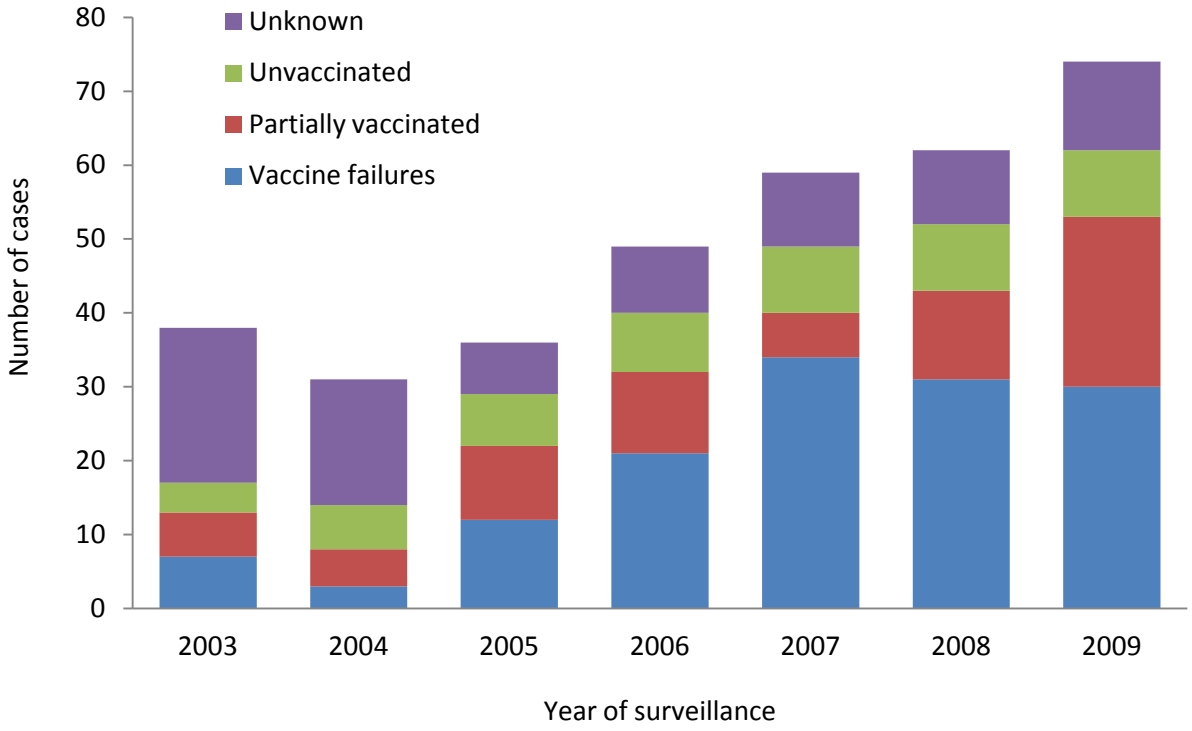

Figure 4.2 Number of children <5 years with confirmed invasive *Haemophilus influenzae* serotype b disease (n=349), by vaccination history and year, South Africa, 2003-2009

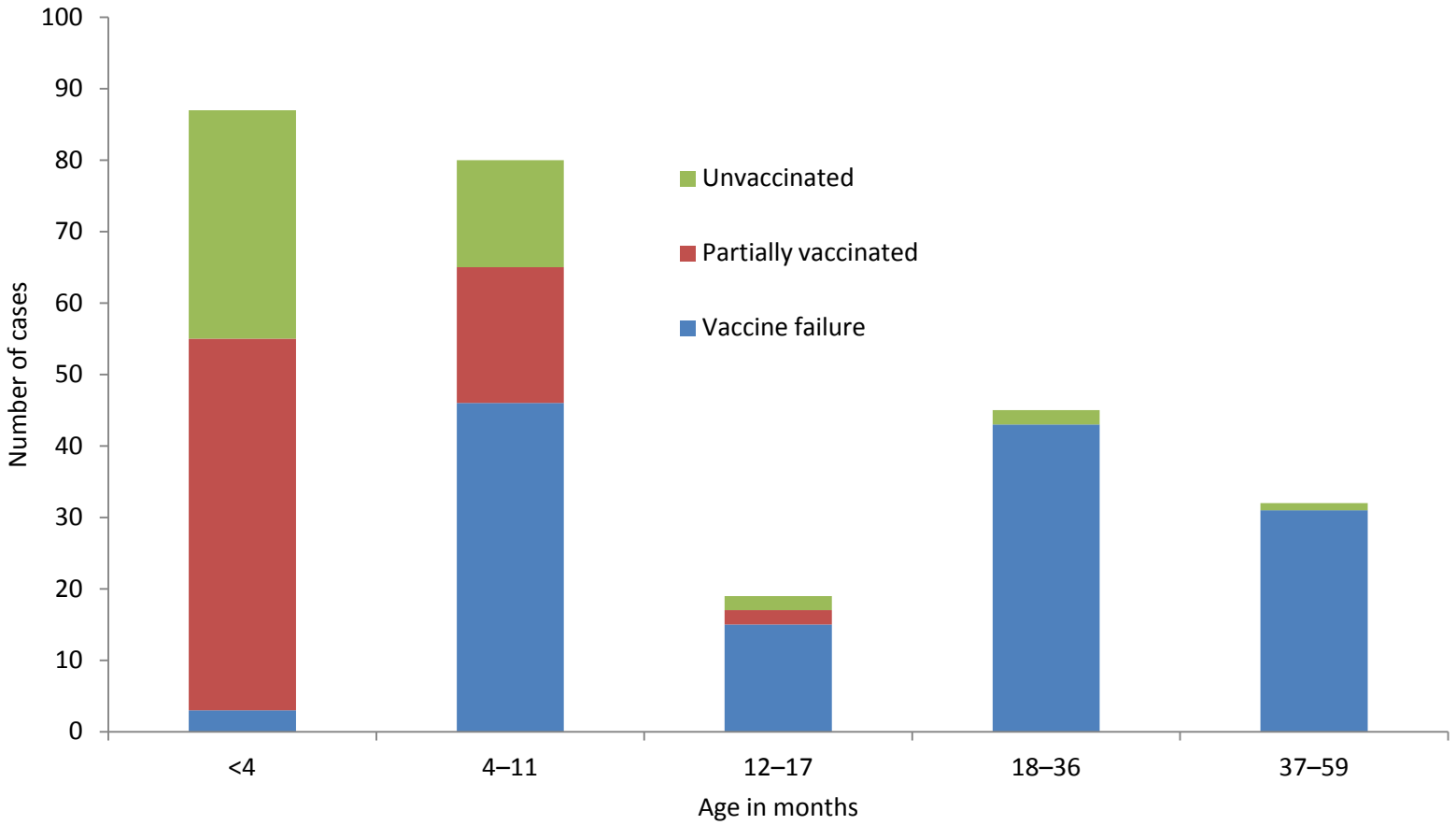

Figure 4.3 Number of children with confirmed invasive *Haemophilus influenzae* serotype b disease, reported by age and known vaccination status (n=263), South Africa, 2003-2009

Among 135 cases of vaccine failure among children at least four months of age, 50 episodes were HIV associated: 42 children with vaccine failures had documented HIV infection, 4 were HIV exposed, uninfected children and 4 were untested children in whom HIV was clinically suspected. Among 90 cases of vaccine failures in children with known HIV status, 48 (53%) occurred in HIV-uninfected children. The prevalence of HIV-infection was higher among 53 case patients ≥18 months (55%) than in 37 case patients <18 months of age (35%; p=0.05) (Figure 4.4). Among 85 cases of vaccine failure not reported to be HIV-associated, other medical conditions were documented in medical records of ten case patients, including kwashiorkor (n=5), valvular heart disease with Down's syndrome (n=1), severe burns requiring intensive care admission (n=1), acute leukaemia (n=1), cerebral palsy (n=1) and unspecified immunotherapy (n=1).

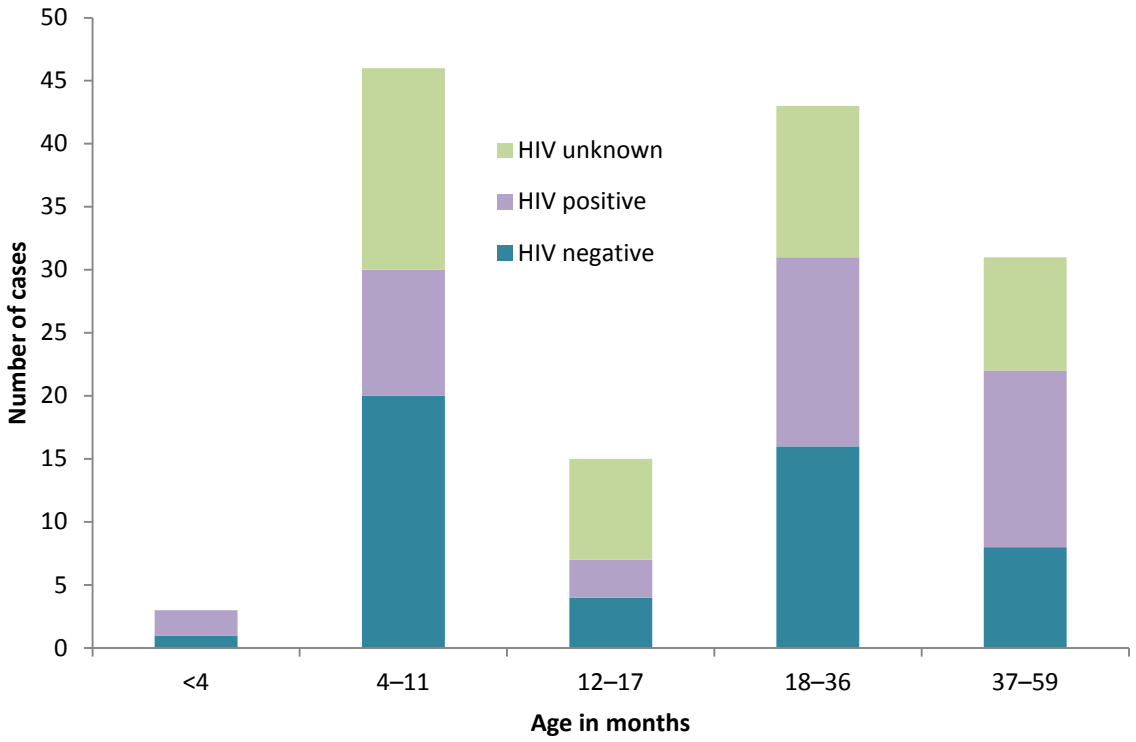

Figure 4.4 Number of *Haemophilus influenzae* serotype b vaccine failures (n=138), by age and HIV infection, South Africa, 2003-2009

4.3.4 Observed versus expected number of HibCV failures

Based on our assumptions, we would have expected 39 vaccine failures to occur in the Soweto population <5 years of age from 2007 through 2009, with 33 (85%) of these occurring among HIV-infected children. Surveillance detected 23 cases of invasive Hib disease in children <5 years from Soweto, 10 of whom were fully vaccinated and classified as vaccine failures, 6 cases were unvaccinated, 3 were partially vaccinated and 4 had unknown vaccination histories. Among the cases of vaccine failure with known HIV status, 38% (3/8) were HIV-uninfected and 63% (5/8) were HIV-infected. While the observed number of HibCV failures in this surveillance area was lower than expected, the proportion of vaccine failures in HIV-uninfected children (38%) was higher than predicted.

4.4 Discussion

HibCV has almost eliminated disease in many countries worldwide, including in Africa, in which they are routinely used.[53-57] Several years after the introduction of a three-dose primary immunisation schedule of HibCV in South Africa, we observed increasing numbers of cases of invasive Hib disease. In this study, 55% of vaccine failures occurred in children 18 months of age and older, an age group that may benefit directly from the booster dose. In 2010, South African children began receiving a booster dose of HibCV as part of the new pentavalent vaccine. While the decision was prompted by the introduction of inactivated polio vaccine, the addition of a booster dose has the potential to substantially reduce the number of HibCV failures in South African children.

Addition of a booster dose may also benefit children who are unvaccinated or incompletely vaccinated against Hib by providing an additional opportunity for vaccination in the second year of life. HibCV prevents nasopharyngeal carriage, the precursor to invasive infection,[116] and widespread vaccine use

may indirectly protect children too young to be completely vaccinated by reducing the pool of infected and colonised individuals who can transmit the disease in the population.[116] Conjugate vaccines may have a greater effect on carriage only after the booster vaccination,[117-119] and booster doses may be required to maximise indirect effects.

This study was prompted by detection of an increasing number of vaccine failures through national, laboratory-based surveillance several years after HibCV introduction. The observation that a substantial proportion of vaccine failures occurred among HIV-uninfected children led to concerns that waning protection and lack of a booster dose were contributing to a true increase in the incidence of Hib disease, as observed in the UK.[120] The schedule in the UK starts at 2 months of age and Hib vaccination was introduced with a catch-up campaign,[100;120] but, similar to South Africa, infant vaccines are given in an accelerated schedule at 4-weekly intervals. The UK has a negligible HIV prevalence in children. Possible reasons for the UK increase included a decrease in indirect protection after the impact of the catch-up campaign waned,[100] a decline in effectiveness of the vaccine in children vaccinated in infancy,[99]and a change to a less immunogenic combination vaccine containing acellular pertussis antigen offered to children.[121;122] A booster dose of HibCV was added to the UK immunisation schedule in 2003, and resulted in reductions in Hib disease in children and adults.[120] In South Africa, limited effects on nasopharyngeal carriage of Hib from HibCV given to infants in an early, accelerated schedule may be contributing to ongoing disease transmission. Other increases in Hib disease have been described in countries with[123] and without[124] booster dose schedules, however these increases were not sustained.

For countries with high HIV prevalence among children, this study adds to available data on the risk of vaccine failures in HIV-infected children. HIV-infected children have an increased risk of Hib disease and this may persist beyond the usual high risk age group of <1 year.[106] In addition, conjugate vaccine-

induced protection may wane more rapidly in HIV-infected children.[125] A previous study in a cohort of South African children estimated that HIV-infected children not on antiretroviral therapy had a 30-fold higher risk of vaccine failure than uninfected children.[79] In this study, the occurrence of vaccine failures among HIV-infected children 2 to 4 years of age suggested the potential benefit of a booster dose at 18 months of age. In our study, more than half of vaccine failures occurred in children 18 months of age or older and of these more than half were HIV uninfected. This contrasts with a national estimate of 4% HIV prevalence among children <5 years during the study period.[107] Surprisingly, long-term data from South Africa are unique in this regard: few or no vaccine failures have been identified in other populations with high HIV prevalence following HibCV introduction.[126] Continued surveillance for HibCV vaccine failures in HIV-infected children in South Africa is especially important to evaluate the impact of a booster dose.

In a setting of high coverage with a vaccine that has 90% to 95% efficacy, a substantial proportion of cases (50% to 60%) are expected to occur in fully vaccinated children.[127] Following introduction of a new vaccine, identification of vaccine failures will increase as more children are fully vaccinated. Projections of expected numbers of vaccine failures in HIV-infected and uninfected children provided some reassurance that the observed number of vaccine failures in HIV-infected children was lower than expected. This could reflect a lack of sensitivity of our system to detect these cases (specimen-taking practices may differ by clinical syndrome or HIV infection) or effects of antiretroviral therapy on incidence of disease or vaccine-induced immunity.[78;106;128] In addition, we used pre-HibCV rates of Hib disease to estimate the expected number of vaccine failures. However, following Hib vaccine introduction, the carriage of Hib in the community likely declined and indirect effects would offer some degree of protection to those who did not mount a proper immune response to the vaccine. Therefore the calculation of expected vaccine failures is likely an overestimate and may help explain the disparity

between the expected and observed HibCV failures. Although we believe the WHO coverage estimates to more closely reflect reality, using a higher coverage estimate ensured that we did not underestimate the projected number of vaccine failures. Despite limitations, similar projections may be useful in settings where cases of vaccine-preventable diseases occur in fully vaccinated children.

The clinical presentation and CFR of invasive Hib disease after vaccine introduction were similar to pre-vaccine cases.[81] The predominance of meningitis likely represents the infrequent use of blood culture in children with pneumonia in South Africa. Bacteraemic pneumonia is more commonly identified in HIV-infected children, which may explain the association between pneumonia and HIV infection in identified Hib cases.[78] The prevalence of HIV among invasive Hib cases in South Africa is higher than reported from other countries with high HIV prevalence such as Malawi,[126] potentially due to differences by clinical syndrome or longer survival of HIV-infected children in South Africa.

Additional effects of the introduction of routine Hib vaccination may be replacement due to other encapsulated or non-typeable (unencapsulated) *H. influenzae*.[129] Initial reports of possible serotype replacement[97;130;131] were not confirmed with ongoing surveillance.[132-134] Where increases of non-Hib disease have been documented,[134-136] ascribing causality to vaccine introduction has been difficult.[43] No sustained increase of non-b *H. influenzae* was identified by our surveillance during the first 10 years of Hib vaccination, both in children (this study) and in adults (data not shown).

Our study has several limitations. Although other diseases reported to our surveillance system did not increase over the same time period, suggesting no major shifts in the surveillance system, it is possible that a proportion of the increase in Hib cases may have resulted from better implementation of the *H. influenzae* surveillance. Data in medical records, including vaccination histories, were not always

complete, and results of HIV testing were not always available. Parental report of vaccination may be unreliable and was the source of 15% of vaccination histories, suggesting that some vaccine failures may not have been fully vaccinated. As ascertainment of vaccination histories improved over the study period, we were also more likely to identify vaccine failures over time. Immune deficiencies, chronic conditions, exposure to HIV, access to antiretroviral drugs and other risk factors were not systematically evaluated by attending physicians and may not have been recorded. The effect of antiretroviral therapy or co-trimoxazole prophylaxis on duration of immunity following vaccination in a setting with high prevalence of HIV infection could not be addressed fully in this study. In addition, we provide a very conservative estimate of the residual burden of Hib disease due to under reporting within a voluntary, laboratory-based surveillance system and differences in specimen-taking practices for the investigation of pneumonia or sepsis. All of these limitations also reduce the generalisability of our results to other settings.

With decreasing incidence of >95% of invasive Hib disease among children following widespread vaccination, remaining cases of Hib disease are of concern, especially when disease affects fully immunised children. In the absence of a booster dose our data suggest that Hib vaccination in a setting of high HIV seroprevalence may not lead to a sustained reduction in Hib disease. This is likely due to a combination of increased risk of Hib in HIV-infected children and waning immunity in this group. Well-designed carriage and/or seroprevalence studies may assist in understanding these observations. However, more than half of all vaccine failures in children aged ≥18 months were among HIV-uninfected children. There may be implications of this study for the addition of a booster dose of HibCV in EPI programmes in other countries, however the prevailing epidemiology of Hib will determine whether or not a booster will be required, the number of booster doses and their appropriate timing. Additional doses of Hib vaccine will have several logistical challenges, including cost implications, choice between

combination vaccines compared to Hib-only vaccines and loss of coverage as vaccines are given to older children.[106] In addition, well-designed studies are needed to address questions of optimal dosing schedules for Hib vaccination in resource-poor and high-HIV-prevalence settings. A booster dose of HibCV added to South Africa's routine childhood immunisation schedule was not primarily intended to prevent Hib disease. However, the introduction of a HibCV booster will likely prevent a substantial proportion of vaccine failures in children who have completed their primary immunisation series, and may provide benefits to unvaccinated and partially-vaccinated children. Impact of the addition of a booster dose on invasive Hib disease in HIV-infected and uninfected children will be monitored.

4.5 Conclusions

Vaccine failures, which occurred in both HIV-infected and -uninfected children, comprised half of the rise in invasive Hib disease detected in South African children 10 years after national introduction of Hib vaccine. These findings suggest that HibCV recommendations may require revision. In November 2010, children in South Africa began receiving a booster dose of HibCV as part of a pentavalent vaccine.

Chapter 5 Emergence of endemic serogroup W135 meningococcal disease associated with a high mortality rate in South Africa

5.1 Introduction

Meningococcal disease remains an important cause of meningitis and sepsis worldwide.[7;11;137;138] Since the early 1970s polysaccharide meningococcal vaccines have been used in industrialised countries and in outbreak settings throughout the world.[139] Recent licensure of conjugate vaccines offers new scope for prevention of disease.[140;141]

Significant prevention challenges still exist for Africa. The African meningitis belt extends from Ethiopia to Senegal and has cyclical epidemics occurring every 5 to 10 years resulting in attack rates of 1000/100,000 or higher.[142] Historically serogroup A disease has been the most common serogroup causing disease in this region.[142] An international outbreak of meningococcal W135 disease in Hajj pilgrims in 2000 and 2001 highlighted the importance of this serogroup.[143-147] Cases of W135 disease were also identified in Burkina Faso in 2001,[148] during an epidemic in the same country in 2002,[149] and in other countries in the belt.[150;151] The causative isolates were characterised as a single clone belonging to the electrophoretic type (ET)-37 complex, ST-11.[146;152]

South Africa does not fall within the meningitis belt, and for many decades has documented endemic meningococcal disease, with seasonal increases during the winter and spring months (May – October).[153] The burden of disease shows a cyclical pattern at intervals of approximately 8 to 10 years.[154]

Incidence of clinical notifications to the Department of Health have declined since the late 1980s, and for the period 1992 to 1997 were between 1 to 2/100,000.[154]

Two provinces have historically been responsible for highest rates of disease and typically had disease due to specific serogroups: serogroup B in Western Cape[155;156] and serogroup A in Gauteng.[157-159] Western Cape has a Mediterranean climate with wet winters and hot, dry summers and Gauteng lies on a plateau and has a temperate climate with summer rainfall.[160]

Most South Africans have good access to health care with 92% of all births assisted by trained health personnel.[161] This ranged from 85% in the Eastern Cape to 93.3% in Western Cape Province and 95.2% in Gauteng Province. In South Africa, it is standard practice to refer all cases of suspected meningitis to hospital and to obtain CSF specimens for laboratory processing. Third-generation cephalosporins are available in hospitals and clinics for empiric treatment of acute bacterial meningitis.[162] No routine or large-scale use of meningococcal vaccines is standard in South Africa.

From 1999 to 2002, serogroup W135 caused <10% of meningococcal disease in South Africa.[159] In this report we describe the replacement of serogroup A disease in Gauteng Province, South Africa, with a hypervirulent clone of W135 causing increased incidence of disease in young infants and higher CFR.

5.2 Materials and Methods

5.2.1 National meningococcal surveillance

Annual laboratory audits and a review of laboratory-confirmed cases reported by clinicians to the Department of Health were performed to identify cases not captured by routine surveillance. Cases

found on audit (up to 10-20% of annual totals) were included, although no associated isolates were available for testing.

5.2.2 Case definition

Cases were defined as residents of South Africa with *N. meningitidis* isolated from normally sterile site specimens (*e.g.*, CSF, blood, joint fluid) from January 2000 through December 2005. In addition, from 2003 the case definition was broadened to include patients with culture-negative specimens that tested positive by latex agglutination and Gram stain microscopy, or by latex agglutination and PCR. Laboratory-confirmed meningococcal meningitis was defined as growth of *N. meningitidis* from CSF (with or without growth from blood cultures). Laboratory-confirmed meningococcaemia was defined as growth of *N. meningitidis* from blood cultures, without growth from CSF.

5.2.3 Strain characterisation

Bacteria were identified according to standardised procedures.[163] Serogroup was determined by slide agglutination using polyclonal antibodies to capsular polysaccharides A, C, X, Y, Z, and W135, and monoclonal to polysaccharide B (Remel, Biotech Limited, Dartford, England). Strains not reacting with these antibodies were sent to the WHO Collaborating Center for Reference and Research on Meningococci, Oslo, Norway; or the Meningitis Laboratory, National Center for Immunisation and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, United States (US), for serogrouping. Two serogroup W135 strains related to the international outbreak in 2000 were obtained from the former institution.[147] Three culture-negative cases diagnosed by latex agglutination had CSF available for serogroup confirmation using PCR.[164] Minimum inhibitory concentrations (MIC) were

determined using Etest® (AB-Biodisk, Solna, Sweden) and broth microdilution methodology, and interpreted using breakpoints recommended by CLSI guidelines.[165]

Pulsed-field gel electrophoresis (PFGE) of *Nhe*I restriction enzyme-digested genomic DNA was performed using a method adapted from Popovic et al.[166] A PFGE cluster was defined as more than three isolates sharing ≥80% similarity on the dendrogram.[166;167] Multilocus sequence typing (MLST) was performed as described by Maiden et al.[168] ST was assigned through the Neisseria MLST website (http://pubmlst.org/ neisseria/).

5.2.4 Incidence

We calculated incidence based on the number of laboratory-confirmed cases reported each year from January 1 through December 31, divided by mid-year population estimates for each year supplied by Statistics South Africa (StatsSA). In 2005, the estimated population of South Africa was 47 million: 9 million in Gauteng Province. Serogroup-specific disease rates for Gauteng and Western Cape provinces were calculated assuming that the distribution of serogroups for cases with missing serogroup data (22% and 32% of cases respectively) was the same as the distribution for cases with serogroup information available.[45] Availability of serogroup data was unlikely to differ by serogroup or epidemiologic features of cases.

5.2.5 Statistical analysis

The χ^2-test for trend was used to assess linear trends over time. Serogroup W135 disease was compared to serogroup A disease alone, as this is a serogroup with specific epidemiological features and a history of predominance in Gauteng. Other serogroups in the province did not vary over time and caused

minimal disease. Univariate assessment of characteristics associated with disease due to serogroup W135 infection and disease resulting in death was performed using the Fisher's exact or Mantel-Haenszel tests for categorical variables, and the Kruskal-Wallis test for continuous variables. Multivariable analyses were limited to cases from Gauteng between 2003 and 2005. Variables available for evaluation as potential risk factors included age group, gender, year of infection, syndrome (laboratory-confirmed meningitis vs. meningococcaemia), HIV infection and non-susceptibility to penicillin. Multivariable logistic regression models were evaluated starting with all variables that were significant at $p<0.1$ on univariate analysis and dropping nonsignificant factors with stepwise backward selection. Independence of data was assumed because the vast majority of cases were considered to be unrelated. All two way interactions in the final multivariable model were evaluated. Univariate and multivariable analysis was performed with Epi Info software, version 6.04d, and Stata version 9 (StataCorp Limited). Two-sided p values of <0.05 were considered significant throughout.

5.3 Results

5.3.1 Trend over time

From 2000 through 2005, 2135 cases of laboratory-confirmed invasive meningococcal disease were reported to the national surveillance system. Cases occurred throughout the year and incidence increased seasonally during the winter and spring months. Rates of disease reported increased from 0.54/100,000 population in 2000 to 0.80/100,000 in 2001 ($p<0.001$) (Table 5.1). In 2005, the incidence increased to 1.16/100,000. The majority of disease nationally over the six years was reported from two provinces, Gauteng (1113/2135 or 52%) and Western Cape (490/2135 or 23%). Rates of disease in Gauteng increased overall from 0.81/100,000 in 2000 to 3.98/100,000 in 2005 ($p<0.001$) (Figure 5.1). Western Cape reported a gradual decline in rates during the same period (2.48/100,000 in 2000 to

1.51/100,000 in 2005, $p<0.001$) (Figure 5.2). The majority of nationally reported cases was laboratory-confirmed meningitis (1667/2135 or 78%); 244 of these cases were culture-positive on both CSF and blood specimens. Twenty-two percent (464/2135) of cases presented as meningococcaemia alone and four were diagnosed from joint fluids. Since 2003, 46 culture-negative cases fulfilling the surveillance case definition were identified: 25 cases in 2003, 11 in 2004 and 10 in 2005.

Table 5.1 Number and incidence of laboratory-confirmed invasive meningococcal cases in South Africa reported for 2000 through 2005, by serogroup

Serogroup	Year of surveillance						
	2000	2001	2002	2003	2004	2005	Total
	n (%[a])						
A	21 (11)	69 (30)	58 (30)	87 (33)	57 (20)	24 (6)	316
B	103 (52)	71 (31)	47 (24)	76 (29)	62 (22)	58 (14)	417
C	17 (9)	16 (7)	20 (10)	31 (12)	31 (11)	21 (5)	136
W135	10 (5)	13 (6)	24 (13)	26 (10)	77 (27)	257 (62)	407
X	4 (2)	2 (1)	1 (1)	1 (0.4)	1 (0.4)	2 (0.5)	11
Y	41 (21)	59 (26)	41 (21)	43 (16)	53 (19)	52 (13)	289
Z	2 (1)	-	-	2 (1)	-	-	4
29E	-[b]	1 (0.4)	-	-	-	-	1
Non-groupable	-	-	1 (1)	-	-	-	1
No isolate available	40 (17[c])	125 (35)	77 (29)	102 (28)	79 (22)	130 (24)	553
Total cases reported	238	356	269	368	360	544	2135
Annual incidence (cases/100,000 population)	0.54	0.80	0.59	0.80	0.78	1.16	NA

[a]Percentage of total isolates tested;

[b]No cases reported;

[c]Percentage of all cases reported;

NA, not applicable.

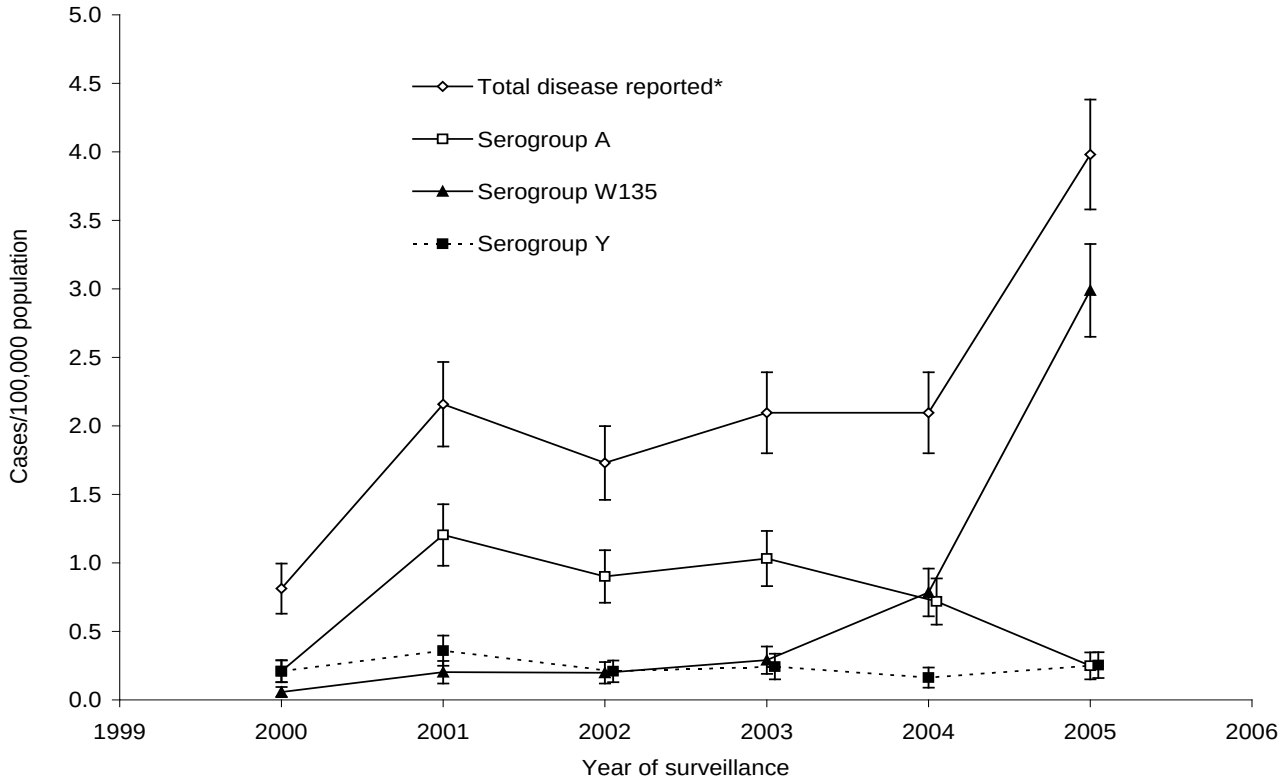

Figure 5.1 Incidence of laboratory-confirmed invasive meningococcal disease in Gauteng Province, South Africa, as reported (2000 – 2005), by serogroup

*Serogroup-specific disease rates were calculated assuming that the distribution of serogroups for cases with missing serogroup data (n=10 (16% of reported cases), 68 (38%), 40 (27%), 33 (18%), 31 (17%) and 64 (18%) for each year respectively) was the same as the distribution for cases with serogroup information available.

Note: Error bars=95% confidence intervals (CI); values offset on the x-axis so that 95% CIs are visible.

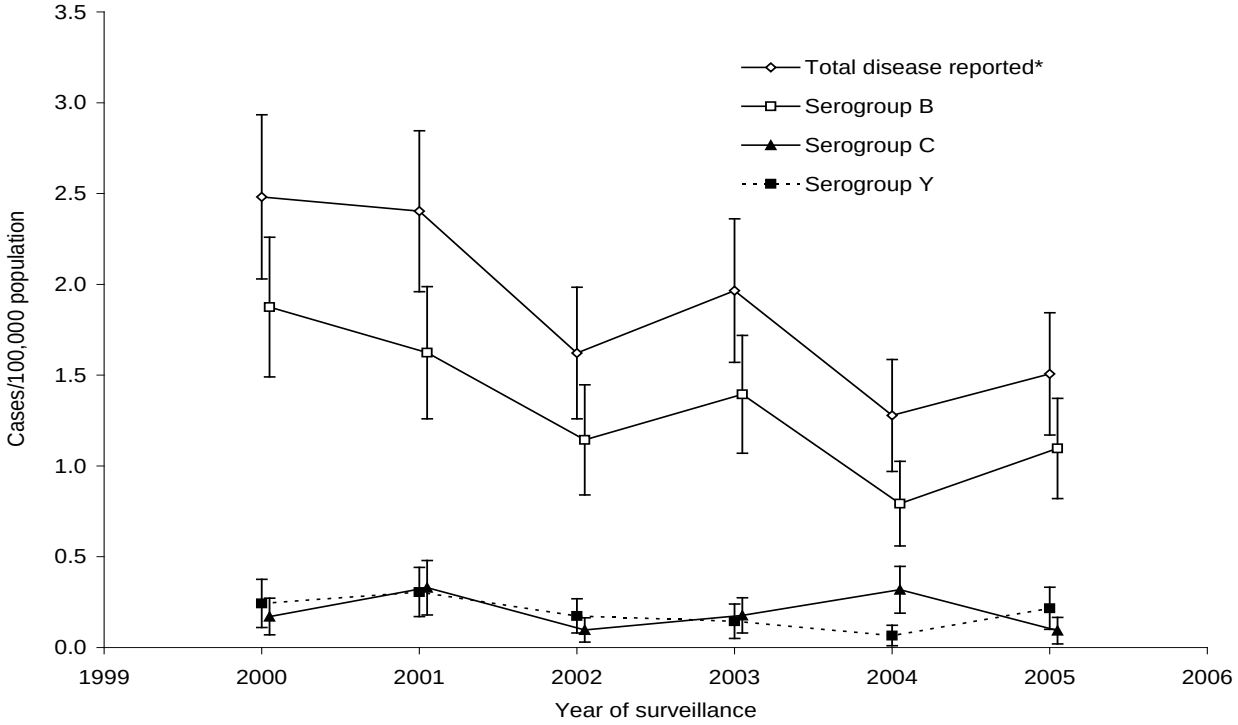

Figure 5.2 Incidence of laboratory-confirmed invasive meningococcal disease in Western Cape Province, South Africa, as reported (2000 – 2005) by serogroup

*Serogroup-specific disease rates were calculated assuming that the distribution of serogroups for cases with missing serogroup data (n=13 (13% of total reported), 34 (34%), 28 (40%), 35 (40%), 15 (26%), and 32 (46%) for each year respectively) was the same as the distribution for cases with serogroup information available.

Note: Error bars=95% confidence intervals (CI); values offset on the x-axis so that 95% CIs are visible.

For all cases from 2000 to 2005, 1582 (74%) had isolates available for serogrouping and other characterisation (Table 5.1). The percentage of disease caused by serogroup W135 increased from 5% (10/198) in 2000 to 62% (257/414) in 2005 ($p<0.001$). The majority of these cases (326/407, 80%) was reported from Gauteng, where the percentage of disease due to serogroup W135 increased from 7% (4/54) of cases in 2000 to 75% (221/295) in the last year. The incidence of W135 disease increased in this province from 0.06/100,000 in 2000 to 2.99/100,000 in 2005 ($p<0.001$) (Figure 5.1). During the same period the incidence of serogroup A disease in Gauteng was lowest in 2000 (0.21/100,000) and increased to approximately 1/100,000 during 2001 to 2003; while rates decreased to 0.25/100,000 in 2005 ($p<0.001$ for 2003-2005). From 2000 through 2003, 48% (201/418) of disease was due to serogroup A, declining to 6% (18/295) in 2005. The incidence of serogroup Y disease remained stable. In Western Cape, the overall incidence of meningococcal disease decreased over time ($p<0.001$): this was mainly due to a decline in the incidence of serogroup B disease ($p<0.001$) (Figure 5.2). The incidence of disease due to serogroup Y and C remained stable.

During the six years analysed, 6% (92/1579) of meningococcal isolates were penicillin non-susceptible (highest MIC was 0.25µg/ml). Percentage of non-susceptible isolates fluctuated by year, from 5/198 or 3% in 2000, to a high of 13% or 33/264 in 2003. Children younger than five years accounted for 51% (45/88) of penicillin non-susceptible isolates and 45% (612/1369) of susceptible isolates (p=0.24). Penicillin non-susceptible isolates were reported from all provinces, and occurred in all serogroups: 7/316, 2% of serogroup A; 28/417, 7% of serogroup B; 9/134, 7% of serogroup C; 25/406, 6% of serogroup W135; and 23/289, 8% of serogroup Y isolates. All isolates tested (n=1578) were susceptible to ceftriaxone, chloramphenicol, and ciprofloxacin. Six isolates were resistant to rifampin.

5.3.2 Characterisation of serogroup W135 isolates

PFGE results were available for 377/406 (93%) serogroup W135 isolates, of which 20 randomly selected strains were further characterised by MLST. The isolates were highly clonal by PFGE with one distinct cluster (Cluster-1) representing 93% (350/377) of all isolates. Eighty percent of isolates within this cluster were indistinguishable or differed by a single band from the pattern demonstrated by two Hajj-outbreak isolates from 2000 (data not shown).[147] All 13 selected isolates from this cluster were ST-11, of the ST-11/ET-37 complex. The percentage of isolates within Cluster-1 increased from the first year, remaining high in the subsequent five years: 5/10 (50%), 12/13 (92%), 18/22 (82%), 19/24 (79%), 70/76 (92%), and 226/232 (97%) for each year respectively, and followed a seasonal pattern (Figure 5.3). The majority of isolates from Cluster-1 were from Gauteng Province (285/350, 81%), although isolates were identified in all nine provinces. In provinces excluding Gauteng, 65 of 75 (87%) serogroup W135 with PFGE results belonged to this cluster, while in Gauteng Province, 95% (285/301) of W135 PFGE patterns were identified as cluster 1 (p<0.001). Seven representative strains from Gauteng Province belonged to the ST-11/ET-37 complex.

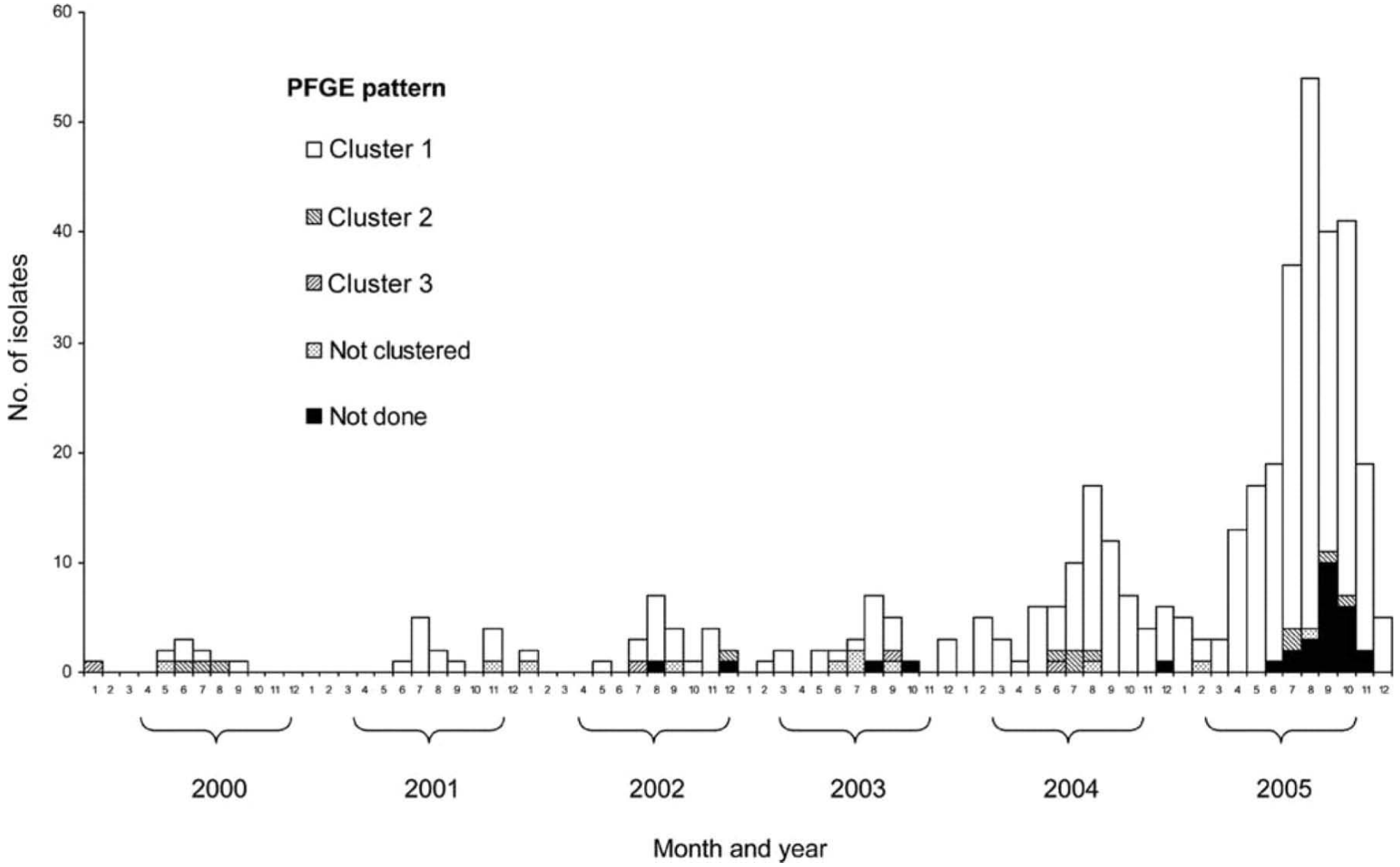

Figure 5.3 Serogroup W135 isolates (n=406) causing invasive meningococcal disease in South Africa by pulsed-field gel electrophoresis (PFGE) pattern and year, 2000-2005

The remaining isolates comprised one small cluster of 3% (12/377) of isolates (Cluster-2), a cluster of four isolates (1%) (Cluster-3), and 11 isolates not belonging to any cluster. One isolate from Cluster-2 was confirmed to be ST-4241 (ST-22 complex); while five non-clustered isolates were ST-175, ST-3687, ST-4079 (ST-103 complex), ST-3181 (ST-22 complex), and ST-5753 (a new ST) respectively. One additional non-clustered isolate was identified as belonging to the ST-1 complex/subgroupI/II.

5.3.3 Descriptive epidemiology of meningococcal disease in Gauteng

In Gauteng over the 6 years reviewed, 80% (889/1113) of cases were laboratory-confirmed meningitis, while 20% (221) were meningococcaemia, and 3 cases were diagnosed from positive culture from joint fluid. Sixty-two percent (658/1066) of cases occurred in males. Median age of patients with serogroup A disease (2000-2005) was 21 years (interquartile range 8-26 years), compared to 5 years (2-23 years) for W135 disease ($p<0.001$). Of those with known age in 2004, highest rates of disease for serogroup A and W135 occurred in infants <1 year of age (Figure 5.4). Eighty-five percent (39/46) of serogroup A disease occurred in persons >4 years of age, as compared to 59% (32/54) of serogroup W135 disease ($p=0.005$). The incidence of serogroup W135 increased in all ages; but most notably among in infants <1 year (5.08/100,000 in 2003 to 21.45/100,000 in 2005, $p<0.001$).

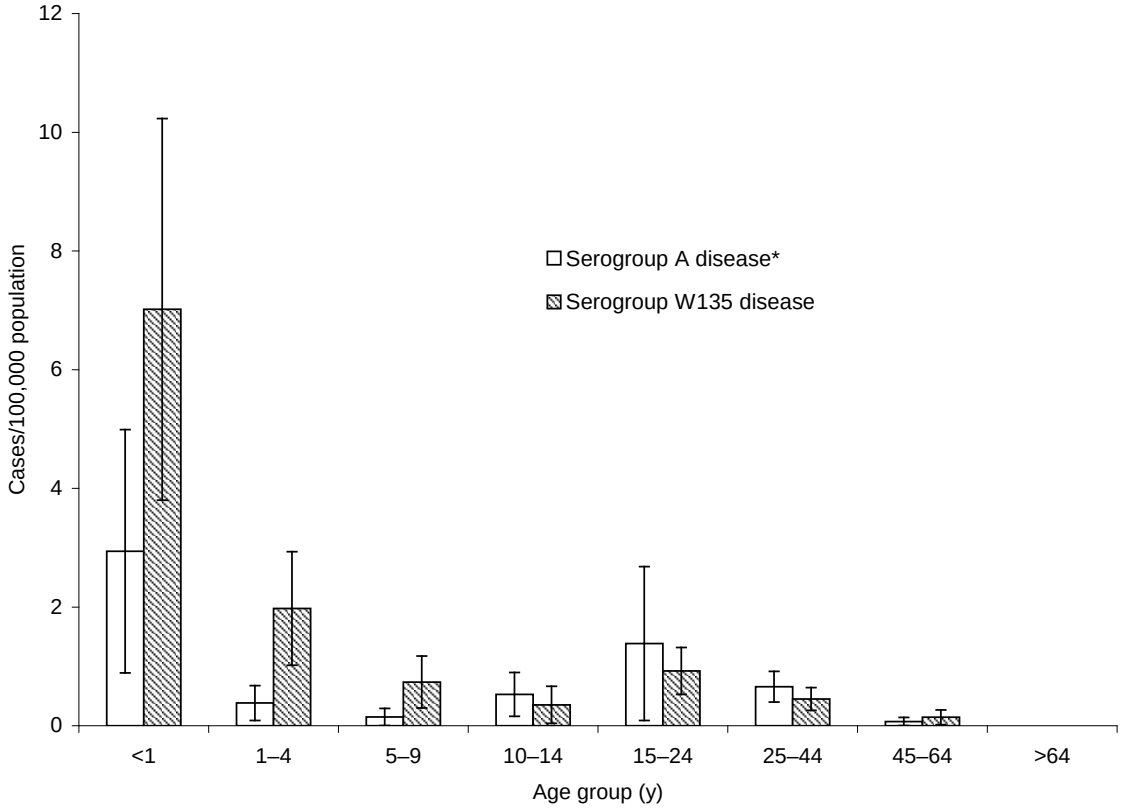

Figure 5.4 Annual age-specific incidence rates for confirmed serogroup A and W135 invasive meningococcal disease in Gauteng Province, South Africa, as reported in 2004

From 2003 through 2005, case report forms were completed on 79% (254/320) cases admitted to enhanced surveillance hospitals in Gauteng. There were no statistically significant demographic differences between cases at sentinel sites with case report forms and those without; and the only difference between cases presenting at sentinel sites in comparison to other hospitals in Gauteng was a greater percentage diagnosed from blood cultures in 2004.

When compared to serogroup A in univariate analysis, serogroup W135 was more likely to affect children <5 years of age and to cause meningococcaemia rather than meningitis (Table 5.2). In

multivariable analysis, year of collection, age <5 years and meningococcaemia were significantly associated with W135 disease.

Table 5.2 Univariate and multivariable analysis of factors associated with serogroup W135 meningococcal cases compared to serogroup A cases in Gauteng, South Africa, 2003-2005

Characteristic		Serogroup W135 (n=299)		Serogroup A (n=141)		Univariate analysis		Multivariable analysis	
		n	%	n	%	OR (95% CI)[a]	P value	OR (95% CI)	P value
Gender (male)		166/291	57	101/140	72	0.51 (0.33-0.80)	0.002		
Age group									
	<5 years	133/280	48	21/127	17	Reference	<0.001	Reference	<0.001
	5-14 years	42/280	15	11/127	9	0.60 (0.27-1.36)		0.18 (0.04-0.93)	
	15-24 years	41/280	15	47/127	37	0.13 (0.07-0.27)		0.17 (0.05-0.58)	
	25-44 years	57/280	20	45/127	35	0.20 (0.11-0.38)		0.26 (0.07-0.88)	
	>44 years	7/280	3	3/127	2	0.37 (0.09-1.56)		0.26 (0.02-4.12)	
Syndrome									
	Meningococcaemia (versus meningitis)	82/297[b]	28	11/141	8	4.51 (2.23-9.31)	<0.001	8.86 (2.16-36.28)	<0.001
HIV-coinfection		41/88	47	12/26	46	1.02 (0.42-2.46)	0.97		
Non-susceptible to penicillin		16/299	5	6/141	4	1.27 (0.49-3.33)	0.62		

Emergence of W135									
Year of infection	2003	21/299	7	72/141	51	Reference	<0.001	Reference	<0.001
	2004	57/299	19	51/141	36	3.83 (2.00-7.33)		6.15 (1.73-21.83)	
	2005	221/299	74	18/141	13	42 (16.01-110.66)		58.70 (15.53-221.76)	

[a]OR=Odds ratio; CI=confidence interval

[b]Excluded 2 cases of arthritis with culture-positive synovial fluids

CFR overall increased from 6/53 (11%) in 2003 to 32/142 (22%) in 2005 (p=0.045). Among patients with invasive serogroup A or W135 disease, factors associated with death in univariate analysis were age group 25-44 years, infection with serogroup W135 and meningococcaemia (Table 5.3). In multivariable analysis, age group and meningococcaemia were significantly associated with death; there was a marginal association between serogroup W135 infection and increased risk of dying (adjusted OR: 3.21, p=0.058).

Table 5.3 Univariate and multivariable analysis of risk factors for death in cases of serogroup W135 and A meningococcal cases at enhanced surveillance sites in Gauteng, South Africa, 2003-2005

Characteristic		Mortality (number of deaths/number of patients)		Univariate analysis		Multivariable analysis	
		n	%	OR (95% CI)[a]	P value	OR (95% CI)	P value
Gender	Female	15/76	20	Reference	0.81		
	Male	20/109	18	0.91 (0.43-1.92)			
Age group	<5 years	8/72	11	Reference	<0.001	Reference	0.002
	5-14 years	5/25	20	2.0 (0.59-6.81)		0.99 (0.25-3.90)	
	15-24 years	5/38	13	1.21 (0.37-4.0)		2.73 (0.69-10.87)	
	25-44 years	17/46	37	4.69 (1.82-12.10)		5.73 (1.87-17.56)	
	>44 years	0/4	0	Undefined		Undefined	
Syndrome[b]	Meningitis	12/135	9	Reference	<0.001	Reference	<0.001
	Meningococcaemia	23/48	48	8.11 (4.02-16.31)		9.69 (3.72-25.27)	
Serogroup	A	4/50	8	Reference	0.01	Reference	0.058
	W135	31/135	23	3.42 (1.14-10.27)		3.21 (0.89-11.56)	

HIV-coinfection	HIV-seronegative	8/61	13	Reference	0.27
	HIV-seropositive	11/53	21	1.74 (0.64-4.70)	
Susceptibility to penicillin	Susceptible	32/177	18	Reference	0.17
	Non-susceptible	3/8	38	2.72 (0.62-11.96)	
Year of infection	2003	4/30	13	Reference	0.1
	2004	5/43	12	1.67 (0.29-4.77)	
	2005	26/112	23	2.30 (0.82-6.44)	

[a]OR=Odds ratio; CI=confidence interval

[b]Excluded 2 cases of arthritis with culture positive synovial fluids

All cases reported to the surveillance network were considered sporadic, with the exception of a cluster of cases in 2005. These cases were part of an institutional outbreak in an overcrowded residence for young adults identified in Gauteng, involving 13 laboratory-confirmed cases over a period of eight months, of which 4 cases had isolates available for serogrouping. All were confirmed as serogroup W135. No other geographic clustering or epidemiologically linked cases were identified.

5.4 Discussion

The rate of invasive meningococcal disease in Gauteng Province doubled from 2003 to 2005. This increase was associated with a decline in serogroup A and the emergence of a clone of serogroup W135. Selected isolates of this clone were confirmed as belonging to the hypervirulent ST-11/ET-37 complex, (W)ET-37 clone, a strain that re-emerged internationally during a Hajj-related meningococcal outbreak in 2000.[152] The emergence of W135 was sustained over subsequent years.[10]

Overall CFR in Gauteng Province doubled from 2003 to 2005. We found the (W)ET-37 clone was associated with meningococcaemia, as has been described in an outbreak in Saudi Arabia, and in sporadic W135 disease in Taiwan.[169;170] In addition, we describe an increased CFR compared to serogroup A. This was of borderline statistical significance on multivariable analysis, while meningococcaemia was independently associated with serogroup W135 disease and with increased risk of death. Meningococcaemia is well described to have a higher case fatality than meningitis.[7] Previous clinical descriptions of disease due to this clone described an association with clinical severity, but not mortality.[169;170]

The increase in incidence of W135 disease, which goes beyond serogroup redistribution, is consistent with the introduction of antigenically distinct meningococci.[171-174] In outbreaks caused by new strains for

which there is little herd immunity, older persons generally are at increased risk of disease.[171;175] This was not seen in our setting, and may reflect immunity to this strain in older persons, or an age-dependent risk factor, for example, viral coinfections in infants.[7] Serogroup-specific differences in age distribution have been described, with W135 meningitis in Burkina Faso peaking in infants compared to serogroup A, which occurs in older children.[176] In contrast sporadic W135 disease in 30 patients in Taiwan was predominantly in adults.[170] Most cases in 2005 in Gauteng were presumed sporadic, with only one outbreak identified. In addition, our observations that infants were most affected, disease was seasonal, and cases occurred throughout the province, suggests that this strain has become endemic.

Although data are limited, to our knowledge, W135 has not been recognised as the predominant cause of sporadic or endemic disease in any of the countries where it has emerged, despite the potential, particularly in the African meningitis belt.[150;176] This may be due to a lack of ongoing laboratory-based surveillance in some regions. W135 has become a recognised cause of epidemic and endemic disease, and a multivalent conjugate vaccine may hold more promise than a monovalent serogroup A conjugate vaccine.[139]

It is of concern that this (W)ET-37 clone is causing severe endemic disease in South Africa. Meningococci belonging to the ST-11/ET-37 complex have been described as a hyperinvasive lineage, and serogroup C isolates belonging to this complex caused increased morbidity and mortality in Europe, Canada and the US in the 1990s, leading to a decision to introduce routine infant vaccination with the conjugate monovalent serogroup C vaccine in the UK.[173;177] It has been postulated that the (W)ET-37 clone may have emerged as a result of a capsular switch from serogroup C strains many years ago, as the clone has been identified since at least 1970.[152] The South African clone was indistinguishable by PFGE from the Hajj-related outbreak strains from 2000, suggesting the possibility of re-introduction of this strain during

the international outbreak. W135 isolates belonging to this complex, however, have been recognised in South Africa in 1986, and elsewhere in Africa at least since 1993.[146;152]

Because our laboratory-based surveillance system excludes disease diagnosed clinically without laboratory confirmation, observed rates represent a minimum estimate of the full burden of disease. However the number of reported cases in Gauteng, before the increase in 2005, are similar to published figures of clinical disease notified to the South African Department of Health, with peak years since 1977 reporting 150 to 200 cases/year.[154] The recent reduction of serogroup B disease in Western Cape Province has also been confirmed by clinical notifications.[178] The increase in reported cases without viable isolates in 2001 may in part have been due to audits identifying non-reporting laboratories and increased awareness of the surveillance network as it became established. Although the number of laboratories increased over the years, most improvements occurred before the disease changes we describe. Audits estimated that more than 80% of all laboratory-confirmed disease was reported to the network. Patients who died before presenting for care would also be missed by the surveillance network. Clinical practice and laboratory diagnostics did not change systematically over the time reviewed.

Serogroup W135 has become the predominant serogroup in Gauteng Province, and caused severe disease in infants. Other countries in Africa, especially those with serogroup A disease, would benefit from routine, inter-epidemic laboratory-based surveillance. The potential importance of W135 disease should be considered in global vaccine development strategies.

5.5 Conclusions

Serogroup W135 has become endemic in Gauteng, South Africa, causing more severe disease than the previous predominant serogroup A strain.

Chapter 6 Emergence of levofloxacin-non-susceptible pneumococci in children associated with treatment for multidrug resistant tuberculosis in South Africa

6.1 Introduction

High-level penicillin and MDR in the pneumococcus first emerged in 1977 among hospitalised children in South Africa.[36;37] Pneumococcal resistance to antibiotics is now a global problem and newer drug classes such as fluoroquinolones have become important, particularly for empiric treatment of community-acquired pneumonia.

To date fluoroquinolones are not indicated for treatment of pneumonia in children.[179] Levofloxacin-non-susceptible *S. pneumoniae* (LNSSP) invasive disease in adults has been reported. The incidence remains low in North America (<3%), while higher prevalence was described for other countries, such as Spain and Hong Kong.[180] Factors associated with LNSSP disease include prior exposure to fluoroquinolones, chronic obstructive pulmonary disease, and nosocomial origin of the bacteria.[181-184] Levofloxacin treatment failures of pneumococcal pneumonia have been described.[185]

Invasive LNSSP disease among children has been confined to two case reports, one from South Africa.[186;187] However, fluoroquinonlones (ofloxacin, ciprofloxacin, levofloxacin and moxifloxacin) are now used globally for treatment of MDR TB in children and adults,[188] and the evaluation of moxifloxacin for treatment of drug susceptible TB is ongoing.[189] Resistance to fluoroquinolones in *Mycobacterium*

tuberculosis led to the recent finding of extensively drug resistant TB (XDR-TB) in a nosocomial setting in South Africa.[190]

We used national, laboratory-based surveillance for IPD to describe the emergence of invasive LNSSP disease in children in South Africa. To investigate transmission dynamics, we also determined the prevalence and risk factors for paediatric nasopharyngeal carriage of LNSSP at two TB hospitals where LNSSP IPD was detected.

6.2 Materials and Methods

6.2.1 Invasive disease surveillance

Cases were defined as patients with *S. pneumoniae* identified in normally sterile site specimens (*e.g.*, CSF, blood, joint fluid) from January 2000 to December 2006. Laboratory-confirmed meningitis was defined as growth of pneumococci from CSF. Cases of LNSSP disease were defined as IPD caused by pneumococci with levofloxacin minimum inhibitory concentrations (MICs) ≥ 4 μg /ml. Children were defined as <15 years of age. We calculated incidence rates for 2006 from January 1 through December 31, using mid-year population estimates for South Africa in 2006 (47,386,792 persons, including 15,157,745 persons <15 years of age) supplied by Statistics South Africa.

6.2.2 Carriage study

We conducted cross-sectional carriage studies at two MDR TB hospitals associated with invasive LNSSP disease (hospital A in Gauteng Province, and hospital B in Western Cape Province). Individuals admitted for >48 hours were eligible for participation. The carriage study at hospital A included all adult and paediatric patients and was conducted during 4 days in August 2006. Based on the finding of no

pneumococcal carriage in adults from hospital A, we modified our protocol for hospital B to swab only children <15 years in May 2007. We collected the following information on participants: demographics; current admission clinical details; TB therapy history; recent antibiotic use; HIV status; antiretroviral use; other immunocompromising conditions; and previous hospitalisations or clinic visits in the past 3 months.

Nasopharyngeal dacron swabs were placed in skim milk-tryptone-glucose-glycerin (STGG),[191] and transported to the laboratory within 4 hours of sampling; long-term storage was at -70°C. Pneumococci were isolated using standard procedures.[191;192]

6.2.3 Susceptibility testing and serotyping

All isolates were screened for fluoroquinolone resistance with 5 µg ofloxacin disks (Mast Diagnostics, Merseyside, UK), and if resistant, ofloxacin, levofloxacin and moxifloxacin MICs were performed using broth microdilution.[165] Isolates were screened for resistance by disk diffusion (Mast Diagnostics, Merseyside, UK).[165] MICs were determined for resistant isolates using agar dilutions or Etest® (AB-Biodisk, Solna, Sweden). Results were interpreted as susceptible or non-susceptible (intermediately resistant and resistant).[165] Pneumococcal MDR was defined as non-susceptibility to three or more antimicrobial classes. Pneumococci were serotyped by the Quellung method using specific antisera (Statens Serum Institut, Copenhagen, Denmark). Isolates were further characterised by PFGE according to previously described methods.[193;194] Isolates were defined as related if they shared ≥80% similarity on the dendrogram.

6.2.4 Statistical analysis

Univariate analysis was performed using the Fisher's exact or Mantel-Haenszel tests for categorical variables, and the Kruskal-Wallis test for continuous variables. Analysis was performed with Epi Info software, version 6.04d[88] and Stata version 9 (StataCorp Limited). Two-sided p values of <0.05 were considered significant throughout. An inherent property of national surveillance systems is the potential for incomplete information. For each univariate analysis, we used all available case information. Age was described as a continuous variable defined as years of age. All other variables are binary (yes/no) defined as the presence or absence of the attribute excluding missing data.

6.3 Results

6.3.1 Invasive disease surveillance

From January 2000 through December 2006, 21,521 cases of IPD were identified; 90% (19,404/21,521) had isolates available for testing. Of 19,572 cases with known age, 44% (8692) were in children. In 2006, the incidence of IPD in children <15 years was 10/100,000 population. Of all cases in children, 93% (8052/8692) had viable isolates for further testing.

Over the 7-year period, 22 (0.1%) of 19,404 isolates were non-susceptible to ofloxacin, of which 12 were LNSSP. LNSSP were obtained from 12 laboratories in 3 of 9 provinces (Gauteng, KwaZulu-Natal, and Western Cape). The first case was identified in 2001,[186] and then from 2003 onwards 2, 4, 3 and 2 cases were identified for each year respectively.

All 12 LNSSP occurred in children (median age 1 year; range 0 to 13 years) (Table 6.1). Three children presented with meningitis and the remainder with lower respiratory tract infections. Nine of 11 patients with documented admission dates had nosocomial infections; an additional patient was hospitalised in

the past three months. Among LNSSP cases with known outcome (n=11), five (45%) died, one with a clinical diagnosis of meningitis. Three of these deaths occurred within 24 hours of the IPD diagnosis. Two LNSSP cases had insufficient information for additional evaluation, limiting results that follow to 10 children. Nine were known to be on TB treatment (including rifampin) and one had no history of TB treatment. Seven developed their acute episode of IPD while admitted to a TB hospital: 2 in TB hospital A (in 2004), 4 in TB hospital B (2003, 2004 and 2005) and 1 in a TB hospital in KwaZulu-Natal (2001). Four of 7 hospitalised TB patients were receiving treatment for MDR TB with ofloxacin at the time of the positive culture. One additional child received ciprofloxacin therapy for nosocomial sepsis within the preceding three months. The remaining five children had no history of fluoroquinolone use. All 10 children were HIV seropositive; two were known to be on antiretroviral therapy, one with a CD4+ T-cell count of 746 cells/mm^3 (14% of lymphocytes). One other child had a CD4+ count of 1109 cells/ mm^3 (24%). CD4+ counts were not available for the remaining children.

For the period of enhanced surveillance (2003-2006), eighty-six percent (2721/3176) of paediatric IPD cases presenting at sentinel sites had viable isolates and sufficient clinical information to assess factors associated with LNSSP infection. Compared to children with fluoroquinolone-susceptible IPD, rifampin resistance, history of TB treatment and nosocomial infection were significantly associated with LNSSP disease (Table 6.1). Fatal outcome, although more common among LNSSP cases, was not significantly associated with LNSSP infection (Table 6.1). This was also true when analysis of fatality was limited to HIV-seropositive children on TB treatment (LNSSP: 3/8 or 38%, vs. non-LNSSP: 48/291 or 16%; relative risk 2.92 (95% CI 0.72-11.82); p=0.12).

Table 6.1 Univariate comparison of levofloxacin-non-susceptible invasive pneumococcal infections compared to -susceptible infections in children <15 years of age, South Africa, 2000-2006

Characteristics	Levofloxacin-non-susceptible	Levofloxacin-susceptible	P value	Relative risk (95% confidence intervals)
2000-2006				
Age (years)*	1 (0-13)	1 (0-15)	0.81	Not available
Male	7/12 (58)	4265/7855 (54)	0.78	1.18 (0.37-3.71)
Isolation from CSF	3/12 (33)	2371/8040 (29)	0.73	0.80 (0.22-2.94)
Penicillin non-susceptible	5/12 (42)	2955/8040 (37)	0.72	1.23 (0.39-3.87)
Rifampin non-susceptible	12/12 (100)	355/8040 (4)	<0.001	Undefined
2003-2006#				
HIV	9/9 (100)	1376/1745 (79)	0.12	Undefined
Nosocomial infection	8/10 (80)	109/2709 (4)	<0.001	88.96 (19.10-414.29)
History of tuberculosis treatment	8/9 (89)	396/2202 (18)	<0.001	35.78 (4.49-285.30)
Case fatality rate (no of deaths/no of cases with known outcome)	4/10 (40)	622/2695 (23)	0.20	2.21 (0.63-7.82)

Data are median (range) or n/n (%)

*Age available for 12 levofloxacin-non-susceptible cases, and 8040 levofloxacin-susceptible cases

#Data for HIV serological status, nosocomial infection, history of tuberculosis treatment, and outcome were only available during enhanced surveillance (2003 onwards) and not available for all cases. Denominators change slightly reflecting those cases with available data.

6.3.2 Carriage study

At hospital A, 116 (83%) of 139 adult patients and all 19 paediatric inpatients were swabbed. Nine of 19 (47%) children and no adults were found to be carriers of pneumococci. All nine isolates were LNSSP. Forty-six (98%) of 47 children were swabbed at hospital B. Of children swabbed, 57% (26/46) were pneumococcal carriers. Among carriers, 22/26 or 85% carried LNSSP.

Among LNSSP carriers (n=31), 17 (55%) were male, 13 (42%) were receiving rifampin, and 17 (55%) were HIV-seropositive, with a median CD4+ T cell count of 228 cells/ mm^3 (range 5-1969). The median number of in-hospital days was 107 (13-614). LNSSP carriers were similar to those not carrying LNSSP (n=34) for the variables available for evaluation. Children carrying LNSSP tended to be younger but the age difference was not significant (median age of 3 years; range 0-11 years vs. 7 years, 0-14 years, p=0.082). LNSSP carriers were not significantly more likely to receive ofloxacin or ciprofloxacin compared to non-carriers at TB hospitals (24/31 or 77% vs. 20/34 or 59%, p=0.11). The median duration of fluoroquinolone therapy was 94 days (43-601) among LNSSP carriers receiving quinolone therapy, compared to 112 days (1-443) among non-carriers (p=0.45).

6.3.3 Characterisation of LNSSP strains

All 12 invasive LNSSP isolates were non-susceptible to trimethoprim-sulfamethoxazole and rifampin (Table 6.2). Ten (83%) of the 12 invasive isolates were serotype 19F, and fell into two clusters by PFGE. Six of the 7 cluster 1 isolates were from cases presenting in the Western Cape Province, including 4 cases known to be from the same hospital. All 3 isolates belonging to cluster 2 were identified in Gauteng Province, two from the same hospital. This penicillin-resistant cluster had higher levofloxacin MICs (Table 6.2).

All nine carriage isolates from hospital A were serotype 19F, levofloxacin MIC > 32 mg/L, and demonstrated antibiograms similar to cluster 2 invasive isolates. PFGE fingerprints (n=7) were indistinguishable from each other and related to the invasive isolates known to be from the same hospital. All 23 LNSSP carriage isolates (one patient carried two LNSSP) from hospital B were non-susceptible to rifampin and trimethoprim-sulfamethoxazole. Twelve isolates were serotype 19F and 11 isolates serotype 23F. PFGE patterns were available for 21 isolates, of which 19 isolates were indistinguishable by PFGE, and were related to the invasive isolates known to be from the same hospital.

Table 6.2 Levofloxacin-non-susceptible pneumococci causing invasive disease in children <15 years in South Africa, 2000-2006

			Minimum inhibitory concentrations (mg/L)				
Isolate	Hospital	Serotype	Ofloxacin	Levofloxacin	Moxifloxacin	Resistance antibiogram	PFGE Cluster
1	C	14	32	16	8	PEN, RIF, SXT	UR
2	D	19F	32	4	2	RIF, SXT	1
3	B	19F	32	4	2	RIF, SXT	1
4	A	19F	>32	>32	8	PEN, TET, ERY, CLI, RIF, SXT	2
5	B	19F	16	4	2	RIF, SXT	1
6	B	19F	16	4	2	RIF, SXT	1
7	A	19F	>32	>32	8	PEN, TET, ERY, CLI, RIF, SXT	2
8	B	19F	32	4	2	RIF, SXT	1
9	E	19F	32	4	2	RIF, SXT	1
10	F	19F	32	4	2	RIF, SXT	1
11	G	19F	>32	>32	8	PEN, TET, ERY, CLI, RIF, SXT	2
12	H	14	4	4	0.25	PEN, TET, ERY, CLI, RIF, SXT	UR

PFGE, pulsed-field gel electrophoresis; PEN, penicillin; TET, tetracycline; ERY, erythromycin; CLI, clindamycin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; UR, unrelated

6.4 Discussion

We describe the emergence of LNSSP among children in South Africa. In 2003, we reported the first case of paediatric invasive LNSSP disease, detected in a child in a TB hospital.[186] We now report the emergence and spread of LNSSP in two additional TB hospitals where fluoroquinolones are used to treat MDR TB. Although the number of cases identified was small, and could thus be seen as a major limitation of this study, bacteraemic pneumonia represents a fraction of the total burden of pneumococcal disease; thus, many more non-bacteraemic infections have probable occurred.

All invasive LNSSP infections in South Africa detected to date have occurred among children, in contrast to elsewhere in the world where LNSSP invasive disease has been reported in adults, and predominantly in the elderly.[181;183;195] Similar to a case-control study in Hong Kong, we found nosocomial acquisition of infection was associated with invasive LNSSP infections.[184]

Among pneumococcal carriers at two TB hospitals in two provinces in South Africa carriage of levofloxacin-resistant pneumococci approached 100% in children <15 years of age. Studies in hospitals in South Africa have previously demonstrated high rates of acquisition of antibiotic-resistant pneumococcal carriage among paediatric ward patients.[37;38] Rates of pneumococcal carriage are lower in children on antibiotics; however, these children are at greater risk of carrying resistant pneumococci.[38] Our observation that 7/31 children carrying LNSSP and 5/10 children with invasive LNSSP disease did not have a history of fluoroquinolone use supports the role of nosocomial spread. The association of carriage with younger age, and lack of carriage among adults, is consistent with pneumococcal epidemiology.[38;196] Resistant isolates may not disseminate to adults in a closed community.[197] However, there is ample evidence that exposure to children is a risk factor for adult IPD[198] and that immunocompromised adults, such as TB hospital patients, are at elevated risk for IPD due to paediatric

pneumococcal serotypes.[199;200] Antibiotic selection pressure in MDR TB hospitals and the lack of routine fluoroquinolone use in other paediatric patients may explain the limited spread outside TB hospitals to date.

The majority of South African LNSSP causing invasive disease in children was due to 2 clones of serotype 19F. In Hong Kong levofloxacin resistance is associated with the MDR Spanish^{23F}-1 clone[195] and several other studies have reported associations with international pneumococcal clones,[201-203] although in Canada a recent molecular analysis of LNSSP found most isolates to be genetically unrelated.[204] Emergence of fluoroquinolone resistance in international clones is predictive of the future potential for rapid spread, particularly if fluoroquinolone use increases.

Chemoprophylaxis is successful at reducing carriage and spread of resistant pneumococci in only limited circumstances.[38] Although infection control procedures can have some impact on transmission in long-term-care facilities, they are less effective in actively mixing patient populations.[205;206] Children may be an especially difficult population to target for infection control interventions. In our study, all of the invasive and carriage isolates were vaccine serotypes 19F, 14 and 23F. The carriage, transmission and disease caused by these organisms may therefore potentially be prevented by routine use of the 7-valent pneumococcal polysaccharide-protein conjugate vaccine on admission of a child to an MDR TB hospital.[207;208] PCV is unlikely to offer long-term protection against LNSSP emergence, because vaccinated children rapidly acquire pneumococcal carriage with non-vaccine serotypes.[208]

The CFR among children with invasive LNSSP disease was high (45%), but we note that among children with IPD that was fluoroquinolone susceptible, the case fatality was also markedly higher (23%) than in most developed countries.[209] This likely stems from the high prevalence of HIV, and among LNSSP cases

is further compounded by MDR TB infection. Fluoroquinolones are not used to treat pneumococcal infections in children in South Africa. However, potential transmission of resistant strains to adults where fluoroquinolones are used for pneumonia could result in treatment failures.[210]

A central limitation of our analysis is the relatively small number of LNSSP IPD cases and carriers. However, these numbers are large given that paediatric LNSSP emergence has not yet been detected globally, and the association with a history of TB treatment is robust.

In South Africa, TB was the most common natural cause of death in 2005, and more than half of adult TB patients may be HIV-infected.[190;211] Fluoroquinolones have become an important part of MDR TB treatment, and the evaluation of fluoroquinolones for shorter course treatment of uncomplicated TB also holds great promise for TB control and prevention. Nonetheless, unintended consequences of fluoroquinolone use for TB control must be monitored carefully. Our data suggest that it promotes emergence of resistance in the pneumococcus. Thirty years ago, the first MDR pneumococci emerged in a limited, nosocomial setting in South Africa very similar to what we describe here. Now MDR pneumococci have achieved worldwide spread. At this time, continued careful monitoring and identification of interventions to prevent acquisition and spread of LNSSP, will be critical.

6.5 Conclusions

Our data suggest that the use of fluoroquinolones to treat MDR TB in children has led to the emergence of LNSSP IPD in children and its nosocomial spread.

Chapter 7 Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine era, South Africa, 2003-2008

7.1 Introduction

The WHO has advocated for PCV immunisation implementation for infants.[51] Globally, an estimated 826,000 deaths occur annually from pneumococcal disease in children <5 years, of which 447,000 occur in Africa.[5] By February 2012, 14 (30%) of 47 African countries had introduced or were planning to introduce PCV for infants.[212] South Africa, a middle-income country with HIV prevalence, introduced PCV-7 into the public immunisation programme since April 2009 and transitioned to PCV-13 in April 2011.

PCV-7 implementation has been highly effective in developed countries. The US, since the introduction of PCV-7 in 2000, has seen a reduction in disease in the age group targeted for immunisation (young children),[45] and also a reduction in disease among adults as a result of decreasing nasopharyngeal acquisition and subsequent reduced transmission of vaccine serotypes from children.[61;213] This vaccine has also been shown to prevent antimicrobial-resistant disease.[64] The UK have documented the benefits of the introduction of the higher-valency vaccines, specifically PCV-13.[214] These studies have highlighted the importance of good quality laboratory-based surveillance data prior to vaccine introduction.

Clinical trial data suggest that PCV is also likely to be beneficial for lower-income and high-risk populations. Clinical trials of a 9-valent PCV documented a 16% reduction in all-cause mortality in The Gambia ,[215] and an 83% and 65% reduction of vaccine-type IPD among HIV-uninfected and HIV-infected

children , respectively, in South Africa.[216] Preliminary data from a population-based surveillance site in Kenya may suggest a reduction in IPD after PCV-10 introduction.[217]

Ongoing surveillance in countries that have introduced PCV-7 has demonstrated a consistent decline of vaccine-serotype disease, but at the same time an increase in non-vaccine-serotype disease, particularly serotype 19A disesase.[218;219] However, other countries have documented increases of serotype 19A disease without use of PCV-7,[220] or have not detected a significant increase in non-vaccine serotypes after the vaccine was introduced.[221] WHO has attempted to define the important surveillance characteristics for monitoring possible serotype replacement, especially looking ahead towards the newer formulations of PCV.[222] These recommendations include having sufficient years of pre-vaccine data and consideration of important non-vaccine factors that may influence interpretation of changes after vaccine introduction.

In this study, we describe the epidemiology of IPD in the pre-vaccine era (2003–2008) caused by serotypes covered by the different vaccine formulations and the burden which they may potentially prevent among HIV-infected and -uninfected children. These analyses also serve as an important baseline from which to monitor the effects of PCV.

7.2 Materials and Methods

7.2.1 Invasive disease surveillance

Surveillance for IPD began in 1999,[66] but was enhanced in 2003 through GERMS-SA (Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa), a nation-wide, active, laboratory-based surveillance system. Over 130 laboratories (representing ~290 hospitals) sent reports of laboratory-

confirmed IPD together with isolates to the NICD in Johannesburg. Demographic details such as age, gender, date of specimen, and source of isolate were captured. Enhanced surveillance at 16 sentinel hospitals located in all nine provinces collected additional information including admission date, HIV serological status, discharge diagnosis and outcome. Annual laboratory audits using a laboratory-based information system (Disa*Lab Laboratory Information Management System) for all public-sector laboratories in 8 provinces was used to identify unreported cases. KwaZulu-Natal did not have an electronic information system during the study years. We added the cases identified by audit to the database.

Cases were defined as patients with known age <5 years with *S. pneumoniae* cultured from normally sterile site specimens (e.g., CSF, blood or joint fluid) from January 2003 through December 2008. Repeat isolates from the same child within 21 days of the initial positive culture were excluded. Specimens yielding *S. pneumoniae* were captured for all national laboratories. Specimen source was defined by the following hierarchical definition: CSF specimen regardless of other specimens; blood specimen regardless of other specimens (excluding CSF); and other *e.g.*, pleural fluid, joint fluid *etc.* without CSF or blood. Clinical syndrome was only captured for enhanced surveillance sites and was defined in the following hierarchical manner: meningitis if the clinical diagnosis of meningitis was noted in the clinical records or the pneumococcus was isolated from CSF; bacteraemic pneumonia if the clinical diagnosis of pneumonia was noted in the clinical records and the pneumococcus was isolated from blood culture; bacteraemia without focus if no localizing clinical diagnosis was noted in the clinical records and the pneumococcus was isolated from blood culture; and other, included any diagnoses not including the preceding three, including localised pneumonia with pneumococcus isolated from pleural fluid only. Predisposing conditions other than HIV were defined as follows: asplenia; chronic illness; other immunocompromising conditions (excluding HIV); other ACIP (Advisory Committee on Immunization

Practices)[223] risk factors; and other risk factors. Malnutrition was defined as the presence of malnutrition as recorded in the medical records.

7.2.2 Estimation of incidence rates

We calculated cumulative annual incidence of IPD by dividing the number of IPD cases identified by mid-year population (in 2008, national population of children aged <5 years was estimated to be 6,108,700 individuals).[224] Incidence by HIV coinfection was estimated for 2008, the year with the most complete HIV data. For estimates of incidence by HIV status, HIV-infected and uninfected population estimates were extracted from the Actuarial Society of South Africa (ASSA) 2008 AIDS and Demographic model. We used multiple imputation to estimate the HIV infection status for pneumococcal cases not tested for HIV using a binomial distribution. The predictors for the multiple imputation model were age; hospital; province; specimen type; penicillin, rifampicin and trimethoprim-sulfamethoxazole susceptibility; serotype; and site (enhanced or non-enhanced).

7.2.3 Serotyping and susceptibility testing

Pneumococci were serotyped by Quellung using specific antisera (Statens Serum Institut, Copenhagen, Denmark). Serotype 6C was distinguished from 6A for the whole study period.[225] PCV-7 included serotypes 4, 6B, 9V, 14, 18C, 19F and 23F; PCV-10 included in addition 1, 5 and 7F; and PCV-13 included in addition 3, 6A and 19A. Isolates were screened for penicillin resistance by oxacillin disc diffusion (Mast Diagnostics, Merseyside, UK).[226] MICs were determined for potentially resistant isolates using agar dilution or Etest® (AB-Biodisk, Solna, Sweden). Results were interpreted as susceptible or non-susceptible (intermediately resistant and resistant) using 2008 CLSI definitions.[226] Isolates were considered to be non-susceptible to penicillin at MICs ≥0.12 mg/L using the oral penicillin breakpoint.

MIC50 was defined as the MIC required to inhibit the growth of 50% of penicillin non-susceptible isolates.

7.2.4 Statistical analysis

Univariate analysis used the χ^2-test for comparison of categorical variables to assess differences within enhanced and non-enhanced site data. Significant trends in incidence by serotype were assessed using Poisson regression. To assess differences between serotypes we evaluated the association of age (age group <1 year, 1–4 years), clinical syndrome, and HIV coinfection for each serotype compared to serotype 14 (the commonest serotype). We used univariate and multivariable multinomial regression models, generating a separate estimate of effect for each predictor on each outcome relative to the base level. The effect measures are the ratios of two relative risks (relative risk ratios) with each relative risk describing the probability of the outcome in the category of interest relative to the baseline category.[227]

All analyses were done using Stata Version 11 (StataCorp Limited). Two-sided p values <0.05 were considered significant. An inherent property of national surveillance systems is the potential for incomplete information. For each analysis, we used all available case information. Variables were binary (yes/no), defined as the presence or absence of the attribute excluding missing data.

7.3 Results

7.3.1 Invasive disease surveillance

GERMS-SA received reports of 8,674 IPD cases among children <5 years of age over the 6-year period. Audits identified approximately 16% of cases, ranging from 14%–19% annually, with no linear trend

(data not shown). Case characteristics are shown in Table 7.1. IPD incidence was highest among children <1 year, and remained stable from 2004 onwards (p=0.2, χ^2-test for trend, 2004–2008), after initial surveillance improvements in 2003 (Figure 7.1). In 2008, reported IPD incidence was 6-fold higher among children <1 year compared to children 1–4 years: 87 per 100,000 population and 14/100,000, respectively. Incidence in 2008 was 48/100,000 (95% confidence interval (CI), 35–63) and 6/100,000 (5–7) among HIV-uninfected children aged <1 and 1–4 years, respectively. Among HIV-infected children, incidence was 1022/100,000 (95% CI, 923–1123) and 198/100,000 (178–220) among children aged <1 and 1–4 years respectively. The relative risk of IPD was 21-fold (95% CI, 19–24) and 34-fold (29–41) greater among HIV-infected compared to HIV-uninfected children in the <1 year and 1–4-year-old age groups, respectively.

Table 7.1 Characteristics of children <5 years of age with invasive pneumococcal disease (IPD), South Africa, 2003-2008

All sites	Characteristic		Age group (years)					
			<1		1–4		Total	
			N=4723		N=3951		N=8674	
			n	%	n	%	n	%
	Year							
		2003	618	13	632	16	1250	14
		2004	782	17	721	18	1503	17
		2005	832	18	722	18	1554	18
		2006	793	17	642	16	1435	17
		2007	810	17	657	17	1467	17
		2008	888	19	577	15	1465	17
	Province							
		Eastern Cape	378	8	238	6	616	7
		Free State	350	7	273	7	623	7
		Gauteng	2324	49	1943	49	4267	49
		KwaZulu-Natal	501	11	514	13	1015	12

	Limpopo		99	2	67	2	166	2
	Mpumalanga		241	5	171	4	412	5
	Northern Cape		54	1	41	1	95	1
	North West		145	3	82	2	227	2.6
	Western Cape		631	13	622	16	1253	14
Gender								
	Female		2143	45	1698	43	3841	44
	Male		2434	52	2177	55	4611	53
	Unknown gender		146	3	76	2	222	2.6
Specimen								
	Cerebrospinal fluid[a]		1875	40	883	22	2758	32
	Blood[b]		2760	58	2914	74	5674	65
	Other		88	2	154	4	242	3
Serotype								
	PCV-7 serotypes	All	2038	57	1811	59	3849	58
		4	66	2	68	2	134	2
		6B	433	12	452	15	885	13
		9V	100	3	113	4	213	3

	14	593	17	435	14	1028	15
	18C	98	3	67	2	165	2
	19F	384	11	287	9	671	10
	23F	364	10	389	13	753	11
PCV-10 serotypes	All	2209	61	2105	68	4314	65
	1	86	2	259	8	345	5
	5	73	2	30	1	103	2
	7F	12	0.3	5	0.2	17	0
PCV-13 serotypes	All	2914	81	2755	89	5669	85
	3	53	1	23	1	76	1
	6A	399	11	402	13	801	12
	19A	253	7	225	7	478	7
Mixed infections[c]		4	0.1	9	0.3	13	0.2
Other serotypes		674	19	325	11	999	15
No serotype data available		1131		862		1993	

Penicillin susceptibility							
	Susceptible	1872	52	1545	50	3417	51
	Non-susceptible	1719	48	1543	50	3262	49
	Not tested	1132		863		1995	
Enhanced sites only							
		N=1921		N=1672		N=3593	
Clinical syndrome							
	Meningitis	762	40	370	22	1132	32
	Bacteraemic pneumonia	826	43	1003	60	1829	51
	Bacteraemia without focus	279	15	220	13	499	14
	Other	49	3	68	4	117	3
	Unknown	5	0.3	11	1	16	0.4
HIV coinfection							
	HIV uninfected	467	37	278	23	745	30
	HIV infected	811	63	938	77	1749	70
	HIV unknown	643		456		1099	

Other predisposing conditions[d]							
	Asplenia	3	0.2	2	0.1	5	0.1
	Chronic illness	75	4	131	8	206	6
	Other immunocompromising condition	13	0.7	29	1.7	42	1.2
	Other ACIP risk factor	15	0.8	17	1.0	32	0.9
	Other risk factor	30	2	17	1	47	1
	Malnutrition only	215	11	221	13	436	12
No HIV or predisposing condition identified		941	49	541	32	1482	41
Outcome							
	Recovered	1344	70	1407	85	2751	77
	Died	565	30	255	15	820	23
	Unknown	12		10		22	

[a]854 cases included additional specimens: blood (n=849), blood and pericardial fluid (n=1), blood and peritoneal fluid (n=1), blood and unspecified fluid (n=1), pleural fluid (n=1) and unspecified fluid (n=1)

[b]47 cases included additional specimens: pleural fluids (n=39), joint fluids (n=2); peritoneal fluids (n=2); pericardial fluid (n=1); unspecified fluids (n=3)

[c]Included only one each, unless otherwise stated: 6A/23F, 6A/14, 6A/19F (n=2), 6B/14, 9V/15A, 8/23F, 15A/19A, 15B/19F, 18C/14, 19A/22, 19F/6B, 23F/22; identified in all years except 2003, 2 to 3 cases for each year

[d]Asplenia included asplenia or sickle cell anaemia; chronic illness included chronic lung, renal, liver, cardiac disease and diabetes; other immunocompromising conditions (excluding HIV) included organ transplant, primary immunodeficiency, immunotherapy and malignancy; other ACIP (Advisory Committee on Immunization Practices) risk factors included head injury with possible CSF leak; other risk factors included neurological disorders, burns, chromosomal abnormalities and prematurity.

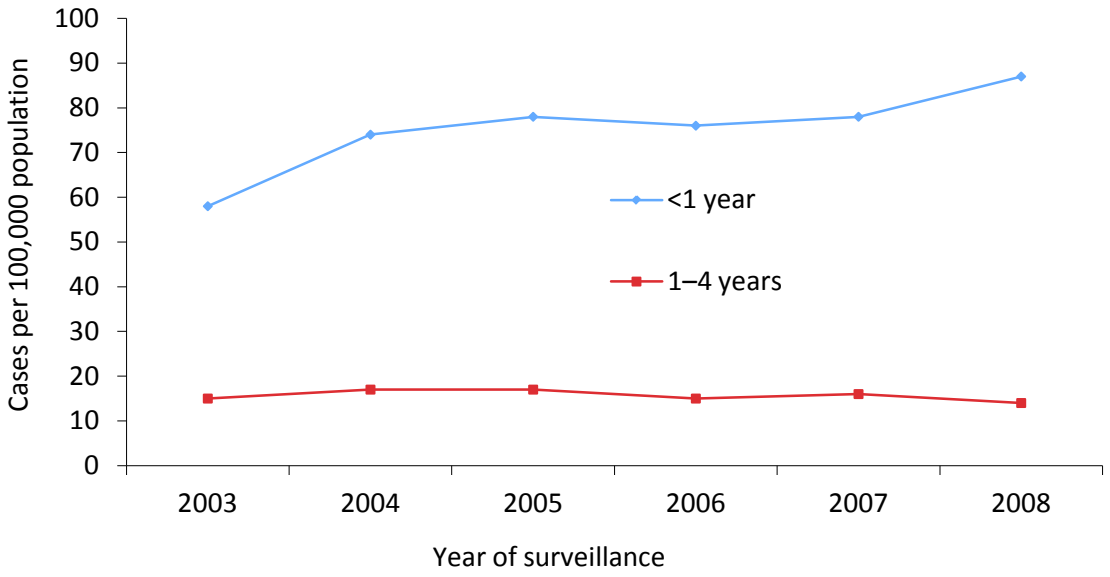

Figure 7.1 National annual incidence of invasive pneumococcal disease as detected through laboratory-based surveillance, by age group, South Africa, 2003-2008

Number of cases reported from non-enhanced sites increased over the study period (Table 7.2). In four provinces, >50% of cases came from enhanced sites compared to non-enhanced sites (Free State, 393/623, 63%; KwaZulu-Natal, 794/1015, 78%; Northern Cape, 55/95, 58% and Western Cape, 911/1253, 73%). IPD cases identified through blood cultures were more common at enhanced sites, and isolates were also more likely to be submitted from these sites (3691/4402, 84% from enhanced sites vs. 2990/4259, 70% from non-enhanced sites, p<0.001). PCV-7 serotypes were more likely to be identified from enhanced sites (1676/2985, 56% vs. 2173/3683, 59%, p<0.02), while penicillin susceptibility did not differ by sites. Overall, 49% of isolates were nonsusceptible to penicillin; among nonsusceptible strains, the MIC50 was 0.25 mg/L (range 0.12–8 mg/L). Using syndrome-specific breakpoints, bacteraemia isolates (susceptible MIC ≤2 mg/L) were almost universally penicillin susceptible (2927/2935, 99.7%), while CSF isolates (susceptible MIC ≤0.06 mg/L) had decreased susceptibility (275/1258, 22% were penicillin susceptible).

Table 7.2 Characteristics of children <5 years of age with invasive pneumococcal disease, South Africa, 2003-2008, by non-enhanced surveillance and enhanced sites

Characteristic		Surveillance site						P value
		Non-enhanced surveillance		Enhanced surveillance		Total		
		N=4264		N=4410		N=8674		
		n	%	n	%	n	%	
Age in years								
	<1	2345	55	2378	54	4723	54	
	1–4	1919	45	2032	46	3951	46	p=0.3
Year								
	2003	590	14	660	15	1250	14	
	2004	648	15	855	19	1503	17	
	2005	717	17	837	19	1554	18	
	2006	754	18	681	15	1435	17	
	2007	758	18	709	16	1467	17	
	2008	797	19	668	15	1465	17	p<0.001
Province								
	Eastern Cape	420	10	196	4	616	7	

	Free State	230	5	393	9	623	7	
	Gauteng	2388	56	1879	43	4267	49	
	KwaZulu-Natal	221	5	794	18	1015	12	
	Limpopo	107	3	59	1	166	2	
	Mpumalanga	328	8	84	2	412	5	
	Northern Cape	40	1	55	1	95	1	
	North West	188	4	39	1	227	3	
	Western Cape	342	8	911	21	1253	14	p<0.001
Gender								
	Female	1860	45	1981	45	3841	45	
	Male	2236	52	2375	54	4611	53	p=1.0[a]
	Unknown gender	168		54		222		
Specimen								
	Cerebrospinal fluid	1581	37	1177	27	2758	32	
	Blood	2574	60	3100	70	5674	65	
	Other	109	3	133	3	242	3	p<0.001
Serotype[b]								
	PCV-7 serotypes	1676	56	2173	59	3849	58	
	PCV-10 additional serotypes	272	9	193	5	465	7	

	PCV-13 additional serotypes	583	20	772	21	1355	20	
	Other serotypes	454	15	545	15	999	15	p<0.001[c]
	Not tested	1274		719		1993		
Penicillin susceptibility								
	Susceptible	1532	51	1885	51	3417	51	
	Non-susceptible	1456	49	1806	49	3262	49	p=0.9
	Not tested	1276		719		1995		

[a]excluding unknown gender

[b]13 mixed infections excluded: 5 from non-enhanced sites and 8 from enhanced sites

[c]excluding not tested

Table 7.3 Percentage of IPD cases in children <5 years that are serotypes included in each vaccine formulation, South Africa, 2003-2008

			PCV-7[a]		PCV-10[b]		PCV-13[c]		Non-PCV-13 serotypes		Total tested
			n	%	n	%	n	%	n	%	n
All sites											
	Age	<1 year	2038	57	2209	62	2914	81	674	19	3588
		1–4 years	1811	59	2105	68	2755	89	325	11	3080
	Penicillin susceptibility	Susceptible	1224	36	1681	49	2505	73	908	27	3413
		Non susceptible	2624	81	2632	81	3162	97	91	3	3253
	Specimen	Cerebrospinal fluid	1225	61	1340	67	1670	83	339	17	2009
		Blood	2551	56	2889	64	3882	86	649	14	4531
		Other	73	57	85	66	117	91	11	9	128
Enhanced sites only											
	Clinical syndrome	Meningitis	633	61	684	66	847	82	183	18	1030
		Bacteraemic pneumonia	938	57	1028	63	1431	88	203	12	1634

	Bacteraemia without focus	246	56	258	59	361	82	79	18	440
HIV coinfection	HIV uninfected	354	53	402	60	541	81	124	19	665
	HIV infected	969	61	1015	64	1371	87	205	13	1576
Outcome	Recovered	1418	58	1551	63	2106	86	340	14	2446
	Died	455	61	482	64	616	82	135	18	751

[a]PCV-7 (7-valent pneumococcal conjugate vaccine) serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F;

[b]PCV-10 serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F;

[c]PCV-13 serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, 19A, 3, 6A.

7.3.2 Serotype changes over time

Overall, 77% (6,681/8,674) of cases had isolates available for serotyping; patients with disease due to >1 serotype (n=13) were excluded from serotype-specific analyses. Eighteen cases were associated with non-typeable isolates. Among the twenty commonest serotypes (number of cases ranged from 41 for serotype 29 to 1028 for serotype 14 over the 6-year period), only 4 serotypes showed significant changes over time (Figure 7.2). Serotypes 19A and 18C increased during the study period, while a peak in serotype 5 was observed in 2004. Among serotype 19A, increases were due to increases in penicillin non-susceptible isolates: 10/56 (18%), 28/71 (39%), 48/94 (51%), 48/87 (55%), 77/86 (90%) and 65/83 (78%) for each year, respectively. Serotype 13 initially remained stable, but then decreased in the last 2 years, although annual numbers were small (range 5–12 cases). Serotype 6C was uncommon, with only 19 cases identified over the study period.

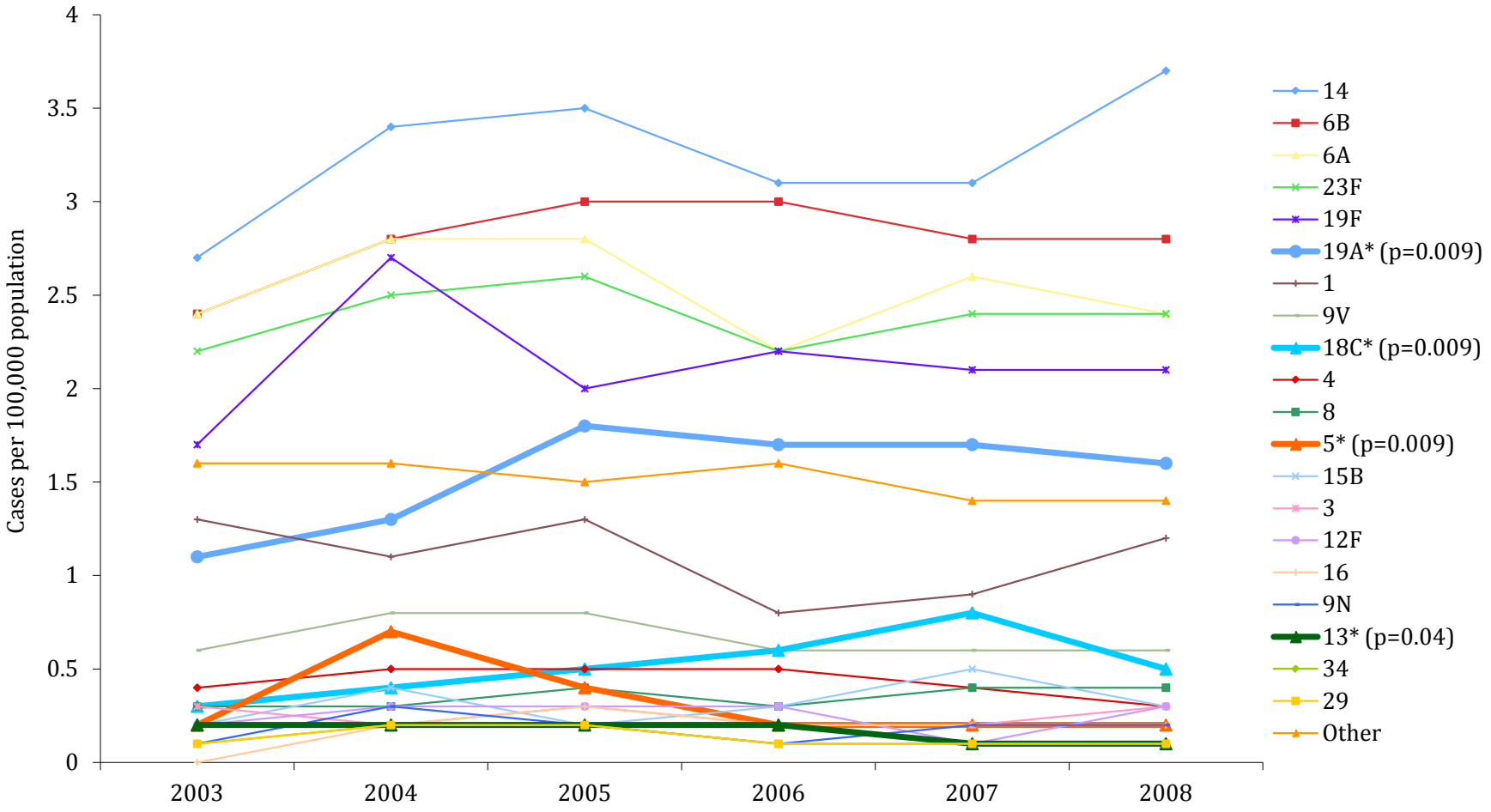

Figure 7.2 Serotype-specific invasive pneumococcal disease rates for children <5 years by the most common 20 serotypes (in descending order of prevalence) by year of surveillance, South Africa, 2003-2008 (n=6668)

*significant trends by poisson regression, lines in bold

During the surveillance period, 58% (3849/6668) of cases reported among children aged <5 years were due to PCV-7 serotypes (Table 7.3). PCV-10 was associated with an additional 3%–10% serotype coverage over PCV-7 stratified by age group, clinical syndrome, HIV co-infection or outcome, while PCV-13 increased coverage by an additional 16%–25% above PCV-10 serotype coverage (Table 7.3). PCV-13 had significantly higher coverage for isolates from blood culture than for isolates from CSF: 3882/4531 (86%) vs. 1670/2009 (83%), p=0.009, but only differed by 3%. Results were reversed for the other vaccine formulations, vaccine serotypes more common in CSF isolates compared to blood culture isolates: p=0.0005 and p=0.02 for PCV-7 and PCV-10 comparisons, respectively, and a difference of 5% and 3%, respectively (Table 7.3). Eighty-one percent of penicillin non-susceptible isolates are serotypes contained in PCV-7. PCV-10 does not add additional cover against non-susceptible serotypes, but PCV-13 increases coverage to 97%, driven by the contributions of serotype 6A (251/3253, 8%) and 19A (276/3253, 8%) to non-susceptible disease. By year the serotype coverage by vaccine formulation did not differ by >4%: PCV-7 coverage ranged from 56%–60%; PCV-10 from 64%–66%; and PCV-13 from 84%–86%.

7.4 Univariate analysis by age, syndrome and HIV co-infection

Serotype 14 was the most common serotype in children <1 year of age, and the second most common in children aged 1–4 years (Figure 7.3). Serotype 14 caused proportionately more disease in infants compared to older children (593/3588, 17% *vs.* 435/3080, 14%, respectively, p=0.007). Analysing the 20 most common serotypes plus serotype 7F, serotypes 6B, 9V, 23F, 1 and 6A were more likely to be isolated from older children compared to serotype 14 (Figure 7.3). Serotypes 5, 3, 8, 13 and 29 were, however, more likely to be identified in infants compared to serotype 14. The frequency of identifying serotype 14 was similar in meningitis (173/1030, 17%) compared to bacteraemia (311/2047, 15%; p=0.2). Serotypes 18C, 6A, 19A, 8 and 16 differed when compared to serotype 14 by clinical syndrome:

serotypes 18C and 8 were more likely to cause meningitis, while serotypes 6A, 19A and 16 were associated with bacteraemia (Figure 7.4). Serotype 14 caused proportionately more disease in HIV-infected (275/1576, 17%) compared to HIV-uninfected children (88/665, 13%; p=0.01). Compared to serotype 14, serotypes 6B, 18C, 1, 5 and 8 were significantly less likely to be identified in HIV-infected children (Figure 7.5).

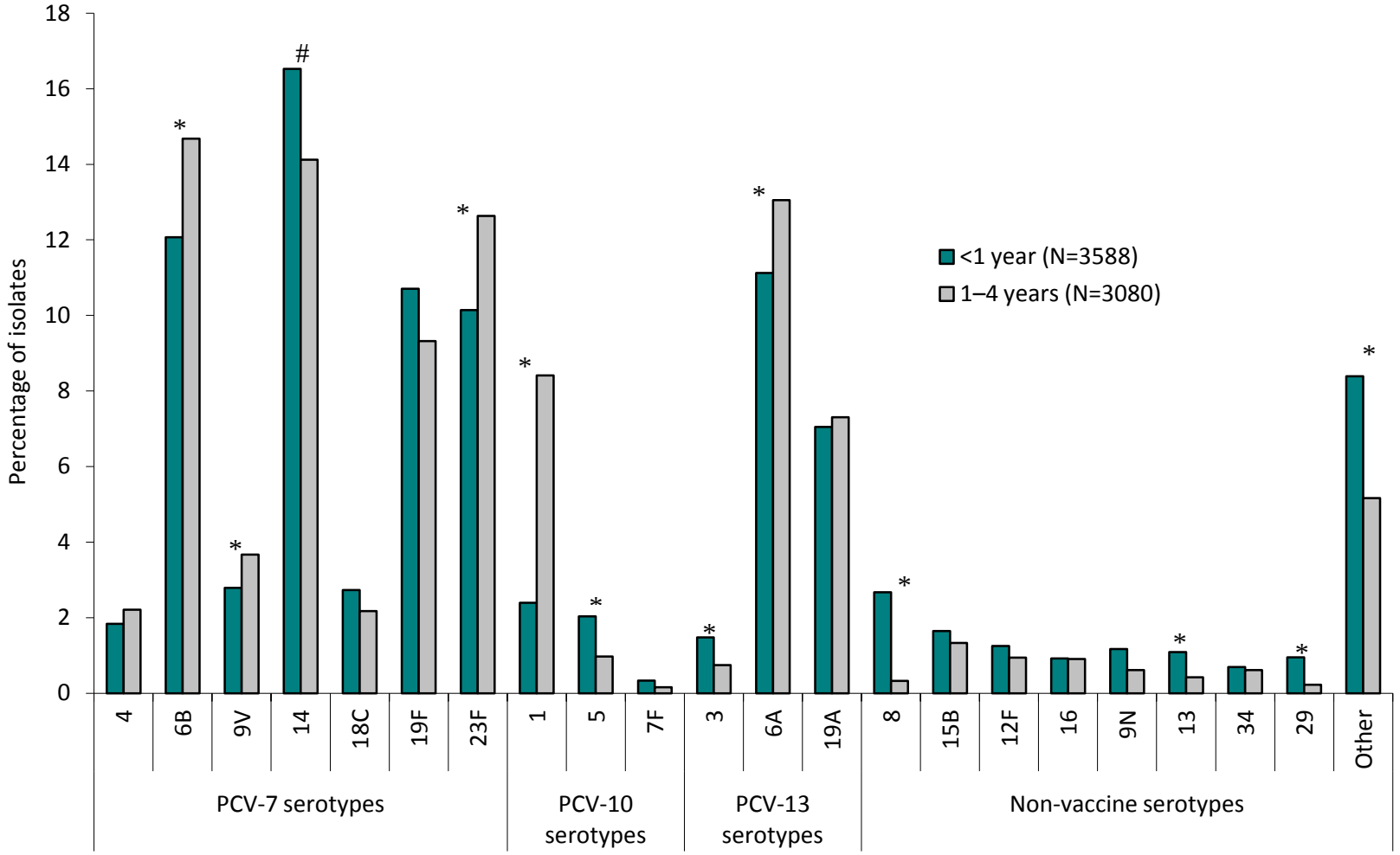

Figure 7.3 Percentage of isolates causing IPD in children <5 years by pneumococcal serotype and age; reported from all sites, South Africa, 2003-2008 (n=6668); # reference group; *p<0.05 on univariate analysis comparing to serotype 14

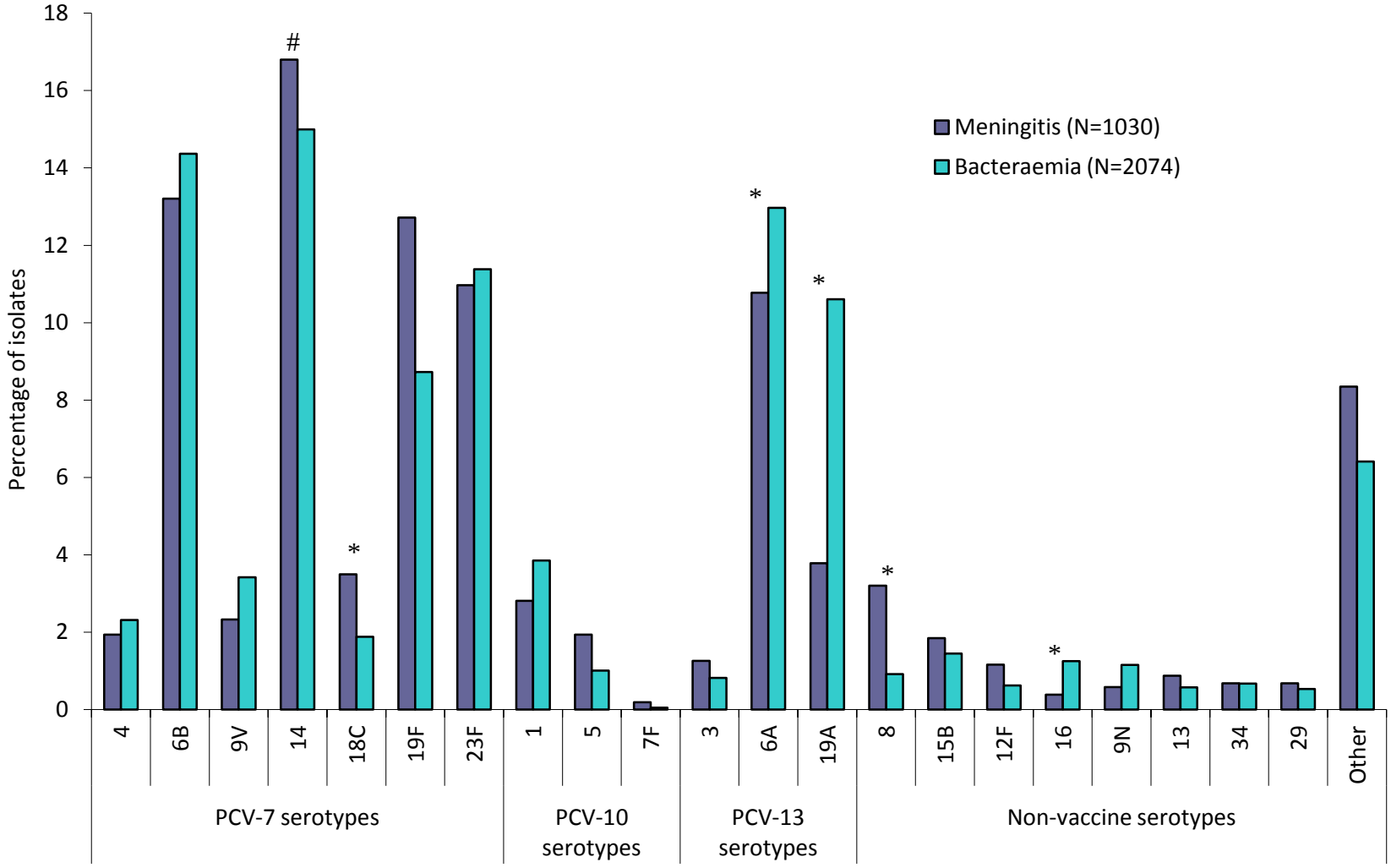

Figure 7.4 Percentage isolates causing IPD in children <5 years by pneumococcal serotype and clinical syndrome; reported from enhanced surveillance sites only, South Africa, 2003-2008 (n=2241); # reference group; *p<0.05 on univariate analysis

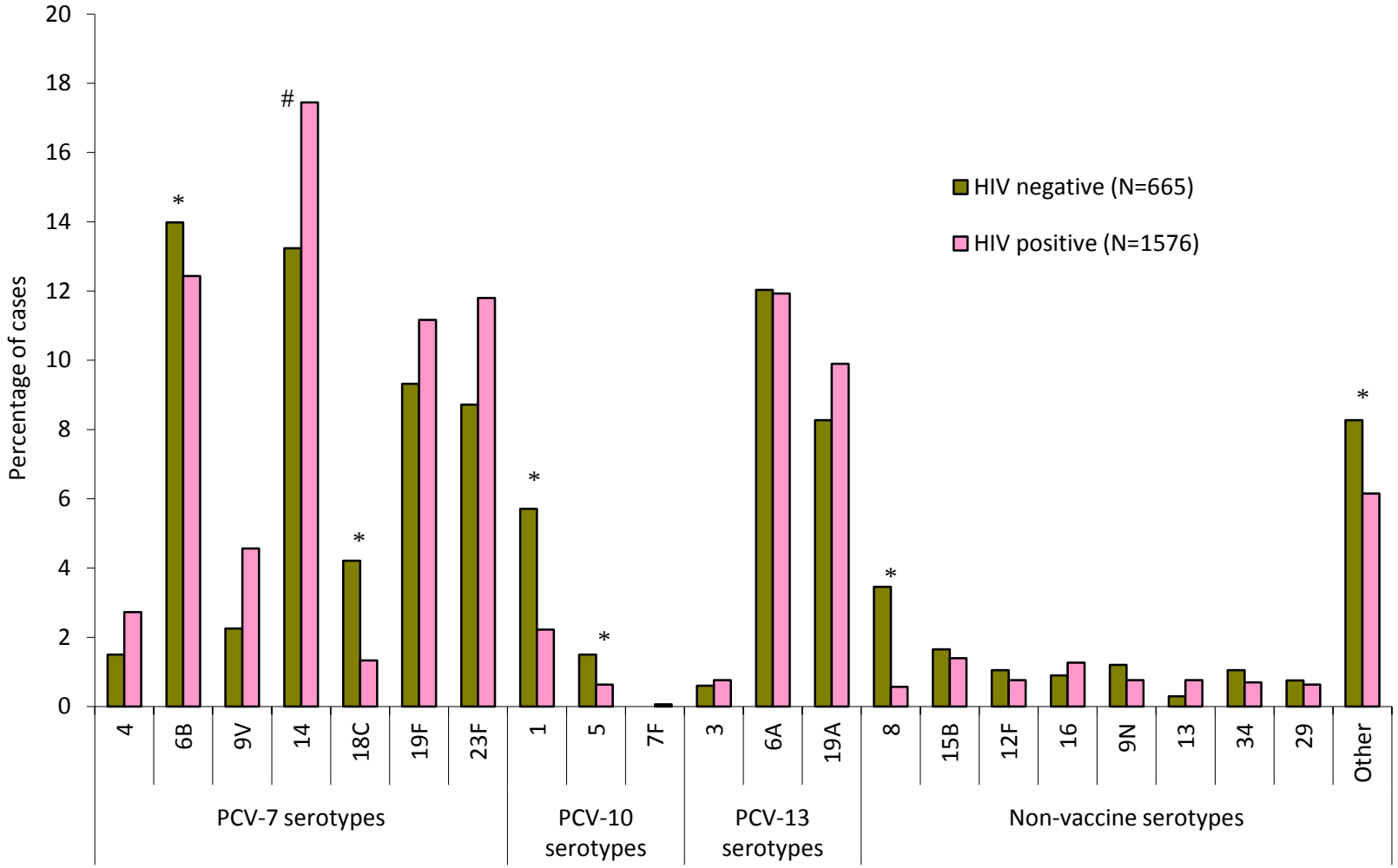

Figure 7.5 Percentage isolates causing IPD in children <5 years by pneumococcal serotype and HIV coinfection; reported from enhanced surveillance sites only, South Africa, 2003-2008 (n=2241); # reference group; *p<0.05 on univariate analysis

On multivariable analysis using serotype 14 as the referent group, older age remained significantly associated with serotypes 6B, 23F, 1, and 6A, while serotype 8 was the only serotype to remain significantly associated with disease in infants (Table 7.4). Only serotypes 19A and 8 remained associated with clinical syndrome: 19A was more likely to cause bacteraemia and serotype 8 more likely to cause meningitis, when compared with serotype 14. Serotypes 6B, 18C, 1 and 8 remained significantly less common in HIV-infected individuals than serotype 14.

Table 7.4 Factors on multivariable analysis associated with invasive pneumococcal disease in children <5 years due to specific pneumococcal serotypes, South Africa, 2003-2008. Bold numbers highlight associations with p<0.05 compared to serotype 14.

Serotype	Age group 1–4 years (*vs*. <1 year) RRR (95% CI)[a]	Clinical syndrome Meningitis (*vs*. bacteraemia) RRR (95% CI)[a]	HIV co-infection HIV infected (*vs*. HIV uninfected) RRR (95% CI)[a]
14	reference	reference	reference
4	1.7 (0.9 to 3.0)	0.7 (0.3 to 1.3	1.2 (0.6 to 2.6)
6B	**1.6 (1.2 to 2.2)**	0.9 (0.7 to 1.3)	**0.7 (0.5 to 0.9)**
9V	1.2 (0.7 to 1.9)	0.7 (0.4 to 1.2)	1.4 (0.7 to 2.6)
18C	1.1 (0.6 to 2.1)	1.4 (0.7 to 2.6)	**0.3 (0.1 to 0.5)**
19F	1.3 (0.9 to 1.8)	1.5 (1.0 to 2.1)	0.9 (0.6 to 1.3)
23F	**1.7 (1.2 to 2.4)**	1 (0.7 to 1.5)	0.9 (0.6 to 1.3)
1	**4.4 (2.4 to 8.0)**	0.7 (0.4 to 1.4)	**0.2 (0.1 to 0.4)**
5	0.5 (0.2 to 1.6)	1.2 (0.4 to 3.1)	0.4 (0.2 to 1.0)
7F	undetermined	undetermined	undetermined
3	0.9 (0.3 to 2.5)	1 (0.3 to 3.0)	0.9 (0.3 to 3.0)
6A	**1.5 (1.1 to 2.1)**	0.8 (0.6 to 1.2)	0.7 (0.5 to 1.0)
19A	1.2 (0.8 to 1.7)	**0.3 (0.2 to 0.5)**	0.8 (0.5 to 1.2)
8	**0.2 (0.1 to 0.8)**	**3.3 (1.5 to 7.5)**	**0.2 (0.1 to 0.4)**
15B	1 (0.5 to 2.1)	1.1 (0.5 to 2.4)	0.6 (0.3 to 1.4)
12F	1.5 (0.5 to 4.2)	2.3 (0.8 to 6.3)	0.5 (0.2 to 1.3)
16	1.3 (0.6 to 3.0)	0.4 (0.1 to 1.2)	0.9 (0.4 to 2.4)
9N	0.4 (0.1 to 1.2)	0.5 (0.2 to 1.5)	0.5 (0.2 to 1.3)
13	0.5 (0.1 to 1.6)	1.1 (0.3 to 3.3)	2.1 (0.5 to 9.9)
34	0.7 (0.3 to 2.1)	0.7 (0.2 to 2.2)	0.5 (0.2 to 1.3)

IPD in preconjugate era

29	0.2 (0.05 to 1)	1.4 (0.5 to 3.9)	0.8 (0.3 to 2.4)
Other	0.8 (0.6 to 1.3)	1.1 (0.7 to 1.6)	**0.6 (0.4 to 0.9)**

[a]RRR – relative risk ratio

7.5 Discussion

Our study provides robust baseline data to monitor the effectiveness of childhood PCV immunisation in South Africa. Rates of disease among children <1 year were up to 6-fold higher than among children 1–4 years of age; HIV-infected children <1 year had a 21-fold greater risk of disease than HIV-uninfected; and PCV-13 could potentially prevent 81% of IPD among children <1 year increasing to 87% among those HIV infected.

Initial increases in reported cases associated with enhancements stabilised after the first year. Reported rates of disease are clearly an underestimation, as not all cases will seek health care, specimens are not taken consistently for different clinical presentations and suboptimal specimen processing and non-reporting by laboratories may also reduce the number of reported cases. Population-based estimates of incidence of IPD among African children <1 year ranged from 242/100,000 in Kenya in 1998-2002,[104] to 554/100,000 in The Gambia in 1989–1991.[228;229] In South Africa, estimated rates were 349/100,000 for IPD among children <1 year in 1996/1997,[230] and, more recently, 264/100,000 among children <2 years of age in 2003/2004.[128] The 20- to 30-fold increased risk of disease among HIV-infected children compared to HIV-uninfected children estimated in 2008 in our national study are very similar to relative risks previously estimated.[128]

In our study, serotype 19A, a serotype not contained in PCV-7 or PCV-10, was the sixth most common serotype causing disease among children <5 years. Serotype 19A was associated with bacteraemic IPD

and caused 10% of disease among infants <1 years. Serotype 1 was an important cause of disease among older children and among HIV-uninfected children, highlighting the possible need for a booster dose to prevent serotypes causing disease in older children.[231] Additional serotypes included in PCV-10 (serotypes 1, 5, and 7F) were rarely penicillin non-susceptible, while serotype 19A accounted for >5% of non-susceptible isolates. Serotypes 3, 5 and 7F caused little disease among South African children. Although there were differences in the vaccine coverage for meningitis and bacteraemia, these were small in magnitude (<5%). For PCV-7 and PCV-10, vaccine serotypes were identified more commonly from CSF, however inclusion of 6A and 19A for PCV-13 made vaccine serotypes more likely from blood cultures for this vaccine formulation. These data suggest that in settings similar to ours, surveillance for meningitis alone may provide representative baseline data and could potentially be used to monitor vaccine impact.[19] In addition, vaccine effectiveness has not been demonstrated to differ by meningitis or bacteraemic pneumonia or other bacteraemia.[232] Previous analyses have shown some serotype differences by syndrome,[233] but different specimen-taking practices, especially for blood cultures, were highlighted as a significant limitation for analyses comparing serotypes and syndromes across different study populations.[233;234] The Global Serotype Project did not report syndrome-specific serotype distribution, but say that future analyses using case-level data may provide insights into serotype distribution by age, syndrome and HIV status.[3]

Serotype 19A disease, in particular antimicrobial resistant strains, will need to be monitored very carefully.[39] Although this serotype is increasing in some populations using PCV, it is unclear whether the increase is due to vaccine introduction, antimicrobial use or other influences.[235] The potential for the bacterium to evade vaccine-induced immunity using techniques such as capsular switching should also be considered.[218] For many countries, the decision for PCV-10 *vs.* PCV-13 may hinge on serotype 19A prevalence, PCV-13 effectiveness against 19A disease, and whether 19F in PCV-10 cross protects against

19A.[236] We did not consider cross-protection of serotype 6B for 6A[232] as it is unclear whether similar effects will occur in our population with the implemented schedule. This may have underestimated the coverage provided by PCV-7 and PCV-10 as calculated in our study.

Our findings again highlight how those with HIV are disproportionately affected by pneumococcal disease. In addition, earlier studies have demonstrated that strains from persons with HIV infection are more often antimicrobial resistant than strains from non-infected persons.[230;237] Although the efficacy of PCV may be lower among HIV-infected children,[238;239] the substantial increased risk of disease in this population means that the potential overall burden of disease prevented may be greater for HIV-infected than -uninfected children.[240;241] In our analysis, disease among HIV-infected children was more likely caused by PCV-7 or PCV-13 serotypes than among HIV-uninfected children, and introduction of any of the vaccine formulations will be useful for prevention of disease, including antimicrobial-resistant disease, in this population.[39]

Our study has some limitations. An inherent property of laboratory-based surveillance for pneumococcal disease is the underestimation of actual burden of pneumococcal disease.[242] Proportions of cases reported from enhanced sites by province differed, reflecting the number of enhanced sites in each province, hospital sizes, patient self-referral patterns, and differing specimen-taking practices compared to non-enhanced sites. Positive blood cultures were more likely from enhanced sites, often large academic hospitals, compared to non-enhanced sites. Enhanced sites were also more likely to submit isolates, likely due to dedicated surveillance staff. Laboratory practices remained stable during the study period. Strengths of our study included the large numbers of isolates and the long time period of observation. Incidence remained stable with minimal fluctuations of vaccine serotype coverage in a 6-year time frame, implying that less years of surveillance may be needed pre-vaccine introduction.

Nonetheless, pneumococcal serotype-specific disease may change over time periods longer than the 6 years we reviewed[22] or over less years if outbreaks of epidemic serotypes are experienced.[243]

7.6 Conclusions

While local data on disease burden and serotype distribution may be preferred when available, data from South Africa may be useful for decision makers in countries with similar populations who lack their own surveillance programmes. The newer vaccine formulations offer additional serotype coverage over PCV-7. Serotype distribution data can provide part of the information policy makers need when making decisions related to vaccine introduction or transitioning to newer vaccines. International data about emerging serotypes under pressure of routine vaccination should also inform vaccine introduction decisions.

Chapter 8 Conclusions

Using an active, national, laboratory-based, population-based surveillance system for *H. influenzae*, *N. meningitidis* and *S. pneumoniae* disease, we were able to document the reduction of Hib disease within the first five years of introduction of HibCV, followed by a small but sustained increase of Hib disease in the subsequent five years. With more than half of the vaccine failures occurring in children 18 months of age and older, an age group that may benefit directly from the booster dose, the introduction of a booster dose of HibCV as part of the new pentavalent vaccine in 2010 may substantially reduce the number of HibCV failures in South African children. These studies have highlighted the strengths of long-term surveillance. Increases of disease under surveillance may only occur several years into a vaccination programme.[120] Reductions of disease in age groups not vaccinated (indirect effects) may also take time and require the surveillance system to include age groups in the case definition outside of the vaccinated age group.[61] Our surveillance system is well placed to do this, especially when considering the future impact of PCV use in South Africa.

We were also able to document in one of our provinces the emergence of a virulent serogroup W-135 meningococcal strain that replaced an endemic serogroup A strain. Early data from the introduction of serogroup A conjugate vaccine in the meningitis belt have been promising,[60] but subsequent outbreaks caused by serogroup W-135[244] have highlighted the potential of other serogroups to replace serogroup A, similar to what happened in South Africa. Other southern African countries have also documented a predominance of serogroup W-135.[245]

As our surveillance includes reference laboratory characterisation, including antimicrobial susceptibility testing, we were able to document the emergence of levofloxacin-non-susceptible pneumococci among

children. A nested carriage study confirmed carriage of these strains among children in MDR TB hospitals and that the strains were vaccine serotypes. Paediatric hospitals in South Africa have previously acted as sentinels of emerging resistance,[37;38] and early detection will assist the global community in assessing possible consequences of new treatment policies. Although PCV may reduce antimicrobial-resistant disease,[64] replacement serotypes may also be resistant[218] and it will require a sensitive surveillance system, testing for both serotypes and resistance to fully monitor the effects of the vaccine in our setting. We have documented the ability of our surveillance system to detect emerging resistance among a specific age group. No increases of levofloxacin-non-susceptible pneumococci have been identified more recently through our surveillance, possibly reflecting the effects of ongoing decreases in IPD in children due to improvements in HIV prevention and care,[128] as well as PCV introduction targeting the resistant serotypes.

A more systematic review of all pneumococcal isolates causing disease in children <5 years summarised the potential for various PCV formulations to prevent disease in our setting and lay the groundwork for rigorous baseline data to monitor the introduction of PCV in South Africa. Although the early generation PCVs were primarily designed to prevent pneumococcal disease in the developed world, serotype distribution data from the developing world, confirming that the majority of disease has the potential to be prevented by PCV, are useful to assist policy makers in making the decision to introduce these expensive vaccines. Future PCV formulations should consider serotype distribution in countries with the greatest disease burden,[3;5] while protein vaccines hold promise of broad or universal coverage for pneumococcal disease.[2]

The introduction of PCV, some years after the introduction of HibCV will change the epidemiology of meningitis in South Africa, with an increase in the relative importance of meningococcal meningitis

contributing to this syndrome, as well as a shift in the median age of individuals presenting with this syndrome.[246] Our surveillance system will be well placed to monitor these changes over time. Although no meningococcal vaccines are currently planned for large-scale use in South Africa in the near future, the epidemiology of disease in our country is being carefully monitored for future interventions[247] and our meningococcal strains are being routinely tested for the prevalence of new vaccine antigens.[248]

The biggest limitation of our surveillance system is the variable specimen-taking practices among doctors working at different hospitals in different provinces.[103] This is most marked for blood cultures leading to substantial underestimation of disease rates. In addition, not all patients may be able to access clinics and hospitals during their acute illness, further reducing our estimates of the true burden of disease. These characteristics of our system may make it impossible for us to estimate true burden of disease, however we have clearly highlighted the ability of our system, with a sentinel component nested within a national surveillance programme, to successfully monitor trends over time. Recent data from the US have also demonstrated that sentinel surveillance systems with an average of more than 30 cases annually at baseline may be able to reflect pneumococcal serotype changes after PCV introduction and changes fell within the 95% confidence interval for the change in incidence that was documented for the corresponding population-based surveillance area.[28]

Our surveillance includes all age groups, is well established with many years of data and includes a strong phenotypic and genotypic characterisation of the disease-causing strains, making it possible to document the epidemiology of the diseases as discussed in the preceding chapters. We have demonstrated the emergence of virulent and resistant strains and monitored the impact of HibCV and the potential impact of PCV. Going forward the programme will continue as a platform to monitor these diseases in a changing climate of PCV use, which is predicted to affect the epidemiology of IPD in all age

groups. Additional challenges in interpreting our data will be other interventions that affect our diseases, e.g., the introduction of highly active antiretroviral therapy (HAART) for children and the improvement of prevention-of-mother-to-child-transmission (PMTCT), both expected to reduce opportunistic infections such as IPD.[128;249;250] Future epidemiological studies nested within our surveillance system may overcome some of the challenges inherent to trend data alone.[251]

Chapter 9 Appendices

GERMS-SA: National Laboratory-based Surveillance for Enteric, Respiratory and Meningeal Bacterial and Fungal Diseases in South Africa
Protocol Version 1.0 (February 2008)
Clinical Case Report Form
National Microbiology Surveillance Unit (NMSU)
TEL: 011 386 6284 OR 011 555 0353 FAX: 0 11 386 6077

Surveillance officer name:		Signature:	Date:
Sources of data: Patient/Guardian ☐	Clinician ☐	Medical records ☐	No record found ☐

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 ☐ URTI ☐ Dysentery ☐ Diarrhoea ☐ Pneumonia/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. jiroveci ☐	Salmonella sp. ☐	Joint Fluid ☐ Other ☐ Specify

Severity of illness for 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: Yes ☐ No ☐ Unk ☐	Dosage:	Date initiated: D D M M Y Y Y Y	Compliant in last month: Yes ☐ No ☐ Unk ☐
TB Treatment: Yes ☐ No ☐ Unk ☐	Drugs: 1. 2.	3. 4.	Date initiated: D D M M Y Y Y Y / Date stopped: 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
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TEL: 011 386 6284 OR 011 555 0858 FAX: 011 386 6077

Laboratory Specimen Number:

Immunocompromising conditions:

Alcohol dependency ☐	Chronic renal failure ☐	Heart failure ☐	Haematological Malignancy ☐	Valvular heart disease ☐	Unknown ☐
Asthma ☐	Current smoker ☐	History of head injury/head surgery ☐	Nephrotic syndrome ☐	Malignancy ☐	Specify:_____
Burns ☐	Coronary Artery Disease ☐	Hydrocephalus with V-P 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 ☐	

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 ☐ Unk ☐ Refused consent ☐ Reason for refusal:_____
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 >10 days ☐ Oral candidiasis ☐ Suspected PCP ☐ None ☐ Kaposi's sarcoma ☐ Tuberculosis ☐ HIV wasting ☐ Unk ☐
Any antiretroviral use: Yes ☐ No ☐ Unk ☐ If yes: Current ☐ Previous ☐ Perinatal ☐ Unk ☐
Current antiretroviral use: STC ☐ D4T ☐ Efavirenz ☐ Nevirapine ☐ AZT ☐ DDI ☐ Kaletra ☐ Unk ☐
Date initiated: D D M M Y Y Y Y Other ARVs:_____
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 admission:
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	

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GERMS-SA: National Laboratory-based Surveillance for Enteric, Respiratory and Meningeal Bacterial and Fungal Diseases in South Africa
Protocol Version 1.0 (February 2008)
Clinical Case Report Form
National Microbiology Surveillance Unit (NMSU)
TEL: 011 386 6284 OR 011 555 0353 FAX: 0 11 386 6077

Laboratory Specimen Number:

Haemophilus spp. and S. pneumoniae ONLY

Vaccination status for *Haemophilus influenzae*:

If <15 years of age, did patient receive *Haemophilus influenzae* type b vaccine: Yes ☐ No ☐ Unk ☐

Dose	Date given	Name of clinic
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	

If patient received vaccine, was there documented proof of vaccine: Yes ☐ No ☐ Unk ☐

Vaccination status for *Streptococcus pneumoniae*:

If <15 years of age, did patient receive pneumococcal conjugate vaccine: Yes ☐ No ☐ Unk ☐

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

Dose	Date given	Name of clinic
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	

If yes, give date most recently given and vaccine name:
i. Most recent date given: D D M M Y Y Y Y
ii. Vaccine name: _____

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_2O Unk ☐

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

Pneumocystis jiroveci ONLY

Admission pulse oximeter reading off oxygen ☐☐☐ % Unk ☐

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:

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Revised Nov 2007

e APPROVAL Vpotect B

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 von Gottberg

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M02-10-42

PROJECT Enhancement of Surveillance for Trimethoprim Sulfamethoxazole Resistant Invasive Respiratory and Diarrhoeal Disease in South Africa

INVESTIGATORS Dr A von Gottberg

DEPARTMENT School of Pathology, NHLS

DATE CONSIDERED 02-10-25

DECISION OF THE COMMITTEE Approved unconditionally

Unles otherwise specified the ethical clearance is valid for 5 years but may be renewed upon application
This ethical clearance will expire on 30 July 2007.

DATE 03-01-14 CHAIRMAN.....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: K Reddy
Dept of School of Pathology, NHLS
Works2\lain0015\HumEth97.wdb\M 02-10-42

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 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 form. I/we agree to inform the Committee once the study is completed.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

SENT BY: WITS; 0116429533; 15-AUG-06 2:59PM; PAGE 1/1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 von Gottberg, et al

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER MQ60634**
PROJECT	Risk Factors for nasopharyngeal carriage of fluoroquinolone-resistant Streptococcus pneumoniae (FQRSP)
INVESTIGATORS	von Gottberg, et al
DEPARTMENT	RMPRU
DATE CONSIDERED	06.06.30
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 06.07.18 **CHAIRPERSON** .....
(Professor A Dhai)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor;

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 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

15-AUG-2006 15:30 FROM: TO:5559437 P.1/1

15-AUG-2006 12:27 FROM: TO:0423553 P.1/4

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