STREPTOCOCCUS PNEUMONIAE SEROTYPES AND MORTALITY IN ADULTS IN SOUTH AFRICA: ANALYSIS OF NATIONAL SURVEILLANCE DATA (2003 – 2008)

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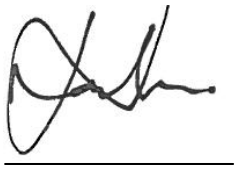
DECLARATION

I declare that this research report is my own work. It is being submitted in partial fulfillment of the requirements for the degree of Master of Science in Epidemiology in the field of Epidemiology & Biostatistics at the University of the Witwatersrand, Johannesburg. It has not been submitted previously for any degree or examination to any other university.



Nireshni Naidoo

Date: January 2014

DEDICATION

I dedicate this work to my loving parents Perumal (Krish) and Utherani (Dolly) Naidoo. Words are simply inadequate to express my gratitude for your tireless love, support, encouragement, prayers and sacrifices that only a parent can understand. May God bless you abundantly and always keep you in the palm of His hand.

ABSTRACT

Introduction

Studies have shown an increased risk of mortality amongst adults with invasive pneumococcal disease (IPD) with certain serotypes but there are no data from South Africa. We aimed to determine the association between serotypes and in-hospital mortality among adults aged 15 years and older with IPD in South Africa.

Methods

IPD cases were identified through the GERMS-SA national laboratory-based surveillance programme. Patient data from 25 enhanced surveillance sites from 2003-2008 (pre-pneumococcal conjugate vaccine introduction) with available data on serotype and in-hospital outcome were used. We assessed the association between the 20 most common serotypes and mortality among patients ≥15 years of age using univariate and multivariable logistic regression models.

Results

From January 2003 through December 2008, there were 3953 cases of IPD amongst adults older than 15 years of age meeting the study inclusion criteria. Amongst the 20 commonest serotypes, the incidence of serotypes 4, 19A, 23F and 18C increased significantly, and serotypes 1, 25 and 5 decreased significantly

from 2003 to 2008. Serotype 1 was the commonest serotype overall (16%, 651/3953), followed by serotype 19A (11%, 443/3953) and serotype 4 (7%, 289/3953). The case-fatality ratio (CFR) was 55% (641/1166) for meningitis and 23% (576/2484) for bacteremia (p<0.001). Serotype 19F had the highest CFR of 48% (100/207), followed by 39% (99/252) for serotype 23F and 38% (246/651) for serotype 1. On multivariable analysis, factors independently associated with mortality were disease caused by serotypes 1 (OR 1.93, 95% CI 1.05–3.53) and 19F (OR 2.89, 95% CI 1.38–6.06) compared to serotype 4; increasing age (25-44 years, OR 1.75, 95% CI 1.03–2.95; 45-64 years, OR 3.56, 95% CI 2.00–6.35; ≥65 years, OR 5.17, 95% CI 1.89–14.14; compared to 15-24 years); living in provinces with intermediate (OR 1.65, 95% CI 1.16–2.35) or high poverty rates (OR 1.72, 95% CI 1.02–2.92) compared to provinces with low poverty rates; having meningitis (OR 4.07, 95% CI 2.98–5.55) compared to bacteremia; prior antibiotic treatment in the last two months (OR 3.93, 95% CI 2.50–6.20); inappropriate antibiotic treatment (OR 2.37, 95% CI 1.74–3.22) and positive HIV status (OR 1.69, 95% CI 1.04–2.75).

Conclusion

Serotypes associated with increased mortality are included in the 10-and-13-valent pneumococcal conjugate vaccine and may be expected to become less common in adults as a result of indirect effects following routine immunization in infants. HIV-infected adults experience increased mortality and the more widespread availability of antiretroviral therapy is likely to substantially improve the quality of

life of HIV-infected individuals in terms of physical and mental health and decrease the incidence of IPD and therefore mortality.

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ABBREVIATIONS

ABCs: The Active Bacterial Core Surveillance

ACIP: Advisory Committee on Immunization Practices

ART: Anti-retroviral therapy

CDC: Centers for Disease Control and Prevention

CFR: Case-fatality ratio

CRFs: Case report forms

CSF: Cerebrospinal fluid

CI: Confidence interval

EPI: Expanded Programme on Immunization

GERMS-SA: The Group for Enteric Respiratory and Meningeal disease Surveillance in South Africa

HAART: Highly-active antiretroviral treatment

HIV: Human immunodeficiency virus

IPD: Invasive Pneumococcal disease

NHLS: National Health Laboratory Service

NICD: National Institute for Communicable Diseases

OR: Odds ratio

PCR: Polymerase chain reaction

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

RRR: Relative risk ratio

CHAPTER 1

INTRODUCTION

This chapter gives an overview of the burden of invasive pneumococcal disease and its public health importance. This chapter also includes the statement of the problem, justification for the study, objectives of the study and a literature review. This study is a secondary retrospective analysis of cross-sectional data to determine the association between serotypes and mortality among adults with IPD, adjusting for confounders, in South Africa using national surveillance data from 2003 to 2008.

1.1 Background

1.1.1 Organism

Streptococcus pneumoniae is a bacterium that causes illnesses that range from mild ear infections to more severe invasive pneumococcal disease (IPD) (1). In most cases, *S. pneumoniae* resides in a harmless carriage state in the nasopharynx of healthy individuals. Therefore, the progression from colonization to invasive disease is a relatively rare event (2). The acquisition and the colonization

of the mucosal surface of the host by *S. pneumoniae* followed by the invasion of the respiratory tract and bloodstream leads to IPD (2;3).

1.1.2 Pneumococcal disease

IPD remains a major cause of mortality and morbidity worldwide (1;4). IPD is defined as *S. pneumoniae* isolated from normally sterile body site specimens, and includes meningitis and bacteremia. Incidence, severity and mortality of IPD are influenced by host- and organism-related factors. The host-related factors include extremities of age, underlying chronic illness and immunosuppression (5-9). Organism-related factors include the polysaccharide capsule which is the basis for determining the serotype (10;11).

1.1.3 Polysaccharide capsule

The polysaccharide capsule of *S. pneumoniae* is a virulence factor (12). It is a highly hydrated shell that surrounds the bacterium. The polysaccharide capsule aids the bacterium in resisting phagocytosis and modulates the movement of ions and molecules to the cell envelope of the bacterium (13). The cell wall components and toxins such as autolysin, peptidoglycan, lipoteichoic and pneumolysin are known to play a role in the inflammatory response to pneumococcal disease (13;14).

1.1.4 Serotypes

The chemically and serologically distinct polysaccharide capsule of *S. pneumoniae* determines the serotype (15). There are 93 known pneumococcal serotypes (16;17). The capsule is covalently linked to the surface of the cell-wall peptidoglycan which represents a virulence determinant and protects *S. pneumoniae* against phagocytic clearance (18;19). The different serotypes display variations with regard to their resistance to phagocytosis (20). Therefore, certain serotypes of *S. pneumoniae* tend to have greater invasive potential than others. Serotypes 3, 6B, 14, and 23F were found to cause more severe meningeal inflammation than serotypes 1, 5, 9 and 7F in experimental studies (21;22). In a murine model of pneumococcal sepsis, serotypes 3, 6A and 6B were more lethal than others (23). Serotypes also differ in their prevalence, age distribution, ability to cause outbreaks (24), and in antibiotic resistance (25).

The reference method for serotyping of *S. pneumoniae* is the Quellung reaction (26). Several alternative methods are also used for serotyping *S. pneumoniae.* These include protein A-mediated staphylococcal (27-29), latex agglutination (30) the dot blot method (31) and the Ouchterlony method (32). However, conventional serotyping is difficult and cannot be performed on culture-negative specimens. Conventional polymerase chain reaction (PCR) assays, targeting serotype-specific genes are used for serotyping clinical specimens (33;34). Real-time PCR is faster and more sensitive (35). More recently, a sequential triplex real-time PCR assay for detecting 21 pneumococcal capsular serotypes has been developed. Some of

the advantages of the triplex real-time PCR assay include greater sensitivity and containment, where amplification products are not potential contaminants for subsequent PCRs (36).

1.1.5 Treatment

The recommended empiric treatment for pneumonia in South Africa is dependent on age and infecting organism and co-morbid illness. Young adults aged 65 years or less with no comorbid illness should be treated with intravenous penicillin G/ampicillin and a macrolide/azalide or a flouroquinolone (37). Adults aged 65 years or older and/or with a comorbid illness should be treated with amoxycillin/clavulanate and a macrolide/azolide or a fluoroquinolone. If the patient presents with severe illness an additional agent should be included in the regimen. According to the Essential Drug List (EDL) the recommendation for empiric treatment for meningitis is based on age. For adults the recommendation is penicillin G/ceftriaxone/cefotaxime. For resistant pneumococcal meningitis, ceftriaxone should be used with vancomycin/moxifloxacin/rifampicin (38).

1.1.6 Pneumococcal vaccines

There are four commercially available pneumococcal vaccines. These include the 23-valent pneumococcal polysaccharide vaccine (PPSV-23) consisting of 23 capsular polysaccharide serotypes, (39) and the pneumococcal conjugate vaccines (PCV-7, PCV-10 and PCV-13).

The 23-valent pneumococcal polysaccharide vaccine (PPSV-23) consists of 23 capsular polysaccharide serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. PPV-23 is stimulates T cell independent response. However, the T cell-*independent* response is not completely developed in children less than 2 years of age (40). Therefore PPSV-23 is recommended for individuals aged 2 years and older who have underlying conditions and are at increased risk of IPD. It is also recommended for use in older adults aged 65 years and older (39;41). The only available guideline for adult pneumococcal vaccination with PPSV-23 in South Africa was published in 1999 (42). However, a double-blinded randomized control trial in Uganda conducted by French et al (43) showed that all-cause pneumonia was significantly more frequent in the vaccine arm (HR 1·89, 95% CI 1·1-3·2) compared to the placebo arm and mortality was unaffected by PPSV-23 vaccination. This suggests that vaccination of HIV-infected individuals with PPSV-23 has little or no public health value in sub-Saharan Africa. A six year follow-up of the clinical trial participants by Watera et al (44) confirmed the excess of all-cause pneumonia in PPSV-23 recipients (HR 1.6, 95% CI 1.0-2.4).

The PCV-7 vaccine is a preparation of capsular polysaccharide from the 7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) that commonly infect children, conjugated to a nontoxic diphtheria cross-reactive material carrier protein (CRM197) which results in enhanced serum antibody response as well as eliciting a T cell-*dependent* memory (booster) response (45). PCV-10 contains the seven serotypes included in PCV-7 and three additional serotypes (1, 5 and 7F). PCV-13

contains the seven serotypes in PCV-7 and six additional serotypes (1, 3, 5, 6A, 7F and 19A). PCV-7 and PCV-13 are recommended for use among children from age 6 weeks to less than 6 years. PCV-10 is recommended for persons up to two years of age (39). In April 2009, PCV-7 was introduced into the Expanded Programme on Immunization (EPI) in South Africa. This was followed by the introduction of PCV-13 in July 2011. The schedule followed for both PCV-7 and PCV-13 in South Africa is vaccination at 6 weeks, 14 weeks and 9 months of age.

Studies in the U.S. have shown the effectiveness of one or more doses of PCV-7 against vaccine serotypes (as well as 6A as a result of cross protection) to be 96% (95% CI 93-98) in healthy children and 81% (95% CI 57-92) in children with underlying illnesses (46). Using an indirect cohort design, Andrews et al (47) used enhanced surveillance data from the Health Protection Agency in the U.K. to show increasing effectiveness of PCV-7 from 56% (95% CI -7-82) for a single dose to 93% (95% CI 70-98) for two doses plus a booster dose in the second year of life. In addition, Pićon et al (48) conducted a case-control study in Uruguay which showed a 91.3% (95% CI 46.4-98.6) vaccine effectiveness of PCV-7 for one or more doses and 94.8% (95% CI 43.1-99.5) for two or more doses.

1.1.7 Surveillance of pneumococcal disease in South Africa

A major event in South Africa was described by Brodie et al (49) where 93 mine workers arrived at a mine in mid-winter and after two weeks only 16 miners were able to work as the remaining miners had contracted respiratory tract infections,

including pneumonia. Eight of the miners died, following which winter employment of miners from tropical countries was banned. In 1912, the South African Institute for Medical Research (SAIMR) was established. This was prompted by major outbreaks of pneumonia among miners as well as other infectious diseases with high mortality rates, such as meningococcal meningitis (50).

Klugman et al (51) analyzed 4766 strains of *S. pneumoniae* isolated from blood and 1157 isolates from cerebrospinal fluid (CSF) from 1979 to 1986. Results showed that resistance increased among isolates from blood (3.8% to 14.1%) and CSF (6.8% to 14.1%). In addition, 19.2% of resistance strains were from strains belonging to serogroups 6, 19 or 14. Huebner et al (52) studied trends in antimicrobial resistance in pneumococci isolates (blood and CSF) between 1991 and 1998 in South Africa. Multiple drug resistance increased significantly from 2.2% to 3.8%. The percentage of serogroups found in the nonavalent pneumococcal conjugate vaccine (1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F) increased significantly from 72% to 91% over the study period.

In 1999, Dr Robyn Huebner and colleagues introduced a national laboratory-based surveillance system for IPD in South Africa. Laboratories were requested to send isolates and reports of laboratory-confirmed IPD cases to the National Institute for Communicable Diseases (NICD) in Johannesburg (53). The system was enhanced in 2003, resulting in an active surveillance system with improved communication and site visits to improve the reporting of cases. Additional clinical and demographic information was collected (54). This national laboratory-based

surveillance system is ongoing and provides a wealth of information for evaluating burden of disease, health risks and health interventions.

1.2 Statement of the problem

S. pneumoniae is a common cause of pneumonia, meningitis, septicemia and otitis media. It is associated with significant morbidity and mortality worldwide. Since the introduction of PCV-7, studies have shown a decline in prevalence of IPD in vaccinated children and unvaccinated adults (55). However, a significant burden of disease exists worldwide due to serotypes that are not included in PCV-7 (56). Previous international studies have shown an association between pneumococcal serotypes and disease outcome, particularly mortality in adults (57;58). These associations could differ in a developing country such as South Africa, where there is a higher HIV prevalence and a greater diversity of serotypes associated with IPD (5;59). von Gottberg et al (54) conducted active laboratory-based, national surveillance for IPD in children <5 years in South Africa to determine the epidemiology of IPD in the pre-conjugate vaccine era. Results showed that 58%, 65% and 85% of cases and 61%, 64% and 82% of deaths were caused by serotypes included in PCV-7, PCV-10 and PCV-13, respectively. It was also shown that compared to serotype 14, serotypes 18C and 8 were more likely to cause meningitis and serotypes 6A, 19A and 16 were more likely to cause bacteremia. Furthermore, compared to serotype 14, serotypes 6B, 18C, 1, 5 and 8 were significantly less likely to be associated with HIV-infected children (54). It

would be interesting to see if similar observations are made in an adult population in South Africa.

1.3 Justification for this study

There are no studies to date in South Africa that have looked at the association of serotypes with mortality amongst adults with IPD. In addition, few studies were able to determine the association between serotypes and mortality in the absence of potential effects of pneumococcal vaccination (58). The study population of this study included adult patients with IPD from 2003 to 2008, which was prior to the widespread introduction of PCV-7 and PCV-13 in South Africa. Understanding serotype-specific disease outcome provides knowledge with regard to the potential benefits of vaccines with serotypes that are associated with increased mortality (57). Therefore, results of this study will provide insight when planning health interventions to control mortality associated with IPD in South Africa.

1.4 Literature review

1.4.1 Serotype epidemiology

1.4.1.1 Association of serotypes with carriage and IPD

Serotypes associated with nasopharyngeal carriage include most of the serotypes found in PCV-7 (except serotype 4), serotype 6A, 19A, 3 and 7F (24). Of the known serotypes, a small number cause the majority of invasive disease (5;60).

Regev-Yochay et al (61) compared the rate of *S. pneumoniae* carriage among adults with that among children ≤6 years from 50 primary care clinics in Israel. *S. pneumoniae* carriage rates differed significantly between adults and children. The most common serotypes among adults were 6A, 14, 6B, 33F, 11A, 3 and 10, which accounted for 47.4% of all isolates from adults and 24.1% of all isolates from children (p=0.02). The most frequently isolated serotypes among children were 6B, 19F, 23F, 6A, 14, 35B and 23A, which accounted for 53.1% of all isolates and only 26.3% of all isolated from adults (p=0.01). Adetifa et al (62) performed a cross sectional survey to describe pneumococcal carriage in Nigeria. Carriage was higher in children (67.4%) compared to adults (26%), was highest in infants <9 months and reduced significantly with age (p<0.001). The most common serotypes were 19F (18.6%) and 6A (14.4%).

Hausdorff et al (5) conducted a review of 73 international studies and showed that serotypes vary depending on geographic location and patient age over time, although serogroups 1, 6, 14, 19 and 23 were predominant in every region. Serotypes 12F, 8, 22F, 15A, 15B and 11A caused a large proportion of disease in the older (adult) age groups (5). In addition, serotypes 1 and 3 were associated with severe disease regardless of age and other factors contributing to disease severity (63). Brueggemann et al (64) compared 150 *S. pneumoniae* isolates causing invasive disease to 351 nasopharyngeal carriage isolates in children <5 years in Oxford. Results showed that serotypes 1, 4 and 7F were associated with a high level of invasiveness. Serotypes 1, 4, 7F and 9V were also found among the invasive isolates in a study performed in Stockholm, Sweden where 273

invasive isolates (257 from adults and 16 from children) and 246 nasopharyngeal isolates were analyzed by serotyping and molecular serotyping (65). This indicates that these pneumococcal serotypes, although less common among carriers, had a high capacity of causing invasive diseases as compared to other serotypes (64;65).

A more recent meta-analysis of serotypes causing IPD among children <5 years of age showed that serotype 14 was the most common in every region and serotypes 1, 5, 6A, 14 19F and 23F were the cause of more than 50% of IPD in every region. Serotype 1 was predominant in Asia, Africa and Europe indicating that this serotype is not only common in developing countries (60). A prospective observational cohort study was conducted in a large UK hospital over two years in patients ≥16 years of age with community-acquired pneumonia. Results indicate that the most prevalent serotypes in the cohort were serotypes 14 (n=45), 1 (n=40), 8 (n=35), 3 (n=20) and 19A (n=20) (66). Another study conducted in Portugal to determine the serotype and antimicrobial susceptibility of 1265 *S. pneumoniae* isolates in adult patients with IPD between 2009 and 2011. The results were compared to previously published data from 1999 to 2008. It was shown that serotypes 3 (12.6%), 7F (10.0%), 19A (9.1%), 14 (8.4%), 1 (6.9%) and 8 (6.2%) were the most common serotypes which accounted for 53.2% of adult IPD. Serotype 1 decreased from 10.7% to 4.1% and serotype 5 decreased from 2.0% to 0% during the study period (2009-2011) (67). Imohl et al (68) evaluated the association of serotypes of *S. pneumoniae* with age in IPD in Germany from 1992 to June 2006. 54.0% of the isolates were from adults ≥16 years of age and

46.0% were from children <16 years of age. Serotypes 3 and 4 were more common in adults while serotypes 14, 6B, 19F and 18C were predominant in children.

1.4.1.2 Association of serotypes with HIV

Hausdorff et al (24) analyzed data from six studies from Africa and North America and demonstrated that HIV-infected adults have more infections with the serotypes present in PCV-7. Studies also showed that the association between the pediatric serotypes and IPD in HIV-infected adults was not a result of increased antibiotic resistance alone (69;70). Moreover, studies also show that HIV-infected women were at greater risk of disease caused by the pediatric serotypes as compared to HIV-infected men. This could be attributed to the fact that mothers are more likely to come into contact with pediatric carriers of pneumococci (71).

1.4.2 Risk factors for mortality in adults with IPD

1.4.2.1 Association of pneumococcal serotypes and mortality

A systematic review and meta-analysis of 9 studies by Weinberger et al (57) determined the association of serotype with risk of death due to IPD. Results suggested that among adult patients presenting with bacteremic pneumonia, the risk of death varies by serotype. However, this was not seen among meningitis patients. Serotypes 1, 7F, and 8 were associated with decreased risk ratios (RRs) of death while serotypes 3, 6A, 6B, 9N and 19F were associated with increased

RRs of death. In addition, it was found that serotypes with increased RRs of death had a higher carriage prevalence and low invasiveness.

A retrospective cohort study by Jansen et al (58) using nationally representative surveillance data from 1075 hospitalized adult patients with IPD in the Netherlands from 1 June 2004 to 31 May 2006 attempted to determine the association among serotypes, disease characteristics and outcome among adult patients with IPD. The serotypes were grouped according to those with a low (1, 5, 7F, 15B, 20 and 33F), intermediate (4, 6A, 8, 9V, 10A, 11A, 12F, 14, 19A, 22A, 22F, 23F and 24F) and high case-fatality ratios (CFR) (3, 6B, 9N, 16F, 18C, 19F and 23A). On multivariable analysis, in comparison to the low-CFR reference group of serotypes, the group of serotypes 3, 6B, 9N, 16F, 18C, 19F and 23A were associated with increased CFR (odds ratio [OR] 2.6, 95% confidence interval [CI] 1.5-4.7). These results indicate that the serotypes were independently associated with outcome of IPD in adults (58).

A more recent study by van Hoek et al (72) investigated the effect of serotype on clinical presentation and outcome from IPD in all ages after the introduction of PCV-7 and PCV-13. Results indicated that there were significant differences between serotypes in the ability to cause meningitis and mortality. Serotypes 31 (CFR of 33%), 11A (CFR of 30%) and 19F (CFR of 21%) had the highest CFR amongst the 5 to 64 years age group. In addition, serotypes 3, 19F, 19A, 6A, 9N, 11A and 31 were associated with higher mortality as compared to serotype 14 (8%) in the 5 to 64 years age group. Nyasulu et al (73) determined the risk factors

for mortality in South African children <5 years of age with IPD. Results showed no association between childhood serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) and risk of death.

1.4.2.2 Age as a risk factor for mortality

Kothe et al (74) conducted a prospective multicentre study initiated by the German Competence Network for Community-Acquired Pneumonia to assess the risk factors influencing community-acquired pneumonia mortality. Overall mortality was significantly higher among elderly patients, ≥65 years (10.3%) than in the younger age group, <65 years (2.2%; p<0.001).

In a prospective cohort study of 404 patients in the USA, Waterer et al showed that increasing age (41 to 80 years) was independently associated with medium-term mortality (75). In addition, Feikin et al (6) used a population-based, active surveillance in North America to assess the epidemiologic factors affecting mortality from pneumococcal pneumonia. Results indicate that increased mortality was associated with increased age (18-64 years, OR 5.1, 95% CI 1.2-21.0; 65-74 years OR 5.8, 95% CI 1.4-25.0; ≥75 years, OR 12, 95% CI 2.8-49.0) compared to ≤17 years of age.

1.4.2.3 Underlying medical conditions, including HIV as a risk factor for mortality

van Hoek et al (76) conducted a study in England where over 22 000 IPD patients aged two years and over were linked to hospital records. Among IPD patients aged 16-64 years, increased odds of developing IPD was associated with liver disease (OR 33.3, 95% CI 30.7-36.1); immunosuppression (OR 17.1, 95% CI 16.0-18.3) and HIV (OR 61.2, 95% CI 51.3-72.9). In addition, within each age-group, the CFRs were higher for those with underlying medical conditions compared to those without underlying medical conditions (OR 2.5 in children, 95% CI 1.2-5.1; OR 3.9 in 16-64 year olds, 95% CI 3.4-4.4; OR 1.2 in ≥65 year olds, 95% CI 1.1-1.3). However, no deaths were seen in the HIV groups as the numbers were too small.

As mentioned previously, Feikin et al (6) used population-based, active surveillance in North America to assess the epidemiologic factors affecting mortality from pneumococcal pneumonia. Results show that increasing mortality was independently associated with underlying medical conditions (OR 2.8, 95% CI 2.0-3.9). In addition, Klemets et al (77) used population-based data reported by Finnish clinical microbiology laboratories to determine risk of mortality associated with underlying medical conditions in patients with IPD. The highest hazard ratios were seen in patients with alcohol-related disease (HR 5.6, 95% CI 3.8-8.1); HIV-infection (HR 4.8, 95% CI 2.1 -10.9) and non-hematological malignancy (HR 4.7, 95% CI 3.3-6.7).

Renee et al (78) conducted a chart review of 147 hospitalized HIV-infected and HIV-uninfected IPD patients in New England to characterize epidemiologic and clinical features, serotypes, as well as antibiotic susceptibility patterns. Mortality rates did not differ by HIV status [13% (n=38) in HIV-infected versus 12% (n=115) in HIV-uninfected] even after controlling for age.

The burden of pneumococcal disease among adults and older children in Africa, with a high HIV prevalence setting, has not been well established. A prospective cohort study by Feikin et al (79) in western Kenya showed a high burden of pneumococcal bacteremia amongst individuals ≥5 years of age. It was also found that the extrapolated rate of pneumococcal bacteremia among HIV- infected adults (≥18 years of age) was 2399 per 100 000 person-years as compared to 122 per 100 000 person-years in HIV- uninfected adults (rate ratio 19.7, 95% CI 12.4–31.1). Studies by Cohen et al performed in the US after PCV-7 and highly-active antiretroviral treatment (HAART) introduction, showed that incidence of IPD in HIV-infected adults was 40-times higher than the incidence of IPD in HIV-uninfected adults (80).

Jones et al (81) determined the burden of bacteremia due to *S. pneumoniae* in HIV-infected children and adults in South Africa. The incidence of bacteremia was 8.2 times increased in HIV-infected adults and 36.9 times increased in HIV-infected children compared to HIV-uninfected individuals.

1.4.3 Impact of antiretroviral therapy (ART) on IPD in South Africa

In Gauteng Province, South Africa, prior to the introduction of PCV-7 into the public sector, Nunes et al (82) aimed to evaluate the trends in IPD hospitalizations in HIV-infected adults after the up-scaling of the HAART program from 2003 to 2008. The periods 2003 to 2004, 2005 to 2006 and 2007 to 2008 were defined as the early-HAART era, intermediate-HAART era and established-HAART era, respectively (82). Despite the introduction of HAART for HIV-infected individuals, the burden of IPD had not decreased in adults that were HIV-infected, while IPD-associated mortality rates in HIV-infected adults increased. Although declines in the burden of IPD in HIV-infected children were associated with the increased availability of HAART and prevention-of-mother-to-child-transmission (PMTCT), these children remained at a high risk for IPD-associated morbidity and mortality (83).

Kourtis et al described national hospital trends of IPD among HIV-infected adults and children after HAART and PCV-7 introduction in the United Stated using a national inpatient cohort. Data was analyzed from 1994-1995 (pre-HAART, pre-PCV era), 1998-1999 (HAART, pre-PCV era) and 2004-2005 (HAART, early PCV era). There was a 49.2% reduction in IPD hospitalizations among HIV-infected individuals between 1994/1995 and 2004/2005. In addition, the adjusted OR for IPD hospitalizations for HIV-infected individuals during 2004/2005 compared to 1994/1995 was 0.64 (95% CI, 0.54-0.77). However, regardless of the overall decreases observed, the rate of IPD hospitalizations remained much higher during

2004-2005 among HIV-infected individuals compared to HIV-uninfected individuals (84).

1.5 Study objectives

1.5.1 To describe trends in incidence of IPD by serotype in South Africa using national surveillance data from 2003 to 2008.

1.5.2 To describe the distribution of serotypes by age, HIV status, clinical syndrome and in-hospital outcome, among adult patients with IPD in South Africa using national surveillance data from 2003 to 2008.

1.5.3 To determine the association between serotypes and mortality rates overall and among adults with meningitis and bacteremia separately, adjusting for confounders, in South Africa using national surveillance data from 2003 to 2008.

CHAPTER 2

MATERIALS AND METHODS

This chapter describes the study population, data sources as well as data management. In addition, variables are defined and a description of the study hypotheses, statistical methods and data analyses are given.

2.1 Study design

This is a secondary retrospective analysis of cross-sectional data from national, population-based surveillance for IPD in South Africa. Although surveillance data could be described as cohort data as all cases within the population are collected on an ongoing basis, for this secondary analysis, data on the exposure and outcome are collected at the same point in time. The study period was from 1 January 2003 to 31 December 2008.

2.2 GERMS-SA: Primary data source

The Group for Enteric Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA) (85) plays a role in describing the epidemiology and estimating the burden of invasive disease caused by several pathogens. Approximately 200 clinical microbiology laboratories in all provinces participate in

the program. These include private, public, mining and military laboratories. Basic demographic information including gender, age, specimen collection date and source of specimen, are collected for all cases using standardized case report forms (CRFs). In 2003, the surveillance system was enhanced to include 25 sentinel enhanced surveillance sites. There is at least one enhanced surveillance site in each province in South Africa. Hospitals at these sites are regional, tertiary academic or referral centres. The National Health Laboratory Service (NHLS) laboratories are well resourced and have the capacity to accommodate laboratory testing for the sites. Information collected at enhanced surveillance sites includes admission date, previous antibiotic usage, HIV status, underlying medical conditions, discharge diagnosis and outcome for all cases. This differentiates the enhanced from the non-enhanced sites (85).

2.3 Study population and sampling

For the national surveillance, laboratories identify organisms under surveillance, and these are submitted with the laboratory reports to the NICD. Limited data are collected at non-enhanced sites. For the enhanced surveillance sites, the laboratories inform the surveillance officers who determine the patient's location and informed consent is obtained from the patient, following which a case report form is filled in. If the surveillance officer is unable to locate the patient, or the patient has been discharged or has died, the surveillance officer completes the case report form retrospectively using the hospital record forms (85).

For the seasonality and outbreaks analysis the study population included adult IPD patients (≥15 years) presenting at enhanced and non-enhanced surveillance sites from 2003 to 2008. For the trend analysis the study population included adult IPD patients (≥15 years) with the 20 most common serotypes presenting at enhanced and non-enhanced surveillance sites from 2003 to 2008. For the descriptive and multivariable analyses the study population included all adult IPD patients (≥15 years), with complete CRFs, known in-hospital outcome and with the 20 most common serotypes presenting at enhanced surveillance sites from 2003 to 2008. The rationale for only using the enhanced surveillance sites in this secondary data analysis, is that these sites include valid data on admission, in-hospital outcome, diagnosis and HIV status that is required for this analysis (85). The top 20 most common serotypes were used for the analyses as patients presenting with these serotypes constituted approximately 84% of the study population. The remaining 16% of serotypes not included in the analysis constituted 51 other serotypes whose prevalence ranged from 0.1% to 1%.

2.4 Data management

The data for the secondary analysis was obtained from the GERMS-SA surveillance database which is in Microsoft-Access. Data from the enhanced surveillance sites was used for the analysis. The database was monitored and cleaned on an ongoing basis with quality assurance processes in place. Before analyzing, validity checks were done to ensure completeness and to identify

transposition, copying, consistency and range errors. This was done by performing frequencies and cross tabulations. Discrepancies were resolved by comparing the database with the hard copy of the forms. The database was then corrected accordingly. The variables of interest were recoded and categorized as necessary.

2.5 Definitions

All variables were defined before the data were analyzed.

2.5.1 Main exposure of interest

The main exposure of interest was serotype. The 20 most common serotypes were included as a categorical variable in multiple levels in the analysis.

2.5.2 Additional potential confounders and risk factors

- HIV status was defined as positive or negative documented form medical records of tests done during current or prior admission, documented by a clinician.
- Disease severity was measured using the Pitt bacteremia score (86;87). The scores resulting from the following parameters were added to form the Pitt bacteremia score:

(i) Oral temperature: 2 points for a temperature of ≤35ºC or ≥40ºC, 1 point for temperature of 35.1ºC-36.0ºC or 39.0ºC–39.9ºC and 0 point for temperature of 36.1ºC-38.9ºC.

(ii) Hypotension: 2 points for systolic blood pressure of less than 90 mm Hg.

(iii) 2 points for receipt of mechanical ventilation.

(iv) 4 points for cardiac arrest.

(v) Mental status: if alert 0 point; disoriented 1 point; stuporous 2 points and comatose 4 points (87).

Acute severe illness was defined as a Pitt bacteremia score of >4 at the time of specimen collection.

- Age-group: Adult patients were categorized into 4 groups. These include 15-24 years, 25-44 years, 45-64 years and ≥65 years.

- Disease syndrome: Pneumococcal meningitis diagnosis was defined by a positive CSF culture or a positive blood culture and clinical diagnosis of meningitis. Pneumococcal pneumonia diagnosis was based on clinician diagnosis of pneumonia with a positive blood or pleural fluid culture (85). Pneumococcal bacteremia without focus was defined by a positive blood culture that had no other known focus of infection (58;88).

- Nosocomial infection was defined as the specimen collection date ≥2 days after hospital admission date.

- Prior antibiotic use (prior 24 hours) was defined as any antibiotic use 24 hours prior to specimen collection.

- Prior antibiotics use (past two months) was defined as use of any antibiotics within two months preceding specimen collection.

- Underlying medical conditions were defined as asplenia, sickle cell anemia; chronic illnesses including lung , liver, renal, cardiac disease and diabetes; other immunocompromising conditions including malignancy, primary immunodeficiency, immunotherapy and organ transplant; as well as other Advisory Committee on Immunization Practices (ACIP) (89) risk factors including head injury with CSF leak, alcohol and smoking. Other risk factors include prematurity, neurological conditions, chromosomal abnormalities and burns. We used a binary variable consisting of the absence or presence of any underlying condition.

- Gender was defined as either male or female sex.

- Province was grouped into 3 categories based on poverty rates (low, intermediate and high) from the Findings of the living conditions survey 2008/2009 conducted by Statistics South Africa (90). The low poverty rate group consisted of Gauteng and Western Cape. The intermediate poverty rate group consisted of KwaZulu-Natal, Free State, Northern Cape and

North West. The high poverty rate group consisted of Eastern Cape, Mpumalanga and Limpopo. This survey was used as it covers a wide range of indicators including household income, household expenditure, minimum income, ownership of assets, self-perceived poverty status as well as access to services and facilities.

- Race was grouped into “Black” and “other”, where “other” consisted of White, Asian and Colored.

- Isolates defined as either intermediately or resistant to penicillin were regarded as non-susceptible using the conservative Clinical and Laboratory Standards Institute meningeal breakpoints (91).

- Multi-drug resistance was defined as non-susceptibility to any three or more different antibiotic classes, according to the 2009 definitions of the Clinical and Laboratory Standards Institute (91).

- Vaccine serotype was defined as the 13 serotypes found in the PCV-13 vaccine i.e 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.

- Appropriate antibiotic use: data on the antibiotics used for data management was compared with the recommended antibiotic guidelines to determine whether the appropriate antibiotic was administered to the patient **(Table 2.1)** (92).

Table 2.1: Criteria to determine appropriate antibiotic use

Antibiotic *	**Meningitis**	**Pneumonia or Bacteremia**	**Other** [#]
1st gen cephalosporin (cefalexin, cefazolin, cefradine)	Inappropriate	Appropriate	Appropriate
2nd gen cephalosporin (cefaclor, cefoxitin, cefuroxime, cefprozil)	Inappropriate	Appropriate	Appropriate
3rd gen cephalosporin (cefotaxime, ceftazidime, ceftriaxone, cefpodoxime)	Appropriate	Appropriate	Appropriate
4th gen cephalosporin (cefepime)	Appropriate	Appropriate	Appropriate
Imipenem	Appropriate	Appropriate	Appropriate
Meropenem	Appropriate	Appropriate	Appropriate
Piperacillin	Inappropriate	Appropriate	Appropriate
Tazobactam	Inappropriate	Appropriate	Appropriate
Amoxicillin	Inappropriate	Appropriate	Appropriate
Augmentin	Inappropriate	Appropriate	Appropriate
Erythromycin	Inappropriate	Appropriate	Appropriate

*Other antibiotics and antifungal agents that were not used to create appropriate antibiotic variable include: amikacin, amphotericin B, chloramphenicol, cipromycin, clindamycin,

cloxacillin, cotrimoxazole (for therapeutic and prophylactic use), flagyl, fluconazole, gentamicin, nalidixic acid, ofloxacin, streptomycin, tetracycline, vancomycin. These antibiotics and antifungal agents were excluded as they are not used individually for the treatment of IPD.

[#]Other illnesses include bacteremia without focus and pneumococcus isolated from other sterile sites (joint aspirate, pleural fluid, peritoneal fluid).

2.5.3 Outcome

Final outcome of the patient was defined as the patient's in-hospital outcome. Mortality was defined as death within 30 days from the first positive result for *S. pneumoniae*. IPD is a severe acute bacterial infection and patients usually die within a day or two of hospitalization (73). If the patient died after 30 days of positive *S. pneumoniae* result, it is likely that the patient died from other causes. The final outcome variable was binary including "died" and "survived." The "survived" category consisted of discharged, transferred, and refused hospital treatment. "Refused hospital treatment" was defined as patients who either refused admission or refused ongoing care in hospital. These patients were given medication before being sent home. The likely outcome of such cases is that they survived.

2.6 Laboratory methods

2.6.1 Pneumococcal serotyping

Pneumococcal serotypes were serotyped by capsule swelling known as the Quellung reaction resulting from the interaction between the pneumococcal capsular polysaccharide and its homologous antibody (93). The pneumococci are typed further by a capsular reaction test using the “Chess-board Method” where pneumococcal groups/types react with one or two pooled antisera. Once a positive reaction is obtained with the pooled antisera, individual group and serotype-specific antisera included in the pooled antisera are tested to determine the serogroup and serotype (94). This was done using sera from the Statens Serum Institut (SSI), Copenhagen, Denmark (95).

2.7 Statistical methods and data analysis

All available data were used for the analysis. The data from Microsoft-Access was exported into Microsoft-Excel 2003 and analyzed using STATA version 12; Stata Corp, College Station, TX.

Proportions and frequencies were used for the descriptive statistics. Significant trends in incidence by serotype were assessed using Poisson regression as it quantifies increases or decreases in incidences and gives *p* values, indicating statistical significance. Seasonality and outbreaks was assessed by evaluating the

incidence of IPD cases by year and month. To assess the association between serotypes and age, HIV co-infection, clinical syndrome and in-hospital outcome for each serotype compared to serotype 4 (the serotype which is third most common serotype (289/3953, 7% and had an intermediate CFR of 29%), we used univariate multinomial regression models and generated a separate estimate of effect for each predictor on each outcome relative to the base level. The effect measures are the relative risk ratios (the ratios of two relative risks) where each relative risk describes the probability of the outcome in the category of interest relative to the baseline category (96).

Descriptive statistics was reported by means of tables and graphs. For the inferential statistics, univariate analysis was done using logistic regression, where crude OR was calculated (with 95% CI and *p* values). Univariate logistic regression was done to assess differences between enhanced and non-enhanced surveillance site data. If patients from enhanced sites differ substantially from patients presenting at non-enhanced sites, this could affect generalizability of the findings.

Univariate logistic regression was done to assess the risk factors (variables) associated with mortality. Multivariable logistic regression was then done to evaluate the risk factors that were statistically significant, $p < 0.1$ in the univariate analysis. This was done using stepwise forward selection and backward elimination. Inferential statistics were reported by means of tables. Potential confounders were controlled for in the multivariable regression model. All

statistical tests excluded missing values. In this study, $p<0.05$ in the multivariable analysis was considered statistically significant.

2.8 Ethical approval

The GERMS-SA obtained ethical clearance from the Human Research Ethics Committee (Medical), University of Witwatersrand Johannesburg (M081117: GERMS-SA: Provision of Strategic Information through Laboratory-based Surveillance for AIDS-associated Bacterial and Fungal Opportunistic Infections in South Africa: Meeting date 28 November 2008 – renewed annually in 2009 and 2010). Ethics clearance was also obtained from the relevant University and Provincial Ethics Committees for the enhanced surveillance sites. Ethics approval was obtained from the Human Research Ethics Committee (Medical), University of Witwatersrand for this secondary analysis (M121050).

CHAPTER 3

Results

This chapter gives an in depth explanation of the results found in the secondary data analysis.

3.1 Overview

From January 2003 through December 2008 we identified 27632 patients of which 15105 had known age and were aged ≥15 years (55%) **(Figure 1)**. Of these, 8962 were ≥15 years presenting with the 20 most common serotypes. These patients were included in the serotype trend analysis.

Of the 15105 patients, 7329 (49%) patients presented at enhanced sites, of which 3407 (46%) were male, 3895 (53%) were female and 27 (1%) were unknown. 2684 patients had known HIV status of which 2401 (89%) were HIV-infected and 283 (11%) were HIV-uninfected. Of the HIV-infected patients 180 (8%) were on ART. Of the 7329 patients presenting at enhanced sites, 6061 patients had a clinical record review of which 6021 (99%) had a known in-hospital outcome.

We included 3953 (84%) patients in the univariate and multivariable analysis of risk factors for mortality that had viable isolates, had known outcome, completed case report forms and presented with the 20 most common serotypes. Of these

patients, 1807 (46%) were male and 2146 (54%) were female and the median age was 36 years. HIV-infected patients constituted 89% (2309/2580), while HIV-uninfected patients constituted 11% (271/2580) of the study population with known HIV status. Of the HIV-infected individuals, 51% (1184/2309) had a $CD4^+$ counts of <200 cells/µL and 178 (8%) were on ART. In addition, the prevalence of HIV was highest among individuals 25-44 years of age (1688/2309, 73%). The prevalence of nonsusceptibility to penicillin was 28% (1089/3953). The majority (99%; 1081/1089) of these isolates displayed intermediate resistance to penicillin, with only 1% (8/1089) showing high-level resistance. The prevalence of multi-drug resistance was 15% (609/3953).

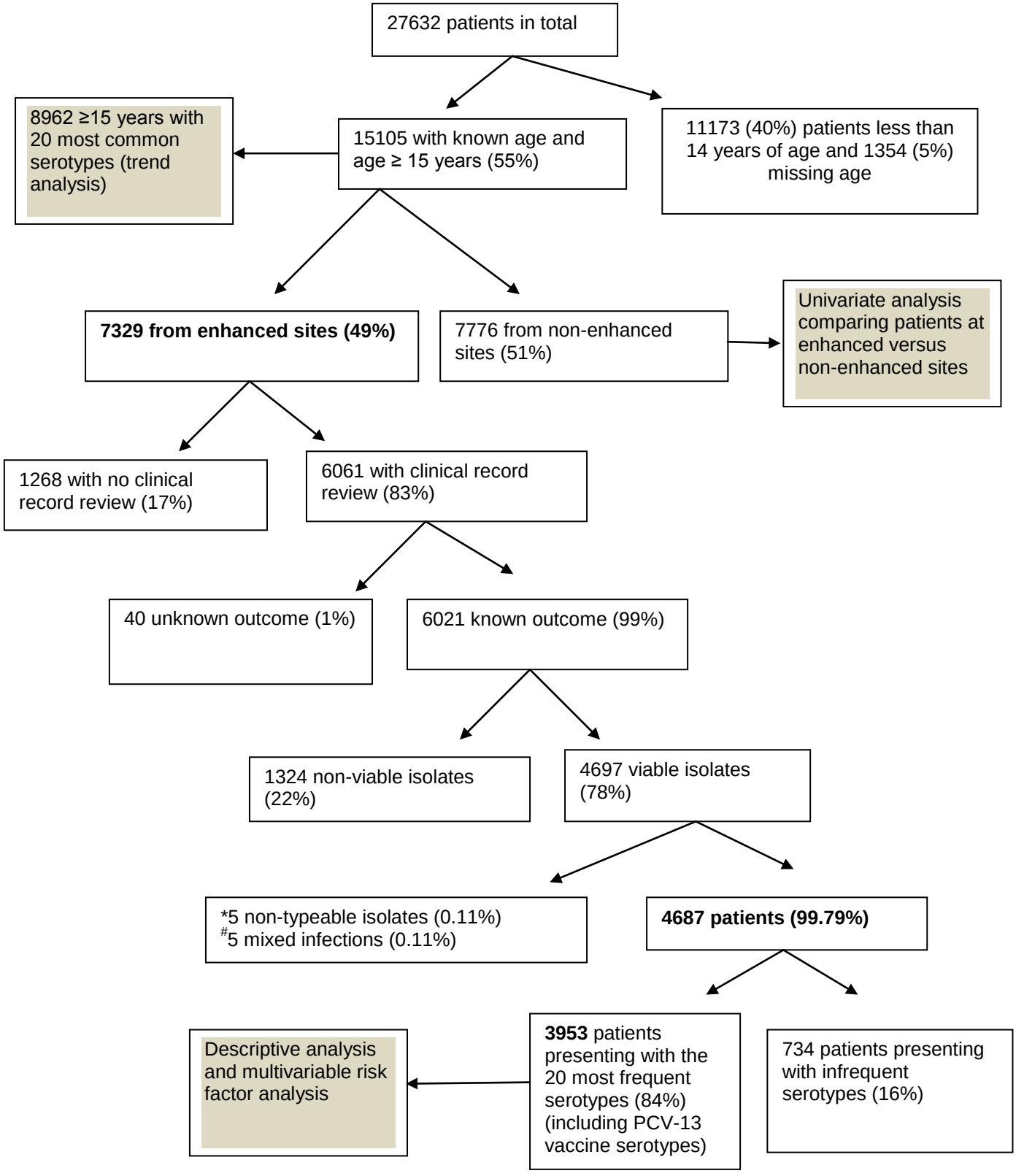

Figure 1: Flow chart of patients enrolled in GERMS-SA from 2003-2008 and included in the analysis.*Pneumococci that did not have a capsule and could not be serotyped. # Isolates from patients that were infected with two strains of pneumococci with different serotypes from the same patient.

3.2 Serotype changes over time

Over the study period there were 8962 patients ≥15 years presenting with the 20 most common serotypes. The incidence of serotypes 4 (p<0.001); 19A (p<0.001); 23F (p=0.003) and 18C (p=0.002) increased significantly from 2003 to 2008. The incidence of serotype 1 (p<0.001); 25 (p=0.001) (97) and 5 (p=0.042) decreased significantly over the study period (2003-2008) **(Figure 2)**.

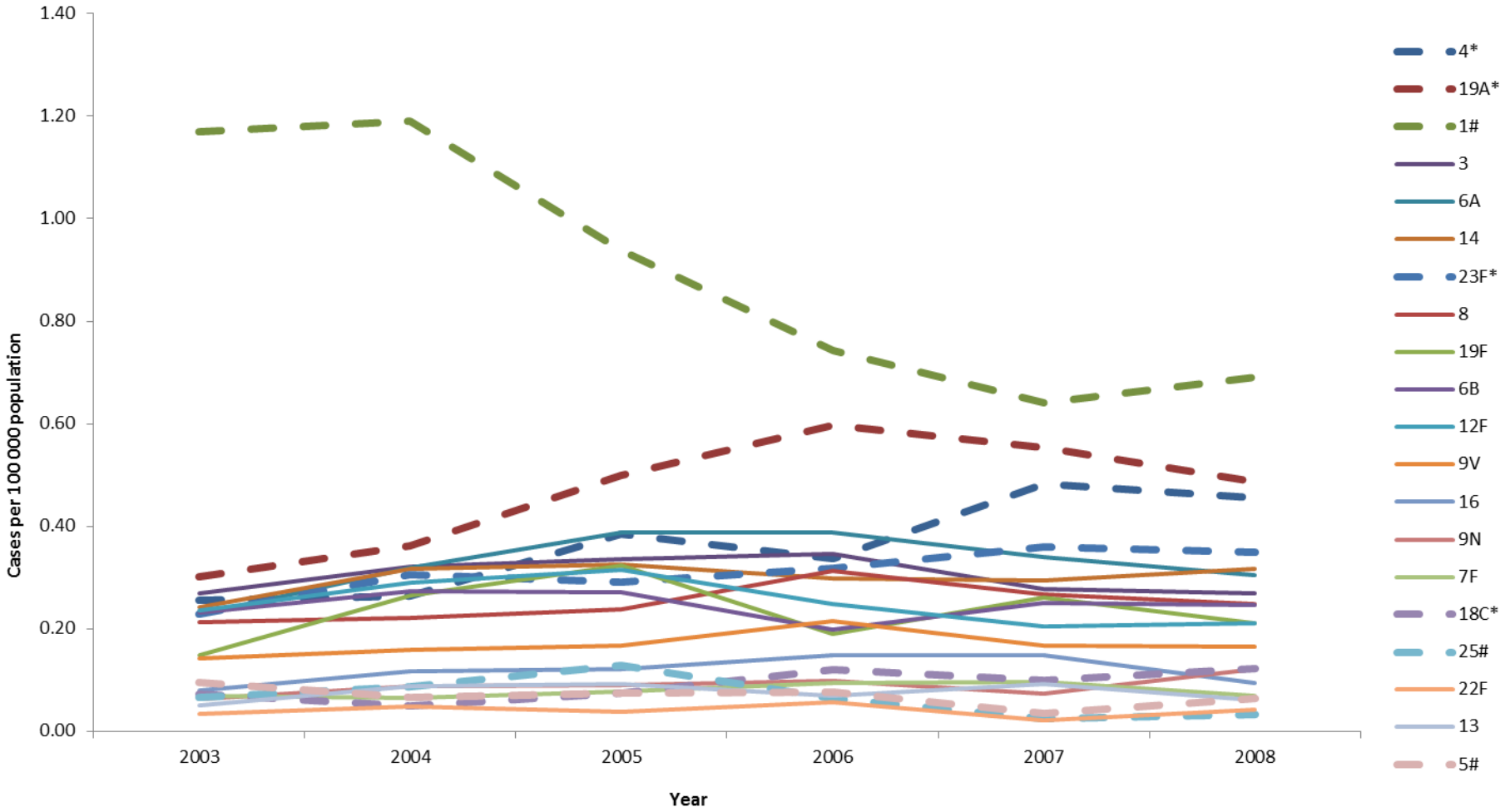

Figure 2: Incidence of invasive pneumococcal disease among persons 15 years and older by the top 20 serotypes causing disease, GERMS-SA, 2003-2008 (n=3988) *Significant increase in trends, broken lines. #Significant decrease in trends, broken lines.

3.3 IPD seasonality and outbreaks

Over the study period there were 15105 patients ≥15 years of age presenting with IPD caused by all serotypes at enhanced and non-enhanced sites **(Figure 3)**. IPD exhibited seasonal variations with the peak incidences during the winter months (May to August). When restricted to the top 20 most common serotypes the seasonal variations of IPD incidences were similar to that of all serotypes causing IPD (data not shown). There was no evidence of obvious outbreaks during the study period.

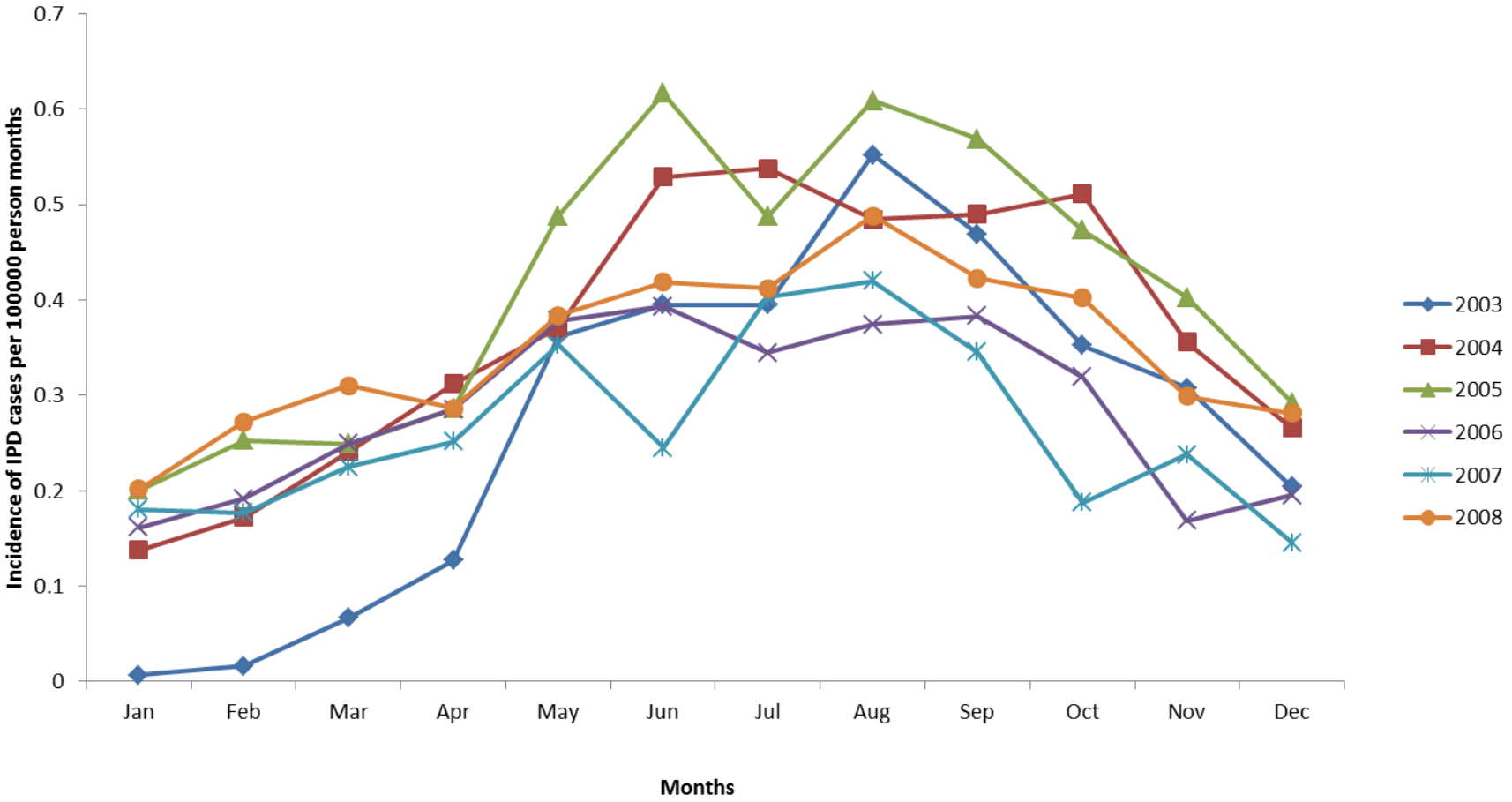

Figure 3: Seasonality of invasive pneumococcal disease among persons 15 years and older by all serotypes causing disease, GERMS-SA, 2003-2008.

3.4 Distribution of IPD serotypes by age group, HIV status, syndrome and mortality

We chose serotype 4 as the reference group as it is the third most common serotype (289/3953, 7%) and has an intermediate CFR (29%). There was no significant difference in the distribution of serotype 4 amongst the different age-groups (p=0.792), HIV status (p=0.812), clinical diagnosis (p=0.265) and CFRs (p=0.062). Analyzing the 20 most common serotypes, using multinomial regression, comparing the age distribution of all other serotypes to the age distribution of serotype 4 (25-44 years as the referent group), serotype 3 was significantly more likely to be identified from patients 45-64 years of age (relative risk ratio [RRR] 1.88, 95% CI 1.26-2.81, p=0.003), serotype 1 was significantly more likely to be isolated from patients 15-24 years of age (RRR 1.79. 95% CI 1.15–2.80, p=0.010) and serotype 19F was significantly more likely to be isolated from patients 45-64 years of age (RRR 1.64 95% CI 1.05–2.56, p=0.030) **(Figure 4)**.

Compared to serotype 4, serotypes 12F (RRR 3.12, 95% CI 1.05-9.31, p=0.041) and 9V (RRR 11.99, 95% CI 1.59-90.30, p=0.016) caused significantly more disease in HIV-infected individuals and serotype 1 (RRR 0.50, 95% CI 0.30-0.83, p=0.008) was significantly less likely to be identified in HIV-infected patients **(Figure 5)**. Serotypes 19A, 3, 6A, 14, 23F, 12F, 9N, 7F, 18C, 25, and 5 differed when compared to serotype 4 by clinical syndrome. Serotypes 6A (RRR 1.47, 95%

CI 1.03-2.10, *p*=0.032) , 23F (RRR 1.59, 95% CI 1.11-2.28, *p*=0.011), 12F (RRR 1.77, 95% CI 1.18-2.65, *p*=0.005), 18C (RRR 2.15, 95% CI 1.20-3.84, *p*=0.010) were more likely to cause meningitis, while serotypes 19A (RRR 2.24, 95% CI 1.58-3.17 *p*<0.001), 3 (RRR 3.31, 95% CI 2.14-5.12, *p*<0.001), 14 (RRR 2.10, 95% CI 1.40-3.15, *p*<0.001), 9N (RRR 2.21, 95% CI 1.21-4.04, *p*=0.009), 7F (RRR 2.14, 95% CI 1.14-4.06, *p*=0.019), 25 (RRR 2.63, 95% CI 1.32-5.28 *p*=0.006) and 5 (RRR 6.31, 95% CI 2.21-18.04, *p*=0.001) were more likely to cause bacteremia **(Figure 6)**. In addition, serotypes 1 (RRR 1.51, 95% CI 1.12-2.04 *p*=0.007), 23F (RRR 1.61, 95% CI 1.12-2.30, *p*=0.010) and 19F (RRR 2.32, 95% CI 1.60-3.37, *p*<0.001) were significantly more likely to cause death in patients compared to serotype 4. Serotype 25 (RRR 0.44, 95% CI 0.22-0.91, *p*=0.027) was significantly less likely to cause death in patients compared to serotype 4 **(Figure 7)**.

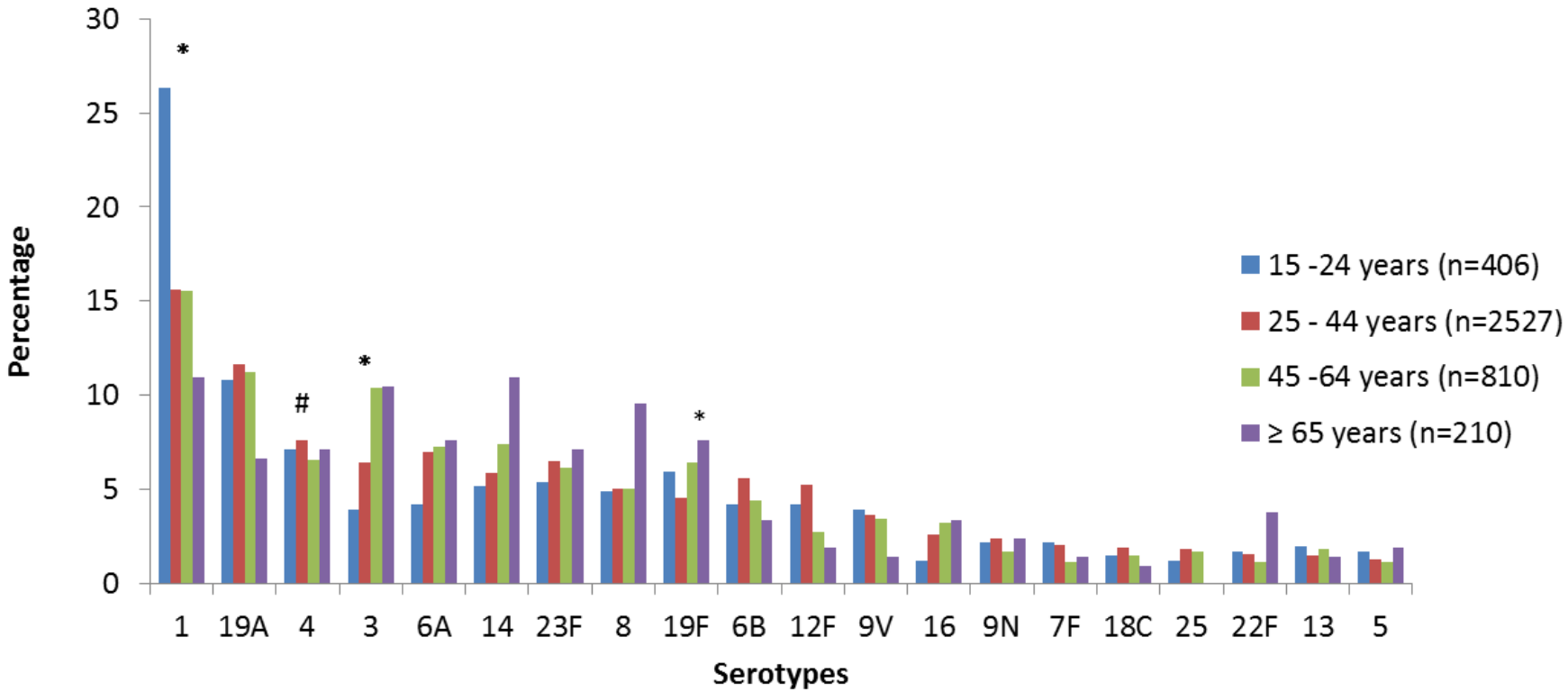

Figure 4: Distribution of IPD serotypes by age groups, GERMS-SA, 2003 to 2008.

[#] Referent group. * Significant serotypes.

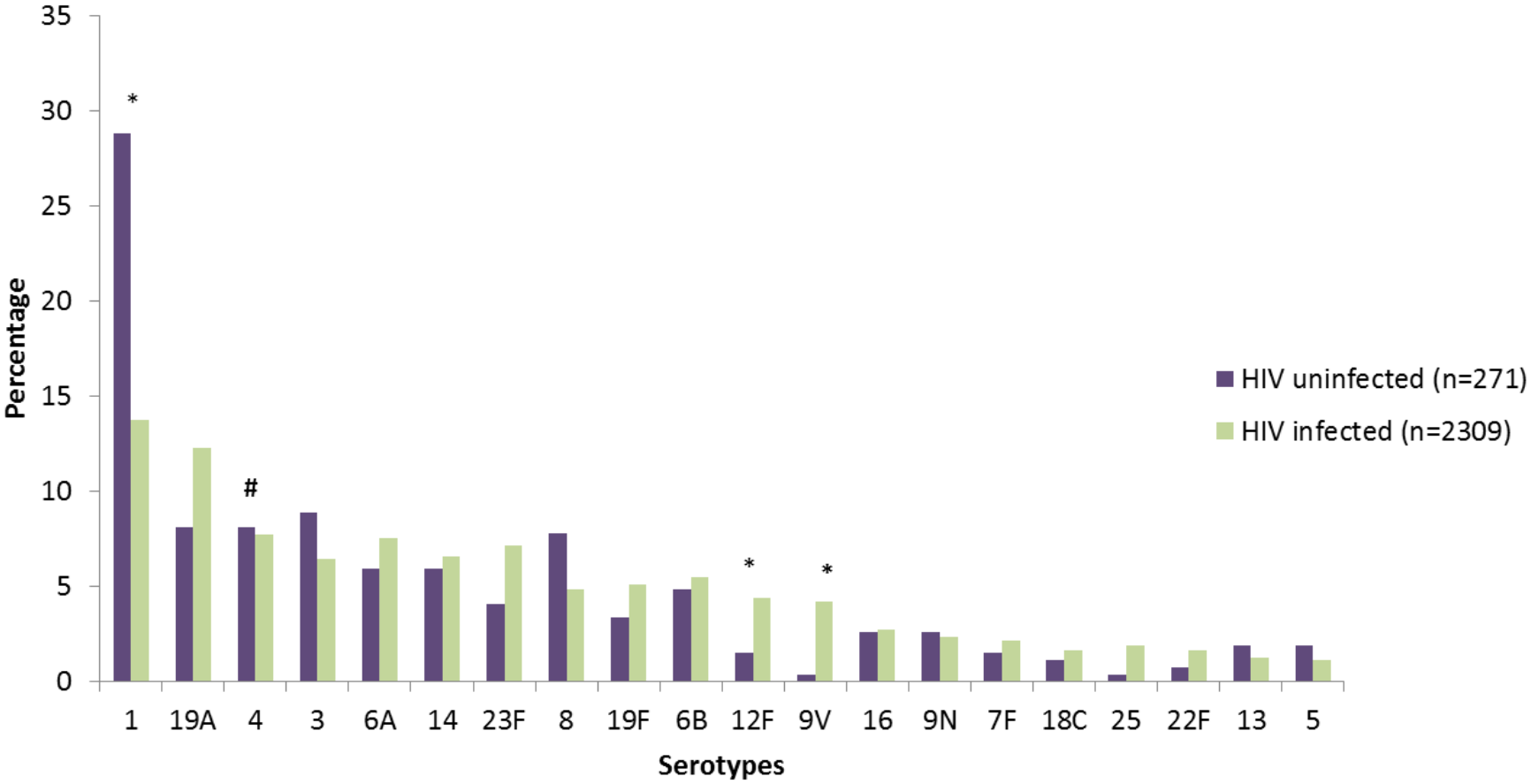

Figure 5: Distribution of IPD serotypes by HIV status, GERMS-SA, 2003 to 2008.

[#] Referent group. * Significant serotypes.

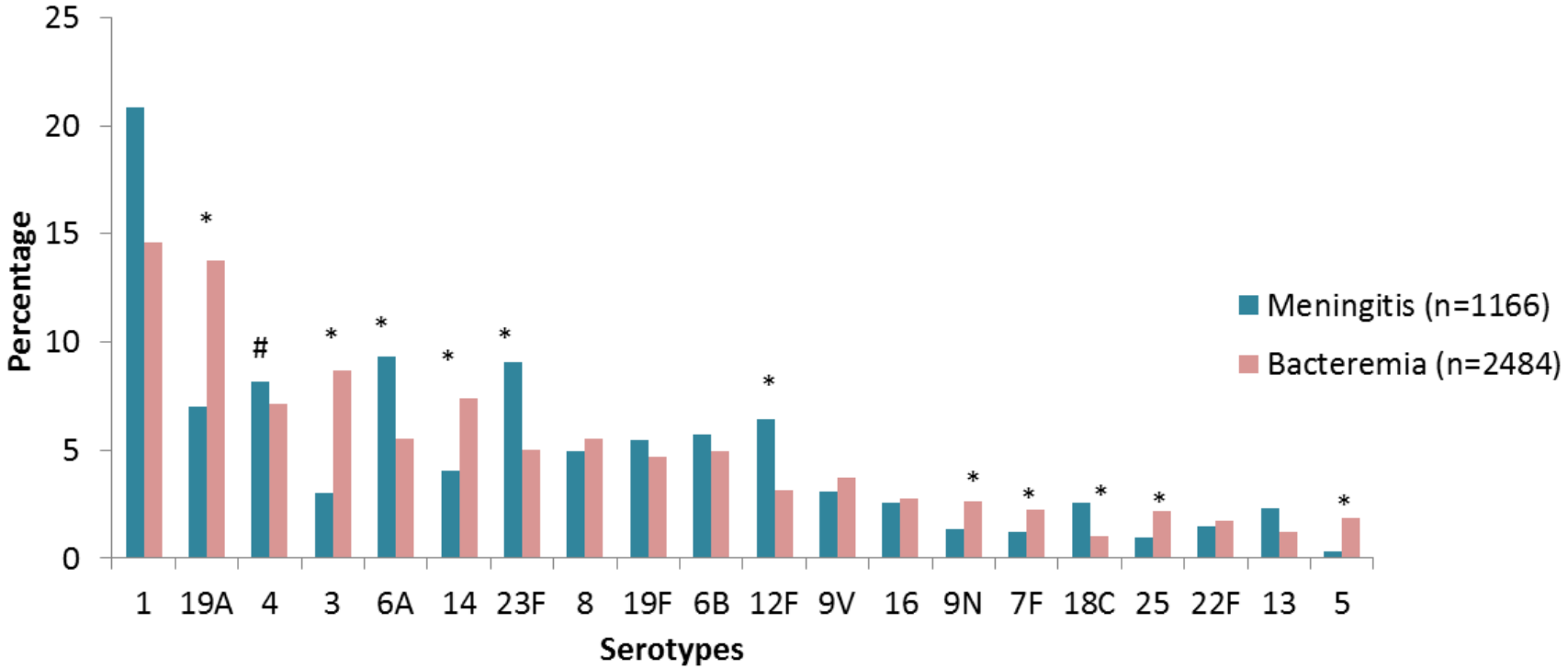

Figure 6: Distribution of IPD serotypes by clinical diagnosis, GERMS-SA, 2003 to 2008.

[#] Referent group. * Significant serotypes.

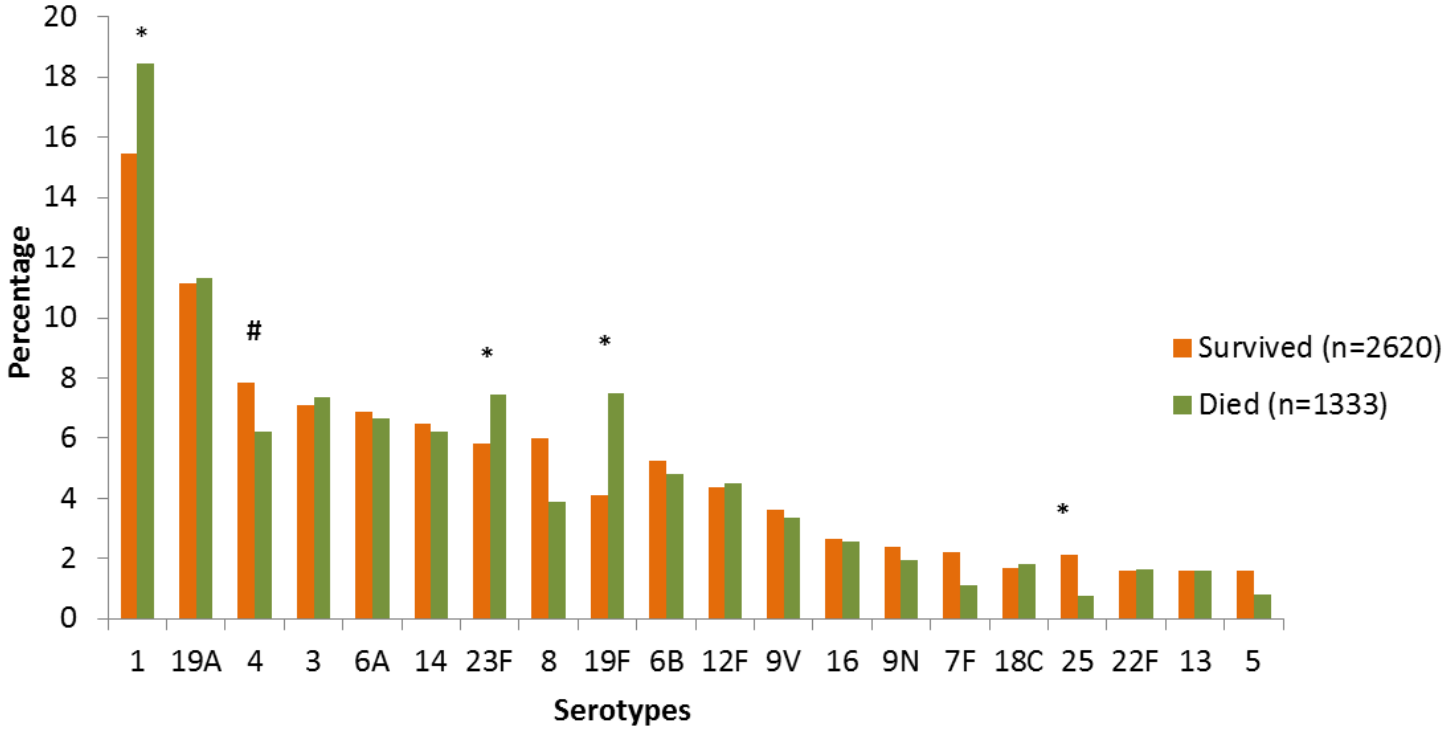

Figure 7: Distribution of IPD serotypes by in-hospital outcome, GERMS-SA, 2003 to 2008.

[#] Referent group. * Significant serotypes.

3.5 Comparison of cases presenting to enhanced and non-enhanced sites

Of the total number of cases 7610/16459 (46%) were from enhanced sites while 8849/16459 (54%) were from non-enhanced sites. On univariate analysis, there were significantly less cases reported from the provinces with intermediate poverty rates (OR 0.78, 95% CI 0.72-0.84) and high poverty rates (OR 0.27, 95% CI 0.23-0.30), compared to those with low poverty rates at enhanced sites. Patients belonging to White, Colored and Indian race groups were more likely to present at enhanced sites compared to non-enhanced sites (OR 4.21, 95% CI 3.37-5.24).

There was a significant difference in the type of specimen collected between enhanced and non-enhanced sites. Positive blood specimens were more likely to be collected from enhanced sites (OR 3.15, 95% CI 2.94-3.38) compared to non-enhanced sites. There were more positive CSF specimens collected at non-enhanced sites [3949/8849 (45%)], compared to enhanced sites [1817/7610 (24%)]. Serotypes 19A (OR 1.27, 95% CI 1.16-1.70), 3 (OR 1.31, 95% CI 1.16-1.79), 9N (OR 1.49, 95% CI 1.18-2.27), 25 (OR 1.53, 95% CI 1.15-2.46) and 22F (OR 27.65, 95% CI 9.16-97.49) were more likely to be isolated from patients presenting at enhanced sites compared to non-enhanced sites **(Table 1)**.

Table 1: Comparison of descriptive demographics and clinical factors for adult patients from GERMS-SA enhanced and non-enhanced sites (2003-2008).

Variable		Enhanced sites	Non-enhanced sites			
		N= 7610	N= 8849			
		n (%)	n (%)	Unadjusted OR	*p*	95% CI
Age group	15-24 years	776/7329 (11)	881/7776 (11)	Ref	Ref	Ref
	25-44 years	4715/7329 (64)	4889/7776 (63)	1.09	0.089	0.99-1.22
	45-64 years	1483/7329 (20)	1633/7776 (21)	1.03	0.616	0.91-1.16
	≥65 years	355/7329 (5)	373/7776 (5)	1.08	0.384	0.91-1.29
	Missing	281	1073			
Gender	Females	4013/7540 (53)	4500/8575 (52)	Ref	Ref	Ref
	Males	3527/7540 (47)	4075/8575 (48)	0.97	0.345	0.91-1.03
	Missing	70	274			
Province	Low poverty	5534/7610 (73)	5144/8849 (58)	Ref	Ref	Ref
	Intermediate	1521/7610 (20)	1814/8849 (21)	**0.78**	**<0.001**	**0.72-0.84**

	poverty					
	High poverty	555/7610 (7)	1891/8849 (21)	**0.27**	**<0.001**	**0.25-0.30**
Race	Black	6402/6946 (92)	4751/4847 (98)	Ref	Ref	Ref
	Other	544/6946 (8)	96/4847 (2)	**4.21**	**<0.001**	**3.37-5.24**
	Missing	664	4002			
Specimen	CSF	1817/7610 (24)	3949/8849 (45)	Ref	Ref	Ref
	Blood	5116/7610 (67)	3531/8849 (40)	**3.15**	**<0.001**	**2.94-3.38**
	Other	677/7610 (9)	1369/8849 (15)	1.07	0.189	0.44-0.49
Serotype	1	775/4651 (17)	1038/5042 (21)	1.10	0.374	0.89-1.36
	19A	498/4651 (11)	438/5042 (9)	**1.27**	**0.019**	**1.16-1.70**
	4	328/4651 (7)	405/5042 (8)	Ref	Ref	Ref
	3	325/4651 (7)	279/5042 (6)	**1.31**	**0.019**	**1.16-1.79**
	6A	317/4651 (7)	368/5042 (7)	0.97	0.751	0.86-1.31
	14	298/4651 (6)	334/5042 (7)	1.10	0.374	0.89-1.36
	23F	312/4651 (7)	338/5042 (7)	1.03	0.761	0.92-1.41

8	234/4651 (5)	261/5042 (5)	1.00	0.968	0.88-1.39
19F	248/4651 (5)	245/5042 (5)	1.13	0.294	0.99-1.57
6B	242/4651 (5)	290/5042 (6)	0.94	0.571	0.82-1.29
12F	223/4651 (5)	291/5042 (6)	0.86	0.203	0.75-1.19
9V	167/4651 (4)	178/5042 (4)	1.05	0.708	0.90-1.50
16	121/4651 (3)	125/5042 (2)	1.08	0.588	0.90-1.60
9N	102/4651 (2)	77/5042 (2)	**1.49**	**0.021**	**1.18-2.27**
7F	84/4651 (2)	73/5042 (1)	0.89	0.152	1.01-2.01
18C	83/4651 (2)	102/5042 (2)	0.91	0.583	0.73-1.39
25	75/4651 (2)	55/5042 (1)	**1.53**	**0.029**	**1.15-2.46**
22F	74/4651 (2)	3/5042 (0.1)	**27.65**	**<0.001**	**9.16-97.49**
13	79/4651 (2)	69/5042 (1)	1.28	0.173	0.99-2.01

5	66/4651 (1)	73/5042 (1)	1.01	0.944	0.78-1.61

Factors statistically significant on univariate logistic regression presented in boldface.

3.6 Common serotypes

Serotype 1 was the most common serotype (651/3953, 16%), followed by serotype 19A (443/3953, 11%), serotype 4 (289/3953, 7%) serotype 3 (284/3953, 7%) and serotype 6A (269/3953, 7%).

3.7 Case-fatality ratios

The CFR for meningitis was 55% (641/1166) and 23% (576/2484) for bacteremia (p<0.001). Serotype 19F had the highest CFR of 48% (100/207), followed by serotype 23F with 39% (99/252) and serotype 1 with 38% (246/651).

3.8 Univariate analysis of risk factors associated with mortality in adults with IPD

On univariate analysis the variables found to be significantly associated with mortality using logistic regression p<0.1 were age-group, province, disease syndrome, disease severity, prior antibiotic use (24 hours), prior antibiotic use (2 months), appropriate antibiotic treatment, HIV status, nosocomial infection, vaccine serotype, multi-drug resistance and serotype **(Table 2)**.

3.9 Multivariable analysis of the association with serotypes and mortality rates among adults with IPD, adjusting for other risk factors

On multivariable analysis factors independently associated with mortality were serotypes 1 (OR 1.93, 95% CI 1.05–3.53) and 19F (OR 2.89, 95% CI 1.38–6.06) compared to serotype 4; increasing age (25-44 years, OR 1.75, 95% CI 1.03–2.95; 45-64 years, OR 3.56, 95% CI 2.00–6.35; ≥65 years, OR 5.17, 95% CI 1.89–14.14; compared to 15-24 years); living in provinces with intermediate (OR 1.65, 95% CI 1.16–2.35) or high poverty rates (OR 1.72, 95% CI 1.02–2.92) compared to provinces with low poverty rates; having meningitis (OR 4.07, 95% CI 2.98–5.55) compared to bacteremia; prior antibiotic use in the last two months (OR 3.93, 95% CI 2.50–6.20); inappropriate antibiotic use (OR 2.37, 95% CI 1.74–3.22) and positive HIV status (OR 1.69, 95% CI 1.04–2.75) **(Table 2)**.

Table 2: Univariate and multivariable analysis of risk factors for mortality amongst adults with IPD.

Risk factors	N=3953	All	CFR	Univariable analysis			Multivariable analysis		
		n/N (%)	n/N (%)	Unadjusted OR	*p*	95% CI	Adjusted OR	*p*	95% CI
Age group	15-24 years	406/3953 (10)	92/406 (23)	Ref	Ref	Ref	Ref	Ref	Ref
	25-44 years	2527/3953 (64)	793/2527 (31)	**1.56**	**<0.001**	**1.22 – 2.00**	**1.75**	**0.037**	**1.03 – 2.95**
	45-64 years	810/3953 (21)	348/810 (43)	**2.57**	**<0.001**	**1.96 – 3.37**	**3.56**	**<0.001**	**2.00 – 6.35**
	≥65 years	210/3953 (5)	100/210 (48)	**3.10**	**<0.001**	**2.17 – 4.43**	**5.17**	**0.001**	**1.89 – 14.14**
Gender	Females	2146/3952 (54)	719/2146 (34)	Ref	Ref	Ref	-	-	-
	Males	1806/3952 (46)	614/1806 (34)	1.02	0.744	0.90 – 1.17	-	-	-
Province	Low poverty	2934/3953 (74)	915/2934 (31)	Ref	Ref	Ref	Ref	Ref	Ref
	Intermediate poverty	780/3953 (20)	301/780 (39)	**1.39**	**<0.001**	**1.18 – 1.63**	**1.65**	**0.005**	**1.16 – 2.35**
	High poverty	239/3953 (6)	117/239 (49)	**2.12**	**<0.001**	**1.62 – 2.76**	**1.72**	**0.042**	**1.02 – 2.92**
Race	Black	3534/3930 (90)	1203/3534 (34)	Ref	Ref	Ref	-	-	-
	Other	396/3030 (10)	121/396 (31)	0.85	0.164	0.68 – 1.07	-	-	-
Disease syndrome	Meningitis	1166/3923 (30)	641/1166 (55)	**4.04**	**<0.001**	**1.16 – 2.19**	**4.07**	**<0.001**	**2.98 – 5.55**

	Bacteremia	2484/3923 (63)	576/2484 (23)	Ref	Ref	Ref	Ref	Ref	Ref
	Other	273/3923 (7)	98/273 (36)	1.86	<0.001	0.29 – 0.54	1.61	0.104	0.91 – 2.85
Disease severity	Pitt bacteremia score <4	2933/3953 (76)	864/2993 (29)	Ref	Ref	Ref	NS	NS	NS
	Pitt bacteremia score ≥4	429/3953 (11)	292/429 (60)	2.35	<0.001	2.03 – 2.73	NS	NS	NS
Prior antibiotic use (24 hours)	Yes	96/2968 (3)	41/96 (43)	2.08	0.001	1.37 – 3.14	NS	NS	NS
	No	2872/2968 (97)	759/2872 (26)	Ref	Ref	Ref	NS	NS	NS
Prior antibiotic use (2 months)	Yes	149/2402 (6)	59/149 (40)	**2.57**	**<0.001**	**1.82 – 3.62**	**3.93**	**<0.001**	**2.50 – 6.20**
	No	2253/2402 (94)	458/2253 (20)	Ref	Ref	Ref	Ref	Ref	Ref
Appropriate antibiotic use	Yes	3005/3923 (77)	900/3005 (30)	Ref	Ref	Ref	Ref	Ref	Ref
	No	918/3923 (23)	415/918 (45)	**1.93**	**<0.001**	**1.66 -2.25**	**2.37**	**<0.001**	**1.74 – 3.22**
Underlying medical conditions	Yes	1022/3953 (26)	328/1022 (32)	0.91	0.201	0.78 – 1.05	-	-	-
	No	2931/3953 (74)	1005/2931 (34)	Ref	Ref	Ref	-	-	-
HIV status	Positive	2309/2580 (58)	687/2309 (30)	**1.46**	**0.013**	**1.08 – 1.97**	**1.69**	**0.035**	**1.04 – 2.75**
	Negative	271/2580 (7)	61/271 (23)	Ref	Ref	Ref	Ref	Ref	Ref
Nosocomial infection	Yes	168/3953 (4)	73/168 (44)	1.54	0.007	1.13 – 2.10	NS	NS	NS
	No	3785/3953	1260/3785	Ref	Ref	Ref	NS	NS	NS

		(96)	(33)						
Penicillin resistance	Resistant	1089/3953 (28)	385/1089 (35)	Ref	Ref	Ref	-	-	-
	Susceptible	2864/3953 (72)	948/2864 (33)	0.90	0.181	0.78 – 1.05	-	-	-
Multi-drug resistant	Yes	609/3953 (15)	230/609 (38)	1.23	0.022	1.03 – 1.47	NS	NS	NS
	No	3344 (85)	1103/3344 (33)	Ref	Ref	Ref	NS	NS	NS
Serotype	1	651/3953 (17)	246/651 (38)	**1.51**	**0.007**	**1.12 – 2.04**	**1.93**	**0.034**	**1.05 – 3.53**
	19A	443/3953 (11)	151/443 (34)	1.28	0.128	0.93 – 1.77	1.79	0.075	0.94 – 3.41
	4	289/3953 (7)	83/289 (29)	Ref	Ref	Ref	Ref	Ref	Ref
	3	284/3953 (7)	98/284 (35)	1.31	0.137	0.92 – 1.86	1.15	0.727	0.53 – 2.51
	6A	269/3953 (7)	89/269 (33)	1.23	0.265	0.86 – 1.76	0.78	0.501	0.37 – 1.63
	14	253/3953 (6)	83/253 (33)	1.21	0.303	0.84 – 1.75	2.05	0.049	1.00 – 4.20
	23F	252/3953 (6)	99/252 (39)	1.61	0.010	1.12 - 2.30	1.89	0.073	0.94 – 3.80
	8	209/3953 (5)	52/209 (25)	0.82	0.342	0.55 – 1.23	1.21	0.637	0.54 – 2.72
	19F	207/3953 (5)	100/207 (48)	**2.32**	**<0.001**	**1.60 – 3.37**	**2.89**	**0.005**	**1.38 – 6.06**
	6B	202/3953 (5)	64/202 (32)	1.15	0.481	0.78 – 1.70	1.35	0.428	0.65 – 2.80
	12F	175/3953 (4)	60/175 (34)	1.30	0.209	0.87 – 1.94	1.16	0.757	0.46 – 2.89
	9V	140/3953 (4)	45/140 (32)	1.18	0.468	0.76 – 1.82	1.81	0.187	0.75 – 4.37
	16	103/3953 (3)	34/103 (33)	1.22	0.414	0.75 – 1.98	0.44	0.201	0.13 – 1.54
	9N	89/3953 (2)	26/89 (29)	1.02	0.928	0.61 – 1.73	0.92	0.888	0.28 – 3.01
	7F	73/3953 (2)	15/73 (21)	0.64	0.163	0.34 – 1.20	0.97	0.965	0.32 – 2.99
	18C	68/3953 (2)	24/68 (35)	1.35	0.288	0.77 – 2.37	1.33	0.611	0.44 – 4.07
	25	66/3953 (2)	10/66 (15)	0.44	0.027	0.22 – 0.91	0.76	0.701	0.19 – 3.06
	22F	64/3953 (2)	22/64 (34)	1.30	0.371	0.73 – 2.31	1.31	0.674	0.37 – 4.62
	13	63/3953 (2)	21/63 (33)	1.24	0.468	0.69 – 2.22	1.95	0.234	0.65 – 5.83
	5	53/3953 (1)	11/53 (21)	0.65	0.235	0.32 – 1.32	0.28	0.291	0.03 – 2.98

Vaccine serotype	Yes	3254/3953 (82)	1116/3254 (34)	1.16	0.099	0.97 – 1.38	-	-	-
	No	669 /3953 (18)	217/699 (31)	Ref	Ref	Ref	-	-	-

Factors statistically significant on multivariable logisitic regression presented in boldface.

- Factors not statistically significant on univariate logistic regression ($p>0.1$) were omitted from the multivariable logistic regression model (except for the serotype-specific comparison).

NS: Factors not statistically significant on multivariable logistic regression ($p\geq0.05$) (except for the serotype-specific comparison).

3.10. Multivariable analysis of the association with serotypes and mortality rates among adults with IPD amongst disease syndromes, adjusting for other risk factors.

On multivariable analysis restricted to patients presenting with bacteremic pneumonia, the factors independently associated with mortality were serotypes 14 (OR 2.20, 95% CI 1.04-4.67) and 19F (OR 3.54, 95% CI 1.58-7.96) compared to serotype 4; increasing age (25-44 years, OR 2.94, 95% CI 1.32-6.56; 45-64 years OR 6.95, 95% CI 3.05-15.88; ≥65 years OR 13.05. 95% CI 5.29-32.19 compared to 15-24 years); prior antibiotic use in the last two months (OR 2.32, 95% CI 1.39-3.87) and inappropriate antibiotic treatment (OR 2.75, 95% CI 1.97-3.83) **(Table 3)**.

Table 3: Univariate and multivariable analysis of risk factors for mortality amongst adults with bacteremic pneumonia.

Risk factors	N=2514	All	CFR	Univariable analysis			Multivariable analysis		
		n/N (%)		Unadjusted OR	*p*	95% CI	Adjusted OR	*p*	95% CI
Age group	15-24 years	227/2514 (9)	25/227 (11)	Ref	Ref	Ref	Ref	Ref	Ref
	25-44 years	1601/2514 (64)	329/1601 (21)	**2.09**	**0.001**	**1.36-3.22**	**2.94**	**0.009**	**1.32-6.56**
	45-64 years	533/2514 (21)	179/533 (34)	**4.09**	**<0.001**	**2.60-6.43**	**6.95**	**<0.001**	**3.05-15.88**
	≥65 years	153/2514 (6)	61/153 (40)	**5.36**	**<0.001**	**3.16-9.07**	**13.05**	**<0.001**	**5.29-32.19**
Gender	Females	1319/2513 (52)	296/1319 (22)	Ref	Ref	Ref	Ref	Ref	Ref
	Males	1194/2513 (48)	298/1194 (25)	1.15	0.138	0.96-1.38	-	-	-
Province	Low poverty	2020/2514 (80)	455/2020 (23)	Ref	Ref	Ref	Ref	Ref	Ref
	Intermediate poverty	432/2514 (17)	123/432 (29)	1.37	0.008	1.08-1.73	NS	NS	NS
	High poverty	62/2514 (3)	16/62 (26)	1.20	0.543	0.67-2.13	-	-	-
Race	Black	2213/2499 (89)	519/2213 (24)	Ref	Ref	Ref	Ref	Ref	Ref
	Other	286 (11)	70/286 (25)	1.06	0.701	0.79-1.41	-	-	-
Disease severity	Pitt bacteremia score <4	1984/2514 (79)	406/1984 (21)	Ref	Ref	Ref	Ref	Ref	Ref
	Pitt bacteremia	530/2514 (21)	188/530 (36)	2.14	<0.001	1.73-2.63	NS	NS	NS

	score ≥4								
Prior antibiotic use (24 hours)	Yes	41/1956 (2)	15/41 (37)	2.75	0.002	1.44-5.25	NS	NS	NS
	No	1915/1956 (98)	332/1915 (17)	Ref	Ref	Ref	Ref	Ref	Ref
Prior antibiotic use (2 months)	Yes	97/1633 (6)	29/97 (30)	**2.72**	**<0.001**	**1.72-4.31**	**2.32**	**0.001**	**1.39-3.87**
	No	1536/1633 (94)	208/1536 (14)	Ref	Ref	Ref	Ref	Ref	Ref
Appropriate antibiotic use	Yes	1976/2484 (80)	397/1976 (20)	Ref	Ref	Ref	Ref	Ref	Ref
	No	508/2484 (20)	179/508 (35)	**2.16**	**<0.001**	**1.75-2.68**	**2.75**	**<0.001**	**1.97-3.83**
Underlying medical conditions	Yes	706/2514 (28)	183/706 (26)	1.19	0.091	0.97-1.45	-	-	-
	No	1808/2514 (72)	411/1808 (23)	Ref	Ref	Ref	Ref	Ref	Ref
HIV status	Positive	1562/1733 (90)	322/1562 (21)	1.01	0.964	0.68-1.49	-	-	-
	Negative	171/1733 (10)	35/171 (21)	Ref	Ref	Ref	Ref	Ref	Ref
Nosocomial infection	Yes	89/2514 (4)	32/89 (36)	1.86	0.006	1.19-2.90	NS	NS	NS
	No	2425/2514 (96)	562/2425 (23)	Ref	Ref	Ref	Ref	Ref	Ref
Penicillin resistance	Yes	696/2514 (28)	192/696 (28)	1.34	0.004	1.10-1.64	NS	NS	NS
	No	1818/2514 (72)	402/1818 (22)	Ref	Ref	Ref	Ref	Ref	Ref
Multi-drug resistance	Yes	375/2514 (15)	111/375 (30)	1.44	0.003	1.13-1.84	NS	NS	NS
	No	2139/2514 (85)	483/2139 (23)	Ref	Ref	Ref	Ref	Ref	Ref

Serotype	1	365/2514 (15)	62/365 (17)	1.02	0.947	0.63-1.64	1.15	0.709	0.55-2.38
	19A	344/2514 (14)	94/344 (27)	1.87	0.008	1.18-2.95	1.52	0.251	0.75-3.05
	4	179/2514 (7)	30/179 (17)	Ref	Ref	Ref	Ref	Ref	Ref
	3	223/2514 (9)	73/223 (33)	2.42	<0.001	1.49-3.91	2.01	0.061	0.97-4.20
	6A	139/2514 (6)	32/139 (23)	1.49	0.164	0.85-2.59	0.72	0.517	0.27-1.95
	14	188/2514 (7)	58/188 (31)	**2.22**	**0.002**	**1.34-3.65**	**2.20**	**0.039**	**1.04-4.67**
	23F	125/2514 (5)	27/125 (22)	1.37	0.288	0.77-2.44	1.52	0.352	0.63-3.65
	8	139/2514 (6)	15/139 (11)	0.60	0.133	0.31-1.17	0.81	0.649	0.32-2.04
	19F	116/2514 (5)	51/116 (44)	**3.90**	**<0.001**	**2.28-6.67**	**3.54**	**0.002**	**1.58-7.96**
	6B	125/2514 (5)	23/125 (18)	1.12	0.711	0.62-2.04	0.97	0.954	0.38-2.46
	12F	80/2514 (3)	16/80 (20)	1.24	0.529	0.63-2.04	1.16	0.778	0.41-3.34
	9V	98/2514 (4)	25/98 (26)	1.70	0.083	0.93-3.10	2.01	0.153	0.77-5.21
	16	69/2514 (3)	20/69 (29)	2.03	0.033	1.06-3.89	0.63	0.508	0.16-2.44
	9N	66/2514 (3)	17/66 (26)	1.72	0.115	0.88-3.39	1.11	0.863	0.35-3.53
	7F	56/2514 (2)	10/56 (18)	1.08	0.849	0.49-2.38	0.89	0.868	0.23-3.45
	18C	27/2514 (1)	7/27 (26)	1.74	0.252	0.68-4.48	1.47	0.583	0.37-5.83
	25	54/2514 (2)	7/54 (13)	0.74	0.505	0.31-1.79	0.54	0.440	0.11-2.60
	22F	43/2514 (2)	11/43 (26)	1.71	0.184	0.78-3.76	1.55	0.498	0.43-5.56
	13	31/2514 (1)	7/31 (23)	1.45	0.434	0.57-3.67	1.64	0.435	0.47-5.68
	5	47/2514 (2)	9/47 (19)	1.18	0.700	0.52-2.69	0.73	0.703	0.15-3.58
Vaccine serotype	Yes	2068/2514 (82)	494/2068 (24)	1.09	0.509	0.85-1.39	-	-	-
	No	446/2514 (18)	100/446 (22)	Ref	Ref	Ref	-	-	-

Factors statistically significant on multivariable logisitic regression presented in boldface.

- Factors not statistically significant on univariate logistic regression ($p>0.1$) were omitted from the multivariable logistic regression model (except for the serotype-specific comparison).

NS: Factors not statistically significant on multivariable logistic regression ($p\geq0.05$) (except for the serotype-specific comparison).

On multivariable analysis restricted to patients presenting with meningitis, the factors independently associated with mortality were ages 45-64 years (OR 6.32, 95% CI 2.53-15.80) compared to 15-24 years; disease severity (OR 2.55, 95% CI 1.51-4.31); prior antibiotic use in the last two months (OR 5.05, 95% CI 2.04-12.53); positive HIV status (OR 4.86, 95% CI 1.83-12.91) and vaccine serotypes (OR 1.39, 95% CI 1.03-1.97). Individual serotypes were not statistically significantly associated with mortality but were included in the multivariable analysis as it is the predictor/exposure variable and was selected a priori **(Table 4)**.

Table 4: Univariate and multivariable analysis of risk factors for mortality amongst adults with meningitis.

Risk factors	N=1196	All	CFR	Univariable analysis			Multivariable analysis		
		n/N (%)		Unadjusted OR	*p*	95% CI	Adjusted OR	*p*	95% CI
Age group	15-24 years	143/1196 (12)	56/143 (39)	Ref	Ref	Ref	Ref	Ref	Ref
	25-44 years	789/1196 (66)	430/789 (55)	1.86	0.001	1.29-2.68	1.80	0.125	0.85-3.83
	45-64 years	232/1196 (19)	149/232 (64)	**2.79**	**<0.001**	**1.81-4.29**	**6.32**	**<0.001**	**2.53-15.80**
	≥65 years	32/1196 (3)	24/32 (75)	4.66	0.001	1.96-11.10	5.91	0.168	0.47-74.18
Gender	Females	693/1196 (58)	379/693 (55)	Ref	Ref	Ref	Ref	Ref	Ref
	Males	503/1196 (42)	280/503 (56)	1.04	0.738	0.83-1.31	-	-	-
Province	Low poverty	730/1196 (61)	388/730 (53)	Ref	Ref	Ref	Ref	Ref	Ref
	Intermediate poverty	298/1196 (25)	174/298 (58)	1.24	0.126	0.94-1.62	-	-	-
	High poverty	168/1196 (14)	97/168 (58)	1.20	0.282	0.86-1.69	-	-	-
Race	Black	1125/1190 (95)	631/1125 (56)	Ref	Ref	Ref	Ref	Ref	Ref
	Other	65/1190 (5)	25/65 (39)	0.49	0.006	0.29-0.82	NS	NS	NS
Disease severity	Pitt bacteremia score <4	822/1196 (69)	408/822 (50)	Ref	Ref	Ref	Ref	Ref	Ref
	Pitt bacteremia score ≥4	374/1196 (31)	251/374 (67)	**2.07**	**<0.001**	**1.60-2.67**	**2.55**	**<0.001**	**1.51-4.31**
Prior antibiotic use (24 hours)	Yes	45/830 (5)	24/45 (53)	1.26	0.458	0.69-2.29	-	-	-
	No	785/830 (95)	374/785 (48)	Ref	Ref	Ref	Ref	Ref	Ref
Prior antibiotic use (2	Yes	36/630 (6)	24/36 (67)	**3.21**	**0.001**	**1.57-6.55**	**5.05**	**<0.001**	**2.04-12.53**

months)									
	No	594/630 (94)	228/594 (38)	Ref	Ref	Ref	Ref	Ref	Ref
Appropriate antibiotic use	Yes	832/1166 (71)	439/832 (53)	Ref	Ref	Ref	Ref	Ref	Ref
	No	334/1166 (29)	202/334 (61)	0.73	0.017	0.56-0.94	NS	NS	NS
Underlying medical conditions	Yes	215/1196 (18)	95/215 (44)	0.56	<0.001	0.43-0.79	NS	NS	NS
	No	981/1196 (82)	564/981 (58)	Ref	Ref	Ref	Ref	Ref	Ref
HIV status	Positive	646/720 (90)	341/646 (53)	**3.02**	**<0.001**	**1.77-5.16**	**4.86**	**0.002**	**1.83-12.91**
	Negative	74/720 (10)	20/74 (27)	Ref	Ref	Ref	Ref	Ref	Ref
Nosocomial infection	Yes	44/1196 (4)	24/44 (55)	0.98	0.940	0.54-1.79	-	-	-
	No	1152/1196 (96)	635/1152 (55)	Ref	Ref	Ref	Ref	Ref	Ref
Penicillin resistance	Yes	320/1196 (27)	168/320 (53)	Ref	Ref	Ref	Ref	Ref	Ref
	No	876/1196 (73)	491/876 (56)	1.15	0.275	0.89-1.49	-	-	-
Multi-drug resistance	Yes	181/1196 (15)	100/181 (55)	1.01	0.965	0.73-1.38	-	-	-
	No	1015/1196 (85)	559/1015 (55)	Ref	Ref	Ref	Ref	Ref	Ref
Serotype	1	245/1196 (20)	176/245 (72)	2.21	0.001	1.36-3.59	1.85	0.149	0.80-4.25
	19A	84/1196 (7)	53/84 (63)	1.48	0.198	0.81-2.69	1.13	0.811	0.41-3.18
	4	97/1196 (8)	52/97 (54)	Ref	Ref	Ref	Ref	Ref	Ref
	3	42/1196 (4)	17/42 (41)	0.59	0.157	0.28-1.23	0.16	0.103	0.02-1.45
	6A	110/1196 (9)	51/110 (46)	0.75	0.299	0.43-1.29	0.46	0.125	0.17-1.24
	14	51/1196 (4)	23/51 (45)	0.71	0.326	0.36-1.40	0.46	0.267	0.11-1.83
	23F	107/1196 (9)	59/107 (55)	1.06	0.826	0.61-1.85	0.86	0.765	0.33-2.28
	8	59/1196 (5)	33/59 (56)	1.10	0.777	0.57-2.11	0.89	0.851	0.26-3.01
	19F	64/1196 (5)	35/64 (55)	1.04	0.893	0.55-1.97	0.69	0.567	0.19-2.49
	6B	69/1196 (6)	39/69 (57)	1.13	0.710	0.60-2.09	1.02	0.975	0.35-2.96
	12F	76/1196 (6)	38/76 (50)	0.87	0.637	0.47-1.58	0.71	0.598	0.20-2.56

	9V	41/1196 (3)	21/41 (51)	0.91	0.797	0.44-1.89	0.14	0.098	0.01-1.44
	16	31/1196 (3)	13/31 (42)	0.63	0.260	0.28-1.42	0.26	0.073	0.06-1.13
	9N	16/1196 (3)	6/16 (38)	0.52	0.238	0.17-1.54	0.41	0.444	0.04-4.03
	7F	14/1196 (1)	3/14 (21)	0.24	0.034	0.06-0.90	0.38	0.271	0.07-2.12
	18C	31/1196 (3)	15/31 (48)	0.81	0.613	0.36-1.82	1.36	0.716	0.26-7.00
	25	11/1196 (1)	3/11 (27)	0.32	0.111	0.08-1.30	0.16	0.155	0.01-2.01
	22F	17/1196 (1)	9/17 (53)	0.97	0.959	0.35-2.73	0.45	0.409	0.07-3.01
	13	27/1196 (2)	11/27 (41)	0.59	0.239	0.25-1.41	1.15	0.851	0.26-5.17
	5	4/1196 (0)	2/4 (50)	0.87	0.887	0.12-6.40	0.83	0.923	0.02-36.02
Vaccine serotype	Yes	981/1196 (82)	555/981 (57)	**1.39**	**0.029**	**1.03-1.87**	**1.39**	**0.029**	**1.03-1.87**
	No	215/1196 (18)	104/215 (48)	Ref	Ref	Ref	Ref	Ref	Ref

Factors statistically significant on multivariable logisitic regression presented in boldface.

- Factors not statistically significant on univariate logistic regression ($p>0.1$) were omitted from the multivariable logistic regression model (except for the serotype-specific comparison).

NS: Factors not statistically significant on multivariable logistic regression ($p\geq0.05$) (except for the serotype-specific comparison).

CHAPTER 4

Discussion

4.1 Overview of study findings

This study done in the pre-vaccine era, serves as an important baseline from which to monitor the effects of PCV. Serotype 1 was the most common serotype followed by serotype 19A, serotype 4, serotype 3 and serotype 6A. The incidence of serotypes 4, 19A, 23F and 18C increased significantly while the incidence of serotype 1, 25 and 5 decreased significantly from 2003 to 2008. Serotype 19F had the highest CFR, followed by serotype 23F and serotype 1. This serotype distribution data could potentially help policy makers when deciding on introducing or transitioning to newer vaccines. *S pneumoniae* showed seasonal patterns with the peak incidences of IPD in the winter months (May to August). There was no evidence of obvious outbreaks during the study period. Increased crowding, temperature, humidity, light intensity and pollution have been suggested to cause annual fluctuations in IPD incidence (98-100).

On multivariable analysis, factors independently associated with mortality were disease caused by serotypes 1 and 19F compared to serotype 4, increasing age, living in provinces with intermediate or high poverty rates, having meningitis, prior antibiotic treatment in the last two months, inappropriate antibiotic treatment and positive HIV status. Serotypes associated with increased mortality are included in

the 10-and-13-valent pneumococcal conjugate vaccine and may be expected to become less common in adults as a result of indirect effects following routine immunization in infants. HIV-infected adults experience increased mortality which may be reduced by more widespread availability of antiretroviral therapy which will in turn substantially improve the quality of life of HIV-infected individuals in terms of physical and mental health and decrease the incidence of IPD and therefore mortality.

4.1.1 Serotypes associated with mortality

On multivariable analysis, serotypes 1 and 19F compared to serotype 4 were independently associated with mortality. Consistent with our findings, previous studies also found 19F to be associated with increased CFRs (57;58;101). Our findings also corroborates studies performed by Martens et al (63) which showed that serotype 1 was independently associated with mortality after controlling for age and other markers of disease severity. However, in contrast to our study, Weinberger et al (57) and Jansen et al (58) showed that serotype 1 was associated with the decreased risk of death. A possible explanation for this could be that the high prevalence of serotype 1 in our setting resulted in a substantial increase in power to detect a significant association of serotype 1 with mortality.

Moreover in previous studies, serotype 1 was found to be among the more invasive serotypes and serotype 19F was found to be among the less invasive serotypes (2;58;65;101). Previous studies also showed that serotypes with

increased RRs of death had a higher carriage prevalence and low invasiveness (57). Differences in our findings compared to other studies could be due to different populations being studied at different time points. Our decision to use serotype 4 as the referent group may also account for differences in our findings compared to other studies.

Consistent with previous studies, serotype 1 was found to be the commonest serotype in our study (5;60;101). Serotype 1 was also found to cause large outbreaks of IPD in the pre-antibiotic era (24). It has more recently been the cause of outbreaks of meningitis in Western Africa (102). However, there is no evidence of serotype 1 outbreaks in our population.

These findings may be useful to similar countries with similar populations who lack surveillance programs. Previous studies have shown that vaccination of young children with PCV-7 and PCV-13 inhibits nasopharyngeal carriage of vaccine serotypes and have the potential of also having indirect effects among older, unvaccinated individuals (103-106). Thus, serotypes 1 and 19F may be expected to become less common in adults as a result of indirect effects following routine immunization in infants. A potential concern is that replacement invasive disease caused by non-vaccine serotypes may occur after vaccination introduction, as seen in previous studies (101;107-109) and this may result in implications for immunocompromised individuals, particularly HIV-infected individuals with regard to disease severity and mortality (110). Continuous surveillance is required to

monitor IPD caused by non-vaccine serotypes to ensure that future vaccines include the relevant serotypes that are associated with mortality.

On multivariable analysis restricted to patients presenting with bacteremic pneumonia, serotypes 14 and 19F compared to serotype 4 were significantly associated with mortality. When restricted to meningitis, no individual serotypes were significantly associated with mortality. This is consistent with findings from the study performed by Weinberger et al (57) where in patients with bacteremic pneumonia caused by serotypes 3, 6A, 6B, 9N and 19F compared to serotype 14 were significantly more likely to die.

It was also found that among patients with meningitis, there were no individual serotypes that were significantly associated with mortality, compared to serotype 14. A possible explanation for the disjunction between serotypes associated with mortality in patients with bacteremic pneumonia and meningitis could be due to the high mortality rates in patients with meningitis, difficultly in diagnosing meningitis, and the fact that meningitis is a serious condition and serotypes may have a different and less important role in this instance.

4.1.2 Age as a risk factor for mortality

Using age-group 15-24 years as the referent group, increasing age remained significantly associated with mortality in our study. This corroborates several other studies (74;75;111). This could be due to the fact that as individuals age, their

immune systems age as well, a phenomenon known as immunosenescence. This makes older individuals more susceptible to have more severe disease and poor outcomes (112).

4.1.3 HIV as a risk factor for mortality

The proportion of IPD cases who were HIV co-infected was high (89%) and more prevalent in the 25-44 year age-group (73%). A minimal proportion of HIV-infected patients were on ART (8%). In addition, the CFR for HIV-infected individuals was high (30%). The high proportion of HIV co-infection and CFR are consistent with other studies where despite large reductions in IPD among HIV-infected adults following PCV-7 and HAART introduction, IPD still causes substantial morbidity and mortality among HIV-infected individuals in whom the burden of HIV and IPD was high (80-82).

In this study HIV-infected patients were 1.69 times more likely to die compared to HIV-uninfected patients. This association has not been seen in other studies. Frankel et al (113) reviewed 147 episodes of IPD patients hospitalized with and without HIV. Results showed that the overall mortality rate was 12% and did not vary by HIV status (HIV-infected, 13%, HIV-uninfected 12%). In addition, several other studies compared mortality between HIV-infected (9%-21%) and HIV-uninfected (3%-15%) patients found no significant differences in mortality between the two populations (114-116). A possible explanation for the statistically

significant association between HIV infection and mortality that is seen in our study and not other studies could be that the high prevalence of HIV-infected individuals (89%) in our study population enabled us to detect a significant association of HIV co-infection with mortality. The CFR (30%) in our study is higher than the CFRs reported in previous studies (113;116). Details on staging of HIV infection was not collected. However, of the HIV-infected individuals, 51% (1184/2309) had $CD4^+$ counts of <200 cells/µL. Therefore, the high CFRs could be due to late presentation of patients to the hospitals, patients presenting with advanced stages of HIV at the time of presentation to the hospital as well as delays in patient admission due to a stressed health care system.

Our findings also show that positive HIV status was independently associated with mortality in patients presenting with meningitis. This is consistent with findings from a previous South African study by Nunes et al (82) where HIV-infected meningitis patients had a higher CFR (55.2%) compared to non-meningitis patients (22.4%, p<0.0001). Another South African study by Nyasulu et al (73) showed that in HIV-infected individuals with meningitis were 5.34 times to die when compared to HIV-uninfected individuals (OR 5.34, 95% CI 2.32-12.29).

This study highlights that HIV-infected patients have a higher risk of mortality resulting from IPD. It is believed that the widespread availability of antiretroviral therapy will substantially improve the quality of life of HIV-infected individuals in terms of physical and mental health and decrease the incidence of IPD and therefore mortality (8). The combined effects of childhood vaccination and access

to ART could also reduce the burden of IPD (110;117). Nunes et al (59) showed that in HIV-infected adults vaccination with PCV induces more functional and durable antibody responses in individuals on ART as opposed to individuals not on ART at the time of vaccination.

4.1.4 Meningitis as a risk factor for mortality

Pneumococcal meningitis was significantly associated with mortality compared to bacteremia. The CFR for meningitis was 55% (641/1166). This is in accordance with previous findings from a Malawian study where the inpatient mortality rate was 65%, 20% and 26% for meningitis, pneumonia and bacteremia respectively and a significant difference in inpatient mortality between patients with meningitis (OR 7.5, 95% CI 3.6-15.5) and bacteremia (OR 5.3, 95% CI 2.3-12.5) compared to patients with pneumonia was seen (118). Harboe et al (101) also demonstrated that in patients >5 years of age, meningitis was independently associated with 30-day mortality compared to bacteremia (OR 2.05 95% CI 1.02-4.09).

4.1.5 Provinces as a risk factor for mortality

When compared to provinces with low poverty rates, provinces with intermediate and high poverty rates remained independently associated with mortality. This has been seen in other studies where mortality rates were much higher in young children in developing countries (10-40%) compared to developed countries,

possibly due to poorer access to health care (101). A more recent population-based study assessing the relationship between IPD and socioeconomic deprivation in North East England showed that the incidence of IPD increased linearly with increasing socioeconomic deprivation from 7.0 per 100 000 population to 13.6 per 100 000 population in the 16-64 age-group. A possible explanation for our findings could be due to the differences in the availability of and access to health care services. This could result in differential outcome in patients presenting with IPD. In addition, specimens are only taken from very ill patients in less resourced provinces thus difference in specimen-taking practices could lead to the difference in the spectrum of patients between provinces. These findings reinforce the urgency to address social inequalities in order to reduce the burden of IPD (119).

4.1.6 Prior antibiotic use within 2 months before admission as a risk factor for mortality

Results of this study show that prior antibiotic use within two months of admission was significantly associated with mortality. Previous studies have utilized data on prior antibiotic use before the patient's admission as a marker for possible antibiotic resistance (9;120). However, our study did not find a significant association between penicillin resistance and mortality or between multi-drug resistance and mortality. A most likely explanation for the statistically significant association found between prior antibiotic use in the past two months and mortality could be that these patients had underlying medical illnesses which often resulted

in them accessing heath care facilities for treatment. Results from a Danish study showed that patients with underlying medical illnesses that required frequent hospitalizations were also at increased risk of being admitted for IPD (aRR: 1.4, 95% CI: 1.1–1.8) (121).

4.1.7 Inappropriate antibiotic treatment associated with mortality

This study shows that inappropriate antibiotic treatment was associated with mortality. This suggests that patients who were not administered the appropriate drug were 2.37 times more likely to die compared to those who were administered the appropriate treatment. This is in accordance with studies by Yu et al (9) which showed that discordant therapy (receipt of a single antibiotic within the first two days of blood collection that was intermediately resistant or resistant in vitro against *S.pneumoniae*) with cefuroxime had a higher mortality rate compared to patients treated with concordant therapy (36.4% [n=4], *p*=0.02). Our findings are also consistent with findings from a study conducted in Barcelona, Spain to determine the risk factors for mortality in patients with community-acquired pneumonia. It was found that discordant antibiotic treatment was significantly associated with mortality (OR 11.28, 95% CI 3.50-36.39) (122). This could be due to the fact that discordant antibiotic therapy could result in failed treatment which in turn could result in an increased risk of mortality. In South Africa, the sale of antibiotics is under control. However, it is imperative that physicians in the public

and private sectors are aware of *S.pneumoniae* resistance and the effective antibiotics to be used in their region.

4.1.8 Factors not associated with mortality

Disease severity (Pitt bacteremia score ≥4) was not found to be associated with mortality. This is contrary to previous studies (8;87;123). This could be due to some limitations in our study, as with all surveillance systems, where detailed data on clinical indicators if disease severity including mechanical ventilation, Glasgow coma score, and other parameters such as white blood cell count were incomplete. Therefore, an in-depth analysis of clinical risk factors associated with mortality could not be assessed.

However, when restricted to meningitis patients, our results found a significant association between disease severity (Pitt bacteremia score ≥4) and an increased risk of dying (OR 4.86, 95% CI 1.83-12.91). A possible reason for this is that meningitis is associated with fever, decreased levels of consciousness and the need for mechanical ventilation. The above mentioned parameters are used to calculate the Pitt bacteremia score. This corroborates other findings as meningitis occurs relatively infrequently and is associated with severe disease resulting in high mortality in adults (CFR=30%, N=352) (124). Previous studies also show that the presence of severe illness that was assessed using the Pitt bacteremia score, was significantly associated with mortality in patients with meningitis and other IPD and may be a representation of overwhelming systemic disease with an inevitable

adverse outcome (6;8;9;125;126). The Glasgow coma scale (GCS) is an objective measurement that is usually used to assess a patient's level of consciousness (127). We tried to collect information on GCS but unfortunately the data collected was incomplete, which is a potential limitation.

Additionally, underlying medical conditions was found not to be associated with mortality. This is inconsistent with other studies (76;77). However, this is could be due to limitations in our study where data on underlying medical conditions may not have been filled out completely or uniformly. Differences in our findings could also be due to the differences in population structure with developed countries having a higher prevalence of underlying medical conditions than developing countries. In contrast to previous studies (76;77) , our underlying medical conditions variable excluded HIV status which was analysed separately and as previously mentioned, HIV-infected patients comprised 89% of our study population.

Penicillin and multi-drug resistance was also found not to be associated with mortality. The most likely explanation for this finding is that our analysis included patients with invasive disease that required hospitalization. Most of these patients would have been treated with high doses of penicillin and this would have been adequate treatment as the prevalence of nonsusceptibility to penicillin was 28%, majority of which displayed intermediate resistance to penicillin (99%) and only 1% showing high-level resistance. Our findings are in accordance with findings by Song et al (128) who conducted a prospective observational study of 233 adult

patients with pneumococcal pneumonia in nine Asian countries. Results showed no statistical differences in mortality among patients with multi-drug resistance (14.9%) or without multi-drug resistance (15.6%) (n=233, OR 0.9, 95% CI 0.3-3.2, *p*=1.0). Additionally, a study conducted by Feikin et al (6) in San Francisco showed no statistically significant association between mortality and penicillin- and cefotaxime-resistant pneumococci. Our study shows that the recent occurrence of widespread antibiotic resistance has not resulted in significant increases in IPD mortality in this study population. We did not compare phenotypic resistance to concordant therapy in our study.

Vaccine serotypes found in PCV-13 were not found to be associated with mortality. However, when restricted to meningitis patients, vaccine serotypes were found to be significantly associated with an increased risk of dying (OR 1.39, 95% CI 1.03-1.97). As previously mentioned, these vaccine serotypes may be expected to become less common in adults as a result of indirect effects following routine immunization in infants. An ecological study performed in the U.S by Tsai et al showed a 32.7% decrease (from 0.10 to 0.07 per 100 000) in the overall pneumococcal meningitis rates and a 43.9% decrease (from 0.34 to 0.19 per 100 000) in mortality rates in individuals ≥65 years of age. In addition, for all age groups, a decrease in pneumococcal meningitis hospitalizations (3330 hospitalizations were prevented) and deaths (394 deaths were prevented) after PCV-7 introduction compared to the baseline years (1994-1999) was seen. It would be interesting to see if similar changes are seen post vaccine introduction in South Africa (129).

Gender, race, prior antibiotic use within 24 hours of admission, nosocomial infections and vaccine serotype were not associated with mortality.

4.2 Representativeness of the study population

The study population included in the multivariable analysis were patients from enhanced sites who had complete case report forms with information regarding in-hospital outcome, HIV status, admission date, prior antibiotic usage and discharge diagnosis. We assessed whether the population at enhanced sites differed from those at non-enhanced sites. There were significantly more cases reported at non-enhanced sites compared to enhanced sites from the provinces with intermediate poverty rates and high poverty rates compared to low poverty rates. This could be attributed to enhanced sites being larger academic hospitals located in well-resourced cities. In addition, patients belonging to White, Colored and Indian race groups were more likely to present at enhanced sites compared to non-enhanced sites. This could be due to the fact that the biggest enhanced sites in some provinces are in urban settings were the hospitals are larger and the population is more dense and diverse. This may have introduced selection bias and may limit the generalizability of our data to the more urban and peri-urban populations.

Positive blood specimens were more likely to be collected from enhanced sites compared to non-enhanced sites as blood cultures are less likely to be taken at smaller, rural hospitals. There were more positive CSF specimens collected at

non-enhanced sites compared to enhanced sites. CSF specimens are usually taken in a standard fashion due to the more severe clinical presentation of meningitis and it is assumed that clinicians will very seldom treat meningitis empirically without taking a CSF specimen.

4.3 Potential study biases

4.3.1 Collection of exposure and outcome data

Systematic differences may have occurred due to differences in interviewing skills between surveillance officers. However, the use of strict case definitions for exposure and outcome may have reduced information bias. The study population includes hospitalized patients nationally. Some patients may die at home without being admitted. This could introduce selection bias to the study. A common limitation with surveillance data is missing information. Data with regards to deaths due to IPD post admission are not available. In addition, patients who refuse to be admitted and treated at the hospital may die at home. This could result in underestimation of mortality. This study used cases from enhanced sites which may introduce selection bias. This may limit the generalizability of the study to populations beyond the enhanced sites.

4.4 Residual confounding

All factors of interest were assessed in a univariate model to find out if they were associated with mortality. Significant factors at $p \leq 0.1$ were included in the multivariable logistic regression model. Each factor was then controlled for the confounding effect of the other. This method helped to control for the confounding effect of several factors at the same time. Factors significant at $p \leq 0.05$ were then considered significant risk factors for mortality. However, residual confounding effect in the association between the risk factors identified and mortality remains a possibility. All factors associated with IPD that could also be risk factors for death such as socioeconomic status and access to health services were not examined due to the lack of data.

4.5 Study strengths

Strength of this study includes the use of national data of laboratory confirmed cases of IPD. The large number of cases and the several years of surveillance data powered the study to evaluate the association between serotypes and mortality, controlling for other risk factors. In addition, serotyping methods remained consistent over the years.

4.6 Study limitations

Our study has limitations. An inherent property of laboratory-based surveillance for pneumococcal disease is the underestimation of the actual burden of pneumococcal disease (130). Surveillance studies represent the proportion of isolates obtained only from patients from whom specimens were taken. The proportion of cases presenting from enhanced sites differed from non-enhanced possibly due to patient self-referral patterns, hospital sizes, specimen-taking practices and more dedicated staff at enhanced sites. This limits generalizability and may have introduced selection bias. Pneumococcal meningitis diagnosis was defined by a positive CSF culture or a positive blood culture and clinical diagnosis of meningitis. This could have resulted in the over diagnosing of patients with meningitis.

Another limitation is that the cohort used for the multivariable analysis comprised 26% of the total cohort. This was after only including patients presenting at enhanced sites who were infected with the top 20 most common serotypes. The reduction in specimen size was also due to the failure to obtain clinical record reviews (17%) and non-viable isolates (22%). Possible explanations for no clinical review could be that patients are discharged or die before the surveillance officers are able to complete the case report forms. Surveillance officers sometimes find it challenging to complete the case report forms or obtain consent after patients have been transferred to other hospitals and patient files are sometimes mislaid

during hospital transfers. Possible reasons for non-viable isolates could be due to contamination or incorrect inoculation and incubation of the Dorset medium.

In addition, we could not be sure that death was due to IPD. Death could have occurred as a result of other conditions. To minimize this we restricted our analysis to death that occurred within 30 days from the first positive result for *S. pneumoniae*.

CHAPTER 5

5.1 Conclusion

In conclusion, we found that amongst the 20 commonest serotypes, the incidence of serotypes 4, 19A, 23F and 18C increased significantly, and serotypes 1, 25 and 5 decreased significantly from 2003 to 2008. Serotype 1 was the commonest serotype overall (16%), followed by serotype 19A (11%) and serotype 4 (7%). The CFR for meningitis was 55% and 23 for bacteremia. Serotype 19F had the highest CFR of 48%, followed by 39% for serotype 23F and 38% for serotype 1.

Analyzing the 20 most common serotypes, using multinomial regression, comparing the age distribution of all other serotypes to the age distribution of serotype 4 (25-44 years as the referent group), serotype 3 was significantly more likely to be identified from patients 45-64 years of age, serotype 1 was significantly more likely to be isolated from patients 15-24 years of age and serotype 19F was significantly more likely to be isolated from patients 45-64 years of age.

In comparison with serotype 4, serotypes 12F and 9V caused significantly more disease in HIV-infected individuals and serotype 1 was significantly less likely to be identified in HIV-infected patients. Serotypes 19A, 3, 6A, 14, 23F, 12F, 9N, 7F, 18C, 25, and 5 differed when compared to serotype 4 by clinical syndrome. Serotypes 6A, 23F, 12F, 18C were more likely to cause meningitis, while serotypes 19A, 3, 14, 9N, 7F, 25 and 5 were more likely to cause bacteremia.

Serotypes 1, 23F and 19F were significantly more likely to cause death in patients compared to serotype 4. Serotype 25 was significantly less likely to cause death in patients compared to serotype 4.

On multivariable analysis, disease caused by serotypes 1 and 19F compared to serotype 4 were independently associated to mortality. In addition, increasing age; living in provinces with intermediate or high poverty rates compared to provinces with low poverty rates; having meningitis compared to bacteremia; prior antibiotic treatment in the last two months; inappropriate antibiotic treatment and positive HIV status were independently associated with mortality.

5.2 Recommendations

There are no studies to date in South Africa that have looked at the association of serotypes with mortality amongst adults with IPD. Serotypes 1 and 19F that were found to be associated with increased mortality are included in the 10-and-13-valent pneumococcal conjugate vaccine and may be expected to become less common in adults as a result of indirect effects following routine immunization in infants.

HIV-infected adults experience increased mortality which may be reduced by more widespread availability of antiretroviral therapy which will in turn substantially improve the quality of life of HIV-infected individuals in terms of physical and

mental health and decrease the incidence of IPD and therefore mortality. Suggestions for future research include evaluating indirect effects of the pneumococcal vaccines on HIV-infected adults. Moreover, it is important that physicians in the public and private sectors are aware of *S.pneumoniae* resistance and the effective antibiotics to be used in their region. Awareness can be raised during conferences and meetings that physicians are likely to attend and during feedback sessions where findings of studies within GERMS –SA are communicated to principle investigators, stakeholders and policy makers.

Data from South Africa may be helpful for policy makers in South Africa and other countries with similar populations who lack their own surveillance. They may assist with regard to decision making concerning additional targeted interventions including adult vaccination with PCV in order to alleviate the burden of IPD among adults. Furthermore, the results from this study again highlight the importance of continuous monitoring and surveillance before and after vaccination introduction in order to evaluate the impact of the new vaccines and potential changes with regard to serotype replacement.

Input can be obtained from policy makers with regard to suggestions on how to adapt and improve the GERMS-SA surveillance so that it can be used for guiding policy. From the laboratory perspective, GERMS-SA surveillance can be improved by strengthening communication between the surveillance officers and laboratory staff, particularly from smaller laboratories. Laboratory information systems should be reliable as technical difficulties could adversely affect laboratory processes.

Clinicians should collect specimens in addition to treating patients empirically. This will in turn improve specimen collection.

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APPENDICES

APPENDIX 1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Nireshni Naidoo

CLEARANCE CERTIFICATE

PROJECT

M121050

Streptococcus Pneumoniae-Serotypes and Mortality in Adults in South Africa: Analysis of National Surveillance Data (2003-2008)

INVESTIGATORS

Ms Nireshni Naidoo

DEPARTMENT

School of Public Health

DATE CONSIDERED

26/10/2012

DECISION OF THE COMMITTEE*

Approved unconditionally

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

DATE 26/10/2012

CHAIRPERSON .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Cheryl Cohen

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

Naidoo

APPENDIX 2

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 6234 OR 011 555 0353 FAX: 011 386 6077

Surveillance officer name: Signature: Date:

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

Lab Specimen No: Laboratory Name:

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

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

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

Patient Surname: Patient First Names:

Address: Town/City: Province:

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

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

ID No. Unk ☐ ARV No. Unk ☐

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

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

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

If yes, name of step down hospital:

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

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

Discharge diagnosis:

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

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

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

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

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

Temp: ℃ 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

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Laboratory Specimen Number: ☐☐☐☐☐☐☐☐☐☐

Immunocompromising conditions:

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

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 >10days ☐ Oral candidiasis ☐ Suspected PCP ☐ None ☐
Kaposis sarcoma ☐ Tuberculosis ☐ HIV wasting ☐ Unk ☐
Any antiretroviral use: Yes ☐ No ☐ Unk ☐ If yes: Current ☐ Previous ☐ Perinatal ☐ Unk ☐
Current antiretroviral use: 3TC ☐ 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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***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 pneumococccal conjugate 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	

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

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_20 Unk ☐

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

***Pneumocystis jirovecii* 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: