THE IMPACT OF HIV ON CLINICAL-MICROBIOLOGIC FEATURES AND MORTALITY AMONG PATIENTS WITH INVASIVE NONTYPHOIDAL *SALMONELLA* INFECTION IN SOUTH AFRICA

Rugola Mtandu

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of

Master of Science in Medicine in Epidemiology and Biostatistics

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DECLARATION

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

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……day of...…….., 2007

In memory of my late grandparents

Rugola, Bahitwa, Wabwiro and Wanyambarya Mkubwa

Dedicated to my wife Irene and son Rugola Jr.

ABSTRACT

Introduction: Nontyphoidal *Salmonella* (NTS) has been associated with HIV from the outset of the HIV pandemic. The few NTS studies done in Africa and America have not documented the impact of HIV on clinical-microbiologic features and mortality in patients with NTS infection. This study determined the association between HIV serostatus and mortality proportion, clinical presentation, length of hospital stay, frequency of invasive NTS infection recurrence, NTS serotypes and estimated the population attributable fraction of mortality due to HIV among patients with invasive NTS infection in South Africa.

Methods: Secondary data from enteric diseases national surveillance in South Africa from 2003 to 2006 were analysed as a cross sectional study. A total of 1 398 subjects with known HIV serostatus were obtained after data cleaning. Data analysis was done in Stata using chi squared test for categorical variables and Wilcoxon rank sum test / Kruskal-Wallis test for continuous variables. Logistic regression models were used to quantify the associations, and adjust for confounders and effect modification. Population attributable fraction was calculated to quantify the impact of HIV on mortality.

Results: Majority (82.26%) of patients were HIV positive. The frequency pattern of HIV positive serostatus in different age groups coincided with that of invasive NTS. The overall mortality was 32.00%. HIV positive patients had a higher proportion (35.79 %) of mortality than HIV negative patients (15.55 %) (P<0.001). Fifty five percent of deaths in this study population were attributed to HIV infection. In multivariate models, HIV positive patients were more likely than HIV negative patients to die (OR = 2.50, 95% CI 1.69- 3.70), to develop lower respiratory tract infection (LRTI) (OR = 1.89, 95% CI,1.34-2.65), to have recurrence of invasive NTS (OR = 3.90, 95% CI 1.41-10.77), to stay less than 16 days in hospitals (OR = 1.61, 95% CI, 1.08-2.40) and to be infected with *Salmonella* serotype Typhimurium infection (OR = 2.59, 95% CI 1.91-3.51). There were

no significant differences in temperature, cardiac arrest, meningitis and site of specimen isolation ($p>0.05$).

Discussion: The major limitation to this study was poor data quality of the surveillance system, including missing HIV serostatus hence the findings cannot be generalized to patients with unknown HIV status.

Conclusion: HIV infection is common among patients with invasive NTS and is associated with excess mortality, LRTI, fewer than 16 days of hospital stay, recurrent invasive NTS infection and *Salmonella* Typhimurium. It is important for clinicians to rule out HIV infection in patients with invasive NTS especially those presenting with LRTI and *Salmonella* Typhimurium infection in addition to recurrent NTS infection, which is a well-known feature associated with HIV.

Recommendation: Since these patients received antimicrobials and had considerable mortality, the first line treatment of invasive NTS should be reviewed especially to HIV positive patients by investigating resistance patterns and conducting a clinical trial of newer and effective antimicrobials.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ARVs	Anti Retroviral (drugs)
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CNS	Central Nervous System
CRF	Case Report File
CSF	Cerebral Spinal Fluid
EDRU	Enteric Diseases Reference Unit
FAO	Food and Agriculture Organisation
GCS	Glasgow Coma Score
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
LRTI	Lower Respiratory Tract Infection
NHLS	National Health Laboratory Services
NICD	National Institute for Communicable Diseases
NTS	Nontyphoidal *Salmonella*
OIs	Opportunistic Infections
OR	Odds Ratio
PCP	*Pneumocystis jiroveci* Pneumonia
TB	Tuberculosis
UN	United Nations
UNICEF	United Nations Children's Fund
USA	United States of America
WHO	World Health Organisation

CHAPTER 1

1.0 INTRODUCTION

Nontyphoidal *Salmonella* (NTS) infection is recognised as one of HIV opportunistic infections causing considerable morbidity and mortality, which has been given relatively little attention. The published literature on HIV and the associated clinical-microbiologic features and mortality of NTS infection is reviewed. The problem statement and the justification for the study are discussed herein. The chapter ends by stating the aim and objectives of the study.

1.1 Overview of HIV opportunistic infections

HIV infection is a risk factor for many if not virtually all infectious diseases (CDC, 1993b; Selik, *et al*., 1995; WHO, 2005). As our knowledge on HIV disease has continued to grow over the past 20 years, we have observed an increase in the frequency of infections associated with HIV disease, some unusual in the sense that they either had low prevalence or would naturally not cause infirmity in human beings in the pre-pandemic era (FAO, 2004; Lange, *et al*., 2005; Levine, *et al*., 1991; Mfinanga, *et al*., 2004; Trevejo, *et al*., 2005). These infections have been given a name "opportunistic" which in simplistic terms means that they seize the "opportunity" of immunodeficiency state, primarily caused by HIV (UNAIDS, 1998).

TB is one of the opportunistic infections which has had much attention and has been shown to be the leading cause of morbidity and mortality in HIV positive individuals worldwide and particularly in sub Saharan Africa where the burden of HIV-TB co infections is relatively heavy (Corbett, *et al*., 2003; Corbett, *et al*., 2006; Lange, *et al*., 2005; Range, *et al*., 2001; Wilkinson & Davies, 1997). Nevertheless other known opportunistic infections particularly of zoonotic nature which might also account for morbidity and mortality in

HIV positive patients have been given little attention (FAO, 2004; Mfinanga, *et al*., 2004). Amongst the zoonotic infections, invasive nontyphoidal *Salmonella* (NTS) infection has been known to be associated with HIV disease since the outset of the pandemic (Hohmann, 2001; Levine, *et al*., 1991). This opportunistic infection is classified under clinical stage 4 in WHO clinical staging for HIV/AIDS (WHO, 2005).

Usually NTS infection is a self-limiting gastrointestinal infection in immunocompetent individuals (Chiu, *et al*., 2004; Gordon, *et al*., 2002). In immunocompromised conditions, in the very young and the elderly there is a high risk of NTS infection becoming invasive and eventually affecting a number of organs (Chiu*, et al*., 2004; Drinković*, et al*., 2004; Fraimow*, et al*., 1990; Hohmann, 2001; Kao*, et al*., 2005). Invasive NTS infection implies isolation of NTS from sites that are usually sterile, including blood, CSF, joint fluid, bone aspirate, pericardial fluid, pleural fluid, peritoneal fluid and deep-seated abscesses.

1.2 LITERATURE REVIEW

1.2.1 HIV as risk factor for NTS infection

The advent of the HIV epidemic has changed the epidemiology of nontyphoidal *Salmonella* infection in human beings. A retrospective study conducted in the USA by Levine, *et al*., (1991) among men and women in states with high prevalence of AIDS, showed a substantial increase in the number of reported blood *Salmonella* isolates from 1978 to 1987. The increase was most remarkable with *Salmonella* Enteritidis (Figure 1.1). Bacteraemia has been shown to be a common sequela of NTS infection in immunodeficiency states. Unpublished data from Massachusetts general hospital found that the common risk factors were corticosteroid use, malignancy and diabetes each at 15% level and HIV, previous use of antimicrobials and other immunosuppressive drugs each at

10% level. Extremes of age have also been identified to be a risk factor for NTS bacteraemia (Hohmann, 2001; Graham, *et al*., 2000b).

In Africa, HIV infection is a major risk factor for NTS infections. Pithie, *et al*., (1993) in Zimbabwe found that NTS bacteraemia as compared to typhoid fever was strongly associated with HIV infection. In recent studies of HIV infected African adults with bacteraemia, NTS were isolated from 7% up to 37% of adults (Gordon, *et al* 2001; Hohmann, 2001; Holmes, *et al*., 2003). In general, bacterial infections were the second commonest OIs after TB whereby NTS was preceded by *Streptococcus pneumoniae* (Holmes, *et al*., 2003).

Conversely, in a study of 100 adults with NTS bacteraemia in Malawi, HIV was confirmed by ELISA in 77 out of 78 tested giving a frequency of 99% (Gordon, *et al*., 2002). The importance of NTS bacteraemia associated morbidity and mortality in African countries where HIV is prevalent has also been elaborated (Gordon, *et al*., 2002; Graham, *et al*., 2000a; Holmes, *et al*., 2003; Kankwatira, *et al*., 2004).

1.2.2 Clinical Features of NTS infection

The clinical presentation of NTS bacteraemia is non specific and usually NTS occurs with other infections. In children it usually presents as a febrile illness with no specific localizing signs (Graham, *et al*., 2000b). A number of studies have elaborated clinical presentation of NTS bacteraemia but failed to elicit the effect of HIV on clinical symptoms. In a Malawian study of 299 children aged from 0 to 14 years old with NTS bacteraemia and without focal sepsis, the common presenting symptoms were fever (96.6%), cough (66.7%) and diarrhoea (47.5%). Out of 264 children, clinical HIV infection was suspected using the WHO clinical case definition for childhood AIDS in 52 children

(19.7%) (Graham, *et al*., 2000a). Due to low sensitivity and specificity of WHO clinical case definition for AIDS (Osmond, 1998) many cases of HIV infection could have been missed in this study.

Another study done in the same setting among 100 adults, found that the presenting symptoms were fever (90%), headache (60%), vomiting (51%), diarrhoea (46%), cough (45%), abdominal pain (36%), dyspnoea (31%), confusion (28%) and chest pain (27%). The median temperature and Glasgow coma score (GCS) were 39°C and 15 with ranges (35.3-41°C) and (3-15) respectively. (Gordon, *et al*., 2002). These two studies did not compare the clinical features between HIV positive and negative patients.

Nevertheless in a study conducted in USA, among 60 adults with *Salmonella enterica* serotype Typhimurium, it was found that there was no significant difference of median duration of hospital stay between HIV positive and negative patients (Fisk, *et al*., 2005). These results could be due to the small sample size and possibly the high standard of care and access to health service resources in USA compared to the developing countries.

Recurrence of NTS infection is also a common clinical feature. Two studies in Africa have associated NTS infection recurrence with HIV infection (Gordon, *et al*., 2002; Kankwatira, *et al*., 2004) but the effect of HIV in the recurrence was not elicited adequately due to inability of these studies to make a comparison between HIV positive and negative patients.

1.2.3 Microbiologic features of NTS infection

The resistance to antimicrobials associated with NTS infection is well established (Hohmann, 2001; Gordon*, et al*., 2002; Graham*, et al*., 2000a; Fisk*, et al*., 2005; Walsh*, et al*., 2000). This study focused only on the distribution of NTS serotypes among HIV positive and negative patients. Most studies have shown *Salmonella enterica* serotype Enteriditis and serotype Typhimurium to be the common isolates in NTS bacteraemia (Gordon, *et al*., 2002; Graham, *et al*., 2000a; Hohmann, 2001; Levine, *et al*., 1991). The distribution between the two serotypes differs between Europe, USA and Africa. Surveillance data for salmonellosis in European union member states show that *Salmonella* Enteritidis is the predominant type (de Jong*, et al*., 2006). A study in Spain found that, *Salmonella enterica* serotype Enteritidis accounted for 70% and serotype Typhimurium accounted for 17% among 172 cases of *Salmonella* bacteraemia in 1982-1992 (Hohmann, 2001) while in USA among culture confirmed *Salmonella* infection serotype Typhimurium was the most frequently identified and accounted for 27% of reported infections to CDC during 1996-1999 (Fisk*, et al*., 2005).

In African NTS studies, *Salmonella enterica* serotype Typhimurium accounted for 75-79% and serotype Enteritidis accounted for 13-19% (Gordon, *et al*., 2002; Graham, *et al*., 2000a). Gordon, *et al*., (2002) in Malawi further showed dual infections of *Salmonella* Typhimurium and *Salmonella* Enteritidis accounted for 1% and other *Salmonella* species accounted for 5% in adults with NTS bacteraemia. None of these studies have compared the prevalence of NTS serotypes between HIV positive and negative patients. Nevertheless, a recent study by Chierakul*, et al*., (2004) in Thailand showed that Group D nontyphoidal *Salmonella* bacteraemia, mainly *Salmonella* Enteritidis raised concurrent with the increase in HIV seroprevalence (Figure 1.2), replicating the findings by Levine*, et al*., (1991) (Figure 1.1). However the methodology employed in these two studies does not

provide evidence for a strong association between HIV infection and *Salmonella* Enteritidis. In South Africa the commonly isolated NTS serotypes in 2002/2003 were *Salmonella* Typhimurium (71.9%), *Salmonella* Enteritidis (8.8%) and *Salmonella* Isangi (6.3%) (Kruger, *et al*., 2004).

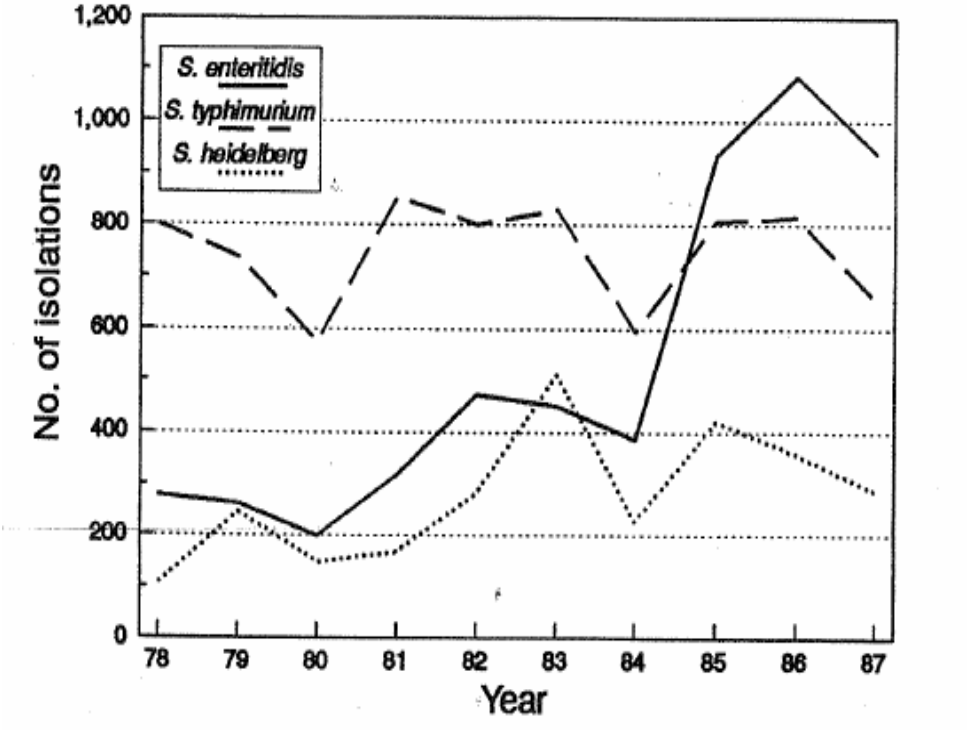

Figure 1.1 Number of reported isolates of three *Salmonella* serotypes in states with high AIDS incidence, 1978-1987 in USA.

(Source: Levine W.C., Buehler J.W., Bean N.H., *et al*., 1991. Epidemiology of nontyphoidal *Salmonella* bacteraemia during the human immunodeficiency virus epidemic. *J Infect Dis*., 164(1), 81-7)

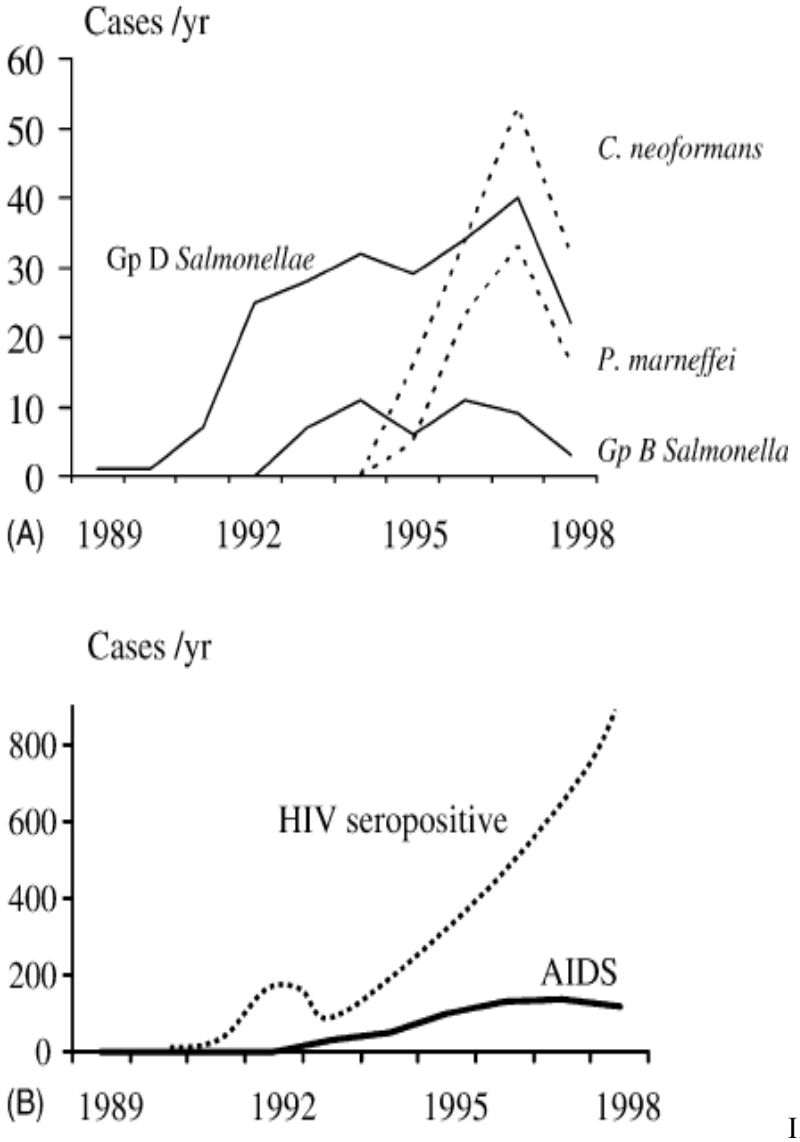

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Figure 1.2 (A) Incidence of bloodstream infections and HIV in Ubon Ratchatani, north-eastern Thailand, during 1989 to 1998. (B) Changes in the incidence of HIV seropositivity, and HIV/AIDS in patients admitted to the regional hospital in Thailand.

(Source: Chierakul W., Rajanuwong A., Wuthiekanun V. *, et al*., 2004. The changing pattern of bloodstream infections associated with the rise in HIV prevalence in north eastern Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 98, 678—686)

1.2.4 Mortality associated with NTS infection

The overall mortality rate associated with NTS bacteraemia is high. Studies conducted in Africa settings with high HIV prevalence show that it ranges from 24% in children (Graham*, et al*., 2000b) to 47% in adults (Gordon*, et al*., 2002). Two studies have compared mortality rates among HIV positive and negative patients with NTS bacteraemia but both have yielded conflicting results. A study by Graham, *et al*., (2000a) conducted among 299 Malawian children found that suspected HIV infection is associated with significantly higher mortality rate of 38.8% compared to mortality rate of 18.3% among children not suspected with HIV infection. Fisk, *et al*., (2005) in USA found that the mortality rate of 15% and 24% among HIV positive and negative NTS bacteraemic patients respectively was not statistically different. The failure to elicit the difference in the latter study could be due to small sample size of 60 patients. Nevertheless the lower median CD4 cell count of 39 cell/µl was associated with death in HIV patients. This finding has been supported by another study among a cohort of HIV infected patients which showed that the CD4 count below 50 cell/µl was strongly associated with risk of death (The PLATO Collaboration 2004).

1.3 PROBLEM STATEMENT AND JUSTIFICATION

South Africa is one of the worst affected countries in the world by the HIV pandemic (Steinbrook, 2004). By the end of 2005 the number of people infected with HIV was estimated to be 5.54 million and the prevalence of HIV in adults aged above 15 years was 16.25% (Department of Health, Republic of South Africa, 2006). Recent estimates show that HIV is 11.2% in the general population (Dorrington, *et al*., 2006). The age group which bears the brunt of HIV attack is from 15 years to 64 years, with women being more affected in the age group 15 to 34 years while men are more affected above 35 years of age (Figure 1.3). The prevalence of NTS infections in South Africa is not known, but since it

has been established that HIV is a risk factor for invasive NTS infection (Thamlikitkul, *et al*., 1996; Fisk, *et al*., 2005) it can be presumed to be significant. There has been an increasing number of NTS isolates from 500 to 1500 in the year 1999 to 2003 at Enteric Diseases Reference Unit (EDRU) (Kruger, *et al*., 2004) and this could be reflecting the HIV epidemic in the country (Keddy, 2005).

Information on the impact of HIV on invasive NTS infection has not been well documented in Africa and South Africa. Most of the previous studies conducted in Africa, have used data from a single hospital, possibly resulting in sampling bias. Therefore in this study a 2003 to 2006 enhanced surveillance dataset of invasive NTS infection from the ten hospital sites (see Appendix I) was used. The dataset consists of approximately 1 398 adults and paediatric patients with known HIV serostatus data, which were collected by surveillance officers. Information of patients with invasive NTS infection was extracted for analysis after data cleaning. By investigating the impact of HIV on clinical-microbiologic features and mortality in patients with invasive NTS infection, the study has provided evidence based information for the need to scale up the management of patients with invasive NTS in South Africa so as to avoid excess mortality.

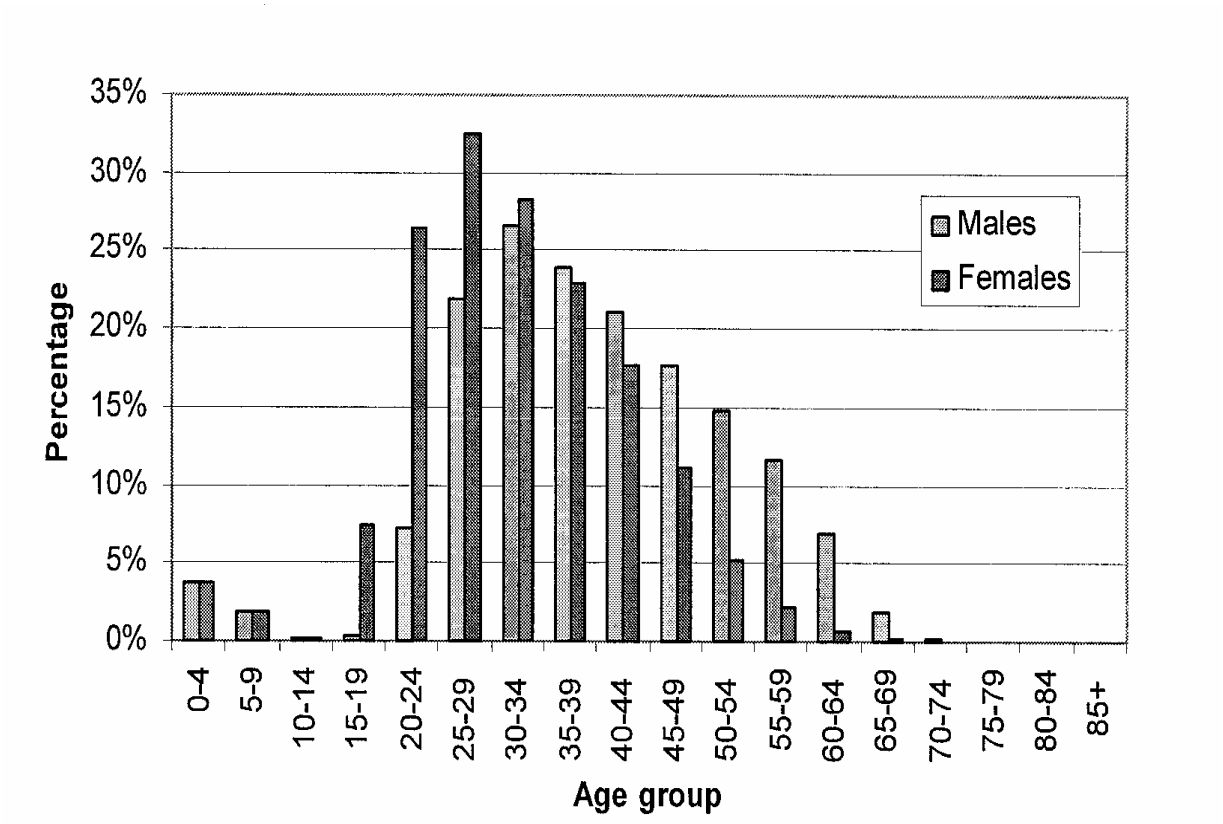


Figure 1.3 HIV prevalence in South Africa by age and sex.

(Source: Dorrington R.E., Johnson L.F., Bradshaw D., *et al*., 2006. *The Demographic Impact of HIV/AIDS in South Africa. National and Provincial Indicators for 2006*. Cape Town: Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa)

1.4 RESEARCH QUESTION

Does HIV status influence the clinical features, nontyphoidal *Salmonella* serotypes and mortality among patients with invasive nontyphoidal *Salmonella* infection in South Africa?

1.5 STUDY AIM AND SPECIFIC OBJECTIVES

1.5.1 Study aim: To determine the impact of HIV on clinical-microbiologic features and mortality among patients with invasive nontyphoidal *Salmonella* infection aged from birth and above admitted in hospitals at 10 surveillance sites in South Africa, 2003-2006.

1.5.2 Specific objectives:

i) To describe socio-demographic characteristics and HIV status of patients with invasive NTS

ii) To determine the distribution and use of ARVs, antimicrobials, mean CD4 counts and median viral loads among HIV positive patients with invasive NTS according to age groups, gender and year of surveillance

iii) To determine immunosuppressive conditions and antimicrobial use in HIV negative patients with invasive NTS according to age groups, gender and year of surveillance

iv) To determine the association between HIV serostatus and mortality proportion, clinical presentation, length of hospital stay, frequency of invasive NTS infection recurrence, site of NTS isolation, number of previous admissions, NTS serotypes, mixed serotypes and mixed infections

v) To estimate the population attributable fraction of mortality due to HIV among patients with invasive NTS

CHAPTER 2

2.0 METHODS

This chapter describes the methods used in the study, which include the study design, study population, sampling strategy, data source, methods for measuring variables, data management and ethical issues.

2.1 Study Design

This study was an analytical cross sectional study on the impact of HIV on clinical-microbiologic features and mortality associated with invasive NTS infection. The study utilised data from the Enteric Diseases Enhanced National Surveillance database in South Africa.

2.2 Study population

The study population comprised of patients with a laboratory confirmed diagnosis of invasive NTS infection aged from birth to 92 years admitted to ten hospital surveillance sites from 2003 to 2006 in South Africa. These patients were interviewed by surveillance officers after taking informed consent.

2.3 Sampling strategy

All surveillance subjects from 2003-2006 with confirmed laboratory diagnosis of invasive NTS infection from enhanced surveillance sites were included in the study based on the following criteria:

2.3.1 Inclusion criteria

Patients aged from one day and above.

2.3.2 Exclusion criteria

Patients with missing HIV serostatus. This variable was considered crucial for the study to be pertinent. Patients with missing final outcome were not excluded because its absence was independent of clinical-microbiologic features.

2.3.3 Sample size

The 2003-2006 dataset contains 1 350 subjects with complete HIV and final outcome information on the discharge date. About 1 112 and 238 patients are HIV positive and negative, respectively. Taking into consideration 36% and 16% mortality proportion in the two groups, the sample size gives a power of 100% with an alpha of 0.05 using a two-sided test, (Stata, version 9).

2.4 Data source

Data collected by Enteric Diseases Reference unit (EDRU) for surveillance purposes was used for analysis. The EDRU is the unit of the National Institute for Communicable Diseases (NICD) that mainly deals with enteric diseases. It has been conducting hospital and laboratory based surveillance in 10 sentinel sites from 2000 to date, representing almost all provinces in the country. The following variables were extracted from the 2003-2006 dataset which is relatively more comprehensive.

2.4.1 Exposure variable

HIV serostatus was confirmed by standard ELISA method at the surveillance sites and the EDRU laboratory.

2.4.2 Outcome variables

2.4.2.1 Primary outcome

Mortality: The mortality proportion between the two groups was measured and compared. Information on mortality was collected by surveillance officers who followed up the patients and recorded the final outcome at the admission hospital or referral hospital if the patient was referred.

2.4.2.2 Secondary outcomes

Clinical features

Clinical features were recorded by surveillance officers from clinical notes of admitted patients. These were then filled in case report files (CRFs) (Appendix II). The information used for the purpose of this study included (a) Type of disease caused by invasive NTS infection (b) Body temperature, blood pressure, cardiac arrest, mental status and Glasgow Coma Score (c) Length of hospital stay (d) Frequency of invasive NTS infection recurrence (e) Site of NTS isolation (f) Number of previous admissions. The information on clinical features was generated by physicians who managed patients with invasive NTS infection.

Number of previous admissions was obtained from the clinical history. Length of hospital stay was deduced from date of admission and date of final outcome, obtained from clinical history and discharge notes respectively. Body temperature, blood pressure, cardiac arrest, mental status and Glasgow Coma Score were obtained from physical examination notes. The first two variables were measured by thermometers and sphygmomanometers, respectively while the last three were assessed by standardized clinical criteria. Cardiac arrest was recorded to be present if the blood pressure was unrecordable or peripheral pulses were impalpable. Mental status was recorded as alert, disorientated, stuporous, comatose or unknown. Glasgow Coma Score was assessed by giving a score of 1 to 4 for

best eye response, 1 to 5 for best verbal response and 1 to 6 for best motor response with the total score ranging from 3 to 15. Type of disease caused by invasive NTS infection was decided by the physicians following clinical history and physical examination findings. Site of NTS isolation was obtained from laboratory result forms positive for NTS. This information was provided by physicians who took the specimen for microbiology investigation. Other clinical features such as antimicrobial use and immunosuppressive conditions were considered as potential confounders. Immunosuppressive conditions included steroid therapy, chemotherapy, radiation, malignancy, diabetes mellitus, chronic renal failure, nephrotic syndrome, liver cirrhosis, kwashiorkor, marasmus, sickle cell anaemia, smoking, TB, PCP and excessive alcohol use.

Microbiologic feature(s)

Salmonella isolates from normally sterile sites were identified by participating laboratories according to the standard operating procedures of the NHLS and referred to EDRU. Microbiologic features were measured by differences in proportions of NTS serotypes, mixed serotypes and mixed infections in the two comparison groups. NTS serotyping was done at EDRU using standard operating techniques following the Kaufmann-White scheme (Brenner and McWhorter-Murlin, 1998) on isolates referred by participating laboratories and results kept in laboratory forms and registers. These results were linked to CRFs for the purpose of determining the relationship between HIV serostatus and NTS serotypes.

2.5 Data management and analysis

2.5.1 Data cleaning

The 2003 to 2006 datasets that are in Epi-Info 6.04c format were exported independently to Microsoft excel in which age, dates of birth, admission and specimen collection were checked and cleaned by use of formula and data validation feature of the program. Age was checked by finding the difference between date of birth and date of admission and was restricted between 0 to 100 years. In order to complement and verify the work done in Excel, the datasets were then transferred to Stata, where duplicate entries and unusual values for age and clinical parameters were identified and cleaned. Duplicate entries were considered in patients with the same type of *Salmonella* isolate who were recorded more than once within three weeks. Patients who were admitted more than once outside the range of three weeks were assigned a new variable of recurrence and double counting in the analysis was avoided by assigning the same unique identifier. Missing information was also identified by use of Stata and a retrieval attempt was done by counterchecking the CRFs and communicating with the surveillance sites.

2.5.1.2 Data appending

The 2003 to 2006 clean datasets were then appended in Stata and a secondary variable "data" was added for identification of the year of the data in the combined dataset.

2.5.1.3 Data extraction

All variables for the study were extracted from the combined dataset.

2.5.1.4 Data coding

Coding was done in Stata whereby categories and secondary variables were generated in order to facilitate data analysis.

2.5.2 Statistical methods

2.5.2.1 Descriptive statistics

The frequency distribution of HIV serostatus was calculated according to socio-demographic characteristics such as age groups, gender, hospital status, province and year of surveillance. Use of ARVs, antimicrobial use, immunosuppressive conditions, mean CD4 counts and median viral loads for HIV positive patients were calculated according to age groups, gender and year of surveillance. Age groups were categorised in accordance with published literature on nontyphoidal *Salmonella* infections (Vugia, *et al*., 2004) which also overlaps with age groups described in HIV/AIDS National and Provincial Indicators for 2006 (Dorrington*, et al*., 2006). In addition immunosuppressive conditions and antimicrobial use associated with HIV negative patients were described using frequency distributions according to age groups and gender. Barcharts and tables for categorical and continuous variables were used accordingly.

2.5.2.2 Analytical statistics

Continuous variables

Differences in means/medians for continuous variables like age and length of hospital stay between HIV positive and negative patients; and differences in mean CD4 counts and median viral loads according to age groups, gender and year of surveillance among HIV positive patients were calculated and analysed using Wilcoxon rank sum test in case of two comparison groups or Kruskal-Wallis test in case of more than two groups due to the skewed distribution of these variables.

Nominal variables

Proportions for nominal variables like mortality proportion, clinical diagnosis, mental status, NTS serotypes between HIV positive and negative patients were calculated and analysed by chi squared test to find out the differences between these two comparison groups.

Associations and Control for confounders

In order to ascertain the association of HIV and clinical-microbiological features and mortality, odds ratios were calculated by logistic regression models. Univariate models were constructed to screen for significant associations at 10% level for HIV serostatus, clinical-microbiologic features and mortality. Significant associations were then adjusted for age, gender, province, hospital status (academic vs. non academic) antimicrobial usage, immunosuppressive conditions and type of diagnosis in multivariate models. Two final multivariate models (one without interaction terms and the other with interaction) were selected for each outcome depending on significance of predictor variables at 5% level, except in case of insignificant interaction whereby only one model was selected. All models have $p<0.001$ for likelihood ratio statistic and $p>0.05$ for Hosmer-Lemeshow goodness of fit test.

Measures of impact

The population attributable fraction (PAF) formula (Levin, 1953) was used to estimate the impact of HIV on mortality among patients with invasive NTS infection as shown below:

$PAF = p\,(OR-1)/p\,(OR-1) + 1$

where by p is the proportion of HIV positive serostatus among patients with NTS infection. OR is odds ratio for mortality in HIV positive patients with invasive NTS infection.

2.6 Ethical clearance

University of the Witwatersrand Committee for Research on Human Subjects approved the study (Protocol number M060916) on 29th September 2006. Permission to use the dataset was granted by the National Institute for Communicable Diseases.

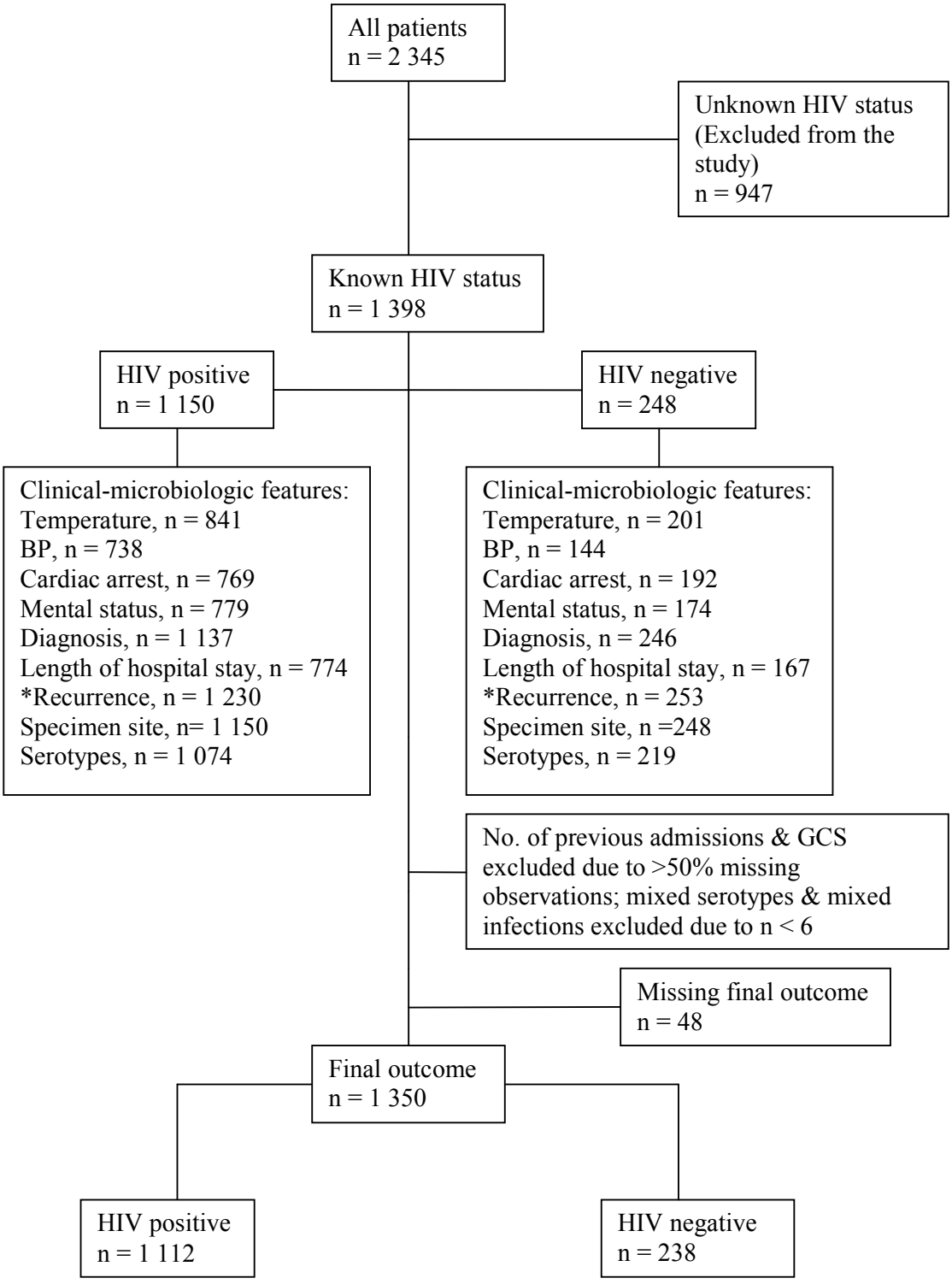
CHAPTER 3

3.0 RESULTS

This chapter elaborates the socio-demographic characteristics of the study population, comparison of HIV serostatus by socio-demographic characteristics, analytical statistics and measure of impact of HIV on mortality among patients with NTS infection.

3.1 Socio-demographic characteristics and HIV serostatus

A total of 2 345 patients with invasive NTS were admitted between 2003 and 2006, among them 947 had unknown HIV status. These were excluded from the study and therefore the following findings cannot be generalized to the unknown HIV status group. An additional 48 patients were excluded in the analysis of HIV and mortality due to missing information on final outcome at the hospitals (figure 3.1). Table 3.1 shows socio-demographic characteristics of 1 398 patients with known HIV status. Most patients (27.25%) were aged between 30-39 years, followed by under one year of age (17.38%) and the smallest number (1.80%) were in the age group 15-19 years. There was no gender difference; both males and females contributed approximately 50% each to the study population. Academic hospitals admitted more patients (88.41%) than non academic hospitals (11.59%). Gauteng and Mpumalanga provinces had, respectively, the highest proportion (73.89%) and lowest proportion (0.79%) of patients. Most of the admitted patients (28.47%) were in the year 2005. The prevalence of HIV in the study population was considerably higher (82.26%) than that of the general population. The proportion of HIV positive serostatus was highest (95%) in the 20-29 and 30-39 years age group and lowest (30%) in the 10-14 years age group. HIV frequency peaked in under one year age group and declined up to 19 years, it then peaked again from 20 to 49 years and finally declined in 50 years and above (Chi squared test for trend = 117.42, $p < 0.001$). This pattern coincided with the frequency of invasive NTS in the same age groups (Figure 3.2).



*Number exceeds the total number due to repeated observations.

Figure 3.1 Flow chart of study subjects.

Table 3.1 Distribution of patients with invasive NTS infection by socio-demographic characteristics and HIV serostatus

Characteristics	Frequency	Percentage
Age groups (years) (n=1 387)		
<1	241	17.38
1-4	107	7.71
5-9	63	4.54
10-14	33	2.38
15-19	25	1.80
20-29	214	15.43
30-39	378	27.25
40-49	220	15.86
50-59	74	5.34
60+	32	2.31
Gender (n=1 371)		
Male	687	50.11
Female	684	49.89
Hospital (n = 1 398)		
Academic*	1 236	88.41
Non academic**	162	11.59

Table 3.1 continued

Characteristics	Frequency	Percentage
Province (n = 1 398)		
Eastern Cape	56	4.01
Free State	51	3.65
Gauteng	1 033	73.89
Kwa Zulu Natal	112	8.01
Limpopo	18	1.29
Mpumalanga	11	0.79
Western Cape	117	8.37
Year (n = 1 398)		
2003	282	20.17
2004	375	26.82
2005	398	28.47
2006	343	24.54
HIV status (n = 1 398)		
Positive	1 150	82.26
Negative	248	17.74

Note that n is not the same throughout, due to missing data in some categories.
*Includes Addington, Baragwanath, Garankuwa, Groote Schuur, Johannesburg, King Edward, Red Cross, Tswane, Tygerberg, Universitas.
**Includes Karl Bremer, Knobel, Mankweng, Pelonomi, Polokwane, Prince Mshiyeni, Public hospital Limpopo, R K Khan, Rob Ferreira, Standerton, Themba, Umtata, Victoria Western Cape.

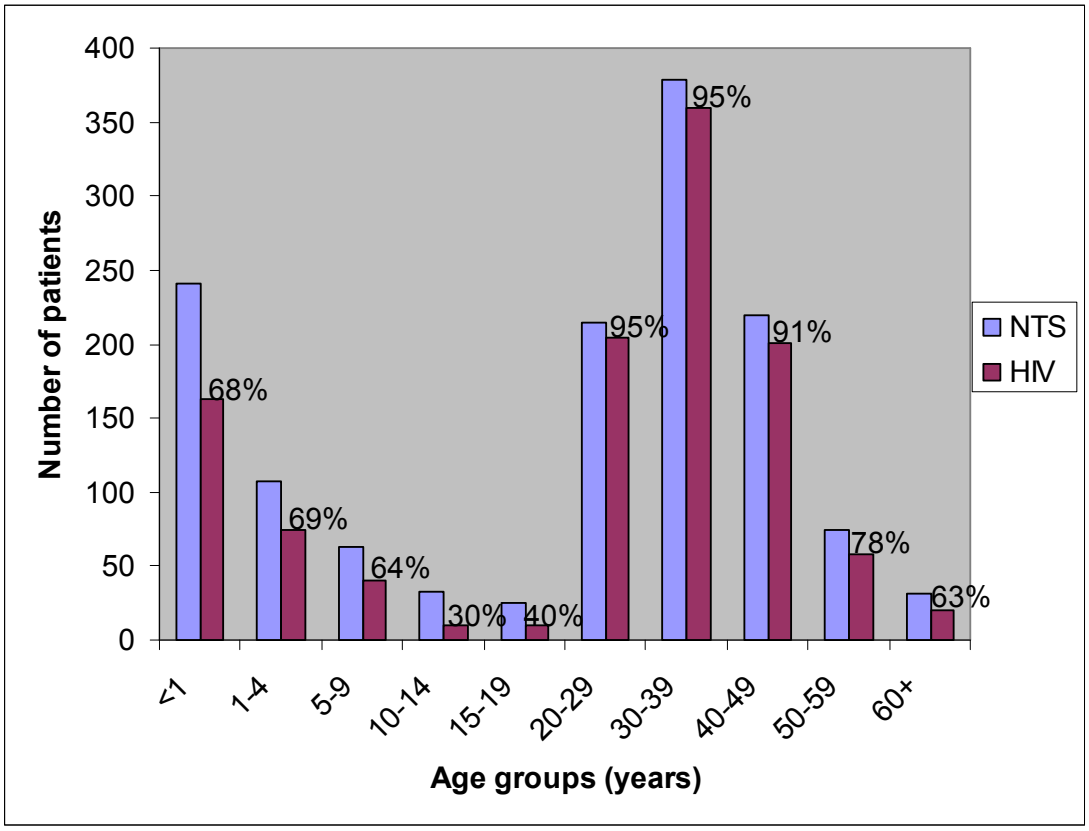


Figure 3.2 Frequency of invasive NTS and (percentage) of HIV positive patients according to age groups.

3.2 Distribution of HIV serostatus by socio-demographic characteristics

HIV positive patients were significantly older than HIV negative. Mean age of HIV positive patients was 28 years old while that of HIV negative patients was 17 years old ($p < 0.001$). Most HIV positive patients (31.58%) were aged between 30 and 39 years while most HIV negative patients (31.58%) were below one year of age. There was no statistical difference ($p > 0.05$) between HIV positive and negative patients in gender. A significant difference ($p < 0.001$) was observed in hospital admissions whereby more HIV positive patients (91.39%) were admitted in academic hospitals than HIV negative patients (74.60%). Most HIV positive (80.70%) and HIV negative patients (42.34%) came from

Gauteng province. There were no statistical differences between HIV positive and negative patients by the years of surveillance (P>0.05) (Table 3.2).

Table 3.2 Distribution of HIV serostatus of patients with invasive NTS infection by socio - demographic characteristics

Characteristics	**HIV status**		P-value*
	Positive %(n)	Negative %(n)	
Age groups (years) (n = 1 387)			0.0001
Mean ± SD (Range)	27.62 ± 17.26 (0-87)	16.93 ± 21.09 (0-83)	0.0001**
<1	14.30(163)	31.58 (78)	
1-4	6.49 (74)	9.31 (23)	
5-9	3.51 (40)	9.31 (23)	
10-14	0.88 (10)	9.31 (23)	
15-19	0.88 (10)	6.07 (15)	
20-29	17.89 (204)	4.05 (10)	
30-39	31.58 (360)	7.29 (18)	
40-49	17.63 (201)	7.69(19)	
50-59	5.09 (58)	6.48 (16)	
60+	1.75 (20)	4.86 (12)	
Gender (n = 1 371)			0.242
Male	49.38 (558)	53.53 (129)	
Female	50.62 (572)	46.47 (112)	
Hospital (n = 1 398)			0.0001
Academic	91.39 (1 051)	74.60 (185)	
Non academic	8.61 (99)	25.40 (63)	

* P-value based on Pearson chi squared test unless otherwise stated.
** P-value based on Wilcoxon rank sum test.

Table 3.2 continued

Characteristics	**HIV status**		P-value*
	Positive %(n)	Negative %(n)	
Province (n=1 398)			0.0001¶
Eastern Cape	2.26 (26)	12.10 (30)	
Free State	2.61 (30)	8.47 (21)	
Gauteng	80.70(928)	42.34 (105)	
Kwa Zulu Natal	7.22 (83)	11.69 (29)	
Limpopo	0.87 (10)	3.23 (8)	
Mpumalanga	0.61 (7)	1.61 (4)	
Western Cape	5.74 (66)	20.56 (51)	
Year (n=1 398)			0.190
2003	21.22 (244)	15.32 (38)	
2004	26.35 (303)	29.03 (72)	
2005	27.91 (321)	31.05 (77)	
2006	24.52 (282)	24.60 (61)	

*P- value based on Pearson chi squared test unless otherwise stated.
¶P-value based on Fisher's exact test.

3.3 Distribution and use of ARVs, antimicrobials and immunosuppressive conditions

The differences in use of ARVs related to age were statistically significant ($p < 0.001$). Table 3.3 shows that among HIV positive patients, those aged below one year had the highest proportion (16.11%) of ARV use. The age group 1-9 years had the second highest proportion (13.04%) of ARV use. The lowest ARV use (2.74%) was in patients aged 50 years and above. The statistically significant difference ($p<0.05$) in use of ARVs was also found in gender and year of surveillance. In gender, males had a higher proportion (10.38%) of use of ARVs while in years there was an increasing trend from 6.19% in 2003 to 13.6% in 2006. Generally, ARVs usage was low (8.44%) compared to the national estimate of 28%.

There were no statistical significant differences in antimicrobial use by age groups and gender ($p>0.05$). More antimicrobials (99.25%) were used in the year 2006 compared to other years ($p<0.001$).

In HIV negative patients there were no statistically significant differences ($p>0.05$) in immunosuppressive conditions by age groups and gender. In age groups, the average proportion of immunosuppressive conditions was 55%. In the year 2005, there was the highest proportion (81.82%) of immunosuppressive conditions that was statistically different ($p<0.001$) from other years. The year 2005 and 2006 had more than 50% of immunosuppressive conditions and the inverse was true for year 2003 and 2004. There were no statistically significant differences ($p>0.05$) in antimicrobial use by age groups and gender. The year 2006 had the highest proportion (100%) of antimicrobial use that was statistically different ($p<0.001$) from other years.

In general, more HIV positive patients (90.64%) used antimicrobials than HIV negative patients (85.77%) and the difference was statistically significant ($p<0.05$).

Table 3.3 Distribution and use of ARVs, antimicrobials in HIV positive patients and immunosuppressive conditions, antimicrobial use in HIV negative patients by age groups, gender and year of surveillance

Age groups (years)	**HIV +** %(n/N)			**HIV -** %(n/N)		
	ARV use*%(n)		ABX use**%(n)	Immuno[§] %(n)		ABX use[¶]%(n)
	Yes	No	Yes	Yes	No	Yes
<1	16.11 (29)	83.89 (151)	88.54 (139)	43.59 (34)	56.41 (44)	84.00 (63)
1-9	13.04 (18)	86.96 (120)	95.54 (107)	57.14 (32)	42.86 (24)	89.09 (49)
10-29	3.08 (6)	96.92 (189)	87.38 (187)	47.92 (23)	52.08 (25)	87.50 (42)
30-49	7.09 (27)	92.91 (354)	90.71 (488)	54.05 (20)	45.95 (17)	82.86 (29)
50+	2.74 (2)	97.26 (71)	95.83 (69)	71.43 (20)	28.57 (8)	84.00 (21)
Gender						
Male	10.38 (49)	89.62 (423)	90.82 (485)	57.36 (74)	42.64 (55)	85.60 (107)
Female	6.20 (30)	93.80 (454)	90.33 (495)	47.32 (53)	52.68 (59)	85.32 (93)
Year						
2003	6.19 (12)	93.81 (182)	60.18 (136)	31.58 (12)	68.42 (26)	12.50 (4)
2004	6.18 (17)	93.82 (258)	98.65 (293)	37.50 (27)	62.50 (45)	92.96 (66)
2005	8.00 (22)	92.00 (253)	97.76 (305)	81.82 (63)	18.18 (14)	98.68 (75)
2006	13.60 (31)	86.40 (197)	99.25 (263)	55.74 (34)	44.26 (27)	100.00 (60)
Total[+]	8.44 (82)	91.56 (890)	90.64 (997)	52.02 (129)	47.98 (119)	85.77 (205)

Age groups (except <1) have been combined due to less than 5 expected observations
ABX, Antimicrobial use.
* Use of ARVs: Fisher's exact test = 0.0001 for age groups; Pearson chi squared = 5.52, p = 0.019 for gender; Pearson chi squared = 11.01, p = 0.012 for year.
** ABX use in HIV positive: Pearson chi squared = 8.89, p = 0.064 for age groups; Pearson chi squared = 0.08, p = 0.780 for gender; Pearson chi squared =311.33, p = 0.0001 for year.
[¶] ABX use in HIV negative: Pearson chi squared = 1.11, p = 0.893 for age groups; Pearson chi squared = 0.04, p = 0.952 for gender; Pearson chi squared = 164.14, p = 0.0001 for year.
[+] ABX use in HIV positive and negative patients: Pearson chi squared = 5.05, p = 0.025.
[§]Immunosuppressive conditions, Pearson chi squared = 7.42, p = 0.115 for age groups; Pearson chi squared = 2.42, p = 0.119 for gender; Pearson chi squared = 41.31, p = 0.0001 for year.

3.3.1 Distribution of mean CD4 counts and median viral loads in HIV positive patients

There was a decrease in mean CD4 count as age increased (Table 3.4). The differences of CD4 counts by age groups were statistically significant ($p<0.001$). The differences in CD4 counts by gender and year of surveillance were not statistically significant ($P>0.05$). Differences in viral loads by age groups and gender were not statistically significant ($P>0.05$) except for year of surveillance ($p<0.001$). Due to more than 50% missing observations these results should be cautiously considered. The number of HIV positive patients with available respective CD4 counts and viral loads were 565 and 102. This differential missing may explain the unexpected finding of insignificant differences in viral loads by age groups.

Table 3.4 Distribution of CD4 counts and viral loads in HIV positive patients with invasive NTS infection by age groups, gender and year of surveillance

	Laboratory parameter	
	CD4 count (mean ± SD, median)	Mean Viral load (mean ± SD, median)
Age groups* (years)		
<1	391 ± 382, 345	150591 ± 177180, 100000
1-4	549 ± 604, 464	139667 ± 202398, 23000
5-9	111 ± 111, 51	343667 ± 351569, 265000
10-14	147.17 ± 112.09, 159	530000 ± 197990, 530000
15-19	319 ± 390, 98	
20-29	89 ± 153, 36	138221 ± 228521, 10000
30-39	97 ± 217, 36	120652 ± 186180, 10000
40-49	59 ± 77, 33	286101.8 ± 356667.7, 67500
50-59	107 ± 112, 63	220100 ± 426624, 10000
60+	70 ± 72, 32	49000 ± 55154, 49000
Gender**		
Male	111 ± 200, 40	189316 ± 276365, 10000
Female	146 ± 302, 42	158536 ± 224884, 13000
Year***		
2003	93 ± 130, 39	------
2004	111 ± 221, 39	------
2005	150 ± 269, 45	309038 ± 268503, 230000
2006	140 ± 302, 40	8933 ± 3051, 10000

* Kruskal-Wallis test: Chi squared = 62.171, $p = 0.0001$ for CD4; Chi squared = 8.258, $p = 0.409$ for viral load.
** Wilcoxon rank sum test: $p = 0.271$ for CD4; $p = 0.841$ for viral load.
*** Kruskal-Wallis test: Chi squared = 1.136, $p = 0.769$ for CD4; Wilcoxon rank sum test, $p = 0.0001$ for viral load.

3.4 Association of HIV serostatus with mortality and clinical-microbiologic features

3.4.1 Mortality

The overall mortality was 32.22% but significantly higher ($p<0.001$) in HIV positive patients (35.79%) than in HIV negative patients (15.55%). After excluding patients who died within three days post admission, the mortality proportion was 29.17% in HIV positive and 12.23% in HIV negative patients but the difference remained statistically significant ($p<0.001$). Mean/median hospital stay before death was not statistically different between HIV positive and negative patients. Most HIV positive patients who died were males (51.79%) aged between 30 and 49 years (51.91%), admitted in academic hospitals in 2005 (28.64%) while most HIV negative patients who died were males (54.05%) aged above 50 years (32.43%), admitted in academic hospitals in 2005 (13.51%).

Table 3.5 Distribution of mortality in HIV positive and negative patients by age groups, gender, hospital status and year of surveillance

	HIV status		P-value
	Positive % (n)	Negative % (n)	
Final outcome [i] (n = 1 350)			0.0001
Died	35.79 (398)	15.55 (37)	
Survived	64.21 (714)	84.45 (201)	
Final outcome [ii] (n = 1 237)			0.0001
Died	29.17 (294)	12.23 (28)	
Survived	70.83 (714)	87.77 (201)	
Mortality within three days of admission	61.90 (104)	40.91 (9)	0.059
Hospital stay before death (days) (mean ± SD, median)	9.38 ± 15.66, 5	10.13 ± 11.38, 5	0.642*
Age groups[§]			
<1	14.50 (57)	27.03 (10)	
1-9	6.62 (26)	8.11 (3)	
10-29	19.34 (76)	10.81 (4)	
30-49	51.91 (204)	21.62 (8)	
50+	7.63 (30)	32.43 (12)	
Gender[§]			
Male	51.79 (202)	54.05 (20)	
Female	48.21 (188)	45.95 (17)	
Hospital			
Academic	90.45 (360)	83.78 (31)	
Non academic	9.55 (38)	16.22 (6)	
Year			
2003	22.36 (89)	21.62 (8)	
2004	26.13 (104)	35.14 (13)	
2005	28.64 (114)	13.51 (5)	
2006	22.86 (91)	29.73 (11)	

* P value based on Wilcoxon rank sum test, otherwise the rest are based on Pearson chi squared test.

[i] Total number of those who died and those who survived.

[ii] Excludes number of patients who died within three days of admission.

[§] Mortality by age groups and gender. Total HIV positive number do not add up to 398 due to missing observations.

3.4.2 Population attributable fraction of mortality due to HIV

It is estimated that 55% of deaths in patients with invasive NTS infection admitted between 2003 and 2006 were attributed to HIV infection using the formula below:

PAF = p (OR- 1)/p (OR-1) + 1

= 0.82(2.50-1)/ 0.82(2.50-1) + 1

= 0.552

= 55.20%

3.4.3 Clinical-microbiologic features

More HIV positive patients (32.86%) than HIV negative patients (19.54%) were not alert on mental status examination (Table 3.5). The differences in lower respiratory tract infections were statistically significant ($p<0.05$) and more common in HIV positive patients (37.73%) than in HIV negative patients (22.76%). Gastroenteritis, bacteraemia without focus and other diagnoses were less common in HIV positive patients than HIV negative patients each at 21.81%, 30.78%, and 1.85% compared to 30.08%, 37.80%, and 4.47% respectively. These differences were statistically significant ($p<0.05$).

HIV positive patients had statistically significant ($p<0.001$) less mean length of hospital stay of 11.18 days compared to 16.54 days of HIV negative patients. This difference was also observed among patients who survived, whose length of hospital stay was 12.18 days in HIV positive patients and 17.56 days in HIV negative patients. HIV positive patients had more recurrence of NTS infections (6.02%) than HIV negative patients (1.98%) ($p<0.05$).

There were no statistical differences ($p>0.05$) in temperature, BP, cardiac arrest and meningitis between HIV positive and negative patients.

Salmonella Typhimurium and other serotypes were found to be significantly different by HIV serostatus ($p<0.001$). More HIV positive patients (67.04%) than HIV negative patients (37.10%) had *Salmonella* Typhimurium and less HIV positive patients (14.70%) had other serotypes than HIV negative patients (44.35%).

Table 3.6 Distribution of clinical-microbiologic features in HIV positive and negative patients

	HIV status		P-value
	Positive % (n)	Negative % (n)	
Temperature [i] (n = 1 042)	7.61 (64)	8.46 (17)	0.203
BP [ii] (n = 882)	24.66(182)	20.14 (29)	0.245
Cardiac arrest * (n = 961)	0.91 (7)	0.52 (1)	1.000
Mental status: (n = 953)			0.001
Alert	67.14 (523)	80.46 (140)	
Not alert [iii]	32.86 (256)	19.54 (34)	
Diagnosis: (n = 1 383)			
Meningitis	7.83 (89)	4.88 (12)	0.107
LRTI	37.73 (429)	22.76 (56)	0.0001
Gastroenteritis [iv]	21.81 (248)	30.08 (74)	0.005
Bacteraemia without focus	30.78 (350)	37.80 (93)	0.032
Other [v]	1.85 (21)	4.47 (11)	0.013
Length of hospital stay (days) [vi] (mean ± SD, median)	11.18 ± 13.74, 8	16.54 ± 22.98, 11	0.0001
Length of hospital stay among survivors (days) [vi] (mean ± SD, median)	12.18 ± 12.50, 9	17.56 ± 24.20, 12	0.003
NTS recurrence [vii] (n = 1 483)	6.02 (74)	1.98 (5)	0.009
Specimen site * (n = 1 398)			0.156
CSF	3.48 (40)	1.21 (3)	
Blood	94.17 (1 083)	96.77 (240)	
Other	2.35 (27)	2.02 (5)	
Serotypes [vii]			
Typhimurium	67.04 (771)	37.10 (92)	0.0001
Enteritidis	8.17 (94)	6.05 (15)	0.258
Isangi	5.39 (62)	7.66 (19)	0.165
Dublin	3.83 (44)	3.23 (8)	0.651
Schwarzengrund *	0.87 (10)	1.61 (4)	0.290
Other§	14.70 (169)	44.35 (110)	0.0001

[i] Temperature was pre-coded in the dataset into three categories 0=36.1-38.9 °C, 1=36 °C or 39 °C and 2= (=>40 °C or <=35 °C). The frequencies displayed here are for the last category (code 2).
[ii] BP was pre-coded in the dataset into two categories of diastolic pressure 0 = >91 mmhg and 2=<90mmhg. The frequencies displayed here are for the last category (code 2).
[iii] Includes disorientated, stuporose and comatose.
[iv] Includes diarrhoea and dysentery.
[v] Other diagnoses include arthritis, burn, abscess, upper respiratory tract infections, pelvic inflammation disease and urinary tract infection.
[vi] P – value calculated by Wilcoxon rank-sum test due to skewed distribution of length of hospital stay.
vii P-value calculated by Pearson chi-squared test unless otherwise stated.
* P-value calculated by Fisher's exact test.

§Include serotypes Aberdeen, Anatum, Bovismorbificans, Bradford, Braenderup, Bredeney, Eppendorf, Essen, Hadar, Heidelberg, Hvittingfoss, Infantis, Irumu, Kibusi, Kisangani, Larochelle, Minnesota, Molade, Muenchen, Newport, Othmarschen, Panama, Powell, Ramsey, Roodepoort, Saintpaul, non-typeable species, Stanleyville, Virchow, Virginia.

3.4.4 Univariate and multivariate logistic regression models for mortality

In univariate analysis, factors associated with mortality were HIV positive serostatus (OR = 3.03, 95% CI 2.089 - 4.390); age (OR = 1.02, 95% CI 1.009 - 1.023); Limpopo + Mpumalanga + Kwa Zulu Natal provinces (OR = 2.57, 95% CI 1.506 - 4.389); Free State + Gauteng provinces (OR = 2.48, 95% CI 1.626 - 3.769); antimicrobial usage (OR = 0.73, 95% CI 0.563 - 0.950) and meningitis (OR = 2.34, 95% CI 1.544 - 3.560).

After adjusting for age, province, antimicrobial usage and meningitis in multivariate analysis, HIV positive patients were more likely to die (OR = 2.50, 95% CI 1.690 - 3.698) than HIV negative patients (Table 3.7).

Table 3.7 Single and multiple modeled risk factors associated with mortality

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model *** OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	3.03 (2.089 - 4.390) 0.000	2.50 (1.690 - 3.698) 0.000
Age	1.02 (1.009 - 1.023) 0.000	1.01 (1.003 - 1.017) 0.004
Gender		
Female	1.00 (reference)	
Male	1.130 (0.897 - 1.423) 0.300	
Province [1]		
EC+WC	1.00 (reference)	1.00 (reference)
LP+MP+KZ	2.57 (1.506 - 4.389) 0.001	2.09 (1.196 - 3.639) 0.010
FS+GA	2.48 (1.626 - 3.769) 0.000	1.55 (0.983 - 2.438) 0.059
Hospital		
Non-academic	1.00 (reference)	
Academic	1.28 (0.884 - 1.845) 0.192	
Antimicrobial usage		
No	1.00 (reference)	1.00 (reference)
Yes	0.73 (0.563 - 0.950) 0.019	0.778 (0.594 - 1.020) 0.070
Immuno [ii]		
Absent	1.00 (reference)	
Present	1.25 (0.985 - 1.585) 0.067	
Diagnosis		
Meningitis	2.34 (1.544 - 3.560) 0.000	2.29 (1.493 - 3.522) 0.000
LRTI	1.10 (0.865 - 1.392) 0.445	
Gastroenteritis	0.86 (0.651 - 1.125) 0.263	
Bacteraemia without focus	0.82 (0.635 - 1.048) 0.112	
Other	0.52 (0.211 - 1.280) 0.154	
Year		
2003	1.00 (reference)	
2004	0.94 (0.674 - 1.308) 0.711	
2005	0.84 (0.602 - 1.158) 0.279	
2006	0.84 (0.601 - 1.185) 0.327	

[1]Provinces were collapsed into three groups according to geographical proximity to allow for efficient control in logistic regression. EC, Eastern Cape; FS, Free State; GA, Gauteng; KZ, Kwa Zulu Natal; LP, Limpopo; MP, Mpumalanga; WC, Western Cape.
[ii]Immuno, immunosuppressive conditions.
* Likelihood ratio statistic chi-squared = 77.09, (*df* = 6, *p* = 0.0000); Pseudo R^2 = 0.0459; Hosmer-Lemeshow goodness of fit chi-squared = 10.06, (*df* = 8, *p* = 0.2608).

3.4.5 Univariate and multivariate logistic regression models for not being alert

In univariate analysis, HIV positive patients were more likely to be not alert than HIV negative patients (OR = 2.02, 95% CI 1.346 - 3.017). After adjusting for province and diagnosis in multivariate model, the association was not significant (OR = 1.47, 95% CI 0.963 - 2.259). Not being alert was rather associated with meningitis (OR = 2.38, 95% CI 1.434 - 3.957) and living in Free state and Gauteng province (OR = 3.11, 95% CI 1.736 - 5.580) (Table 3.8).

Table 3.8 Single and multiple modeled risk factors associated with not being alert

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model***
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	2.02 (1.346 - 3.017) 0.001	1.47 (0.963 - 2.259) 0.074
Age	1.01 (1.006 – 1.021) 0.001	
Province		
EC+WC	1.00 (reference)	1.00 (reference)
LP+MP+KZ	0.904 (0.414 – 1.976) 0.801	
FS+GA	3.465 (1.968 – 6.100) 0.000	3.11 (1.736 - 5.580) 0.000
Hospital		
Non-academic	1.00 (reference)	
Academic	3.19 (1.846 – 5.499) 0.000	
Antimicrobial usage		
No	1.00 (reference)	
Yes	0.475 (0.280 – 0.804) 0.006	
Diagnosis		
Meningitis	2.31 (1.416 – 3.779) 0.001	2.38 (1.434 - 3.957) 0.001
LRTI	0.93 (0.697 – 1.245) 0.632	
Gastroenteritis	0.86 (0.602 – 1.236) 0.421	
Bacteraemia without focus	0.99 (0.741 – 1.316) 0.931	
Other	0.264 (0.061 – 1.150) 0.076	

* Likelihood ratio statistic chi-squared = 59.89, ($df = 4$, $p = 0.0000$); Pseudo $R^2 = 0.0511$; Hosmer-Lemeshow goodness of fit chi-squared = 0.12, ($df = 3$, $p = 0.9897$).

3.4.6 Univariate and multivariate logistic regression models for LRTI

In univariate model, HIV positive patients were more likely than HIV negative patients to have LRTI (OR = 2.06, 95% CI 1.491 - 2.835). After adjusting for province and immunosuppressive conditions in multivariate model, the association was almost similar (OR = 1.89, 95% CI 1.342- 2.647). Immunosuppressive conditions were an effect modifier in association between HIV serostatus and LRTI. The association between HIV positive serostatus and LRTI was observed in presence of immunosuppressive conditions (OR =2.43, 95% CI 1.518 - 3.887) but not in its absence (OR = 1.48, 95% CI 0.893 - 2.467) (Table 3.9).

Table 3.9 Single and multiple modeled risk factors associated with LRTI

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model***
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	2.06 (1.491 - 2.835) 0.000	1.89 (1.342- 2.647) 0.000
Immuno absent		1.48 (0.893 - 2.467) 0.128
Immuno present		2.43 (1.518 - 3.887) 0.000
Age	1.00 (0.993 – 1.006) 0.907	0.99 (0.988 – 1.001) 0.071
Province		
EC+WC	1.00 (reference)	1.00 (reference)
LP+MP+KZ	1.24 (0.756 – 2.045) 0.390	
FS+GA	1.71 (1.187 – 2.457) 0.004	1.54 (1.037 - 2.282) 0.032
Antimicrobial usage		
No	1.00 (reference)	
Yes	1.18 (0.910 – 1.538) 0.209	
Immuno		
Absent	1.00 (reference)	1.00 (reference)
Present	1.50 (1.188 – 1.888) 0.001	1.40 (1.103 - 1.767) 0.005

***** Likelihood ratio statistic chi-squared = 36.09, (*df* = 5, *p* = 0.0000); Pseudo R^2 = 0.0201; Hosmer-Lemeshow goodness of fit chi-squared = 7.82, (*df* = 8, *p* = 0.4515).

3.4.7 Univariate and multivariate logistic regression models for NTS recurrence

In univariate model, HIV positive patients were more likely than HIV negative patients to have recurrent NTS infections (OR = 3.18, 95% CI 1.270 - 7.935). After adjusting for year of surveillance in multivariate model, the magnitude of association increased (OR = 3.90, 95%CI 1.409 - 10.769) (Table 3.10).

Table 3.10 Single and multiple modeled risk factors associated with NTS recurrence

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model*** OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	3.18 (1.270 - 7.935) 0.013	3.90 (1.409 - 10.769) 0.009
Diagnosis		
Meningitis	1.39 (0.620 – 3.099) 0.426	
LRTI	0.82 (0.504 – 1.348) 0.441	
Gastroenteritis	0.80 (0.315 – 2.007) 0.627	
Bacteraemia without focus	1.00 (0.617 – 1.635) 0.986	
Other	0.63 (0.085 – 4.691) 0.652	
Year		
2003	1.00 (reference)	1.00 (reference)
2004	1.70 (0.891 – 3.247) 0.108	1.76 (1.096 – 2.833) 0.019
2005	1.11 (0.557 – 2.203) 0.770	
2006	0.65 (0.289 – 1.445) 0.288	

***** Likelihood ratio statistic chi-squared = 15.39, ($df = 2$, $p = 0.0005$); Pseudo $R^2 = 0.0255$; Hosmer-Lemeshow goodness of fit chi-squared = 0.20, ($df = 1$, $p = 0.6561$).

3.4.8 Univariate and multivariate logistic regression models for hospital stay of less than 16 days

In univariate model, HIV positive patients were more likely than HIV negative patients to stay less that 16 days in the hospital (OR = 1.98, 95% CI 1.361 - 2.876). After adjusting for age, antimicrobial usage and immunosuppressive conditions in multivariate model, the association slightly decreased (OR = 1.61, 95% CI 1.075 – 2.403). Patients who used antimicrobials (OR = 0.43, 95% CI 0.223 – 0.847) and had immunosuppressive conditions (OR = 0.65, 95% CI 0.431 -0.984) were more likely to stay longer than 16 days in the hospital (Table 3.11).

Table 3.11 Single and multiple modeled risk factors associated with hospital stay of less than 16 days [*]

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model**[**] OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	1.98 (1.361 - 2.876) 0.000	1.61 (1.075 - 2.403) 0.021
Age	1.03 (1.018 – 1.036) 0.000	1.02 (1.014 – 1.033) 0.000
Province		
EC+WC	1.00 (reference)	
LP+MP+KZ	1.31 (0.720 – 2.365) 0.380	
FS+GA	2.068 (1.365 – 3.133) 0.001	
Hospital		
Non-academic	1.00 (reference)	
Academic	1.44 (0.927 – 2.230) 0.105	
Antimicrobial usage		
No	1.00 (reference)	1.00 (reference)
Yes	0.620 (0.429 – 0.898) 0.011	0.43 (0.223 – 0.847) 0.014
Immuno		
Absent	1.00 (reference)	1.00 (reference)
Present	0.73 (0.509 – 1.048) 0.088	0.65 (0.431 -0.984) 0.041

[*]Length of hospital stay was categorized into below and above 16 days in accordance with published literature (Helms *et al*., 2006).

[**]Likelihood ratio statistic chi-squared = 53.47, (*df* = 6, *p* = 0.0000); Pseudo R^2 = 0.0556; Hosmer-Lemeshow goodness of fit chi-squared = 2.73, (*df* = 8, *p* = 0.9500).

3.4.9 Univariate and multivariate logistic regression models for *Salmonella* Typhimurium

In univariate model, HIV positive patients were more likely than HIV negative patients to be infected with *Salmonella* Typhimurium (OR = 3.45, 95% CI 2.593 – 4.589). After adjusting for age, type of hospital, diagnosis and year of surveillance in multivariate model, the association remained the same (OR = 2.59, 95% CI 1.910 - 3.507). LRTI was associated with *Salmonella* Typhimurium (OR = 1.38, 95% CI 1.081 – 1.767) (Table 3.12).

Table 3.12 Single and multiple modeled risk factors associated with *Salmonella* Typhimurium

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model*** OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	3.45 (2.593 - 4.589) 0.000	2.59 (1.910 - 3.507) 0.000
Age	1.03 (1.020 – 1.032) 0.000	1.02 (1.013 – 1.026) 0.000
Hospital		
Non-academic	1.00 (reference)	1.00 (reference)
Academic	2.72 (1.945 – 3.801) 0.000	1.89 (1.314 – 2.717) 0.001
Diagnosis		
Meningitis	1.08 (0.708 – 1.641) 0.726	
LRTI	1.50 (1.187 – 1.887) 0.001	1.38 (1.081 – 1.767) 0.010
Gastroenteritis	0.60 (0.465 – 0.769) 0.000	
Bacteraemia without focus	1.07 (0.845 – 1.345) 0.592	
Other	0.61 (0.304 – 1.236) 0.171	
Year		
2003	1.00 (reference)	1.00 (reference)
2004	1.02 (0.743 – 1.395) 0.912	
2005	0.99 (0.725 – 1.349) 0.942	
2006	1.46 (1.048 – 2.024) 0.025	1.68 (1.182 – 2.389) 0.004

***** Likelihood ratio statistic chi-squared = 147.82, (*df* = 7, *p* = 0.0000); Pseudo R^2 = 0.0802; Hosmer-Lemeshow goodness of fit chi-squared = 13.14, (*df* = 8, *p* = 0.1071).

3.4.10 Univariate and multivariate logistic regression models for Gastroenteritis

In the respective univariate and multivariate models, HIV positive patients were less likely than HIV negative patients to suffer from gastroenteritis (OR = 0.65, 95% CI 0.477 - 0.881) (OR = 0.66, 95% CI 0.468 - 0.920) (Table 3.13).

Table 3.13 Single and multiple modeled risk factors associated with gastroenteritis

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model*** OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	0.65 (0.477 - 0.881) 0.006	0.66 (0.468 - 0.920) 0.014
Age	0.988 (0.981 – 0.995) 0.000	0.99 (0.982 - 0.996) 0.003
Province		
EC+WC	1.00 (reference)	
LP+MP+KZ	0.89 (0.544 – 1.455) 0.641	
FS+GA	0.63 (0.441 – 0.900) 0.011	
Antimicrobial usage		
No	1.00 (reference)	
Yes	0.50 (0.383 – 0.662) 0.000	
Immuno		
Absent	1.00 (reference)	
Present	0.60 (0.467 – 0.773) 0.000	
Year		
2003	1.00 (reference)	1.00 (reference)
2004	0.71 (0.511 - 0.974) 0.034	0.70 (0.504 - 0.972) 0.033
2005	0.30 (0.211 - 0.431) 0.000	0.28 (0.197 - 0.409) 0.000
2006	0.12 (0.074 - 0.191) 0.000	0.119 (0.073 - 0.193) 0.000

***** Likelihood ratio statistic chi-squared = 136.13, (*df* = 5, *p* = 0.0000); Pseudo R^2 = 0.0922; Hosmer-Lemeshow goodness of fit chi-squared = 11.95, (*df* = 8, *p* = 0.1534).

3.4.11 Univariate and multivariate logistic regression models for bacteraemia without focus

In the respective univariate and multivariate models, HIV positive patients were less likely than HIV negative patients to suffer from bacteraemia without focus (OR = 0.73, 95% CI 0.549 - 0.975) (OR = 0.66, 95% CI 0.505 - 0.943) (Table 3.14).

Table 3.14 Single and multiple modeled risk factors associated with bacteraemia without focus

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model*** OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	0.73 (0.549 - 0.975) 0.033	0.66 (0.505 - 0.943) 0.011
Age	1.01 (1.001 – 1.014) 0.017	1.01 (1.003 – 1.016) 0.005
Gender		
Female	1.00 (reference)	
Male	1.27 (1.009 – 1.593) 0.042	
Antimicrobial usage		
No	1.00 (reference)	1.00 (reference)
Yes	2.03 (1.512 – 2.712) 0.000	0.67 (0.430 – 1.040) 0.074
Year		
2003	1.00 (reference)	1.00 (reference)
2004	2.82 (1.837 – 4.327) 0.000	3.84 (2.188 – 6.743) 0.000
2005	4.23 (2.789 – 6.420) 0.000	5.93 (3.371 – 10.426) 0.000
2006	6.99 (4.594 – 10.649) 0.000	9.12 (5.201 – 15.986) 0.000

***** Likelihood ratio statistic chi-squared = 116.73, (*df* = 6, *p* = 0.0000); Pseudo R^2 = 0.0678; Hosmer-Lemeshow goodness of fit chi-squared = 3.73, (*df* = 8, *p* = 0.8806).

3.4.12 Univariate and multivariate logistic regression models for other diagnosis

In univariate model, HIV positive patients were less likely than HIV negative patients to have other diagnosis (OR = 0.40, 95% CI 0.191 - 0.845). In multivariate model the association was not significant (OR = 0.64, 95% CI 0.276 - 1.46) (Table 3.15).

Table 3.15 Single and multiple modeled risk factors associated with other diagnosis

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model*** OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	0.40 (0.191 - 0.845) 0.016	0.64 (0.276 - 1.46) 0.286
Age	1.01 (0.990 – 1.029) 0.334	1.02 (1.005 – 1.044) 0.014
Province		
EC+WC	1.00 (reference)	1.00 (reference)
LP+MP+KZ	0.54 (0.184 – 1.597) 0.266	
FS+GA	0.22 (0.101 – 0.484) 0.000	0.16 (0.062 – 0.409) 0.000
Hospital		
Non-academic	1.00 (reference)	
Academic	0.32 (0.147 – 0.709) 0.005	
Year		
2003	1.00 (reference)	1.00 (reference)
2004	1.75 (0.711 – 4.315) 0.224	
2005	0.70 (0.244 – 2.028) 0.515	
2006	0.23 (0.048 – 1.118) 0.069	0.22 (0.045 – 1.091) 0.064

***** Likelihood ratio statistic chi-squared = 32.73, (*df* = 7, *p* = 0.0000); Pseudo R^2 = 0.1075; Hosmer-Lemeshow goodness of fit chi-squared = 5.88, (*df* = 8, *p* = 0.6605).

3.4.13 Univariate and multivariate logistic regression models for other *Salmonella* serotypes

In univariate and multivariate models, HIV positive patients were less likely than HIV negative patients to be infected with other *Salmonella* serotypes (OR = 0.22, 95%CI 0.160 – 0.291) and (OR = 0.99, 95% CI 0.982 – 0.997) respectively. Gastroenteritis was associated with other *Salmonella* serotypes (OR = 1.42, 95% CI 1.034 – 1.948) (Table 3.16).

Table 3.16 Single and multiple modeled risk factors associated with other *Salmonella* serotypes

Factor	**Univariate Model** OR (95% CI) p-value	**Multivariate model*** OR (95% CI) p-value
HIV status		
Negative	1.00 (reference)	1.00 (reference)
Positive	0.22 (0.160 - 0.291) 0.000	0.28 (0.206 - 0.389) 0.000
Age	0.98 (0.971 – 0.986) 0.000	0.99 (0.982 – 0.997) 0.009
Hospital		
Non-academic	1.00 (reference)	1.00 (reference)
Academic	0.26 (0.184 – 0.366) 0.000	0.35 (0.244 – 0.513) 0.000
Immuno		
Absent	1.00 (reference)	
Present	0.704 (0.540 – 0.918) 0.01	
Diagnosis		
Meningitis	1.20 (0.738 – 1.947) 0.463	
LRTI	0.54 (0.402 – 0.732) 0.000	
Gastroenteritis	1.53 (1.136 – 2.047) 0.005	1.42 (1.034 – 1.948) 0.030
Bacteraemia without focus	1.14 (0.866 – 1.511) 0.343	
Other	1.85 (0.868 – 3.961) 0.111	

***** Likelihood ratio statistic chi-squared = 141.15, (*df* = 4, *p* = 0.0000); Pseudo R^2 = 0.1020; Hosmer-Lemeshow goodness of fit chi-squared = 8.75, (*df* = 8, *p* = 0.3635).

CHAPTER 4

4.0 DISCUSSION

Information on the effect of HIV on opportunistic diseases is important for managing patients with dual HIV and NTS infection and recommending for HIV care and treatment policy. This study was carried out to determine the impact of HIV on clinical-microbiologic features and mortality among patients with invasive nontyphoidal *Salmonella* infection in South Africa.

The study found that among 1 398 patients with invasive nontyphoidal *Salmonella*, the overall mortality was 32% and HIV infection was independently associated with higher mortality and 55% of these deaths were attributable to HIV infection. It was also found that LRTI, recurrence of invasive NTS, shorter duration of hospital stay of less than 16 days and *Salmonella* serotype Typhimurium infection were associated with HIV infection. Furthermore, HIV positive patients were less likely than HIV negative patients to suffer from gastroenteritis, bacteraemia without focus and other *Salmonella* serotypes.

The HIV prevalence in this study population was higher than that of the general population (Dorrington, *et al*., 2006). The frequency pattern of HIV positive status by age groups coincided with that of invasive NTS infection, with age groups 20-29 and 30-39 having the highest proportions of HIV positive status. Despite the rise in the ARV usage trend by year of surveillance, ARV usage was generally low compared to the recent national figure (WHO, 2006) and gender differences were observed with more males using ARVs than females. Lastly, a higher proportion of HIV positive patients used antimicrobials than HIV negative patients.

Impact of HIV on mortality

The association of HIV with mortality in patients with NTS has also been demonstrated by a previous study conducted among paediatric patients in Africa (Graham, *et al*., 2000a). To the contrary, one study conducted by Fisk, *et al*., (2005) among adult patients in the United States of America showed no association of HIV and mortality in patients with NTS. However the study was limited by a small sample size that led to failure in eliciting the HIV-mortality association.

The current study combined both children and adults and quantified the magnitude of HIV-mortality association. Moreover the impact of HIV on mortality was shown by the use of population attributable faction, which has not been considered before. The study found that 55% of deaths would have been avoided in patients with invasive NTS, if HIV transmission could have been prevented in this study population.

Generally invasive NTS is independently associated with high mortality (Graham, *et al*., 2000b; Gordon, *et al*., 2002). Nevertheless patients with dual HIV-invasive NTS infection have excess mortality due to immunosuppression caused by HIV infection resulting into increased disease severity and probably resistance to antimicrobials by invasive NTS and eventually death. Another study is currently underway, looking at the relationship between HIV serostatus and antimicrobial resistance patterns in patients with NTS.

Association of HIV with clinical features

Most previous studies have reported clinical presentation of invasive NTS in general (Gordon, *et al*., 2000; Graham, *et al*., 2000a) with the exception of the study by Gordon, *et al*., (2002) which reported clinical presentation of NTS in a 99% HIV positive study population. The current study compared clinical features of invasive NTS between HIV positive and negative patients despite the limited amount of collected information. This

study compared and quantified the association between HIV infection with LRTI, duration of hospital stay and invasive NTS recurrence.

The association of HIV and LRTI in patients with invasive NTS could be due to the NTS predilection to damaged tissues (Casado, *et al*., 1997; Charles, *et al*., 1998; Lin, *et al*., 2006). This was shown in the study by the effect modification of immunosuppressive conditions, whose presence gave odds ratio of two in the association of HIV and LRTI while its absence gave odds ratio of 1.5 that was statistically not significant. This implies that respiratory conditions like TB and PCP (collapsed into immunosuppressive conditions) in HIV positive patients played a major role in predisposing the lungs to NTS infection. It is most unlikely that the LRTI was directly due to PCP or TB rather than NTS itself because the two OIs were controlled for in the multivariate analysis. Since the diagnosis of NTS was confirmed through blood in this study population, the LRTI was due to lung invasion by NTS through the haematogenous route. Others have also reported lung involvement by NTS (Casado, *et al*., 1997; Mankhambo, *et al*., 2006; Ridha, *et al*., 1996; Zaidi, *et al*., 1999). This study however was limited in clinical and laboratory information in ascertaining whether it was pneumonia or lung abscess and confirmation by chest x-ray, isolation of NTS from sputum and pleural fluid, ruling out co-infections by other organisms like *Streptococcus pneumoniae* respectively.

The shorter duration of hospital stay of less than 16 days among HIV positive patients was an unexpected finding and in contrast to other studies that found no differences in hospital duration by HIV status in invasive NTS patients (Fisk*, et al*.,2005) and general patients (Arthur, *et al*.,2000; Bhagwanjee, *et* al., 1997; Palmer, *et al*., 2000). It was expected that HIV patients would stay longer in hospitals due to the associated severity of clinical conditions. It was thought that the higher mortality in HIV positive patients was responsible for the shorter duration of hospital stay but this was unlikely because in the sub-analysis among all survivors, HIV positive patients continued to have a shorter

duration of hospital stay. Probably HIV positive patients are discharged early or transferred to HIV care units after the diagnosis of HIV is confirmed. It could also be due to a short term effect of quicker clinical improvement from either more effective antimicrobials prescribed or the synergistic action of ARVs and antimicrobials. Zidovudine has been shown to have activity against *Salmonella* (Casado, *et al*., 1999). A further research on doctors' practices towards discharge of patients, antimicrobial prescribing patterns in relation to HIV serostatus and combined ARVs-antimicrobials effects is warranted.

This study replicates the findings by Gordon, *et al*., (2002) that invasive NTS recurrence is a common feature of HIV by a comparative analysis of HIV positive and negative patients, which was not considered in the previous study. However Gordon, *et al*., (2002) described the pattern of recurrence and showed it was mainly due to recrudescence rather than re-infection. The differentiation of these two features associated with recurrence was not considered in the current study due to limitation in amount of collected information.

The finding that HIV positive serostatus is less likely to be associated with gastroenteritis and bacteraemia without focus adds to the fact that HIV infection is associated with more severe and not less severe clinical conditions. There was a limitation in objective assessment of severity of different clinical conditions. Ideally, this should have been achieved by a comparative analysis of severity scores such as GCS by HIV serostatus but information on GCS were extensively missing and therefore were excluded from analysis. Kariuki, *et al*., (2006) in Kenya have reported gastroenteritis and bacteraemia without focus in paediatric NTS patients with unknown HIV status.

Association of HIV with microbiologic features

Many previous studies have not considered the association of HIV infection and *Salmonella* serotypes. This study found that HIV positive serostatus is associated with *Salmonella* serotype Typhimurium which is contrary to the previous studies that showed a relationship between HIV and *Salmonella* serotype Enteritidis (Chierakul, *et al*., 2004; Levine, *et al*., 1991). The design of these studies was ecological and therefore the evidence for the association was weak. Studies conducted in African populations with high HIV prevalence have shown that *Salmonella* Typhimurium is the predominant invasive serotype (Gordon, *et al*., 2002; Graham, *et al*., 2000a; Kruger, *et al*., 2004). This implies that *Salmonella* Typhimurium has a propensity to infect HIV patients, which has been confirmed by the current study through controlling for other serotypes.

HIV prevalence

High prevalence of HIV among patients with invasive NTS has also been reported by Gordon, *et al*., (2002). This is because HIV is a risk factor for NTS infection (Hohmann, 2001; Levine, *et al*., 1991; Pithie, *et al*., 1993). It is most unlikely that the prevalence estimate in this study is far from the true value because most patients with unknown HIV status could be HIV positive as well.

For the first time, this study has described the pattern of HIV positive serostatus among patients with invasive NTS according to age groups which showed that the age groups 20-29 and 30-39 are the most affected, reflecting HIV prevalence in the general population (Dorrington, *et al*., 2006). The correlation of frequency of HIV positive pattern and that of invasive NTS according to age groups suggests that the latter can be used as a proxy in estimating HIV prevalence, but this needs further modeling and control of all known OIs.

Antiretroviral and antimicrobial usage

The rise in ARV usage trend by years reflects an improvement in their roll out in the country although it might reflect improvement in data collection as well. The lower ARV usage than the national estimate (WHO, 2006) could be due to missing data. Otherwise, it could be reflecting the fact that most of these HIV patients did not take ARVs initially and eventually got infected with invasive NTS.

The finding of more men accessing ARVs than women is different from the previous study by Conradie, *et al*., (2005) conducted among HIV positive patients in a tertiary hospital in South Africa. The current study showed that the dynamics of HIV-NTS patients may be different from the general HIV patients' population. In general, studies conducted in various settings, have found either no gender differences or differences in ARV usage (WHO, 2006). The reasons for observed gender differences could be social-cultural which need to be researched further.

More antimicrobial usage in HIV positive patients is due to the higher burden and severity of opportunistic infections, which is not the case in HIV negative patients. Palmer, *et al*., (2000), has also reported the use of more antimicrobials in HIV positive than HIV negative patients.

Limitations

The major limitation of this study was missing information, which is common in surveillance data. The absence of information has affected the study in a number of ways. Firstly, patients with unknown HIV status differed in mean age by being three years younger than patients with known HIV status and other social demographic characteristics like provinces, hospitals and year of surveillance. Therefore, these findings cannot be generalized to the former group. Secondly, differential missing by HIV status contributed to insignificant association for variables like temperature and cardiac arrest. Thirdly,

absence of more than 50% led to the exclusion from analysis of variables like number of previous admissions and GCS. Other variables excluded in the analysis were the diagnosis of mixed serotypes and infections due to small sample size resulting from a non routine procedure for their laboratory diagnosis.

Although the analyzed variables had also missing observations, these were neither greater than 50% nor were there differential by HIV serostatus except for CD4 count. In principle, methods of dealing with missing data were put in place (D'agostino and Rubin, 2000; Faris*, et al*., 2002; Lubeck*, et al.,* 1999; Norris*, et al.,*2000; Rothman and Greenland, 1998; Schoenbach and Rosamond, 2000) but they are laborious and complex (Rothman and Greenland, 1998; Schoenbach and Rosamond, 2000). Therefore, it was decided to exclude the missing observations in the analysis. Although this is simple and commended by some authorities (Rothman and Greenland, 1998) it might have introduced selection bias (Schoenbach and Rosamond, 2000) and again the results cannot be generalized to patients with unknown HIV status. It is most unlikely that information bias affected this study because the collection of other clinical information preceded that of HIV status and serotyping was done without prior knowledge of HIV status.

Other limitations include the categorization of temperature and BP in the dataset. Temperature was pre-coded into three categories 0=36.1-38.9 °C, 1=36 °C or 39 °C and 2= (=>40 °C or <=35 °C) and BP was pre-coded into two categories of diastolic pressure 0 = >91 mmHg and 2=<90mmHg. This might have led to the wastage of their specificity. The variables should have been left as continuous and comparison of means by HIV status would have been attempted, despite the already mentioned differential missing.

Information on NTS symptoms and rural-urban placement was not available in the dataset. The importance of symptoms was to detail the clinical presentation and the rural-urban

placement was to control for differential mortality, nevertheless controlling for provinces may have accommodated the rural-urban differential mortality.

The use of a cross sectional design undermined the determination of sequence of events. Although it is known that invasive NTS is an HIV disease opportunistic infection, this study does not assert that HIV preceded NTS infection in the sequence of events. The study design did also not allow for elucidating the sequence of events with regard to TB or PCP exposing the lungs to NTS infection in HIV positive patients. In addition, the collapse of immunosuppressive conditions into a single variable, while allowing for effective adjustment of confounding and effect modification, it caused the loss of specificity to conditions responsible for NTS in HIV negative patients.

Despite these limitations, the study used data collected from almost all provinces in South Africa with most data (74%) coming from Gauteng province resulting in a larger sample size compared to previous studies. It can be argued that a considerable number of patients in the Gauteng province could have come from other provinces by either referral or self-referral and thus it is most unlikely that this affected the representativeness of other provinces. The larger sample size allowed for comparative analysis between HIV positive and negative patients by use of multivariate logistic regression and thus adjustment of confounders and effect modification was achieved resulting into eliciting the independent effect of HIV on mortality and clinical-microbiologic features.

CHAPTER 5

5.0 CONCLUSION

This study underscores the importance of HIV in patients with invasive NTS by quantifying its impact on clinical-microbiologic features and mortality. The study has shown that HIV infection is common among patients with invasive NTS and is associated with higher mortality, LRTI, fewer than 16 days of hospital stay, recurrent invasive NTS infection and *Salmonella* Typhimurium. It is important for clinicians to rule out HIV infection in patients with invasive NTS especially those presenting with LRTI and *Salmonella* Typhimurium infection in addition to recurrent NTS infection, which is a well known feature associated with HIV.

5.1 RECOMMENDATIONS

5.1.1 HIV prevention and management

The excess mortality in HIV positive patients with invasive NTS calls for scaled up efforts in HIV prevention so that these deaths can be avoided. Due to HIV- NTS association, HIV testing and counselling should be offered routinely to patients with NTS with subsequent provision of HAART depending on results.

5.1.2 TB and PCP

Since TB or PCP could be antecedents for LRTI in HIV positive patients with invasive NTS, the two diseases should be actively investigated and managed in case of LRTI presentation, even in the absence of a suggestive clinical history.

5.1.3 Treatment for invasive NTS

The overall mortality of 32% and high frequency of recurrence in HIV positive patients is considerable notwithstanding antimicrobial use in these patients admitted to tertiary hospitals. Therefore, there is a need to revisit the first line treatment of invasive NTS especially to HIV positive patients. This can be achieved by investigating resistance

patterns and conducting a clinical trial of newer and effective antimicrobials with long term effects, important for avoiding recurrence.

5.1.4 Completeness of surveillance data

Surveillance data is crucial for health planning. A mechanism for ensuring the completeness of information should be devised. This can be in form of incentives such as workshops and short term training on data management whereby the importance of complete data will be re-emphasized.

Surveillance teams should also play their part by taking efforts to collect information such as CD4 count and viral load to allow for monitoring studies on the ARV roll out in the country.

In addition there is a need to enhance the data collection tool for surveillance to include major symptoms and their duration that will be combined with complete data on GCS, temperature and cardiac arrest to allow for objective assessment of severity of NTS infection associated with HIV.

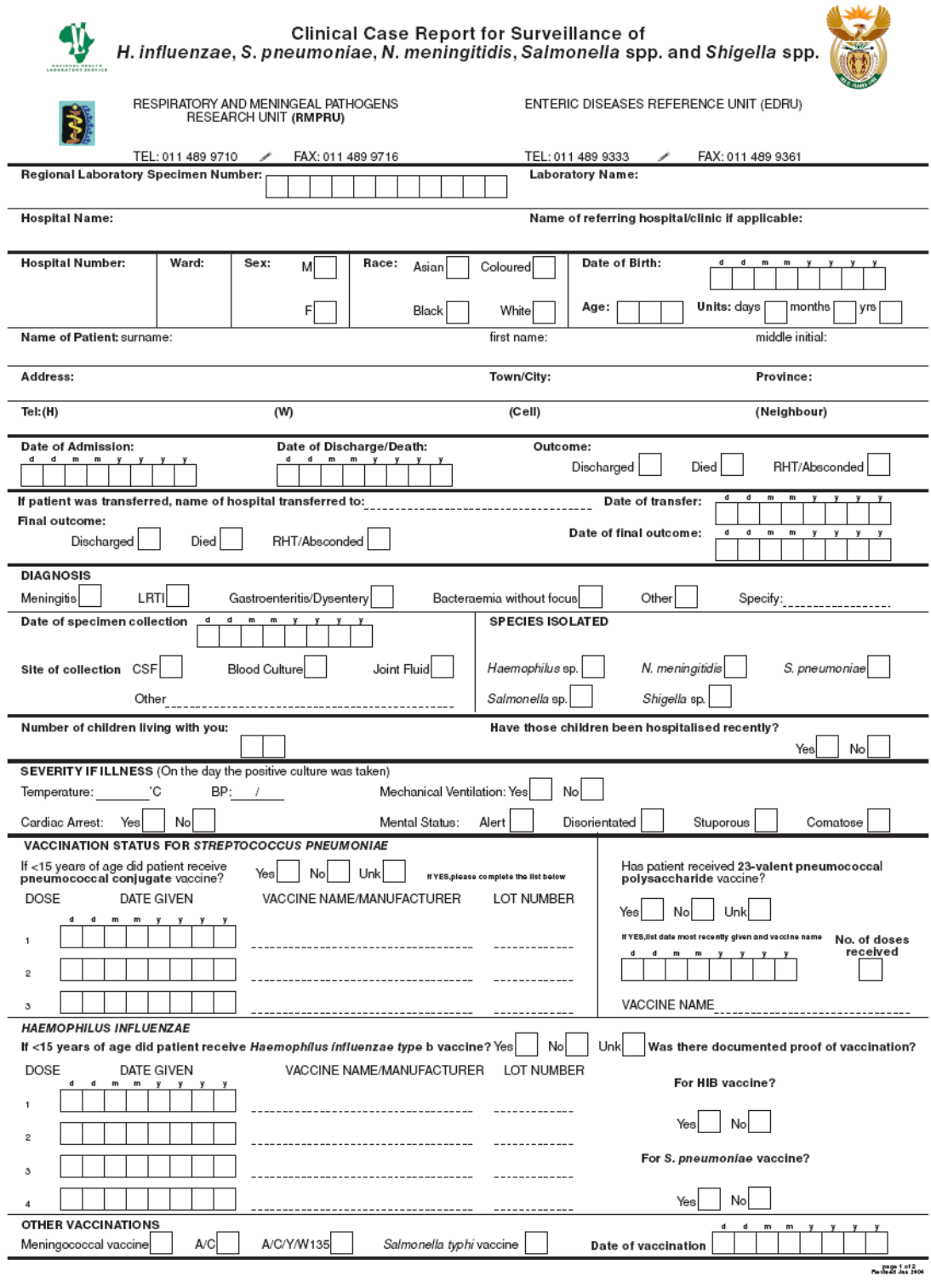
APPENDICES

Appendix I

Surveillance sites

Province	Hospital
Eastern Cape	Umtata Provincial Hospital, Umtata,
Free State	Pelonomi Hospital and Universitas Hospital, Bloemfontein
Gauteng	o Chris Hani Baragwanath Hospital, Soweto o Johannesburg General Hospital, Johannesburg o GaRankuwa Hospital and Tswane, Pretoria
Kwazulu Natal	King Edward VIII Hospital, Durban, Addington and R K Khan
Mpumalanga	Rob Ferreira Hospital, Themba Hospital and Barberton Hospital, Nelspruit
Limpopo	Polokwane-Mankweng Complex, Polokwane; Knobel; Standerton
Western Cape	Red Cross Hospital, Groote Schuur Hospital, Victoria Hospital and Tygerberg Hospital, Cape Town complex

Appendix II



Clinical Case Report for Surveillance of *H. influenzae*, *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp. and *Shigella* spp.

RESPIRATORY AND MENINGEAL PATHOGENS RESEARCH UNIT **(RMPRU)**

TEL: 011 489 9710 FAX: 011 489 9716

ENTERIC DISEASES REFERENCE UNIT (EDRU)

TEL: 011 489 9333 FAX: 011 489 9361

Regional Laboratory Specimen Number: **Laboratory Name:**

Hospital Name: **Name of referring hospital/clinic if applicable:**

Hospital Number: **Ward:** **Sex:** M F **Race:** Asian Coloured Black White **Date of Birth:** d d m m y y y y **Age:** **Units:** days months yrs

Name of Patient: surname: first name: middle initial:

Address: **Town/City:** **Province:**

Tel:(H) **(W)** **(Cell)** **(Neighbour)**

Date of Admission: d d m m y y y y **Date of Discharge/Death:** d d m m y y y y **Outcome:** Discharged Died RHT/Absconded

If patient was transferred, name of hospital transferred to: **Date of transfer:** d d m m y y y y

Final outcome: Discharged Died RHT/Absconded **Date of final outcome:** d d m m y y y y

DIAGNOSIS

Meningitis LRTI Gastroenteritis/Dysentery Bacteraemia without focus Other Specify:

Date of specimen collection d d m m y y y y

Site of collection CSF Blood Culture Joint Fluid Other

SPECIES ISOLATED

Haemophilus sp. *N. meningitidis* *S. pneumoniae* *Salmonella* sp. *Shigella* sp.

Number of children living with you: **Have those children been hospitalised recently?** Yes No

SEVERITY IF ILLNESS (On the day the positive culture was taken)

Temperature: °C BP: / Mechanical Ventilation: Yes No

Cardiac Arrest: Yes No Mental Status: Alert Disorientated Stuporous Comatose

VACCINATION STATUS FOR *STREPTOCOCCUS PNEUMONIAE*

If <15 years of age did patient receive **pneumococcal conjugate** vaccine? Yes No Unk **If YES,please complete the list below**

DOSE	DATE GIVEN (d d m m y y y y)	VACCINE NAME/MANUFACTURER	LOT NUMBER
1			
2			
3			

Has patient received **23-valent pneumococcal polysaccharide** vaccine? Yes No Unk

If YES,list date most recently given and vaccine name d d m m y y y y **No. of doses received**

VACCINE NAME

HAEMOPHILUS INFLUENZAE

If <15 years of age did patient receive *Haemophilus influenzae type* b vaccine? Yes No Unk **Was there documented proof of vaccination?**

DOSE	DATE GIVEN (d d m m y y y y)	VACCINE NAME/MANUFACTURER	LOT NUMBER
1			
2			
3			
4			

For HIB vaccine? Yes No

For *S. pneumoniae* vaccine? Yes No

OTHER VACCINATIONS

Meningococcal vaccine A/C A/C/Y/W135 *Salmonella typhi* vaccine **Date of vaccination** d d m m y y y y

page 1 of 2

Clinical Case Report for Surveillance of *H.influenzae*, *S.pneumoniae*, *N. meningitidis*, *Salmonella* spp. and *Shigella* spp.

RESPIRATORY AND MENINGEAL PATHOGENS RESEARCH UNIT (RMPRU)
TEL: 011 489 9710 / FAX: 011 489 9716

ENTERIC DISEASES REFERENCE UNIT (EDRU)
TEL: 011 489 9333 / FAX: 011 489 9361

Regional Laboratory Specimen Number:

UNDERLYING DISEASES

PREVIOUS HIV STATUS
Positive ☐ Negative ☐ Unknown ☐

CD4 count:
Absolute _____ ; _____ %

CD4 count date taken (d d m m y y y y)

THIS ADMISSION: Pre and Post test counselling offered: Yes ☐ No ☐

If NO HIV taken, is there clinical suspicion of HIV?
Yes ☐ No ☐

HIV consent given: Yes ☐ No ☐ **Result:** Positive ☐ Negative ☐

OTHER IMMUNOCOMPROMISE: *(Tick all that apply)* ☐ None ☐ Unknown

- ☐ TB
- ☐ Current Smoker
- ☐ Sickle Cell Anaemia
- ☐ Splenectomy/Asplenia
- ☐ Immunoglobulin Deficiency
- ☐ Immunoglobulin Therapy (Steroids,Chemotherapy,Radiation)
- ☐ Kwashiorkor/Marasmus
- ☐ Asthma
- ☐ Emphysema/COPD
- ☐ Diabetes Mellitus
- ☐ Nephrotic Syndrome
- ☐ Renal Failure/Dialysis
- ☐ Systemic Lupus Erythematosus(SLE)
- ☐ Cirrhosis/Liver Failure
- ☐ Alcohol Abuse
- ☐ Coronary artery disease
- ☐ Valvular disease
- ☐ Heart Failure
- ☐ Burns
- ☐ Cerebral Vascular Accident (CVA) /Stroke
- ☐ CSF Leak (2° trauma/surgery)
- ☐ Hydrocephalus with VP shunt
- ☐ Malignancy *(specify)* _____
- ☐ Organ Transplant *(specify)* _____
- ☐ Other *(specify)* _____

PREVIOUS ADMISSIONS in last 12 months: Yes ☐ No ☐ **Number of admissions** ☐

Admission 1	Diagnosis	Admission 2	Diagnosis	Admission 3	Diagnosis	Admission 4	Diagnosis
☐ Meningitis	☐ LRTI	☐ Meningitis	☐ LRTI	☐ Meningitis	☐ LRTI	☐ Meningitis	☐ LRTI
☐ Gastro	☐ TB	☐ Gastro	☐ TB	☐ Gastro	☐ TB	☐ Gastro	☐ TB
☐ Other_____		☐ Other_____		☐ Other_____		☐ Other_____	

ANTIBIOTIC USE IN HOSPITAL ON THIS ADMISSION

	Name of Antibiotic	Dose	Route	Date Initiated (d d m m y y y y)	Duration
1					
2					
3					
4					

PREVIOUS USE OF ANTIBIOTICS (ABX) OR ANTIRETROVIRALS

Cotrimoxazole prophylaxis: Yes ☐ No ☐ Unk ☐
Dose: **Route:**
Date initiated: (m m y y y y)
Compliant in last month: Most ☐ Some ☐ None ☐

ABX in 24 hours before specimen: Yes ☐ No ☐
Names:
1 _____
2 _____
3 _____
4 _____
Dose: **Route:**
Date initiated: (m m y y y y)

Other ABX within 2 months: Yes ☐ No ☐ Unk ☐
Names: 1 _____ 2 _____
In the last 30 days: Yes ☐ No ☐
In the last 30 to 60 days: Yes ☐ No ☐

TB Rx Yes ☐ No ☐ Unk ☐
Drugs: 1 _____ 2 _____
Dose:
Date initiated: (m m y y y y)
Compliant in last month: Most ☐ Some ☐ None ☐

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

Antiretroviral therapy received: 3TC ☐ D4T ☐ Efavarenz ☐ Nevirapine ☐ AZT ☐ DDI ☐ Kaletra ☐

Sources of antibiotic data:
Patient ☐ Clinician ☐ Medical Records ☐

Has regional laboratory sent isolate to Central? Yes ☐ No ☐
Date isolate sent (d d m m y y y y)

SURVEILLANCE OFFICER NAME: _____

CONTACT DETAILS: Tel: _____ Fax: _____ Cell: _____

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