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Alimia, Shinny Eskander

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PROFILE OF NON-TYPHOIDAL SALMONELLA BACTERAEMIA IN ADULTS WITH HIV INFECTION ADMITTED TO CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

Shinny Eskander Alimia

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Master of Medicine.

Johannesburg 2020

DECLARATION

I, Shinny Eskander Alimia declare that this research report (protocol with extended literature review and submissable article), being submitted for the Degree of Master of Medicine in the University of the Witwatersrand, is my own unaided work and has not been previously submitted for any other degree or examination at this or any other university.

Signature of candidate: _____

This _____day of _____, 2020

DEDICATION

For my beloved parents, Eskander and Anawara Alimia, who have supported me through it all.

ABSTRACT

Background: In sub-Saharan Africa, non-typhoidal salmonella (NTS) has emerged as an important cause of invasive bloodstream infections, especially among human immunodeficiency virus (HIV) infected adults. South Africa has one of the highest burdens of HIV disease in Africa. Literature shows invasive NTS as a well-established disease amongst HIV infected adults with mortality rates of 20-47% in sub-Saharan Africa. However, limited data exists for NTS bacteraemia in South Africa.

Aim: Profile NTS bacteraemia in HIV infected adults admitted to Chris Hani Baragwanath Academic Hospital from 2014-2015 examining demographic and clinical profile, sites of infection, antibiotics used, CD4 counts, viral loads and outcomes.

Methods: Retrospective review of patient records with positive NTS isolates on blood culture.

Results: A total of 69 patients (53.6% male, n=37) were included in the study and consisted predominantly of Black Africans (98.5%, n=68). The mean age (± SD) at presentation was 44 years ±12.39. A total 73.5% (n=50) of patients were empirically started on appropriate antibiotics on admission prior to blood culture results. Ceftriaxone was the most used antibiotic (56.5%, n=39), followed by ciprofloxacin (36%, n=25) and co-amoxiclav (33%, n=23). Trimethoprim/ sulfamethoxazole was used in 20% (n=14) of patients for empiric *Pneumocystis jiroveci pneumonia* (PJP) cover. Of the 69 patients, 71% (n=49) had CD4 counts < 200 cells/μL, of which 39.1% (n=27) had CD4 counts < 50 cells/ μL. Of note, 75.4% (n=52) were newly diagnosed with HIV and anti-retroviral therapy naive. The mortality rate was 25% (n=18). A GCS<15 and presence of renal dysfunction were associated with mortality. In multivariable analyses, a GCS<15 independently predicted death, with patients with GCS<15 having 15.7 times greater likelihood of dying than fully conscious patients [adj OR 15.69 (3.43- 71.78) p= <0.001]. Men were 4.7 times more likely to die compared to women [adj OR 4.69 (1.09 – 20.99) p=0.043]. Patients treated with inappropriate antibiotics (n=7) were shown to have 3.2 times greater likelihood of dying than those treated with appropriate antibiotics [adj OR 3.20 (0.75- 13.61) p= 0.117].

Conclusion: Invasive NTS was more prevalent in advanced HIV infected patients with a 25% mortality rate.

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ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ALT	Alanine transaminase
ART	Anti-retroviral therapy
AST	Aspartate transaminase
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
CED	Center for Enteric Diseases
CHBAH	Chris Hani Baragwanath Academic Hospital
EFV	Efavirenz
FTC	Emtricitabine
GCS	Glasgow Coma Scale
HIV	Human immunodeficiency virus
IV	intravenous
IQR	Interquartile range
MDR	Multi-drug resistant
NADPH	Nicotinamide adenine dinucleotide phosphate
NHLS	National Health Laboratory Services
NTS	Non-typhoidal salmonella
PJP	*Pneumocystis jiroveci* pneumonia
SAS	Statistical Analysis System
SD	Standard deviation
TDF	Tenofovir
TMP-SMX	Trimethoprim-sulfamethoxazole

CHAPTER 1: PROTOCOL WITH EXTENDED LITERATURE REVIEW

1.1 Introduction and reason for study

Salmonella causing disease in humans are divided into typhoidal serovars (*S typhi and S paratyphi*) and thousands of non-typhoidal serovars. Non typhoidal salmonella (NTS) predominantly cause a self-limiting diarrheal illness in healthy individuals from high income countries.[1] By contrast, in sub-Saharan Africa, NTS has emerged as an important cause of invasive bloodstream infections, especially among immunocompromised human immunodeficiency (HIV) infected adults.[2] South Africa has one of the highest burdens of HIV disease in Africa with invasive NTS being a well-established disease amongst HIV infected individuals with mortality rates of 35-60% in Acquired immune deficiency syndrome (AIDS) patients in sub-Saharan Africa.[3] The incidence of invasive NTS has been declining with the roll out of anti- retroviral therapy (ART) in 2004. [4] Very little epidemiological data exists for NTS isolates identified in South Africa and their association with HIV.[3] A review of this topic will provide vital data about the South African setting.

1.2 Global burden of disease

The two main NTS serovars causing invasive disease in sub Saharan Africa are Typhimurium and Enteritidis.[1, 2] A review conducted by Reddy *et al* involving 22 studies looking at bloodstream infections in febrile patients admitted to hospitals across the African continent identified *S. enterica* as the commonest isolate among adults, of which, 58% were non typhoidal serovars (mainly Typhimurium and Enteritidis).[2, 5]

A global systematic review of the literature from January 1990 to December 2012 by Feasey *et al* estimated an annual incidence of 3.4 million cases of invasive NTS with an overall incidence of 49 cases per 100 000 population and 681 316 deaths.[6] Enteric NTS illnesses accounted for approximately 93.7 million cases with an overall 155 000 deaths. In comparison, invasive NTS cases were considerably lower but had significantly higher mortality rates compared to enteric cases. Africa had the highest incidence of invasive NTS with 227 cases per 100 000 population with Asia and Oceania having the least number of cases (0.8 per 100 000 population) [1,2,6]. Another review showed an estimated annual incidence of 1800-9000 cases per 100 000 population of invasive NTS among retroviral positive and treatment naive adults in Africa.[2] Data has shown that invasive NTS is a

relatively rare event in industrialized populations.[2] A study conducted in Finland, Australia, Denmark and Canada from 2000- 2007 reported an overall crude incidence of 1.02 cases per 100 000 population and observed a seasonal pattern, with increased rates of invasive NTS in the autumn.[2] In Africa, invasive NTS has also shown to be highly seasonal with peaks of infection during the rainy season in both adults and children coinciding with increased incidences of malaria and malnutrition.[1]

1.3 Demographics

A discrete bimodal age distribution of invasive NTS exists in Africa with peaks occurring in children aged 6-36 months and adults aged 25-40 years.[1, 2, 4, 6-8] In Africa, the incidence of invasive NTS is higher among men compared to women predominantly due to men seeking less access to HIV testing and ART programs.[4,8] This is due to HIV stigma and perceived effects on masculinity amongst men. [4,8] In contrast, a study by Laupland *et al* conducted in high income countries (Finland, Denmark, Canada & Australia) found increasing age and male gender to be associated with a significantly higher rate of invasive NTS ($p< 0.0001$). The excess risk was notable in males aged 70 to 89 years with a markedly increased incidence above 90 years and older.[9]A study conducted in Asia also found a male preponderance for invasive NTS and attributed the presence of intra- venous drug use to be one of the risk factors more common among men. [10]

1.4 Underlying risk factors

Several predisposing risk factors, including malaria, malnutrition and HIV infection, have been identified in the literature for invasive NTS in Africa. In South Africa, the major contributing factor among adults is undoubtedly HIV infection, with patients with advanced HIV (CD4 cell counts < 200 cells/μL) demonstrating more invasive disease.[1, 2, 4, 6, 7, 11-15]

1.4.1 Malaria and malnutrition

The presence of malaria, malnutrition, co-infection with schistosomiasis and sickle cell disease are notable identifiable risk factors associated with invasive NTS among children particularly in Africa compared to other regions [16, 17] In South Africa, the association

between malnutrition and mortality due to invasive NTS is not significant.[4] Severe anaemia secondary to malaria is associated with invasive NTS in children in Africa but not adults. Its hypothesized that the association is due to hemosiderin loading by macrophages causing impaired intracellular killing of NTS.[18] Clinical malaria studies show that in the acute phase of malaria there is evidence of malabsorption and loss of epithelial tight junction integrity resulting in alterations to the intestinal architecture with oedema, mononuclear infiltrates and shortening of villi, ultimately resulting in increased risk of invasive NTS disease.[19]

1.4.2 Immunosuppression and immunodeficiency states

Immunosuppressive states such as diabetes, chronic steroid use, haematological and solid malignancies, auto-immune disease, liver disease (especially alcoholics), renal transplantation and patients on immunosuppressive drugs can predispose to invasive NTS. Haemoglobinopathies like sickle cell disease and thalassaemia can predispose to invasive NTS. [18] In higher income countries, certain primary immunodeficiencies syndromes, such as Chronic Granulomatous Disease (CGD), caused by a genetic defect in Nicotinamide adenine dinucleotide phosphate (NADPH) phagocyte oxidase with loss of intracellular killing of NTS and Mendelian susceptibility to mycobacterial disease (MSMD) caused by defects in genes or receptors encoding for interleukin 12 and 23 (IL-12/ 23) predispose individuals to invasive NTS. [18,19]

1.4.3 HIV infection

Advanced HIV infection is undoubtedly the main risk factor in adults in sub-Saharan Africa. Several case series show that more than 95% of patients are infected with HIV, of which 80% have CD4 counts less than 200 cells/μL. [1,11] There is also increased rate of recurrences despite adequate antimicrobial therapy with increased recrudescence rather than reinfection as a cause for the recurrences. [20] Three key immunological defects have been described in contributing to the invasive pathogenesis of NTS in HIV-infected adults in Africa. Firstly, there is loss of gut mucosal Interleukin-17 producing cells leading to loss of intestinal epithelial cell barrier integrity and loss of neutrophil chemotaxis with resultant translocation and invasion of NTS species into the bloodstream. Secondly, dysregulated cytokine production allows intracellular persistence of NTS species leading to recurrence of infections with recrudescence of NTS bacteraemia. Lastly, there is a humoral defect in HIV-infected patients with the presence of IgG antibodies against *Salmonellae* lipopolysaccharides causing impaired killing. [21-23]

1.5 Association with CD4 count and ART

Studies have shown that HIV infected adults with CD4 cell counts less than 200 cells/ µL have more severe invasive NTS disease versus patients with CD4 cell counts greater than 200cells/ µL.[1, 11, 24] A study done in Uganda reported NTS bacteraemia to be rare among adults with CD4 cell counts greater than 500cells/ µL. However in patients with CD4 cell counts between 200-500 cells/µL the incidence was 500 cases per 100 000 person- years and 7500 cases per 100 000 person- years among patients with CD4 cell counts less than 200 cells/µL, a 15-fold increase.[24]

Limited data at a population level is available looking at the impact of ART on the incidence of NTS among HIV infected patients especially in Africa.[2] However, in South Africa, there has been a decline in the incidence of invasive NTS noted since the roll out of Anti- retroviral therapy (ART) in 2004. [4] The study by Keddy *et al* conducted in Gauteng Province, South Africa from 2003-2013 showed a significant association between decreasing incidence of invasive NTS, especially *Salmonella typhimurium*, with increased ART utilization. It showed the incidence of invasive NTS per 100 000 population per year declined from 5.0 to 2.2 and the age group with the most notable decrease in incidence was 25 to 49 years old, the age group most burdened by HIV infection in South Africa.[4] The same study confirmed the observation that adult men accessed ART programs less than women resulting in higher incidence of invasive NTS in this group.[4]

1.6 Clinical presentation

NTS bacteraemia most commonly presents as a non- specific, febrile illness in HIV infected patients, making diagnosis difficult. [1,10,11,18] Diarrhoea is infrequent in these patients and fever, without a focus, is a common presentation occurring in up to 95% of cases. Of note in Africa, respiratory signs and symptoms in keeping with a lower respiratory tract infection occur in up to 30% of cases and is commonly due to co- infection with *Mycobacterium tuberculosis* or *Streptococcus pneumoniae.* Hepatomegaly and splenomegaly occur in up to half of cases (15-35%, and 30-45% respectively). The presence of high fever with splenomegaly can be used as a useful clinical predictor of invasive NTS disease in the African setting. Severe anaemia is present in 40-50% of adult cases. Invasive NTS can seed to the meninges, especially in children, and is associated with a significantly higher mortality

of up to 80% in adults.[1,10,11,18] The presence of anaemia and confusion are the most reliable indicators of poor prognosis in invasive NTS disease. [1,10,11,18]

1.7 Focal sites of NTS infection

Cohen *et al* conducted a study in New York demonstrating that underlying immunosuppressive states (excluding HIV infection**)** and structural abnormalities, both predispose to focal and suppurative NTS infections. [18, 25] Septic arthritis and osteomyelitis are associated with underlying heterozygous haemoglobinopathies whereas immunosuppression from malignancies or chronic steroid use are more important in focal soft tissue and chest NTS infections. Focal renal infections are associated with renal transplant patients on immunosuppressive therapy and abdominal foci are associated with underlying hepatobiliary structural abnormalities or presence of malignancy and alcoholism.[18, 25]

In contrast, amongst HIV infected individuals, focal NTS infection can occur with or without invasive NTS disease. It is uncommon to see focal disease without bloodstream infection. In addition, it also does not explain the frequent recurrences of blood stream infections that is commonly seen with HIV. The most common focal site of infection in HIV positive patients is the lung, particularly in the setting of cavitary lung disease. [18]

1.8 Salmonella enterica serovar Typhimurium ST313 strain

S. typhimurium and *S. enteritidis* are the two most identified serotypes causing invasive NTS disease in Africa. However, the ST313 strain is emerging as a dominant serotype in HIV infected patients especially in sub-Saharan Africa. [1,15,16] This strain has several genetic differences compared to other strains of this serotype. The most important characteristic of this ST313 strain is a degraded genome capacity in the form of pseudogenes and deletions, 60% of which are also seen in the *S. typhi* serotype. This convergence between *S. Typhimurium* ST313 and *S. Typhi* raises the possibility that the ST313 strain has adapted to occupy a unique niche in Africa with a different host range. [1, 15, 16] The ST313 strain has a multi-drug resistant (MDR) phenotype with resistance to ampicillin, chloramphenicol,

streptomycin, sulphonamides and trimethoprim. [19] Surveillance studies of antimicrobial resistance from the Democratic Republic of Congo and Kenya shows this strain to have decreased susceptibility to ciprofloxacin and third generation cephalosporins. [19, 26] If these strains spread further, it may reduce antibiotics options available for the effective treatment of invasive NTS.

1.9 Antimicrobial therapy and resistance

In the past, antimicrobials like chloramphenicol, ampicillin and trimethoprim were commonly used as treatment options for invasive NTS.[18] However, the emergence of multi-drug resistance since the 1980s has required the use of fluoroquinolones and extended-spectrum, third generation cephalosporins as effective alternative therapies.[18] Resistance to traditional first line drugs like ampicillin, co-trimoxazole and chloramphenicol is now common among invasive NTS strains in South Africa.[3] In sub-Saharan Africa, data suggests that *S. typhimurium* ST313 has developed multiple drug resistance mechanisms to conventional first line antibiotics.[19, 27-29] The emergence of resistance has necessitated the use of drugs as empirical management of sepsis, such as third generation cephalosporins and fluoroquinolones, like ciprofloxacin.[3]

World Health Organization (WHO) guidelines advise that in the presence of substantial resistance to first-line antimicrobials the use of a third-generation cephalosporin may be appropriate. The Center for Disease Control and Prevention (CDC) recommends 4-6 weeks of ciprofloxacin as treatment for NTS bacteraemia in HIV infected adults with CD4 cell counts less than 200 cells/μL, followed by long term prophylaxis.[24] The Infectious Diseases Society of America guidelines however recommends 2-6 weeks of treatment with fluoroquinolones. [30] Data to guide duration of therapy and prophylaxis is lacking in Africa.[24]

A study conducted in Ghana, among children, showed reduced ciprofloxacin susceptibility to *S. enteritidis* serotypes compared to *S. typhimurium* serotypes. It's uncertain whether these findings can be extrapolated to adults.[31] A bacteraemia study done in Tanzania shows that most *Salmonella* species are susceptible to ceftriaxone while being highly resistant to chloramphenicol, ampicillin and co-trimoxazole, with 8% resistance rate to ciprofloxacin.[11]

Another study done in Pemba shows resistance rate of 1.2% to ciprofloxacin. This emergence in resistance to ciprofloxacin is attributed to the overuse of ciprofloxacin in the primary health care facilities. [7] Studies also show that rapid commencement of ART reduce the incidence and recurrence of invasive NTS disease.[1, 3, 4, 6, 11] There isn't any resistance data from South Africa.

1.10 Mortality

The mortality rate of patients with NTS vary between studies from 20-47% with significantly higher mortality rates among advanced HIV infected individuals who have more invasive NTS disease.[1, 4, 6, 7, 11, 13, 14, 24] A study conducted in Malawi and another in Nairobi, Kenya, showed improving case fatality from invasive NTS to approximately 20% and attributed it to earlier health seeking behaviour and recognition and empiric treatment of sepsis. [18, 20, 32]

1.11 Aim

Non-typhoidal salmonella causes significant morbidity and mortality with more invasive disease being documented amongst the HIV-infected population. The literature has shown a paucity of information on this topic in Africa and South Africa. I aim to profile NTS in adults with HIV infection admitted to Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa.

1.12 Objectives

1. To describe the clinical and demographic profile of patients with NTS bacteraemia and HIV infection.
2. To describe the sites of infection and the choice of antibiotics prescribed in patients with NTS bacteraemia and HIV infection.
3. To evaluate the CD4 cell counts and viral loads in patients with NTS bacteraemia and HIV infection.

4. To evaluate the outcomes in patients with NTS bacteraemia and HIV infection

1.13 Methodology

Study design and population

This was a retrospective study of clinical records with an analytical and descriptive element. Convenience sampling was used, examining NTS bacteraemia in HIV infected adults admitted to CHBAH between January 2014 to December 2015.

Study sampling and sample size

The Trak care Laboratory Information System database from National Health Laboratory Services (NHLS) and surveillance data from the Centre for Enteric Diseases (CED) identified approximately 90 cases of NTS isolates from CHBAH during 2015. A similar figure was extrapolated for 2014 resulting in a sample size estimate of 170 cases. NHLS identified 164 NTS cases from 2014- 2015. (see figure 1). However, these cases were not all from blood cultures and included specimens from other sites such as stool, urine, cerebrospinal fluid, joint or abdominal aspirations etc. All the non-blood culture specimens were excluded as were patients who were subsequently found to be HIV negative and/or less than 18 years old resulting in a final sample size consisting of 69 adult patients.

Data collection

Once permission was granted by the hospital to retrieve and use the file data, a request to retrieve patients' files was submitted to the records department and data retrieved from the files was transferred to a data collection sheet. Each patient was allocated a reference number and no names or surnames were used, to maintain anonymity. Data collected included: age, gender, ethnicity, date of admission, length of hospital stay, whether the patients needed escalation of treatment with ventilation or dialysis and outcome, date of culture, culture isolates, other possible sites of infection, clinical parameters, blood parameters including full blood count, renal function and liver function, CD4 cell count and viral load, antibiotics used, duration of treatment and prophylaxis. Data about the anti-retroviral therapy was also collected. Additional data was obtained from the NHLS results database using the Trak Care® system.

1.14 Statistical analysis

Data was transferred to Epidata, a Statistical Analysis System (SAS) ®, for analysis. Frequencies and proportions were used to describe patient characteristics. For continuous variables such as age, CD4 count and HIV viral load, median and interquartile ranges (IQR) were used for non-normally distributed data and mean and standard deviations for normally distributed data. CHBAH NHLS laboratory normal range values were used to classify blood parameters as low, normal and high. Proportions of missing data were reported for all variables. Tables and horizontal bar graphs were used to present the data.

Demographic factors, clinical features, antibiotics given, and blood parameters were explored for their association with in hospital mortality using Chi-squared tests or Fisher's exact tests and in unadjusted logistical models. Variables with a p value <0.2 were considered for inclusion in the multivariable model. Gender, inappropriate use of antibiotics and Glasgow Coma Scale (GCS) <15 was included in the final model.

1.15 Ethics

Ethics approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand (see Ethics clearance certificate number M180276). Permission was also obtained from the management of CHBAH and NHLS to conduct the research. Confidentiality of the patients was maintained throughout as all data collected remained anonymous and no patient details were used in the study. Only the primary researcher had access to the data. Since this was a retrospective study no harm was done to the patients.

1.16 Limitations of study

The study population was small as per inclusion criteria as only patients who cultured NTS on blood were included. If NTS was cultured elsewhere and not on blood, these had to be excluded. Also, this was a retrospective study and the data obtained from the patients' records was dependent on the doctors attending to them during that period and may not have been

accurate or complete at times. There was also the issue of missing or misplaced patient records and files.

1.17 Timing

	May- July '17	Aug-Oct '17	November '17	Feb- June '18	July '18- Mar '19	April- Jun '19	July- Dec '19	Jan- June '20
Literature review	■							
Preparing protocol		■						
Protocol assessment			■					
Ethics application				■				
Collecting data					■			
Data analysis						■		
Writing up - thesis							■	
Writing up - paper								■

Figure 1: Timeline

1.18 Funding

The expenses for this research were funded by myself and included printing costs, stationery, and internet costs.

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CHAPTER 2: SUBMISSIBLE ARTICLE

TITLE: PROFILE OF NONTYPHOIDAL SALMONELLA BACTERAEMIA IN ADULTS WITH HIV INFECTION ADMITTED TO CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

Authors:

Alimia SE[1]

Tsitsi JML[1,2]

Menezes C[1,2]

Affiliations:

1. Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand

2. Division of Infectious Diseases, Department of Medicine, Chris Hani Baragwanath Academic Hospital

Short Title: Profile of NTS bacteraemia in HIV infected adults at Chris Hani Baragwanath Academic Hospital.

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Abstract

Background: In sub- Saharan Africa, NTS has emerged as an important cause of invasive bloodstream infections, especially among HIV infected adults. South Africa has one of the highest burdens of HIV disease in Africa. Literature shows invasive NTS as a well-established disease amongst HIV infected adults with mortality rates of 20-47% in sub-Saharan Africa. However, limited data exists for NTS bacteraemia in South Africa.

Aim: Profile NTS bacteraemia in HIV infected adults admitted to Chris Hani Baragwanath Academic Hospital from 2014-2015 examining demographic and clinical profile, sites of infection, antibiotics used, CD4 counts, viral loads and outcomes.

Methods: Retrospective review of patient records with positive NTS isolates on blood culture.

Results: A total of 69 patients (53.6% male, n=37) were included in the study and consisted predominantly of Black Africans (98.5%, n=68). The mean age (± SD) at presentation was 44 years ±12.39. A total 73.5% (n=50) of patients were empirically started on appropriate antibiotics on admission prior to blood culture results. Ceftriaxone was the most used antibiotic (56.5%, n=39), followed by ciprofloxacin (36%, n=25) and co-amoxiclav (33%, n=23). Trimethoprim/ sulfamethoxazole was used in 20% (n=14) of patients for empiric *Pneumocystis jiroveci pneumonia* cover. Of the 69 patients, 71% (n=49) had CD4 counts < 200 cells/µL, of which 39.1% (n=27) had CD4 counts < 50 cells/ µL. Of note, 75.4% (n=52) were newly diagnosed with HIV and anti-retroviral therapy naive. The mortality rate was 25% (n=18). A GCS<15 and presence of renal dysfunction were associated with mortality. In multivariable analyses, a GCS<15 independently predicted death, with patients with GCS<15 having 15.7 times greater likelihood of dying than fully conscious patients [adj OR 15.69 (3.43- 71.78) p= <0.001]. Men were 4.7 times more likely to die compared to women [adj OR 4.69 (1.09 – 20.99) p=0.043]. Patients treated with inappropriate antibiotics (n=7) were shown to have 3.2 times greater likelihood of dying than those treated with appropriate antibiotics [adj OR 3.20 (0.75- 13.61) p= 0.117].

Conclusion: Invasive NTS was more prevalent in advanced HIV infected patients with a 25% mortality rate.

Introduction

Salmonella causing disease in humans are divided into typhoidal serovars (*Typhi* and *Paratyphi*) and thousands of non-typhoidal serovars. Non-typhoidal salmonella (NTS) predominantly causes a self-limiting diarrheal illness in healthy individuals from high income countries.[1] By contrast, in sub-Saharan Africa, NTS has emerged as an important cause of invasive bloodstream infections, especially among human immunodeficiency virus (HIV) infected adults.[2] South Africa has one of the highest burden of HIV disease in Africa with invasive NTS being a well-established disease amongst HIV infected individuals with mortality rates of 35-60% in sub-Saharan Africa.[3] The incidence of invasive NTS has been declining since the roll out of anti-retroviral therapy (ART) in 2004. [4] Very little epidemiological data exists for NTS isolates identified in South Africa and its association with HIV.[3] A review of this topic will provide data about the South African setting.

A global systematic review of the literature from January 1990 to December 2012 by Feasey *et al* estimated an annual incidence of 3.4 million cases of invasive NTS with an overall incidence of 49 cases per 100 000 population and 681 316 deaths.[6] Enteric NTS illnesses accounts for approximately 93.7 million cases with an overall of 155 000 deaths. In comparison, invasive NTS cases are considerably lower but have significantly higher mortality rates compared to enteric cases. Africa has the highest incidence of invasive NTS with 227 cases per 100 000 population.[1, 2, 6]

A discrete bimodal age distribution of invasive NTS exists in Africa with peaks occurring in children aged 6-36 months and adults aged 25-40 years.[1, 2, 4, 6-8] In Africa, the incidence of invasive NTS is higher among men compared to women predominantly due to men seeking less access to HIV testing and ART programs.[4,8] This is due to HIV stigma and perceived effects on masculinity amongst men. [4,8]

Several predisposing risk factors, including malaria, malnutrition and HIV infection, have been identified in the literature for invasive NTS in Africa. In South Africa, the major contributing factor among adults is undoubtedly HIV infection, with patients with advanced HIV (CD4 cell counts < 200 cells/μL) demonstrating more invasive disease.[1, 2, 4, 6, 7, 11-15]

NTS bacteraemia most commonly presents as a non-specific, febrile illness in HIV infected patients, making diagnosis difficult. [1,11,18] Diarrhoea is infrequent in these patients and fever, without a focus, is a common presentation occurring in up to 95% of cases. Of note in Africa,

respiratory signs and symptoms in keeping with a lower respiratory tract infection occur in up to 30% of cases and is commonly due to co-infection with *Mycobacterium tuberculosis* or *Streptococcus pneumoniae* [1,18]

Resistance to traditional first line drugs like ampicillin, co-trimoxazole and chloramphenicol are now common among invasive NTS strains in South Africa.[3] In sub-Saharan Africa, data suggests that *Salmonella typhimurium* ST313 has developed multiple drug resistance.[6, 27] This emergence of resistance has necessitated the use of drugs for empirical management of sepsis, such as third generation cephalosporins and fluoroquinolones, like ciprofloxacin.[3]

The mortality rate of patients with NTS vary between studies from 20–47% with significantly higher mortality rates among advanced HIV infected individuals who have more invasive NTS disease.[1, 4, 6, 7, 11, 13, 14, 24]

Aim

Due to the paucity of information in the South African setting, this study aims to profile NTS bacteraemia amongst HIV infected adults in CHBAH.

Methods

Study design and population

This is a retrospective study of clinical data with an analytical and descriptive element. Convenience sampling is used, examining NTS bacteraemia in HIV infected adults admitted to CHBAH between January 2014 to December 2015.

Study sampling and sample size

The Trak care Laboratory Information System database from National Health Laboratory Services (NHLS) and surveillance data from the Centre for Enteric Diseases (CED) identified approximately 90 cases of NTS isolates from CHBAH during 2015. A similar figure was extrapolated for 2014 resulting in a sample size estimate of 170 cases. NHLS identified 164 NTS cases from 2014- 2015. (see figure 1) These cases included specimens from other sites such as stool, urine, cerebrospinal fluid, joint or abdominal aspirations and these were

excluded as were patients who were subsequently found to be HIV negative and/or less than 18 years old resulting in a final sample size consisting of 69 patients.

Data collection

Once permission was granted by the hospital to retrieve and use the file data a request to retrieve the patients' files was submitted to the records department and data retrieved from the files was transferred to a data collection sheet. Each patient was allocated a reference number and no names or surnames were used, to maintain anonymity. Data collected included: age, gender, ethnicity, date of admission, length of hospital stay, whether the patients needed escalation of treatment with ventilation or dialysis and outcome, date of culture, culture isolates, other possible sites of infection, clinical parameters, blood parameters including full blood count, renal function and liver function, CD4 cell count and viral load, antibiotics used, duration of treatment and prophylaxis. Data about the anti-retroviral therapy was also collected. Additional data was obtained from the NHLS results database using the Trak Care® system.

Statistical analysis

Data was then transferred to Epidata, a Statistical Analysis System (SAS) ®, for analysis. Frequencies and proportions were used to describe patient characteristics. For continuous variables such as age, CD4 count and HIV viral load, median and interquartile ranges (IQR) were used for non-normally distributed data and mean and standard deviations for normally distributed data. CHBAH NHLS laboratory normal range values were used to classify blood parameters as low, normal and high. Proportions of missing data were reported for all variables. Tables and horizontal bar graphs were used to present the data.

Demographic factors, clinical features, antibiotics given, and blood parameters were explored for their association with in hospital mortality using Chi-squared tests or Fisher's exact tests and in unadjusted logistical models. Variables with a p value <0.2 were considered for inclusion in the multivariable model. Gender, inappropriate use of antibiotics (defined as incorrect empirical antibiotics prescribed from admission and continued despite having blood culture results with appropriate antibiotic susceptibility) and Glasgow Coma Scale (GCS) <15 was included in the final model.

Results

Patient demographics

The study consisted predominantly of Black African patients (98.5%, n=68) and there was a higher proportion of male patients (53.6%, n=37). (See table 1)

The sample size was normally distributed for age with +0.04 skewness, ranging from 18 to 72 years. The highest rates of NTS bacteraemia were seen between the ages of 36 to 52 years. The mean age at diagnosis was 44 years (SD ±12.39). (See figure 3) Of the 69 patients, six were further immunocompromised with an underlying malignancy or end stage renal disease (see figure 2).

Clinical presentation

The median length of stay in hospital was 10 days (IQR 6- 20). Of the 69 patients, three required further escalation of care and high care admission for haemodynamic support for hypotension. Of the three patients, two required escalation with ventilatory support and dialysis. Of the two patients who received dialysis one had concomitant severe malaria with renal dysfunction. (see Table 1)

The most common symptoms reported were fever, cough and diarrhoea (52.2%; 52.2% and 40.6% respectively). A total 42% (n=29) of patients were found to have pyrexia on presentation and 23.2% (n=16) had a GCS < 15. Respiratory crackles were found in 36.2% (n=25) of patients on presentation indicating a lower respiratory tract infection. A third of these patients were concomitantly diagnosed with pulmonary *Mycobacterium tuberculosis* and started on treatment accordingly. Hepatomegaly was more common than splenomegaly (27.5%, n=19 versus 5.8%, n=4). (see Figure 4)

All the patients had NTS confirmed on blood culture, however four patients (5.6%) also cultured NTS from other sites. Whether these were the primary sites of infection with seeding into the blood or they were secondary sites is difficult to establish. (see Table 1)

Blood parameters

A total 94.2% (n=65) of patients had a low haemoglobin on admission. In contrast, 56.5% (n=39) of patients had normal white blood cell counts and 52.2% (n=32) had normal platelet counts on admission. Of the 69 patients, 75.4% (n=52) had a low sodium on blood chemistry. This hyponatraemia was most likely hypovolemic in origin and due to the gastrointestinal

losses from diarrhoea which was reported in 40% (n=28) of the patients. A total 62 % (n=43) of the patients had a normal potassium with 83% (n=57) of patients having a metabolic acidosis with low bicarbonate values. A high urea was seen in 61% (n=42) of patients and 49% (n=34) had a high creatinine value indicating renal dysfunction. A total 32% (n=22) of patients lacked a full liver function test from admission which possibly under evaluated abnormal liver function findings with 55% (n=38) of the patients having a low albumin. A hepatocellular pattern with raised ALT and AST was seen in 35% and 58% of patients respectively versus a cholestatic pattern with raised ALP and GGT in 13% and 23% of patients, respectively. (See Table 2)

Choice of antibiotics

A total 73.5% (n=50) of patients were started empirically on appropriate antibiotics from admission with ceftriaxone, ciprofloxacin and co- amoxiclav being the most commonly prescribed antibiotics (56.5%; 36.2%; 33.3% respectively) This finding was attributed to the fact that patients presented to hospital clinically with features of sepsis and thus were empirically treated with antibiotics based on their clinical presentation. Patients who had diarrhoea as a presenting feature, were empirically treated with ciprofloxacin and metronidazole whereas those with confusion (GCS<15) were empirically treated with ceftriaxone. In both this instances NTS bacteraemia was appropriately empirically treated by this choice of antibiotics prior to obtaining blood culture results. (see Figure 6)

The remaining 26.5 % (n=18) of patients were prescribed incorrect empirical antibiotics from admission and remained on the same antibiotics throughout hospital stay. There was no documentation of the blood culture results in these patients' clinical notes, suggesting that these may not have been obtained prior to discharge, step down or death. Of the 18 patients, three patients demised before the blood cultures were available, five patients were prematurely discharged prior to checking blood culture results and five patients were stepped down to a different health care facility.

Co- amoxiclav was prescribed for 33% (n=23) of patients because of signs and symptom of a concomitant lower respiratory tract infection on admission and 20% (n=14) of the patients received high dose trimethoprim/ sulfamethoxazole (TMP-SMX) as empiric *Pneumocystis jiroveci pneumonia (PJP)* treatment based on the clinical picture. Also 71% (n=49) of the study population had CD4 cell counts less than 200 cells/µL thus were given prophylactic doses of TMP-SMX.

Of the 69 patients, 58% (n=40) were treated with ≤ 7 days of antibiotics and 23.5% (n=16) were given secondary prophylaxis (defined as oral ciprofloxacin 500mg per os twice daily) on discharge. The median duration for which prophylaxis was given was 14 days (IQR 10-21). (see Table 3 and 6)

CD4 and viral load

A total 71% (n=49) of the study population had CD4 cell counts less than 200 cells/µL, of which 39.1% (n=27) had CD4 cell counts less than 50 cells/µL. The CD4 cell count distribution was skewed (skewness +1.87). The median CD4 cell count was 55 cells/µL (IQR 18 – 100). The median Log viral load was 12.5 (IQR 10.7- 13.5). (see Figure 5)

Of the 69 patients, 75.4% (n=52) were ART naïve and new diagnosis of HIV infection at the time of presentation, with 7 of the 52 patients (13.5%) having had previously defaulted ART. A total 5/17 patients on ART had viral loads less than 40 copies/ml. Most of the patients were on first line ART and the most used regimen was Tenofovir/Emtricitabine/Efavirenz. (See Table 1)

Outcomes and association with mortality

There was a 25% (n=18) mortality rate amongst patients in this study. We explored associations between clinical features and in hospital mortality using Chi-squared / Fisher's exact tests and in unadjusted logistical models. A GCS<15 and renal dysfunction were associated with mortality. Renal dysfunction could not be explored in binary logistical models as it perfectly predicted mortality (i.e. none of the patients with normal renal function died – see Table 4).

In multivariable analyses, a GCS<15 independently predicted death, with patients with GCS<15 having 15.7 times greater odds of dying than fully conscious patients [adj OR 15.69 (3.43-71.78) p= <0.001]. Men were 4.7 times more likely to die compared to women [adj OR 4.69 (1.09 –20.99) p=0.043]. In addition, patients treated with inappropriate antibiotics were shown to have 3.2 times greater odds of dying than those treated with appropriate antibiotics [adj OR 3.20 (0.75-13.61) p= 0.117]. (see Table 5 and Figure 7)

Discussion

The main reason for doing this study is because data on NTS bacteraemia is limited in sub-Saharan Africa, despite it being a significant cause of mortality especially in the advanced HIV infected population. There are multiple similarities in our study findings with literature, however there are also some differences.

Our study has a mean age at presentation of 44 years with the highest rates of NTS bacteraemia seen amongst the ages of 36-52 years old. This is mainly due to this age category having the highest burden of HIV disease [(1)].

Despite a relatively small sample size, 54% of the study population were male. The study conducted by Keddy *et al* in Gauteng Province, South Africa, shows that men are less likely to seek access to ART compared to women hence having higher prevalence of invasive NTS disease leading to a higher mortality rate.[(4)]. A large multicentre study conducted by Marks *et al* involving 13 surveillance sites across the African continent including South Africa demonstrated that men are at high risk of developing NTS disease compared to women[(8)]. Similarly, studies conducted in high income countries also show male gender to be associated with high incidence rates of invasive NTS disease [(9)]. In contrast, studies conducted in high income countries show increasing age to be associated with invasive NTS disease [(9)]. This could be because high income countries have a low burden of HIV disease and increasing age is associated with other co- morbidities with increased risk of underlying malignancies.

The literature shows that invasive NTS most commonly presents as a febrile illness with non-specific signs and symptoms making diagnosis difficult without microbiological confirmatory testing [(1, 18).] Our study population presented similarly however the presence of diarrhoea was more common in our study than has been described in the literature with 40% (n=28) of our patients reporting to have diarrhoea on presentation. [(1, 18)]

A total 36% (n= 25) of the patients had signs and symptoms of a lower respiratory tract infection on presentation with a third of these patients being concomitantly diagnosed with pulmonary *Mycobacterium tuberculosis* and started on treatment accordingly. This is in line with studies done previously which showed similar findings [(1, 18)]. The finding of concomitant tuberculosis is due to 71% of the patients having CD4 cell counts less than 200 cells/µL predisposing them to opportunistic infections like tuberculosis.

All the study patients had NTS isolated on blood culture as this was part of the inclusion criteria. Only 4 of the 69 patients grew NTS at different sites in addition. Whether these were the primary sites with seeding or secondary sites is difficult to establish. The review by Gordon MA showed that in HIV- infected adults, invasive NTS disease could occur with or without other focal sites of infection but is not seen commonly[18]. Seeding into meninges can occur, but is more commonly seen in children and is associated with very high mortality rates [1, 18].

With respect to antibiotic use, the literature has shown that invasive NTS has become resistant to traditional first line drugs like ampicillin and trimethoprim [11]. In this study, the use of antibiotics like co-amoxiclav and trimethoprim/sulfamethoxazole for concomitant respiratory tract infections, empiric and prophylactic PJP cover respectively, is justified based on the clinical picture. More research is needed to ascertain if this is contributing to the emergence of resistance.

In addition, 73.5 % (n= 50) of the patients were started on appropriate empirical broad-spectrum antibiotics on admission based on their clinical presentation with either ceftriaxone or ciprofloxacin. Studies from the literature advocate the use of third generation cephalosporins and fluoroquinolones for invasive NTS due to increasing emergence of highly resistant strains [1, 3, 18].

A total 58% (n=40) of the patients received ≤ 7 days of antibiotic therapy with less than a quarter (n=16) of patients given secondary prophylaxis on discharge. This was mainly because patients improved clinically and biochemically from admission. This, however, highlights poor knowledge of guidelines as the culture results were known. It was only in three cases that the Infectious Diseases team was consulted for advice on the appropriate duration of treatment. This was a significant undertreatment in both duration of therapy and duration of secondary prophylaxis when compared to what most guidelines suggest. The Centers for Disease Control and Prevention (CDC) recommends 4-6 weeks of ciprofloxacin as treatment for NTS bacteraemia in HIV infected adults with CD4 cell counts less than 200 cells/µL, followed by long term prophylaxis[24]. (see Table 6) Whereas, the Infectious Diseases Society of America guidelines recommend 2-6 weeks of treatment with fluoroquinolones [30]. Unfortunately, there are no South African guidelines.

Most patients were given secondary prophylaxis for an average of 14 days. With regards to relapses, it would be interesting to follow up and see how many of these patients represented

with relapses of NTS bacteraemia and how many of these relapses were attributable to previously inappropriately treated infection versus re- infection

The literature has extensively shown HIV as the most significant risk factor associated with invasive NTS disease. Multiple studies across Africa have shown that invasive NTS disease is more prevalent among HIV- infected adults with CD4 cell counts less than 200 cells/µL [1, 11, 24]. Morpeth *et al* conducted a study in Uganda showing a 15- fold increase in the incidence of invasive NTS amongst patients with CD4 cell counts less than 200 cells/µL [24]. Interestingly, the study by Keddy *et al* in South Africa demonstrated the decline in incidence of invasive NTS disease since the extensive ART program rollout from 2004[4]. This might have contributed to fewer cases of invasive NTS resulting in our sample size of 69 patients. Concerningly, despite this, 75% (n=52) of the study population were ART naïve at presentation. However, almost all were initiated on ART upon discharge. Literature also shows that there are higher rates of recurrence amongst HIV infected patients versus recrudescence [1, 24]. Whether this higher recurrence rate is due to patient factors such as non-compliance with ART is not elaborated.

Finally, factors affecting clinical outcome and mortality are explored and show male gender, presence of confusion, renal dysfunction and the inappropriate use of antibiotics is associated with mortality. Our study has a mortality rate of 25% similar to studies done elsewhere in sub-Saharan Africa of 20- 47%. [1,4,6-7,11,13-14,24] Male patients have a five times higher chance of mortality compared to women. This is mostly attributed to men being less likely to seek access to ART and more likely to default treatment. The study by Keddy *et al* in Gauteng province demonstrated this[4]. The studies by Feasey *et al* and Gordon MA both showed anaemia and confusion to be poor prognostic factors in invasive NTS disease [1, 18]. In our study despite 94.2% (n=65) of patients presenting with anaemia, it was not associated with a significant mortality (p=0.687). Whereas, the presence of confusion on presentation, independently predicted death. This may be because presence of confusion indicates a marker for multi- organ dysfunction which is associated with higher mortality rates [1,18]. In addition, the presence of renal dysfunction is associated with higher mortality rates as well. The possible reason for this is that 40% (n=28) of our patients had diarrhoea on presentation and were dehydrated with a pre-renal uraemia. In the literature, diarrhoea is not commonly seen among invasive NTS patients and resultant renal dysfunction not being a common finding. Previous studies have usually associated *Salmonella typhi* strains with diarrhoea [1, 18]. Despite 71% of the study population having CD4 cell counts less than 200 cells/µL,

interestingly, it is not significantly associated with a higher chance of mortality (p=0.313). This could be attributed to the fact that almost all patients were initiated on ART therapy whilst in hospital with no delay. This is of importance as previously mentioned that the ART rollout has directly impacted the decline in incidence of invasive NTS disease.

Limitations of the study:

The main limitation of the study is the small sample size. This can be attributed to the extensive ART rollout program initiated in South Africa since 2004 resulting in fewer cases of NTS bacteraemia seen. In addition, being a retrospective study, some questions cannot be answered because of missing data.

Conclusion

This study demonstrated numerous similarities with previous studies regarding invasive NTS disease. It confirmed that invasive NTS is more prevalent among HIV infected adults with advanced disease causing mortality rates like those seen elsewhere in Africa. Previous studies have highlighted the decline in invasive NTS cases since the rollout of ART and our study highlights this too. Our study highlights the current use of broad-spectrum antibiotics in treatment of sepsis in our setting is appropriate empiric treatment for invasive NTS disease and is associated with positive outcomes. Lastly, a compelling finding of this study is the poor guideline compliance regarding secondary prophylaxis. The impact of appropriate secondary prophylaxis on rates of recurrence of invasive NTS is an area for further research which, in conjunction with the success of the ART programme, may impact disease incidence and prevention.

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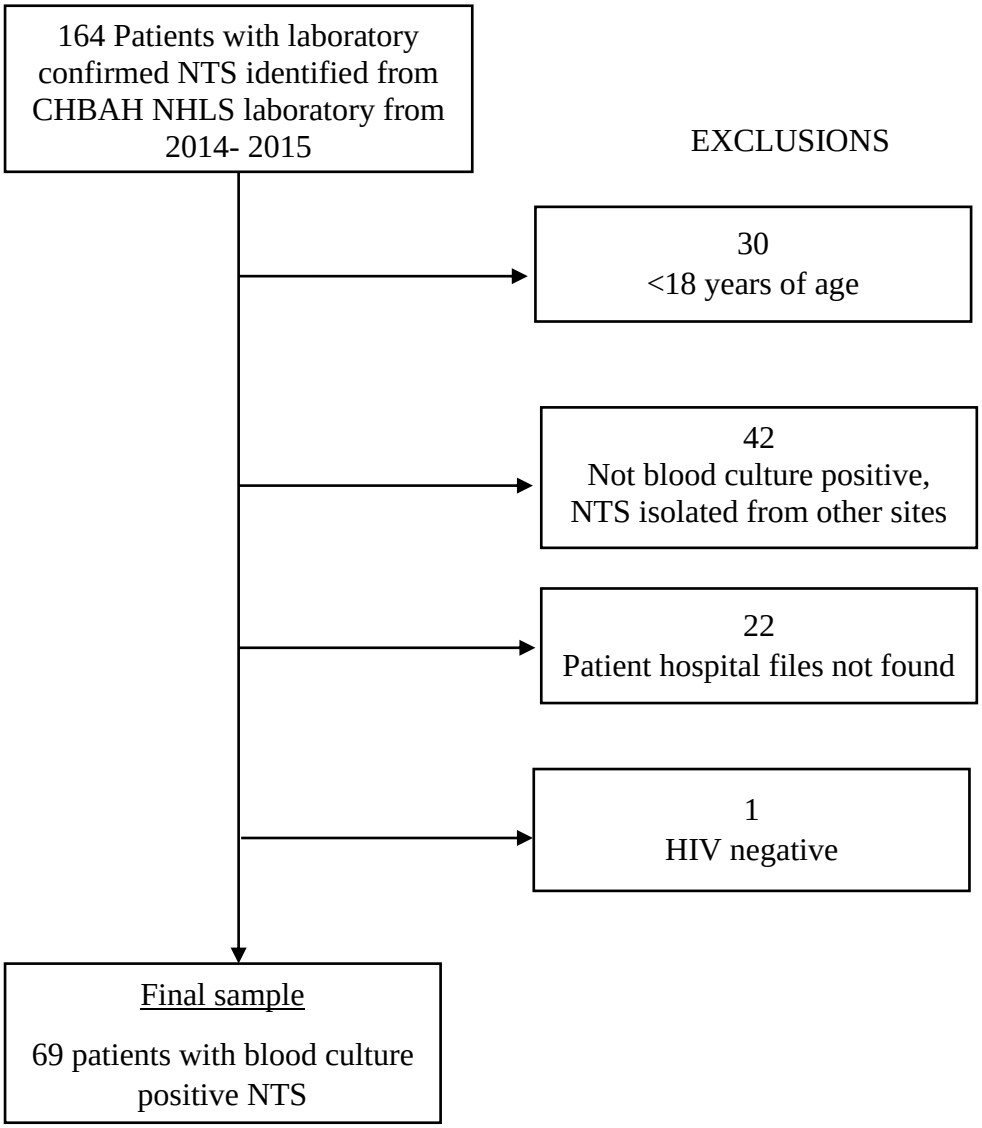


Figure 1: Flowchart of patients included in study

Concomitant immunosuppressive condition	Number
Acute leukaemia	1
Kaposi sarcoma	1
Burkitt's lymphoma	1
Hodgkin's lymphoma	1
Hepatocellular carcinoma	1
End stage renal disease	1

Figure 2: Associated immunosuppressive condition

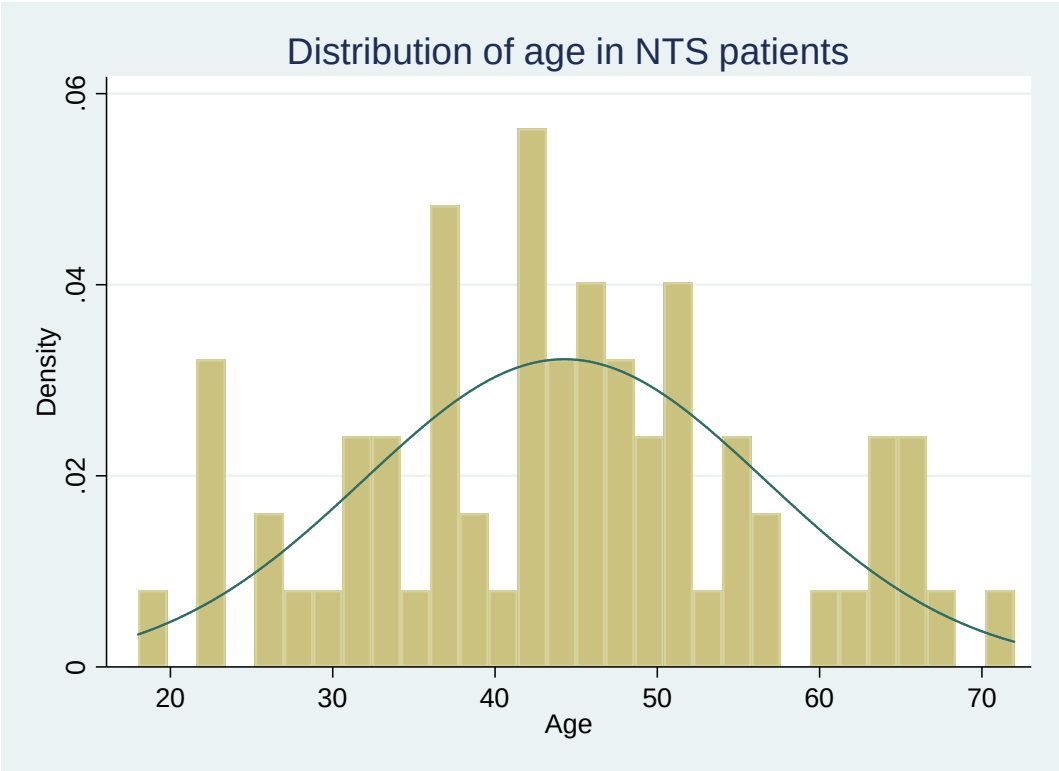


Figure 3: Distribution of age at diagnosis in NTS patients (skewness +0.04)

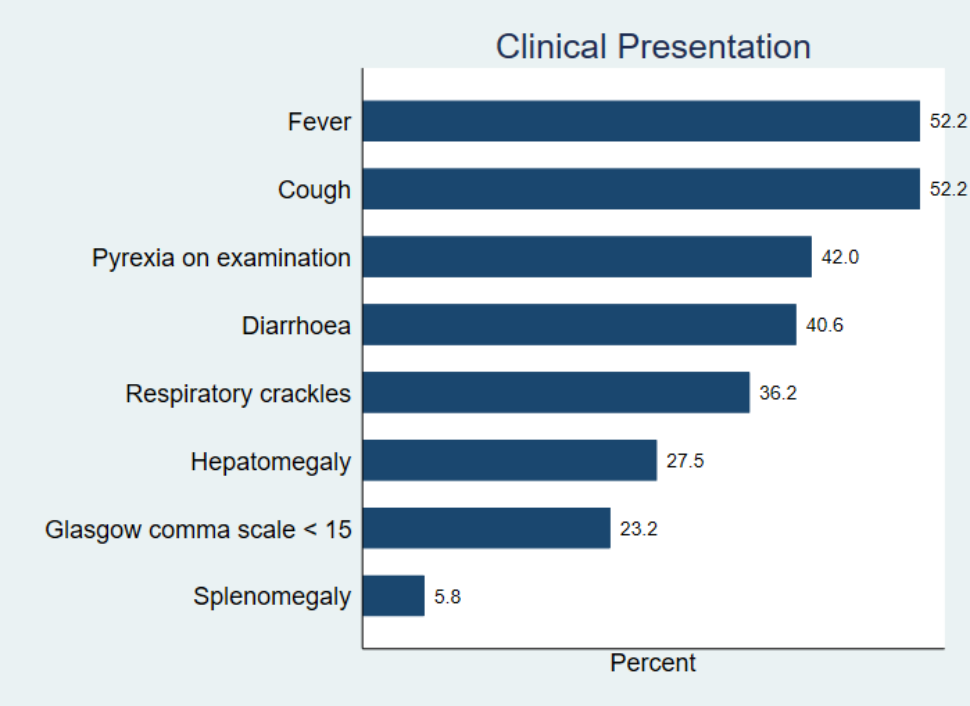


Figure 4: Clinical signs and symptoms in patients with NTS bacteraemia

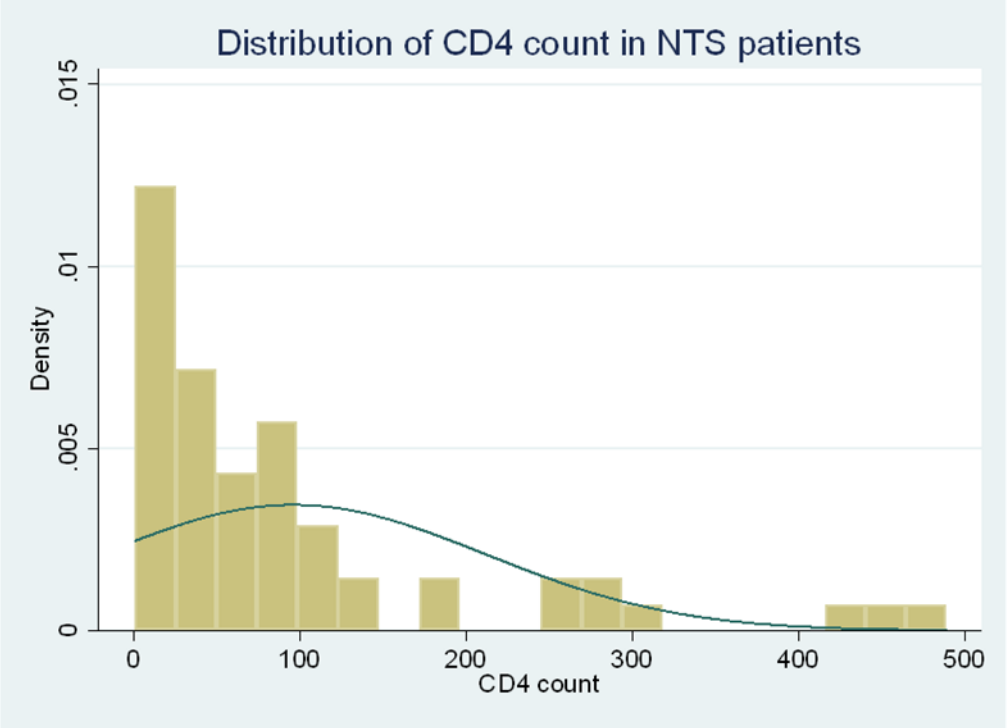


Figure 5: Distribution of CD4 count in NTS patients (skewness +1.87)

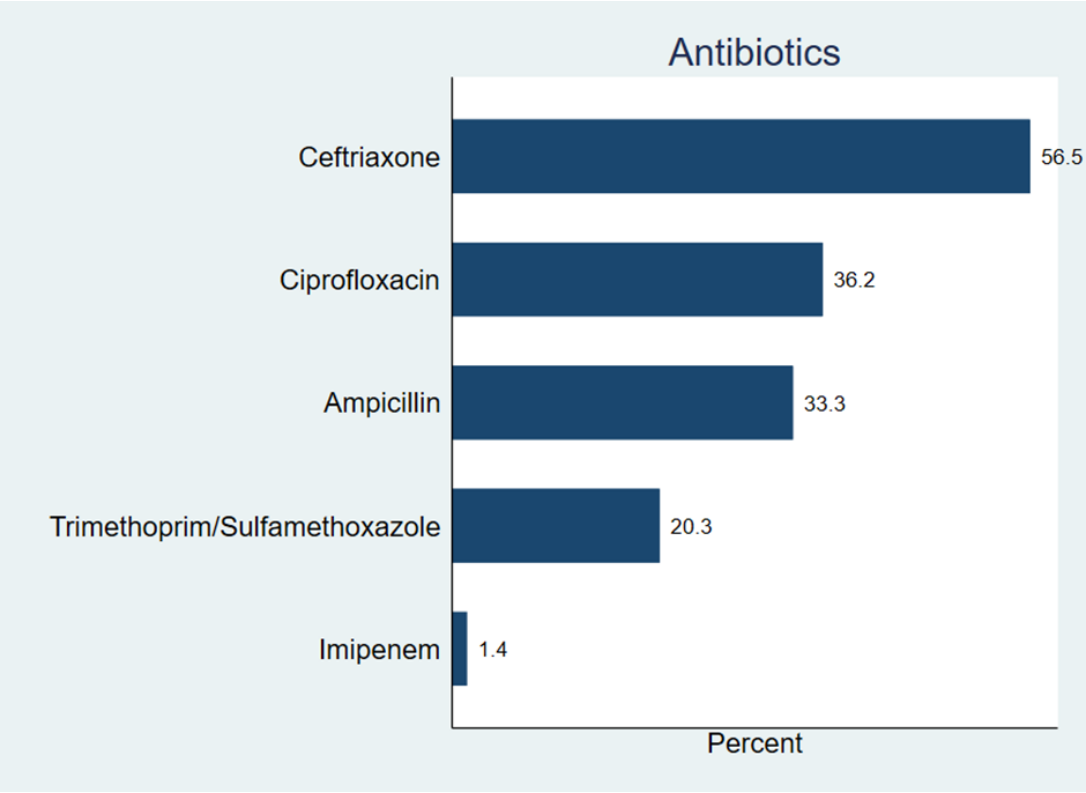


Figure 6: Antibiotics prescribed in NTS patients

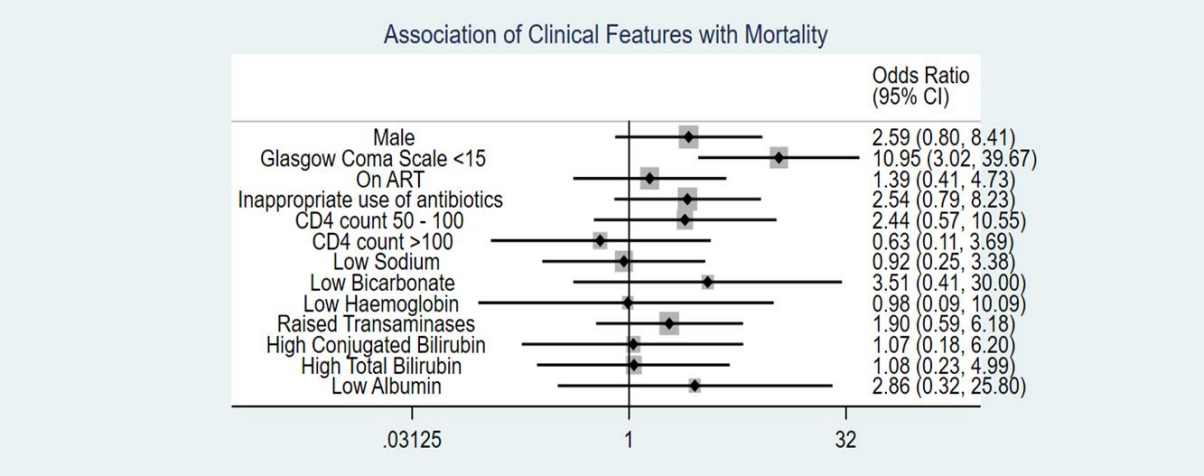


Figure 7: Exploration of the association of clinical features with mortality in unadjusted logistical models

Table 1: Characteristics of patients with NTS bacteraemia

Patient Characteristics		**N**	**%**
Demographic characteristics			
Age			
Overall age in years (mean, SD)	44 (± 12.39)		
Self-reported race			
Black		68	98.5
Coloured/ mixed race		1	1.5
Gender			
Male		37	53.6
Female		32	46.4
Clinical features			
Severity of Illness			
Length of stay in days (median, IQR)	10 (6 – 20)		
Escalation of care during admission		3	4.4
Requiring ventilation		2	2.9
Requiring dialysis		2	2.9
Died		17	24.6
Other sites of infection			
Lungs		1	1.4
Skin & soft tissues		1	1.4
Meninges		1	1.4
Gastrointestinal tract		1	1.4
Clinical presentation			
Symptoms			
Fever		36	52.2
Diarrhoea		28	40.6
Cough		36	52.2
Signs (on examination)			
Pyrexia		29	42.0
Glasgow coma scale <15		16	23.2
Respiratory crackles		25	36.2
Splenomegaly		4	5.8
Hepatomegaly		19	27.5
Markers of HIV Disease Progression			
CD4 count (median, IQR)	55 (18 – 100)		
CD4 count categories (cells/µL)			
<50		27	39.1
50 – 99		14	20.3
100 – 199		8	11.6
200 – 349		5	7.2
>350		3	4.4
Missing		12	17.4

HIV viral load (copies/mL) (median, IQR)	258933(43775 – 705564)		
Log viral load (median, IQR)	12.5 (10.7 – 13.5)		
Virally suppressed (<40 copies/ mL)		5	7.2
Antiretroviral treatment			
On ART		17	24.6
Not on ART		52	75.4
ART Regimen (N=17)			
TDF/FTC/EFV		11	64.7
ABC/FTC/EFV		1	5.9
AZT/FTC/LPVr		2	11.8
On ART but regimen not specified		3	17.6

* TDF= Tenofovir/ FTC= Emtricitabine/ EFV= Efavirenz/ ABC= Abacavir/ AZT= Zidovudine/ LPVr= Lopinavir+ Ritonavir

Table 2: Blood parameters on admission

Blood Parameters	**N**	**%**
Full Blood Count		
White blood cell count (x10^9/l)		
Low	19	27.5
Normal	39	56.5
High	11	19.9
Missing	-	-
Haemoglobin (g/L)		
Low	65	94.2
Normal	4	5.8
High	-	-
Missing	-	-
Platelet count (x10^9/l)		
Low	26	37.7
Normal	36	52.2
High	7	10.1
Missing	-	-
Blood Chemistry		
Sodium (mmol/l)		
Low	52	75.4
Normal	13	18.8
High	2	2.9
Missing	2	2.9
Potassium (mmol/l)		
Low	17	24.6
Normal	43	62.3
High	7	10.1
Missing	2	2.9
Bicarbonate (mmol/l)		
Low	57	82.6
Normal	10	14.5
High	-	-
Missing	2	2.9
Urea (mmol/l)		
Low	2	2.9
Normal	23	33.3
High	42	60.9
Missing	2	2.9
Creatinine (µmol/l)		
Low	10	14.5
Normal	23	33.3
High	34	49.3
Missing	2	2.9

Liver Function Tests		
Total protein (g/l)		
Low	7	10.1
Normal	20	29.0
High	18	26.1
Missing	24	34.8
Albumin (g/l)		
Low	38	55.1
Normal	9	13.0
High	-	-
Missing	22	31.9
Total bilirubin (µmol/l)		
Low	2	2.9
Normal	33	47.8
High	11	15.9
Missing	23	33.3
Conjugated bilirubin (µmol/l)		
Low	-	-
Normal	8	11.6
High	38	55.1
Missing	23	33.3
Alanine transaminase (U/l)		
Low	3	4.3
Normal	20	29.0
High	24	34.8
Missing	22	31.9
Aspartate transaminase (U/l)		
Low	-	-
Normal	7	10.1
High	40	58.0
Missing	22	31.9
Alkaline phosphatase (U/l)		
Low	6	8.7
Normal	32	46.4
High	9	13.0
Missing	22	31.9
Gamma- glutamyl transferase (U/l)		
Low	-	-
Normal	31	44.9
High	16	23.2
Missing	22	31.9

Table 3: Choice of antibiotics

Patient Characteristics		**N**	**%**
Antibiotic treatment			
Appropriate empiric antibiotic use on admission			
Yes		50	73.5
No		18	26.5
Antibiotics Given			
Co- amoxiclav		23	33.3
Trimethoprim/sulfamethoxazole		14	20.3
Ciprofloxacin		25	36.2
Imipenem		1	1.4
Ceftriaxone		39	56.5
Treatment Duration categories			
≤ 7 days		40	58.0
≤ 10 days		12	17.4
≤ 14 days		17	24.6
Secondary Prophylaxis			
Given		16	23.5
Treatment duration in days (median, IQR)	14 (10 – 21)		

Table 4: Associations with mortality

Factor	Alive at discharge N (%)	Died N (%)	P Value*
Gender			
Male	25 (67.6)	12 (32.4)	
Female	27 (84.4)	5 (15.6)	0.106
Inappropriate use of antibiotics			
No	40 (80.0)	10 (20.0)	
Yes	11 (61.1)	7 (38.9)	0.113
GCS <15			
No	46 (86.8)	7 (13.2)	
Yes	6 (37.5)	10 (62.5)	<0.001
On ART			
No	40 (76.9)	12 (23.1)	
Yes	12 (70.6)	5 (29.4)	0.599
CD4 Count			
<50	22 (81.5)	5 (18.5)	
50 - 100	9 (64.3)	5 (35.7)	
>100	14 (87.5)	2 (12.5)	0.313
Low Sodium			
No	11 (73.3)	4 (26.7)	
Yes	39 (75.0)	13 (25.0)	0.569
Low Bicarbonate			
No	9 (90.0)	1 (10.0)	
Yes	41 (71.9)	16 (28.1)	0.213
Low Haemoglobin			
No	3 (75.0)	1 (25.0)	
Yes	49 (75.4)	16 (24.6)	0.687
Renal Dysfunction			
No	10 (100.0)	0 (0)	
Yes	42 (71.2)	17 (28.8)	0.047
Raised Transaminases			
No	23 (82.1)	5 (17.9)	
Yes	29 (70.7)	12 (29.3)	0.215
High Conjugated Bilirubin			
No	6 (75.0)	2 (25.0)	
Yes	28 (73.7)	10 (26.3)	0.657
High Total Bilirubin			
No	26 (74.3)	9 (25.7)	
Yes	8 (72.7)	3 (27.3)	0.601
Low Albumin			
No	8 (88.9)	1 (11.1)	
Yes	28 (73.7)	10 (26.3)	0.313

*P value for Chi-squared test or Fischer's exact (where cells have n<5). Renal Dysfunction (high creatinine, high urea and low bicarbonate), raised transaminases (raised AST and ALT)

Table 5: Risk factors for mortality in patients with NTS

Factor	**Unadjusted OR (95% CI)**	**P Value**	**Adjusted OR (95% CI)**	**P Value**
Gender				
Female	Reference		Reference	
Male	2.59 (0.80 – 8.41)	0.113	4.69 (1.05 – 20.99)	0.043
Inappropriate use of antibiotics				
No	Reference		Reference	
Yes	2.54 (0.79 – 8.23)	0.119	3.20 (0.75 – 13.61)	0.117
GCS <15				
No	Reference		Reference	
Yes	10.9 (3.02 – 39.67)	<0.001	15.69 (3.43 – 71.78)	<0.001

Table 6: CDC guidelines for treatment of NTS disease

Opportunistic infection: Bacterial enteric infections- Salmonellosis	
Preferred therapy:	• Ciprofloxacin 500-750mg PO BD (or 400mg IV BD)
Duration of therapy:	• If CD4 ≥200: 7-14 days • If CD4 <200: 2-6 weeks • If recurrent symptomatic septicaemia- may need 6 months or more therapy
Alternative therapy:	• Levofloxacin 750mg or moxifloxacin • TMP- SMX PO or IV • Third generation cephalosporin like ceftriaxone or cefotaxime- if susceptible

Adapted from Centers for Disease Control and Prevention. (2009, April 10). Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents. *Morbidity and Mortality Weekly Reports.* Retrieved from https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5804a1.htm#tab2)

CHAPTER 3: APPENDIX A: Ethics approval certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Shinny Eskander Alimia

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180276

NAME: (Principal Investigator)	Dr Shinny Eskander Alimia
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Profile of non-typhoidal salmonella bacteraemia in adults with HIV infection admitted to Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	23/02/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr J Tsitsi and Prof Colin Menezes
APPROVED BY:	Prof C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/05/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

18/05/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B: Data collection sheet

DATA COLLECTION FORM

PROFILE OF NON- TYPHOIDAL SALMONELLA BACTERAEMIA IN HIV INFECTED

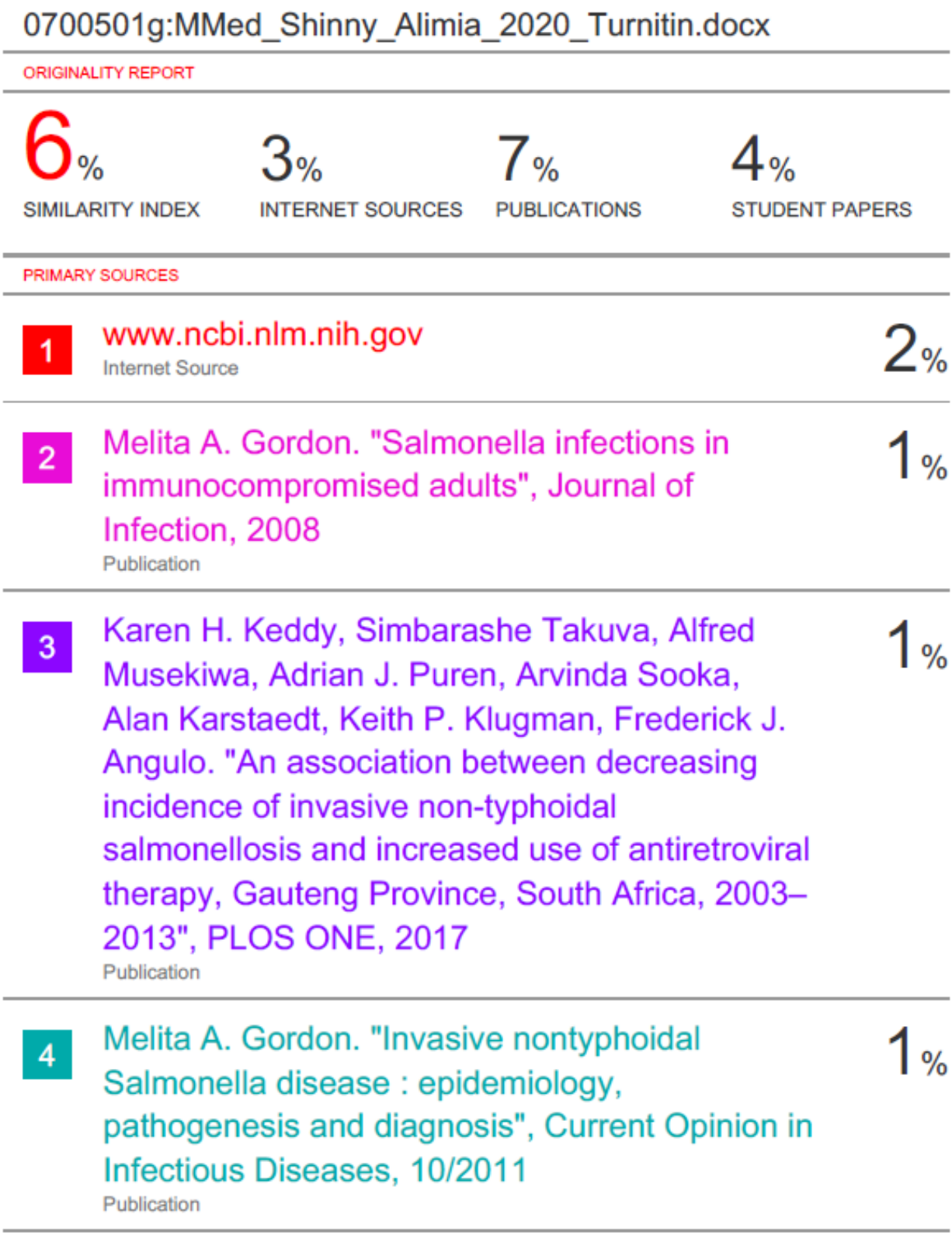
ADULTS ADMITTED TO CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

1. Patient unique number				
2. Demographics				
· Age				
· Gender	Male	Female		
· Race	Black	White	Indian	Coloured
3. Hospital stay & outcomes				
· Date of admission				
· Length of stay in days				
· Previous NTS	Yes	No		
· Escalation during admission	Yes	No		
· Ventilation requiring	Yes	No		
· Dialysis requiring	Yes	No		
· Discharged alive	Yes	No		
· Died	Yes	No		
5. Blood culture positive:	Yes	No		
· Date				
· Organism				
6. Other sites of infection:				
· Lungs:	Yes	No		
· Skin & Soft Tissues:	Yes	No		
· Bones & Joints:	Yes	No		
· Meninges:	Yes	No		
· Urinary tract:	Yes	No		
· GIT:	Yes	No		
7. Clinical characteristics:				
· Fever:	Yes	No		
· Diarrhoea:	Yes	No		
· Cough:	Yes	No		
· Temp (degrees Celsius):				
· GCS:	15	<15		
· Respiratory crackles:	Yes	No		
· Splenomegaly:	Yes	No		
· Hepatomegaly:	Yes	No		
8. Blood Parameters:	Day 0	Day 1-3	Day 4-7	
· White blood cell count (x109/l)				
· Haemoglobin (g/dl)				

· Platelet count(x109/l)				
· Sodium (mmol/l)				
· Potassium (mmol/l)				
· Bicarbonate (mmol/l)				
· Urea (mmol/l)				
· Creatinine (µmol/l)				
· Total protein (g/l)				
· Albumin (g/l)				
· Total bilirubin (µmol/l)				
· Conjugated bilirubin (µmol/l)				
· Alanine transaminase (U/l)				
· Aspartate transaminase (U/l)				
· Alkaline phosphatase (U/l)				
· Gamma- glutamyl transferase (U/l)				
9. HIV:				
· CD4 (cells/µL)				
· VL (copies/mL)				
· On ART:	Yes	No		
10. ARV regimen used:				
· Tenofovir (TDF):	Yes	No		
· Lamivudine (3TC):	Yes	No		
· Zidovudine (AZT):	Yes	No		
· Didanosine (ddI):	Yes	No		
· Abacavir (ABC):	Yes	No		
· Stavudine (D4T):	Yes	No		
· Efavirenz (EFV):	Yes	No		
· Nevirapine (NVP):	Yes	No		
· Lopinavir/ ritonavir:	Yes	No		
· Atazanavir (ATZ):	Yes	No		
· Raltegravir:	Yes	No		
· Darunavir:	Yes	No		
11. Antibiotics:				
· Appropriate antibiotic based on culture result	Yes	No		
12. Antibiotic given:				
o Ampicillin:	Yes	No		
o Trimethoprim/sulfamethoxazole:	Yes	No		
o Chloramphenicol:	Yes	No		
o Streptomycin:	Yes	No		
o Tetracycline:	Yes	No		
o Ciprofloxacin:	Yes	No		
o Imipenem:	Yes	No		

o Ertapenem:	Yes	No		
o Kanamycin:	Yes	No		
o Cefepime:	Yes	No		
o Ceftazidime:	Yes	No		
o Ceftriaxone:	Yes	No		
o Treatment duration:	≤7 days	≤10 days	≤14 days	
13. Prophylaxis:				
· Given	Yes	No		
· Antibiotic given:				
· Route administered:	Oral	IVI	Other	
· Duration (in days):				

APPENDIX C: Turn it in Report



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ORIGINALITY REPORT

6%	3%	7%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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