Clinical and microbiological characterisation of invasive enteric pathogens in a South African population:

The interaction with HIV

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Declaration

I, Karen Helena Keddy, Student number 81-1138/4, declare that this thesis is my original work. Where contributions have been made by others, this has been duly acknowledged. It is being submitted for the degree of Doctor of Philosophy in Public Health in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any other University.

I was integral to the original development of the GERMS-SA national laboratory-based surveillance system, developing the national reference system for the bacterial enteric pathogens and the contributing the enteric surveillance protocols to that combined protocol for all pathogens of public health importance currently under surveillance. As such, although this thesis represents a secondary data analysis, it is based on my own original work. For each publication, I was responsible for development of the study, cleaning and analysis of the surveillance data and the primary draft and final submission of the manuscript. I am first author on each of the manuscripts included in this thesis.

This thesis is submitted by publication and follows the European style, presenting an integrating narrative of the research projects undertaken, with the resultant publications included in the appendices.

Signed: 

Date: 23 December 2016

Abstract

Introduction

Human immunodeficiency virus (HIV) has been associated with invasive enteric infections in HIV-infected patients, since it was first described in the 1980s. In South Africa, HIV remains an important health challenge, despite the introduction of antiretroviral therapy (ART) in 2003. In association with this, is an ongoing problem of invasive enteric infections, including those due to *Shigella* and *Salmonella*, including *Salmonella enterica* serovar Typhi (*Salmonella* Typhi). There are few South African data available as to the incidence of invasive disease due to these pathogens and how these data may contrast with the presentation and outcome in HIV-uninfected patients. The associated risk factors for mortality due to invasive enteric pathogens and whether there has been a response with ART as an intervention also needs further elucidation.

Aims

This work was undertaken to better describe the burden of invasive enteric infections (*Shigella*, nontyphoidal *Salmonella* and *Salmonella* Typhi) in association with HIV, define risk factors for mortality and establish whether the introduction of ART has impacted on disease burdens due to these pathogens.

Methods

Laboratory-based surveillance for enteric pathogens was initiated in 2003. Basic demographic details (age and gender) were collected on all patients where possible. In 25 hospital sites in all nine provinces, additional clinical information was collected by trained surveillance officers, including HIV status, data reflecting severity of illness, other immune suppressive conditions, antimicrobial and antiretroviral usage and outcome (survival versus death). Laboratories were requested to transport all isolates to the Centre for Enteric Diseases (CED) at the National Institute for Communicable Diseases of the National Health

Laboratory Service (NHLS) in Johannesburg for further characterisation, including serotyping, antimicrobial susceptibility testing and molecular typing where relevant (whether isolates could respectively be classified as *Salmonella* Typhimurium ST313 and *Salmonella* Typhi H58). Additional cases were sought through audits of the Central Data Warehouse (CDW) of the NHLS.

Annual incidence rates were calculated according to published estimates of population by age group by the Actuarial Society of South Africa for the Department of Statistics of the South African government. Analyses were specifically directed at invasive shigellosis, *Salmonella* meningitis, typhoid fever in South Africa and nontyphoidal salmonellosis in Gauteng Province, South Africa. Data were recorded in an Access database and analysed using chi-squared test to establish differences between HIV-infected and uninfected individuals and univariate and multivariate analysis to compare risk factors for mortality. Data in the number of patients accessing ART were derived through audits of the CDW, by using the numbers of patients on whom viral loads were done annually as a proxy.

Results

Between 2003 and 2013, a total of 10111 invasive enteric isolates were received by CED. For patients for whom sex was recorded, 3283/6244 (52.6%) of patients presenting with invasive enteric infections were male; invasive disease was predominantly observed in children less than five years of age (1605/6131; 26.2%) and those who were aged between 25 and 54 years (3186/6131; 52.0%), with the exception of typhoid fever where the major burden was in patients aged 5 to 14 years (302/855; 35.3%).

More HIV-infected adult women were observed with invasive shigellosis (P=0.002) and with typhoid fever compared with adult men (P=0.009). Adults aged ≥ 15 years were more likely to die than children aged < 15 years (invasive shigellosis, odds ratio [OR]=3.2, 95% confidence interval [CI]=1.6 – 6.6, P=0.001; *Salmonella* meningitis, OR=3.7, 95% CI=1.7 – 8.1, P=0.001; typhoid fever, OR=3.7, 95% CI=1.1 – 14.9, P=0.03; invasive nontyphoidal salmonellosis, OR=2.0, 95% CI=1.6 – 2.5, P<0.001).

HIV-infected patients had a significantly higher risk of mortality compared with HIV-uninfected patients (invasive shigellosis, OR=4.1, 95% CI=1.5 – 11.8, P=0.008; *Salmonella* meningitis OR=5.3, 95% CI=1.4-20.0, P=0.013; typhoid fever, OR=11.3, 95% CI=3.0 – 42.4, P<0.001; invasive nontyphoidal salmonellosis OR=2.5, 95% CI=1.7 – 3.5, P<0.001). In all patients, severity of illness was the most significant factor contributing to mortality (invasive shigellosis, OR=22.9, 95% CI=2.7 – 194.2, P=0.004; *Salmonella* meningitis OR=21.6, 95% CI=3.5 – 133.3, P=0.01; typhoid fever, OR=10.8, 95% CI=2.9 – 39.5, P<0.001; invasive nontyphoidal salmonellosis OR=5.4, 95% CI=3.6 – 8.1, P<0.001). Between 2003 and 2013, ART was significantly associated with decreasing incidence rates of invasive nontyphoidal salmonellosis in adults aged 25 - 49 years (R=-0.92; P<0.001), but not in children (R=-0.50; P=0.14).

Conclusion

Decreasing incidence rates of invasive nontyphoidal salmonellosis and shigellosis suggest that ART is having an impact on opportunistic enteric disease in HIV. Further work is necessary however, to fully understand the associations between age, sex and invasive enteric pathogens. Specifically, this work would include typhoid fever, *Shigella* transmission from child to adult carer, development of invasive enteric infections in HIV-exposed children and whether the decreasing incidence rates can be sustained. Moving forward, an understanding

of invasive enteric infections in the HIV-uninfected patient may assist in targeting severity of illness as a risk factor for mortality.

Acknowledgments

I would like to start by thanking my supervisor, Keith Klugman, who not only saw something in me, to give me my amazing career in medical microbiology, but also patiently nurtured me through two postgraduate degrees and who was the primary instigator in developing the GERMS-SA study, on which this work is based. I am equally grateful to my co-supervisor, Fred Angulo, whose invaluable experience and extensive knowledge of *Salmonella* epidemiology and in the international field of scientific publication, he generously made available for this thesis and continues to do so, through my ongoing association with the Global Disease Detection of the Centers for Disease Control. I am eternally indebted to Alfred Musekiwa, biostatistician and teacher extraordinaire for his endless patience with my biostatistical queries and blunders. Without the support of the finest laboratory manager I know, Arvinda Sooka, who both assisted in the project, ran the laboratory and patiently encouraged me to continue moving forward, and my senior scientist, Anthony Smith, who stepped up and stepped in whenever needed, to build our laboratory into an internationally recognised Centre, so that this work would be undertaken, this PhD would not have been finished. My wonderful staff in Centre for Enteric Diseases who have assisted in the laboratory aspects of this work, with good humour and patience, and all those in GERMS-SA, past and present, were invaluable partners.

Lastly this work could not have been completed without the generosity of the funders: the National Institute for Communicable Diseases (NICD) South Africa, President's Emergency Plan for AIDS Relief (PEPFAR), through the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, and the various international programmes of CDC.

Dedication

I dedicate this thesis to my sons, Anthony Ryan and Stuart Russell. I hope, by my work, I have brightened the path to your futures and made your world a better place.

Contents

List of Figures

List of Tables

Prologue

The original desire to study medicine came through a number of influences, most significant of these being my father, who as a medical physicist, had spurred my interest and as the father of daughters, believed a woman, in those days could function well in what was a predominantly male profession. In addition, I had a younger sister who developed an osteosarcoma at the age of 8 years. Although she survived, her right arm was amputated and she received long courses of radiotherapy and chemotherapy as part of her treatment. She was finally declared cancer-free after 30 years. My intention, at the innocent age of 15 years, was therefore to study medicine so that I could become a paediatric oncologist, and through medical research, find new, better and less traumatic ways of treating children with cancer.

I enrolled as a medical student at the University of the Witwatersrand (Wits) in 1981, but discovered that the early, preclinical years, were a little dry and although the workload was tremendous, much of the work, particularly in the second year of anatomy, involved large quantities of rote learning and less of analytical thinking. This was somewhat against my nature and I broke away from the traditional medical path for a year to study and earn additional degree of BSc (Med), giving me my first taste of scientific research. It was an extremely stimulating and constructive time and through exposure to some extraordinary scientists, including Prof Philip Tobias in the Department of Anatomy and Prof Ann Andrews in Microanatomy, I learnt much about experimental rigour and critical review of scientific studies. Another lecturer, who became the first to try to teach me biostatistics, was Prof Allen in the Department of Anatomy – little did I realise how important these lectures were then!

I joined the staff of Sizwe (then Rietfontein) Hospital as a medical officer in 1990. The exposure to infectious disease and an extraordinarily stimulating year of gaining my DTM&H

while I was working at Rietfontein in 1991 had enthused me to study medical microbiology. The Head of Department and person who had the final say in my appointment as a microbiology registrar, was Professor Keith Klugman. It was the start of a long and extremely (for myself at least) productive association with one of my current PhD supervisors.

I was fortunate to spend three months as a guest researcher in the Foodborne Diseases Branch at the Centers for Disease Control and Prevention in Atlanta, USA, in 1997, following my graduation as a Medical Microbiologist. At the CDC, I was given my first introduction on the importance of setting up a reference laboratory, at a time when molecular diagnostics and epidemiological techniques were just being introduced at the highest levels and had not yet become the norm. Although I was primarily laboratory-based, I had interaction with the epidemiologists while there as well, and met Dr Fred Angulo, a senior epidemiologist in the food-borne diseases branch at the time; later he was to become the second supervisor on my PhD.

It took another 10 years or more, before I was ready to embark on my PhD. In the intervening years, my supervisor and mentor from my Masters years, Keith Klugman, had emigrated to the USA. I had been invited to Geneva a few years earlier, to sit on a WHO expert committee to describe the global burden of foodborne diseases, and was introduced to a group of passionate and dedicated scientists with a deep and sincere concern in gathering well-researched, relevant and accurate data for this project. Here, I again met with Fred Angulo, one of the major forces involved in the project. Sitting at a dinner table that year, I was chatting with an Australian colleague who had just embarked on his PhD; I discussed my desire to do a PhD with him: he suggested that Fred would “see me through”.

Shortly after my return from that meeting, I met with Keith, who was visiting South Africa. In discussions with him, he pointed out that he had been supervising a colleague of mine who was just finishing her PhD, and asked if I was ready to embark on the same. I replied: "Funny, Keith, I was just about to ask you about that…"

List of original papers

- Keddy KH, Sooka A, Crowther-Gibson P, Quan V, Meiring S, Cohen C, Nana T, Sriruttan C, Seetharam S, Hoosen A, Naicker P, Elliott E, Haffejee S, Whitelaw A, Klugman KP; for the Group for Enteric, Respiratory, and Meningeal Disease Surveillance in South Africa (GERMS-SA). Systemic shigellosis in South Africa. Clin Infect Dis. (2012) 54(10): 1448-1454.
    - The candidate was responsible for developing the surveillance protocol, , the laboratory characterisation of *Shigella* in the reference laboratory, creating the analytical databases and cleaning the data, analysing the surveillance data, writing the original manuscript draft and final submission of the manuscript.
- Keddy KH, Sooka A, Musekiwa A, Smith AM, Ismail H, Tau NP, Crowther-Gibson P, Angulo FJ, Klugman KP; Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Clinical and microbiological features of *Salmonella* Meningitis in a South African Population, 2003-2013. Clin Infect Dis. (2015) Nov 1;61 Suppl 4:S272-282.
    - The candidate was responsible for developing the surveillance protocol, , the laboratory characterisation of *Salmonella* in the reference laboratory, creating the analytical databases and cleaning the data, analysing the surveillance data, writing the original manuscript draft and final submission of the manuscript.
- Keddy KH, Sooka A, Musekiwa A, Tau N, Klugman KP, Angulo FJ for GERMS-SA. Typhoid fever in South Africa in an endemic HIV setting. PLOS One: (2016) Oct 25;11(10):e0164939. doi: 10.1371/journal.pone.0164939.

- The candidate was responsible for developing the surveillance protocol, , the laboratory characterisation of *Salmonella* in the reference laboratory, creating the analytical databases and cleaning the data, analysing the surveillance data, writing the original manuscript draft and final submission of the manuscript.

- Keddy KH, Takuva S, Musekiwa A, Puren AJ, Sooka A, Karstaedt A, Angulo FJ, Klugman KP. An ecological association between decreasing incidence of invasive non-typhoidal salmonellosis and increased use of highly active antiretroviral therapy, Gauteng Province, South Africa, 2003 – 2013: submitted.
  - The candidate was responsible for developing the surveillance protocol, , the laboratory characterisation of *Salmonella* in the reference laboratory, creating the *Salmonella* analytical databases and cleaning these data, analysing the *Salmonella* surveillance data and in part the data related to viral loads, writing the original manuscript draft and final submission of the manuscript.
- Keddy KH, Musekiwa A, Sooka A, Karstaedt A, Trusha Nana T, Seetharam S, Nchabaleng M, Ruth Lekalakala R, Angulo FJ, Klugman KP for GERMS-SA. Clinical and microbiological features of invasive nontyphoidal *Salmonella* in Gauteng Province, South Africa, 2003 – 2013: submitted.
  - The candidate was responsible for developing the surveillance protocol, , the laboratory characterisation of *Salmonella* in the reference laboratory, creating the analytical databases and cleaning the data, analysing the surveillance data, writing the original manuscript draft and final submission of the manuscript.

List of conference proceedings

- Keddy KH*, Sooka A, Crowther-Gibson P for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Microbiological characteristics of *Salmonella* meningitis in a South African population, 2003 – 2010. (Poster presentation). International Conference on Emerging Infectious Diseases, Atlanta, GA, USA. 11 – 14 March, 2012.
- Tau NP, Smith AM, Keddy KH*, for the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA). Development and evaluation of a multiple-locus variable-number tandem-repeats analysis assay for subtyping *Salmonella* Typhi strains from Sub-Saharan Africa. (Poster presentation). 16th International Conference on Infectious Diseases, Cape Town, South Africa. 2 – 5 April, 2014.
- Keddy KH*, Quan V, Meiring S, Sooka A. Changing burden and clinical features of invasive *Salmonella* Enteritidis and *Salmonella* Typhimurium in South Africa (Poster presentation). 9th International Conference on Typhoid and invasive NTS Disease, Bali Indonesia, 30 April – 3 May 2015.

*Presenting author

List of abbreviations

AOR	Adjusted odds ratio
ART	Antiretroviral therapy
ASSA	Actuarial Society of South Africa
CD4+	CD4-positive T-lymphocyte
CDW	Central data warehouse
CED	Centre for Enteric Diseases
CFR	Case fatality rate / case fatality ratio (see in text)
CI	Confidence interval
DNA	Deoxyribonucleic acid
ESBL	Extended spectrum β-lactamase
GCS	Glasgow coma scale
GERMS-SA	Group for Enteric Respiratory and Meningeal pathogens Surveillance – South Africa
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
IFN	Interferon
IgA	Immunoglobulin A
IgG2	Immunoglobulin G2
IL	Interleukin
iNTS	Invasive non-typhoidal *Salmonella*
IRR	Incidence rate ratio
M-cells	Microfold cells
MIC	Minimum inhibitory concentration

MLST	Multi-locus sequence typing
MSM	Men who have sex with men
NDoH	National Department of Health
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
NTS	Non-typhoidal *Salmonella*
OR	Odds ratio
PBS	Pitt bacteraemia score
PCR	Polymerase chain reaction
PEPFAR	United States President's Emergency Plan for AIDS Relief
PYO	Person-years of observation
SCV	*Salmonella*-containing vacuoles
SPI	*Salmonella* pathogenicity island
ST	Sequence type
STATS-SA	Statistics South Africa
T3SS	Type III secretion system
TNF	Tumour necrosis factor
USD	United States dollar
WaSH	Water, sanitation and hygiene

1. Review of the literature

1.1. HIV, opportunistic infections and the introduction of antiretroviral therapy

Globally, HIV remains a severe problem and South Africa has one of the highest rates of HIV infection in the world, different sources estimating that between 5.2 and 5.7 million people are infected (1-3). There has been major impact on life expectancy, with a major negative effect on the economically active sector of the population, due to morbidity and mortality in this age group (1;4;5). The infant mortality rate in South Africa, which previously could be partially ascribed to HIV, was one of the highest in the world in 2005 and had increased since the millennium (6), but has decreased substantially since (7;8). Nonetheless, HIV uninfected infants who were HIV exposed in utero or at birth remain at risk for a number of infectious diseases (9). Highly active antiretroviral therapy (HAART) was introduced in South Africa in 2004 (10), and has been phased in over a number of years, the coverage being estimated at 40.2% for those with CD4+ less than 200 cells per µl of blood or World Health Organization stage 4 criteria (11). However, following the current South African HIV Clinicians Society guidelines, coverage is only 22.2% (11) and may thus be deemed inadequate to prevent opportunistic infection.

The full impact on the introduction of HAART in South Africa is not completely elucidated: the role that it has played in morbidity and mortality due to opportunistic and acute bacterial infections is only partially described. If we accept that coverage with HAART may be inadequate, it becomes more critical to measure the effects of acute bacterial opportunistic infections on disease (11).

It is well recognised that HIV co-infection is a high risk factor for both common human pathogens as well as opportunistic pathogens and this remains relevant, even in the era of HAART (12). African studies have shown that this includes, amongst others, acute bacterial infections, co-infections with other opportunistic pathogens and chronic infections including cryptococcosis, tuberculosis and *Pneumocystis* pneumonia (13). The association of these last three pathogens with HIV is well-elucidated, but that of the acute invasive bacterial pathogens, including the enteric bacteria, is less easily described, due to the occurrence of these in association with other clinical conditions in Africa, as well as the occurrence in healthy individuals (13-23).

1.2 The enteric bacteria and the association with acute invasive disease

Enteric pathogens are important causes of morbidity and mortality in the developing world (24;25) and may be associated with serious consequences in these patients. Among the most common of the pathogenic enteric bacteria are non-typhoidal *Salmonella*, *Shigella* and *Campylobacter*, all of which had been described as associated with HIV (26-29), including other immune suppressed or immune deficient states (14-18;20;21;30;31).

Although not included in this work, *Campylobacter* has a strong history of causing invasive infections in the presence of HIV. As one of the most ubiquitous of the pathogenic enteric bacteria (32-34), the association of *Campylobacter* diarrhoea with HIV, particularly in men who have sex with men (MSM), is not unexpected (35-37). In addition, *Campylobacter* bacteraemia may occur both in HIV-infected individuals as well as other comorbidities, but

appears rare with a prevalence of 0.004 per cent compared with the numbers of reported diarrhoea cases in Denmark (37).

Conversely, *Shigella* and *Salmonella* share a common ancestry, and are similar in that both pathogens invade the intestinal mucosa, causing mucosal damage, diarrhoea and dysentery (38). The breaching of the mucosal barrier potentially conveys an advantage, compared with other intestinal pathogens, in causing systemic disease; this may well be potentiated in the immune suppressed patient. Previously, meta-analysis has described both of these pathogens as causes of community-acquired bacteraemias in Africa (23).

In Africa, shigellae are recognised as one of the commonest causes of diarrhoeal morbidity and mortality in all age groups (24;25;39). Invasive shigellosis was recognised as an unusual, but important manifestation of disease in HIV, early in the course of the HIV epidemic, with reports emanating from the developed world (40-43), but has also been associated with malnutrition in children (44;45). *Shigella* have also been associated with invasive disease in HIV-infected patients in South Africa (46), but the full impact of the disease in South Africa, where major health disparities exist, is poorly understood. The epidemiology of invasive shigellosis in association with HIV in South Africa differs from that previously described: as a pathogen of MSM (47); given the differing epidemiology of HIV, which is typically due to heterosexual transmission in South Africa (2;48), and was previously described in young children in association with HIV and malnutrition (49). Recommendations addressing the risk factors may decrease morbidity and mortality in systemic disease, as well as decreasing the incidence rates in gastroenteritis in these patients (27;44;50) given the ubiquitous nature of this pathogen.

Typically, common acute infectious diseases, such as invasive pneumococcal disease, present more commonly in HIV-infected patients (51;52) in a setting where HIV infection is common. Typhoid fever, due to *Salmonella enterica* serovar Typhi, remains an important public health problem (53;54). The association between typhoid fever and HIV is also not fully elucidated, and even confusing, and there are limited reports in the literature regarding the association of *Salmonella* Typhi with HIV (55-57). Gotuzzo *et al* found that HIV-infected patients had a 60-fold increased likelihood of developing typhoid fever when compared with the general population in Lima, Peru (55), but few researchers have been able to confirm this. Conversely, Crump *et al* have recently shown that HIV appears protective against typhoid fever in northern Tanzania, although the reasons for this are not well understood (13), and confirmed observations of a meta-analysis of causes of bacteraemia in Africa (23): further work may better clarify the association, which appears to be based primarily on fever studies (recorded temperature in a patient >38°C) . Antimicrobial resistance appears to be increasing in this pathogen in South Africa (58-65), with the emergence of quinolone resistance a major cause for concern (66). It is apparent that in HIV-infected individuals, currently available typhoid vaccines are associated with a poor immune response (67). A better understanding of the role of HIV in typhoid fever would assist in preparation of vaccine guidelines in these patients, antimicrobial management and an appropriate public health approach. Of further concern is the spread of the virulent haplotype, *Salmonella* Typhi H58, which is frequently associated with multi-drug resistance, from South East Asia to Africa and the potential impact this may have on an already vulnerable, immune-suppressed population (68;69).

The role of invasive nontyphoidal salmonellosis as an opportunistic infection in HIV-infected patients has been well studied elsewhere (70-74), but aspects of this disease remain poorly understood. The importance of this problem in Africa is well recognised, and resulted in a

consortium being formed, to better understand the role, impact and potential interventions for this disease in Africa (74;75). The impact of HIV on *Salmonella* meningitis (76-79), including a high mortality in HIV-infected infants in some studies (79), conflicting with the results of others (76), may be due to different management protocols. The role of co-infection due to other pathogens or co-morbidity, the potential association of meningitis with certain serovars, the impact of multidrug resistance on outcome remains to be elucidated and the relevance of unusual serovars, particularly in a nosocomial setting, needs to be elucidated. Given the morbidity and mortality associated with meningitis, a better understanding of these associations may advance patient management and decrease mortality (77;78).

Changing incidence rates of invasive nontyphoidal salmonellosis since the introduction of HAART into South Africa in 2004 (2), have not been examined. A defective immune response in HIV-infected adults appears central to progression to invasive disease due to *Salmonella*, as a result of both abnormal cell mediated and humoral immunity (80;81). Invasive disease in HIV-uninfected patients has not been extensively researched in South Africa and there may be aspects that differentiate this from disease in HIV-infected patients (20;76;82-84). The importance of non-invasive *Salmonella* as a common cause of diarrhoea globally has been recently described and the organism is responsible for 93.8 million cases and 155 000 deaths (76;85). It is likely, given the prevalence of this pathogen, that even in the HIV-uninfected patient, invasive disease may play a role in South Africa: invasive disease has been described at the extremes of age or in association with other immunosuppressive conditions (20;82;83;86;87). The nosocomial nature of *Salmonella* infections in South Africa has been previously described, with devastating effects on hospitalised patients (72;83) and adding to the current health care burden. Unpublished evidence suggests that there may be an association with invasive disease due to non-typhoidal *Salmonella* in HIV- infected patients,

which may affect homes and communities. These organisms appear to be multidrug resistant (72;83;88) and an unusual serovar, *Salmonella enterica* serovar Isangi (*Salmonella* Isangi) appears an uncommon cause of disease beyond Africa (83;88-92).

1.2. Pathogenesis of shigellosis and salmonellosis

Enteric infections remain common in both children and adults in the developing world, an estimated 1.7 billion episodes occurring in children under five years of age in low to middle income countries (93) and two billion cases occurring overall in 2010 (94). Against this background, certain factors must nonetheless be in place for the organism to cause invasive disease, both host- and pathogen- specific. Both *Shigella* and *Salmonella* are able to invade the gut epithelium to cause diarrhoea, but only *Salmonella* Typhi and Paratyphi (including *Salmonella* Paratyphi A, *Salmonella* Paratyphi B and *Salmonella* Paratyphi C) are typically able to cause systemic disease, due to specific adaptations to overcome the human host's immune system in the immune-competent host.

1.2.1. *Shigella* and the host response

The pathogenesis of *Shigella* diarrhoea has been the subject of many studies and there are some excellent reviews. *Shigella* have a very small infectious dose and are easily transmitted through person-to-person spread, by flies and via environmental contamination, being specifically associated with poor sanitation and hygiene (95;96). The organisms gain entry in the epithelial cells of the large intestine, utilising a type III secretion system, (T3SS), preferentially via the M cells (microfold cells) through macropinocytosis (figure 1) (97;98). From the M cells, they are passed by transcytosis to resident macrophages, but evade intracellular killing by inducing apoptosis (99). Shigellae have thus evolved to utilise the

primary defence to subvert the resultant inflammatory process, such that the intestinal tissue is destabilised. This permits invasion of the gut epithelial cells from the basolateral aspect: on contact with the epithelial cell, T3SS effector proteins trigger bacterial uptake within vacuoles, but the vacuolar membrane is disrupted by the pathogen and the organisms multiply within the cytoplasm (97). Shigellae induce actin polymerisation in the epithelial cells at one pole, providing the propulsive force to move within and between cells (figure 1) (97;99). Massive and rapid colonization of the epithelial cells is followed by reprogramming of these cells to preferentially express interleukin-8 (IL-8), recruiting polymorphonuclear cells (98), with extensive epithelial cell destruction (100).

By inducing apoptosis in macrophages, the shigellae also subvert the acute inflammatory response through the T3SS effectors in the Peyer's patches of the gut (101). There is recruitment of T-cells (101), with specific targeting of CD4+ Th17 cells by *Shigella* (102), the priming of which conferring only limited protection against reinfection (102). Shigellae invade B cells, which undergo apoptosis (103), there is impairment of dendritic cells recruitment (104) and CD8+ T-cells fail to respond to infection (105).

Studies on *S. sonnei* have shown that the O antigen (OAg) lipopolysaccharide capsule, absent from *S. flexneri* 2a, which assists *S. sonnei* to resist complement-mediated killing. In contrast, experimental knockout of the capsule increased invasiveness in rabbit gut epithelial cells, increasing the inflammatory response and reducing spread to peripheral organs through enhanced complement-mediated killing (106). Goh *et al* have confirmed a role for complement-mediated protection against invasive nontyphoidal salmonellosis in HIV-uninfected Africans, but in HIV-infected Africans, Immunoglobulin A (IgA) and IgG2 have poor ability to activate complement (107;108). Similar factors may be at play in invasive

shigellosis, and *Shigella* may cause bacteraemias in immune suppressed patients; however, immunity to different *Shigella* serotypes may be cross-protective (109). Lower case numbers of invasive *Shigella* would thus occur, whereas invasive *Salmonella* and the presence of non-specific antibodies (discussed below) (110), may contribute to a greater burden of invasive disease.

Specifically therefore, *Shigella* has adapted to causing disease in the presence of a number of factors, through the subversion of host immunity via the T3SS and molecular cross-talk: impaired host immunity and immunologic cross-protection may be disadvantageous in association with invasion and systemic shigellosis in HIV. The development of invasive shigellosis is a rarer event compared with the occurrence of invasive non-typhoidal *Salmonella* in HIV-infected individuals (111;112).

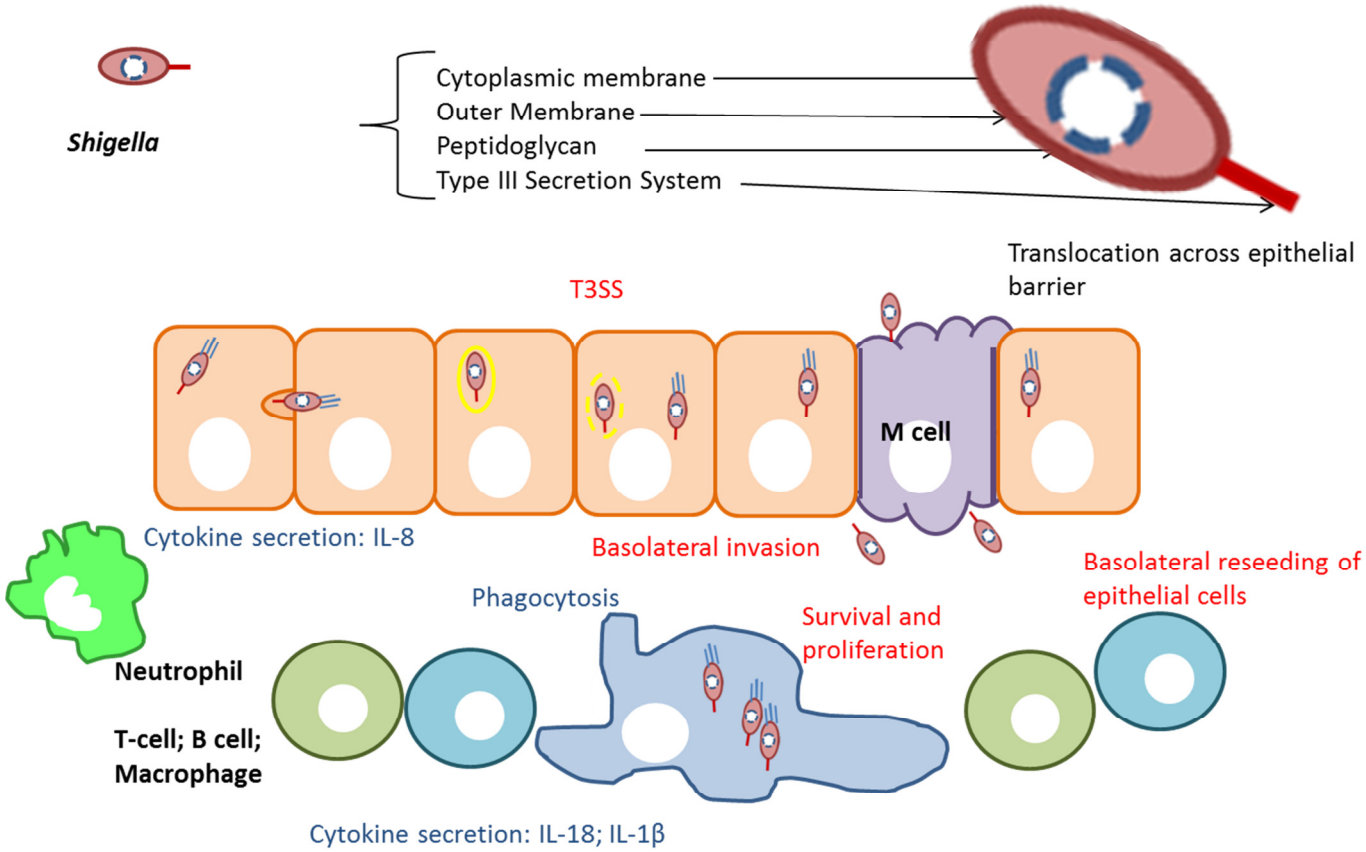


Figure 1. Pathogenesis of *Shigella* infection and the immune response.

Intracellular survival and basolateral invasion allow *Shigella* to penetrate the gut epithelium, adapted from Ashida *et al* (97).

1.2.2. *Salmonella* and the host response

Salmonella enterica has a similar ability to invade the gut as *Shigella* species – and also employs a type III secretion system (figure 2). Non-typhoidal *Salmonella* have evolved differently from the typhoidal group, and show a range of host adaptation: some are highly host-adapted, similar to *Salmonella* Typhi, with the ability to invade the animal host, whereas others are "generalists" and typically cause enteric disease in the immune-competent individual (113).

The non-typhoidal *Salmonella* (NTS) have less ability to invade the human host, although certain serovars have a higher invasiveness potential than others, including *Salmonella* Dublin and *Salmonella* Choleraesuis (114). Irrespective, the major pathogen that has emerged in Africa as a cause of invasive nontyphoidal salmonellosis, is *Salmonella* Typhimurium (115;116), in association with the HIV epidemic on the continent (112;113).

The pathogenesis of NTS diarrhoea is dependent on the presence of pathogenicity islands, acquired through the horizontal transfer of genes, and located either chromosomally or on the bacterial genome (117). They function to both promote host invasion, intracellular survival and to induce host immunity (Table 1) (117). Once ingested, these pathogens successfully compete with the gut microbiota, multiplying through the preferential utilisation of nutrients, at a cost to competing pathogens (118). *Salmonella* invade both non-phagocytic (119) and preferentially, the phagocytic microfold (M)-cells (120) of the intestinal epithelium. Within the gut epithelial cells, the organisms exist with membrane-bound *Salmonella*-containing vacuoles (SCV), distinct from the phagosome (117), avoiding killing by preventing lysosome fusion (120) (Figure 2). Salmonellae can survive and replicate within the SCV, through the production of various effector proteins enabling the pathogen to translocate into the host

cytoplasm leading to rearrangements of the actin cytoskeleton (117;118). Salmonellae invade basolaterally through the epithelial lining, reaching the Peyer's patches, stimulating an immune response (117;118;120;121). Proinflammatory cytokines including interleukin (IL)-1β, IL-6, interferon (IFN)-γ and tumour necrosis factor (TNF)-α are secreted, promoting systemic inflammation (117;118;121). Both CD4+ and CD8+ T-cells are important for protective immunity (121;122), although protective immunity requires the presence of functional B-cells (110;123). Bloodstream invasion by NTS in immune-suppressed individuals therefore results from three primary defects: loss of IL-17 producing CD4+ T-cells, attenuation and dysregulation of cytokine production (118;121) and humoral defects in pathogen-specific IgG, due to B-cell defects (110;123) in HIV-infected individuals, associated with reduced IgG specific to *Salmonella* lipopolysaccharide (124).

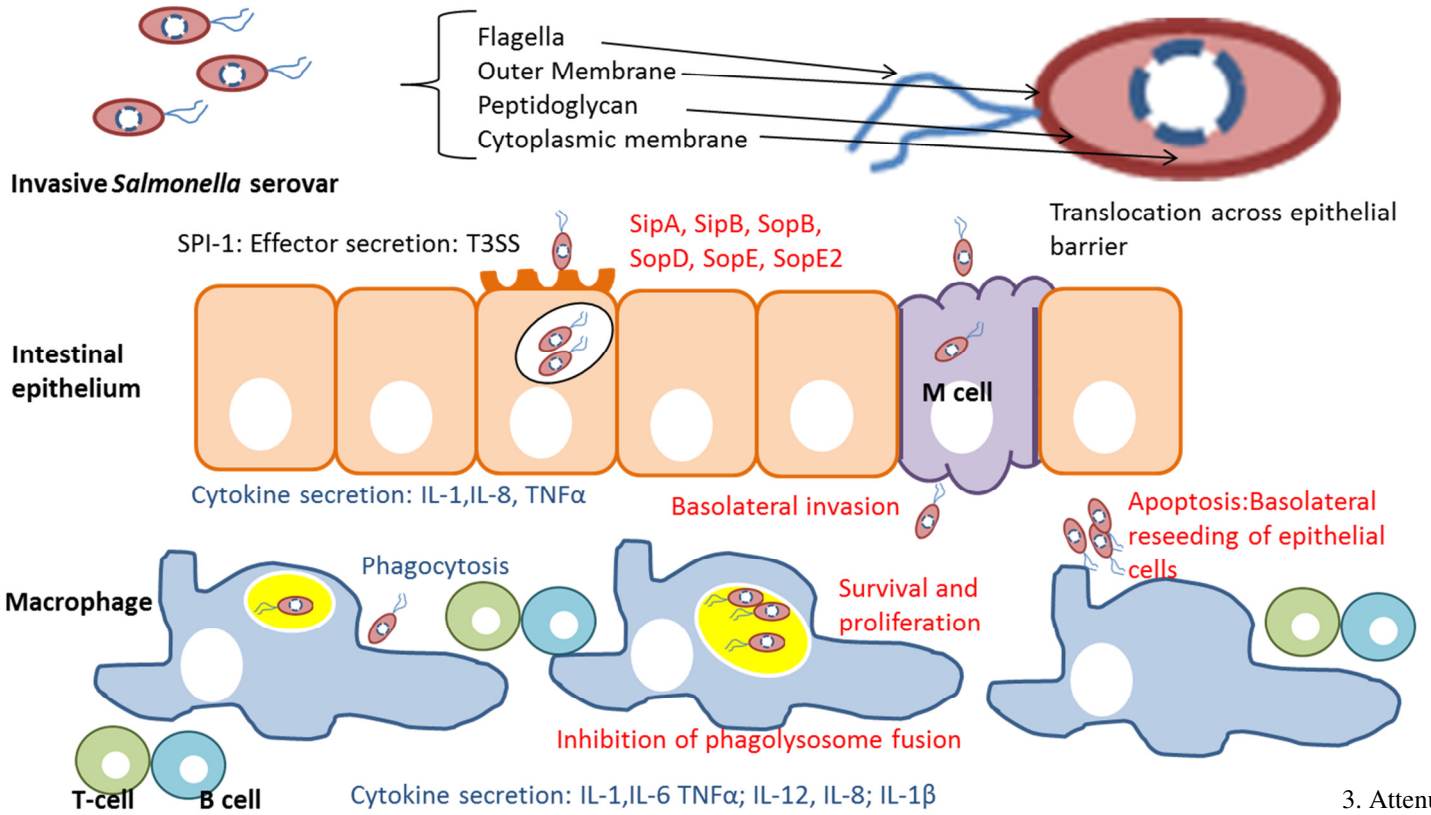
As stated above, certain NTS appear to have a greater ability to invade (invasiveness index) than others, calculated by comparing the number of non-invasive to invasive cases (114). In association with the HIV epidemic, *Salmonella* Typhimurium has evolved to take advantage of the disease in Africa, with the emergence of *Salmonella* Typhimurium ST313 (115;116). The pathogen has become so common it has replaced *Streptococcus pneumoniae* as the most frequent cause of bacteraemia in parts of sub-Saharan Africa (125;126). Whole genome sequencing analysis of representative isolates from a number of African countries suggests it emerged temporally in close association with the HIV pandemic (116). However, recent evidence has also indicated that *Salmonella* Typhimurium ST313 has retained its ability to cause intestinal inflammation in both mice and macaque monkeys, highlighting the role of defective immunity, over intrinsic pathogen virulence, in its role in iNTS in Africa (127).

Table 1. Functions of major *Salmonella* pathogenicity islands (SPI).

Pathogenicity Island	Approximate size (kilobases)	Type secretion system	Features/Functions
SPI-1	40	Type III secretion system (T3SS)	Invasion of intestinal epithelium; rearrangement of actin cytoskeleton; membrane ruffling; induction of interleukin-8
SPI-2	40	Type III secretion system (T3SS)	Intracellular survival in phagocytic cells; inhibition of phagosome-lysosome fusion; avoidance of intracellular killing;
SPI-3	17		Intramacrophage survival
SPI-4	27	Type I secretion system (T1SS)	Mediation of epithelial cell adhesion; role in oral virulence
SPI-5	8		Encodes virulence protein secreted by T3SS

SPI-1 through SPI-5 are common among *Salmonella* serovars, adapted from Hurley *et al* (117).

Molecular analysis suggests that it has emerged through partial selective genome degradation and the acquisition of pseudogenes, comparable to the emergence of *Salmonella* Typhi as a human pathogen (115). *Salmonella* Typhimurium ST313 shares a root with *Salmonella* Typhimurium ST19 (115), which is common in Europe and associated with diarrhoea in humans (115;128). Interestingly, the former stimulates less inflammasome activation (129;130), a feature typically associated with the invasive, human adapted serovars of *Salmonella* Typhi and *Salmonella* Paratyphi (131). This information has highlighted why invasive *Salmonella* Typhimurium has been associated with human-to-human, rather than zoonotic spread (132), including association with nosocomial bacteraemia in South Africa (88). In contrast, *Salmonella* Typhimurium ST19 has been associated with a nosocomial diarrhoeal outbreak in this country, but was rarely invasive, although the outbreak occurred in a vulnerable population (133). The ability of certain Salmonella Typhimurium strains to cause invasive disease, whereas others appear to cause diarrhoea only, even in severely immune suppressed HIV-infected patients, has also been documented in the developed world (134).



1. Loss of interleukin 17-producing CD4 T cells in HIV results in failure to maintain the epithelial cell barrier with additional loss of antimicrobial peptides, failure of neutrophil recruitment and translocation and invasion of salmonellae.

2. Humoral defects in Serum IgG against *Salmonella* lipopolysaccharide inhibits effective serum killing of salmonellae.

3. Attenuation of proinflammatory responses and dysregulation of cytokine production results in intracellular persistence of salmonellae and recrudescence of bacteraemia.

Figure 2. Pathogenesis of *Salmonella* infection and the immune response.

This diagram indicates key defects contributing to the development of invasive non-typhoidal *Salmonella* disease in HIV (113;117); adapted from Hurley et al (117).

Similarly to the non-typhoidal serovars, *Salmonella* Typhi has evolved over the past 30 000 (135) years to bypass the gut immune system through the acquisition of a number of virulence factors, permitting it to survive in human macrophages. The core genome is approximately 90% common including the primary pathogenicity islands 1 – 5, but there are a further 300 to 400 genes that are associated with specific phages or SPIs (136). Differences thus emerge in the failure of this pathogen to elicit a high inflammatory response in healthy individuals during the primary invasion (137). *Salmonella* Typhi invades via the small intestine (137), but in contrast to NTS, there is minimal neutrophil recruitment (136). Failure to elicit the marked inflammatory response observed with other enteric pathogens may be due to innate characteristics of *Salmonella* Typhi, which is associated with elevated pyrogenic cytokines, including TNF- α and IL1-β, through expression of various virulence mechanisms (138). In contrast, macrophage-like cells infected with *Salmonella* Typhi have reduced expression of IL-8 (138), the neutrophil chemoattractant which is additionally induced by bacterial flagellin. *Salmonella* Typhi then gain access to the underlying lymphoid tissues, where they are taken up by macrophages and possibly phagocytosed by dendritic cells, in which they replicate (136). Infection then spreads via the mesenteric lymph nodes through the common bile duct to the blood stream, resulting in a primary bacteraemia (136;137). Salmonellae are taken up by phagocytic cells of the reticuloendothelial system (liver, spleen and bone marrow); seeding of bacteria from here causes the secondary bacteraemia (136;137).

Over the past 30 years, *Salmonella* Typhi H58 has emerged to dominate in Asia and Africa, at a well-described cost to antimicrobial-susceptible strains (139). In contrast to the HIV-associated emergence of multidrug resistant *Salmonella* Typhimurium ST313 (116), which appears rather to be the result of widespread use of broad-spectrum antimicrobials for the

treatment of typhoid fever (68), in combination with enhanced fitness associated with multidrug resistance and the fluoroquinolones in particular (140).

1.4 Public health implications of invasive enteric pathogens associated with HIV-infection

The Global Burden of Disease study, updated in 2013, was undertaken in order to identify public health priorities for potential policy development and interventions at global, regional and country levels for all major causes of morbidity and premature mortality (141;142). In tandem with these efforts, systematic reviews examining the burden of food and waterborne pathogens were undertaken (85;94). Despite focussed attempts in these studies to address the association of invasive enteric pathogens, and particularly invasive NTS, in Africa, major data gaps have remained (112).

Recently, concerted efforts have been made to identify these gaps at a special meeting, convened to address a selection of these in Blantyre, Malawi, on 18 and 19 November 2014 (143). A comprehensive collection of publications that reviewed disease incidence due to iNTS in a number of African countries confirmed that burden of disease is decreasing in a number of African countries. Muthumbi et al confirmed a decrease in incidence rates from 50 cases per 100 000 person-years of observation (PYO) to 10 cases per 100 000 PYO, in children aged < 15 years in Kilifi, Kenya, over a 15 year period but ascribed this reduction primarily to reduced rates of malaria (144). They did not however note a similar decrease in adults over time, in whom iNTS disease was primarily associated with HIV: in HIV-infected patients the incidence was 13.2 per 100 000 PYO (144). Similarly, a decrease in iNTS disease in Mozambican children in Manhiça province, from 200 to 25 per 100 000 between 2001 and 2014 was primarily ascribed to malaria control, although malnutrition remained a major risk factor (145). Feasey *et al* have previously shown that a multipronged approach, including

improving nutritional status, controlling malaria and management of HIV contributed to decreasing iNTS incidence in Malawian children in Blantyre by ~12% per annum between 2001 and 2010 (146). While fewer reports specifically examine iNTS in adults, undoubtedly controlling HIV has had an impact on the number of blood stream infections as well as the case fatality rates (CFR): culture-confirmed bacteraemias decreased from 16% to 10% of suspected cases and CFR fell from 40% to 14% from 1997 to 2010 in Malawi (147). Where antiretroviral therapy is less accessible, cases numbers have remained constant: in adults aged ≥15 years in Kilifi, Kenya, case rates have remained unchanged at 1.7 per100 000 person years (144). Conversely to older, popular conceptions, the role of humans as reservoirs for the transmission of NTS in Africa has emerged as critical in maintaining disease in HIV-infected populations and *Salmonella* Typhimurium ST313 in particular has become adapted as a human pathogen, through lineage-specific genome degradation, with some similarities to those observed in *Salmonella* Typhi (148).

Salmonella Typhi remains an important cause of morbidity and mortality in Africa and burden of disease calculations have suggested that it is common in Africa (149), although South African estimates used in this study were taken from surveillance conducted around a vaccine trial in the 1980s (150). Updated burden estimates are required to better understand the role of water and sanitation programmes in current incidence rates, as well as the role of the emerging multidrug resistant haplotype, *Salmonella* Typhi H58 (139), in disease causation among HIV-infected and uninfected patients in South Africa.

Data regarding systemic shigellosis are less complete. A limited case series describing invasive shigellosis reported from Chris Hani-Baragwanath Hospital in Johannesburg, South Africa, described all adult patients as HIV-infected, compared with 30% of children (46).

Reddy *et al* undertook a systematic review and calculated that <1% of bacteraemias in Africa are due to invasive *Shigella* (23), but did not comment on the association with HIV. Nonetheless, the burden of shigellosis in developing countries is high and causes an estimated 4% of cases in children aged from 12 – 24 months (151). As rates of person-to-person transmission are estimated as being responsible for ~50% of cases (152), risk factors for acquiring disease in HIV-infected adults would be expected to be proportionately high. At the same time, however, there is a major impetus to develop vaccines for *Shigella*: the predominance of *S. sonnei*, *S. flexneri* 2a, *S. flexneri* 3a, and *S. flexneri* 6 means a vaccine against these strains would protect against ~90% of *Shigella* infections, should a vaccine be developed (153). Despite this, additional public health considerations, including provision of potable water and adequate sanitation should not be ignored in control of disease (109).

The burden of invasive enteric infections has hence taken a massive social and financial toll in South Africa. Socially, due to HIV deaths in South Africa, there were an estimated 122 000 children living in 60 000 child-only households in 2006, representing 0.67% of children in South Africa (154). To further complicate matters, misclassifications of HIV-related deaths on death certificates (excluding HIV as a cause of death) suggests an excessive mortality due to food-borne disease salmonellosis (155). The introduction of ART should impact on invasive enteric infections: in total, the programme will have averted 900 000 orphans in South Africa by 2020 (156), should it be successful. More recently, new evidence has suggested that there is a serious lag in the numbers of adult men accessing ART, compared with the numbers of women: between 2003 and 2011, the ratio between HIV-related mortality in adult women (≥15 years of age) compared with adult men in Hlabisa, in rural Kwazulu-Natal, South Africa, declined significantly from 0.93 to 0.73 (P=0.046) (157).

Inpatient treatment costs for invasive enteric infections in association with HIV cannot be calculated in the absence of burden information. Long *et al*, however, recently calculated that at a large Johannesburg hospital in 2010, acute invasive bacterial infections accounted for 12% of all admissions, the majority (65%) being in HIV-infected patients (158). The cost of management per patient for an in-hospital stay, averaging 9 to 10 days, was estimated at ~USD1800, or USD180 to USD 200 per day (158). Critically, in this series, none of the patients received any antiretroviral therapy although all were purportedly on an ART programmes, implying that they either continued on their own ART or that their treatment was disrupted (158). A previous study, at another Johannesburg hospital in 2005 found similar costs: in-patient care for HIV-infected patients for a period of 8 to 9 days cost USD1114 – most patients were not receiving ART (159); ten years previously, prior to the introduction of ART, in-patient costs for HIV-infected patients were estimated at USD1175 (160). Little improvement has occurred therefore over 20 years, in the costs of managing opportunistic pathogens in HIV-infected patients.

In order to optimise management, it has become necessary to better define prevalence of HIV-associated opportunistic infections, including those due to the enteric pathogens. Due to its public health significance, the interaction of *Salmonella* Typhi with HIV needs to be better defined, to assess the potential of this pathogen to add to the burden of typhoid fever and whether this may change in a post-HIV era. Data on the actual disease incidence, as well as response to ART over an extended period, will define the success of antiretroviral treatment campaigns as well as provide guidance for policy makers and health economists to motivate for funds for disease management. Additionally, such information can provide baseline estimates for numbers of patients presenting with invasive enteric pathogens in a post-HIV era. Burgeoning antimicrobial resistance has additionally accelerated the need for effective

vaccines against *Shigella* and non-typhoidal *Salmonella* and improved vaccines for *Salmonella* Typhi.

In 2013, a passionate plea was made regarding the proposed decrease in the funds provided by the President's Emergency Plan for Aids Relief (PEPFAR) (161), which provided a large proportion of the funds for this project (see section 3.5 below). One of the arguments provided was how PEPFAR had done more for health in Africa than simply provide a channel for ART (162). This work additionally provides data that tests and confirms the validity of this statement, as it tells a story of an evolving laboratory-based surveillance network, which has provided extensive data on enteric pathogens in South Africa for public health action, beyond simply the association with HIV.

2. Aims

This thesis aims to define the relationship of HIV and invasive disease due to enteric pathogens in South Africa (Figure 3):

- To define the burden of invasive shigellosis in HIV-infected patients: Elaborate on the clinical and microbiological aspects of systemic shigellosis in a South African population and the relationship to HIV, including clinical outcome, age and sex related differences and relevance of different serotypes, from 2003 to 2009, using quantitative analysis;
- To describe the association between HIV and meningitis due to non-typhoidal *Salmonella*: Investigate the impact of HIV on *Salmonella* meningitis in South African patients, and whether the outcome is affected by age or sex; to elaborate on the distribution of *Salmonella* serovars and antimicrobial resistance associated with *Salmonella* meningitis, the impact of the microbiological features on outcome including the presence of *Salmonella* Typhimurium ST313, from 2003 to 2013, using quantitative analytical techniques;
- To examine the association of typhoid fever with HIV: review the role of HIV in the presentation, clinical course and outcome of typhoid fever in South Africa from 2003 to 2013, using quantitative analysis and describe whether multidrug resistance and *Salmonella* Typhi haplotype H58 has a role in typhoid fever in South Africa and relate these findings to age and sex distribution in HIV-infected and HIV-uninfected patients;
- To measure the impact of antiretroviral treatment (ART) programmes on invasive nontyphoidal salmonellosis (NTS) in HIV-infected patients: elucidate the role of ART

in relation to invasive NTS from 2003 to 2013, and establish whether this has impacted on incidence rates and outcome of invasive NTS in South Africa;

- To compare these data with those from HIV-uninfected patients over the same time period, from 2003 to 2013, using quantitative analysis; and discover whether clinical and microbiological characteristics of isolates from these patients potentially play a role in disease presentation and outcome.

These analyses are presented as a series of five papers (Appendix I).

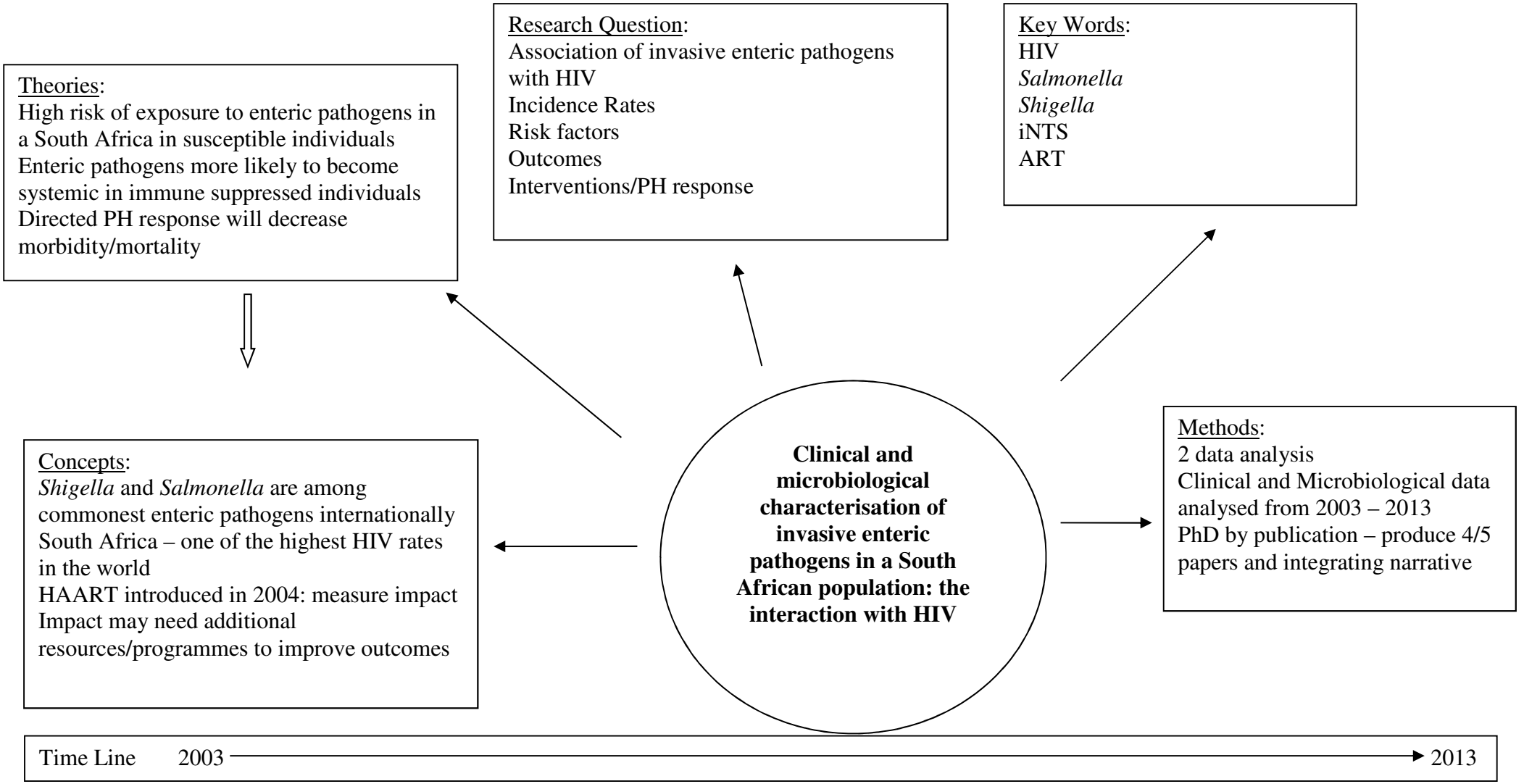


Figure 3. Conceptual framework developing analysis protocols for thesis.

3. Methods

We conducted active laboratory-based surveillance for invasive cases of *Shigella* and *Salmonella enterica*, including *Salmonella enterica* serovar Typhi (*Salmonella* Typhi) in South Africa between 2003 and 2013. For completeness of results here additional analyses for invasive shigellosis from 2003 – 2013 are included. An invasive case was defined as the isolation of one of the afore-mentioned pathogens from a normally sterile body site. All clinical laboratories (both National Health Laboratory Service [NHLS] and private pathology laboratories) were requested to submit isolates to the Centre for Enteric Diseases (CED), National Institute for Communicable Diseases, for further characterisation, including biochemical confirmation of identity, antimicrobial susceptibility testing and limited molecular characterisation. In addition, we conducted audits for these pathogens of the NHLS laboratories through the Central Data Warehouse (CDW) of the NHLS, a repository for all pathology tests done at the NHLS (163), and conducted training during regular site visits to laboratories submitting isolates for surveillance (Figure 4).

Figure 4. Site visit to Upington laboratory, Northern Cape, 2007.

3.1. Enhanced surveillance sites

In addition, at selected sites around South Africa, we conducted enhanced sentinel surveillance for additional clinical information on these pathogens. These sites were selected to represent the population of South Africa as widely as possible (Figure 5).

Figure 5. Sentinel surveillance sites selected for additional clinical data collection, South Africa, 2003 - 2013.

These include Polokwane (Limpopo Province), Nelspruit (Mpumalanga Province), Pretoria and Johannesburg (Gauteng Province), Rustenburg and Klerksdorp (North West Province), Bloemfontein (Free State Province), Durban and Pietermaritzburg (Kwazulu-Natal Province), Kimberley (Northern Cape Province), Mthatha (Eastern Cape Province) and Cape Town (Western Cape Province). These sites were additionally selected on the basis of the hospital

laboratories being able to perform microbiology, as well as basic haematological and biochemical analysis of patients' specimens. Tertiary care facilities and teaching hospitals were preferentially included as these would provide additional support for the surveillance programme.

Trained surveillance officers identified relevant cases based on the isolation of *Shigella* or *Salmonella* from a normally sterile body site (e.g. cerebrospinal fluid, blood culture, plural fluid, synovial fluid) at each of these sites and interviewed patients based on standardised questionnaires, to capture information on age; sex; HIV status and risk factors for infection, including other immune compromising conditions and access to antiretroviral therapy (ART). Severity of illness (either Pitt bacteraemia score [PBS] (Table 2) (164) or Glasgow Coma Scale [GCS] where relevant), current and past antimicrobial exposure; CD4+ lymphocyte counts and viral load data, if available for HIV-infected patients, and outcome, including survival or death was obtained through record review. In addition, the surveillance officers were trained to test for HIV status, including in the provision of pre- and post-test HIV counselling, to patients for whom the HIV status was unknown at the time of admission. Surveillance officers recorded whether HIV status was known at admission and the outcome of HIV testing as part of the surveillance protocol (Appendix II). Where patients may have been too ill or too young to respond to questions, consent was obtained from relatives, to whom the questionnaire was administered, or permission was sought from local hospital authorities to conduct a record review. Incomplete data was marked as such on the case information form (CIF). Incomplete data and data gaps primarily occurred on record review after patients were lost to follow up, or where patients were obtunded or comatose and unable to respond. Where patients had been discharged or died before enrolment, permission was obtained from local hospital authorities to conduct a record review. Surveillance officers were regularly retrained at biannual meetings, to optimise clinical data collection.

Table 2. Elements evaluated in the Pitt bacteraemia score and corresponding values for analysis. These data should be routinely collected on all patients presenting with suspected sepsis at South African hospitals.

Criteria for scoring are derived from Feldman *et al* (164).

Clinical presentation	**Numerical Score**	**Criteria for scoring**
Temperature	Fever (oral temperature) - 2 (≤35°C or ≥40°C) - 1 (35.1–36.0°C or 39.0–39.9°C) - 0 (36.1–38.9°C)	Patient's temperature in ºC on the day the specimen was taken
Blood pressure	- 2 (≤90mmHg) - 0 (>91mmHg) - U (Unknown)	Systolic blood pressure reading on the day the specimen was taken
Mechanical ventilation	- 2 (Yes) - 0 (No) - U (Unknown)	Whether patient was on mechanical ventilation at the time the specimen was taken
Cardiac arrest	- 4 (Yes) - 0 (No) - U (Unknown)	Whether patient suffered cardiac arrest on the day the specimen was taken
Mental State	- 0 (Alert) - 1 (Disorientated) - 2 (Stuporous) - 4 (Comatosed) - S (Sedated) - U (Unknown)	Mental state of the patient on the day the specimen was taken

3.2. Microbiological characterisation

Shigella and *Salmonella* isolates from normally sterile sites were subcultured by the diagnostic laboratory of origin onto Dorset egg transport media (NHLS, Johannesburg, South Africa) and transported to CED, NICD, Johannesburg. All isolates submitted to CED were characterised according to standard operating procedures (SOPs) of the NHLS. Briefly, biochemical characterisation was undertaken utilising either standard biochemical tube tests (NHLS, Johannesburg, South Africa) (2003 – 2009) or the Vitek ® 2 automated diagnostic platform (bioMérieux, Marcy l'Étoile, France). Antimicrobial susceptibility testing was conducted using Etests ® (bioMérieux) or the Vitek ® 2 (bioMérieux), according to SOPs of CED and the manufacturer's instructions. Minimum inhibitory concentrations were recorded for the following antimicrobials: ampicillin, trimethoprim-sulphamethoxazole, tetracycline, nalidixic acid, ciprofloxacin, streptomycin, kanamycin or amikacin, ceftriaxone, ceftazidime, cefepime and imipenem (165). In addition, the double-disk method (MAST Diagnostics, Bootie, England) was used to identify extended spectrum β-lactamase (ESBL) production (165). Multidrug resistance (MDR) was defined as resistance to three or more antimicrobials or the combination of trimethoprim-sulphamethoxazole and two other antimicrobials.

Molecular characterisation using multi-locus sequence typing (MLST) of *Salmonella* Typhimurium for the *Salmonella* meningitis study was undertaken, as described at the *Salmonella* MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica), including DNA sequencing the following housekeeping genes: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*. DNA sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and an Applied Biosystems 3500 Genetic Analyzer. DNA sequences were collated and analysed using the DNASTAR Lasergene (version 8.0) Software (DNASTAR, Inc., Madison, WI, USA), followed by analysis at the *Salmonella*

MLST database where allele numbers and a MLST sequence type (ST) were assigned. DNA sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and an Applied Biosystems 3500 Genetic Analyzer. DNA sequences were collated and analyzed using the DNASTAR Lasergene (version 8.0) Software (DNASTAR, Inc., Madison, WI, USA), followed by analysis at the *Salmonella* MLST database where allele numbers and a MLST sequence type (ST) were assigned.

Molecular characterisation of *Salmonella* Typhi isolates to define whether these were the H58 haplotype was undertaken as follows: all isolates from known HIV-infected patients were amplified, using primers developed targeting the H58 gene, using a conventional polymerase chain reaction (PCR) (166). Each of these isolates was matched as closely as possible to one to two isolates from patients of the same age and from the same geographic location. A total of 185 isolates were tested.

3.3. Statistical analysis

Data were recorded in EpiInfo (Centers for Disease Control, Atlanta, GA, USA) and later into Access 2007 (Microsoft Corp, Redmond, WA, USA). Data were then extracted and imported into STATA version 11 or version 13 (StataCorp, College Station, TX, USA), for further statistical analysis. Incidence rates for papers I through III were calculated using population projections developed by Actuarial Society of South Africa (ASSA) (167). Incidence measures were minimum incidence as there was no correction for underascertainment. Incidence rates for paper IV were calculated based on annual data published by Statistics South Africa, to make these data more comparable with data derived by NDoH (Appendix III) (www.statssa.gov.za). Basic analyses calculated odds ratios (ORs), 95% confidence intervals (CIs) and P values for age range, sex, HIV status, PBS, other comorbidities, serotype for *Shigella* and nontyphoidal *Salmonella* serovars, multidrug

resistance and ESBL production. For multivariate analysis, logistic regression was used to calculate adjusted odds ratios (AORs), including non-collinear variables, with an observed cut-off of $P<0.1$ for the univariate analysis. Patients with missing data were excluded from the analyses. The $\chi 2$ test was used to compare clinical and microbiological features where relevant. Additional statistical analysis including incidence rate ratios and Pearson's correlation were used when applicable. Two-sided P values of <0.05 were considered significant throughout.

The burden of disease was assessed for each of the disease manifestations investigated, that is invasive shigellosis (South Africa), *Salmonella* meningitis (South Africa), typhoid fever (South Africa) and iNTS (Gauteng Province only, due to large case numbers as well as the strength of the data in that province). For invasive shigellosis, *Salmonella* meningitis and typhoid fever; population data were used based on tables developed by the Actuarial Society of South Africa (ASSA) (167). For the iNTS study in Gauteng Province, data published by the National Department of Statistics were utilised for burden of disease calculations (www.statssa.gov.za), as these would act as a comparator for published data from National Department of Health (NDoH) for the numbers of adults accessing ART from 2004 to 2012 (168). To calculate changing incidence rates of iNTS, viral load data were extracted from the CDW (as above) as a proxy for the numbers of patients accessing antiretroviral therapy (ART) in Gauteng Province and compared with published data from NDoH (168).

3.4. Ethics

Ethics approvals were obtained for the University of the Witwatersrand Human Research Ethics Committee (HREC) (M110601, granted 24 June 2011). Prior ethics for the Enhanced Surveillance for cotrimoxazole [trimethoprim-sulphamethoxazole] resistance in South Africa project was obtained with the following clearance numbers: M02-10-42 and M081117, and

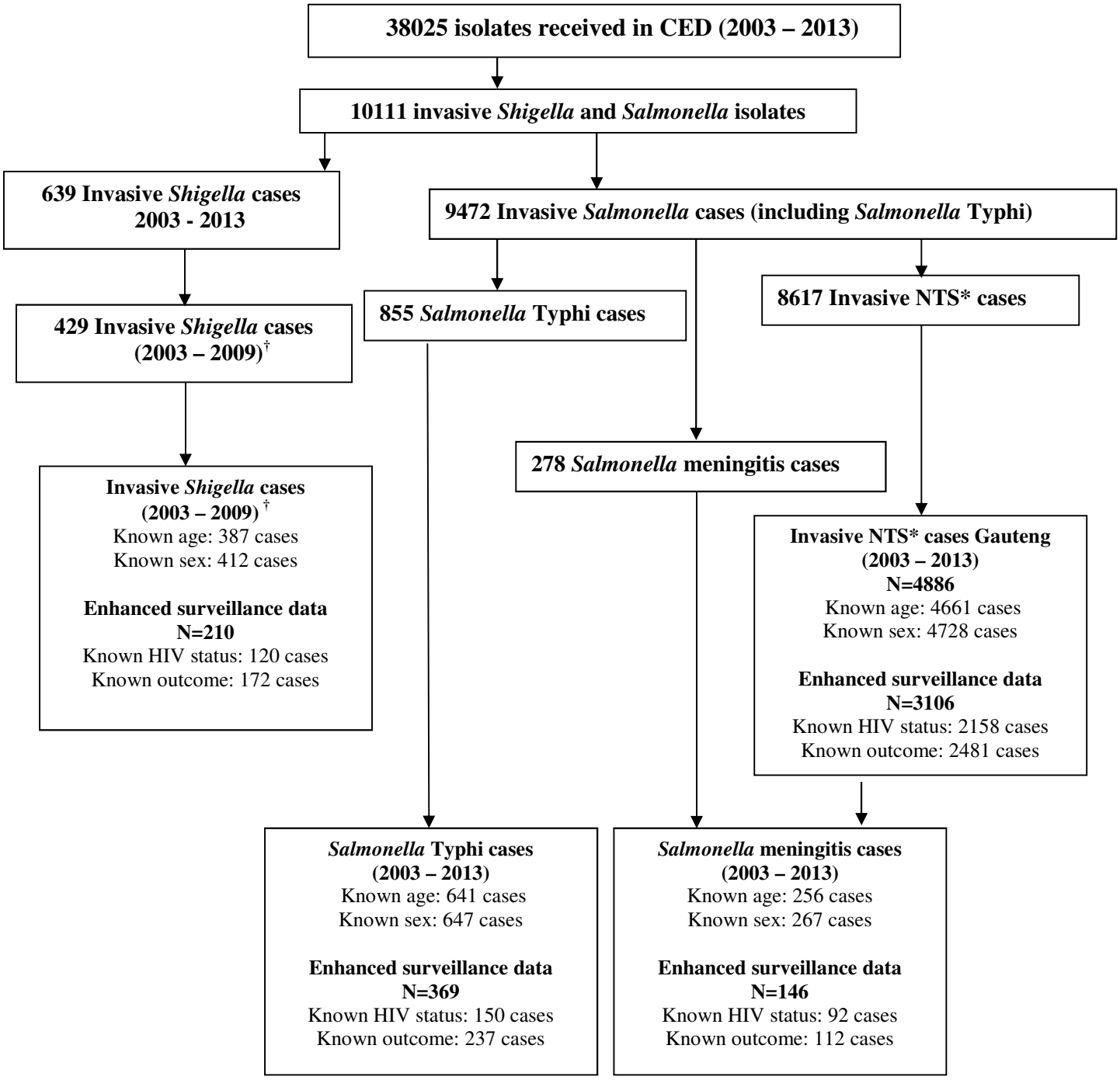
M060449 for general surveillance (Appendix IV). Additional institutional ethics were obtained where relevant, at each of the sentinel sites. All patients who were interviewed provided informed consent (and assent in the case of minors capable of understanding and providing assent), or in the case of minors or those unable to provide consent due to their mental status, consent was provided by the parent or guardian. For those patients for whom data were gathered from review of clinical records, consent could not be obtained, but this was acknowledged and accepted in the ethics clearance process.

3.5. Funding

This research has been supported by NICD/NHLS and the President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention (CDC) under the terms of [5U2GPS001328] and, in part, for 2003-2006 by funds from the United States Agency for International Development's Antimicrobial Resistance Initiative, transferred via a cooperative agreement [number U60/CCU022088] from the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. For 2007 - 2009, it was supported by the HHS Centers for Disease Control and Prevention (CDC), National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (NCHHSTP), Global AIDS Program (GAP) Cooperative Agreement [U62/PSO022901].

4. Results

4.1. Invasive enteric infections 2003 – 2013, South Africa



*NTS, Nontyphoidal *Salmonella*

†Paper I was published before 2013, when this study ended, thus the data presented in this paper do not reflect all the invasive *Shigella* cases from 2003 - 2013

Figure 6. Flow chart indicating the breakdown of number of cases available for analysis and primary data for analysis for Papers I - V.

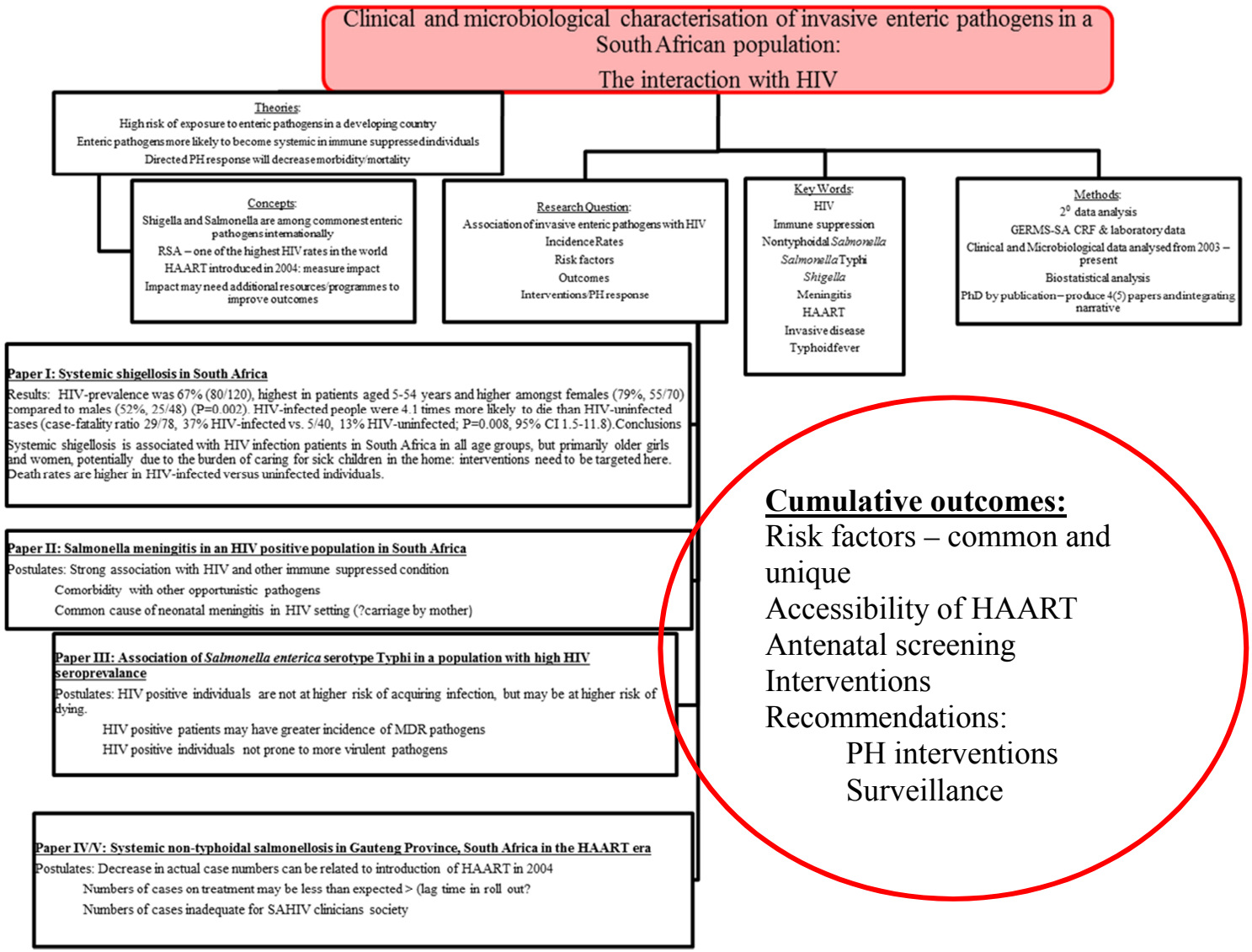


Figure 7. Evolution of the conceptual framework based on data analysis.

Between 2003 and 2013, a total of 38025 enteric isolates were received by CED for characterisation, of which 10111 (26.6%) were invasive. Of these, 1397 (13.8%) isolates were identified on audit of the CDW. Data extraction for this work is reflected in Figure 6. Full data for the period 2003 – 2013 were not included for patients with invasive shigellosis for the publication of Paper I: this analysis covered the period from 2003 – 2009 only, as this manuscript was published before the study ended. For the remaining analyses (Papers II to V) and illustrative purposes for incidence and age range in this monograph, data were extracted for the full period, to include invasive shigellosis from 2003 to 2013.

Results of this work are published as five papers (Figure 7). Paper I analyses the association of HIV infection and invasive shigellosis in South Africa; Paper II describes the connection between HIV infection and meningitis in South Africa; Paper III examines the links between HIV infection and typhoid fever in South Africa and Papers IV and V analyse the association between HIV infection and iNTS in Gauteng Province, South Africa, the former specifically focussing on the correlation between increasing access to antiretroviral treatment (ART) and the latter primarily discussing clinical and microbiological aspects of iNTS and HIV.

Annual incidence rates are reflected in Figure 8. While incidence rates for invasive shigellosis, typhoid fever and *Salmonella* meningitis were comparable, averaging from 0.05 to 0.15 per 100,000 over the 11 year period, incidence rates for iNTS infection in Gauteng were much higher, peaking at 5.8 per 100,000 in 2004, thereafter decreasing to 2.2 per 100,000 in 2013 (Figure 8). Higher incidence rates of typhoid fever were noted in 2005 and 2006, in association with documented typhoid fever outbreaks (0.39 and 0.23 per 100,000 respectively).

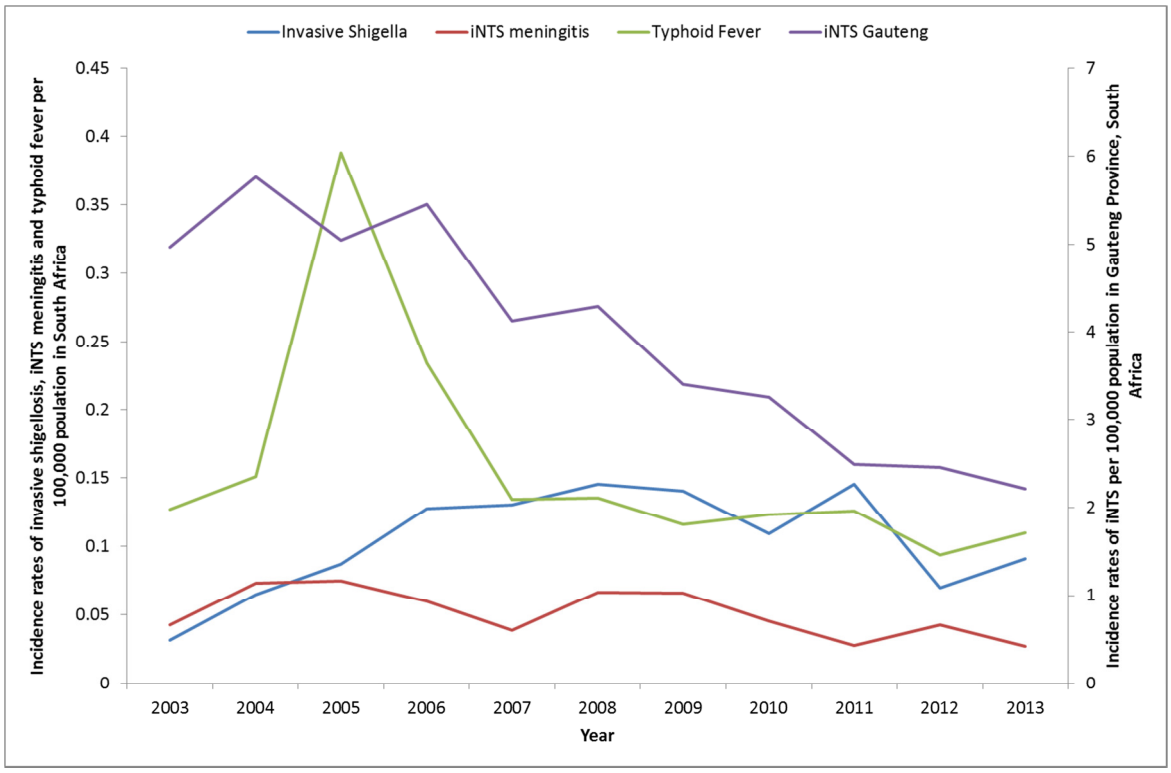
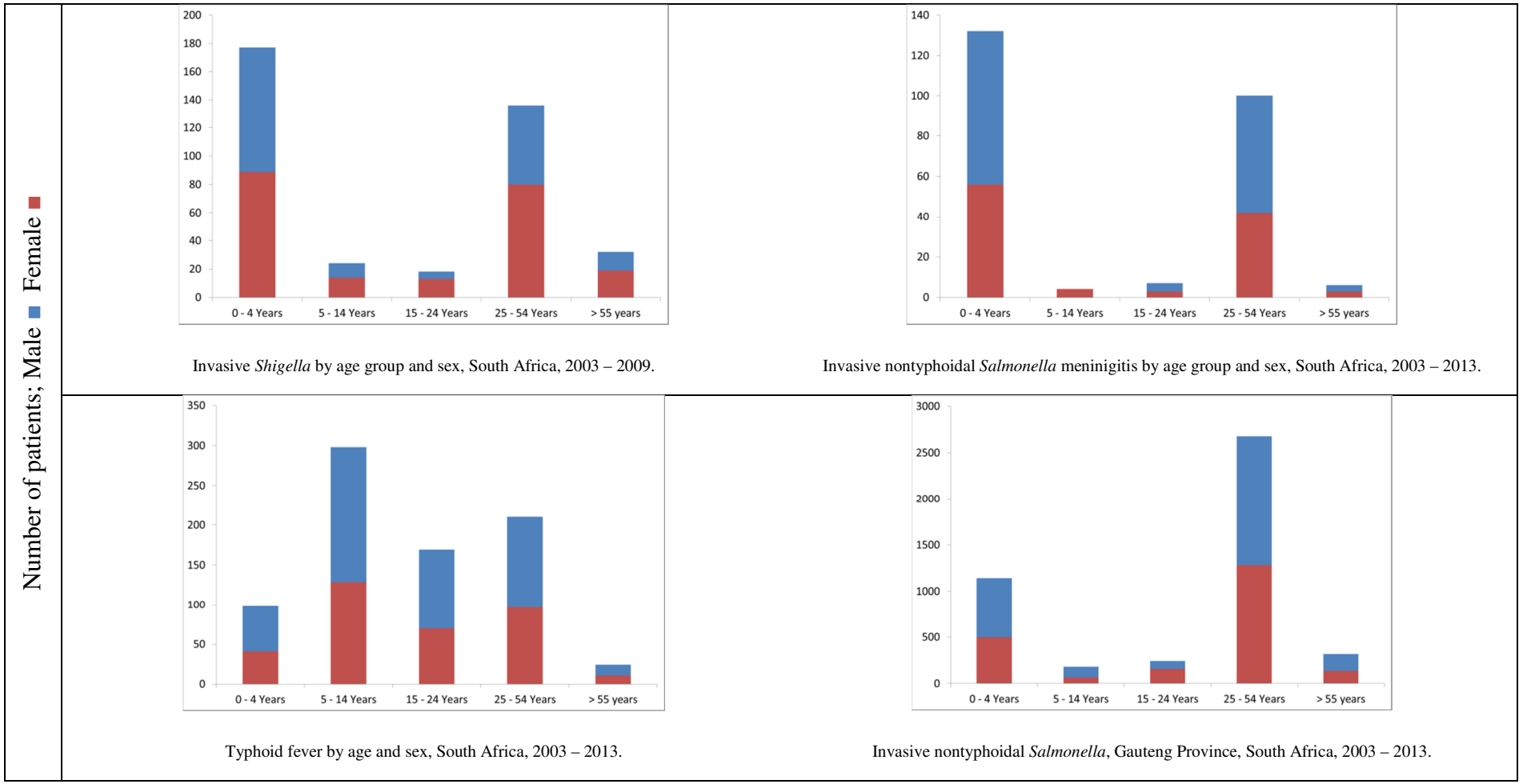


Figure 8. Comparative incidence rates per 100,000 population of invasive shigellosis, *Salmonella* meningitis, typhoid fever (South Africa) and invasive nontyphoidal *Salmonella* (iNTS) infection (Gauteng Province, South Africa) 2003 – 2013.

Scales on the vertical axes reflecting incidence rates differ for iNTS incidence rates, due to higher overall numbers for iNTS.

Of 6244 patients for whom sex was known, 3283 (52.6%) patients were male; where age was known, 1605/6131 (26.2%) were less than five years of age, 517 (8.4%) were aged between 5 and 14 years; 439 (7.2%) were aged between 15 and 24 years; 3186 (52.0%) were aged between 25 and 54 years and 384 (6.3%) aged 55 years and older. For invasive shigellosis and iNTS, the majority of cases occurred in children < 5 years and adults aged from 25 to 54 years, but for typhoid fever, the highest incidence rates were noted in the 5 to 14 year age band (Figure 9).



Invasive *Shigella* by age group and sex, South Africa, 2003 – 2009.

Invasive nontyphoidal *Salmonella* meninigitis by age group and sex, South Africa, 2003 – 2013.

Typhoid fever by age and sex, South Africa, 2003 – 2013.

Invasive nontyphoidal *Salmonella*, Gauteng Province, South Africa, 2003 – 2013.

Figure 9. Age range and sex distribution in patients with invasive enteric infections in South Africa, 2003 – 2013 (data for invasive *Shigella* are for 2003 – 2009 only).

4.2. Differences in HIV prevalence and mortality associated with age and sex in patients presenting with invasive enteric infections

This work confirmed that HIV infection was a significant risk factor predisposing patients to invasive disease due to *Shigella* and nontyphoidal *Salmonella* (NTS), including *Salmonella* meningitis (Table 3). Prevalence rates of HIV infection in patients presenting with invasive enteric infection were higher in invasive shigellosis, *Salmonella* meningitis and iNTS in Gauteng (Papers I, II and V respectively).

Data for typhoid fever were less conclusive: although HIV infection appeared to be a risk factor for typhoid fever in adult women, this could not be shown for adult men and neither was it observed in children. Additionally, in this series, excluding those patients with typhoid fever, between 95% and 100% of adults presenting with invasive enteric infection were HIV-infected (Table 3).

Comparing patients presenting with typhoid fever with those presenting with iNTS in Gauteng, at admission, 335/369 (90.8%) of patients with typhoid fever had unknown HIV status, compared with 1917/3106 (61.7%) of those presenting with iNTS. Post admission, 219/369 (59.3%) of patients with typhoid fever still had unknown HIV status, compared with 951/3106 (30.6%) of patients with iNTS (χ^2=8.3; P=0.004).

Table 3. Incidence of invasive enteric infections in known HIV- infected individuals[†] with known age, and in Gauteng for invasive nontyphoidal salmonellosis, compared with average national prevalence of HIV in South Africa, 2003 – 2013.

	Total	HIV-infected	% HIV-infected	Odds ratio	95% Confidence interval	P
*National data** (referent)						
▪ Children < 15 years	15493078	385955	2.5	-	-	-
▪ Males ≥ 15 years	16161807	1986415	12.3	-	-	-
▪ Females ≥ 15 years	17823516	2803527	15.7	-	-	-
Study						
• Invasive shigellosis						
▪ Children < 15 years	61	23	37.7	23.7	(13.5 - 40.8)	<0.001
▪ Males ≥ 15 years	15	15	100	-	-	-
▪ Females ≥ 15 years	44	42	95.5	112	(29.3 - 959.3)	<0.001
• *Salmonella* meningitis						
▪ Children < 15 years	46	24	52.2	42.7	(22.9 - 79.9)	<0.001

▪ Males ≥ 15 years	23	23	100	-	-	-
▪ Females ≥ 15 years	21	21	100	-	-	-
• Typhoid fever						
▪ Children < 15 years	63	3	4.8	2.0	(0.4 – 6.0)	0.2
▪ Males ≥ 15 years	44	7	15.9	1.4	(0.5 - 3.1)	0.5
▪ Females ≥ 15 years	43	19	44.2	4.2	(2.2 - 8.1)	<0.001
• Invasive nontyphoidal salmonellosis (Gauteng)						
▪ Children < 15 years	504	322	63.9	69.3	(57.5 - 83.4)	<0.001
▪ Males ≥ 15 years	824	780	94.7	150	(108.2 - 215.2)	<0.001
▪ Females ≥ 15 years	816	779	95.5	94.8	(70.0 - 131.6)	<0.001

[†]Data reflect HIV status only for those patients for whom it was known at sentinel surveillance sites and not all cases identified.

*Data averaged from ASSA calculations for population and HIV prevalence in South Africa, and in Gauteng for invasive nontyphoidal salmonellosis (167).

In Paper I, all adults (≥15 years) with invasive shigellosis were HIV-infected, but the burden of disease of invasive shigellosis in adults predominantly occurred in adult females (55/70; [78.6%] versus 25/48 [52.1%]; P=0.00]), but this predominance was not seen in the other studies (Papers II to V). Significantly, however, more adult women with typhoid fever were HIV-infected compared with adult men (19/44 [43.2%] versus 7/43 [16.3%]; P=0.009). In all the studies, adults aged ≥ 15 years were more likely to die than children aged < 15 years (Table 4). There were additionally differences in the incidence of HIV infection between females and males in association with *Salmonella* meningitis (35/41 [85.4%] versus 33/49 [67.4%]; P=0.04) and a trend was observed for iNTS infection in Gauteng Province (916/1026 [89.3%] versus 962/1111 [86.6%]; P=0.06) (Paper V).

Papers I, III and V confirmed that children aged < 15 years with invasive enteric infections were also less likely to be HIV–infected, compared with adults (Table 5). In paper II, all patients aged ≥ 15 years were HIV-infected: statistical analysis was therefore not possible on this cohort. Additionally, Paper IV confirmed that as access to ART increased over the period 2003 – 2013, annual incidence rates of iNTS across all age groups decreased in Gauteng Province (R=-0.94; P<0.001).

In association with these findings, paper IV showed that ART had had a significant impact in decreasing incidence rates of invasive nontyphoidal salmonellosis on adults aged 25 - 49 years than on children aged < 5 years in Gauteng Province between 2003 and 2013 (R=-0.92; P<0.001 versus R=-0.50; P=0.14). Similarly, adults aged 25 – 49 years had a significantly greater negative correlation between decreasing incidence rates of *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium) compared with children aged < 5 years (R=-0.91; P<0.001 versus R=-0.67; P=0.04).

Table 4. Comparative risk factors for mortality due to invasive enteric infections between children aged < 15 years and adults ≥ 15 years.

Study	Cases	Deaths	Case fatality ratio	Odds ratio	95% Confidence Interval (CI)	P
Children < 15 years (referent)						
• Invasive shigellosis	90	16	17.8	1	-	-
• *Salmonella* meningitis	57	19	33.3	1	-	-
• Typhoid fever	111	3	2.7	1	-	-
• Invasive nontyphoidal salmonellosis	624	126	20.2	1	-	-
Adults ≥ 15 years						
• Invasive shigellosis	82	34	41.5	3.2	(1.6 – 6.6)	0.001
• *Salmonella* meningitis	71	41	57.7	3.7	(1.7 – 8.1)	0.001
• Typhoid fever	126	13	10.3	4.1	(1.1- 14.9)	0.03
• Invasive nontyphoidal salmonellosis	1854	632	34.1	2.0	(1.6 – 2.5)	<0.001

Table 5. Comparative risk of invasive enteric infections associated with HIV infection between children aged < 15 years and adults.

Study	Children < 15 years N (%)		Adults ≥ 15 years N (%)		Odds ratio	95% Confidence Interval (CI)	P
	HIV-uninfected	HIV-infected	HIV-uninfected	HIV-infected			
Invasive shigellosis	38 (62.3)	23 (37.7)	2 (3.4)	57 (96.6)	47.1	(10.3 - 419.7)	<0.001
Salmonella meningitis	22 (47.8)	24 (52.2)	0 (0.0)	45 (100.0)	-	-	-
Typhoid fever	60 (95.2)	3 (4.8)	61 (70.1)	26 (29.9)	8.4	(2.4 – 45.0)	<0.001
Invasive nontyphoidal salmonellosis	182 (36.1)	322 (63.9)	81 (4.9)	1569 (95.1)	10.9	(8.1 – 14.8)	<0.001

Reviewing male versus female case numbers for iNTS in Gauteng over the period, in adults, there was a significant difference in males versus females between the years 2003 - 2005 and 2006 – 2013 (509/1,109 [45.9%] and 600/1,109 [54.1%] versus 1,139/2,122 [53.7%] and 983/2,122 [46.3%] respectively; $\chi 2=17.6$, $P<0.001$) (Figure 10), which was not apparent in male and female children between the years 2003 - 2005 and 2006 – 2013 (207/371 [55.8%] and 164/371 [44.2%]) versus 545/948 [57.5%] and 403/948 [42.5%] respectively; $\chi 2=0.3$, $P=0.6$) (Figure 11) (Table 6). The correlation however between adult males and females accessing ART and male and female children accessing ART between 2004 and 2013 was very good (Figure 12). Paper IV confirmed the rate ratio between adult men and women presenting with iNTS in 2003 and 2004 (rate ratio=0.73 and 0.89, respectively) reversed in 2005 (rate ratio=1.07) and this trend was maintained until 2013 (rate ratio=1.44), in conjunction with consistently lower numbers of men accessing ART.

Table 6. Numbers and incidence per 100 000 population of male and female patients, adults, aged ≥ 15 years, and children, aged < 15 years, accessing ART and presenting with invasive nontyphoidal *Salmonella* (iNTS), in Gauteng Province, South Africa, between 2003 and 2013. Results for prevalence of ART usage and iNTS incidence are calculated from STATSSA population based numbers (Appendix III; www.statssa.gov.za).

Year	Patients accessing ART					Patients presenting with iNTS					Prevalence of ART usage per 100 000					Incidence of iNTS per 100 000				
	Adult		Child		Total	Adult		Child		Total	Adult		Child		Total	Adult		Child		Total
	Male	Female	Male	Female		Male	Female	Male	Female		Male	Female	Male	Female		Male	Female	Male	Female	
2003	-	-	-	-	-	137	195	54	46	432	-	-	-	-	-	3.6	5.1	4.1	3.4	4.2
2004	2282	3921	607	604	7414	185	218	83	63	549	58.4	100.5	45.6	44.4	70.6	4.7	5.6	6.2	4.6	5.2
2005	9924	18135	2437	2318	32814	187	187	70	55	499	247.6	454.8	179.9	168.0	305.8	4.7	4.7	5.2	4.0	4.7
2006	23106	43521	4826	4925	76378	221	181	94	62	558	561.9	1066.5	350.5	353.0	696.6	5.4	4.4	6.8	4.4	5.1
2007	35017	65670	6938	6842	114467	138	150	86	62	436	830.7	1573.7	495.2	484.2	1021.8	3.3	3.6	6.1	4.4	3.9
2008	53428	99637	9047	9179	171291	179	139	92	57	467	1235.8	2334.1	635.5	641.8	1496.6	4.1	3.3	6.5	4.0	4.1
2009	63445	120259	10153	10719	204576	157	108	66	50	381	1430.7	2753.7	702.6	740.7	1749.4	3.5	2.5	4.6	3.5	3.3
2010	65343	128330	11201	11882	216756	138	115	69	56	378	1436.5	2872.1	764.2	811.9	1814.5	3.0	2.6	4.7	3.8	3.2
2011	80277	161494	11443	12360	265574	97	105	47	42	291	1719.9	3531.2	771.2	836.5	2176.4	2.1	2.3	3.2	2.8	2.4
2012	108134	217413	12814	14151	352512	111	95	48	45	299	2257.5	4643.0	853.6	949.6	2828.3	2.3	2.0	3.2	3.0	2.4
2013	138174	272753	14011	15425	440363	98	90	43	29	260	2809.4	5683.5	925.6	1030.2	3459.7	2.0	1.9	2.8	1.9	2.0

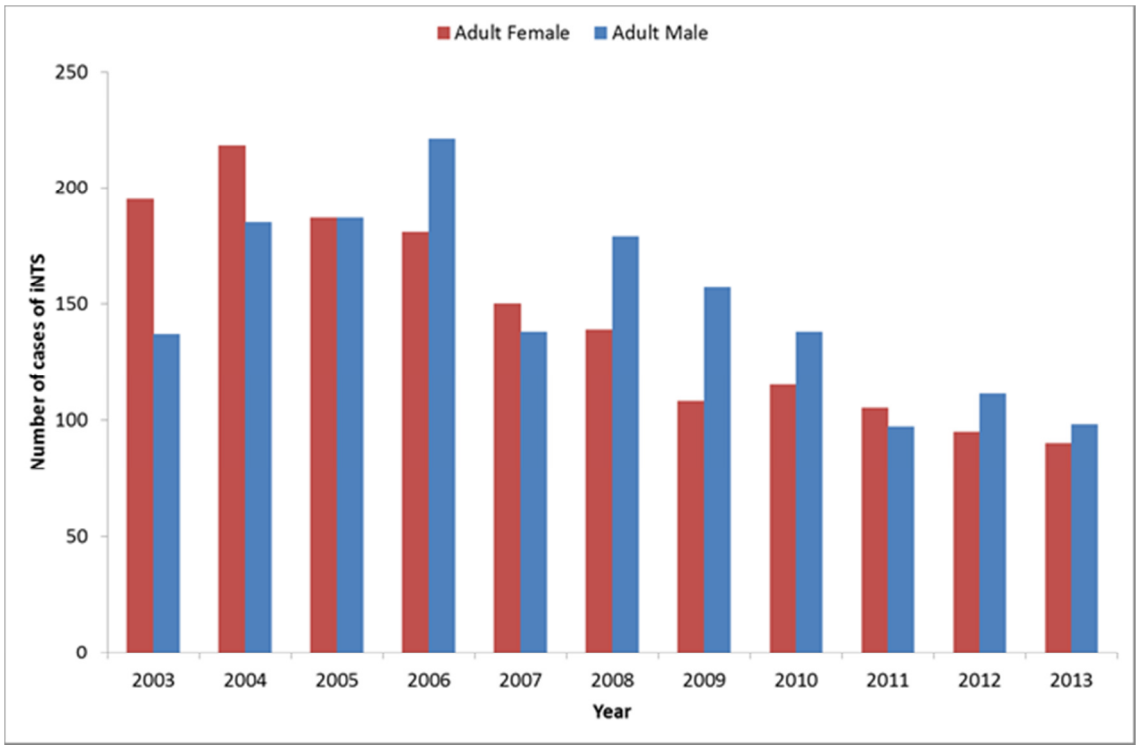


Figure 10. Number of adults aged ≥ 15 years, male versus female, presenting with invasive nontyphoidal *Salmonella* (iNTS) in Gauteng Province, South Africa, 2003 – 2013.

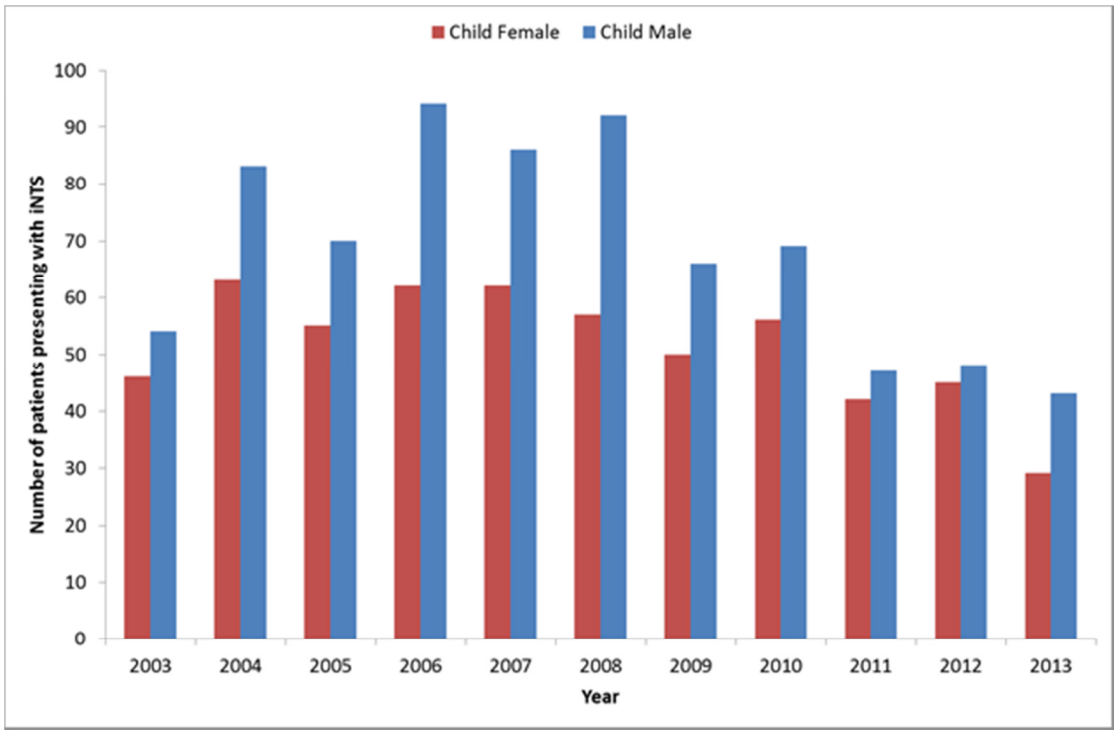


Figure 11. Number of children aged < 15 years, male versus female, presenting with invasive nontyphoidal *Salmonella* (iNTS) in Gauteng Province, South Africa, 2003 – 2013.

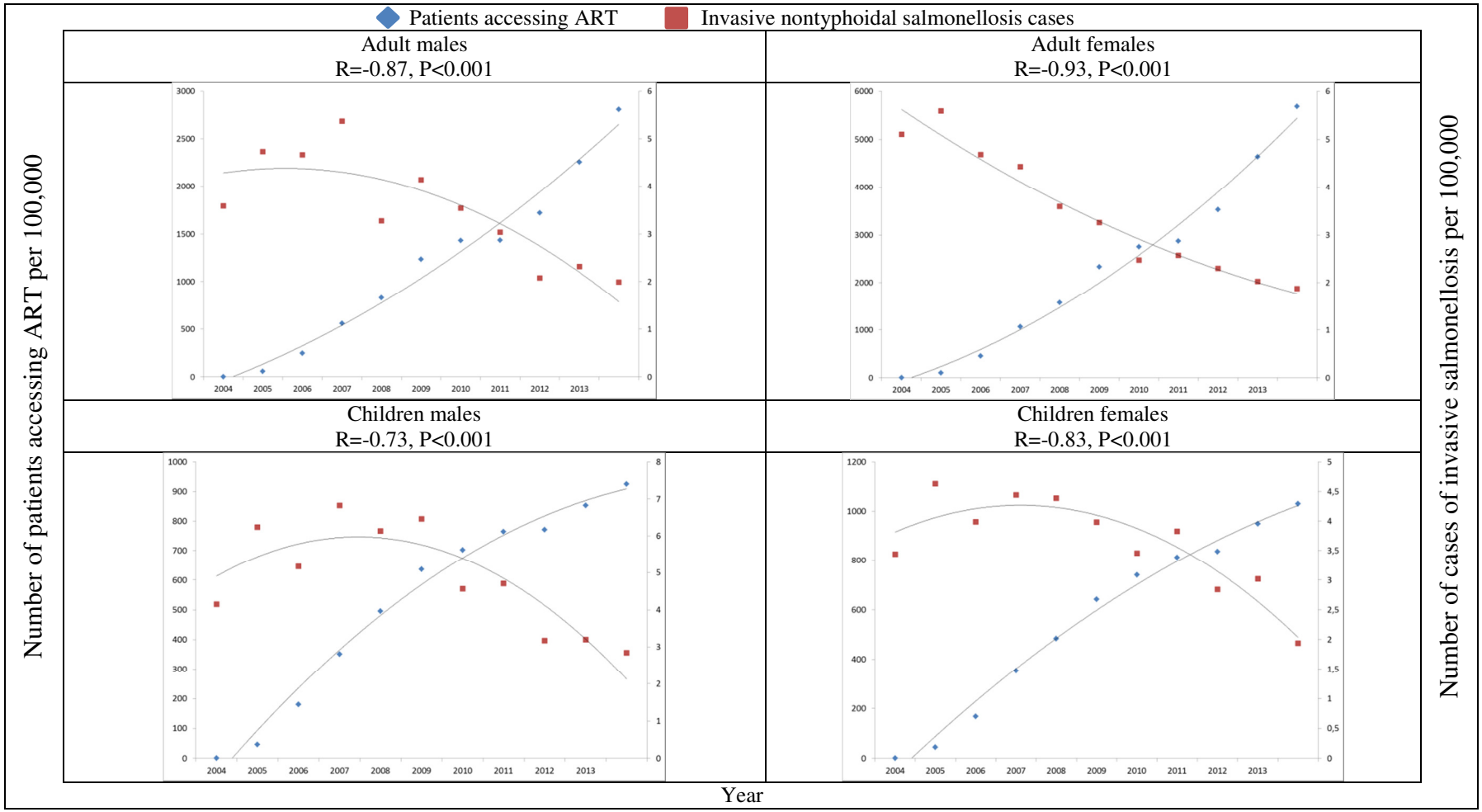


Figure 12. Comparison of incidence of number of patient presenting with invasive *Salmonella* (iNTS) per 100,000 population per year by sex and age group (adult versus child) and prevalence of number of patients accessing antiretroviral therapy (ART) per 100,000 population by age range, Gauteng Province, South Africa, 2004 – 2013.

4.3. Invasive enteric infections in HIV-infected patients, markers of HIV, access to antiretroviral treatment and HIV as a risk factor for mortality

In addition, Papers IV and V showed mortality decreased as access to ART increased. Most adult patients aged ≥15 years had CD4+ counts ≤50 cells: 18/24 (69.2%) for invasive shigellosis; 14/27 (51.9%) for *Salmonella* meningitis and 663/1308 (50.7%) for patients with iNTS infection in Gauteng province, but only 3/16 (18.8%) for adults with typhoid fever. A total of 8/80 (10.0%) patients with invasive shigellosis, 10/70 (14.3%) with *Salmonella* meningitis, 4/29 (13.8%) with typhoid fever and 291/1454 (20.0%) with iNTS infection in Gauteng were on antiretroviral treatment. A total of 1/76 (1.3%) infants with invasive shigellosis, 4/22 (18.2%) with *Salmonella* meningitis, 1/60 (1.7%) with typhoid fever and 20/182 (11.0%) with iNTS infection in Gauteng children had a history of being HIV-exposed (born to HIV-infected mothers), but were HIV-uninfected.

Papers I, II, III and V found a significantly increased risk of mortality among HIV-infected patients compared with HIV-uninfected individuals (Table 7). The highest CFR was in HIV-infected patients with *Salmonella* meningitis (30/32 [93.8%]), and the lowest in those with typhoid fever (7/25 [28.0%]).

In contrast to the findings described above, correlating the decreasing incidence rates of iNTS infection, and specifically *Salmonella* Typhimurium infection in Gauteng, compared with increasing access to ART (R=-0.94; P<0.001 and R=-0.93; P<0.001, respectively), there was an unexpected significant correlation between increasing incidence rates of invasive *Salmonella* Enteritidis and ART access across all age groups (R=0.95; P<0.001).

Table 7. HIV infection as a risk factor for mortality due to invasive enteric pathogens.

Study	Cases	Deaths	Case fatality ratio	Odds ratio	95% Confidence Interval (CI)	P
HIV-uninfected (referent)						
• Invasive shigellosis	40	5	12.5	1	-	-
• *Salmonella* meningitis	17	3	17.6	1	-	-
• Typhoid fever	120	4	3.3	1	-	-
• Invasive nontyphoidal salmonellosis	260	41	15.9	1	-	-
HIV-infected						
• Invasive shigellosis	78	29	37.2	4.1	(1.5 – 11.8)	0.008
• *Salmonella* meningitis	32	30	93.8	5.3	(1.4 - 20.0)	0.013
• Typhoid fever	25	7	28.0	11.3	(3.0 - 42.4)	<0.001
• Invasive nontyphoidal salmonellosis	1806	570	31.6	2.5	(1.7 – 3.5)	<0.001

4.4. Invasive enteric infections and severity of illness as a risk factor for mortality.

All clinical papers found severity of illness, (Pitt bacteraemia score [PBS] ≥ 4 for predominantly bacteraemic infections or Glasgow coma scale [GCS] ≤ 13 for *Salmonella* meningitis) was a risk factor for mortality on univariate analysis (Table 8). Critically, severity of illness remained a significant risk factor contributing to mortality in all the analyses on multivariate analysis, when adjusting for age, HIV status, other comorbid features, time period (2003 – 2005; 2006 – 2009; 2010-2013) analysed for typhoid fever and iNTS in Gauteng, or microbiological risk factors (specifically multidrug resistance or *Salmonella* serovar).

In Paper II and III, on multivariate analysis, correcting for age, comorbidity and microbiological risk factors for mortality, severity of illness for *Salmonella* meningitis (GCS≤13) and for iNTS infection in Gauteng (PBS≥4), remained significant (AOR, 18.7; 95% CI, 3.0 – 118.5; P=0.002 and AOR, 6.3; 95% CI, 3.8 – 10.5; P<0.001, respectively), although for HIV infection, the adjusted analysis did not (AOR, 0.9; 95% CI 0.1 – 15.7; P=0.987 and AOR, 1.5; 95% CI 0.9 – 2.4; P=0.1, respectively). For typhoid fever (Paper III), however, correcting for other factors contributing to mortality, both HIV infection (AOR, 10.8; 95% CI, 2.3 - 50.3; P=0.002) and severity of illness (PBS≥4) (AOR, 9.8; 95% CI, 1.6 - 60.0; P=0.01) remained significant. Numbers in the invasive *Shigella* cohort (Paper I) were too small to analyse for severity of illness.

Table 8. Severity of illness as a risk factor for mortality due to invasive enteric pathogens.

Study	Cases	Deaths	Case fatality ratio	Odds ratio	95% Confidence Interval (CI)	P
Severity of illness (referent)						
PBS<4; GCS>13						
• Invasive shigellosis‡	124	29	23.3	1	-	-
• *Salmonella* meningitis†	5	33	15.2	1	-	-
• Typhoid fever‡	177	11	6.2	1	-	-
• Invasive nontyphoidal salmonellosis‡	1731	460	26.6	1	-	-
Severity of illness						
PBS≥4; GCS≤13						
• Invasive shigellosis‡	8	7	87.5	22.9	(2.7 – 194.2)	0.004
• *Salmonella* meningitis†	10	8	80.0	21.6	(3.5 – 133.3)	0.01
• Typhoid fever‡	12	5	41.7	10.8	(2.9 – 39.5)	<0.001
• Invasive nontyphoidal salmonellosis‡	109	72	66.1	5.4	(3.6 – 8.1)	<0.001

‡Severity of illness defined as a Pitt bacteraemia score (PBS) ≥4.
†Severity of illness defined as Glasgow coma scale (GCS) ≤13.

4.5. Microbiological risk factors associated with HIV infection, invasive enteric pathogens and mortality

Microbiological risk factors for mortality were not consistent through this study. Paper I showed there were no differences between the occurrence of multidrug resistant (MDR) *Shigella* serotypes in HIV- infected and uninfected individuals (P=0.3) and HIV-infected patients were not more likely to die if infected with a MDR *Shigella* strain (P=0.2). Additionally, in Paper III, there were no differences between the occurrence of MDR *Salmonella enterica* serovar Typhi (*Salmonella* Typhi) between HIV-infected and HIV-uninfected individuals (P=0.5) or the isolation of *Salmonella* Typhi H58 (P=0.3). HIV-infected patients infected with MDR *Salmonella* Typhi were, however, more likely to die than HIV-uninfected patients (P=0.02).

A trend was noted however among patients with *Salmonella* meningitis (Paper II): infection with *Salmonella* Typhimurium was a risk factor for mortality (Odds Ratio [OR], 3.0; 95% Confidence Interval [CI], 0.05 – 1.04; P=0.06). Additionally, *Salmonella* Typhimurium ST313 occurred more frequently in HIV-infected patients with *Salmonella* meningitis compared with other sequence types (P=0.03). In Paper V, in patients in Gauteng Province with iNTS infection, HIV-infected patients were more likely to be infected with *Salmonella* Typhimurium (χ^2=120.1; P<0.001) or MDR *Salmonella* isolates (χ^2=12.2; P<0.001).

Additionally, as described in sections 4.3 and 4.4 above, Paper IV showed that iNTS infections and invasive *Salmonella* Typhimurium decreased as access to ART increased between 2003 and 2013, but incidence of *Salmonella* Enteritidis infection increased at a comparable rate to numbers of patients accessing ART.

5. Discussion

While the relationship between HIV and invasive enteric diseases has long been described in Africa, a full understanding of clinical features, including the role of age, sex, severity of illness and microbiological features including organism serotype and antimicrobial resistance profiles in South African patients, has been lacking. This work was undertaken to fill these data gaps and to provide additional information that could assist in both the prevention of disease as well as the management of patients presenting with invasive enteric infections. This is the accumulation of eleven years of data from a unique national laboratory-based surveillance system, developed to optimise data collection in a resource-limited emerging economy, representing a wide range of sentinel sites around South Africa (Figure 13).

Figure 13. View overlooking Edendale Hospital, an enhanced surveillance site.

5.1. Clinical characteristics

Whereas this work has highlighted the association between HIV-infected patients and invasive shigellosis in adults, as well as invasive nontyphoidal salmonellosis in causing bacteraemic infections and meningitis in adults, these associations were less clear in children. Rather, it appears that children under the age of 15 years, and particularly young children, may acquire disease not only in association with HIV infection, but also due to HIV exposure, due to maternal HIV infection, or in association with malnutrition (18;49). By contrast, the role of HIV in adult infections is undisputed (29;46;169-172).

Novel findings have been highlighted in this work: *Shigella* is a common cause of diarrhoea and dysentery in children in developing countries (153). HIV-infected women have been shown to be at a greater risk of acquiring invasive shigellosis, possibly in association with their having the burden of child care of children with diarrhoea. Conversely, while sick children may place HIV-infected adults at risk, HIV-infected mothers may be placing their newborn infants at risk of *Salmonella* meningitis, possibly due to an inability to clear the pathogen from the gut and a failure to pass on maternal protective antibodies. Excessive numbers of young children, particularly those from disadvantaged backgrounds, presenting with invasive nontyphoidal salmonellosis, has been highlighted in the United States (173). This work confirmed both their vulnerability and that maternal HIV may impact even HIV-uninfected infants (Figure 14). Interventions in known HIV-infected mothers have been shown to reduce neonatal mortality in Zambia (174): although, given the prevalence of trimethoprim sulfamethoxazole resistance in the South African nontyphoidal *Salmonella* isolates (>40%) would render this unfeasible, other antimicrobials may be appropriate in preventing mother-to-child transmission of disease.

Figure 14. The paediatric ward at Edendale Hospital, an enhanced surveillance sentinel site.

This work could not resolve the risks of HIV-infected individuals for acquiring typhoid fever however, primarily due to data gaps regarding HIV status in patients with typhoid fever. While it appears that children and adult males were not at a greater risk, it suggested that adult females were: 43% of adult women with typhoid fever were HIV-infected compared with 16% of adult women in the general population. It is also well described in South Africa that women exhibit better healthcare seeking behaviour in relation to suspected HIV-related infections (157), and women who knew they were HIV-infected may have preferentially sought medical care at tertiary care facilities. As current HIV programmes in South Africa include antenatal testing of all pregnant women presenting to government antenatal clinics (175), most women of childbearing age who have had children are aware of their HIV status. This contradiction requires further clarification In addition, reviewing the proportion of patients who still had an unknown HIV status after admission for typhoid fever versus those patients admitted for iNTS in Gauteng Province, it appears that there is a clear and significant

bias towards testing patients with iNTS for HIV infection, compared with testing patients with typhoid fever.

Of concern is the role that malnutrition plays in association with HIV infection in children. In a Zambian series, it was noted that HIV infection negatively affected the nutritional status of children with intestinal infections (44) and a similar result was noted here. The incidence of invasive nontyphoidal salmonellosis decreases significantly in association with interventions including both HIV treatment programmes and nutritional interventions (146).

5.2. Mortality

Figure 15. A school child walks past a graveyard with numerous fresh graves, Upington, Northern Cape.

This thesis has highlighted again the powerful association between HIV as a risk factor for invasive enteric disease and additionally for mortality due to invasive enteric disease in South

Africa (Figure 15). Irrespective of whether invasive disease is due to *Shigella*, *Salmonella* Typhi or the non-typhoidal *Salmonella*, excessive mortality due to these pathogens is a cause for concern and odds ratios ranged from 2.5 for invasive nontyphoidal salmonellosis to 11.3 for typhoid fever, comparing HIV-infected with HIV-uninfected individuals. Medrano *et al* have additionally described a higher frequency mortality in relation to HIV infection in patients in an intensive care facility (176). Adults were also between two and three times more likely to die than children less than 15 years of age, reflecting the excessive burden of HIV infection, particularly among adults aged 25 to 45 years of age (167).

Additionally, severity of illness, including Glasgow coma scale (GCS) ≤13 in those patients presenting with *Salmonella* meningitis or Pitt bacteraemia score (PBS) ≥4, for those patients with bacteraemic infections, remains a critical aspect that must be considered in managing any patient with an invasive enteric infection, irrespective of whether they are HIV-infected or HIV-uninfected, due to excessive mortality associated with these patients. In fact, on multivariate analysis, HIV remained a significant risk factor for mortality only in those patients presenting with typhoid fever, compared with severity of illness. Interestingly, recent data suggest that irrespective of HIV or nutritional status, children presenting with certain cytokine profiles affecting neutrophil recruitment were more likely to die (177), highlighting additional host factors besides premorbid conditions.

Mortality has nonetheless decreased significantly over the time period: this appears to be primarily due to the introduction of antiretroviral therapy (ART) in 2004 (10). This trend is particularly clear for iNTS disease and has been described by other authors in association with ART as an intervention for HIV-associated bacteraemias (147). This supports the critical role of ART in Africa, in controlling mortality due to HIV.

5.3. Microbiological characteristics

There was no significant difference between *Shigella* serotype and association with HIV infection or *Shigella* serotype and mortality: rather the predominant *Shigella* serotypes appeared to reflect those that are currently circulating in South Africa (58-64). Conversely, specific *Salmonella* serovars, and in particular *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium) ST313, was associated with HIV infection. Given the well-described evolution of this latter pathogen in Africa, in association with HIV (115;116;148), the finding that this was significantly associated with HIV-infected patients with iNTS meningitis is unsurprising. Although sequence typing was not undertaken for those isolates that caused iNTS bacteraemia in Gauteng Province, these data strongly suggest that this sequence type is responsible for the majority of HIV-associated infections here. *Salmonella* Enteritidis appears to be associated primarily with HIV-uninfected individuals and has a lower case fatality ratio compared with *Salmonella* Typhimurium, but overall incidence rate is increasing.

The incidence of invasive nontyphoidal salmonellosis is almost 40 times the incidence of invasive shigellosis: this may be due to intrinsic differences in the way these pathogens invade the gut epithelium. Specifically, *Salmonella enterica* has adapted to invading macrophages, benefitting from the additionally protective phagolysosome (117), compared with *Shigella*, which survive in the cytosol (97) (see section 1). Additionally, however, *Salmonella* appear to have a unique ability to evolve to favour causing invasive disease: *Salmonella* Typhi diverged from other *Salmonella* serovars through the loss of primarily protein-encoding gene function as well as horizontal acquisition of several *Salmonella* pathogenicity islands (178). A similar evolutionary tale has been described for the host

adaptation of *Salmonella* Typhimurium ST 313 (115;116;148) and new evidence suggests that a lineage of *Salmonella* Enteritidis is evolving to cause infection in humans in Africa through the loss of selected areas of the genome as well (179). Whether this *Salmonella* Enteritidis clade has spread from central and East Africa to South Africa is unknown, thus whether it has a role in the increasing incidence of invasive *Salmonella* Enteritidis infection between 2003 and 2013 cannot yet be confirmed.

5.4. The role of antiretroviral treatment

While the critical role of ART in decreasing incidence rates of iNTS is well-recognised and undeniable, data on the effectiveness of this intervention in South Africa are difficult to obtain. A partial publication by the National Department of Health (NDoH) referred to ART uptake in adults only (168). Otherwise, publications from South Africa (180) have relied on estimates of the Actuarial Society of South Africa (167). To ascertain whether iNTS incidence had decreased with the introduction of ART in 2004, this work utilised a previously evaluated methodology (181). Viral load tests in HIV-infected patients attending HIV clinics around Gauteng served as a proxy for the numbers of patients accessing ART. The highly correlated significant effect the ART programme has had on iNTS incidence rates, specifically invasive *Salmonella* Typhimurium, in adults aged between 25 and 49 years, is remarkable and testimony to the programme's success in Gauteng Province and certainly confirms the programme is working under the more optimal conditions of South Africa's wealthiest province (182). Whether the programme has been as successful in other provinces remains to be elucidated: these data have not been comprehensively analysed.

The year 2006 appears to have been a watershed year for the ART programme, with both declining mortality in typhoid fever and iNTS and changing patterns in incidence rates. The

ratio of adult women to men presenting with iNTS in Gauteng, reversed between the early (2003 to 2005) and later (2006 to 2013) years of ART, greater numbers of women were observed in the former period. This may be ascribed to better health care utilisation in women (157). Both groups appeared to have a comparably good correlation between ART access and declining iNTS infection, however, indicating the success of ART as an intervention to decrease iNTS incidence rates.

Where questions remain, these are around the contradictory results, indicating that invasive *Salmonella* Enteritidis has increased as *Salmonella* Typhimurium has decreased, despite the introduction of ART. It is possible that the human-adapted *Salmonella* Enteritidis strain described by Feasey et al (179), migrated into South Africa over the past few years, but additional complexities, such as food insecurity in a country that is becoming poorer, may also be contributory (183). Whatever the reasons, further work is mandatory in order to combat a new scourge and identify and implement appropriate interventions.

Despite the introduction of a highly effective method to decrease opportunistic enteric infection in HIV, it is estimated that the disease still has a high cost of treatment averaging ZAR27,000 (USD1,800) per admission, primarily due to patients who were not on ART (158). In Kwazulu-Natal, high ART coverage (30 to 40% of all HIV-infected individuals receiving ART) is associated with HIV-uninfected individuals being nearly 40% less likely to acquire HIV, than someone living in a community where ART coverage was low (<10% of all HIV-infected individuals receiving ART) (184). As this work specifically examined the decrease in iNTS in association with the number of people accessing ART in Gauteng, it is fair to conclude that ART is highly successful as an intervention for decreasing opportunistic

infection, but in those patients failing or unable to access ART programmes, the risk of succumbing to an opportunistic infection remains real.

5.5 Public health relevance of these data

This work has shown that HIV is intrinsically associated with invasive enteric infections, more specifically, invasive shigellosis and salmonellosis. More specifically it appears that invasive enteric pathogens, whether transmitted person-to-person, or acquired through exposure to contaminated food and water, have a considerable burden on morbidity and mortality in association with HIV. Figure 16 summarises the hypotheses and results of this thesis and highlights a number of achievable interventions. These would include optimizing antenatal screening protocols and ensuring that HIV-infected mothers obtain perinatal antiretrovirals, to ensure that the foetus is protected, and should additionally perinatal antimicrobials be considered, for those pregnant mothers who may be carrying *Salmonella* or *Shigella* in their gut. Additionally, this study has provided additional evidence that a treatment lag in HIV-infected adult males may be contributing to higher burdens of invasive *Salmonella* infections in the later years of the surveillance, supporting calls for additional HIV testing and improving access to HIV-related care among this population (157). New vaccines are under development for both *Shigella* (153) and nontyphoidal *Salmonella* (185), and once these become available, these may play a role in preventing person-to-person transmission, as well as protecting patients in the event of food or waterborne disease.

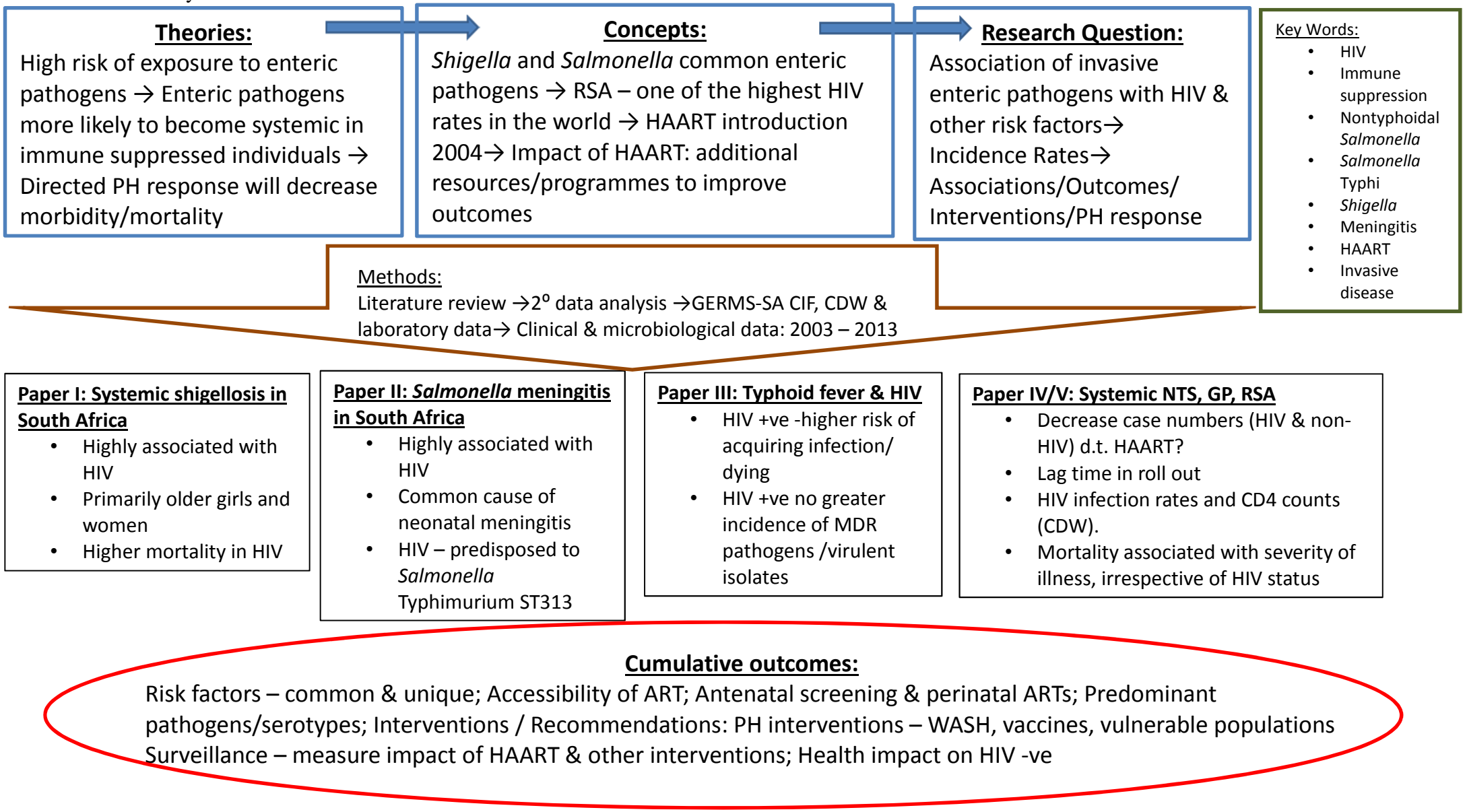


Figure 16. Final conceptual framework integrating findings from thesis.

In addition, patient education on the value of accessing and remaining in these programmes are an important part of management. Further education programmes would include advising on the importance of "WaSH" - water, sanitation and hygiene, which has previously been shown to be valuable in preventing infections in HIV-infected patients (186). Given that most new infections in South Africa appear to be in young women (187), WaSH would be a critical intervention, particular for decreasing invasive shigellosis.

Although this thesis was primarily examining the association between invasive enteric infections and HIV, it has highlighted a number of vulnerable HIV-uninfected patients that would need to be managed as well. Specifically, childhood nutrition programmes, management of diseases including malignancy or organ failure and the elderly may all be included in interventions to decrease disease.

Given the exceptionally strong association between increasing ART usage and decreasing iNTS incidence, particularly with reference to *Salmonella* Typhimurium, this study makes a strong argument for utilising data on new iNTS infections as an indicator of how successful the ART programme will continue to be. As iNTS infection is an acute disease, changing incidence rates will provide better indicator data than chronic infections such as tuberculosis (180) or cryptococcosis (188).

Lastly, this work has described an evolving epidemic due to HIV in association with invasive enteric infections using laboratory-based surveillance data. This has provided powerful arguments for the extended value of the PEPFAR programme (161;162), which provided

additional value in developing the laboratory-based surveillance system for enteric pathogens. It is a testament to a farsighted and generous international public health programme and has argued strongly for a multifaceted approach in detailing and understanding challenges in public health, to provide viable answers and solutions to questions that such challenges may raise.

6. Limitations

Despite this being one of the larger studies of its kind conducted, there were a number of limitations in this thesis. Inadequate data, particularly pertaining to HIV status was collected for the studies that included the smaller data sets (Paper I: systemic shigellosis; Paper II: *Salmonella* meningitis; Paper III: Typhoid fever), which prevented a complete analysis of the association of HIV status in susceptibility to and mortality due to these diseases. As this study was conducted at sentinel sites, it is also possible that there may have been a selection bias in these patients. The sentinel sites in this study are all tertiary level hospitals, and most are academic centres as well: it is possible that patients who are aware that they are HIV-infected may preferentially refer themselves to such hospitals, or are already in HIV treatment programmes at these institutions.

This may particularly be true in the case of the excessive numbers of adult women presenting with typhoid fever: women are preferentially tested for HIV infection, through the antenatal screening programmes in South Africa (175). Moreover, adult women, knowing their HIV status, may preferentially seek medical care at tertiary care facilities, thereby exhibiting Berkson's bias (189). Low numbers of HIV results in this group of patients in particular make Berkson's bias difficult to exclude. There is in addition the possibility that Berkson's bias operated in reverse: once a clinician realised that a patient had typhoid fever, signs and symptoms would have been ascribed to this disease alone and the patient may not have been tested for HIV. This is borne out by the proportion of typhoid fever patients who still had unknown HIV status following admission, compared with those presenting with iNTS.

Given the large numbers of patients included in the study on iNTS in Gauteng, which represented approximately half of the iNTS cases identified in South Africa during the study

period, and the consistency of the major findings, that HIV, disease severity and older age (adult patients) impact mortality, Berkson's bias would have been less likely to have had a major impact or significantly alter the findings with regard to invasive nontyphoidal salmonellosis. Moreover, as the study patients were included based on a positive culture result from a normally sterile body site for invasive enteric infections, following which surveillance officers were requested to perform HIV testing in all patients for whom HIV status was unknown, this study did attempt to mediate the effect of Berkson's bias through promoting testing of all patients who were enrolled.

Other limitations include the fact that only a very small selection of the *Salmonella* Typhimurium received were sequence types, and only for those patients presenting with *Salmonella* meningitis. This work therefore cannot confirm that *Salmonella* Typhimurium ST313 had the same significant association with HIV in South Africa as it does in other African countries (116). Given the international data regarding the prevalence of this pathogens in association with HIV on the African continent and it's prevalence in causing *Salmonella* meningitis in South African HIV-infected patients, it is reasonable to suppose that most of the *Salmonella* Typhimurium isolates we observed were *Salmonella* Typhimurium ST313. More detailed information on potential limitations can be found in each of the five papers.

7. Conclusions

This study initially started as a project to examine the prevalence of trimethoprim-sulphamethoxazole resistance in HIV-infected patients, before it was even thought that antiretroviral treatment (ART) programmes would be introduced into South Africa. When these were phased in from 2004, this provided an extraordinary opportunity to evaluate a large data set over a longitudinal period, both to describe the incidence of invasive enteric infections as well as to evaluate the response to ART. The work has highlighted that much needs yet to be done: besides the public health interventions suggested above, answers need to be sought for critical questions:

- Invasive shigellosis appeared to decrease between 2003 and 2009, but updated information reviewed after the publication of Paper I suggests that it may be plateauing or on the rise: further information is required to understand these trends.
- Better information is required as to why certain patients succumb to *Salmonella* meningitis, particularly to define if there is any associated infection such as cryptococcal disease or tuberculous meningitis; this may additionally need to be tied into potential predisposing host factors, such as genetic predisposition to invasive *Salmonella* disease (190).
- The association between HIV and typhoid fever remains problematic: low patient numbers meant that a conclusive result could not be obtained in this work and further surveillance is necessary to define whether HIV is in fact associated with higher disease rates.
- The increase in the incidence of invasive *Salmonella* Enteritidis needs to be understood: whether this has been sustained since 2013, what the patient exposures are and what interventions can be done to counteract this increase.

- Access to ART as an intervention is problematic in certain groups, primarily adult men and potentially due to poorer health care utilisation: ongoing surveillance for iNTS will alert to defects in the ART programme and permit remedial action.

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Appendix I: Original papers

I. Systemic shigellosis in South Africa.

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For this publication, the student was responsible for development of the study, cleaning and analysis of the surveillance data and the primary draft and final submission of the manuscript. The student is first author on this manuscript.

MAJOR ARTICLE

Systemic Shigellosis in South Africa

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Background. Systemic disease due to shigellae is associated with human immunodeficiency virus (HIV), malnutrition, and other immunosuppressed states. We examined the clinical and microbiologic characteristics of systemic shigellosis in South Africa, where rates of HIV infection are high.

Methods. From 2003 to 2009, 429 cases of invasive shigellosis were identified through national laboratory-based surveillance. At selected sites, additional information was captured on HIV serostatus and outcome. Isolates were serotyped and antimicrobial susceptibility testing performed.

Results. Most cases of systemic shigellosis were diagnosed on blood culture (408 of 429 cases; 95%). HIV prevalence was 67% (80 of 120 cases), highest in patients aged 5–54 years, and higher among females (55 of 70 cases; 79%) compared with males (25 of 48 cases; 52%; $P = .002$). HIV-infected people were 4.1 times more likely to die than HIV-uninfected cases (case-fatality ratio, 29 of 78 HIV-infected people [37%] vs 5 of 40 HIV-uninfected people [13%]; $P = .008$; 95% confidence interval [CI], 1.5–11.8). The commonest serotype was *Shigella flexneri* 2a (89 of 292 serotypes [30.5%]). Pentavalent resistance occurred in 120 of 292 isolates (41.1%). There was no difference in multidrug resistance between HIV-infected patients (33 of 71 [46%]) and uninfected patients (12 of 33 [36%]; 95% CI, .65–3.55).

Conclusions. Systemic shigellosis is associated with HIV-infected patients, primarily in older girls and women, potentially due to the burden of caring for sick children in the home; interventions need to be targeted here. Death rates are higher in HIV-infected versus uninfected individuals.

Shigella are among the most ubiquitous of enteric pathogens and are a major cause of bacillary dysentery worldwide [1, 2]. *Shigella flexneri* serotypes are particularly common in the developing world, although changing patterns in serotype prevalence over prolonged periods of surveillance have been observed in some settings [3, 4]. Nearly 70% of episodes and 60% of deaths involve children under the age of 5 years. Infectious dose is low, and although the disease is primarily waterborne, fecal-oral transmission is an important route for acquisition of disease [2]. The organisms are highly adapted to mucosal invasion in the human host and have been associated with systemic disease [1]. Reports of resistance, including to the fluoroquinolones, are becoming more frequent [2, 5].

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Meta-analysis of published reports from Africa suggests that <1% of cause-specific bacteremias on the continent are due to *Shigella* [6]. *Shigella* bacteremia and systemic disease are nonetheless recognized as a problem in children, neonates being at an increased risk [7–14]. Since the advent of human immunodeficiency virus (HIV), cases in adults are increasingly recognized [7, 15–23]. Inadequate host immunity appears to play an important role in the development of systemic shigellosis, and factors besides HIV that have a role in development of disease include malnutrition in children, diabetes, and malignancy [9, 12, 13, 15, 24].

There are few reports in the literature that examine systemic shigellosis, and much of the knowledge is gleaned from case reports rather than analyses of series of patients [10, 13, 19, 21, 23, 25–30]. We aim to describe the epidemiology, clinical features, and the microbiology of patients with systemic shigellosis from 2003 through 2009 in South Africa, a country with an emerging economy and where seroprevalence rates for HIV are high, and the antenatal HIV prevalence among pregnant women in 2008 was 29.3% in women between 15 and 49 years of age [31].

METHODS

Case Definition

National active laboratory-based surveillance for invasive shigellosis has been performed by the Enteric Diseases Reference Unit (EDRU) of the National Institute for Communicable Diseases in South Africa since 2003, as described elsewhere [32, 33]. A case is defined as any patient from whom *Shigella* species are isolated from a normally sterile site such as blood and cerebrospinal fluid (CSF). All diagnostic microbiology laboratories in South Africa are encouraged to submit isolates from patients fulfilling the case definition to EDRU, and audits are conducted wherever possible to identify missing cases, which are then included in the database. Audits could not be conducted in KwaZulu-Natal or for all laboratories in the remaining provinces in 2003 and 2004, because not all laboratories were on a common laboratory information system. Additional clinical information is collected on cases at 24 sentinel hospitals in all 9 provinces, through patient interview, interviewing relatives, or bed letter review. This includes data on HIV status (HIV enzyme-linked immunosorbent assay results in older children and adults; HIV polymerase chain reaction [PCR] results in infants), other immunosuppressive conditions, admission and discharge dates, antibiotic exposure, and disease outcome. Outcome data (whether the patient survived hospitalization or died) were recorded on follow-up of the current hospitalization; no long-term follow-up was undertaken. Severity of acute illness was assessed by using the Pitt bacteremia score, a previously validated scoring system that is based on mental status, vital signs, requirement for mechanical ventilation, and recent cardiac arrest [34–36].

Laboratory Characterization

All *Shigella* isolates received are serotyped by the reference unit according to established methods (Mast Diagnostics, Bootie, England). Minimum inhibitory concentrations were determined for the following antimicrobials: ampicillin, amoxicillin-clavulanate, chloramphenicol, trimethoprim, sulfamethoxazole, co-trimoxazole, tetracycline, streptomycin, kanamycin, nalidixic acid, ciprofloxacin, ceftriaxone, and ceftazidime, using E test strips, according to the manufacturers instructions (AB-Biodisk, Solna, Sweden). Production of extended spectrum β-lactamase is tested for using the MAST laboratories double disk method, according to the manufacturer's instructions (MAST Diagnostics, Bootie, England).

Statistical Analysis

Univariate analysis was performed using the χ^2, Fisher exact, or Mantel-Haenszel test for comparison of categorical variables. The χ^2 test for trend was used to examine the number of isolates received year-on-year. Analysis was performed with Epi Info software, version 6.04d [37] and Stata software, version 9 (StataCorp Ltd, College Station, TX). Two-sided *P* values of <.05 were considered significant throughout.

RESULTS

All Cases

From January 2003 through December 2009, we identified 429 laboratory-confirmed cases of invasive shigellosis, for whom data on age were available for 387, and 292 were available to the reference unit for serotyping and susceptibility testing. Including audit cases, blood cultures were positive for *Shigella* for 408 of 429 patients (95%), the remaining patients had *Shigella* isolated from CSF (4 of 429 patients [1%]) or other sterile sites, including tissue biopsy or pleural fluid (17 of 429 patients [4%]). Eight patients had positive fecal cultures for *Shigella* in addition to the isolate from a sterile site.

The incidence of invasive *Shigella* increased from 0.11 to 0.13 per 100 000 population from 2005 through 2009, peaking in 2006 at 0.18 ($P = .935$). Data from 2003 to 2004 were excluded from this analysis as laboratory audits could not be done for these years. The incidence was highest in children younger than 1 year of age followed by children aged 1–4 years, with a second peak in persons aged 15–54 years (Figure 1). Patient ages ranged from 1 day to 84 years (median, 6 years), and 227 of 412 (55%) of patients for whom sex was recorded were female (sex was not recorded for 17 patients).

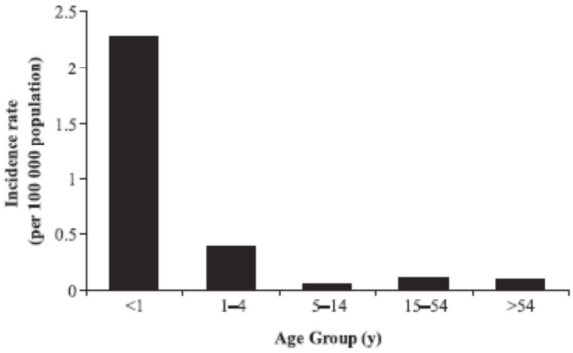

Figure 1. Incidence rates of all cases of invasive *Shigella* in South Africa in 2006 by age group (each year followed the same distribution).

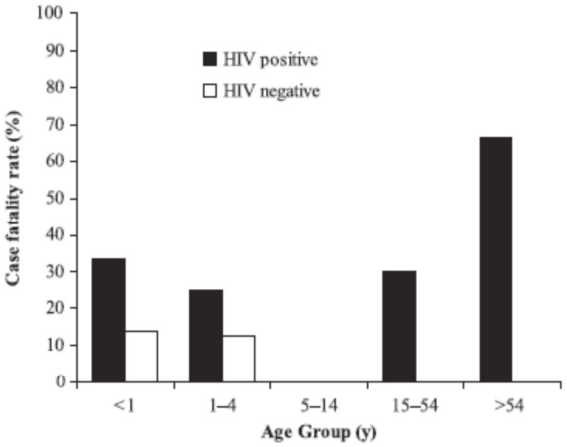

Figure 2. Case fatality rate of patients from sentinel sites with invasive shigellosis in South Africa by age group.

Clinical and Epidemiologic Features at Enhanced Surveillance Sites

HIV status was established for 120 of 210 patients (57%) admitted at enhanced or sentinel surveillance sites; 80 of these patients (67%) were HIV infected. The HIV prevalence among cases at sentinel sites was highest in patients aged 5–54 years and was significantly higher (55 of 70 cases [79%]) among females than males (25 of 48 cases [52%]; $P = .002$). HIV status was unknown in 38 of 86 (44%) of females and 48 of 86 (56%) of males at enhanced surveillance sites. There was no significant increase in HIV prevalence among tested patients by year (2003: 2 of 3 [67%]; 2004: 8 of 13 [62%]; 2005: 8 of 12 [67%]; 2006: 11 of 17 [65%]; 2007: 20 of 24 [83%]; 2008: 13 of 21 [62%]; 2009: 18 of 30 [60%]; $P = .602$). Other immunosuppressive states identified at sentinel sites that could have contributed to invasive shigellosis included kwashiorkor (protein energy malnutrition) in 19 children between birth and 5 years (6 of whom were HIV infected), diabetes mellitus (n = 2), renal impairment (n = 3), and malignancy (n = 1; adult patient). Presenting diagnoses at sentinel sites included diarrhea or dysentery (42 of 210 [20.0%]), bacteremia (122 of 210 [58.0%]), lower respiratory tract infection (34 of 210 [16.0%]), meningitis (3 of 210 [1.4%]) and other diagnoses (2 of 210 [1.0%]), with 7 of 210 diagnoses (3.0%) unknown.

Information on the length of hospital stay was available for 169 of 210 patients (80%). Time in hospital ranged from 0 to 106 days, with a median of 7 days. Fifty of 172 patients (29%) died; 50% of deaths occurred within the first 3 days of treatment, the remainder during the current hospitalization. One hundred twenty patients (70%) recovered or were transferred to chronic care/step-down facilities, and 2 patients (1%) refused hospital care. In children (age <15 years) the case fatality rate was 17% (15 of 86). Twenty-eight percent (5 of 18) of the HIV-infected children <5 years died as compared with 14% (5 of 37) of the HIV-uninfected children <5 years ($P = .236$) (Figure 2). Although protein energy malnutrition was associated with death in 4 of 5 HIV-uninfected children (80%), no contributing factor was recorded for 1 of 5 children (20%). There were no deaths in HIV-uninfected adults (0 of 2), but 39% (22 of 56) of HIV-infected adults died (Figure 2). Death was 3.2 times more likely to occur in adults than in children (32 of 79 adults [41%] vs 15 of 86 children [17%]; $P = .001$, 95% confidence interval [CI], 1.6–6.6). Overall, HIV-infected cases were 4.1 times more likely to die than HIV-uninfected cases (29 of 78 HIV-infected [37%] vs 5 of 40 HIV-uninfected [13%]; $P = .008$, 95% CI, 1.5–11.8) (Figure 2).

Severity of illness scores were fully recorded for a small number of patients only. Of the 135 patients with a calculated Pitt bacteremia score, 8 patients (6%) had a score ≥4, whereas the other 127 patients (94%) had a score <4. Having a Pitt bacteremia score of ≥4 was significantly associated with death (7 of 8 Pitt ≥4 [88%] vs 29 of 124 Pitt <4 [23%]; OR, 23; $P = .004$, 95% CI, 2.7–194.2). On presentation, the commonest sign of sepsis included pyrexia in 28 of 158 patients (18%), 37 of 144 patients (26%) were disorientated, and 3 of 144 patients (2%) were comatose. Mechanical ventilation was required for 8 of 158 patients (5%).

CD4 lymphocyte cell counts were available for 33 of 80 HIV-infected patients (41%), 9 (27%) of whom were children (<15 years); all children were 6 years of age or younger. Eight (89%) of these children had CD4 counts ≤750, and one (11%) had a CD4 count >1500. Of the adults, 18 (70%) had CD4 counts ≤50, 4 (15%) had CD4 counts between 51 and 200, and 4 (15%) had CD4 counts ≥350. One death

occurred in an infant in whom the CD4 count was >350, the remaining patients survived. Data on access to co-trimoxazole prophylaxis was obtained for 125 patients, irrespective of HIV status. Of the 80 patients in whom HIV status was positive, 18 (23%) were on co-trimoxazole prophylaxis. Only 15 patients (10%) were receiving highly active antiretroviral therapy; 13 were <10 years of age.

Microbiological Findings

The numbers of isolates received increased from 11 in 2003 to 39 in 2005 and 54 isolates in 2009. In total, 292 viable isolates (68%) were received from 429 cases. The most common *Shigella* serotype isolated from invasive cases was *S. flexneri* 2a (30%), followed by *S. flexneri* 1b (16%) (Table 1). Pentavalent resistance occurred in 120 of 292 isolates (41%). Of the *S. flexneri* 2a isolates, 48 isolates (54%) were resistant to ≥5 antimicrobials and 2 isolates (2.3%) produced extended spectrum β-lactamase (ESBL; Table 1). Thirty-seven isolates (77%) of *S. flexneri* 1b were resistant to ≥5 antimicrobials, and 1 isolate (2.1%) was an ESBL producer. Pentavalent resistance was less common in the remaining serotypes, and only one other isolate had ESBL production (*S. sonnei* phase II). Resistance to the fluoroquinolone antibiotics in invasive *Shigella* was not documented, although nalidixic acid resistance occurred in 4 isolates (Table 1). No difference was noted in the occurrence of multidrug-resistant isolates between HIV-infected (33 of 71; 46%) and -uninfected patients (12 of 33 patients [36%]; $P = .334$; 95% CI, .65–3.55).

DISCUSSION

Invasive shigellosis is an uncommon complication of gastrointestinal disease. Previous studies have highlighted the predominance of the disease in children, as well as the role of protein energy malnutrition in children [12, 14, 27, 30, 38] and the association of the disease with HIV infection in adults [7, 19–21, 30, 39, 40]. There are rare reports of invasive shigellosis occurring in immunocompetent individuals [30, 41, 42]. Cell-mediated immunity has been postulated as being important in prevention of disease, and this is supported by current literature [7, 12, 16, 17, 19, 22, 26, 27, 43], but despite apparently high mortality, invasive shigellosis is infrequently reported from Africa [7, 14, 44]. We collected clinical and microbiological data on 279 patients who were treated around South Africa over an 8-year period, to better understand the features of this disease and compare the disease between HIV-infected and HIV-uninfected patients. Our study has confirmed previous findings and emphasizes the importance of this disease in older girls and women, as well as highlighting the higher death rates in HIV-infected versus HIV-uninfected patients.

Table 1. Antimicrobial Resistance Patterns in Major *Shigella* Serotypes Associated With Invasive Shigellosis

Serotype	Ampicillin	Chloramphenicol	Streptomycin	Sulfamethoxazole	Trimethoprim	Tetracycline	Nalidixic Acid	Ciprofloxacin	ESBL Production
S. flexneri type 2a (n = 89)	59 (66.3)	42 (47.2)	62 (69.7)	81 (91.0)	84 (94.4)	54 (60.7)	1 (1.1)	0 (0)	2 (2.3)
S. flexneri type 1b (n = 48)	41 (85.4)	37 (77.1)	41 (85.4)	36 (75.0)	48 (100)	43 (89.6)	0 (0)	0 (0)	1 (2.1)
S. sonnei phase I/II (n = 45)	6 (13.3)	1 (2.2)	38 (84.4)	42 (93.3)	44 (97.8)	32 (71.1)	0 (0)	0 (0)	1 (2.2)
S. flexneri type 3a (n = 33)	8 (24.2)	0 (0)	5 (15.2)	33 (100)	33 (100)	4 (12.1)	1 (3.0)	0 (0)	0 (0)
S. flexneri type 6 (n = 14)	6 (42.9)	2 (14.3)	5 (35.7)	14 (100)	14 (100)	2 (14.3)	0 (0)	0 (0)	0 (0)
S. flexneri variant X (n = 17)	11 (64.7)	5 (29.4)	8 (47.1)	16 (94.1)	17 (100)	11 (64.7)	0 (0)	0 (0)	0 (0)
S. flexneri variant Y (n = 9)	4 (44.4)	2 (22.2)	5 (55.6)	8 (88.9)	8 (88.9)	4 (44.4)	0 (0)	0 (0)	0 (0)
S. flexneri type 3b (n = 8)	4 (50.0)	2 (25.0)	5 (62.5)	7 (87.5)	7 (87.5)	4 (50.0)	1 (12.5)	0 (0)	0 (0)

Data are no. (%) unless otherwise indicated.

Abbreviation: ESBL, extended spectrum β-lactamase production.

In this study, invasive disease was primarily associated with children under the age of 5 years, and adults aged from 15 to 54 years. This supports, in part, others' findings of disease prominence in malnourished and immunosuppressed children [7–11, 28, 38], as well as the role of HIV in adults [7, 16, 22, 23, 27, 30, 39, 40, 45], and mirrors the age-related distribution of HIV in South Africa. It emphasizes the importance of HIV status in affecting childhood mortality, as deaths in HIV-infected versus uninfected children <15 years of age were nearly doubled. The increased numbers identified over the 8-year period may potentially reflect improved collection practices by GERMS-SA rather than increasing numbers of cases of systemic shigellosis, due to challenges in conducting full audits in all provinces between 2003 and 2009. In those children aged <1 year who tested negative by PCR for HIV, we did not collect data on HIV exposure; that is, whether the mother was HIV infected but the child did not seroconvert. Proxy data, such as the use of perinatal nevirapine, were also not available for this subset of patients.

The strong correlation of HIV with invasive shigellosis in patients aged >15 years was expected, but it is notable that the disease predominated in this age group in South African patients, and 98% of patients identified in this study in this age group were HIV infected. These patients were frequently severely immunosuppressed, as evidenced by low CD4 counts. No other immunosuppressive condition, as previously described, was individually associated with invasive shigellosis, supporting the observation that it is a rare disease in adults, in the absence of HIV, even given the prevalence of other immunosuppressive conditions such as diabetes, malignancy, and renal failure [27, 30, 46]. We did not attempt to extrapolate the HIV data to those patients for whom the HIV status was unknown, as the numbers were small and there may have been selection bias among the patients who had blood cultures taken. Nonetheless, these data do emphasize the strong association between HIV and invasive shigellosis.

In common with other literature reports, most patients presented with clinical sepsis, which frequently followed or was associated with a history of gastroenteritis. There were no outstanding clinical features that would otherwise have given an indication of the bacteriological diagnosis. The Pitt bacteremia score of ≥4 was significantly associated with death. Previous authors have discussed the relevance of this clinical marker in association with mortality [34–36], and we found it to be a useful predictor for mortality in this group of patients.

The predominance of older women who were HIV infected with systemic shigellosis is noteworthy and may reflect the role of women in child-care practices in South Africa. The predominance of women who were HIV infected with invasive shigellosis in this study (79%) was greater than published figures for rates of HIV-infected women in South Africa [47]. Women may be more likely to be exposed to gastrointestinal pathogens that the child may carry, and in the presence of immunosuppression may hence be more prone to developing invasive disease. Moreover, although numbers are small, invasive shigellosis occurred in some HIV-uninfected elderly women, but there was only one case reported from an HIV-infected man over 54 years of age, supporting the argument for the burden of child care. This finding highlights a number of imperatives. First, as part of patient education in HIV clinics, interventions such as handwashing, which has been shown to be protective in preventing diarrhea, should be stressed, given the association in our patients with gastrointestinal disease [48]. Second, as almost no adults were receiving antiretrovirals in this study, it argues strongly that antiretrovirals should become more accessible to the South African population in need [49, 50].

It seems unlikely that serotype plays a role in invasion, as serotype distribution corresponds with the national data, which primarily reflect the serotype distribution in noninvasive shigellosis [32, 33]. Similarly, the occurrence of antimicrobial resistance is comparable to reports from other countries [51–54] and is unlikely to be related to the HIV seroprevalence in South Africa, but rather to antimicrobial usage patterns in the country, given that there was no significant difference in multidrug resistance between HIV-infected patients and HIV-uninfected patients with invasive disease. We are currently characterizing the ESBL produced by certain isolates [55], but postulate that these enzymes may have been acquired due to the number of patients who spend extended periods in the hospital environment, in the association with HIV and other immunosuppressive conditions. Although the serotypes associated with shigellosis in South Africa are well characterized, prevention of enteric infection through vaccination is not a consideration for the near future [2, 51, 56], and hence systemic infection in the immunosuppressed individual is likely to remain a problem in the absence of improved sanitation.

This study had a number of limitations. First, clinical data were collected at selected sites only and may not be relevant to all the cases. Second, clinical data may have been incomplete, where surveillance officers could not access the patient and relied on family members or patients' bed letters to complete the case report forms. Not all patients at enhanced sites had HIV results. It is possible that had these results been available, the differences in HIV infection between the sexes would have been less marked, but as the ratio of females to males in this group was 0.8, we believe this would not have seriously affected our results. As cases were based on a laboratory surveillance system rather than a bacteremia study, this study had limited influence on clinical

management and patients therefore may not have had comprehensive access to laboratory diagnoses, such as having stool samples taken. This may also have influenced the numbers of cases in whom pyrexia was recorded or those who had dysentery symptoms.

In conclusion, we found that systemic shigellosis is primarily associated with HIV-infected patients in South Africa in all age groups, although other immune compromising features may contribute in children. Disease in adults particularly affects women of child-bearing age, who probably have the larger burden of caring for sick children in the home, and interventions need to be targeted at this group.

Notes

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II. Clinical and microbiological features of *Salmonella* meningitis in a South African population, 2003 – 2013.

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SUPPLEMENT ARTICLE

Clinical and Microbiological Features of *Salmonella* Meningitis in a South African Population, 2003–2013

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Background. The clinical and microbiological characteristics of nontyphoidal *Salmonella* (NTS) meningitis in South Africa, where human immunodeficiency virus (HIV) prevalence is high (approximately 15% in persons ≥15 years of age), were reviewed.

Methods. From 2003 through 2013, 278 cases were identified through national laboratory-based surveillance. Clinical information (age, sex, outcome, Glasgow Coma Scale [GCS], and HIV status) was ascertained at selected sites. Isolates were serotyped; susceptibility testing and multilocus sequence typing on *Salmonella enterica* serovar Typhimurium isolates was performed. Multivariable logistic regression was used to determine factors associated with mortality outcome, using Stata software, version 13.

Results. Where age was ascertained, 139 of 256 (54.3%) patients were <15 years. Males represented 151 of 267 (56.6%). Mortality outcome was recorded for 112 of 146 (76.7%) enhanced surveillance patients; 53 of 112 (47.3%) died. Death was associated with GCS ≤13 (adjusted odds ratio [OR], 18.7; 95% confidence interval [CI], 3.0–118.5; $P = .002$) on multivariable analysis. Where data were available, all 45 patients aged >15 years were HIV infected, compared with 24 of 46 (52.2%) patients aged <5 years. Neonates were less likely to be HIV infected than infants aged 2–12 months (OR, 4.8; 95% CI, 1.1–21.1; $P = .039$).

Salmonella Typhimurium represented 106 of 238 (44.5%) serotyped isolates: 65 of 95 (68.4%) were ST313 vs ST19, respectively, and significantly associated with HIV-infected patients ($P = .03$) and multidrug resistance (OR, 6.6; 95% CI, 2.5–17.2; $P < .001$).

Conclusions. NTS meningitis in South Africa is highly associated with HIV in adults, with neonates (irrespective of HIV status), and with *Salmonella* Typhimurium ST313. GCS is the best predictor of mortality: early diagnosis and treatment are critical. Focused prevention requires further studies to understand the sources and transmission routes.

Keywords. *Salmonella*; meningitis; HIV; *Salmonella* Typhimurium ST313.

Bacterial meningitis in Africa remains an important disease with a high associated mortality [1–7]. Meningitis in human immunodeficiency virus (HIV)–infected persons is frequently associated with cryptococcosis, tuberculosis, and *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib), and *Neisseria meningitidis* [3, 4, 8–12]. Numerous other pathogens have been described as a cause of meningitis among HIV-infected persons [1, 7, 9, 12, 13]. Nontyphoidal *Salmonella* (NTS) is emerging as a significant meningeal pathogen among HIV-infected persons, following the decline in incidence of *S. pneumoniae* and Hib with the introduction

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of pneumococcal conjugate and Hib conjugate vaccines [1, 6, 9, 12–14]. In previous series, in adults from South African institutions, NTS meningitis represented 16% of acute bacterial meningitis cases among HIV-infected patients [7]. An estimated 8% of all meningitis cases for which a microbiological diagnosis was made was attributed to acute bacterial meningitis [15]. NTS meningitis was not reported in pediatric series [16, 17].

The association between HIV and invasive NTS infections, including meningitis, was described early in the AIDS epidemic [18]. Case reports of adults with NTS meningitis frequently describe an association with HIV [7, 18–20], although other immunosuppressive conditions have been described [21–23]. Molyneux et al described NTS meningitis in a cohort of 105 Malawian children, aged between 2 months and 16 years, over a 10-year period [2]. Approximately half were HIV infected, and 12.4% were infected with malaria; mortality rates were >50% [2]. There are rare reports of NTS meningitis in previously healthy individuals [24], suggesting that comorbidity is common, but not an absolute prerequisite to infection.

A meta-analysis of published African studies suggests that *Salmonella* bacteremia accounts for 21.4% of all bacteremias [25], with an incidence rate of 227 per 100 000 [26]. In contrast, *Salmonella* meningitis accounted for <10% of all-cause meningitis (incidence rate of 20/100 000) in Malawian patients in 2012 [6], suggesting approximately 1% of NTS bacteremias results in meningitis. *Salmonella enterica* serovars Typhimurium and Enteritidis account for 65.2% and 33.1% of all invasive NTS infections, respectively [25], and similar observations have been made regarding the frequency with which these serovars occur in NTS meningitis [2]. In the past 30 years, the emergence of invasive *Salmonella* Typhimurium ST313, a sequence type associated with the African AIDS epidemic, has been highlighted [27, 28].

We describe the clinical and microbiological data associated with a series of patients presenting with NTS meningitis in South Africa, a country that is largely malaria-free, with high HIV prevalence affecting approximately 15% of the population ≥15 years of age [29], to better understand the association with HIV, potential predisposing conditions, and the role of *Salmonella* Typhimurium ST313.

METHODS

Case Definition

National active laboratory-based surveillance for invasive salmonellosis, defined as the isolation of NTS from a normally sterile body site, including *Salmonella* meningitis, was performed by the Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases in South Africa from 2003 through 2013, as previously described [30]. A case of NTS meningitis was defined as any patient from whom NTS was isolated from cerebrospinal fluid (CSF). A nosocomial infection was defined as NTS meningitis infection in a patient in whom the diagnosis of meningitis was made ≥48 hours after the patient had been admitted to a medical or long-term-care facility. We used the annualized South African population from 2003 to 2013, which increased from 45.80 million to 52.98 million over the period, to calculate incidence (http://www.statssa.gov.za/publications).

All diagnostic microbiology laboratories in South Africa were requested to submit *Salmonella* isolates from patients fulfilling the case definition, for serotyping and susceptibility testing, supplemented by audits from 2005 to identify isolates not received by the reference laboratory. Additional clinical information was collected on cases at 24 sentinel hospitals in 9 provinces through the Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa network, a national surveillance system sharing resources to monitor diseases of public health importance. This included data on HIV status, other immunosuppressive conditions, admission and discharge dates, antibiotic exposure, timing of meningitis diagnosis (number of hours after patient admission), and disease outcome.

Laboratory Characterization

All *Salmonella* isolates received were serotyped by CED according to established protocols (Mast Group, Merseyside, United Kingdom; Bio-Rad, Marnes-la-Coquette, France; Remel, Kent, United Kingdom; Statens Serum Institut, Copenhagen, Denmark). Minimum inhibitory concentrations (MICs) were determined for the following antimicrobials: ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole (cotrimoxazole), tetracycline, ciprofloxacin, ceftriaxone, and ceftazidime, using Etest strips according to the manufacturer's instructions (bioMérieux, Marcy-l'Étoile, France). Multidrug resistance was defined as resistance to ≥3 of these antimicrobials. Production of extended-spectrum β-lactamase was tested for using the Mast Laboratories double disk method, according to the manufacturer's instructions (Mast Diagnostics, Bootie, England).

Genotypic Characterization of *Salmonella* Typhimurium

Genotyping of *Salmonella* Typhimurium isolates was performed using multilocus sequence typing (MLST), as described at the *Salmonella* MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica), including DNA sequencing analysis of the following 7 housekeeping genes: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*. DNA sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and an Applied Biosystems 3500 Genetic Analyzer. DNA sequences were collated and analyzed using the DNASTAR Lasergene (version 8.0) software (DNASTAR, Inc, Madison, Wisconsin), followed by analysis at the

Salmonella MLST database, where allele numbers and a MLST sequence type (ST) were assigned.

Ethical Approval

Ethical approval for this study was granted by the Human Research Ethics Committee of the University of the Witwatersrand (M110601).

Statistical Analysis

Variables analyzed included age, sex, HIV status, Glasgow Coma Scale (GCS), nosocomial infection, cotrimoxazole prophylaxis, use of antiretrovirals (ARVs), $CD4^+$ count, other comorbid conditions, isolation of *Salmonella* Typhimurium or multidrug-resistant *Salmonella*, and *Salmonella* sequence type. Clinically relevant groupings were created for continuous variables, to aid interpretation of results. Univariate and multivariate logistic regression were used to determine factors associated with mortality. Multivariate analysis using a manual forward stepwise progression was used, with a cutoff of $P < .1$ for the univariate analysis, dropping nonsignificant factors. Model fit was assessed by using the Hosmer–Lemeshow goodness-of-fit test. Analysis was performed using Stata version 13 (StataCorp, College Station, Texas). Two-sided P values of <.05 were considered significant throughout. Due to the nature of the study, important variables had missing data. For both univariate and multivariate analyses, a complete case analysis was conducted in which patients with missing data were excluded from the analysis.

RESULTS

Cumulative Data

Figure 1 summarizes case numbers, including relevant clinical and microbiological data pertaining to these. We identified a total of 278 cases of laboratory-confirmed NTS meningitis in South African hospitals from 2003 through 2013, representing 3.3% of all invasive salmonellosis (Figure 1). Bimodal peaks of disease incidence were observed in children <5 years of age and adults >15 years of age (Figure 2*A*). NTS was isolated from the CSF only in 196 (70.5%) patients; NTS was additionally isolated from blood, stool, or other body sites of 82 (29.5%) patients. The average incidence of NTS meningitis during the period was 5 per 10 000 000 per year. Clinical data are summarized in Table 1.

Clinical Data

Additional clinical information was available from 146 (52.5%) patients with NTS meningitis: 112 had a known outcome (76.7%) (Figure 1), and 111 (99.1%) and 82 (73.2%) had an available age and HIV status, respectively. Sex was recorded in 267 patients: 151 of 267 (56.6%) were male. Table 2 summarizes clinical risk factors for mortality. Nineteen of 58 (32.8%) children aged <15 years died, compared with 34 of 53 (64.2%) patients aged ≥15 years ($P = .001$; Table 2, Figure 2*B*). HIV status was recorded for 92 of 146 (63.0%) patients; 70 (76.1%) were HIV infected (Figure 2*C*). Among patients with recorded age and HIV status, all 45 patients aged ≥15 years were HIV infected, compared with 24 of 46 (52.2%) patients aged <5 years (Figure 2*C*). Children aged between 5 and 15 years had no outcome or HIV data recorded. Nine of 11 (81.9%) infants aged >1 year for whom data were available had a history of HIV exposure at birth.

Neonates were significantly less likely to be HIV infected than infants aged between 2 months and 1 year (3/12 [25.0%] vs 19/31 [61.3%]; odds ratio [OR], 4.8; 95% confidence interval [CI], 1.1–21.1; $P = .039$); however, there was no difference in mortality between the 2 groups (7/18 [38.9%] vs 12/38 [31.6%]; OR, 0.7; 95% CI, .2–2.3; $P = .590$). Nosocomial NTS meningitis was diagnosed in 13 of 118 (11.0%) patients for whom admission dates were available.

The length of hospital stay varied between 0 and 77 days (median of 12 days). Cases were 7 times more likely to die within the initial 10 days of their hospital stay (OR, 7.1; 95% CI, 3.1–16.4; $P < .001$). There was no significant association between nosocomial vs community-acquired infections and mortality (Table 2).

$CD4^+$ cell counts were available for 33 of 146 (22.6%) patients. A $CD4^+$ count <200 cells/μL was not significantly associated with mortality (Table 2). Whether the HIV-infected patients received ARVs was recorded for 41 patients with NTS meningitis; 10 (24.4%) received ARVs at the time of diagnosis of NTS meningitis. There was no significant effect on the mortality between HIV-infected persons on ARVs and those not on ARVs ($P = .7$; Table 2).

The GCS was recorded for 43 patients: 7 of 43 (16.3%) had a GCS ≤13. For the 35 (81.4%) patients with GCS recorded for whom HIV status was known, 9 of 10 HIV-uninfected patients had a GCS >13 (90.0%), compared with 18 of 25 (72.0%) HIV-infected patients. Multivariable analysis confirmed that mortality was associated with a GCS ≤13 (adjusted OR, 18.7; 95% CI, 3.0–118.5; $P = .002$; Table 2). The Hosmer–Lemeshow goodness-of-fit test confirmed the fitness of the model ($\chi^2 = 0.04$, $P = .8411$).

Comorbidities potentially associated with NTS meningitis were recorded in 45 of 124 (36.2%) patients. Laboratory-confirmed cryptococcal meningitis was diagnosed in 3 of 45 (6.7%); 5 of 45 (11.1%) had a history of head injury; and 29 of 45 (64.4%) were receiving therapy for tuberculosis or had a history of active tuberculosis, but the site of tuberculosis infection was not stated (ie, pulmonary vs extrapulmonary or central nervous system infection). One HIV-uninfected 3-month-old infant receiving treatment for tuberculosis had a GCS of 1. No patients were coinfected with other etiological agents of acute bacterial meningitis (*S. pneumoniae*, *N. meningitidis*, or Hib). None of the patients had malaria.

In addition, 81 of 146 (55.5%) patients had data on cotrimoxazole prophylaxis. Cotrimoxazole prophylaxis was not significantly

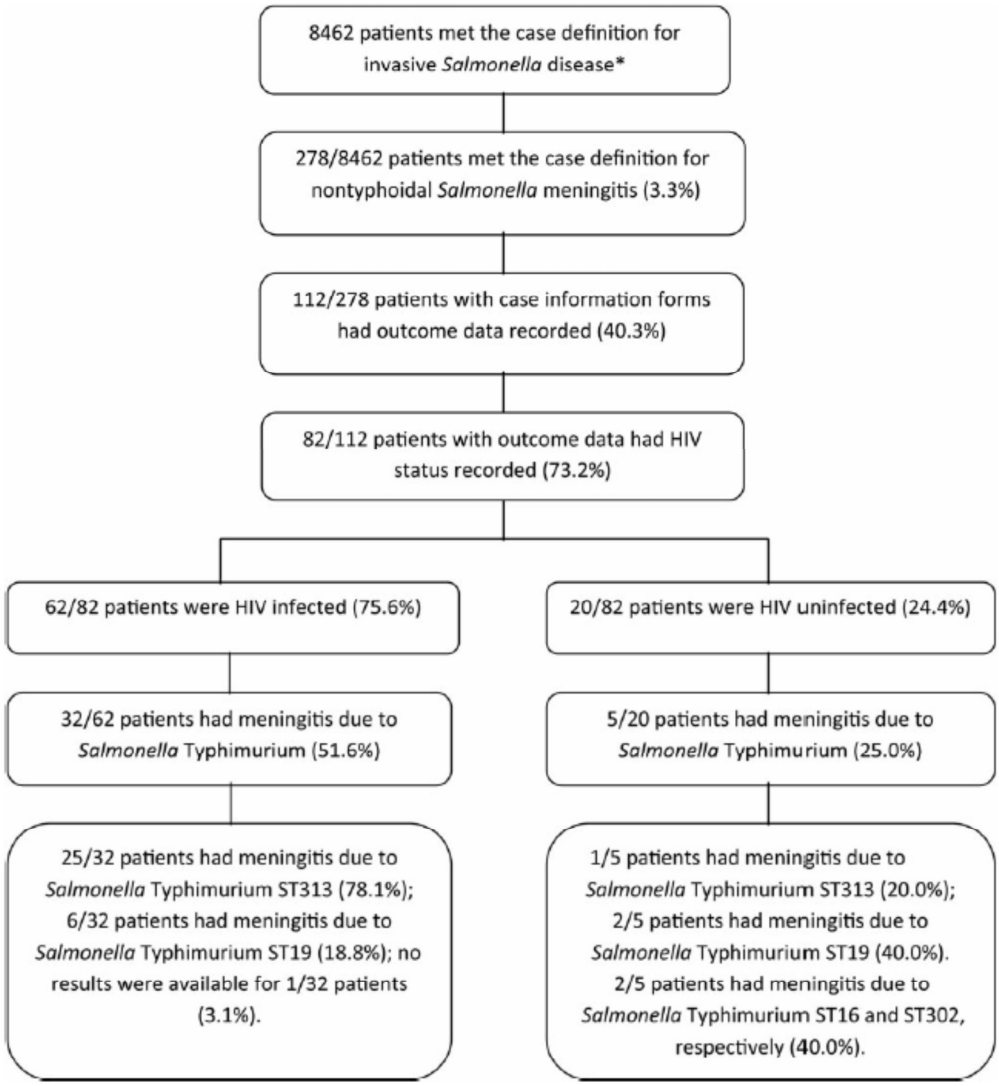

Figure 1. Patients with nontyphoidal *Salmonella* (NTS) meningitis identified in South Africa, 2003–2013. *Invasive disease was defined as the isolation of NTS from a normally sterile body site. Abbreviations: HIV, human immunodeficiency virus; ST, sequence type.

associated with outcome ($P = .3$; Table 2). Individual patient records suggested that all patients received appropriate antimicrobial therapy for NTS meningitis on admission or were changed to appropriate antimicrobial therapy once susceptibility data were available for the *Salmonella* isolated from the patients' CSF.

Microbiological Data

Microbiological characterization was undertaken on 247 of 278 (88.8%) isolates. *Salmonella* Typhimurium predominated (Table 3).

Patient outcome information was available for 111 of 247 (44.9%) cases with NTS serovar and antimicrobial susceptibility data. Patients infected with *Salmonella* Typhimurium were more likely to die compared with other serovars on univariate analysis ($P = .006$); this was not significant on multivariable analysis (Table 2). Death rates were not significantly higher in those patients who had multidrug-resistant isolates.

Where HIV status was known, 39 of 70 (55.7%) HIV-infected patients had meningitis due to *Salmonella* Typhimurium, compared with 31 of 70 (44.3%) HIV-infected patients with NTS

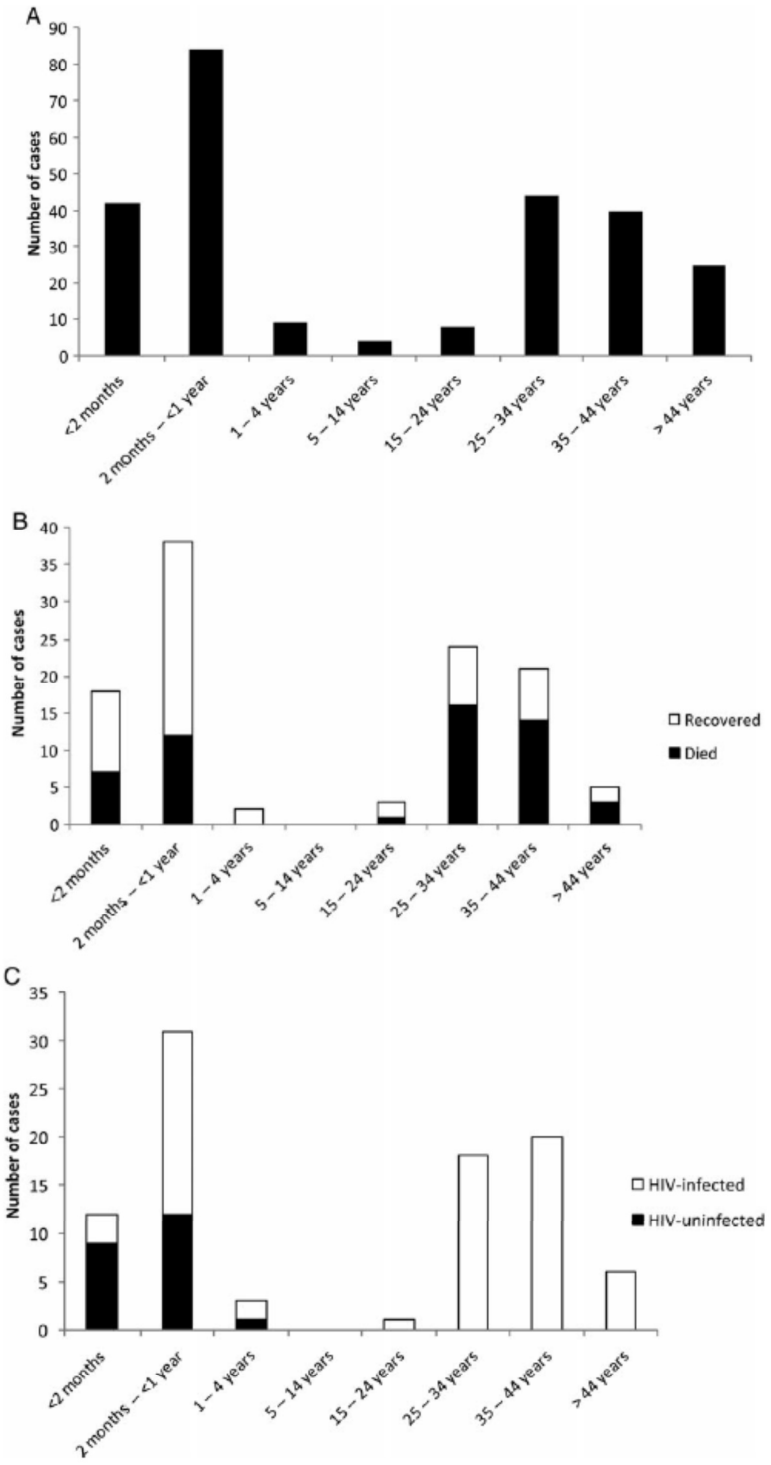

Figure 2. *A*, Number of cases of nontyphoidal *Salmonella* (NTS) meningitis (n = 256) by age group, 2003–2013. *B*, Number of cases of NTS meningitis (n = 111) by age group and mortality, 2003–2013. One patient who recovered did not have an age recorded. *C*, Number of cases of NTS meningitis (n = 91) by age group and human immunodeficiency virus (HIV) status, 2003–2013. One HIV-infected patient did not have an age recorded.

Table 1. Clinical Characteristics Associated With Patients With *Salmonella* Meningitis in South Africa, 2003–2013

Characteristic	Outcome: Survived, No. (%)	Outcome: Died, No. (%)	Total,[a] No. (%)	*P* Value
Data from all cases			278 (100)	
Age				
<2 mo	11 (26.2)	7 (16.7)	42 (16.4)	. . .
2 mo to <1 y	26 (30.9)	12 (14.3)	84 (32.8)	.5
1–4 y	2 (22.2)	0 (0.0)	9 (3.5)	.2
5–14 y	. . .	. . .	4 (1.6)	. . .
15–24 y	2 (25.0)	1 (12.5)	8 (3.1)	.9
25–34 y	8 (18.2)	16 (36.4)	44 (17.2)	.07
35–44 y	7 (17.5)	14 (35.0)	40 (15.6)	.08
>44 y	2 (8.0)	3 (12.0)	25 (9.8)	.4
Sex				
Female	20 (17.2)	26 (22.4)	116 (43.4)	. . .
Male	37 (24.5)	26 (17.2)	151 (56.6)	.1
Sentinel site data	59 (52.7)	53 (47.3)	112 (52.5)	
HIV status				
Uninfected	17 (77.3)	3 (13.6)	22 (23.9)	. . .
Infected	32 (45.7)	30 (42.9)	70 (76.1)	.008
Glasgow Coma Scale				
≤13	2 (20.0)	8 (80.0)	10 (23.3)	. . .
>13	27 (81.8)	5 (15.2)	33 (76.7)	.3
Nosocomial infection				
No	52 (51.5)	45 (44.6)	101 (88.6)	. . .
Yes	6 (46.2)	6 (46.2)	13 (11.4)	.8
Cotrimoxazole prophylaxis				
No	40 (65.6)	20 (32.8)	61 (75.3)	. . .
Yes	10 (50.0)	9 (45.0)	20 (24.7)	.3
Antiretrovirals				
No	31 (57.4)	22 (40.7)	54 (78.3)	. . .
Yes	9 (60.0)	5 (33.3)	15 (21.7)	.7
CD4 count, cells/μL				
≤200	10 (34.5)	16 (55.2)	29 (87.9)	. . .
>200	2 (50.0)	2 (50.0)	4 (12.1)	.7
Other comorbidity				
No	40 (50.6)	29 (25.3)	79 (63.7)	. . .
Yes	19 (42.2)	24 (53.3)	45 (36.3)	.2
Salmonella serotype				
Enteritidis	21 (55.3)	12 (31.6)	38 (26.0)	. . .
Typhimurium	17 (27.4)	29 (46.8)	62 (42.5)	.02
Other	21 (45.7)	12 (26.1)	46 (31.5)	.2
Salmonella multidrug resistance				
No	40 (46.5)	27 (31.4)	86 (63.7)	. . .
Yes	16 (32.7)	22 (44.9)	49 (36.3)	.08
Salmonella Typhimurium sequence type				
ST19	6 (35.3)	4 (23.5)	17 (30.9)	. . .
ST313	8 (21.1)	23 (60.5)	38 (69.1)	.05

Abbreviations: HIV, human immunodeficiency virus; ST, sequence type.

[a] Total includes those patients for whom outcome was unknown.

meningitis due to other serovars, although this was not significant ($P = .08$).

Multidrug resistance (defined above) was detected in 103 of 247 (41.7%) of the isolates; 56 of 247 (22.7%) were resistant or intermediately resistant to ciprofloxacin (Table 3). *Salmonella enterica* serovar Isangi and serovar Virchow isolates were more likely to be extended-spectrum β-lactamase producers than other serovars (20/30 [66.7%] for *Salmonella* Isangi and *Salmonella* Virchow vs 17/247 [6.9%] for other serovars; OR, 46.1; 95% CI, 15.4–138.3). *Salmonella* Typhimurium was more likely to be multidrug resistant than other serovars (62/104 [59.6%] vs 34/143 [23.8%] for other serovars; OR, 4.7; 95% CI, 2.7–8.2; $P < .001$).

MLST of *Salmonella* Typhimurium

Ninety-seven of 104 *Salmonella* Typhimurium isolates (93.3%) were MLST subtyped (Table 4). There was a trend toward association of outcome with meningitis due to *Salmonella* Typhimurium ST313 on univariate analysis ($P = .056$; Table 2). HIV-infected patients were significantly more likely to be infected by *Salmonella* Typhimurium ST313 ($P = .03$) compared with other sequence types (Table 4). *Salmonella* Typhimurium ST16 and ST302 were isolated from HIV-uninfected patients.

Multidrug resistance was significantly associated with *Salmonella* Typhimurium ST313 (48/65 [73.8%]) compared with *Salmonella* Typhimurium ST19 (9/30 [30.0%]) (OR, 6.6; 95% CI, 2.5–17.2; $P < .001$).

DISCUSSION

To our knowledge, this is the largest series of *Salmonella* meningitis described. Molyneux et al [2] described a large series in children aged 2 months to 16 years in Malawi. Predisposing conditions in Malawi included HIV infection and malaria [2]. An earlier publication has highlighted NTS as a cause of meningitis in HIV-infected South African adult patients and described the associated characteristics [7], NTS meningitis representing approximately 1.3% of all-cause meningitis in this age group [15]. Previously, we have compared South African and Malawian data, reviewing the role of NTS in invasive disease in these 2 countries. The countries both showed a bimodal age distribution, with disease occurring primarily in young children and adults aged 20–50 years, confirming the importance of these ages in association with invasive disease due to NTS [31].

Our report again highlights the vulnerability of children aged <5 years to NTS meningitis. All the adults in our series and 58% of children <5 years of age were HIV infected, although none had malaria. A similar distribution in patients' ages occurs with invasive shigellosis in South Africa, with excessive case numbers occurring in the very young and a second peak from early adulthood [30].

Table 2. Univariate and Multivariate Analysis of Risk Factors Associated With Mortality in Patients With *Salmonella* Meningitis in South Africa, 2003–2013

	Univariate Analysis			Multivariate Analysis		
Characteristic	OR	(95% CI)	*P* Value	AOR	(95% CI)	*P* Value
Age						
<15 y	1	. . .	. . .	1	. . .	. . .
≥15 y	3.7	(1.7–8.1)	.001	2.3	(.4–12.1)	.338
Sex						
Male	1	. . .	. . .			
Female	1.9	(.9–4.0)	.117			
HIV status						
Uninfected	1	. . .	. . .	1	. . .	. . .
Infected	5.3	(1.4–20.0)	.013	0.9	(.1–15.7)	.987
Glasgow Coma Scale						
≤13	1	. . .	. . .	1	. . .	. . .
>13	21.6	(3.5–133.3)	.001	18.7	(3.0–118.5)	.002
Nosocomial infection						
No	1	. . .	. . .			
Yes	1.1	(.3–3.7)	.844			
Cotrimoxazole prophylaxis						
No	1	. . .	. . .			
Yes	1.8	(.6–5.1)	.272			
Antiretrovirals						
No	1	. . .	. . .			
Yes	0.8	(.2–2.7)	.695			
$CD4^+$ count, cells/μL						
≤200	1	. . .	. . .			
>200	0.6	(.8–5.2)	.663			
Other comorbid conditions						
No	1	. . .	. . .			
Yes	1.7	(.8–4.0)	.157			
Salmonella serotype						
Other serotypes	1	. . .	. . .	1	. . .	. . .
Typhimurium	3.0	(1.3–6.5)	.006	0.6	(.1–4.8)	.659
Salmonella multidrug resistance						
No	1	. . .	. . .	1	. . .	. . .
Yes	2.0	(.9–4.6)	.084	0.6	(.1–5.5)	.648
Salmonella Typhimurium sequence type						
ST313	1	. . .	. . .	1	. . .	. . .
ST19	0.2	(.05–1.04)	.056	1.0	(.04–23.0)	.994

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio; ST, sequence type.

In addition to our series, the strong association of NTS meningitis with HIV infection in adults is suggested by case reports in the literature [18–20, 32]. Predisposing conditions, which we did not observe, include autoimmune conditions or other coinfections [21–23]; rarely, patients may have no predisposing conditions [24].

In the follow-up of the Malawian pediatric meningitis cases, impaired consciousness was significant in the outcome of cases of NTS meningitis [1]. We had similar findings in adults and children. Patients presenting with a GCS ≤13 were at a greater risk of death, irrespective of HIV status. Of note, none of the HIV-uninfected patients who had a recorded GCS >13 died. Previous reports from South Africa on meningitis due to *N. meningitidis* and *S. pneumoniae* emphasize the significance of severity of illness at presentation [3, 4].

Death rates in children in our series were lower than those described in the Malawian series [1, 2]; almost half of the deaths were in adults. Wall et al reviewed meningitis due to all causes in adolescents and adults in Malawi and similarly found that

Table 3. Commonest *Salmonella* Serotypes, 2003–2013, and Antimicrobial Resistance Profiles (n = 247) Associated With *Salmonella* Meningitis in South Africa

	Selected Antimicrobials Tested Against Strains of Nontyphoidal *Salmonella*					
Serotype (No. Tested)	Ampicillin[a]	Chloramphenicol[a]	TMP-SMX[a]	Tetracycline[a]	Ciprofloxacin[a]	ESBL Production[a,b]
Typhimurium (104)	72 (69.2)	39 (37.5)	67 (64.4)	47 (45.1)	30 (28.8)	9 (8.6)
Enteritidis (66)	5 (7.6)	3 (435)	3 (4.5)	8 (12.1)	14 (21.2)	1 (1.5)
Isangi (15)	14 (93.3)	13 (86.7)	14 (93.3)	15 (100.0)	9 (60.0)	12 (80.0)
Virchow (10)	9 (90.0)	7 (70.0)	8 (80.0)	8 (80.0)	0 (0.0)	8 (80.0)
Dublin (10)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other (42)	10 (23.8)	9 (21.4)	12 (28.6)	17 (40.5)	3 (7.1)	6 (14.3)

Data are presented as No. (%). Complete antimicrobial resistance data were not available for all serotypes tested.

Abbreviations: ESBL, extended-spectrum β-lactamase; TMP-SMX, trimethoprim-sulfamethoxazole.

[a] No. of intermediate and fully resistant isolates.

[b] Production of extended-spectrum β-lactamase.

GCS was the strongest independent predictor of mortality, using a cutoff of 11 rather than 13 [5].

Irrespective of age, HIV infection was a major contributing factor for death in the univariate model. In South Africa, excessive deaths due to meningococcal and pneumococcal meningitis in association with HIV infection have been reported [3, 4], emphasizing the vulnerability of these patients to severe infections. In Malawian adults, mortality due to bacterial meningitis increased over time [5, 6]: we did not follow patients after discharge, but longer hospital stay was associated with improved survival in our series.

Antiretroviral therapy did not impact mortality in this study, stressing the importance of early diagnosis and treatment of NTS meningitis. Our patients also received appropriate antimicrobial therapy; incorrect treatment did not play a major role in the outcome. We postulated that other comorbidities may have contributed to the development of NTS meningitis, but data were too scanty to develop definitive conclusions.

Neonates and young children are particularly vulnerable groups warranting further consideration. We did not have access to information posthospitalization, but the Malawian study suggested that infants and young children who recover from the acute infection are susceptible to neurological sequelae [1, 2]. Although mortality was independent of HIV status in our series, a greater proportion of infants who acquired NTS meningitis were HIV exposed at birth. Absence of maternal immunity in HIV-infected mothers may increase the risk in infants to acquiring NTS meningitis, which should be included in the differential diagnosis of causes of neonatal and infant meningitis in settings of high HIV seroprevalence [13, 33–36]. Previously, we described the role of childhood *Shigella* infections predisposing HIV-infected women to invasive shigellosis [30]. In this instance, the reverse appears to be true: Maternal HIV infection may predispose neonates to invasive salmonellosis, confirming the importance of maternal health and a competent immune system in decreasing infant mortality in South Africa [37].

In immunosuppressed patients, whether due to HIV infection or extreme youth, innate characteristics of *Salmonella* may predispose the organism to invading the central nervous system. *Salmonella* Typhimurium has the ability to adhere to, penetrate, and invade the brain microvascular endothelium, in association with a proinflammatory immune response [38]. Our understanding of the organism may need to be altered to adapt to new management paradigms; following the introduction of

Table 4. Association of *Salmonella* Typhimurium Multilocus Sequence Type, Human Immunodeficiency Virus Infection, and Multidrug Resistance (Resistance to ≥3 Antimicrobials)

Salmonella Typhimurium Sequence Type (n = 97)	HIV-Uninfected Patients (n = 7)	HIV-Infected Patients (n = 38)	Nonresistant Isolates or Isolates With Limited Resistance	Multidrug-Resistant Isolates[a]
16 (n = 1)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
19 (n = 30)	4 (28.6)	10 (71.4)	21 (70.0)	9 (30.0)
302 (n = 1)	1 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)
313 (n = 65)	1 (3.4)	28 (96.6)	17 (26.1)	48 (73.8)

Data are presented as No. (%).

Abbreviation: HIV, human immunodeficiency virus.

[a] Includes intermediate resistance.

new vaccines to prevent childhood meningitis, new pathogens associated with meningitis may be seen to emerge [9, 12, 13].

Salmonella serovar was a better predictor of outcome than multidrug resistance: *Salmonella* Typhimurium was more highly associated with death. *Salmonella* Typhimurium ST313 is well associated with HIV [27]; we found that the organism contributes significantly to NTS meningitis in HIV-infected patients. Besides ST313, representing 67% of typed *Salmonella* Typhimurium isolates, ST19 represented 31% of typed isolates; ST19 is commonly described worldwide, including from South Africa [39]. A stronger association between HIV infection and *Salmonella* meningitis due to *Salmonella* Typhimurium ST313, compared with *Salmonella* Typhimurium ST19, was noted. In our previous report of predominantly noninvasive *Salmonella* Typhimurium ST19 infection, most of the patients were HIV uninfected [39].

To prevent NTS meningitis infections, further studies are needed regarding the source of infections in South Africa. We demonstrated that nosocomial NTS meningitis was rare; infections were likely community acquired. *Salmonella* are ubiquitous, and the association between foodborne disease transmission and human-to-human transmission with invasive disease is recognized [24, 40, 41]; although transmission is often assumed to be foodborne, actual routes are not always clear. Attention should be paid to preventing mother-to-child transmission, including maternal screening for fecal pathogens, and ensuring maternal health in a potentially disadvantaged subset of HIV-infected individuals [37, 42–45].

This study had limitations. Clinical data were collected at selected sites only and may not be relevant to all the cases, and clinical data were often incomplete. Not all patients at enhanced sites had outcome data, HIV results, $CD4^+$ counts, and access to ARV treatment. Data on tuberculous meningitis, prior treatment for cryptococcal meningitis, or acute bacterial meningitis were not collected. Insufficient data were collected on maternal HIV status in pediatric cases, and this impact could not be fully assessed. Fecal cultures were not performed on the mothers of infants in association with meningitis; we cannot comment on whether maternal carriage of NTS contributed to infection in infants. Incomplete clinical data meant that the ubiquity and consequences of *Salmonella* Typhimurium ST313 in NTS meningitis could not be fully elucidated. We elected not to do imputations for the missing data, believing that on univariate analysis at least, sufficient association was shown between outcome, age, HIV status, GCS, *Salmonella* Typhimurium, and *Salmonella* Typhimurium sequence types to highlight critical factors associated with *Salmonella* meningitis. Considering the high proportion of missing data in our data set, imputation (especially of binary data) could lead to biased inference. The complete case analysis that was conducted in this study assumes that data are missing completely at random. Our data may not be missing completely at random—but rather missing at random where relevant information regarding outcome, HIV result, or consent at a sentinel site was not collected—or not missing at random: Sentinel hospitals are typically referral hospitals where HIV-infected persons may preferentially present. We do believe we identified the majority of NTS meningitis cases over the period; typically in South Africa, patients with suspected meningitis will have lumbar punctures performed and CSF samples submitted to the laboratory for culture.

In conclusion, we describe a national series of laboratory-confirmed meningitis cases due to NTS over an 11-year period, highlighting the importance of disease severity as measured by GCS, HIV status in adults, and infection in neonates and infants, and the association of outcome and HIV status with specific serovars and sequence types. Early diagnosis and appropriate therapy may decrease death rates, and optimizing maternal health may lower case numbers in neonates and infants. Better understanding of the role of *Salmonella* Typhimurium, specifically *Salmonella* Typhimurium ST313, may also assist in controlling invasive disease due to NTS.

Notes

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III. Typhoid fever in South Africa in an endemic HIV setting.

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RESEARCH ARTICLE

Typhoid Fever in South Africa in an Endemic HIV Setting

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Abstract

Background

Typhoid fever remains an important disease in Africa, associated with outbreaks and the emerging multidrug resistant *Salmonella enterica* serotype Typhi (*Salmonella* Typhi) haplotype, H58. This study describes the incidence of, and factors associated with mortality due to, typhoid fever in South Africa, where HIV prevalence is high.

Methods and Findings

Nationwide active laboratory-based surveillance for culture-confirmed typhoid fever was undertaken from 2003–2013. At selected institutions, additional clinical data from patients were collected including age, sex, HIV status, disease severity and outcome. HIV prevalence among typhoid fever patients was compared to national HIV seroprevalence estimates. The national reference laboratory tested *Salmonella* Typhi isolates for antimicrobial susceptibility and haplotype. Unadjusted and adjusted logistic regression analyses were conducted determining factors associated with typhoid fever mortality. We identified 855 typhoid fever cases: annual incidence ranged from 0.11 to 0.39 per 100,000 population. Additional clinical data were available for 369 (46.8%) cases presenting to the selected sites. Among typhoid fever patients with known HIV status, 19.3% (29/150) were HIV-infected. In adult females, HIV prevalence in typhoid fever patients was 43.2% (19/44) versus 15.7% national HIV seroprevalence (P < .001); in adult males, 16.3% (7/43) versus 12.3% national HIV seroprevalence (P = .2). H58 represented 11.9% (22/185) of *Salmonella* Typhi isolates tested. Increased mortality was associated with HIV infection (AOR 10.7; 95% CI 2.3–50.3) and disease severity (AOR 9.8; 95% CI 1.6–60.0) on multivariate analysis.

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Conclusions

Typhoid fever incidence in South Africa was largely unchanged from 2003–2013. Typhoid fever mortality was associated disease severity. HIV infection may be a contributing factor. Interventions mandate improved health care access, including to HIV management programmes as well as patient education. Further studies are necessary to clarify relationships between HIV infection and typhoid fever in adults.

Introduction

Typhoid fever, infection with *Salmonella enterica* serotype Typhi (*Salmonella* Typhi), remains an important infectious disease in developing countries; the estimated annual disease burden is 10 to 13 million cases [1] with 190 000 deaths globally [2]. Typhoid fever mortality in Africa has been estimated at 7.2 per 100 000 person-years, double that of south Asia and 25–70 times that of the rest of the world in a recent systematic review [1]. Typhoid fever appears particularly problematic in emerging economies, with high burdens reported from Kenya [3], Pakistan, India and Indonesia [3], where disease is associated with urban informal settlements [3,4]. Numerous data gaps remain regarding typhoid fever in low and middle income countries including factors associated with death among typhoid fever patients [5]. The association of HIV infection with typhoid fever also remains unclear: an increased risk of acquiring typhoid fever among HIV-infected persons was described in Peru [6], whereas a Tanzanian study found HIV infection was associated with a decreased risk of typhoid fever [7]. HIV infection is a particular public health problem in South Africa where 15% of the population ≥15 years of age were HIV-infected in 2013 [8].

Recently, a multidrug resistant haplotype of *Salmonella* Typhi, H58, has emerged in South East Asia [9–11], and Africa [12,13]. This emergence may be related to increased virulence or selective pressures created by antimicrobial use [14]. We report on the incidence of typhoid fever in South Africa from 2003 to 2013, the clinical and microbiological features of typhoid fever that may drive mortality, including association with HIV infection and the prevalence of *Salmonella* Typhi H58.

Methods

Surveillance

Nationwide active laboratory-based surveillance for culture-confirmed typhoid fever, (isolation of *Salmonella* Typhi from a normally sterile site), was performed by the Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases in South Africa from 2003 through 2013, as previously described [15]. Specifically, we requested that all diagnostic laboratories in South Africa send all *Salmonella* Typhi isolates to CED and performed audits to identify additional laboratory-confirmed cases of typhoid fever for which *Salmonella* Typhi isolates were not received using the Central Data Warehouse (CDW), a national databank derived from the laboratory information system storing basic demographic and laboratory data for all typhoid fever cases diagnosed by the National Health Laboratory Service [15] (active case finding). Ethical approval for this study was granted by the Human Research Ethics Committee of the University of the Witwatersrand (M110601).

At 25 hospitals selected to represent major centres in the nine South African provinces, additional information was collected from medical records of typhoid fever patients through

GERMS-SA, a national surveillance network sharing resources to characterise diseases of public health importance in South Africa. Written informed consent from the patient or (care provider, in the case of minors) was obtained for those patients for whom interviews were conducted. Where clinical data was obtained solely from clinical record review, no written informed consent was obtained from the patient. Patient records could not be anonymised as patient information was required to cross reference each case with those cases that were notified through audit of the NHLS CDW, to avoid duplicating case counts. Patient records were reviewed for clinical presentation, HIV infection and other co-morbidities, and outcome (survival or death). A patient was considered to have co-morbid disease if they had any of the following diagnoses: tuberculosis, malignancy, autoimmune disease, renal disease, liver failure, diabetes mellitus, protein energy malnutrition (children) or congested cardiac failure. A Pitt bacteraemia score (PBS) was determined for patients with typhoid fever by assigning values between 0 and 2 for presence of fever or hypopyrexia, mechanical ventilation and hypotension or 0 and 4 for cardiac arrest and mental status. Higher scores corresponded with increased severity of illness [15,16].

HIV prevalence among male and female typhoid fever patients ≥15 years of age were compared to national HIV seroprevalence estimates from the 2008 Actuarial Society of South Africa (ASSA) [8].

Laboratory

All clinical diagnostic laboratories in South Africa were requested to submit *Salmonella* Typhi isolates to CED for confirmation and further characterisation in accordance with GERMS-SA network guidelines. At CED, *Salmonella* serotype was confirmed according to the White-Kauffmann-Le Minor scheme and antimicrobial susceptibility to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, ciprofloxacin and ceftriaxone was determined using E-tests, according to the manufacturer's instructions (BioMérieux, Marcy-l'Étoile, France). Multidrug resistance (MDR) was defined as resistance to three or more of these antimicrobials.

The presence of the H58 haplotype was confirmed using conventional PCR [17].

Statistical analysis

Typhoid fever incidence calculations used national population estimates from ASSA [8]. Univariate and multivariate logistic regression were used to determine factors (age, sex, HIV status, PBS, other comorbidities, multidrug resistance and H58 haplotype) associated with mortality among typhoid fever patients. Analyses were performed using Stata version 13 (StataCorp Limited, College Station, TX, USA). Two-sided P values of $< .05$ were considered significant throughout. To assess changes in typhoid fever mortality over time, corresponding to changes in the proportion of the population of HIV-infected persons with access to antiretroviral treatment [8], we defined early (2003–2005), middle (2006–2010) and late (2011–2013) study periods. The Fisher Exact test and Chi-squared (in adult women) test were used to compare HIV prevalence among typhoid fever patients and national seroprevalence estimates.

Results

Surveillance

From 2003 to 2013, we ascertained 855 cases of culture-confirmed typhoid fever in South Africa: 67 (7.8%) were identified on audit of the CDW: the number of cases annually ranging from 48 to 187. With the exception of increases in culture-confirmed typhoid cases in 2005

Table 1. Number of cases and incidence rates per 100,000 population of culture-confirmed typhoid fever cases in South Africa 2003–2013 (N = 855).

Year	Number of culture-confirmed typhoid fever cases	South African Population*	Incidence rate of culture-confirmed typhoid fever cases
2003	60	47150661	0.13
2004	72	47676795	0.15
2005	187	48155945	0.39
2006	114	48605035	0.23
2007	66	49034069	0.13
2008	67	49459134	0.14
2009	58	49905065	0.12
2010	62	50371513	0.12
2011	64	50840589	0.13
2012	48	51304466	0.09
2013	57	51759127	0.11

*Data derived from ASSA tables [8].

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and 2006, the incidence in South Africa was largely unchanged from 2003 to 2013, ranging from 0.11 per 100,000 population in 2013 to 0.39 per 100,000 population in 2005 (Table 1). Age was available for 814 cases, ranging from 0 days (birth) to 81 years with a median of 15 years. Sex was available for 841 cases; 470 (55.9%) were male. Sex and age were available for 803 cases (Fig 1). Among 855 typhoid fever cases, *Salmonella* Typhi was isolated from blood in 816 (95.4%), CSF in 4 (0.5%) and other body sites in 35 (4.1%) cases.

Laboratory

Isolates were received at CED from 788 (92.2%) of the 855 cases: 760 (96.4%) were viable and confirmed as *Salmonella* Typhi. Antimicrobial susceptibility testing on 758 (99.7%) indicated 175 (23.1%) were resistant to ampicillin, 165 (21.8%) to co-trimoxazole, 91 (12.0%) to chloramphenicol, 111 (14.6%) to tetracycline, 69 (9.1%) resistant to ciprofloxacin and 1 (0.1%) to ceftriaxone (two isolates died before susceptibilities could be confirmed). Multidrug resistance was recorded in 158 (20.8%). *Salmonella* Typhi H58 represented 22 (11.9%) of 185 isolates tested; 14/22 (63.6%) H58 isolates were multidrug resistant compared with 26/163 (16.0%) non-H58 isolates ($P < .001$).

Selected sites

Additional clinical data including outcome (recovery or death) were available for 369 (46.8%) cases presenting to the selected sites. Presence or absence of fever was recorded for 215 (59.1%) cases; 130 (60.5%) presented with fever (temperature ≥38°C) and 18 (8.4%) were hypopyrexial (temperature <36.5°C). Of the 196 cases with sufficient clinical information to derive a PBS, 12 (6.1%) had a PBS ≥4; patients with PBS ≥4 reported later to hospital compared with patients with PBS <4 (data not shown). HIV status at time of typhoid fever diagnosis was recorded for 150 (41.2%) cases; 29 (19.3%) were HIV-infected (Table 2). Record review suggested no differences in patient management between 29 HIV-infected patients compared with 121 HIV uninfected patients. Among 63 persons <15 years of age with recorded HIV status, 3 (4.8%) were HIV-infected. Among 87 persons ≥ 15 years of age with recorded HIV status, 26 (29.9%) were HIV-infected. The proportion of typhoid fever patients ≥15 years of age who were HIV-infected at the time of typhoid fever diagnosis was 36.4% (8/22) in 2003–2005, 28.1% (9/32) in 2006–2010, and 27.3% (9/33) in 2011–2013. Among female patients ≥15 years of age with

recorded HIV status, 19/44 (43.2%) were HIV-infected at time of typhoid fever diagnosis versus 7/43 (16.3%) males (p = .009).

According to ASSA estimates from 2003 to 2013, average national HIV seroprevalence in females ≥15 years of age was 15.7%; female typhoid fever patients ≥15 years of age had a significantly higher HIV seroprevalence (19/44; 43.2%) than national estimates for females ≥15 years of age (15.7%) (p < .001). Average national HIV seroprevalence from 2003 to 2013 in males ≥15 years of age was 12.3%; male typhoid fever patients ≥15 years of age had a higher HIV seroprevalence (7/43; 16.3%) than national estimates for males ≥15 years of age (12.3%), although this was not significant (p = .2).

The presence or absence of co-morbidities for typhoid cases was determined from medical records available 362 (98.1%) of 369 cases available from these sites; 26 (7.2%) had co-morbidities including diabetes mellitus, malignancy, protein-energy malnutrition, congestive cardiac failure and hepatic failure. No patients, including HIV-infected patients, had other bacterial co-infections. Eight HIV-infected patients refused hospital treatment; further clinical information was unavailable from these patients.

Final patient outcome (hospital discharge or death) was available for 237/369 (65.1%) cases from these sites; 16 (6.8%) died (Table 2). Among the patients who died, sex was known for 15; 7 (46.7%) were male. The ages of these 16 patients ranged from 7 to 65 years, with a median of 19.5 years. Of the 237 cases with final hospital outcome data, *Salmonella* Typhi was isolated from blood or CSF in 232 cases; 196 of these had a recorded PBS (median PBS 1; range 0–7), of whom 12 (6.1%) had a PBS ≥4.

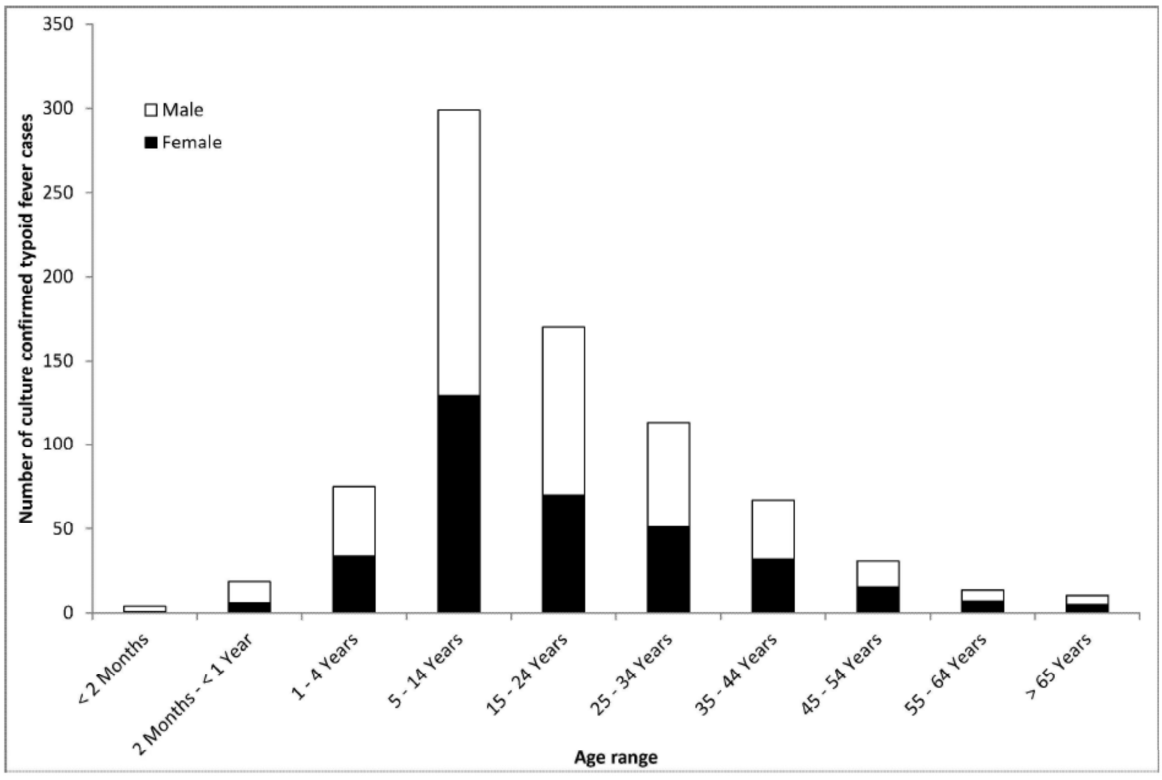

Fig 1. Number of culture-confirmed typhoid fever cases (N = 803) by age range and sex in South Africa, 2003–2013.

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Table 2. Clinical and laboratory characteristics associated with patients with culture-confirmed typhoid fever in South Africa (N = 369), 2003–2013, based on data from sentinel sites.

Characteristic	Total n (%)
Age	361*
< 2 Months	1 (0.3)
2 Months—< 1 Year	10 (2.8)
1–4 Years	38 (10.5)
5–14 Years	132 (36.6)
15–24 Years	84 (23.3)
25–34 Years	46 (12.7)
35–44 Years	28 (7.8)
45–54 Years	11 (3.0)
55–64 Years	6 (1.6)
> 65 Years	5 (1.4)
Sex	367*
Male	196 (53.4)
Female	171 (46.6)
Outcome	237*
Death	16 (6.8)
Recovery	221 (93.2)
HIV status	150*
Uninfected	121 (80.7)
Infected	29 (19.3)
Antiretroviral access period	369*
Early 2003–2005	141 (38.3)
Mid 2006–2009	140 (37.9)
Late 2010–2013	88 (23.8)
Pitt bacteraemia score (severity of illness)	196*
<4	184 (93.9)
≥4	12 (6.1)
Other comorbidity	362*
No	336 (92.8)
Yes	26 (7.2)
Multidrug resistance	330*
No	236 (71.5)
Yes	94 (28.5)
Salmonella Typhi haplotype	176*
Non-H58	155 (88.1)
H58	21 (11.9)

*Total excludes those patients for whom results were unknown.

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Final hospital outcome data were known for 55 of 158 (34.8%) typhoid fever patients infected with multidrug resistant *Salmonella* Typhi; 5 (9.1%) of whom died. Outcome was known for 19 (90.5%) of the 21 typhoid fever patients infected with H58, of whom one (5.3%) died.

On unadjusted analysis, age ≥15 years (Odds Ratio [OR] 4.1; 95% Confidence Interval [CI] 1.1–14.9), HIV infection (OR 11.3; 95% CI 3.0–42.4), and PBS ≥4 (OR 10.8; CI 2.9–39.5) were associated with death among typhoid fever patients (Table 3). On adjusted analysis, HIV infection (Adjusted OR [AOR] 10.8; 95% CI 2.3–50.3) and PBS ≥4 (AOR 9.8; 95% CI 1.6–60.0)

Table 3. Univariate and multivariate analysis of risk factors associated with mortality in patients with *Salmonella* Typhi in South Africa, 2003–2013.

	Unadjusted analysis			Adjusted analysis		
Characteristic	**OR**	**(95% CI)**	***P***	**AOR**	**(95% CI)**	***P***
Age						
<15 years	1	-	-	1	-	-
≥15 years	4.1	(1.1–14.9)	.03	2.0	(0.2–19.3)	.6
Sex						
Male	1	-	-			
Female	1.4	(0.5–3.8)	.5			
HIV status						
Uninfected	1	-	-	1	-	-
Infected	11.3	(3.0–42.4)	< .001	10.8	(2.3–50.3)	.002
Antiretroviral access period						
Early 2003–2005	1	-	-			
Mid 2006–2009	1.7	(0.6–5.4)	.3			
Late 2010–2013	0.4	(0.1–2.3)	.3			
Pitt bacteraemia score						
<4	1	-	-	1	-	-
≥4	10.8	(2.9–39.5)	< .001	9.8	(1.6–60.0)	.01
Other co-morbid conditions						
No	1	-	-			
Yes	2.1	(0.5–8.0)	.2			
Multidrug resistance						
No	1	-	-			
Yes	1.7	(0.6–5.3)	.3			
Salmonella Typhi haplotype						
Non-H58	1	-	-			
H58	0.7	(0.09–5.8)	.8			

OR, odds ratio; CI, confidence interval; AOR, adjusted odds ratio

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remained associated with mortality. Neither multidrug resistance nor *Salmonella* Typhi H58 was associated with mortality among typhoid fever patients (Table 3); there were additionally no differences between HIV-uninfected and HIV-infected individuals in the occurrence of multidrug resistance *Salmonella* Typhi or the isolation of *Salmonella* Typhi H58 (P = 0.5and P = 0.3, respectively).

Discussion

Typhoid fever remains an important public health challenge in South Africa [18–20]. Incidence rates of culture-confirmed typhoid fever were between 0.11 and 0.39 per 100,000 population; peaking in 2005 and 2006, due to typhoid outbreaks of over 600 clinically diagnosed cases, of which approximately 160 were culture-confirmed and captured in our surveillance [19].

Factors associated with mortality among persons with typhoid fever on multivariate analysis were HIV-infection and disease severity as measured by PBS (Table 3). When we reviewed individual patient records from the sentinel sites, no differences were identified between the management of HIV-infected and uninfected patients: i.e. all patients were appropriately and timeously treated, based on a positive culture result of invasive *Salmonella* Typhi at the presenting hospital. Independently of HIV status, PBS score was significantly associated with

mortality. Specifically, in our series, late access to health care appeared critical in contributing to mortality due to severity of illness in typhoid fever patients, rather than belated diagnosis and treatment delays in hospital or inappropriate treatment.

Among the clinical records we reviewed, only HIV-infected typhoid fever patients refused hospital treatment. This, and the observation that typhoid fever patients may delay accessing health care, suggest patient and population education is paramount in managing typhoid fever from a public health and an individual patient perspective.

Our finding that 16.3% of adult male patients versus 43.2% of adult females with typhoid fever were HIV-infected may be related to the antenatal testing programme for HIV status in pregnant women within the country [21]: most South African women who have borne children would know their HIV status, which would be recorded on hospital admission, in contrast to South African men. Sex, moreover, was not significantly associated with mortality. Previously we observed a female predominance among HIV-infected adults with systemic shigellosis, which included patients from selected and non-selected sites and was ascribed to the burden of childcare of children with *Shigella* diarrhoea [15]. Here, no general female predominance was observed: 53.4% culture-confirmed cases in sentinel sites were male: the marked HIV predominance recorded among women in this series may potentially be indicative of testing biases. HIV infection is also more common in women of child-bearing age than in men [8,21], thus, a combination of factors, both clinical and due to sampling methods, may have influenced the numbers of HIV-infected women presenting with typhoid fever, as limited numbers of patients from the sentinel sites had HIV status determined.

HIV prevalence observed among women and men ≥15 years of age with typhoid fever, was higher than the comparable national seroprevalence, significantly so in women [8], suggesting HIV-infected adults may be at greater risk of acquiring typhoid fever. This apparent predominance contrasts observations by Crump *et al* [7], who found HIV-infected adolescents and adults less likely to acquire typhoid fever compared with HIV-uninfected patients. This earlier study was a clinically-based fever study: only patients with documented fever >38°C were included. Our study used laboratory-based surveillance enrolling all patients with culture-confirmed disease, irrespective of clinical characteristics, including fever. Approximately 40% of our patients were hypopyrexial or apyrexial on admission, possibly contributing to these observed differences. As the immune response is necessary for the development of fever [22], if this is defective, it is feasible the classic fever curves associated with typhoid fever may not be observed. Moreover, functional CD4 cells are necessary to combat typhoid fever in healthy adults [23] and transient defects in these cells have previously been associated with opportunistic typhoid fever infection [24].

Undefined parameters may nonetheless make women more susceptible to typhoid fever: Khan *et al* described a series from Durban, South Africa, in which women were more likely to have severe disease, although they excluded HIV status in the analysis [25]. Other literature series examining the association of HIV and typhoid fever have looked at populations where HIV is predominantly a male disease, in men who have sex with men [6,26], where sexual practices may constitute an additional risk factor, or the male-to-female ratio for typhoid fever was not reported [7]. Not all reports from African countries have highlighted a female predominance [4,27], although increased numbers of women were reported from large outbreaks of typhoid fever in Zimbabwe [28] and Malawi [29]: HIV status was not documented in these reports. In Zambia, male-to-female ratios were equal in a large outbreak from 2010 to 2012, but most cases were reported in children less than 15 years of age [12].

In children, typhoid fever is typically associated with the 5 to 15 year age group in South Africa [18], in which the HIV prevalence was calculated at 3% in 2003 [8]; in the absence of ART, few HIV-infected children survive beyond 5 years of age. It is likely in this age group,

very few HIV-infected children with typhoid fever would have unknown HIV status, given current testing recommendations in the country [30]. Most children with typhoid fever in this series under five years of age would have benefited from the perinatal antiretroviral programme introduced in 2004 [31], very few HIV-infected children would be expected among this age group, in which typhoid fever incidence is also low. It is therefore unsurprising that typhoid fever was not significantly associated with HIV infection in children under 15 years of age.

The presence of multidrug resistant (MDR) *Salmonella* Typhi H58 in South Africa has previously been reported [32]. This virulent, MDR clade affected both HIV-infected and uninfected patients equally, irrespective of these two groups of patients' susceptibility to typhoid fever. This virulent strain appears not to be specifically adapted to a vulnerable population group, as has been reported with *Salmonella* Typhimurium ST313 in Africa [33], including an association with *Salmonella* meningitis in South Africa [34], but probably emerged as a result of global population movements and wide-spread use and misuse of antimicrobial therapy [32].

This study had limitations. Primarily, missing data for certain clinical parameters may have affected the data analyses and results. Secondly, not all cases of typhoid fever over the study period were included: the majority of cases related to the 2005 typhoid fever outbreak did not have blood cultures done due to resource constraints [19]. In non-outbreak years, cases may similarly have been missed, thus burden of disease estimates could not be calculated. A lack of robustness of the data with year-on-year variation in case numbers and incompleteness of the HIV results meant that imputation of data for patients with unknown HIV status could lead to biased inference. From 2004, ART was rolled out in South Africa [31]. We attempted to overcome the data insufficiencies regarding HIV status through analysing the data based on early, mid and late ART periods, showing no association between HIV and typhoid fever in children, possibly due to decreasing HIV seroprevalence following the introduction of ART [30] and the preponderance of children aged 5 to 14 years with typhoid fever [18]. Limited numbers of cases with HIV results and the increased prevalence of HIV infection in adults and in women in particular may account for apparent associations between HIV-infected women and typhoid fever. HIV seroprevalence in adults was nonetheless greater than the overall prevalence of HIV-infected adults in South Africa [8]: the role of HIV as a risk factor for the development of typhoid fever still needs further exploration.

Conclusions

In summary, typhoid fever in South Africa remains a public health challenge. Persons with typhoid fever who are HIV-infected or severely ill are more prone to mortality. Disease severity may be affected by access to health care and treatment delays; multidrug resistance and H58 haplotype were not associated with mortality. *Salmonella* Typhi H58 has not specifically emerged in Africa in response to the HIV pandemic, but has probably expanded as a clone in association with population movements and antimicrobial usage practices on the African continent. Public health interventions should include patient and population education and enhanced management of HIV infection, including testing patients with typhoid fever, who may be at risk for HIV infection, in order that they may benefit from national treatment plans.

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IV. An association between decreasing incidence of invasive non-typhoidal salmonellosis and increased use of antiretroviral therapy, Gauteng Province, South Africa, 2003 – 2013.

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An association between decreasing incidence of invasive non-typhoidal salmonellosis and increased use of antiretroviral therapy, Gauteng Province, South Africa, 2003 – 2013

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Abstract

Background

HIV-infected persons are at increased risk of invasive nontyphoidal *Salmonella* (iNTS) infections; antiretroviral therapy (ART) reduces this risk. We explored changing iNTS incidence at a population-based level associated with increasing ART availability in Gauteng, South Africa.

Method

Laboratory-based surveillance for iNTS was conducted in Gauteng between 2003 and 2013. Isolates were serotyped at the Centre for Enteric Diseases. The National Health Laboratory Service's Central Data Warehouse (CDW) provided HIV viral load measurements data to estimate numbers of HIV-infected patients receiving ART. The association between iNTS incidence and ART use from 2004 to 2013 was described using Pearson's correlation.

Findings

The annual incidence per 100,000 population of iNTS decreased from 5·0 to 2·2 from 2003 to 2013 ($p<0·001$), while HIV viral load testing increased from 75·2 to 3,620·3 per 100,000 from 2004 to 2013 ($p<0·001$). A strong correlation was observed between decreasing iNTS incidence and increasing ART use from 2004 to 2013 ($r=-0·94$, $p<0·001$). Similarly, decreasing incidence of invasive *Salmonella enterica* serotype Typhimurium (*Salmonella* Typhimurium) infection correlated with increasing ART use ($r=-0·94$, $p<0·001$). Incidence of invasive *Salmonella* Enteritidis infection increased ($r=0·95$, $p<0·001$). Rates of iNTS in HIV-infected men decreased less rapidly than in HIV-infected women; men accessed ART consistently less ($p<0·001$).

Interpretation

The incidence of iNTS infections including *Salmonella* Typhimurium decreased significantly in Gauteng associated with increased ART utilisation. Increasing incidence of invasive

Salmonella Enteritidis infections needs further elucidation. Population-based monitoring of iNTS incidence and viral load data may guide ART programmes, enhancing enrolment of HIV-infected men.

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Introduction

Despite the introduction and rapid uptake of antiretroviral therapy (ART) in South Africa in 2004,[1] South Africa has among the highest prevalence of HIV infection in the world.[2] In 2013, the estimated national seroprevalence of HIV infection in South Africa was 15% in adults aged 15 – 49 years,[3] with an estimated 1000 new infections daily.[4] High prevalence of HIV infection in South Africa places a tremendous burden on the health systems within the country, due in largely to opportunistic infections, including invasive non-typhoidal salmonellosis (iNTS). In parts of South Africa where ART coverage is high, declining HIV transmission rates and improvements in health-related quality of life have been noted.[5] Previous researchers have attempted to measure the impact of increased ART use among HIV-infected persons on the incidence of opportunistic infections in South Africa, with mixed results. Nunes et al showed increased ART use among HIV-infected persons was associated with declining morbidity and mortality in HIV-infected children due to invasive pneumococcal disease,[6] but were unable to replicate these findings in HIV-infected adults.[7] Nanoo et al described a temporal decrease in the incidence of microbiologically confirmed tuberculosis with increase antiretroviral use among HIV-infected individuals in South Africa,[8] based on estimates of ART use, derived by the Actuarial Society of South Africa (ASSA).[3]

The impact of increased ART use among HIV-infected persons on the incidence of iNTS in South Africa has not been defined, despite the well-described association of *Salmonella enterica* serotype Typhimurium (*Salmonella* Typhimurium) ST313 with iNTS among HIV-infected persons in Africa.[9-12] At the Queen Elizabeth Central Hospital in Malawi, following the introduction of ART, a 36% reduction in iNTS incidence in adults was described:[13] however, it was unclear how much of the reduction in iNTS incidence in children in Malawi could be ascribed to increased ART use since there were also improvements in nutrition

status and declines in prevalence of malaria during this period.[14] Researchers in other African countries have also recently described declines in iNTS incidence, but have not specifically related this decline to increased ART use.[15,16] Concerns have also been raised that adult men may not be accessing ART programmes: Bor et al reported HIV-related mortality in a rural area of South Africa declined significantly more in women than men (p=0·05).[17] This study was undertaken to describe associations between iNTS incidence and ART use among HIV-infected persons in Gauteng Province, South Africa, a malaria-free province with an urbanised population with good health care access,[4] low prevalence of malnutrition, and an HIV-seroprevalence of 11%.[3]

Methods

Invasive non-typhoidal Salmonella

Between 2003 and 2013, the Centre for Enteric Diseases (CED) undertook active laboratory-based surveillance for invasive non-typhoidal *Salmonella* (iNTS) at clinical diagnostic laboratories in Gauteng Province. Laboratories were requested to submit all *Salmonella* isolated from normally sterile sites to CED for further characterization, including serotyping. We defined iNTS infection as the isolation of non-typhoidal *Salmonella* from a normally sterile body site. Data at the Central Data Warehouse (CDW) of the National Health Laboratory System (NHLS) were reviewed to confirm reporting of all iNTS infections: CDW is a repository for all public sector laboratory results in South Africa (representing over 80% of all facilities, >43 million people) which includes all microbiology and HIV-related laboratory tests conducted across public health laboratories. We recorded data on age and sex for all patients presenting with iNTS infection. All *Salmonella* isolates received were serotyped following CED standard operating procedures (Mast Group, Merseyside, UK; BioRad, Marnes-la-Coquette, France; Remel, Kent, UK; Statens Serum Institute,

Copenhagen, Denmark). Data were recorded in an Access 2007 database (Microsoft Corp, Redmond, WA, USA).

Antiretroviral use among HIV-infected persons

Since the availability of ART in 2004, routine management of HIV-infected persons in South Africa includes ART and measurement of HIV viral load at least annually; most patients on ART in South Africa have viral load testing performed at a NHLS laboratory. We therefore used CDW's data on HIV viral load measurement tests to estimate the number of persons in Gauteng Province using ART from 2004 to 2013. Viral load data were de-duplicated by removing repeat viral load tests performed on the same patient in the same calendar year (supplementary table 1). As a sensitivity analysis, we compared our estimate of the number of HIV-infected persons ≥15 years of age on ART to an unpublished report from the South African National Department of Health (NDoH).[18]

Statistical Analysis

To derive estimates of the incidence of iNTS and estimates of the incidence of the number of persons on ART, population denominators were derived from mid-year data estimates data published annually by the national Department of Statistics (www.statssa.gov.za). Incidence was also estimated for the following age groups: <5 years; 5 – 14 years; 15 – 24 years; 25 – 49 years; and ≥50 years.

A Poisson regression model was used to measure the change in incidence of iNTS infection from 2003 to 2013, and change in incidence of ART use from 2004 to 2013. We additionally determined the correlation between the incidence of iNTS and ART use from 2004 to 2013 using Pearson's correlation co-efficient. Analyses were performed using Stata version 13 (StataCorp Limited, College Station, TX, USA).

Results

Invasive non-typhoidal Salmonella

From January 2003 – December 2013, we identified 4,886 cases of iNTS from Gauteng Province, South Africa. Sex was recorded for 4,728 (96·8%) patients: 2,478 (52·4%) were male. Ages were available for 4,661 (95·4%) patients, ranging from 0 days (newborn) to 93 years, with a median of 32 years. The incidence of iNTS (cases per 100,000 population) per year increased from 5·0 in 2003 to 5·8 in 2004, after which there was a steady decrease to 2·2 in 2013 (table 1). The highest incidence of iNTS was in children <5 years of age; the lowest incidence was in children aged from 5 to 14 years (figure 1).

Serotyping was completed on 4,459 (91·2%) of isolates: the most common serotypes were *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium) 2,469 (55·4%) and *Salmonella* Enteritidis, 1,156 (25·9%).

The incidence of iNTS decreased from 2003 to 2013 (incidence rate ratio [irr]=0·91, 95% confidence interval [CI]=0·90 – 0·.92, p<0·001) (figure 1; supplementary table 2). By serotype, invasive *Salmonella* Typhimurium infections also decreased between 2003 and 2013 (irr=0·79, 95% CI=0·78 – 0·81, p<0·001) (supplementary table 2). Invasive *Salmonella* Enteritidis infections, however increased over the same period (irr=1·14, 95% CI 1·12 – 1·22, p<0·001) (supplementary table 2).

ART use

After removing repeat viral load tests on the same patient during the same calendar year, 1,940,203 viral load tests were done in Gauteng between 2004 and 2013. Of these, 677,246 (35·3 %) were done on male patients (19,511 had no gender stated) and 174,477 (9·0%) were done on children <15 years of age (supplementary table 1). Annually, the number of viral load tests increased as follows: 7,849 tests in 2004 (75·2 per 100,000 population); 2005: 35,059 tests (326·7 per 100,000 population); 2006: 79,671 tests (726·6 per 100,000

population); 2007: 118,550 tests (1058·3 per 100,000 population); 2008: 176,950 tests (1,546·0 per 100,000 population); 2009: 209,445 tests (1,791·1 per 100,000 population); 2010: 220,217 tests (1,843·4 per 100,000 population); 2011: 271,179 tests (2,222·4 per 100,000 population); 2012: 360,432 tests (2,891·8 per 100,000 population); 2013: 460,806 tests (3,620·3 per 100,000 population).

Association between incidence of iNTS and incidence of ART use

There was a correlation between increased use of ART and decreased incidence of iNTS infection per 100,000 population per year from 2004 to 2014· This correlation was observed in all age groups ≥5 years of age: (5 – 14 years, r=-0·69, p=0·03; 15 – 24 years, r=-0·84, p=0·002; 25 – 49 years, r=-0·92, p<0·001; ≥50 years, r=-0·70, p=0·02) (Figure 2a; supplementary table 3). Specifically analysing invasive disease due to *Salmonella* Typhimurium, there was a significant decrease in incidence which correlated with the increased use of ART from 2004 to 2013 (r=-0·93, p<0·001) (Figure 2b; supplementary table 4). This correlation was not observed with invasive disease due to *Salmonella* Enteritidis, which increased significantly over the period (r=0·95, p<0·001) (Figure 2c; supplementary table 5).

Comparing rates of iNTS infection against numbers of HIV-infected adult men versus those in HIV-infected adult women, between 2003 and 2004, fewer men presented with iNTS (2003, 29·9 versus 41·0, rate ratio=0·73; 2004, 38·7 versus 43·3 per 100,000, rate ratio=0·89 respectively), but this was reversed from 2005 through 2013 (2005, 38·2 versus 35·5, rate ratio=1·07; 2006, 44·6 versus 33·1, rate ratio 1·36; 2007, 27·0 versus 25·3, rate ratio=1·06, 2008, 35·3 versus 23·0, rate ratio=1·53; 2009, 31·3 versus 17·6, rate ratio=1·78; 2010, 27·7 versus 18·4, rate ratio=1·78; 2011, 19·6 versus 16·5, rate ratio=1·51; 2012, 22·6 versus 14·8, rate ratio=1·53; 2013, 20·0 versus 13·7, rate ratio=1·44) (supplementary table 6). Regarding

access to antiretrovirals, adult men in Gauteng Province accessed ART consistently less than adult women, ranging from a rate ratio of 0·61 in 2004 to 0·67 in 2013 (2004 to 2013: p<0.001) (supplementary table 6).

In the sensitivity analysis, there was a good correlation between number of HIV-infected persons ≥15 years of age on ART in the ASSA estimate and in our estimates (r=0·93, p<0·001) compared with the unpublished data from NDoH.[18]

Discussion

There is a high incidence of invasive salmonellosis in Africa, due to several important predisposing factors, including malaria, malnutrition and HIV infection[11,19]. In South Africa, the major contributing factor to invasive salmonellosis is HIV infection:[20] the introduction of ART is thus critical to preventing iNTS infections. We examined the incidence of iNTS in Gauteng Province, which has a predominantly urbanised population, and documented a significant decrease in the incidence of iNTS cases in a period of increased ART utilisation which followed the introduction of ART in 2004.

A decrease in iNTS incidence has been described in other countries in Africa where malaria is endemic.[14-16] Malaria is an uncommon disease in South Africa, and Gauteng Province is malaria-free; therefore, we conclude that the decrease in iNTS incidence in South Africa is not due to malaria control efforts but due to the introduction of ART. In South Africa, almost all HIV-infected persons obtain ART through government HIV clinics. Our novel method for estimating the number of patients using ART takes advantage of the widely implemented HIV management protocol in South Africa that HIV-infected patients on ART should have viral load testing done at least annually to monitor their response to antiretrovirals.[1] Therefore, our study utilized data collected in a province where the population has good

access to healthcare, and included almost comprehensive HIV viral load data from HIV-infected persons in this province. Other studies examining the association of tuberculosis and HIV in South Africa[8] utilised the less complete ART use estimates from ASSA which are based on prospective statistical modelling.[3] In our sensitivity analysis, we showed that our estimate of ART use, based on HIV viral load data, correlated well with the incomplete ASSA ART use estimates. However, because of the more robust data on viral load measurement tests used for our ART use estimates, we believe our estimate on ART is a better indication of the true number of patients accessing ART.

Tanser et al showed a decline in HIV acquisition in HIV-discordant couples in South Africa, supporting the importance of the national antiretroviral programme in controlling HIV.[21] We demonstrate the additional significant impact of ART on prevention of iNTS infection. Although we only examined data from a single province, ART use has been implemented in South Africa and these findings can likely be extrapolated to other provinces.

We elected to separate children under five years from those aged 5 – 14 years for two reasons: firstly, as has previously been shown, children in the former age group are predisposed to a high mortality due to diarrheal diseases[22] and may thus have different predisposing factors for iNTS. Secondly, there are differences in the HIV rates and clinical presentation between these two age groups; HIV-infected children living beyond five years were an unusual event prior to the introduction of perinatal ART and the ART roll-out.

Our data showed a significant correlation between the declining incidence of *Salmonella* Typhimurium and increasing incidence of patients accessing ART, suggesting this serotype specifically may act as an indicator pathogen for the response to ART in South Africa. More specifically, this decrease was observed across individual age groups and most notably in patients aged 25 to 49 years, who bear the highest burden of HIV infection in South Africa.

An excessive burden of *Salmonella* Typhimurium, representing 85% of iNTS isolates, associated with multidrug resistance, has been described from other African studies and has been partly associated with HIV status.[23] We have previously described the predominance of *Salmonella* Typhimurium ST313, associated with iNTS meningitis in HI- infected patients:[12] we suspect this particular pathogen, which emerged in Africa with the HIV epidemic,[9] was equally responsible for much of the invasive disease we identified here.

The incidence of invasive disease due to *Salmonella* Enteritidis has increased in South Africa. This was observed across all age groups over the time period, despite the ART roll-out, and is not easily explainable. We suspect that this have been due in part to ill-defined associations between food safety and food security: the economic outlook of the country has decreased dramatically over the time period, with the gross domestic product (GDP) growth rate halving during the period (http://www.africaneconomicoutlook.org/en/statistics/table-2-real-gdp-growth-rates-2003-2013/), although the population increased by approximately 50% (www.statssa.gov.za). In addition, new evidence suggests that some strains of *Salmonella* Enteritidis, similar to *Salmonella* Typhimurium ST313, may have become adapted in Africa to human-to-human transmission.[24] Interestingly, Muthumbi et al conversely found *Salmonella* Enteritidis was replaced by *Salmonella* Typhimurium over the comparable time period.[16] *Salmonella* Typhimurium appears primarily associated with nosocomial transmission in South Africa.[25] We suspect that as HIV transmission is controlled through various management programmes, the importance of other factors associated with iNTS in South Africa (Keddy et al, submitted), where malaria plays an insignificant role, will become more apparent: in the future, we may see disease trends comparable with those of industrialised countries in patients presenting with iNTS.[11]

It is concerning that adult men appeared to access ART at a slower rate than adult women, which may be translating into a greater risk of opportunistic iNTS. Previously we showed approximately 90% of iNTS cases in adults are HIV-associated (Keddy et al, submitted) and assumed that this would impact on the rates of HIV-infected men versus women presenting with iNTS from 2003 to 2013. In 2003 and 2004, when ART was introduced, iNTS incidence rates per 100,000 HIV-infected adult men were less than those in adult women, but this reversed in 2005, and has remained so through to 2013, as ART became more accessible to the Gauteng population. Excess HIV-associated mortality in adult men, in association with a delayed ART access has previously been described in South Africa:[17] adult men may benefit from targeted interventions for HIV management programmes.

This study has limitations. Firstly, although we attempted to comprehensively record Gauteng iNTS cases during the study period, some may have been were missed by our surveillance system. However, due the large numbers of patients with iNTS and extensive ART data from 2004 to 2013, any potential effect of missed cases on our analyses would be minimal. Secondly, some HIV clinics possibly were not following HIV-management guidelines in obtaining HIV viral load estimates for patients on ART, resulting in our underestimating the number of HIV-infected persons on ART; we judge this to have limited effect on our findings since such underestimations are more likely to have occurred in more recent years, when greater numbers of HIV-infected persons were on ART.[4] The most important limitation of this study is the ecological nature of the study design. However, the strong scientific evidence that ART prevents opportunist infections among HIV-infected persons clearly supports the plausibility of our finding that increased use of ART among HIV-infected persons is associated with a decreased incidence of iNTS infections, and our methodology was validated by our sensitivity analysis based on unpublished NDoH data.

In conclusion, we showed iNTS decreased dramatically in Gauteng Province, South Africa, particularly iNTS infections caused by *Salmonella* Typhimurium, during a period of increased ART utilisation. Continual monitoring of iNTS and *Salmonella* Typhimurium in particular, may act as an early alert to further successes or potential failures in the HIV treatment programme. We also noted a concerning increase in invasive *Salmonella* Enteritidis cases, for poorly understood reasons, needing further elucidation. Adult men may not be accessing ART programmes optimally and should be the focus of targeted interventions. Our data should significantly enhance current global efforts to define more accurate burdens of disease for invasive salmonellosis. It will also contribute to current understanding of how ART is accessed in countries with high burdens of HIV infection and allow better understanding how this access has decreased iNTS rates, independently of other interventions, such as malaria control, in Africa.

Competing Interests

KHK: Nil Declared

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Ethics

Ethical approval for this study was granted by the Human Research Ethics Committee of the University of the Witwatersrand (M110601).

Authors' contributions

All authors contributed to the writing of this manuscript and reviewed the final content. KHK, FJA and KPK designed the study; KHK and AS developed the database; KHK and AK were responsible for reviewing clinical data; AS and KHK were responsible for the laboratory characterization of *Salmonella* isolates; ST and AJP developed the protocols and were responsible for cleaning and analysing viral load data; KHK, ST and AM completed the data analysis.

Disclaimer

The findings and conclusions in this report are solely the responsibility of the authors and do not necessarily represent the official position of the National Institute for Communicable Diseases / National Health Laboratory Service or US Centers for Disease Control and Prevention (CDC).

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Table 1. Population and number (incidence per 100,000 population) of invasive non-typhoidal *Salmonella* (iNTS) cases per year, Gauteng Province, South Africa, 2003 – 2013.

Year	Population*	Number of iNTS cases (iNTS incidence)
2003	10,273,446	510 (5·0)
2004	10,500,732	605 (5·8)
2005	10,730,594	541 (5·0)
2006	10,964,701	598 (5·5)
2007	11,202,290	463 (4·1)
2008	11,445,709	491 (4·3)
2009	11,693,933	398 (3·4)
2010	11,946,060	389 (3·3)
2011	12,202,306	304 (2·5)
2012	12,463,886	306 (2·5)
2013	12,728,438	281 (2·2)

*www.statssa.gov.za

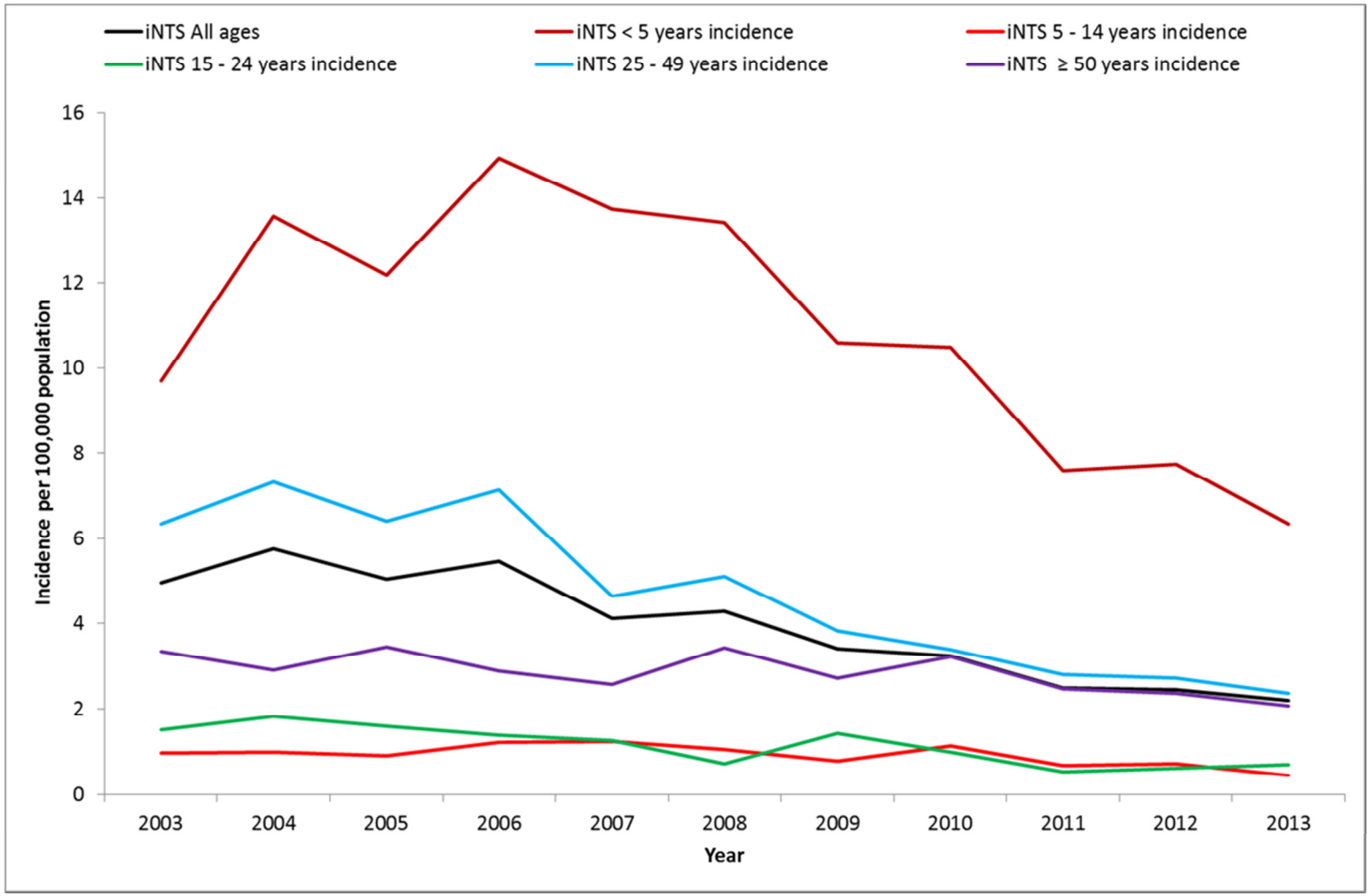

Figure 1. Incidence of invasive *Salmonella* per 100,000 population per year in Gauteng Province, South Africa, by age group, 2003 – 2013. (Test for trend: All ages, incidence rate ratio (IRR)=0.91, 95% CI=0.90 – 0.92, p<0.001; <5 years, IRR=0.95, 95% CI=0.93 – 0.96, p<0.001; 5 – 14 years, IRR=0.95, 95% CI=0.91 – 0.99, p=0.03; 15 – 24 years, IRR=0.90, 95% CI=0.87 – 0.94, p<0.001; 25 – 49 years, IRR=0.89, 95% CI=0.88 – 0.90, p<0.001; ≥50 years, IRR=0.96, 95% CI=0.94 – 0.99, p=0.007).

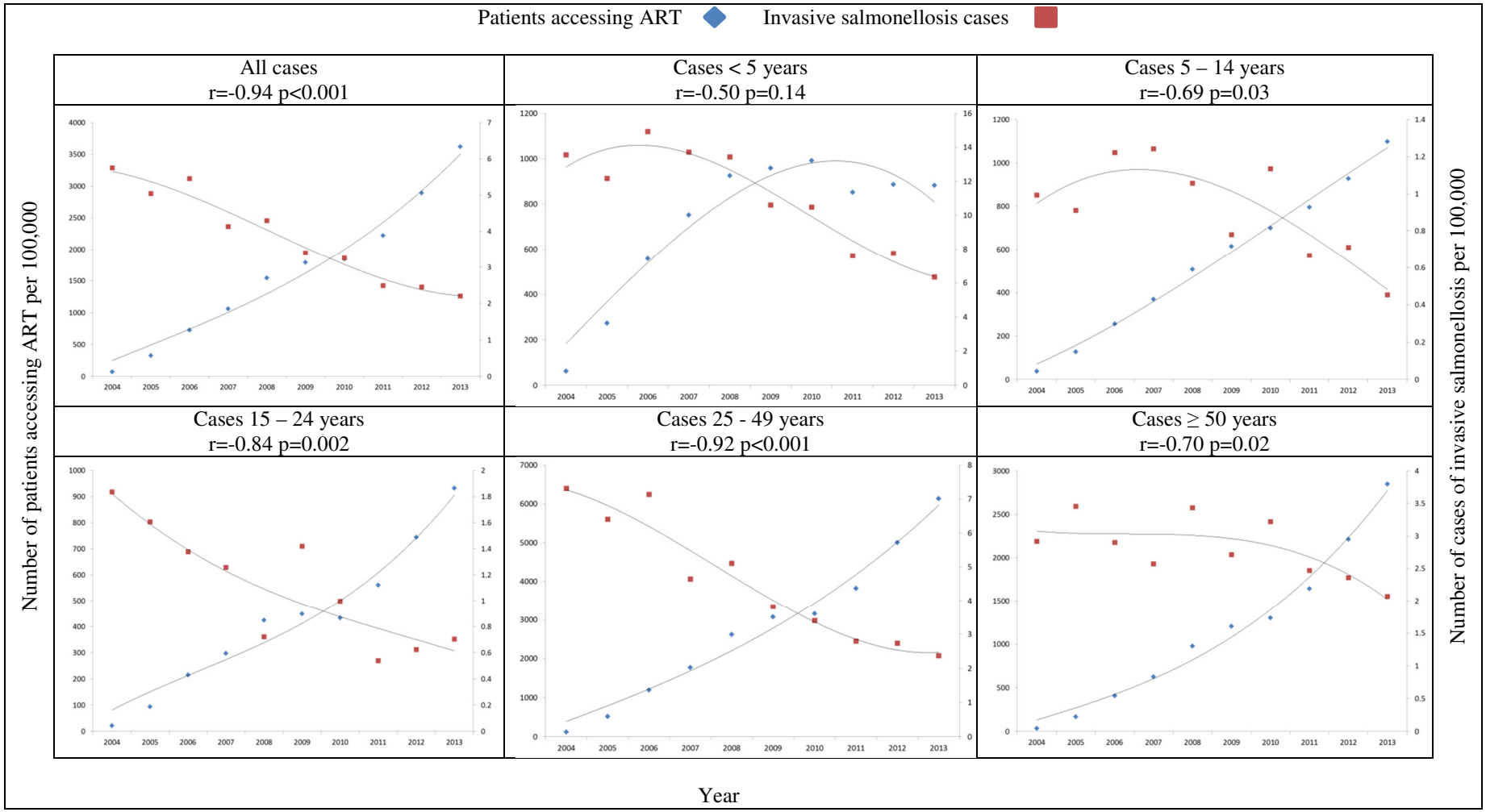

Figure 2a. Comparison of incidence of invasive *Salmonella* (iNTS) per 100,000 population per year by age range and incidence of number of patients accessing antiretroviral therapy (ART) per 100,000 population by age range, Gauteng Province, South Africa, 2004 – 2013.

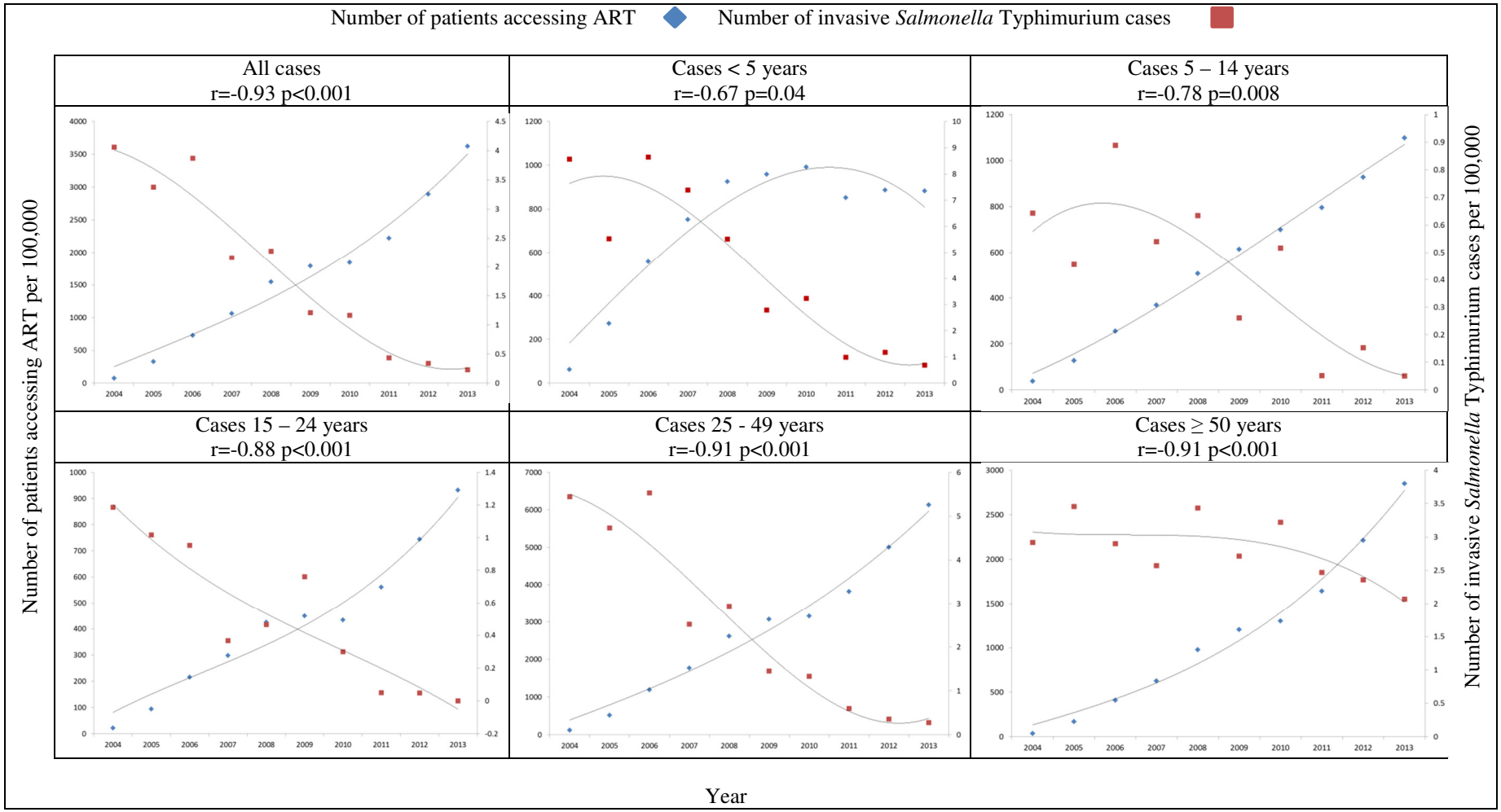

Figure 2b. Comparison of incidence of invasive *Salmonella* Typhimurium per 100,000 population per year by age range, and incidence of number of patients accessing antiretroviral therapy (ART) per 100,000 population per year by age range, Gauteng Province, South Africa, 2004 – 2013.

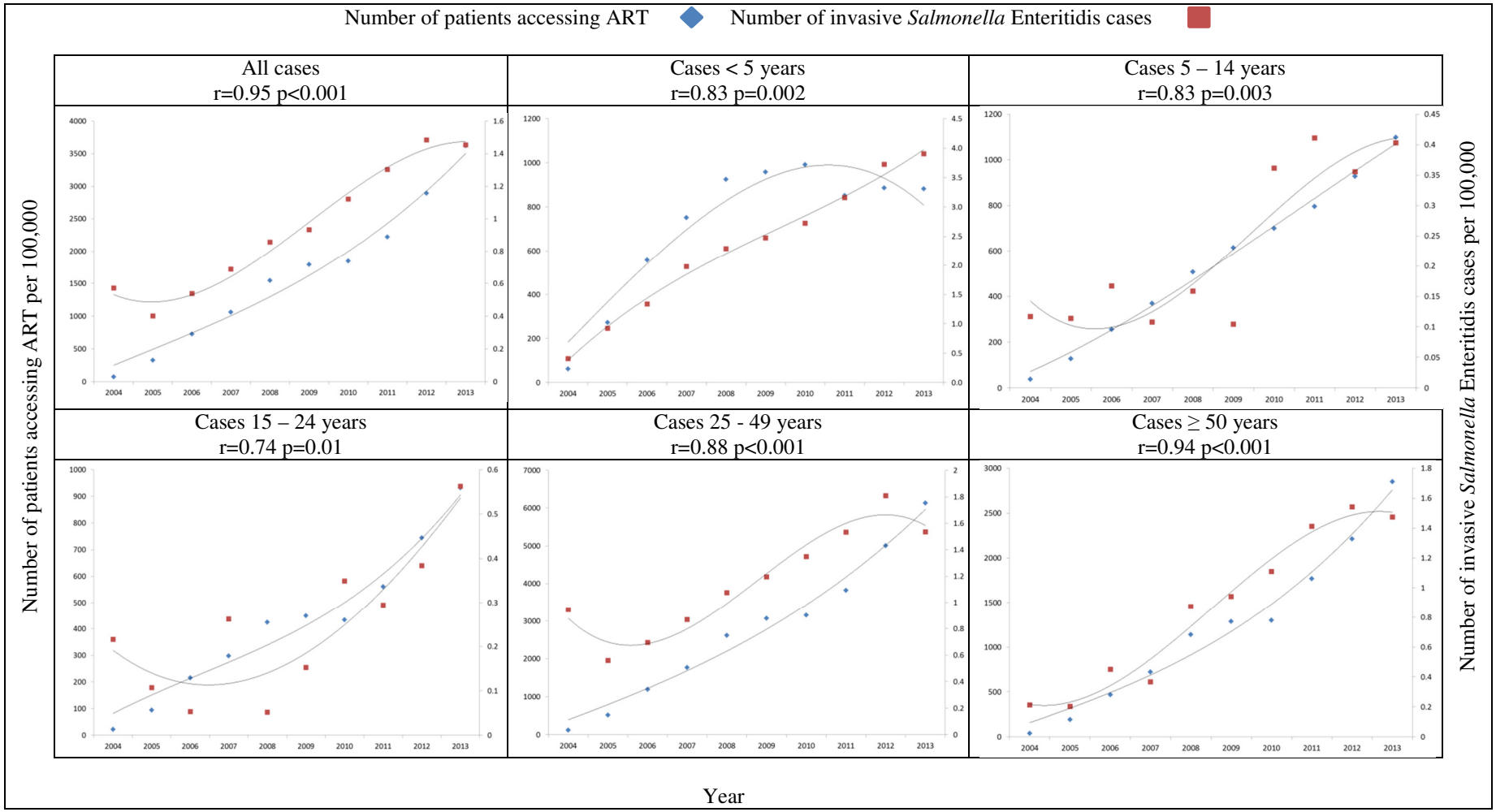

Figure 2c. Comparison of incidence of invasive *Salmonella* Enteritidis per 100,000 population per year by age range, and incidence of number of patients accessing antiretroviral therapy (ART) per 100,000 population per year by age range, Gauteng Province, South Africa, 2004 – 2013.

Supplementary table 1. Population by age group (www.statssa.gov.za) per year, and number of persons (and incidence per 100,000 population) accessing antiretroviral therapy (ART) per year based on viral load testing extracted from the Central Data Warehouse, Gauteng Province, South Africa, 2004 -2013.

Year	<5 years			5 - 14 years			15 – 24 years			25 – 49 years			≥50 years		
	Population	Population accessing ART (incidence)		Population	Population accessing ART (incidence)		Population	Population accessing ART (incidence)		Population	Population accessing ART (incidence)		Population	Population accessing ART (incidence)	
2004	980,499	606	(61.8)	1,709,956	649	(38.0)	1,853,453	392	(21.1)	4,552,587	5,396	(118.5)	1,404,237	474	(33.8)
2005	977,015	2,669	(273.2)	1,757,046	2,234	(127.1)	1,868,051	1,758	(94.1)	4,653,273	24,231	(520.7)	1,475,210	2,488	(168.7)
2006	971,544	5,413	(557.2)	1,800,450	4,587	(254.8)	1,887,926	4,065	(215.3)	4,752,778	56,850	(1,196.1)	1,552,003	6,340	(408.5)
2007	961,731	7,229	(751.7)	1,852,191	6,823	(368.4)	1,911,049	5,691	(297.8)	4,843,434	85,640	(1,768.2)	1,633,885	10,187	(623.5)
2008	960,768	8,892	(925.5)	1,892,937	9,580	(506.1)	1,940,398	8,235	(424.4)	4,932,900	128,965	(2,614.4)	1,718,706	16,768	(975.6)
2009	971,248	9,302	(957.7)	1,920,887	11,789	(613.7)	1,972,974	8,845	(448.3)	5,023,297	154,129	(3,068.3)	1,805,526	21,670	(1,200.2)
2010	991,519	9,835	(991.9)	1,937,694	13,550	(699.3)	2,006,540	8,677	(432.4)	5,116,721	161,513	(3,156.6)	1,893,586	24,610	(1,299.7)
2011	1,013,711	8,628	(851.1)	1,947,754	15,505	(796.0)	2,039,462	11,439	(560.9)	5,217,804	199,613	(3,825.6)	1,983,575	32,539	(1,640.4)
2012	1,020,369	9,040	(886.0	1,970,982	18,290	(928.0)	2,085,959	15,529	(744.5)	5,309,141	265,938	(5,009.1)	2,077,435	45,953	(2,212.0)
2013	1,025,336	9,049	(882.5)	1,985,733	21,810	(1,098.3)	2,134,067	19,883	(931.7)	5,410,194	331,955	(96,135.7)	2,173,107	61,899	(2,848.4)

Supplementary table 2. Test for trend of incidence of invasive nontyphoidal *Salmonella* (all serotypes, *Salmonella* Typhimurium, and *Salmonella* Enteritidis) per 100,000 population per year, by age group, in Gauteng Province, South Africa. 2003 – 2013.

Characteristic		IRR	(95% CI)	*P*
All nontyphoidal *Salmonella*		0.91	(0.90 – 0.92)	<0.001
	Age group			
	<5 years	0.95	(0.93 – 0.96)	<0.001
	5 - 14 years	0.95	(0.91 – 0.99)	0.03
	15 - 24 years	0.90	(0.87 – 0.94)	<0.001
	25 - 49 years	0.89	(0.88 – 0.90)	<0.001
	≥50 years	0.96	(0.94 – 0.99)	0.007
Salmonella Typhimurium		0.79	(0.78 – 0.81)	<0.001
	Age group			
	<5 years	0.85	(0.82 – 0.87)	<0.001
	5 - 14 years	0.85	(0.79 – 0.90)	<0.001
	15 - 24 years	0.78	(0.72 – 0.82)	<0.001
	25 - 49 years	0.78	(0.77 – 0.80)	<0.001
	≥50 years	0.82	(0.78 – 0.85)	<0.001
Salmonella Enteritidis		1.14	(1.12 – 1.17)	<0.001
	Age group			
	<5 years	1.17	(1.12 – 1.22)	<0.001
	5 - 14 years	1.22	(1.10 – 1.36)	<0.001
	15 - 24 years	1.18	(1.08 – 1.30)	0.001
	25 - 49 years	1.11	(1.08 – 1.14)	<0.001
	≥50 years	1.23	(1.16 – 1.30)	<0.001

IRR, incidence rate ratio; CI, confidence interval

Supplementary table 3. Incidence of invasive nontyphoidal *Salmonella* per 100,000 population per year by age group Gauteng Province, South Africa, 2004 – 2013.

Year	<5 years		5 - 14 years		15 – 24 years		25 – 49 years		≥50 years	
	Number of invasive *Salmonella* cases	(incidence)	Number of invasive *Salmonella* cases	(incidence)	Number of invasive *Salmonella* cases	(incidence)	Number of invasive *Salmonella* cases	(incidence)	Number of invasive *Salmonella* cases	(incidence)
2004	133	(13.56)	17	(0.99)	34	(1.83)	333	(7.31)	41	(2.92)
2005	119	(12.18)	16	(0.91)	30	(1.61)	298	(6.40)	51	(3.46)
2006	145	(14.92)	22	(1.22)	26	(1.38)	339	(7.13)	45	(2.90)
2007	132	(13.73)	23	(1.24)	24	(1.26)	225	(4.65)	42	(2.57)
2008	129	(13.43)	20	(1.06)	14	(0.72)	252	(5.11)	59	(3.43)
2009	103	(10.60)	15	(0.78)	28	(1.42)	192	(3.82)	49	(2.71)
2010	104	(10.49)	22	(1.14)	20	(1.00)	174	(3.40)	61	(3.22)
2011	77	(7.60)	13	(0.67)	11	(0.54)	146	(2.80)	49	(2.47)
2012	79	(7.74)	14	(0.71)	13	(0.62)	145	(2.73)	49	(2.36)
2013	65	(6.34)	9	(0.45)	15	(0.70)	128	(2.37)	45	(2.07)

Supplementary table 4. Incidence of invasive *Salmonella* Typhimurium per 100,000 population per year by age group, Gauteng Province, South Africa, 2004 – 2013.

Year	<5 years		5 - 14 years		15 – 24 years		25 – 49 years		≥50 years	
	Number of invasive *Salmonella* Typhimurium cases	(incidence)	Number of invasive *Salmonella* Typhimurium cases	(incidence)	Number of invasive *Salmonella* Typhimurium cases	(incidence)	Number of invasive *Salmonella* Typhimurium cases	(incidence)	Number of invasive *Salmonella* Typhimurium cases	(incidence)
2004	84	(8.57)	11	(0.64)	22	(1.19)	248	(5.45)	30	(2.14)
2005	54	(5.53)	8	(0.46)	19	(1.02)	220	(4.73)	41	(2.78)
2006	84	(8.65)	16	(0.89)	18	(0.95)	263	(5.53)	29	(1.87)
2007	71	(7.38)	10	(0.54)	7	(0.37)	122	(2.52)	23	(1.41)
2008	53	(5.52)	12	(0.63)	9	(0.46)	145	(2.94)	31	(1.80)
2009	27	(2.78)	5	(0.26)	15	(0.76)	73	(1.45)	17	(0.94)
2010	32	(3.23)	10	(0.52)	6	(0.30)	68	(1.33)	21	(1.11)
2011	10	(0.99)	1	(0.05)	1	(0.05)	31	(0.59)	9	(0.45)
2012	12	(1.18)	3	(0.15)	1	(0.05)	19	(0.36)	6	(0.29)
2013	7	(0.68)	1	(0.05)	0	(0.00)	15	(0.28)	5	(0.23)

Supplementary table 5. Incidence of invasive *Salmonella* Enteritidis per 100,000 population per year by age group, Gauteng Province, South Africa, 2004 – 2013.

Year	<5 years		5 - 14 years		15 – 24 years		25 – 49 years		≥50 years	
	Number of invasive *Salmonella* Enteritidis cases	(incidence)	Number of invasive *Salmonella* Enteritidis cases	(incidence)	Number of invasive *Salmonella* Enteritidis cases	(incidence)	Number of invasive *Salmonella* Enteritidis cases	(incidence)	Number of invasive *Salmonella* Enteritidis cases	(incidence)
2004	4	(0.41)	4	(0.12)	4	(0.22)	43	(0.94)	3	(0.21)
2005	9	(0.92)	9	(0.11)	2	(0.11)	26	(0.56)	3	(0.20)
2006	13	(1.34)	13	(0.17)	1	(0.05)	33	(0.69)	7	(0.45)
2007	19	(1.98)	19	(0.11)	5	(0.26)	42	(0.87)	6	(0.37)
2008	22	(2.29)	22	(0.16)	1	(0.05)	53	(1.07)	15	(0.87)
2009	24	(2.47)	24	(0.10)	3	(0.15)	60	(1.19)	17	(0.94)
2010	27	(2.72)	27	(0.36)	7	(0.35)	69	(1.35)	21	(1.11)
2011	32	(3.16)	32	(0.41)	6	(0.29)	80	(1.53)	28	(1.41)
2012	38	(3.72)	38	(0.36)	8	(0.38)	96	(1.81)	32	(1.54)
2013	40	(3.90)	40	(0.40)	12	(0.56)	83	(1.53)	32	(1.47)

Supplementary table 6. Comparison of number of HIV-infected adult men and women (> 15 years), invasive nontyphoidal salmonellosis incidence rates in adult men and women and adult men and women accessing antiretroviral therapy (ART) in Gauteng Province, South Africa, 2004 – 2013.

Year	Number of HIV-infected patients*		Number of invasive salmonellosis cases				Male-to-female rate ratio	95% Confidence interval	*P*	Patients accessing ART				Male-to-female rate ratio	95% Confidence interval	*P*
	Male	Female	Male	iNTS/ 100,000 HIV-infected	Female	iNTS/ 100,000 HIV-infected				Male	ART/ 1,000 HIV-infected	Female	ART/ 1,000 HIV-infected			
2003	457,906	475,113	137	29.9	195	41.0	0.73	0.58 – 0.91	0.002	-	-	-	-	-	-	-
2004	478,250	503,872	185	38.7	218	43.3	0.89	0.73 – 1.09	0.1	2,282	4.77	3,921	7.78	0.61	0.58 – 0.64	<0.001
2005	490,114	526,817	187	38.1	187	35.5	1.07	0.87 – 1.32	0.2	9,924	20.24	18,135	34.42	0.59	0.57 – 0.60	<0.001
2006	495,452	546,123	221	44.6	181	33.1	1.35	1.10 – 1.64	0.002	23,106	46.6	43,521	79.7	0.58	0.57 – 0.59	<0.001
2007	511,784	592,102	138	27.0	150	25.3	1.06	0.83 – 1.35	0.2	35,017	68.4	65,670	110.9	0.62	0.61 – 0.62	<0.001
2008	506,694	603,934	179	35.3	139	23.0	1.53	1.22 – 1.93	<0.001	53,428	105.4	99,637	165.0	0.64	0.63 – 0.64	<0.001
2009	501,142	615,333	157	31.3	108	17.6	1.78	1.39 – 2.30	<0.001	63,445	126.6	120,259	195.4	0.65	0.64 – 0.65	<0.001
2010	497,477	626,112	138	27.7	115	18.4	1.51	1.17 – 1.95	<0.001	65,343	131.3	128,330	205.0	0.64	0.63 – 0.65	<0.001
2011	494,379	635,183	97	19.6	105	16.5	1.19	0.89 – 1.58	0.1	80,277	162.4	161,494	254.2	0.64	0.63 – 0.64	<0.001
2012	491,862	642,927	111	22.6	95	14.8	1.53	1.15 – 2.03	0.001	108,134	219.8	217,413	338.1	0.65	0.64 – 0.65	<0.001
2013	489,469	649,234	98	20.0	90	13.7	1.44	1.07 – 1.94	0.006	138,174	282.3	272,753	420.1	0.67	0.67 – 0.68	<0.001

*Based on Actuarial Society of South Africa (ASSA) models[3].

V. Clinical and microbiological features of invasive nontyphoidal *Salmonella* in Gauteng Province, South Africa, 2003 – 2013.

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For this publication, the student was responsible for development of the study, cleaning and analysis of the surveillance data and the primary draft and final submission of the manuscript. The student is first author on this manuscript.

Clinical and microbiological features of invasive nontyphoidal *Salmonella* in Gauteng Province, South Africa, 2003 – 2013

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Abstract

Background

In Africa, HIV infection, malnutrition and malaria are important predisposing factors for invasive non-typhoidal *Salmonella* (iNTS) infections; factors associated with mortality among persons with iNTS infections are not well described.

Methods

Laboratory-based surveillance for iNTS, including sentinel site data (age, sex, HIV status, severity of illness and clinical outcome) was conducted in Gauteng, South Africa, from 2003 to 2013. Isolates were serotyped and antimicrobial resistance profiles recorded. Clinical and microbiological differences between HIV-infected and uninfected patients were defined. Risk factors for mortality were calculated using univariate and multivariate analyses.

Results

Of 4,886 iNTS infections in Gauteng from 2003 to 2013, 3,106 (63.5%) were diagnosed at sentinel sites. Among persons with iNTS infections, more HIV-infected persons were aged ≥5 years ($\chi 2$=417.6; p<0.001) and more HIV-infected children were malnourished ($\chi 2$=5.8; P=0.02). On univariate analysis, mortality among persons with iNTS infections was associated with patients aged 25-49 years (odds ratio [OR]=2.2; 95% confidence interval [CI]=1.7 – 2.7; p<0.001) and ≥50 years (OR=3.0; 95% CI=2.2-4.1; p<0.001) compared with children aged <5 years, HIV-infected patients (OR=2.4; 95% CI=1.7-3.4; p<0.001) and severe illness (OR=5.4; 95% CI=3.6-8.1; p<0.001). On multivariate analysis, mortality was associated with patients ≥50 years (adjusted OR [AOR]=3.6, 95% CI=2.1 – 6.1, p<0.001) and severe illness (AOR=6.3; 95% CI=3.8 – 10.5; p<0.001).

Conclusions

Mortality due to iNTS in Gauteng remains high primarily due to disease severity, irrespective of HIV status, indicating the need for interventions aimed at predisposing conditions, including HIV, other immune-suppressive conditions and malignancy.

Funding

PEPFAR, GAP (CDC) USA; NICD/NHLS (RSA)

Introduction

Invasive non-typhoidal *Salmonella* (iNTS) infections are an important cause of morbidity and mortality in Africa. According to recent WHO Foodborne diseases Epidemiology Reference Group (FERG) estimates, there were approximately 600,000 iNTS infections, resulting in 63,000 deaths, globally in 2010 (1); FERG estimated that 78% of iNTS infections occurred in the WHO Africa Region.

Recent studies in several countries in Africa report high numbers of iNTS infections, commonly in association with malaria and HIV (2-6). The multicentre Typhoid Fever Surveillance in Africa Programme (TSAP) study concluded that malaria is associated with a five times higher odds of iNTS disease (6). A number of studies have highlighted the high health burden of iNTS in children, frequently associated with malaria and malnutrition (3;5). Much work has also focussed on microbiological aspects of iNTS, including serotype and multidrug resistance (4;5), or iNTS disease burden (7). Several have called for development of nontyphoidal *Salmonella* vaccines (8). Although there have been reports of decreasing incidence of iNTS infections in some parts of Africa (7;9), mortality rates among persons with iNTS infections remain high (4;5;9) and factors associated with mortality among persons infected with iNTS are not well understood. A better understanding of disability and associated mortality of iNTS infections will inform decisions for control and prevention of iNTS infections (8).

This study was undertaken to describe factors associated with mortality among persons infected with iNTS in Gauteng Province, South Africa, a largely industrialized urban

population in a malaria-free area, with an HIV seroprevalence of 11% (10). We also explored the differences in clinical and microbiological features of iNTS infections in HIV-uninfected versus HIV-infected individuals as these data may inform further management of iNTS disease.

Methods

Laboratory-based surveillance for invasive non-typhoidal Salmonella

Between 2003 and 2013, the Centre for Enteric Diseases (CED) conducted active laboratory-based surveillance for iNTS in all clinical diagnostic laboratories in Gauteng Province, South Africa. Invasive disease was defined as the isolation of non-typhoidal *Salmonella* from a normally sterile body site. Laboratory audits for additional iNTS cases in Gauteng Province were conducted by reviewing data in the Central Data Warehouse (CDW) of the National Health Laboratory System (NHLS), which stores results for all microbiology tests done by the NHLS (Keddy *et al*, in preparation). In selected sites, patients with iNTS infections had additional clinical information collected regarding HIV status, other co-morbid conditions (prematurity, protein-energy malnutrition [PEM], malignancy, autoimmune disorders, hepatic or renal disease), antimicrobial management, severity of illness (utilising the Pitt bacteraemia score [PBS] ≥4 to define severe infection (11)) and outcome (recovery versus death). If the patient with iNTS infection acquired iNTS infection after hospitalization for another reason, the iNTS infection was defined as a nosocomial-acquired infection. For HIV-infected patients, we additionally documented cotrimoxazole prophylaxis, antiretroviral therapy (ART) and CD4+ counts. Laboratories were requested to submit all *Salmonella* isolated from normally sterile sites to CED for serotyping and antimicrobial susceptibility testing. All *Salmonella* isolates received were serotyped following standard operating procedures (Mast

Group, Merseyside, UK; BioRad, Marnes-la-Coquette, France; Remel, Kent, UK; Statens Serum Institute, Copenhagen, Denmark). Minimum inhibitory concentrations (MICs) were determined for ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole (cotrimoxazole), tetracycline, ciprofloxacin, and ceftriaxone, using E-test strips, according to the manufacturer's instructions (BioMérieux, Marcy-l'Étoile, France). Multidrug resistance (MDR) was defined as resistance to three or more of these antimicrobials (12). Extended spectrum β-lactamase (ESBL) production was measured using double disk testing, according to the manufacturer instructions (MAST Diagnostics, Bootie, England). Data were recorded in an Access 2007 database (Microsoft Corp, Redmond, WA, USA).

Statistical Analysis

Incidence was calculated using population denominators derived from data published annually by the National Department of Statistics (www.statssa.gov.za). To estimate risk factors for mortality due to iNTS, we calculated odds ratios (ORs), 95% confidence intervals (CIs) and p-values for age group, sex, HIV status, PBS, other comorbidities, serotype, multidrug resistance and ESBL production. For multivariate analysis, we used logistic regression to calculate adjusted odds ratios (AORs). Patients with missing data were excluded from the analyses. The $\chi 2$ test was used to compare clinical and microbiological features of HIV-uninfected with HIV-infected patients with iNTS. Two-sided P values of <0.05 were considered significant for all analyses. All statistical analyses were performed using STATA version 13 software.

Results

We recorded 4,886 laboratory-confirmed iNTS infections in Gauteng Province from January 2003 – December 2013, of which 334 (6.8%) were identified using CDW data. Of 4,728

(96.8%) iNTS-infected patients for whom sex was recorded, 2,478 (52.4%) were male; 4,661 (95.4%) patients had age recorded, ranging from 0 days (newborn) to 93 years, with a median of 32 years. The highest incidence of iNTS infection was in children aged <5 years, averaging 10.9 per 100,000 population per year during 2003-2013. The lowest incidence rate was in children aged 5 to 14 years (averaging 0.9 per 100,000 population per year).

Serotyping was completed on 4,459 *Salmonella* isolates: the most common serotypes were *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium) (2,469 [55.4%]); *Salmonella* Enteritidis (1,156 [25.9%]); *Salmonella* Isangi (175 [3.9%]) and *Salmonella* Dublin (170 [3.8%]). Antimicrobial susceptibility testing results were available for 4,347 (97.5%) isolates: 1,035 (23.8%) isolates were MDR, of which 381 (36.8%) were ESBL producers (Table 1). *Salmonella* Isangi (155/171 [90.6%]) had the highest prevalence of MDR among the most common serotypes, followed by *Salmonella* Typhimurium (808/2,240 [36.1%]). MDR among *Salmonella* Enteritidis (20/1,149 (1.7%]) and *Salmonella* Dublin (1/165 [0.6%]) isolates was low.

Sentinel site data

Additional data from the sentinel sites were available for 3,106 (63.5%) iNTS-infected patients. Outcome data were available for 2,481 (79.9%) iNTS-infected patients: 760 (30.6%) patients died (Table 2). Mortality decreased over the time period (2003: 97/263 [36.9%]; 2004: 114/301 [37.9%]; 2005: 107/302 [35.4%]; 2006: 113/311 [36.3%]; 2007: 61/248 [24.6%]; 2008: 90/304 [29.6%]; 2009: 54/192 [28.1%]; 2010: 36/170 [21.2%]; 2011: 39/147 [26.5%]; 2012: 23/123 [18.7%]; 2013: 26/120 [21.7%]). Comparing 2003-2005 with 2006-2013, there was a significant decline in mortality among iNTS-infected persons (2003-2005: 318/866 [36.7%] versus 2006-2013: 442/1,615 [27.3%]; $\chi 2=18.6$; $p<0.001$).

Of the patients with iNTS infections with available data, 1,900/2,158 (88.0 %) were HIV-infected, 113/1,945 (5.8%) had a PBS≥4, 372/2,599 (14.3%) had a nosocomial-acquired iNTS infection, 458/2,218 (20.6%) presented with other co-morbidities and 127/638 (19.9%) children presented with PEM. Among 1,900 patients with iNTS infections who were HIV-infected, CD4 counts were available for 1,308 (68.8%) patients: 663 patients (50.7%) had a CD4+ count between 0 and 50 cells per mm^3, 217 (16.6%) had a CD4+ count between 51 and 100, 239 (18.3%) had a CD4+ count between 101 and 200 and 93 (7.1%) had a CD4+count between 201 and 350 ; 293/1,457 (20.1%) patients had a previous or current history of ART use, including perinatal antiretrovirals (15 patients [5.1%]) and 426/1,354 (31.5%) patients were on cotrimoxazole.

HIV-infected versus HIV-uninfected patients

Among persons infected with iNTS, comparing clinical features of HIV-uninfected with HIV-infected individuals, there were significant differences in age range: a significantly smaller proportion of HIV-infected patients were aged <5 years (HIV-infected: 256/1,896 [13.5%] versus HIV-uninfected: 170/258 [65.9%]; $\chi 2=417.6$; $p<0.001$) (Table 2). HIV-infected individuals were less likely to present with nosocomially acquired iNTS infection (HIV-infected: 235/1,881 [12.5%] versus HIV-uninfected: 59/253 [23.3%]; $\chi 2=22.0$; $p<0.001$) and were less likely to have other comorbid conditions (HIV-infected: 302/1,898 [15.9%] versus HIV-uninfected: 96/258 [37.2%]; $\chi 2=68.4$; $p<0.001$) (Table 2). HIV-infected children presenting with iNTS were also more likely to be diagnosed with PEM (HIV-infected: 81/322 [25.2%] versus HIV-uninfected: 29/182 [15.9%]; $\chi 2=5.8$; $P=0.02$). Among persons infected with iNTS, there was no significant difference, however, between severity of illness (PBS score≥4) between the two groups (HIV-infected: 79/1,415 [5.6%] versus HIV-

uninfected: 9/204 [4.4%]; $\chi 2$= 0.5; P=0.5) (Table 2). HIV-infected patients were also more likely to present with invasive *Salmonella* Typhimurium compared with other serotypes (HIV-infected: 1,110/1,773 [62.6%] versus HIV-uninfected: 73/232 [31.5%]; $\chi 2$=118.0, p<0.001) or to present with MDR *Salmonella* isolates (HIV-infected: 491/1,753 [28.0%] versus HIV-uninfected: 38/224 [16.9%]; $\chi 2$=12.4; p<0.001) (Table 2). HIV-infected individuals were less likely to be infected with invasive *Salmonella* Enteritidis (HIV-infected: 422/1,773 [23.8%] versus HIV-uninfected: 76/232 [32.8%]; $\chi 2$=8.8, p=0.003).

Risk factors for mortality

On univariate analysis, mortality among persons with iNTS infection was associated with patients aged 25 – 49 years compared with children aged< 5 years, (odds ratio [OR]=2.2; 95% confidence interval [CI]=1.7 – 2.7; p<0.001) and with those ≥50 years of age compared with children aged< 5 years (OR=3.0; 95% CI=2.2-4.1; p<0.001), HIV-infected patients (OR=2.4; 95% CI=1.7-3.4; p<0.001) compared with HIV-uninfected patients, more severe illness (PBS≥4) (OR=5.4; 95% CI=3.6-8.1; p<0.001) and infection with a MDR *Salmonella* (OR=1.9; 95% CI=1.5-2.3; p<0.001) (Table 3). On multivariate analysis, mortality among iNTS-infected persons was associated with patients ≥ 50 years of age (adjusted OR [AOR]=3.6, 95% CI=2.1 – 6.1, p<0.001), PBS ≥4 (AOR=6.3; 95% CI=3.8 – 10.5; p<0.001) and infection with MDR *Salmonella* (AOR=1.7, 95% CI=1.2-2.3, p=0.001), but not associated with being HIV-infected (AOR=1.4; 95% C=0.9-2.3; p=0.2) (Table 3). Analysing data on PEM in children, these patients were not significantly more likely to die compared with children who did not have PEM (OR=1.5; 95 % CI=1.0- 2.4; p=0.07) (Table 3). Among HIV-infected patients, those with CD4+ counts ≤350 were significantly more likely to die (OR=0.4; 95% CI=0.2-0.7; p=0.004), but a history of ART use did not significantly impact outcome (OR=0.8; 95% CI=0.6-1.1, p=0.1)

Discussion

Invasive salmonellosis remains a challenge in Africa, due to a number of significant predisposing factors, including malaria, PEM in children and HIV infection (5;13;14). In South Africa, the major contributing factor to invasive salmonellosis is HIV infection (15). However, factors associated with mortality in patients infected with iNTS have not previously been well described.

We examined disease burdens of iNTS in the predominantly urbanised population of Gauteng between 2003 and 2013, recording a significant decrease in the numbers of iNTS cases, following the roll out of ART in 2004 (Keddy *et al*, in preparation). Incidence for iNTS were highest among children <5 years and adults aged 25 to 49 years, highlighting the vulnerability of the former and the predominance of HIV-associated infections in the latter, supporting data from previous studies and systematic analyses (14;16). Similar to other African studies, the predominant serotypes were *Salmonella* Typhimurium and *Salmonella* Enteritidis, but we additionally identified increased numbers of *Salmonella* Isangi and *Salmonella* Dublin. *Salmonella* Enteritidis and *Salmonella* Dublin isolate were predominantly susceptible to the antimicrobials tested, but in common with other African studies, multidrug resistance was common in *Salmonella* Typhimurium (4;5) and *Salmonella* Isangi. Reports from Mali indicate that multidrug resistance occurred in >90% and 40% of *Salmonella* Typhimurium and *Salmonella* Enteritidis respectively (4), whereas reports from Kenya suggest that 36% to 77% of invasive *Salmonella* Typhimurium (5;17) and 30% of invasive *Salmonella* Enteritidis are multidrug resistant (17). In the Democratic Republic of the Congo multidrug resistance occurs in >80% of these two serotypes (18). Interestingly, multidrug resistance was low in *Salmonella* Dublin in Mali (4), similar to our findings. The

predominance of *Salmonella* Typhimurium and *Salmonella* Enteritidis in Africa has highlighted these pathogens as primary serotypes for *Salmonella* vaccine development; the rapidly burgeoning multidrug resistance is making vaccine development an important imperative.

There were additional differences between HIV-infected and HIV-uninfected individuals presenting with iNTS. Children aged less than five years with iNTS infections were less likely to be HIV infected (19). Additionally, more HIV-uninfected patients with iNTS had comorbidities including chronic organ failure or malignancy, highlighting non-communicable diseases as potential risk factor for iNTS infection. Although nosocomial-acquired iNTS infections appeared more frequently in HIV-uninfected patients, this study did not examine the role of HIV clinics in patients with iNTS. Data on long term care facilities were additionally not always forthcoming, thus this finding may not be truly reflective, as some HIV-infected patients in particular may have acquired iNTS while in these care facilities. Nosocomial salmonellosis is well described in South Africa, often affecting children (20;21) and nonetheless warrants careful monitoring. In contrast to invasive shigellosis (22), we did not see an excess of adult women presenting with iNTS: intrinsic characteristics of *Salmonella*, in HIV-infected patients in particular, appear to be at play in the context of invasive disease, beyond human behavioural factors. This has already been shown for *Salmonella* Typhimurium ST313 (23;24) and *Salmonella* Enteritidis (4;25). Further differences between HIV-infected versus uninfected individuals included *Salmonella* serotype: a significantly higher proportion of HIV-uninfected individuals presented with invasive *Salmonella* Enteritidis. This lesser association of *Salmonella* Enteritidis with HIV infection may partly explain why mortality due to this pathogen was significantly lower

compared with *Salmonella* Typhimurium. This contrasts with observations of Tapia *et al* (4), who found a significantly higher case fatality of 27.8% associated with *Salmonella* Enteritidis compared with *Salmonella* Typhimurium in Mali, possibly in relation to a highly virulent clone in that country (4). HIV-infected patients in our series were more likely to be infected with multidrug resistant pathogens, which could be ascribed to their multiple exposures to antibiotics associated with management of other opportunistic infections (26). Severity of illness (PBS≥4) however was comparable between the two groups: irrespective of the primary illness, immune-suppressive conditions predispose patients to iNTS.

Other researchers have highlighted the importance of underlying immunosuppressive disease, other than HIV, as the most important risk factor in adult patients for iNTS. Older patients, neonates and isolation of invasive serotypes were identified as risk factors for iNTS infection in HIV-uninfected patients (27). Malaria and malnutrition are important risk factors for iNTS in children (9). The excessive numbers of children in our series with iNTS who were HIV-infected with PEM is concerning. HIV infection has been associated with undernourishment in children aged <5 years in South Africa (28;29), and clearly remains problematic. This may be directly due to an HIV-associated effect in our study or due to other factors such as maternal illness or death (29). This may also be indicative of ongoing failures in nutritional programmes in primary health care (30). Feasey *et al* have clearly shown that iNTS rates decrease when childhood nutrition programmes are introduced (9) and greater emphasis may be needed on such programmes in South Africa .

We elected to analyse data for children aged less than five years separately from those aged 5 to 14 years to correspond to incidence data that have been calculated for iNTS and ART programmes in Gauteng Province, South Africa (Keddy *et al*, in preparation). Firstly, the

younger group is predisposed to higher mortality due to foodborne diseases, including salmonellosis (1). Secondly, differences in the HIV rates and presentation with iNTS between these two age groups may impact analyses: HIV-infected children surviving beyond five years of age was an unusual observation before the ART roll-out. Many of these patients in our series had associated malignancies or other predisposing immune-suppressive conditions as opposed to HIV infection. We have shown iNTS incidence rates in children aged <5 years correlates poorly with ART treatment programmes (Keddy *et al*, in preparation). Additional factors, such as HIV exposure in HIV-uninfected infants born to HIV-infected mothers, may also be at play, as has been postulated for neonatal *Salmonella* meningitis (23).

We characterised risk factors for mortality due to iNTS, with a view to highlighting interventions to decrease mortality rates. Mortality among persons with infected with iNTS has decreased significantly after the introduction of ART, in other African studies (31) and we have observed a similar trend, but clinical risk factors needed clarification. HIV infection on univariate analysis, and the severely ill (PBS≥4), adults ≥25 years, and those with MDR isolates were associated with excessive mortality on multivariate analysis. Older age and disease severity were the most significant predictors of a poor outcome: excessive mortality occurs in patients with severely immunosuppressive conditions predisposing to iNTS, besides HIV infection, including, malignancy, organ failure and prematurity (27). In HIV-infected persons, we found mortality was associated with patients with a low CD4+ count, who also bore the greatest burden of illness, but not with ART use, supporting global initiatives for earlier initiation of ART (32).

This study had some limitations. Firstly, although we attempted to comprehensively identify the majority of the Gauteng iNTS cases during the study period, it is possible that some were not identified by our surveillance system, which focussed primarily on patients presenting to public hospitals. Indications and use of blood cultures are unlikely to have changed over the period, however, and the majority of cases (~60%) were identified in sentinel surveillance hospitals. This study also did not look at the impact of delayed or inappropriate antimicrobial therapy on mortality. Complete clinical information was not available for all cases at sentinel hospitals, but total case numbers were large. This should minimize the effect on the statistical analysis. Similarly, missing isolates constituted <10% of those characterised; we believe that the serotyping and drug resistance data are representative and it is unlikely those iNTS cases that were identified on audit would have significantly altered our findings.

In conclusion, we showed although mortality due to iNTS in South Africa has decreased it remains high irrespective of the patients' HIV status. We additionally noted a concerning increase in invasive cases of *Salmonella* Enteritidis across all age groups (Keddy *et al*, in preparation), for reasons that are not well understood and needing further elucidation. These observations, in combination with the prevalence of multidrug resistant *Salmonella* Typhimurium in Africa, support the need for the development of vaccines for these serotypes. As ART becomes even more readily available in South Africa, with more patients and older patients accessing antiretroviral programmes, we may see a change in disease patterns of iNTS to more closely resemble those of the developed world (14).

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Competing Interests

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Ethics

Ethical approval for this study was granted by the Human Research Ethics Committee of the University of the Witwatersrand (M110601).

Authors' contributions

All authors contributed to the writing of this manuscript and reviewed the final content. KHK, FJA and KPK designed the study; KHK and AS developed the database; KK and AK were responsible for reviewing clinical data; AS, TN, SS, MN, RL and were responsible for the laboratory characterization of *Salmonella* isolates: KHK and AM completed the data analysis.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention (CDC).

Research in context

Evidence before this study

We conducted a literature review of the PubMed database for the terms "invasive *Salmonella*" AND "HIV" AND "malaria" AND "malnutrition", refining the searches further by adding "Africa" , "antiretroviral treatment", "antiretroviral therapy" or "ART" and "mortality" to the search terms. We identified a number of studies that discussed risk factors for invasive salmonellosis and decreasing mortality rates, but there was a dearth of publications examining risk factors for mortality. There were also no studies directly comparing HIV-infected with HIV-uninfected individuals in Africa.

Added value of this study

We believe that this may be the largest study published from Africa and the only one representing a national surveillance system, examining invasive nontyphoidal salmonellosis. This study has highlighted that although incidence and mortality rates due to invasive salmonellosis may be decreasing, patients have remained at high risk for mortality, particularly in association with severity of illness, irrespective of HIV status, and in the elderly. Mortality remains at ~20%. We have moreover highlighted an added vulnerability in HIV-infected children, who are more likely to be malnourished and confirmed that it is a disease of severe immunosuppression in HIV, with more than 50% of patients having a CD4+ count of 0 to 50 cells per mm^3.

Implications of all the available evidence

In context with other studies from Africa, we have shown that a number of interventions may benefit the management of invasive salmonellosis, including childhood nutrition programmes, HIV management and treatment of other comorbidities that may place HIV-uninfected patients at risk of invasive Salmonella infection. We have uniquely highlighted risk factors for mortality, which will assist in individual case management and provided hard data to support early intuition of antiretroviral treatment. We have confirmed that multidrug resistance in invasive *Salmonella* is an urgent and continent-wide challenge. In addition, the predominant invasive serotypes we identified included *Salmonella* Enteritidis and *Salmonella* Typhimurium, which supports current initiatives for vaccine development programmes against these two pathogens.

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Table 1. Serotyping and antimicrobial resistance for invasive non-typhoidal *Salmonella* isolates (N=4,347), Gauteng Province, South Africa, 2003 – 2013.

Serotype and number of isolates tested (n)	Ampicillin n (%)	Chloramphenicol n (%)	Trimethoprim-Sulphamethoxozole n (%)	Tetracycline n (%)	Ciprofloxacin Intermediate n (%)	Ciprofloxacin Resistant n (%)	Ceftriaxone n (%)	ESBL* producers n (%)	Multidrug resistance n (%)
Typhimurium (n=2,420)	1,651 (68.2)	933 (38.6)	1,491 (61.6)	1,048 (43.3)	784 (32.4)	63 (2.6)	232 (9.5)	324 (13.4)	808 (33.3)
Enteritidis (n=1,149)	43 (3.7)	30 (2.6)	38 (3.3)	127 (11.1)	344 (29.9)	5 (0.4)	6 (0.5)	7 (0.6)	20 (1.7)
Isangi (n=171)	165 (96.5)	163 (95.3)	157 (91.8)	167 (97.7)	130 (76.0)	7 (4.1)	113 (66.1)	104 (60.8)	155 (90.6)
Dublin (n=166)	5 (3.0)	5 (3.0)	6 (3.6)	5 (3.0)	3 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
Other (n=441)	138 (31.3)	91 (20.6)	144 (32.7)	167 (37.9)	56 (12.7)	11 (2.5)	41 (9.3)	39 (8.8)	51 (11.6)
Total (n=4,347)	2,002 (46.1)	1,222 (28.1)	1,836 (42.2)	1,514 (34.8)	1,137 (26.2)	86 (1.9)	392 (9.0)	474 (10.9)	1,035 (23.8)

*ESBL, Extended Spectrum β-lactamase

Table 2. Clinical and microbiological features associated with HIV infection and invasive salmonellosis in Gauteng province South Africa, 2003 – 2013.

	HIV uninfected n (%)	HIV infected n (%)	χ^2	p
Sex				
Male	147 (13.2)	963 (86.8)	4.1	0.04
Female	107 (10.4)	920 (89.6)	-	-
Age				
<5 years	170 (39.9)	256 (60.1)	417.6	<0.001
5 - 14 years	12 (15.4)	66 (84.6)	-	-
15 - 24 years	10 (8.6)	106 (91.4)	-	-
25 - 49 years	40 (3.0)	1,281 (97.0)	-	-
≥50 years	26 (12.2)	187 (87.8)	-	-
Pitt bacteraemia (severity of illness) score				
< 4	195 (12.7)	1,336 (87.3)	0.5	0.5
≥ 4	9 (10.2)	79 (89.8)	-	-
Nosocomial infection				
No	194 (10.5)	1,646 (89.5)	22.0	<0.001
Yes	59 (20.1)	235 (79.9)	-	-
Comorbidity				
No	162 (9.2)	1,596 (90.8)	68.4	<0.001
Yes	96 (24.1)	302 (75.9)	-	-
Protein energy malnutrition (children<15 years)				
No	153 (38.8)	241 (61.2)	5.8	0.02
Yes	29 (26.4)	81 (73.6)	-	-
Salmonella *serotype*				
Typhimurium	73 (6.2)	1,110 (93.8)	118.0	<0.001
Enteritidis	76 (15.3)	422 (84.7)	-	-
Isangi	18 (28.1)	46 (71.9)	-	-
Dublin	10 (13.2)	66 (86.8)	-	-
Other serotypes	55 (29.9)	129 (70.1)	-	-
Salmonella *multidrug resistance*				
No	186 (12.9)	1,262 (87.1)	12.4	<0.001
Yes	38 (7.2)	491 (92.8)	-	-
Salmonella *ESBL*[†] *production*				
No	201 (11.6)	1,529 (88.4)	1.1	0.3
Yes	23 (9.3)	224 (90.7)	-	-

[†]Extended spectrum β-lactamase

Table 3. Risk factors for mortality among persons with invasive non-typhoidal *Salmonella* with known outcome (N=2481) in Gauteng Province, South Africa, 2003 – 2013.

	Cases	Deaths	CFR*	Univariate analysis			Multivariate analysis**		
Characteristic	n	n	(%)	OR	(95% CI)	p	AOR	(95% CI)	p
Sex									
Male	1,301	416	32.0	-	-	-			
Female	1,156	334	28.9	0.9	0.7 – 1.0	0.1	0.8	0.6 – 1.1	0.2
Age									
<5 years	534	101	18.9	-	-	-	-	-	-
5 - 14 years	90	25	27.8	1.6	1.0 – 2.7	0.05	1.4	0.6 – 3.3	0.4
15 - 24 years	132	34	25.8	1.5	1.0 – 2.3	0.08	1.8	0.9 – 3.5	0.1
25 - 49 years	1,435	480	33.5	2.2	1.7 – 2.7	<0.001	2.7	1.7 – 4.2	<0.001
≥50 years	287	118	41.1	3.0	2.2 – 4.1	<0.001	3.6	2.1 – 6.1	<0.001
HIV status									
Uninfected	255	41	16.1	-	-	-	-	-	-
Infected	1,811	572	31.6	2.4	1.7 – 3.4	<0.001	1.4	0.9 – 2.3	0.2
Pitt bacteraemia (severity of illness) score									
< 4	1,731	460	26.6	-	-	-	-	-	-
≥ 4	109	72	66.1	5.4	3.6 – 8.1	<0.001	6.3	3.8 – 10.5	<0.001
Nosocomial infection									
No	2,099	639	30.4	-	-	-			
Yes	355	112	31.5	1.1	0.8 – 1.3	0.7			
Other co-morbid conditions									
No	1,679	506	30.1	-	-	-			
Yes	437	132	30.2	1.0	0.8 – 1.3	1.0			
*Protein energy malnutrition (children<14 years)***									
No	503	96	19.1						
Yes	124	33	26.6						

CD4+ count (HIV-infected patients only)[‡]									
CD4+ ≤350	1,193	365	30.6						
CD4+ >350	94	15	16.0						
Antiretroviral treatment (HIV-infected patients only)[‡]									
No	1,106	389	27.7						
Yes	287	66	23.0						
Salmonella *serotype*									
Typhimurium	1,329	465	35.0	-	-	-	-	-	-
Enteritidis	567	137	24.2	0.5	0.5 – 0.7	<0.001	0.8	0.6 – 1.1	0.2
Isangi	84	30	35.7	1.0	0.7 – 1.6	0.9	1.7	0.8 – 3.9	0.2
Dublin	94	20	21.3	0.5	0.3 – 0.8	0.008	0.5	0.2 – 1.1	0.07
Other serotypes	224	108	26.5	0.8	0.6 – 1.1	0.2	1.3	0.8 – 2.1	0.3
Salmonella *multidrug resistance*									
No	1,678	464	27.7	-	-	-	-	-	-
Yes	588	245	41.7	1.9	1.5 – 2.3	<0.001	1.7	1.2 – 2.3	0.001
Salmonella *ESBL*[†] *production***									
No	1,995	606	30.4	-	-	-			
Yes	271	103	38.0	1.4	1.1 – 1.8	0.01			

CI, confidence interval; OR, odds ratio; AOR, adjusted odds ratio
*Case fatality rate
**Excluded from multivariate analysis: multivariate analysis includes all age groups; multidrug resistance includes ESBL producing isolates
[‡]Excluded from multivariate analysis: multivariate analysis includes both HIV-infected and HIV-uninfected patients
[†]Extended spectrum β-lactamase

Appendix II: Case Investigation Form: GERMS-SA

NATIONAL HEALTH LABORATORY SERVICE
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

GERMS-SA: National Laboratory-based Surveillance for Enteric, Respiratory and Meningeal Bacterial and Fungal Diseases in South Africa
Clinical Case Report Form 2013
National Microbiology Surveillance Unit (NMSU)
TEL: 011 555 0412 OR 011 386 6234
FAX: 011 386 6221

CRF start date: ☐☐/☐☐/☐☐☐☐ (dd/mm/yyyy) | **CRF finalised date:** ☐☐/☐☐/☐☐☐☐ (dd/mm/yyyy)

CRF completed by Surveillance Officer name: | **Signature:** | **CRF checked by Surveillance Officer name:**

Sources of data: Patient/ Guardian ☐ Clinician ☐ Medical Records ☐ No record found ☐ Not admitted ☐ Informed consent refused ☐

Was a telephonic interview done: Yes ☐ No ☐

Lab specimen No: | **Laboratory name:**

Hospital Name: | **Hospital Number:** | **Ward:** Adult ward ☐ Paed ward ☐

Gender: Male ☐ Female ☐ Unk ☐ | **Race:** Asian ☐ Black ☐ Coloured ☐ White ☐ Unk ☐

Date of Birth: ☐☐/☐☐/☐☐☐☐ (dd/mm/yyyy) DOB Unk ☐ | **Age:** ____ Days ☐ Months ☐ Years ☐ Age Unk ☐

Identity No: ☐☐☐☐☐☐☐☐☐☐☐☐☐ Identity No Unk ☐

Patient surname: | **Patient first names:**

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

Telephone No: (H) (Work) (Cell) (Neigbour)

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

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

Number of days in above facility before transfer to acute hospital: N/A ☐ 0-2 days ☐ 3-4 days ☐ >4 days ☐ Unk ☐

Date of admission to acute hospital: ☐☐/☐☐/☐☐☐☐ Date Unk ☐

Was patient transferred to another hospital? Yes ☐ No ☐ Unk ☐ **If yes, name of other hospital:**

Date of transfer: ☐☐/☐☐/☐☐☐☐

Final outcome of patient: Discharged ☐ Died ☐ RHT/ Absconded ☐ Unk ☐ **Outcome date:** ☐☐/☐☐/☐☐☐☐

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

Diagnosis related to the organism isolated: Meningitis ☐ LRTI ☐ Dysentery ☐ Diarrhoea ☐ Fungaemia/ Bacteraemia without focus ☐ Other ☐ Specify:

Organism isolated:
S. pneumoniae ☐ *Haemophilus* sp. ☐ *N. meningitidis* ☐
Salmonella sp. ☐ *Shigella* sp. ☐ *Cryptococcus* sp. ☐
Candida sp. ☐

Date of specimen collection: ☐☐/☐☐/☐☐☐☐

Site of specimen collection:
CSF ☐ Blood ☐ Joint Fluid ☐ Other ☐
Specify:

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

Temp: °C Unk ☐ | **BP** / Unk ☐ | **Mechanical ventilation** Yes ☐ No ☐ Unk ☐ | **Cardiac Arrest:** Yes ☐ No ☐ Unk ☐

GCS: /15 Unk ☐ | **Mental status:** Alert ☐ Responds to verbal commands (Disoriented) ☐ Responds to painful stimuli (Stuporous) ☐ Unresponsive/unconscious (Comatose) ☐ Sedated/Post ictal ☐ Unk ☐

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

Cotrimoxazole prophylaxis (not current treatment): Yes ☐ No ☐ Unk ☐ **Dosage:** **Frequency:**

Date initiated: ☐☐/☐☐/☐☐☐☐ Date unk ☐ | **Compliant in last month:** Yes ☐ No ☐ Unk ☐

TB treatment (from the last 3 months and current): Yes ☐ No ☐ Unk ☐ **If Yes, is it:** Pulmonary ☐ Meningeal ☐ Other ☐ Unk ☐

Drugs : 1. 2. 3. 4. | **Date initiated:** ☐☐/☐☐/☐☐☐☐ **Date stopped:** ☐☐/☐☐/☐☐☐☐

Version – 28/11/2012 1 of 4

NATIONAL HEALTH LABORATORY SERVICE
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

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

Clinical Case Report Form 2013

National Microbiology Surveillance Unit (NMSU)
TEL: 011 555 0412 OR 011 386 6234
FAX: 011 386 6221

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

Conditions predisposing patient to infections: Yes ☐ No ☐ Unknown (if yes, please specify)

Chronic lung disease incl Asthma/ COPD/ Cystic Fibrosis ☐	Chronic renal disease incl nephrotic syndrome/ Fanconi Anaemia ☐	CVA/ Stroke neuromuscular diseases/ cerebral palsy ☐	Chronic liver conditions incl cirrhosis/ liver failure ☐	Head injury/ CSF leak/ head surgery/ ventricular shunt/ cochlear implants ☐
Connective tissue diseases incl SLE ☐	Functional/ anatomic asplenia incl sickle cell disease ☐	Malaria ☐	Pregnancy ☐	Prematurity ☐ Age at birth:
Burns ☐	Alcohol dependency ☐	Current smoker ☐	Gastric acid suppression ☐	Aplastic anaemia ☐
Primary Immuno-deficiency conditions ☐	Immunosuppressive Rx (steroids, chemoRx) ☐	Metabolic diseases incl diabetes mellitus ☐	Chromosomal conditions incl Down Syndrome ☐	Protein Energy Malnution (PEM) ☐
Cardiac conditions incl valvular disease, heart failure ☐ Specify:		Malignancy ☐ Specify:	Organ transplant ☐ Specify:	Other ☐ Specify:
Surgery: Abdominal surgery ☐		Non-abdominal surgery ☐	Pancreatitis ☐	

Information regarding HIV status

HIV-related counselling offered by SO: *(to patient/family/guardian)* Yes ☐ No ☐

HIV test performed by SO: Yes ☐ No ☐

HIV status prior to this admission: Pos ☐ Neg ☐ Unk ☐

If **HIV unknown**, is there clinical suspicion of HIV? Yes ☐ No ☐ Unk ☐

HIV status at this admission: Pos ☐ Neg ☐ **Unk** ☐

If **HIV unknown**, why was patient not tested?

For children <18 months: HIV PCR done: N/A ☐ Yes ☐ No ☐ Unk ☐

Patient died ☐ Patient confused/ comatose ☐

If <5 year, was the child exposed to HIV ? N/A ☐ Yes ☐ No ☐ Unk ☐

Patient not seen ☐ Patient referred for VCT elsewhere ☐

No guardian ☐ Refused consent ☐ Reason:

Clinical markers of HIV: N/A ☐ Yes ☐ No ☐ Unk ☐

Most recent CD4 count: N/A or not done ☐

Diarrhoea > 10 days ☐ HIV wasting ☐ Oral candidiasis ☐

Absolute: Percentage % ☐☐/☐☐/☐☐☐

Suspected PCP ☐ Kaposis sarcoma ☐ Tuberculosis ☐

Most recent viral load: N/A or not done ☐

Current cryptococcal meningitis ☐

<400 ☐ 400-10 000 ☐ >10 000 ☐ ☐☐/☐☐/☐☐☐☐

Any antiretroviral use? N/A ☐ Yes ☐ No ☐ Unk ☐

If yes, is it: Current ☐ Previous ☐ Perinatal ☐ Unk ☐

If current, what was the date of initiation of HAART? ☐☐/☐☐/☐☐☐☐ Date unk ☐

If HIV positive and no current ARV use, has the patient been referred to an ARV clinic? N/A ☐ Yes ☐ No ☐ Died ☐ Unk ☐

PLEASE COMPLETE RELEVANT SECTIONS FOR SPECIFIED ORGANISMS

***Cryptococcus* spp. ONLY**

Antifungals prior to this admission:

Fluconazole: Yes ☐ No ☐ Unk ☐ If yes, date initiated: ☐☐/☐☐/☐☐☐☐ **Dose:** Daily ☐ BD ☐

Amphotericin B: Yes ☐ No ☐ Unk ☐ If yes, date initiated: ☐☐/☐☐/☐☐☐☐ **Dose:**

Is this the first episode of cryptococcosis? Yes ☐ No ☐ Unk ☐ **Weight:** ☐☐☐.☐ kg Unk ☐

Management during this admission: Antifungal therapy unknown ☐ Antifungal therapy not prescribed ☐

	Dose	Route	Frequency	Date initiated	Total number of doses/ number of days
Fluconazole		PO ☐ IVI ☐	Daily ☐ BD ☐	☐☐/☐☐/☐☐☐☐	
Amphotericin B			Daily ☐ BD ☐	☐☐/☐☐/☐☐☐☐	
Rifampicin	Yes ☐	No ☐	Unk ☐		

Was opening intracranial pressure documented at any time? Yes ☐ No ☐ Unk ☐

If yes, what was the recorded opening pressure? cm H_20 Unk ☐

On discharge, was patient given fluconazole? Yes ☐ No ☐ Unk ☐ Died ☐

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TEL: 011 555 0412 OR 011 386 6234
FAX: 011 386 6221

Laboratory specimen Number: ☐☐☐☐☐☐☐☐☐☐☐

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

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

Have any of these children been hospitalised in the last 3 months? N/A ☐ Yes ☐ No ☐ Unk ☐

Antibiotic use prior to this specimen collection date:

ABX in 24hr before specimen: Yes ☐ No ☐ Unk ☐ **Date initiated:** ☐☐/☐☐/☐☐☐☐

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

Other ABX in last 2 months (excluding last 24hrs): Yes ☐ No ☐ Unk ☐ **In last 30 days** Yes ☐ No ☐ Unk ☐

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

Antibiotic use in hospital during this admission (excluding TB therapy and cotrimoxazole prophylaxis) **Weight:** ☐☐☐.☐ kg Unk ☐ Antimicrobial therapy unknown: ☐ Antimicrobial therapy not prescribed ☐

Name of antimicrobial	Dose	Route			Freq	Date initiated	Total doses given	No of days
		PO	IVI	IMI				
1.						☐☐/☐☐/☐☐☐☐		
2.						☐☐/☐☐/☐☐☐☐		
3.						☐☐/☐☐/☐☐☐☐		
4.						☐☐/☐☐/☐☐☐☐		
5.						☐☐/☐☐/☐☐☐☐		

***Haemophilus* spp. and *S. pneumoniae* ONLY**

Vaccination status for *Haemophilus influenzae*:

If **<15 years old**, did patient receive *Haemophilus influenzae* type b (Hib) vaccine? N/A ☐ Yes ☐ No ☐ Unk ☐

Dose	Dose given			Date given
Dose 1 (6 weeks)	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐
Dose 2 (10 weeks)	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐
Dose 3 (14 weeks)	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐
Dose 4 (18 month booster – Pentaxim)	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐

Vaccination status for *S. pneumoniae*:

If **<15 years old**, did patient receive conjugate vaccine for *S. pneumoniae*? N/A ☐ Yes ☐ No ☐ Unk ☐

Dose	Dose given			Date given
Dose 1 (6 weeks)	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐
Dose 2 (14 weeks)	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐
Dose 3 (9 months)	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐
Catch up dose	Yes ☐	No ☐	Unk ☐	☐☐/☐☐/☐☐☐☐

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

If yes, give date most recently given: ☐☐/☐☐/☐☐☐☐

Source of vaccine status information:

Road to health card seen by S. officer ☐ Verbal report from caregiver/ patient ☐ Notes in medical record from RTHC ☐

Notes in medical record ☐ Directly from the clinic ☐ Other ☐ Specify:

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National Microbiology Surveillance Unit (NMSU)
TEL: 011 555 0412 OR 011 386 6234
FAX: 011 386 6221

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

***Candida* spp. ONLY**

Did this patient have a prior episode of candidaemia? Yes ☐ No ☐ Unk ☐ **If yes, date of specimen collection:** ☐☐/☐☐/☐☐☐☐

Did patient require hospitalisation in the 90 days before the first positive culture for *Candida* was drawn? Yes ☐ No ☐ Unk ☐

Was patient ever in an ICU during this hospital admission? Yes ☐ No ☐ Unk ☐

Did the patient have a central venous catheter (CVC) before or while the first positive culture was drawn? Yes ☐ No ☐ Unk ☐

Were CVCs removed or changed after the date of candidaemia? N/A ☐ Yes ☐ No ☐ Unk ☐

Did the patient receive any of the following in the 14 days before initial culture date?

Systemic antibacterials: Yes ☐ No ☐ Unk ☐ Total parenteral nutrition (TPN): Yes ☐ No ☐ Unk ☐

Did the patient receive systemic antifungal medication in the 14 days before the date the first positive culture was drawn? Yes ☐ No ☐ Unk ☐

If yes, please specify antifungals:

Antifungal drug can't be specified – unk ☐ Amphotericin B, any formulation ☐ Itraconazole (Sporanox) ☐ Caspofungin (Cancidas) ☐

Posaconazole (Noxafil) ☐ Fluconazole (Diflucan) ☐ Voriconazole (Vfend) ☐ Other:

Was systemic antifungal therapy given to treat candidaemia during this admission? Yes ☐ No ☐ Unk ☐

If yes, please specify antifungals: Antifungal drug can't be specified – unk ☐ **Weight:** ☐☐.☐kg Unk ☐

Name of antimicrobial	Dose	Route			Freq	Date initiated	Date stopped
		PO	IVI	Other			
Amphotericin B, any formulation						☐☐/☐☐/☐☐☐☐	☐☐/☐☐/☐☐☐☐
Fluconazole (Diflucan)						☐☐/☐☐/☐☐☐☐	☐☐/☐☐/☐☐☐☐
Caspofungin (Cancidas)						☐☐/☐☐/☐☐☐☐	☐☐/☐☐/☐☐☐☐
Voriconazole (Vfend)						☐☐/☐☐/☐☐☐☐	☐☐/☐☐/☐☐☐☐
						☐☐/☐☐/☐☐☐☐	☐☐/☐☐/☐☐☐☐
						☐☐/☐☐/☐☐☐☐	☐☐/☐☐/☐☐☐☐

Has *Candida* disseminated to the deep organs? Yes ☐ No ☐ Unk ☐

If yes, specify: CNS ☐ Cardiac ☐ Osteoarticular ☐ Hepatosplenic ☐ Renal ☐ Ocular ☐

Is this specimen thought by the clinicians to be causing a true infection? Yes ☐ No ☐ (thought to be a contaminant)

Is patient ≤90 days of age at the time of candidaemia? Yes ☐ No ☐ Unk ☐ **If no or unknown, questionnaire is complete**

Infant and neonatal candidaemia (≤90 days)

Is patient ≤30 days of age at the time of candidaemia? Yes ☐ No ☐ Unk ☐

What kind of delivery did the patient have? Vaginal delivery ☐ Caesarean section ☐ Unk ☐

What was the 5-minute Apgar score? ☐☐ Unk ☐ **What was the birth weight?** ☐☐☐☐ grams Unk ☐

What was the gestational age at birth? ☐☐ weeks Unk ☐ **What is the current weight?** ☐☐☐☐ grams Unk ☐

Were H2 blockers used before the date of the first positive culture? Yes ☐ No ☐ Unk ☐

How was the baby fed before the date of the first positive blood culture? Breastfeeding ☐ Formula ☐ Mixed ☐ Unk ☐ N/A (TPN only) ☐

At what age were the feeds introduced? ☐☐ days Unk ☐

Underlying conditions prior to candidaemia (check all that apply): None ☐ Unk ☐

Abdominal pathology (e.g. gastroschisis or exomphalos) ☐ Congenital malformation ☐ Surgery ☐

Postnatal steroid exposure ☐ Hyperglycaemia ☐

Appendix III: Population data used for incidence rate analysis for Paper IV from Statistics South Africa (www.statssa.gov.za)

Age	2003		2004		2005		2006		2007		2008	
(years)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0 - 4	484385	494252	488029	492470	489522	487493	490222	481321	487864	473867	488628	472140
5 - 14	817509	843329	841892	868064	865000	892046	886478	913972	913134	939057	934874	958063
15 - 24	911834	937732	917758	935696	929508	938542	944686	943240	959879	951171	978377	962021
25 - 49	2290029	2155126	2345814	2206773	2397531	2255741	2448049	2304729	2496769	2346665	2545039	2387861
≥50	614325	724924	646684	757553	681745	793465	719425	832578	758760	875124	799862	918844
Total	5118082	5155364	5240176	5260556	5363306	5367288	5488861	5475840	5616406	5585884	5746780	5698929
Grand total	10273446		10500732		10730594		10964701		11202290		11445709	

Age	2009		2010		2011		2012		2013	
(years)	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0 - 4	493955	477293	503307	488212	513229	500483	515322	505047	517229	508108
5 - 14	951069	969818	962400	975294	970593	977161	985785	985197	996499	989234
15 - 24	998644	974330	1019368	987172	1034659	1004802	1055748	1030211	1077401	1056666
25 - 49	2593653	2429644	2643534	2473187	2702271	2515533	2757022	2552119	2815959	2594235
≥50	842313	963213	885778	1007808	930625	1052949	977165	1100270	1024965	1148142
Total	5879634	5814299	6014387	5931673	6151378	6050928	6291042	6172844	6432053	6296385
Grand total	11693933		11946060		12202306		12463886		12728438	

Appendix IV: Ethics clearance certificates

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Karen Helena Keddy

CLEARANCE CERTIFICATE	**M110601**
PROJECT	Clinical and Microbiological Charaterisation of Invasive Enteric Pathogens in a South African Population from 2003 to 2010: The Interaction with HIV
INVESTIGATORS	Dr Karen Helena Keddy.
DEPARTMENT Surveillance	Divisionof Virology & Communicable Dis.
DATE CONSIDERED	24/06/2011
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 24/06/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof KP Klugman

DECLARATION OF INVESTIGATOR(S)

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

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Govender

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M081117**
PROJECT	GERMS-SA: Provision of Strategic Information through Laboratory-Based Surveillance for AIDS-Associated Bacterial and Fungal Opportunistic Infections in South Africa
INVESTIGATORS	Dr N Govender
DEPARTMENT	NICD
DATE CONSIDERED	08.11.28
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 08.12.12 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Schoub

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M060449**
PROJECT	Essential Communicable Disease Surveillance Activities of the National Institute for Communicable Disease (NICD)....
INVESTIGATORS	Prof B Schoub
DEPARTMENT	NICD
DATE CONSIDERED	06.05.05
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 06.05.08 **CHAIRPERSON** .....
pp (Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof B Schoub

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

e I APPROVAL Vpotect B

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

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

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

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

INVESTIGATORS Dr A von Gottberg

DEPARTMENT School of Pathology, NHLS

DATE CONSIDERED 02-10-25

DECISION OF THE COMMITTEE Approved unconditionally

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

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

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

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

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress form. I/we agree to inform the Committee once the study is completed.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix V: Turn-it-in Report

KHKeddy8111384PhDfinal.docx

ORIGINALITY REPORT

26%	23%	20%	14%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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Similarities are to references, common phrases, and occasional descriptions of standard methods. No plagiarism of results or discussion.

4/7/16 Keith Klugman

KEITH KLUGMAN SUPERVISOR