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Is there an association between trimethoprim-sulfamethoxazole use as prophylaxis and multi-drug resistant non-typhoidal salmonella? A secondary data analysis of antibiotic co-resistance surveillance data in South Africa - 2003-2005

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IS THERE AN ASSOCIATION BETWEEN TRIMETHOPRIM-SULFAMETHOXAZOLE USE AS PROPHYLAXIS AND MULTI-DRUG RESISTANT NON-TYPHOIDAL SALMONELLA?
A SECONDARY DATA ANALYSIS OF ANTIBIOTIC CO-RESISTANCE SURVEILLANCE DATA IN SOUTH AFRICA – 2003 – 2005

Ananta Nanoo

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Science in Medicine in Epidemiology and Biostatistics

Johannesburg, 2010

DECLARATION

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

_____

01 day of November, 2010

DEDICATION

I dedicate this work to my family, especially my husband, Daryl, and my children, Anjita and Prianca, for their patience, support and understanding, and to my parents for instilling in me the value of education.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Nanoo A. Is there an association between trimethoprim-sulfamethoxazole use as prophylactic and multi-drug resistance in non-typhoidal salmonella? GERMS-SA Principle Investigator Meeting, National Institute of Communicable Diseases; 07 November 2006, Johannesburg, South Africa.

ABSTRACT

Introduction

Given the increasing prevalence of non-typhoidal salmonella in humans, especially as an opportunistic illness associated with HIV, enhanced surveillance for non-typhoidal salmonella (NTS), including screening for antibiotic resistance, is conducted annually in South Africa. We aimed to determine whether there is an association between trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis and multi-drug resistant NTS infection, to establish whether various factors modify the relationship between TMP-SMX resistance and invasive NTS infection, to examine whether these associations vary by province, and to quantify the resistance rates of NTS to a range of antibiotics.

Methods

This study was a secondary analysis of enhanced surveillance data on NTS collected between 2003 and 2005. We used descriptive methods to assess the prevalence of NTS by year, province and serotype, and to determine the prevalence of four MDR patterns. Univariate and multivariate regression models were used to investigate the relationships between TMP-SMX prophylaxis and MDR NTS. Univariate logistic regression was used to assess the relationship between invasive NTS and TMP-SMX resistance.

Results

TMP-SMX prophylaxis is associated with the ACKSSuT pattern (OR 1.91, 95% CI 1.14 – 3.19, p=0.0080) and the AKSSuT MDR pattern (OR 2.00, 95% CI 1.26 – 3.15, p=0.0015). Being on TMP-SMX prophylaxis is associated with an increased odds of having at least one of the four MDR patterns investigated (OR 1.43, 95% CI 1.00 – 2.04, p=0.0388). We also found high rates of resistance to all antibiotics tested except for ciprofloxacin and imipenem. The highest resistance rate was observed for sulfamethoxazole (>75.85%). *S. enterica* Isangi isolates showed the highest levels of resistance, with 94.43% having at least one MDR pattern. Other factors significantly associated with MDR NTS were ESBL production, prior treatment with antibiotics, HIV status and resistance to TMP-SMX.

Discussion and conclusions

Isolates from patients on TMP-SMX prophylaxis were associated with an increased odds of having the ACKSSuT and AKSSuT MDR patterns, not taking into account other explanatory factors. These associations did not remain significant when possible

confounders were taken into account. Despite the threat of increased multi-drug resistance, TMP-SMX prophylaxis remains important in certain clinical settings.

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NOMENCLATURE

ACSSuT – multi-drug resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline

ACKSSuT– multi-drug resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, sulfonamides, tetracycline

ACSSuTNx– multi-drug resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, nalidixic acid

AKSSuT– multi-drug resistance to ampicillin, kanamycin, streptomycin, sulfonamides, tetracycline

CLSI – Clinical Laboratory Standards Institute

EDRU – Enteric Disease Reference Unit

ESBL – expanded spectrum beta-lactamase

HIV/AIDS – Human immune-deficiency virus / Acquired immune deficiency syndrome

MIC – minimum inhibitory concentration

NICD – National Institute for Communicable Diseases

NTS – non-typhoidal salmonella

TMP-SMX – trimethoprim-sulfamethoxazole (also known as cotrimoxazole)

WHO – World Health Organisation

1.0 INTRODUCTION

This section presents a brief overview of *Salmonella spp*, in particular non-typhoidal salmonellae (NTS) and antibiotic resistance. The section ends with a review of pertinent and recent literature and a statement of the aim and objectives of the research undertaken.

1.1 Background

1.1.1 Salmonellae

Salmonellae are Gram-negative, rod-shaped bacteria. There are more than 1800 serotypes (variants) of salmonellae. NTS serotypes (those which do not cause typhoid fever, i.e. all except *S enterica*, serotypes Typhi and Paratyphi) are primarily animal pathogens and their increasing prevalence in humans is therefore of interest (EMEA, 1999; Gianella, 2005). These organisms are food- or water-borne and transmission is usually faecal-oral, brought about by poor hygiene, inferior sanitation and poor-quality water supply. Whilst salmonellae are ubiquitous, they pose a particular public health problem in developing countries.

NTS infections are increasingly reported in patients with Acquired Immune Deficiency Syndrome (AIDS), who often present with recurrent NTS infections, bacteraemia and septicaemia (Arthur et al, 2001; Hohmann, 2001; Pasquali, 2004; Yen et al, 2007). HIV/AIDS is considered a risk factor for NTS infection as NTS was isolated from 35% of HIV-infected adults in a recent study (Hohmann, 2001). NTS may therefore be an opportunistic illness associated with AIDS. Other risk factors for salmonella infection include extremities of age, immune suppression, exposure to contaminated food and water (especially poultry and eggs), travel to other countries and contact with domestic / agricultural / wild animals (Hohmann, 2001; Doré et al, 2004; Pasquali, 2004).

Salmonellae may be invasive or non-invasive – this refers to the ability of the bacteria to cross the intestinal epithelium and cause systemic disease (Gianella, 2005). As immune suppression is thought to be a contributory factor to the development of invasive salmonellosis and as HIV/AIDS and/or immune suppression is a known risk factor for salmonellosis itself, this disease is of particular interest in developing countries with high HIV prevalence rates (Gianella, 2005).

1.1.2 Antibiotic resistance

Antibiotic resistance is not a new phenomenon brought about solely by antibiotic use; rather it has been in existence in some instances even before the development of antibiotics for therapeutic use. Drug resistance can therefore be attributed to a combination of two factors, namely the antibiotic, which exerts a selection pressure for the resistant organism, and the organism itself, which may be genetically predisposed to the resistance selected for by that antibiotic. Continual use of an antibiotic to which certain strains of bacteria are resistant leads to an amplification of those strains and a decreased growth of susceptible strains (Levy and Marshall, 2004). In this manner, the drug-resistant strains become the predominant ones.

Organisms may be multi-drug-resistant, meaning that they are resistant to more than one antibiotic or more than one class of antibiotics Single-drug resistance was noted quite early on in the use of antibiotics and multi-drug resistance in enteric bacteria was first noted in the 1950's (Levy and Marshall, 2004). A common pattern of resistance has been identified in salmonellae: the classic penta-resistance pattern, abbreviated as ACSSuT (Glynn et al, 1998; Helms et al, 2005). Organisms exhibiting this resistance pattern are not susceptible to the antibiotics ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines. Further resistance patterns have been observed, namely AKSSuT (with kanamycin replacing chloramphenicol), ACKSSuT and ACSSuTNx (with nalidixic acid added to the classic penta-resistance pattern) (Helms et al, 2002; Rabatsky-Ehr et al, 2004).

The problem of resistance in salmonellae is exacerbated by the burgeoning HIV epidemic and its treatment regimens, including the use of trimethoprim-sulfamethoxazole (TMP-SMX) to prevent opportunistic infections. Two major factors implicated in the development of antibiotic resistance in bacteria are the use of antibiotics and the presence (in the bacteria) of a resistance gene (EMEA, 1999). Over-prescribing of antibiotics, utilization at sub-therapeutic dosages and inappropriate selection of antibiotics for therapy have all been identified as factors associated with the development of antibiotic resistance (EMEA, 1999; School of Public and Community Health, Washington, 2000; Tambic and Andrasevic, 2002). While considerable progress has been made in understanding the biochemical / molecular mechanisms of antibiotic resistance, few studies have examined the factors associated with antibiotic resistance and quantified these associations.

1.2 Literature review

1.2.1 Prevalence of Resistant NTS Internationally

Numerous studies have acknowledged that antibiotic resistance is a burgeoning global public health problem. Levy and Marshall highlighted the increasing scope of drug resistance in organisms in terms of the drugs to which bacteria are becoming resistant, the organisms becoming resistant and the geographical distribution of antibiotic resistance (Levy and Marshall, 2004). They point out that resistance mechanisms are known to exist to all classes of antibiotics.

Helms and others examined the prevalence of *S. enterica* Typhimurium DT104 infections as well as antimicrobial resistance in this organism (Helms et al, 2005). Their work was based on surveillance data from 29 countries collected between 1992 and 2001, and noted an increase in the prevalence of DT104 infections from 8.7% in 1992 to 33% in 2001. They also reported on the increase in the proportion of resistant DT104 isolates from 15% to 42% respectively. Helms et al also found a slight decrease in the prevalence of MDR DT104 isolates from 99% to 94% but an increase in resistance to quinolones and TMP over the study period.

Vugia and co-workers examined the population-based FoodNet dataset to assess the incidence, clinical outcome and predominant serotypes of invasive salmonellae infections for the period 1996 – 1999 (Vugia et al, 2004). They found an overall annual incidence of 0.9 cases of invasive salmonellosis per 100 000 population. Vugia et al also found that 74% of cases were caused by eight salmonellae serotypes including S. *enterica* Typhimurium and S. *enterica* Enteritidis. The authors acknowledged the limitations of the study including missed cases, lack of complete data on HIV and other underlying illnesses, and lack of representativeness / generalisability to the US population. The authors also acknowledge that there is little published work on invasive salmonellosis, despite its prevalence in children, the elderly and immune-compromised people (including HIV-positive people) even given the severity of the condition.

Glynn and others also analysed the data collected by the FoodNet surveillance programme. This case-control study aimed to assess risk factors for non-outbreak-related *S. enterica* Typhimurium DT104 in the United States (Glynn et al, 2004). Glynn et al found an overall incidence of 4.3 *S. enterica* Typhimurium DT104 cases per 100 000 people. They also found that a large proportion (69%) of MDR isolates were *S. enterica* Typhimurium

DT104, and that 88% of MDR isolates exhibiting a penta-resistance pattern were *S. enterica* Typhimurium DT104.

A common finding in the study was that participants were likely to have been treated with an antibiotic (in particular one of the ACKSSuT antibiotics) to which the infecting strain of *S. enterica* Typhimurium was resistant, with an odds ratio (OR) of 2.86 (95%CI 1.3-6.1) for a matched case-control study comparing all infected individuals with healthy controls, and 5.7 (95% CI 1.8-17.4) when participants infected with MDR NTS were compared with healthy controls (Glynn et al, 2004). This OR increased to 19.7 (3.7-105.7) when individuals infected with MDR NTS were compared with people infected with NTS susceptible to all antibiotics. The case-control study design used by Glynn et al was appropriate in order to achieve the authors' goals. The authors claim that cases are similar to controls, having been chosen from the same population, but acknowledge the limitations of the study in terms of similarity of cases to those not included in the case-control study, recall bias and generalisability to the entire US population.

The Alliance for the Prudent Use of Antibiotics (APUA) reported that S. *enterica* Enteritidis and Dublin were less likely to be resistant than S. *enterica* Typhimurium isolates (APUA, 2003).

Rabatsky-Ehr and co-workers (Rabatsky-Ehr et al, 2004) conducted a review of data collected by the National Antimicrobial Resistance Monitoring System (NARMS) to determine resistance patterns and describe the spread of S. *enterica* Typhimurium phage types in the US, between 1997 and 1998. They found that S. *enterica* Typhimurium constituted 25% of NTS isolates tested. These isolates were very resistant to sulfamethoxazole (58%), streptomycin (51%), tetracycline (48%) and chloramphenicol (35%). Fifty eight percent of S. *enterica* Typhimurium isolates were resistant to at least one drug, 54% to two or more drugs and 48% to five drugs. The MDR resistance patterns (i.e. to five or more drugs) were:

- ACSSuT – 67% of isolates
- ACKSSuT – 8% of isolates
- AKSSuT – 20% of isolates

ACSSuT phenotypes were more likely to be isolated from a sterile site (i.e. invasive) as compared to the other resistance phenotypes or pansusceptible strains ($p<0.01$). This study

was quite comprehensive, with a clear and appropriate study design, and provides a good picture of the descriptive epidemiology of S. *enterica* Typhimurium R-type ACSSuT, but does not examine co-resistance factors such as immune-compromise. The study is limited to the 14 states that participated, and it is therefore questionable how generalisable this is to all of the US. The authors acknowledge this limitation, but do not address the issues of sample bias and representativeness.

1.2.2 Prevalence of Resistant NTS in South Africa / Africa

There is a paucity of information on the prevalence of NTS in Southern Africa. However, the National Institute for Communicable Diseases (NICD) has been collecting data as part of a national antimicrobial resistance surveillance programme. Kruger and others found that 59% of isolates were invasive NTS producing a positive blood culture (Kruger et al, 2004). Of the NTS isolates analysed for this period, 15.6% were expanded spectrum beta-lactamase (ESBL) producers and also demonstrated multi-drug resistance to ampicillin, TMP-SMX, chloramphenicol and nalidixic acid. Resistance to the latter implies that these infections may not be ideally treated with floroquinolones. This study focussed on characterizing the molecular epidemiology of NTS in South Africa and did not identify risk factors for co-resistance.

More recently, the NICD recorded 1874 NTS isolates for 2006, of which 52.2% were invasive (GERMSSA, 2006). They also found that 50% of 1751 isolates tested exhibited resistance to five or more antibiotics, while the prevalence of resistance to TMP-SMX was 50.7%. The most common serotype amongst both invasive and non-invasive NTS was S. *enterica* Typhimurium (68%), followed by S. *enterica* Isangi (19%) and S. *enterica* Enteritidis (8%). During the same period, the NICD also found that the highest incidence rate of invasive NTS was in the <1year age group (NICD, 2006). However, there was no analysis of what may have been associated with the antimicrobial resistance pattern observed.

Helms et al (2005) reported that S. *enterica* Typhimurium was cultured from 51.3% of NTS isolates submitted for phage typing in South Africa in 2000-2001, of which 11.2% were multidrug-resistant, while 74% of S. *enterica* Typhimurium isolates tested in Southern Africa were multidrug-resistant. As South African data was only submitted for one year, it was not possible to look at trends in prevalence of resistance over time.

Furthermore, this study was a survey of annual data and the role of co-resistance and other factors in the development of multidrug-resistance were not explored.

Mwansa and others examined the prevalence and antimicrobial resistance patterns of intestinal bacteria in HIV-infected patients in Zambia. They found that the prevalence of NTS was (Mwansa et al, 2002):

- 5% in adults with persistent diarrhoea
- 20% in children with persistent diarrhoea
- <1% in asymptomatic adults

In addition, there appeared to be an overall decrease in sensitivity to antimicrobials during the period of the study, with only 22% of 158 NTS isolates susceptible to SMX. Based on the resistance patterns observed, Mwansa et al concluded that NTS would be best treated with expensive floroquinolones as opposed to more cost-effective antibiotics.

Kariuki and others analysed the resistance profiles of 342 NTS isolates from adults admitted to hospitals in Nairobi, Kenya, between 1994 and 2003 (Kariuki et al, 2005). They reported that only 16% of isolates tested between 1994 and 1997 were susceptible to all eleven antibiotics, while 47.9% were resistant to at least three of the eleven antibiotics tested. They did not observe a significant difference in resistance by each of the serotypes identified during this study. Resistance to ciprofloxacin was not observed during the period this study was undertaken. Kariuki et al also documented an increase in the prevalence of resistance to several antibiotics, including ampicillin, streptomycin, TMP-SMX and chloramphenicol.

1.2.3 Prevalence of NTS in HIV-positive people

HIV is known to increase the frequency and severity of salmonella infection as well as the development of resistance to antibiotics by suppression of natural host defences against salmonellosis (Gianella, 1996). NTS infections are increasingly reported in patients with Acquired Immune Deficiency Syndrome (AIDS), who often present with recurrent NTS infections, bacteraemia and septicaemia (Gianella, 1996). HIV/AIDS is considered a risk factor for NTS infection: Keddy et al state that the NTS bacteraemia-specific mortality rate in HIV infection may be between 23 and 47% (Keddy et al, 2005). Also, NTS, considered an opportunistic illness associated with AIDS (especially recurrent bacteraemic NTS), was isolated from 35% of HIV-infected adults in a recent study (Doré et al, 2004).

Pegues and Miller reported that S. *enterica* Enteritidis bacteraemia affected HIV-positive individuals disproportionately (though less so recently, possibly because of the use of zidovudine which is known to act against S. *enterica* Enteritidis), a fully functioning immune system is required to effectively combat salmonellae (Pegues and Miller, 1994). They also reported that there is a genetic basis to the ability of salmonellae to invade, especially S. *enterica* Typhimurium.

Although they did not explore these issues in terms of NTS resistant to antibiotics, the factors pertaining to immune compromise and HIV status would be useful to consider as possible confounders in the relationship between exposure and outcome in this study.

1.2.4 Association between TMP-SMX prophylaxis and MDR NTS

The effectiveness of TMP-SMX as prophylaxis for opportunistic disease in HIV patients has been well documented (Anglaret et al, 1999; Wiktor et al, 1999; Grimwade et al, 2004; Grimwade et al, 2005). This strategy has been embraced by many countries to reduce morbidity and mortality in HIV-infected people by reducing their chances of contracting an opportunistic disease. The benefits of TMP-SMX prophylaxis must be weighed against the risks of developing resistance to other antibiotics and anti-malarials such as sulfadoxine-pyrimethamine (Anglaret et al, 1999; Boeree, 1999; Gill et al, 2004).

Studies such as that conducted by Martin et al have established a link between TMP-SMX usage as prophylaxis against opportunistic illnesses and an increase in TMP-SMX resistance in various bacteria (Martin et al, 1999). However, it would appear that little consideration has been given to assessing the effects of prophylactic TMP-SMX usage on bacterial co-resistance, along with other possible and/or known co-factors.

Hoge et al investigated antibiotic resistance trends in surveillance data from Thailand and found that 40% of NTS isolates were resistant to TMP-SMX (Hoge et al, 1998). However, the data was not analysed to determine the prevalence of multi-drug resistance in NTS or what other risk factors were associated with MDR NTS.

A WHO expert panel recognised that TMP-SMX, although effective as a prophylactic against opportunistic disease, could still contribute to high rates of bacterial resistance and recommended that another antibiotic be used to treat diarrhoeal infections in HIV-infected

patients on TMP-SMX prophylaxis while continuing the prophylactic treatment (WHO, 2006).

It is therefore important to determine whether there is an association between TMP-SMX usage and antibiotic resistance in NTS; and to assess whether other factors modify this relationship. An understanding of these issues may facilitate the development of strategies to minimize the impact (if any) of TMP-SMX on antibiotic resistance in NTS.

1.2.5 Laboratory-based surveillance for NTS

The use of surveillance programs to monitor antimicrobial resistance is well documented (APUA, 2003). Surveillance for antimicrobial resistance may include sentinel, comprehensive routine and enhanced surveillance methods. Each of these systems has their advantages and disadvantages and these are summarized in Table 1.

Table 1: Comparison of surveillance methodologies

SURVEILLANCE METHOD	ADVANTAGES	DISADVANTAGES
Sentinel	▪ Relatively inexpensive	▪ Does not provide prevalence information – cannot be used to influence policy
Routine	▪ Relatively inexpensive ▪ Easily maintained ▪ Data can be used to document epidemiology of disease and antimicrobial resistance ▪ Can analyse data for trends over time	▪ Data needs to be interpreted cautiously ▪ Data collection is prone to bias
Enhanced	▪ Allows for bias to be limited (with careful study design and sampling methodology) ▪ Allows for more detailed information (e.g. risk factors, clinical outcomes, hospitalization period) to be collected	▪ More costly than routine / sentinel ▪ Targeted nature, therefore cope of information collected is narrow ▪ Subject to bias introduced by sampling and testing

Surveillance data is prone to bias, especially as regards patient data collection and sampling; laboratory data (serotyping and antimicrobial susceptibility), being subject to quality assurance procedures, may be more reliable (APUA, 2003; WHO, 2002).

In addition, not all cases of disease are detected during routine or enhanced surveillance: Alos and co-workers and Hardnett et al described the "burden of illness pyramid" which illustrates how the proportion of cases detected and reported in surveillance is a fraction of the actual number of people exposed to enteric pathogens (Alos et al, 2004; Hardnett et al, 2004).

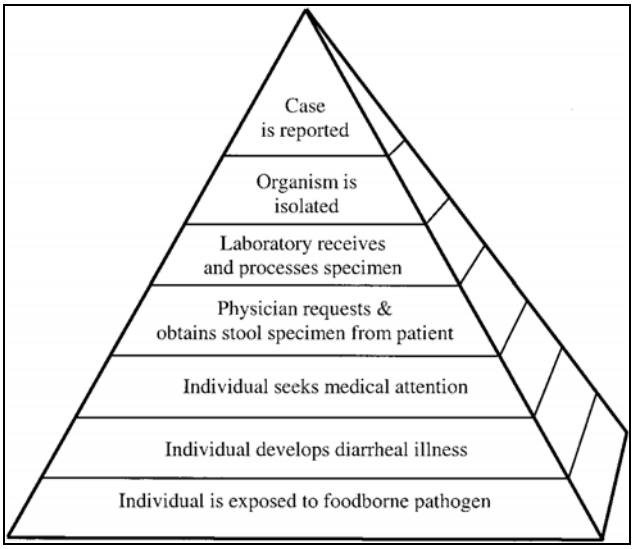


Fig. 1 The "burden of illness pyramid" used by FoodNet to assess the burden of foodborne disease in the United States (Alos et al, 2004; Hardnett et al, 2004)

Several countries have embarked on national surveillance programmes to ascertain the prevalence and sources of antibiotic resistance and some of the key findings of these programs have been previously addressed in paragraphs 1.2.1 and 1.2.2.

Blomberg et al examined the implementation of an antimicrobial resistance surveillance program at a tertiary hospital in Tanzania (Blomberg et al, 2004). They reported that laboratory-based surveillance is a good method of collecting antimicrobial resistance data, especially for purposes of applying this data locally, i.e. in choosing what would be an effective treatment against a particular organism. They affirmed that the data furnished by surveillance programs are also useful for assessing trends in antimicrobial resistance, but also pointed out certain inherent limitations in antimicrobial resistance surveillance such as selection bias and standardization of tests and data collection methodologies. Blomberg et al also found laboratory-based surveillance coupled with effective software to be a useful and cost-effective tool for gauging antimicrobial resistance prevalence and patterns.

The National Health Laboratory Services (NHLS) of South Africa established the Enteric Diseases Reference Unit (EDRU) to conduct routine surveillance for a range of enteric pathogens and to monitor antimicrobial resistance in diarrhoeagenic pathogens (von Gottberg et al, 2002). In addition, ten surveillance centres across the nine provinces of South Africa were identified and strengthened to conduct enhanced surveillance for diarrhoeal disease – this would include collecting additional information such as HIV serostatus, usage of cotrimoxazole prophylaxis, etc. This is of particular relevance in the face of widespread use of antimicrobials in general and in the treatment of the HIV / AIDS in adults and children.

It must be emphasized that the information gained in antimicrobial resistance surveillance programmes is used to quantify resistance rates of various bacteria to a range of antibiotics and to assess the prevalence of antibiotic resistance amongst the various bacterial serotypes. Few of these studies have examined the actual associations between various factors and antibiotic resistance.

1.3 Aim

This study was a secondary analysis of data collected by the EDRU as part of a national antibiotic surveillance programme for the years 2003, 2004 and 2005. The primary aim of this analysis was to show whether there is an association between the use of TMP-SMX prophylaxis and multidrug-resistance in NTS in participants in the enhanced surveillance programme for invasive diarrhoeal disease in South Africa from 2003 to 2005.

1.4 Objectives

The objectives of this study were to:

- Determine whether there is an association between prophylactic use of TMP-SMX and multi-drug resistant NTS infection
- Establish whether immune-compromised status and other factors modify the relationship between TMP-SMX resistance and invasive / non-invasive type of NTS infection
- Establish whether these associations vary by province
- Quantify the resistance rates of NTS, in total and by serotype, to a range of antibiotics (to establish a baseline prevalence of antibiotic resistance in NTS in South Africa)

1.5 Problem statement

Antibiotic resistance is an increasing public health problem for various reasons, ranging from its ease of availability and the frequency of its use in agriculture to over-prescribing and misuse (EMEA, 1999; School of Public and Community Health, Washington, 2000; Tambic and Andrasevic, 2002). This has far-reaching implications as the development of new antibiotics has not kept pace with the emerging resistance problem, and fewer effective treatments are available. In turn, the costs of healthcare are being pushed up as people require more expensive antibiotics and even hospitalization for infections which could previously have been treated with simpler, less expensive antibiotics (EMEA, 1999; School of Public and Community Health, Washington, 2000; Tambic and Andrasevic, 2002).

There is also an increasing trend of nosocomial and community-acquired infection patterns in both developed and developing countries, which further impacts on public healthcare systems (Tambic and Andrasevic, 2002; Yalcin et al, 2003). It is therefore important to determine whether there is an association between TMP-SMX usage and antibiotic resistance in NTS; and to assess whether other factors modify this relationship. An understanding of these issues may facilitate the development of strategies to minimize the impact (if any) of TMP-SMX on antibiotic resistance in NTS.

As far as can be ascertained, this was the first study of its kind on NTS in South Africa, and given the increasing reliance on antibiotics for the treatment of tuberculosis and HIV, and the emergence of hospital-acquired resistant infections, the results could form the basis of research that would have far-reaching implications for public health policy and treatment regimens.

2.0 METHODS

2.1 Study population

For the analysis of resistance prevalence, the study population was drawn from patients in over one hundred public and private hospitals, across all nine provinces in South Africa, who have a positive culture for salmonella as per the EDRU Enhanced Surveillance Protocol, Appendix A (von Gottberg et al, 2002). For the case-control analysis assessing the association of TMP-SMX prophylactic use and MDR-NTS, data from the enhanced surveillance sites was used. These sites were expanded over the period of data collection and Table 2 lists the enhanced sites that contributed isolates per surveillance year that were analysed in this study.

Table 2: Enhanced surveillance sites in South Africa between 2003 and 2005

ENHANCED SURVEILLANCE SITES CONTRIBUTING ISOLATES	2003	2004	2005
Charlotte Maxeke Johannesburg	✓	✓	✓
Chris Hani Baragwanath	✓	✓	✓
King Edward VIII	✓	✓	✓
Groote Schuur / Red Cross	✓	✓	✓
Tygerberg /	✓	✓	✓
Dr George Mukhari / Medunsa	✓	✓	✓
Universitas / Pelonomi	✓	✓	✓
Rob Ferreira / Themba	✓	✓	✓
Mthatha	✓	✓	✓
Polokwane		✓	✓
Tshwane / University of Pretoria		✓	✓
Addington / RK Khan / Prince Mshiyeni		✓	✓
Mankweng		✓	

2.2 Study design

The study design was a cross-sectional study, with outcome defined as persons infected with multi-drug resistant or susceptible NTS.

2.3 Sampling strategy

This study was a secondary analysis of data collected by the EDRU as part of a national antibiotic surveillance programme for the years 2003, 2004 and 2005. The surveillance programme has both a routine passive component for serotyping and antimicrobial susceptibility testing of all positive culture salmonella specimens obtained from hospital admissions in South Africa, as well as an enhanced component for invasive specimens.

For 2003, nine enhanced sites operated in eight provinces, expanding to sixteen sites in all nine provinces by the end of 2005.

2.4 Variables

2.4.1 Exposure

The exposure of interest was the use of TMP-SMX as prophylaxis. This variable was initially recorded as Yes / No and was recoded as a binary variable (0=No, 1=Yes) for this study.

2.4.2 Outcome

Tabulating the primary exposure against isolates resistant to more than one antibiotic showed that all patients for whom prophylactic TMP-SMX usage was known were resistant to more than one antibiotic (meaning that there would have been no controls for the analysis if MDR was defined as "being resistant to more than one antibiotic). It would therefore not have been feasible to consider multi-drug resistance in this manner.

Accordingly, the definition of multi-drug resistance was refined in terms of the multi-drug resistance patterns for salmonella that were identified in earlier research (Glynn et al, 1998; Helms et al, 2002; Rabatsky-Ehr et al, 2004; Helms et al, 2005). Multi-drug resistant isolates were defined as those isolates exhibiting one of four resistance patterns: ACSSuT, ACKSSuT, ACSSuTNx or AKSSuT. In addition, a category for all isolates that exhibited at least one of these MDR patterns was created. Binary variables were created for each of the resistance patterns examined, and these were coded as 0=No for isolates susceptible to at least one but not all, of the antibiotics in the pattern and 1=Yes for isolates resistant to all antibiotics in the pattern.

For the case control analysis, cases were defined as a culture-confirmed diagnosis of NTS infection, made at the referring diagnostic laboratory as per the EDRU's enhanced surveillance protocol, exhibiting one or more of the MDR patterns. Controls would be defined as culture-confirmed NTS isolates without any of these four resistance patterns.

2.4.3 Confounders / other explanatory variables

2.4.3.1 Demographic factors

Age was recalculated in years, using dates of birth and specimen collection preferentially, followed by age information recorded in the absence of a date of birth. Age was then

recoded into groups starting with <1 year, 1 to 4 years and then in ten-year groups up to age 64, with ≥65 years being the last group.

Sex was recoded from M and F to 0=male and 1=female respectively.

Province was recoded from one to nine as a nominal variable, to facilitate statistical analysis. Isolates were received from each province throughout the period of surveillance.

2.4.3.2 Clinical variables

HIV status was recoded from Yes / No / Positive / Negative / Unknown to a binary variable (0=Negative, 1=Positive, Unknown=.)

Similarly prior antibiotic use in the preceding two months was recoded from No / Yes to a binary variable following the convention 0=No, 1=Yes.

ESBL production was also recoded as a binary variable (0=No, 1=Yes)

During data collection and entry, invasive specimens were classified as those isolated from cerebrospinal fluid, blood culture, pleural fluid, joint fluid or pus (if from a retro-peritoneal abcess). For the purposes of this analysis, specimens were recoded into a binary variable (1=Yes/0=No) based on the existing variable for invasive isolates.

For other immune compromise, a wide range of conditions were recorded and this variable was recoded as follows:

- 0= none
- 1= chronic disease (known to have diabetes, renal failure, cardiac failure, coronary artery disease, heart disease or systemic lupus erythematosus)
- 2= known to have tuberculosis
- 3= other (known to be on immunosuppressive treatment, having cancer or immunosuppressed for organ transplant purposes, or malnourished, i.e. having kwashiorkor or marasmus)

2.4.3.3 Laboratory data

Antimicrobial susceptibility was ascertained using Clinical and Laboratory Standards Institute (CLSI) resistance testing and susceptibility breakpoints for the minimum inhibitory concentrations of antibiotics measured using E-tests (AB-Biodisk, Solna, Sweden) and disk diffusion diameters (Mast Diagnostics, Mersey, UK) for measurement

of ESBL production. The variables with susceptibility information was recoded by classifying isolates as susceptible (S) or resistant (R) according to CLSI MIC breakpoints (Wayne, 2003). Isolates with intermediate levels of resistance were recoded as resistant.

During 2003 to 2005, more than eighty salmonella serotypes were identified according to the Kaufmann-White scheme used by the reference laboratory (Kauffman, 1951). The five most prevalent serotypes were retained as individual categories (1=Typhimurium, 2=Isangi, 3=Enteritidis, 4=Species, 5=Dublin), with all the remaining serotypes being reclassified as "6=Other".

2.5 Data collection

Surveillance officers collected data on case report forms (CRFs) compiled from laboratory and clinical records (see Appendix B) at specified sites around South Africa as set out in the enhanced surveillance protocol. The data was supplemented by information from patient and family interviews, where possible, for the isolates from the enhanced surveillance sites. These CRFs were routed with the isolates to the central reference laboratory in Johannesburg. The data included on these case report forms were basic demographic data (age, sex, place of residence / province). In addition, clinical data such as HIV status, TMP-SMX use, information on other immune-compromising conditions such as tuberculosis (TB), cancer, preparation for or recent transplant, laboratory confirmation of serotype, and recent antibiotic use was recorded. Resistance to antibiotics quantified in terms of minimum inhibitory concentrations and disk diffusion diameters reflecting extended spectrum beta-lactamase (EBSL) production were recorded on separate laboratory cards at the reference laboratory and attached to the CRFs along with other relevant information (such as the patients' chart notes).

2.6 Data entry

The information in the CRFs and the accompanying laboratory cards for each year were manually entered into an EpiInfo version 6.0 database (Centers for Disease Control and Prevention, Atlanta, Georgia). Single data entry was performed (i.e. each record was only entered once, no second entry was done for comparison and error-checking). Any errors detected during quality control were corrected in writing on the CRFs and laboratory cards and manually corrected on the EpiInfo databases.

2.7 Data cleaning

The EpiInfo databases for each of the years of interest were exported in DBIV format and then re-imported into a blank MS Access database for cleaning. Relevant fields were identified and selected for inclusion in the final table for analysis. Data from the three tables were merged into one.

Using a series of queries, range checks were carried out to identify possible errors and recoding was carried out. Certain logic checks such as recoding of resistance / susceptibility based on MIC breakpoints and making sure that specimen collection date did not pre-date a patient's date of birth were done.

Duplicate, recurrent and mixed serotype isolates were identified, categorised and coded as such. The records for each of these observations were reviewed. True duplicates were identified as isolates with the same hospital number, same collection date or collected within a 21-day period of each other, same/different specimen, serotype and susceptibility testing results. Recurrent episodes were identified as isolates from patients with the same hospital number, name and serotype, collected more than 21 days apart. Mixed serotypes were defined as isolates from the same patient (as identified by hospital number and name), with the same collection date or within a 21-day period, same or different specimen from which more than one serotype was cultured with different susceptibility test results.

As recurrent episodes made up a small proportion (3.11%) of all isolates, they were treated as individual cases for the analysis of resistance prevalence. This is important because re-admissions add to the burden on the health care system. They were excluded for the case-control and regression analyses and the calculation of annual disease incidence.

Resistance to imipenem has not yet been observed in NTS in South Africa (personal communication, K Keddy, 2009), therefore the laboratory cards of isolates with MICs which implied imipenem resistance were examined – all but one (which was excluded from analysis) were errors due to data entry.

Isolates for which antimicrobial susceptibility results for a particular year were incomplete were excluded from the analysis.

2.8 Statistical analysis

Univariate analysis was carried out for each variable, to build a description of the study sample. For continuous or discrete variables, means, medians, interquartile ranges, minimum and maximum values were determined. Histograms with normal density plots were produced for age and the key antibiotic MICs. Categorical and binary data were tabulated and proportions were calculated. Appropriate graphs were used to present variables of interest. Annual NTS incidence rates (calculated as annual diagnosis rates per 100000 population) were determined using mid-year population estimates for South Africa for each year of surveillance (Statistics South Africa, 2003; Statistics South Africa, 2004; Statistics South Africa, 2005). Standard 95% confidence intervals for these incidence rates were determined using immediate commands in Stata.

Bivariate analyses of the outcome variables with the primary exposure, i.e. TMP-SMX prophylaxis, as well as with age (grouped), sex, province, immune-compromise status, antibiotic use in the last two months were carried out using χ^2 tests to determine if there were any significant differences in proportion of outcome with exposure at the 95% significance level. Fisher's exact p values were reported for tabulations with cells having an n of five or less.

Student's t-tests were used to determine if there were any differences in age between controls and each of the case types.

Mantel-Haenszel methods were used to test for interaction between exposure variables or effect modification of the primary associations by covariates, at the 95% level of significance.

Univariate logistic regression models of outcome (each of the MDR patterns) with each exposure factor in turn were run. Multivariate logistic regression models, including all exposure variables significant at the 10% level in the unvariate analysis, were developed. Likelihood ratio tests at the 5% level of significance were used to assess the fit of each multivariate model.

To investigate the relationship between resistance to TMP-SMX and invasive NTS, a univariate regression model was constructed. I attempted to fit a multivariate regression model to investigate the relationship between TMP-SMX resistance and invasive disease. However, running the Stata command for this model yielded an error message, because a

positive outcome (i.e. resistant to TMP-SMX=yes) predicted the data perfectly, i.e. all invasive isolates were resistant to TMP-SMX.

All statistical analysis was carried out using Stata version 10. Graphs were prepared using either Stata 10 or MS Excel 2003.

2.9 Ethical clearance

An application for clearance of the enhanced surveillance protocol from the Wits Health Research Ethics Committee was applied for and originally granted under protocol clearance number. M02 – 10 – 42. A separate application was made to conduct this secondary analysis, this was granted and allocated protocol number M071028. Copies of both letters from the ethics committee are attached (Appendix C).

3.0 RESULTS

3.1 Study population – Number of isolates analysed

For the period 01/01/2003 to 31/12/2005, 4765 viable salmonella (both typhoidal and non-typhoidal) isolates were received at the EDRU's reference laboratory in Sandringham. Of these, 4402 non-typhoidal salmonella (NTS) isolates were included in the analyses for antimicrobial resistance prevalence, after excluding isolates for which MICs were incomplete / not available and duplicate isolates.

TMP-SMX usage was ascertained for 772 patients and contributed in part or total to the case-control analyses done. Figure 3.1 shows how the final number of isolates included in these analyses was arrived at. The case control and regression analyses excluded recurrent episodes of infection and therefore the maximum sample size for this part of the analysis was 4265. As susceptibilities to kanamycin were not determined during 2003, the maximum sample size for the ACKSSuT and AKSSuT MDR patterns was 552. To facilitate reading of this paper, the total sample size for each analysis is stipulated in each table in which results are presented.

3.2 Missing data

Also presented in Table 3.1, is information on the extent of missing data within the dataset. From the demographic variables, both age and sex have missing values, which make up less than 10% of the data for each variable and is within the acceptable limit for missing data (personal communication, Dr R Kellerman, 2008).

The variables measuring HIV status, TMP-SMX prophylactic use, other immune compromise and prior antimicrobial use had a high percentage of missing data (80.61-88.25%) on initial inspection. However, it must be borne in mind that data for these variables is not collected for all isolates and should only be available for the isolates from the enhanced sites. For these variables it was ascertained that the majority of data came from the enhanced surveillance sites as opposed to the routine surveillance sites:

- Other immune compromise – 493/501 (98.40%) of responses were from records of isolates from the enhanced surveillance sites at which this information was routinely sourced
- HIV status – 803/827 (97.10%) of responses were from the enhanced surveillance sites
- Prior antibiotic usage – 722/742 (97.30%) of responses were from the enhanced surveillance sites

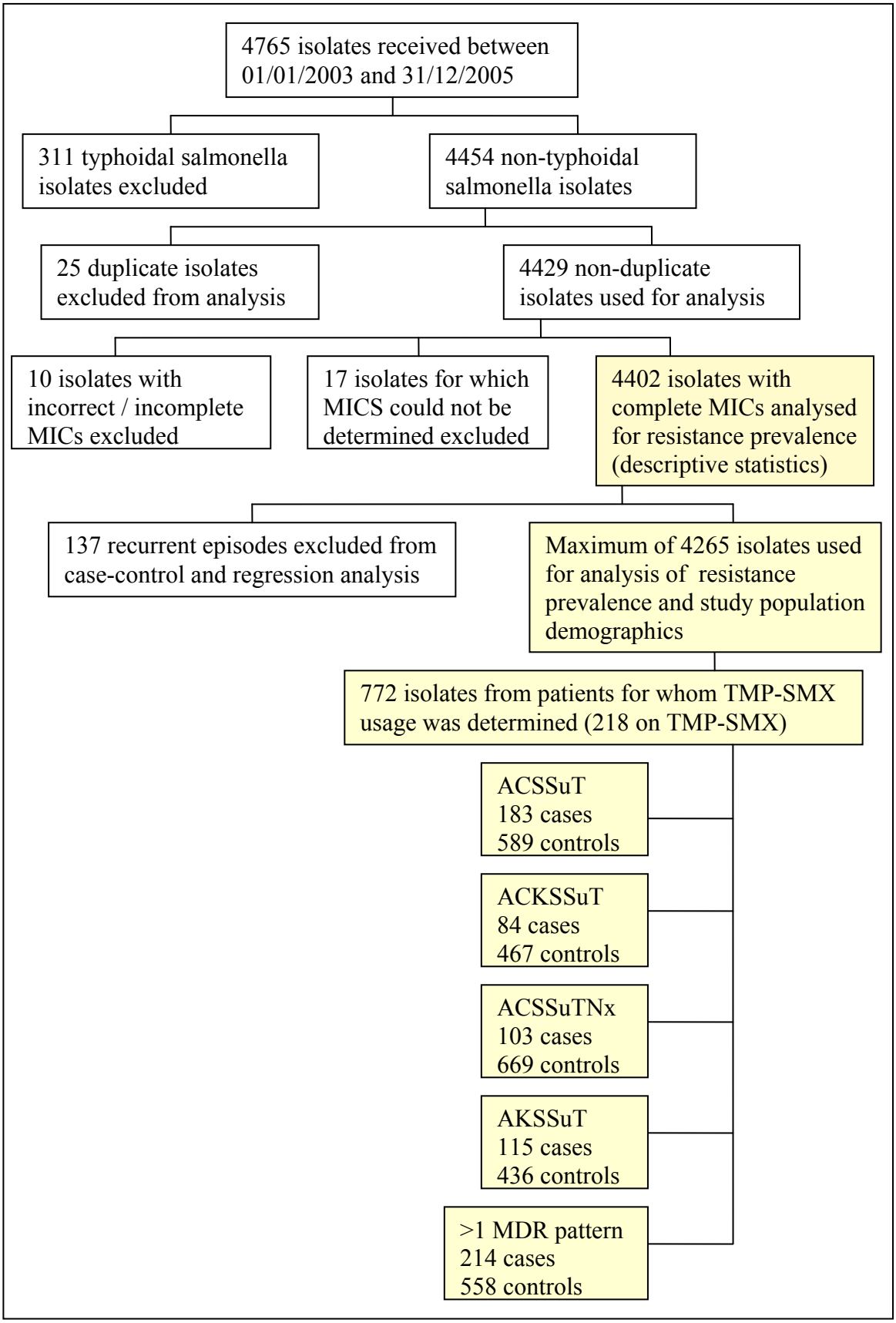


Fig. 3.1 Flow diagram showing derivation of analysis samples

- TMP-SMX prophylactic use – 750/772 (97.15%) of responses were from the enhanced surveillance sites

As such, the percentage of missing data is reduced to between 42% for HIV status and 64% for other immune compromise (since the denominator is now reduced to 1386 isolates from the enhanced sites instead of 4265 for the entire surveillance programme). This is still a high proportion of missing data, but it is important to note that for these variables, responses recorded as "Unknown" were recoded as "Missing" in order to create dichotomous variables for the analyses performed. Table 3.1 shows the proportion of missing data for each variable in the dataset.

3.3 Study population – Demographics of patients contributing isolates

In order to develop a picture of the overall study population, univariate analysis was conducted to obtain frequencies/percentages of the demographic, clinical and laboratory data. These findings are presented in Table 3.1.

3.3.1 Age

The mean age of patients from whom NTS isolates were obtained was 19.92 years (95% CI 19.27 – 20.57) and 50% of patients, for whom age was ascertained, were aged 13 years or less, as expected. The age distribution of patients from whom isolates were obtained was positively skewed (Figure 3.2).

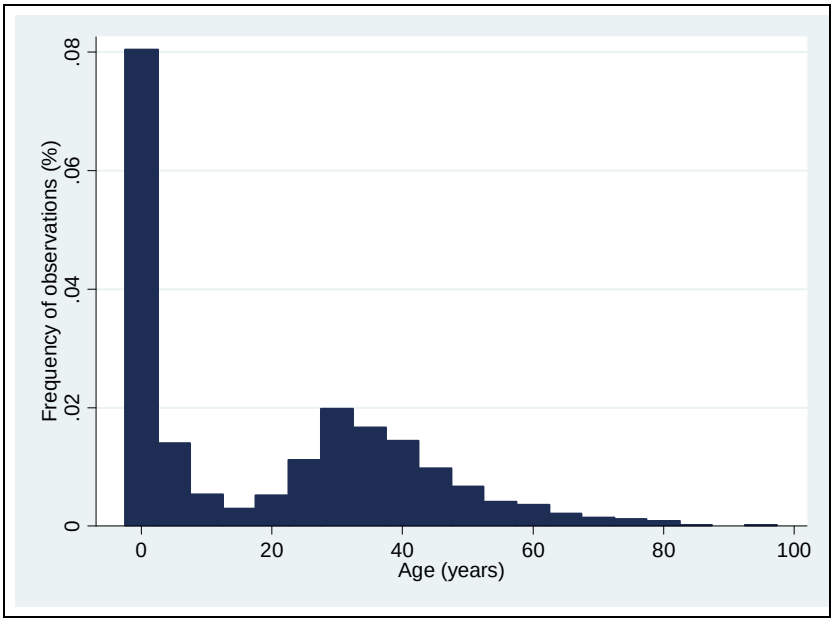


Figure 3.2 Frequency distribution of age (with normal density plot)

3.3.2 Gender

1983 of 3917 isolates (49.37%) were from female patients.

3.3.3 Province

The majority of isolates 2119 out of 4265 (49.68%) came from the Gauteng province, followed by the Western Cape (15.97%) and KwaZulu-Natal (13.51%).

3.3.4 On TMP prophylaxis

Of the 772 patients (18.10% of total dataset) for whom information was ascertained on prophylactic usage of TMP-SMX, 28.24% were confirmed to be using TMP-SMX for prophylactic purposes. In addition, 99.49% of patients on TMP-SMX prophylaxis were known to be HIV positive.

3.3.5 HIV status

Of the 827 patients for whom HIV status was determined, 750 (90.69%) were HIV positive.

3.3.6 Prior antibiotic treatment

Of the 742 patients for whom prior antibiotic usage was assessed, 160 (21.56%) were treated with antibiotics in the two months preceding admission.

3.3.7 Site of infection

Forty eight percent (2047/4265) of isolates were classified as being from patients with invasive NTS.

3.3.8 Other immune compromise

Tuberculosis was the next most prevalent immune compromise factor other than HIV status, and 97.24% of patients with TB were also HIV positive. The majority, 68.04%, of patients with other immune compromise factors (including TB) were HIV positive. Consequently, although the proportion of each type of non-HIV immune compromise is reported in subsequent tables of the results of the bivariate analyses for each MDR pattern, this factor was not included in the regression analysis.

3.3.9 Resistance to TMP-SMX

Of 4265 isolates tested, 55.71% were resistant to TMP-SMX, while 167 of 218 (76.61%) patients on TMP-SMX prophylaxis produced isolates that were resistant to it.

3.3.10 ESBL-producers

30.88% of isolates were ESBL-producers.

3.3.11 Serotype

The most prevalent serotype was S. *enterica* Typhimurium (49.14% of isolates over the period of surveillance), while the next most common serotype was S. *enterica* Isangi (1023/4265 isolates or 23.99%). S. *enterica* Dublin was the least prevalent serotype, making up just 2.98% of the 4265 isolates tested.

Table 3.1: Demographic and clinical characteristics of all participants

	All participants n (%)
Mean age (n=3930)	19.92 (95% CI 19.27 – 20.57)
Age group (n=4265)	
<1 year	1114 (26.12)
1-4 years	635 (14.89)
5-14 years	235 (5.51)
15-24 years	230 (5.39)
25-34 years	667 (15.64)
35-44 years	553 (12.97)
45-54 years	263 (6.17)
55-64 years	137 (3.21)
>=65 years	96 (2.25)
Missing	335 (7.85)
Sex (n=4265)	
Female	1983 (46.49)
Male	1934 (45.35)
Missing	348 (8.16)
Province (n=4265)	
Eastern Cape	417 (9.78)
Free State	124 (2.91)
Gauteng	2119 (49.68)
KwaZulu-Natal	576 (13.51)
Limpopo	67 (1.57)
Mpumalanga	169 (3.96)
Northern Cape	11 (0.26)
North West	101 (2.37)
Western Cape	681 (15.97)
TMP-SMX prophylaxis (n=4265)	
No	554 (12.99)
Yes	218 (5.11)
Missing	3493 (81.90)
Antibiotic treatment in previous 2 months (n=4265)	
No	582 (13.65)
Yes	160 (3.75)
Missing	3523 (82.60)
HIV status (n=4265)	
Negative	77 (1.81)
Positive	750 (17.58)

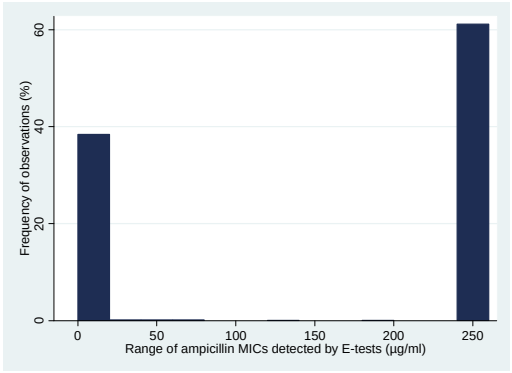
Missing	3438 (80.61)
Invasive (n=4265)	
No	2218 (52.00)
Yes	2047 (48.00)
Other immune compromise (n=4265)	
TB	294 (6.89)
Chronic disease	25 (0.59)
Other immune suppression	120 (2.81)
None	62 (1.45)
Missing	3764 (88.25)
Resistant to TMP-SMX (n=4265)	
Yes	2376 (55.71)
No	1889 (44.29)
ESBL-producers (n=4265)	
No	2948 (69.12)
Yes	1317 (30.88)
Serotype (n=4265)	
Typhimurium	2096 (49.14)
Isangi	1023 (23.99)
Enteritidis	273 (6.40)
Species	202 (4.74)
Dublin	127 (2.98)
Other	544 (12.75)

3.4 Annual incidence of NTS for 2003-2005

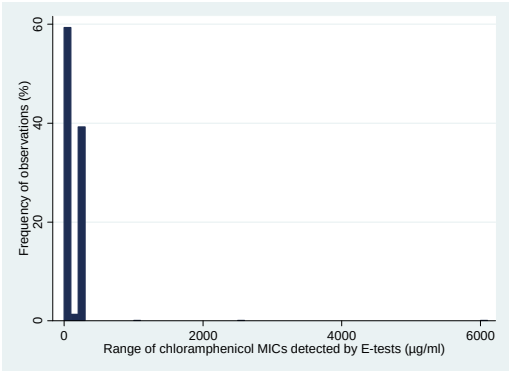
A total of 1070, 1505 and 1690 single (i.e. non-recurrent) NTS isolates were received by the reference laboratory for the years 2003, 2004 and 2005 respectively. Of these, 521(48.69%) 732 (48.64%) and 794 (46.98%) isolates were invasive in the years 2003, 2004 and 2005 respectively. This translated to annual incidence rates of 1.12 invasive non-typhoidal salmonella cases per 100000 (95% CI 1.03-1.22/100000) people for 2003, 1.57/100000 for 2004 (1.46-1.69/100000) and 1.69/100000 (1.58-1.82/100000) for 2005.

3.5 Prevalence of resistance rates of NTS to antibiotics tested

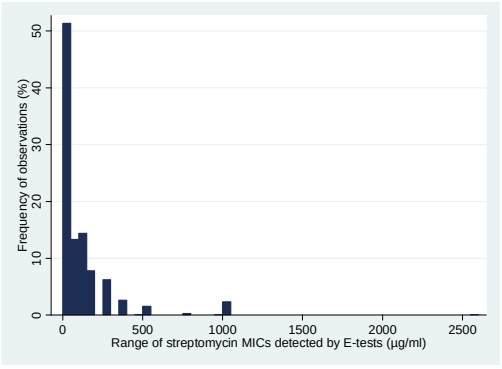
Histograms with normal density plots revealed that the MICs of the antibiotics tested followed either skewed or bimodal (with observations peaked around the breakpoint values) distributions. These graphs are presented in Figure 3.3.


Frequency of observations (%)
0
20
40
60
0
50
100
150
200
250
Range of ampicillin MICs detected by E-tests (μg/ml)

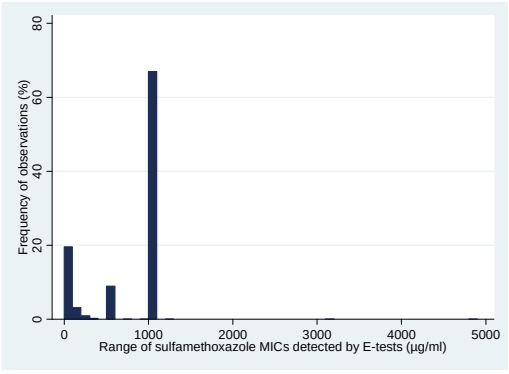
(a)


Frequency of observations (%)
0
20
40
60
0
2000
4000
6000
Range of chloramphenicol MICs detected by E-tests (μg/ml)

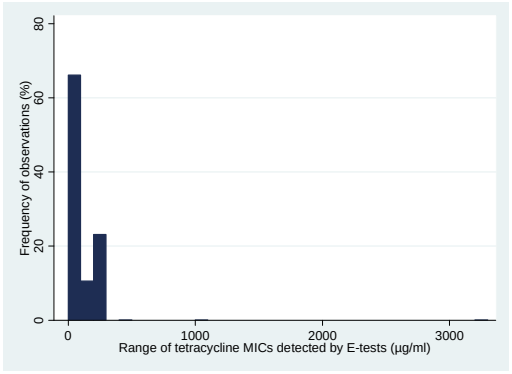
(b)


Frequency of observations (%)
0
10
20
30
40
50
0
500
1000
1500
2000
2500
Range of streptomycin MICs detected by E-tests (μg/ml)

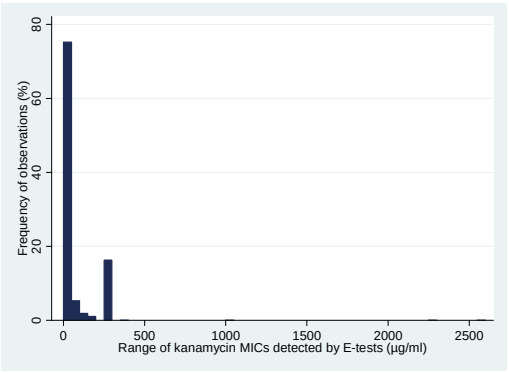
(c)


Frequency of obsenvations (%)
0
20
40
60
80
0
1000
2000
3000
4000
5000
Range of sulfamethoxazole MICs detected by E-tests (μg/ml)

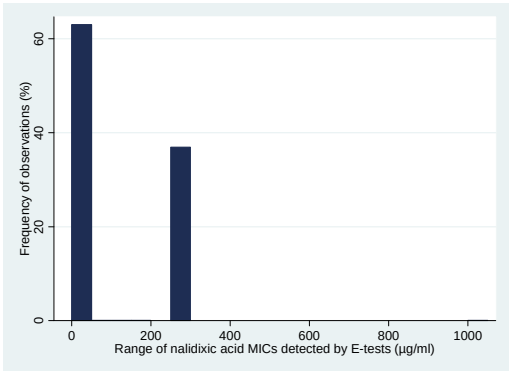
(d)


Frequency of observations (%)
0
20
40
60
80
0
1000
2000
3000
Range of tetracycline MICs detected by E-tests (μg/ml)

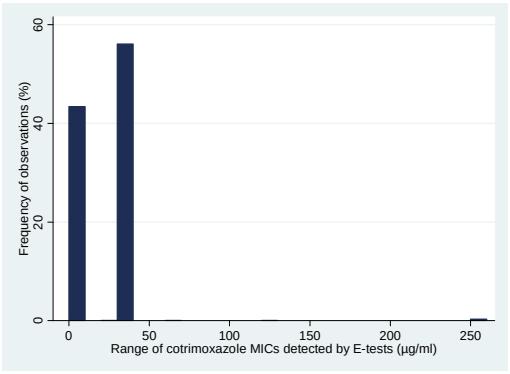
(e)


Frequency of observations (%)
0
20
40
60
80
0
500
1000
1500
2000
2500
Range of kanamycin MICs detected by E-tests (μg/ml)

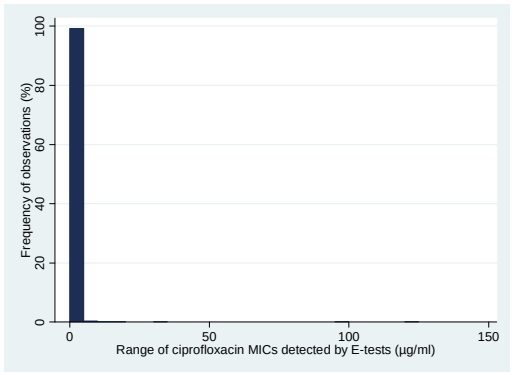
(f)


Frequency of observations (%)
0
20
40
60
0
200
400
600
800
1000
Range of nalidixic acid MICs detected by E-tests (μg/ml)

(g)


Frequency of observations (%)
0
20
40
60
0
50
100
150
200
250
Range of cotrimoxazole MICs detected by E-tests (μg/ml)

(h)



(i)

Figure 3.3 Frequency distributions of MICs for antibiotics tested
(a) – Ampicillin, (b) – Chloramphenicol, (c) – Streptomycin, (d) – Sulfamethoxazole, (e) – Tetracycline, (f) –Kanamycin, (g) – Nalidixic acid, (h) – Cotrimoxazole, (i) – Ciprofloxacin

Basic descriptive statistics of the minimum inhibitory concentrations of the antibiotics tested during the period of surveillance are presented in Table 3.2. The NTS isolates demonstrated low rates of resistance to ciprofloxacin and high rates of resistance to ampicillin and sulfamethoxazole for the period surveyed. Imipenem resistance was not observed during the period surveyed.

Table 3.2 MICs using E-tests for NTS isolates received during 2003 – 2005

Antibiotic	Mean µg/ml	Median	Range	Resistance rate (%)	N
Ampicillin	155.06	256	0.023-256.30	60.87	4265
Augmentin	8.05	4	0.032-632	28.16	4265
TMP-SMX	18.64	32	0.002-256	55.71	4265
Trimethoprim	19.45	32	0.002-3200	55.90	4265
Sulfamethoxazole	748.26	1024	0.064-4824	75.85	4265
Chloramphenicol	107.04	6	0.012-6024	43.87	4265
Nalidixic acid	96.08	4	0.012-1024	37.47	4265
Ciprofloxacin	0.24	0.016	0.003-125	0.98	4265
Tetracycline	88.30	12	0.012-3256	55.43	4265
Kanamycin	58.33	4	0.19-2566	38.25	3195
Streptomycin	111.25	48	0.125-2596	48.04	4265
Imipenem	0.25	0.19	0.008 - 4	0	3195
Ceftriaxone	34.86	0.19	0.004-256	20.42	4265
Cefepime	31.30	8	0.016-600	43.29	1587
Ceftazidime	77.81	0.50	0.019-256	33.76	4265

3.6 Distribution of resistance rates by province, serotype and year

3.6.1 Distribution of resistance by province

The distribution of resistance rates by province shows consistently lowest resistance rates (0.98%) to ciprofloxacin across all provinces in South Africa (see Table 3.3). Overall, the highest rates of resistance observed across all provinces were to sulfamethoxazole, with 75.85% of isolates submitted exhibiting resistance to it. 30.88% of NTS isolates were ESBL-producers, and 55.71% of isolates were resistant to TMP-SMX. Resistance to imipenem was not observed in the 3195 isolates tested from 2003 to 2005.

Table 3.3 Distribution of antibiotic resistance rates by province

Antibiotic / Province	Ampicillin (n=4265)	Augmentin (n=4265)	TMP-SMX (n=4265)	Trimethoprim (n=4265)	Sulfamethoxazole (n-4265)	Chloramphenicol (n=4265)	Nalidixic acid (n=4265)	Ciprofloxacin (n=4265)	Tetracycline (n-4265)	Kanamycin (n=3195)	Streptomycin (n=4265)	Ceftriaxone (4265)	Cefepime (n=1587)	Ceftazidime (n=4265)	ESBLs (4265)
Eastern Cape	62.83	17.03	61.39	61.63	77.94	58.27	39.09	0.24	68.35	40.92	56.83	25.42	44.39	52.76	49.16
Free State	45.97	13.71	40.32	40.32	66.13	43.55	40.32	1.61	43.55	22.58	41.13	27.42	45.95	31.45	28.23
Gauteng	68.29	39.08	62.48	62.62	80.08	42.80	39.03	0.47	53.23	46.82	50.26	22.98	44.64	30.91	27.37
KwaZulu-Natal	56.60	25.00	53.47	53.65	74.48	39.58	34.38	3.82	56.94	39.81	42.71	20.66	49.75	32.47	30.38
Limpopo	32.84	11.94	31.34	31.34	56.72	32.84	5.97	1.49	38.81	21.54	28.36	7.46	60.00	20.90	20.90
Mpumalanga	34.32	14.79	24.26	25.44	56.21	18.93	5.33	0	25.44	14.29	13.02	2.37	28.57	2.96	2.37
Northern Cape	36.36	9.09	36.36	36.36	72.73	27.27	9.09	0	27.27	30.00	36.36	27.27	66.67	27.27	27.27
North West	58.42	19.80	50.50	50.50	76.24	44.55	28.71	0	50.50	33.77	44.55	34.65	57.14	39.60	34.65
Western Cape	53.01	12.78	47.14	47.28	71.07	49.49	46.55	0.88	65.49	20.31	52.86	11.45	31.79	40.68	39.06
Total	**60.87**	**28.16**	**55.71**	**55.90**	**75.85**	**43.87**	**37.47**	**0.98**	**55.43**	**38.25**	**48.04**	**20.42**	**43.29**	**33.76**	**30.88**

3.6.2 Distribution of resistance by serotype

Table 3.4 shows the resistance rates by serotype for the period 2003 to 2005, generally, low rates of resistance to ciprofloxacin were observed. The *S. enterica* Isangi isolates had very high resistance rates to all antibiotics tested except for ciprofloxacin (0.39%). This holds true for *S. enterica* Typhimurium as well with 1.53% of isolates resistant to ciprofloxacin.

Table 3.4: Distribution of antibiotic resistance rates by NTS serotype (%)

Antibiotic / Serotype	Ampicillin (n=4402)	Augmentin (n=4402)	TMP-SMX (n=4402)	Trimethoprim (n=4402)	Sulfamethoxazole (n=4402)	Chloramphenicol (n=4402)	Nalidixic acid (n=4402)	Ciprofloxacin (4402)	Tetracycline (n=4402)	Kanamycin (n=3303)	Streptomycin (n=4402)	Ceftriaxone (n=4402)	Cefepime (n=1664)	Ceftazidime (n=4402)	ESBLs (n=4402)
Typhimurium	65.46	32.16	57.06	57.35	80.68	34.40	21.37	1.53	50.48	46.13	42.56	14.89	27.66	18.03	17.89
Isangi	97.75	42.62	95.41	95.41	98.04	98.14	90.13	0.39	98.14	56.11	96.09	49.07	51.06	95.70	84.65
Enteritidis	9.16	1.47	9.89	9.52	36.63	7.33	46.15	0.73	17.22	0.48	7.69	2.20	18.18	1.83	2.20
Species	47.52	22.28	39.60	40.10	67.33	23.27	21.29	1.49	40.59	35.82	28.71	12.38	54.55	14.85	12.87
Dublin	5.51	1.57	6.30	6.30	20.47	3.94	3.94	0	6.30	1.11	6.30	0	0	0	0
Other	17.65	7.35	16.36	16.73	51.29	13.60	9.93	0.18	30.33	4.95	15.99	4.78	40.68	8.82	8.09

3.6.3 Distribution of resistance by year of surveillance

Fig 3.4 shows the distribution of serotypes per year of surveillance from 2003 to 2005. *S. enterica* Typhimurim was the most predominant serotype identified, making up 49.14% of the total isolates serotyped over that period. *S. enterica* Dublin was the least prevalent serotype, identified from 2.98% of isolates tested between 2003 and 2005.

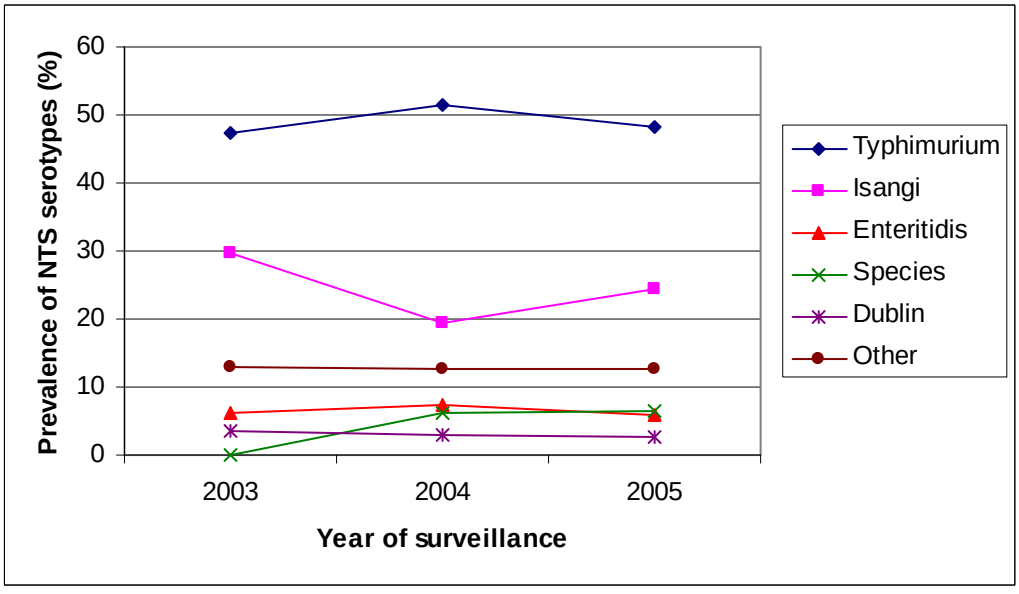


Fig 3.4 Distribution of NTS serotypes by year for the period studied

3.6.4 Prevalence of MDR patterns per year of surveillance

The prevalence of each MDR resistance pattern was plotted by year and this is presented in Fig 3.5. There were slight decreases in the prevalence of the ACSSuT resistance pattern from 38.13% to 32.13% between 2003 and 2005, and this was significant at the 95% level (χ^2 p=0.003). Prevalence of ACSSuTNx multi-drug resistance also decreased from 29.35% in 2003 to 23.59% in 2004, but rose again to 26.33% in 2005; these differences in the prevalence of the ACSSuTNx pattern were also significant (χ^2 p=0.004). The prevalence of ACKSSuT and AKSSuT resistance in NTS decreased significantly from 23.72% in 2004 to 16.69% in 2005 (p=0.000) and 26.51% to 20.71% respectively (p=0.000).

Prevalence of the ACKSSuT and AKSSuT resistance patterns was not calculated for 2003, as susceptibility testing of isolates for that year excluded the antibiotic kanamycin. Although the prevalence of isolates with one or more MDR patterns is seen to decrease from 38.13% to 35.68 between 2003 and 2004, then increases slightly to 36.15% in 2005, these differences were not statistically significant (p=0.417).

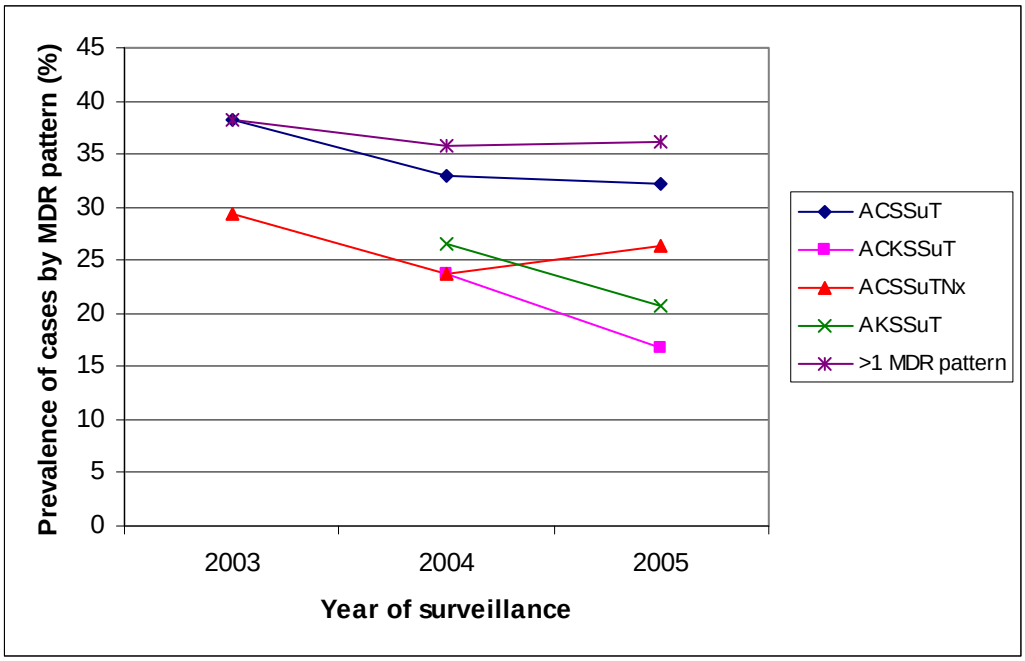


Fig 3.5 Distribution of MDR patterns per year of surveillance

3.7 Univariate analysis of TMP-SMX usage and MDR NTS

3.7.1 Relationship between ACSSuT and prophylactic use of TMP-SMX

A summary of the univariate analysis of the ACSSuT multi-drug resistance pattern with each risk factor / explanatory variable is presented in Table 3.5. Findings in respect of each of these factors are highlighted below.

3.7.1.1 Demographic factors

- Age: Unpaired t-tests revealed that the mean age of ACSSuT cases was 10.20 years (9.27 – 11.13) and was significantly lower than that of controls, who had an average age of 24.76 years (23.97 – 25.55), p=0.0000.
- Using Chi-squared tests, age and province of origin of isolate were significantly associated with the ACSSuT resistance pattern at $p \leq 0.05$

3.7.1.2 Clinical factors

- On TMP-SMX prophylaxis: Of the 772 cases for whom prophylactic use of TMP-SMX was ascertained, 60 (32.79%) were on TMP-SMX prophylaxis. Although being on TMP-SMX was not significantly associated with outcome (χ^2 $p>0.05$).
- Prior antibiotic treatment and site of infection were also significantly associated with ACSSuT resistance (χ^2 p=0.000).

3.7.1.3 Microbiological factors

- TMP-SMX resistance, ESBL-production and serotype were associated with ACSSuT resistance at $p \leq 0.05$ during univariate analysis using Pearson's Chi-squared test.

Table 3.5 Characteristics of cases and controls with the ACSSuT resistance pattern

	Controls n (%)	Cases n (%)	p value
Mean age	24.76 (23.97-25.55)	10.20 (9.27-11.13)	0.0000
Age group (n=3930)			0.0000
<1 year	484 (18.45)	630 (48.24)	
1-4 years	344 (13.11)	291 (22.28)	
5-14 years	175 (6.67)	60 (4.59)	
15-24 years	195 (7.43)	35 (2.68)	
25-34 years	542 (20.66)	125 (9.57)	
35-44 years	459 (17.49)	94 (7.20)	
45-54 years	228 (8.69)	35 (2.68)	
55-64 years	115 (4.38)	22 (1.68)	
>=65 years	82 (3.13)	14 (1.07)	
Sex (n=3917)			0.417
Female	1309 (49.83)	625 (48.45)	
Male	1318 (50.17)	665 (51.55)	
Province (n=4265)			0.000[1]

Eastern Cape	205 (7.27)	212 (14.65)	
Free State	84 (2.98)	40 (2.76)	
Gauteng	1455 (51.63)	664 (45.89)	
KwaZulu-Natal	390 (13.84)	186 (12.85)	
Limpopo	53 (1.88)	14 (0.97)	
Mpumalanga	160 (5.68)	9 (0.62)	
Northern Cape	9 (0.32)	2 (0.14)	
North West	68 (2.41)	33 (2.28)	
Western Cape	394 (13.98)	287 (19.83)	
TMP-SMX prophylaxis (n=772)			0.118
No	431 (73.17)	123 (67.21)	
Yes	158 (26.83)	60 (32.79)	
Prior antibiotic treatment (n=742)			
Yes	105 (18.62)	55 (30.90)	0.001
No	459 (81.38)	123 (69.10)	
HIV positive (n=827)			0.439
Yes	575 (91.13)	175 (89.29)	
No	56 (8.87)	21 (10.71)	
Site of infection (n=4265)			0.000
Invasive	1637 (58.09)	410 (28.33)	
Non-invasive	1181 (41.91)	1037 (71.67)	
Other immune compromise (n=501)			0.336[2]
TB	218 (56.77)	76 (64.96)	
Chronic disease	22 (5.73)	3 (2.56)	
Other immune suppression	96 (25.00)	24 (20.51)	
None	48 (12.50)	14 (11.97)	
Resistant to TMP-SMX (n=4265)			0.000
Yes	1000 (35.49)	1376 (95.09)	
No	1818 (64.51)	71 (4.91)	
ESBL-producer (n=4265)			0.000
Yes	149 (5.29)	1168 (80.72)	
No	2669 (94.71)	279 (19.28)	
Serotype (n=4265)			0.000[1]
Typhimurium	1701 (60.36)	395 (27.30)	
Isangi	59 (2.09)	964 (66.62)	
Enteritidis	259 (9.19)	14 (0.97)	
Species	170 (6.03)	32 (2.21)	
Dublin	127 (4.51)	0	
Other	502 (17.81)	42 (2.90)	

[1] Stata could not calculate Fisher's exact p value because of memory requirements
[2] Fisher's exact p value

3.7.2 Relationship between ACKSSuT and prophylactic use of TMP-SMX

Tabulating isolates with the ACKSSuT MDR pattern with each risk factor produced results summarized in Table 3.6.

3.7.2.1 Demographic factors

- Age: The mean age of ACKSSuT cases was 11.04 years (9.65 – 12.44) compared to 22.19 years (21.35 – 23.03) for controls, this difference was significant at the 95% level (unpaired t-test p=0.0000). There were significant differences in the proportion

of cases in each age group (χ^2 p=0.000), and the majority of cases were from patients aged 4 years or younger.

- There were also significant differences in the distribution of cases and controls between the provinces (χ^2 p=0.000),

3.7.2.2 Clinical factors

- Being on TMP-SMX prophylaxis, HIV status, site of infection, other immune compromise and prior antibiotic treatment were significantly associated with ACKSSuT resistance (χ^2 p≤0.05).

3.7.2.3 Microbiological factors

- TMP-SMX resistance, ESBL-production and serotype were significantly associated with ACKSSuT resistance in univariate analysis (χ^2 p≤0.05).

Table 3.6: Characteristics of cases and controls with the ACKSSuT resistance pattern

	Controls n (%)	Cases n (%)	p value
Mean age	22.19 (21.35-23.03)	11.04 (9.65-12.44)	0.0000
Age group (n=3028)			0.000
<1 year	582 (24.07)	281 (46.07)	
1-4 years	355 (14.68)	133 (21.80)	
5-14 years	158 (6.53)	30 (4.92)	
15-24 years	158 (6.53)	14 (2.30)	
25-34 years	440 (18.20)	66 (10.82)	
35-44 years	367 (15.18)	52 (8.52)	
45-54 years	189 (7.82)	18 (2.95)	
55-64 years	98 (4.05)	8 (1.31)	
>=65 years	71 (2.94)	8 (1.31)	
Sex (n=3103)			0.550
Female	1222 (49.16)	295 (47.81)	
Male	1264 (50.84)	322 (52.19)	
Province (n=3195)			0.000[1]
Eastern Cape	212 (8.30)	113 (17.66)	
Free State	79 (3.09)	14 (2.19)	
Gauteng	1207 (47.24)	288 (45.00)	
KwaZulu-Natal	421 (16.48)	114 (17.81)	
Limpopo	58 (2.27)	7 (1.09)	
Mpumalanga	145 (5.68)	2 (0.31)	
Northern Cape	8 (0.31)	2 (0.31)	
North West	59 (2.31)	18 (2.81)	
Western Cape	366 (14.32)	82 (12.81)	
TMP-SMX prophylaxis (n=551)			0.008
Yes	118 (25.27)	33 (39.29)	
No	349 (74.73)	51 (60.71)	
Antibiotic use in last 2 months (n=527)			0.000
Yes	73 (16.33)	31 (38.75)	

No	374 (83.67)	49 (61.25)	
HIV status (n=613)			0.064[2]
Negative	54 (10.49)	5 (5.10)	
Positive	461 (89.51)	93 (94.90)	
Site of infection (n=3195)			0.000
Invasive	1335 (52.25)	191 (29.84)	
Non-invasive	1220 (47.75)	449 (70.16)	
Other immune compromise (n=416)			0.008[2]
TB	178 (51.59)	49 (69.01)	
Chronic disease	17 (4.93)	0	
Other immune suppression	101 (29.28)	11 (15.49)	
None	49 (14.20)	11 (15.49)	
Resistant to TMP-SMX (n=3195)			0.000
Yes	1137 (44.50)	632 (98.75)	
No	1418 (55.50)	8 (1.25)	
ESBL-producer (n=3195)			0.000
Yes	355 (13.89)	539 (84.22)	
No	2200 (86.11)	101 (15.78)	
Serotype (n=3195)			0.000[1]
Typhimurium	1362 (53.31)	227 (35.47)	
Isangi	324 (12.68)	380 (59.38)	
Enteritidis	207 (8.10)	0	
Species	182 (7.12)	19 (2.97)	
Dublin	90 (3.52)	0	
Other	390 (15.26)	14 (2.19)	

[1] Stata could not calculate Fisher's exact p value because of memory requirements
[2] Fisher's exact p value

3.7.3 Relationship between ACSSuTNx and prophylactic use of TMP-SMX

Table 3.7 summarises results from the univariate analysis of the ACSSuTNx resistance pattern with each risk factor.

3.7.3.1 Demographic factors

- Age: ACSSuTNx cases were younger than controls, with a mean age of 8.11 years (7.13-9.09) compared to 23.90 (23.15-24.65) for controls. This difference was significant at the 95% level (unpaired t-test p=0.0000).
- There were also significant differences in the distribution of cases and controls between the provinces (χ^2 p=0.000) with the majority of cases (41.70%) and controls (52.51%) coming from Gauteng.

3.7.3.2 Clinical factors

- On TMP-SMX prophylaxis: 34.95% of cases were on TMP-SMX prophylaxis compared to 27.20% of controls. This was not a statistically significant difference (χ^2 p=0.104).
- Prior antibiotic treatment, other immune compromise and site of infection were significantly associated with ACSSuTNx resistance (χ^2 p≤0.05).

3.7.3.3 Microbiological factors

- TMP-SMX resistance, ESBL-production and serotype as they were significantly associated with ACSSuTNx resistance (χ^2 p≤0.05)

Table 3.7: Characteristics of cases and controls with the ACSSuTNx resistance pattern

	Controls n (%)	Cases n (%)	p value
Mean age	23.90 (23.15-24.65)	8.11 (7.13-9.09)	0.0000
Age group (n=3930)			0.000
<1 year	597 (20.31)	517 (52.17)	
1-4 years	395 (13.44)	240 (24.22)	
5-14 years	189 (6.43)	46 (4.64)	
15-24 years	211 (7.18)	19 (1.92)	
25-34 years	592 (20.14)	75 (7.57)	
35-44 years	502 (17.08)	51 (5.15)	
45-54 years	245 (8.34)	18 (1.82)	
55-64 years	122 (4.15)	15 (1.51)	
>=65 years	86 (2.93)	10 (1.01)	
Sex (n=3917)			0.423
Female	1461 (49.74)	473 (48.27)	
Male	1476 (50.26)	507 (51.73)	
Province (n=4265)			0.000[1]
Eastern Cape	263 (8.35)	154 (13.81)	
Free State	87 (2.76)	37 (3.32)	
Gauteng	1654 (52.51)	465 (41.70)	
KwaZulu-Natal	414 (13.14)	162 (14.53)	
Limpopo	66 (2.10)	1 (0.09)*	
Mpumalanga	167 (5.30)	2 (0.18) *	
Northern Cape	10 (0.32)	1 (0.09) *	
North West	78 (2.48)	23 (2.06)	
Western Cape	411 (13.05)	270 (24.22)	
TMP-SMX prophylaxis (n=772)			0.104
No	487 (72.80)	67 (65.05)	
Yes	182 (27.20)	36 (34.95)	
Antibiotic use in last 2 months (n=742)			0.014
No	513 (79.91)	69 (69.00)	
Yes	129 (20.09)	31 (31.00)	
HIV status (n=827)			0.270
Negative	63 (8.86)	14 (12.07)	
Positive	648 (91.14)	102 (87.93)	
Site of infection (n=4265)			0.000

Invasive	1799 (57.11)	248 (22.24)	
Non-invasive	1351 (42.89)	867 (77.76)	
Other immune compromise (n=501)			0.086[2]
TB	254 (58.93)	40 (57.14)	
Chronic disease	25 (5.80)	0*	
Other immune suppression	102 (23.67)	18 (25.71)	
None	50 (11.60)	12 (17.14)	
TMP-SMX resistant (n=4265)			0.000
Yes	1289 (40.92)	1087 (97.49)	
No	1861 (59.08)	28 (2.51)	
ESBL-producer (n=4265)			0.000
Yes	367 (11.65)	950 (85.20)	
No	2783 (88.35)	165 (14.80)	
Serotype (n=3195)			0.000[1]
Typhimurium	1924 (61.08)	172 (15.43)	
Isangi	130 (4.13)	893 (80.09)	
Enteritidis	271 (8.60)	2 (0.18) *	
Species	178 (5.65)	24 (2.15)	
Dublin	127 (4.03)	0*	
Other	520 (16.51)	24 (2.15)	

[1] Stata could not calculate Fisher's exact p value because of memory requirements
[2] Fisher's exact p value

3.7.4 Relationship between AKSSuT and prophylactic use of TMP-SMX

A summary of results from the univariate analysis of the AKSSuT resistance pattern with each risk factor in turn is presented in Table 3.8.

3.7.4.1 Demographic factors

- Age: AKSSuT cases were younger than controls, with a mean age of 13.52 years (12.14-14.91) compared to 21.92 (21.06-22.78) for controls. This difference was statistically significant (unpaired t-test for equality of means p=0.0000). There were significant differences in the proportion of AKSSuT cases in each age group (χ^2 p=0.000), with 61.44% of cases aged 4 years or younger compared to 39.44% of controls.
- Province of origin of isolate was also significantly associated with AKSSuT resistance (χ^2 $p \leq 0.05$).

3.7.4.2 Clinical factors

- Being on TMP-SMX prophylaxis, prior antibiotic treatment, HIV status, site of infection and other immune compromise were significantly associated with the AKSSuT resistance pattern (χ^2 $p \leq 0.05$).

3.7.4.3 Microbiological factors

- TMP-SMX resistance, ESBL-producers and serotype were also significantly associated with AKSSuT resistance (χ^2 p 0.05).

Table 3.8: Characteristics of cases and controls with the AKSSuT resistance pattern

	Controls n (%)	Cases n (%)	p value
Mean age	21.92 (21.06-22.78)	13.52 (12.14-14.91)	0.0000
Age group n (%) (n=3028)			0.000
<1 year	565 (24.41)	298 (41.80)	
1-4 years	348 (15.03)	140 (19.64)	
5-14 years	153 (6.61)	35 (4.91)	
15-24 years	149 (6.44)	23 (3.23)	
25-34 years	414 (17.88)	92 (12.90)	
35-44 years	351 (15.16)	68 (9.54)	
45-54 years	177 (7.65)	30 (4.21)	
55-64 years	88 (3.80)	18 (2.52)	
>=65 years	70 (3.02)	9 (1.26)	
Sex (n=3103)			0.836
Female	1165 (48.99)	352 (48.55)	
Male	1213 (51.01)	373 (51.45)	
Province (n=3195)			0.000[1]
Eastern Cape	210 (8.59)	115 (15.33)	
Free State	79 (3.23)	14 (1.87)	
Gauteng	1128 (46.13)	367 (48.93)	
KwaZulu-Natal	400 (16.36)	135 (18.00)	
Limpopo	56 (2.29)	9 (1.20)	
Mpumalanga	143 (5.85)	4 (0.53)	
Northern Cape	8 (0.33)	2 (0.27)	
North West	58(2.37)	19 (2.53)	
Western Cape	363 (14.85)	85 (11.33)	
TMP-SMX prophylaxis (n=551)			0.000
Yes	106 (24.31)	45 (39.13)	
No	330 (75.69)	70 (60.87)	
Prior antibiotic treatment (n=527)			0.000
Yes	69 (16.59)	35 (31.53)	
No	347 (83.41)	76 (68.47)	
HIV status (n=613)			0.022
Negative	53 (11.06)	6 (4.48)	
Positive	426 (88.94)	128 (95.52)	
Site of infection (n=3195)			0.000
Invasive	1252 (51.21)	274 (36.53)	
Non-invasive	1193 (48.79)	476 (63.47)	
Other immune compromise (n=416)			0.046[2]
TB	164 (51.57)	63 (64.29)	
Chronic disease	16 (5.03)	1 (1.02)	
Other immune suppression	93 (29.25)	19 (19.39)	
None	45 (14.15)	15 (15.31)	
Resistant to TMP-SMX (n=3195)			0.000
Yes	1028 (42.04)	741 (98.80)	
No	1417 (57.96)	9 (1.20)	
ESBL-producer (n=3195)			0.000
Yes	346 (14.15)	548 (73.07)	
No	2099 (85.85)	202 (26.93)	
Serotype (n=3195)			0.000[1]

Typhimurium	1265 (51.74)	324 (43.20)
Isangi	322 (13.17)	382 (50.93)
Enteritidis	207 (8.47)	0
Species	172 (7.03)	29 (3.87)
Dublin	90 (3.68)	0
Other	389 (15.91)	15 (2.00)

[1] Stata could not calculate Fisher's exact p value because of memory requirements
[2] Fisher's exact p value

3.7.5 Relationship between ≥1 MDR pattern and prophylactic use of TMP-SMX

A summary of results from the univariate analysis of isolates with at least one MDR pattern with each risk factor in turn is presented in Table 3.9.

3.7.5.1 Demographic factors

- Age and province of origin of isolate were significantly associated with at least one MDR pattern (χ^2 $p \leq 0.05$).

3.7.5.2 Clinical factors

- Chi-squared tests show that being on TMP-SMX prophylaxis, prior antibiotic treatment and site of infection were all significantly associated with at least one of the four identified MDR patterns (χ^2 $p \leq 0.05$).

3.7.5.3 Microbiological factors

- TMP-SMX resistance, ESBL-production and serotype were significantly associated with at having at least one of the four MDR patterns (χ^2 $p \leq 0.05$).

Table 3.9: Characteristics of cases and controls with more than one MDR pattern

	Controls n (%)	Cases n (%)	p value
Mean age	24.62 (23.81-25.43)	11.52 (10.58-12.45)	0.0000
Age group (n=3930)			0.000
<1 year	467 (18.52)	647 (45.92)	
1-4 years	337 (13.37)	298 (21.15)	
5-14 years	170 (6.74)	65 (4.61)	
15-24 years	186 (7.38)	44 (3.12)	
25-34 years	516 (20.47)	151 (10.72)	
35-44 years	443 (17.57)	110 (7.81)	
45-54 years	216 (8.57)	47 (3.34)	
55-64 years	105 (4.17)	32 (2.27)	
>=65 years	81 (3.21)	15 (1.06)	
Sex (n=3917)			0.582
Female	1252 (49.70)	682 (48.78)	
Male	1267 (50.30)	716 (51.22)	
Province (n=4265)			0.000[1]
Eastern Cape	203 (7.50)	214 (13.74)	
Free State	84 (3.10)	40 (2.57)	

Gauteng	1376 (50.81)	743 (47.72)	
KwaZulu-Natal	369 (13.63)	207 (13.29)	
Limpopo	51 (1.88)	16 (1.03)	
Mpumalanga	158 (5.83)	11 (0.71)	
Northern Cape	9 (0.33)	2 (0.13)	
North West	67 (2.47)	34 (2.18)	
Western Cape	391 (14.44)	290 (18.63)	
TMP-SMX prophylaxis (n=772)			0.039
Yes	146 (26.16)	72 (33.64)	
No	412 (73.84)	142 (66.36)	
Prior antibiotic treatment (n=742)			0.006
Yes	101 (18.95)	59 (28.23)	
No	432 (81.05)	150 (71.77)	
HIV status (n=827)			0.915
Negative	55 (9.24)	22 (9.48)	
Positive	540 (90.76)	210 (90.52)	
Site of infection (n=4265)			0.000
Invasive	1554 (57.39)	493 (31.66)	
Non-invasive	1154 (42.61)	1064 (68.34)	
Other immune compromise (n=501)			0.453[2]
TB	204 (57.14)	90 (62.50)	
Chronic disease	21 (5.88)	4 (2.78)	
Other immune suppression	88 (24.65)	32 (22.22)	
None	44 (12.32)	18 (12.50)	
Resistant to TMP-SMX (n=4265)			0.000
Yes	891 (32.90)	1485 (95.38)	
No	1817 (67.10)	72 (4.62)	
ESBL-producer (n=4265)			0.000
Yes	140 (5.17)	1177 (75.59)	
No	2568 (94.83)	380 (24.41)	
Serotype (n=4265)			0.000[1]
Typhimurium	1604 (59.23)	492 (31.60)	
Isangi	57 (2.10)	966 (62.04)	
Enteritidis	259 (9.56)	14 (0.90)	
Species	160 (5.91)	42 (2.70)	
Dublin	127 (4.69)	0	
Other	501 (18.50)	43 (2.76)	

[1] Stata could not calculate Fisher's exact p value because of memory requirements
[2] Fisher's exact p value

3.8 Multivariate analyses and adjustments for possible confounders / effect modifiers

3.8.1 Unadjusted odds ratios

Case-control analyses showed that isolates with the ACKSSuT and AKSSuT MDR patterns had significant odds of association with the primary exposure (i.e. patient on TMP-SMX prophylaxis). Table 3.10 shows the unadjusted odds ratios yielded by these

analyses as 1.91 (95% CI 1.14 – 3.19) and 2.00 (1.20 – 3.15) for the MDR patterns ACKSSuT and AKSSuT respectively.

This means that isolates from patients who were on TMP-SMX prophylaxis were 1.91 times more likely to have the MDR pattern ACKSSuT, and 2.00 times more likely to exhibit the AKSSuT MDR pattern. Both of these associations had narrow confidence intervals and were significant at the 95% level (p= 0.0080 and 0.0015 respectively).

Although isolates with the ACSSuT and ACSSuTNx resistance patterns were 1.33 and 1.44 times more likely to have come from patients on TMP-SMX prophylaxis, these associations were not statistically significant.

NTS isolates having at least one MDR pattern were 1.43 (1.00 – 2.04) times more likely to have come from patients who were on TMP-SMX prophylaxis as compared to isolates without any of the NTS MDR patterns identified, this relationship was statistically significant (p=0.0388).

Table 3.10 Unadjusted odds ratios for the association between MDR NTS and TMP-SMX prophylaxis

MDR categories	n	OR	95% CI	χ^2 (df)	p values
ACSSuT	772	1.33	0.91 – 1.93	2.45 (1)	0.1176
ACKSSuT	551	1.91	1.14 – 3.19	7.03 (1)	0.0080
ACSSuTNx	772	1.44	0.90 – 2.27	2.64 (1)	0.1040
AKSSuT	551	2.00	1.26 – 3.15	10.04 (1)	0.0015
>=1 MDR pattern	772	1.43	1.00 – 2.04	4.27 (1)	0.0388

3.8.2 Stratified analysis

Mantel-Haenszel pooled estimates of odds ratios for each explanatory variable were obtained and the results of these analyses are presented in Tables 3.11 – 3.15.

3.8.2.1 Mantel-Haenszel analysis of ACSSuT MDR pattern

The analysis of the association between the ACSSuT MDR pattern and TMP-SMX prophylaxis stratified by each explanatory factor is presented in Table 3.11.

The stratum-specific odds ratios for the association of ACSSuT with TMP-SMX prophylaxis use, taking into account age, province, sex, HIV status, ESBL production, site of infection, antibiotic use in the preceding two months and TMP-SMX resistance, do not differ significantly from each other (tests of homogeneity p>0.05), therefore the pooled odds ratios are reported. The ACSSuT MDR pattern is associated with TMP-SMX

prophylaxis independent of patient's age, sex, province of origin, HIV status, other immune compromise, whether the patient was on antibiotics in the two months preceding admission, whether the isolate was invasive and whether the serotype isolated was an ESBL-producer or resistant to TMP-SMX.

The test of homogeneity for the association between ACSSuT and prophylactic use of TMP-SMX controlling for serotype yielded a significant p value (0.0253), meaning that there were differences in the odds ratios for this association stratified by each category of serotype. Although the stratum-specific odds ratios show that isolates of the S. *enterica* Enteritidis serotype had a much higher odds of 6.88 for the association of ACSSuT with TMP-SMX prophylaxis, the association was not statistically significant (95% CI 0.36 – 130.47).

Table 3.11: Odds of association of exposure with MDR pattern ACSSUT and adjustment for possible confounders (Mantel-Haenszel odds ratios)

		Pooled OR (95% CI)	p	Stratum-specific OR (95% CI)	p (Test for homogeneity)
Unadjusted OR: 1.33 (0.91 – 1.93)					
Possible confounders	Age group	1.36 (0.94 – 1.98)	0.1035		0.9963
	Province	1.30 (0.90 – 1.86)	0.1583		0.6507
	Sex	1.33 (0.92 – 1.91)	0.1241		0.3553
	HIV status	1.27 (0.87 – 1.85)	0.2192		0.7747
	Antibiotic use in last 2 months	1.11 (0.75 – 1.66)	0.5959		0.7128
	TMP-SMX resistance	1.17 (0.54 – 1.19)	0.2790		0.1027
	ESBL-producing	0.81 (0.48 – 1.35)	0.4138		0.2203
	Other immune compromise	1.25 (0.87 – 1.80)	0.2188		0.2921
	Site of infection	1.33 (0.93 – 1.90)	0.1221		0.6374
	Serotype	1.19 (0.80 – 1.77)	0.3992		0.0253
	Typhimurium			1.09 (0.70 – 1.70)	
	Isangi			1.04 (0.18 – 5.95)	
	Enteritidis			6.88 (0.36 -130.47)	
	Species			0.53 (0.08 – 3.58)	
	Dublin			D*	
	Other			NUC**	

*D – no cases in stratum
**NUC – no unexposed cases in stratum

3.8.2.2 Mantel-Haenszel analysis of ACKSSuT MDR pattern

The results of the stratified analysis for the association of the ACKSSuT MDR pattern with TMP-SMX prophylaxis are presented in Table 3.12.

The tests of homogeneity for the stratum-specific odds ratios of the association between ACKSSuT and TMP-SMX prophylaxis controlling for age, province of origin of isolate,

sex, HIV status, ESBL production, site of infection, antibiotic use in the two months preceding admission, TMP-SMX resistance and serotype were all non-significant.

This means that the stratum-specific odds ratios did not differ from each other statistically and that these factors did not confound the association between the ACKSSuT MDR pattern and TMP-SMX prophylaxis. As a result, the pooled odds ratios for this association taking into account each explanatory variable are reported.

This analysis showed that the MDR pattern ACKSSuT is associated with TMP-SMX prophylaxis independent of all demographic, clinical and microbiological explanatory factors examined. The pooled odds ratios and the unadjusted odds ratio for the association between the ACKSSuT MDR pattern and TMP-SMX prophylaxis stratified by age, province, sex, site of infection and serotype do not differ from each other by more than 10% (10.99% for province and serotype). In addition, the confidence intervals for these odds ratios overlap with that of the unadjusted odds ratio, consequently, the pooled odds ratios do not differ significantly from the unadjusted odds ratio.

Table 3.12: Odds of association of exposure with outcome ACKSSUT and adjustment for possible confounders (Mantel-Haenszel odds ratios)

		Pooled OR (95% CI)	p	p (Test for homogeneity)
Unadjusted OR: 1.91 (1.14 – 3.19)				
Possible confounders	Age group	1.85 (1.13 – 3.05)	0.0135	0.8906
	Province	1.70 (1.04 – 2.77)	0.0330	0.3613
	Sex	1.98 (1.21 – 3.23)	0.0055	0.2208
	HIV status	1.49 (0.91 – 2.44)	0.1140	0.2806
	Antibiotic use in last 2 months	1.32 (0.78 – 2.22)	0.3010	0.4029
	TMP-SMX resistance	1.22 (0.72 – 2.04)	0.4596	0.4950
	ESBL-producing	1.87 (0.98 – 3.58)	0.0545	0.4369
	Other immune compromise	1.59 (0.98 – 2.58)	0.0599	0.0871
	Site of infection	1.92 (1.17 – 3.12)	0.0080	0.4864
	Serotype	1.70 (1.02 – 2.84)	0.0399	0.4163

3.8.2.3 Mantel-Haenszel analysis of ACSSuTNx MDR pattern

The analysis of the association between the ACSSuTNx MDR pattern and TMP-SMX prophylaxis stratified by other explanatory factors are presented in Table 3.13. The tests of homogeneity for the stratum-specific odds ratios of the association between ACSSuTNx and TMP-SMX prophylaxis taking into account all explanatory variables except for province of origin of isolate were non-significant ($p>0.05$). This means that the stratum-specific odds ratios did not differ from each other statistically. As a result, the pooled odds

ratios for the association between ACKSSuT and TMP-SMX taking into account these explanatory variables are reported.

The test for homogeneity of stratum-specific odds ratios for this association taking into account province of origin of isolate yielded a significant p value of 0.0034. The stratum-specific odds ratios are reported in this case, as province of origin of isolate is acting as a confounder in this association.

The association between the ACSSuTNx resistance pattern and TMP-SMX prophylaxis for isolates from the KwaZulu-Natal province was 9.33 (1.49 – 58.60), this was higher than that of other provinces and statistically significant. Although there were differences in the stratum-specific odds ratios for other provinces, these were not statistically significant.

The odds ratio for the relationship between the ACSSuTNx MDR pattern and TMP-SMX prophylaxis for isolates from the Eastern Cape province is 0 as there were no isolates with the ACSSuTNx resistance pattern from that province for the period of surveillance.
The Northern Cape and North West provinces of the country did not contribute data to the analysis, as there were no isolates with this MDR pattern from patients on TMP-SMX prophylaxis in these provinces.

Table 3.13: Odds of association of exposure with outcome ACSSuTNx and adjustment for possible confounders (Mantel-Haenszel odds ratios)

		Pooled OR (95% CI)	p	Stratum-specific OR (95% CI)	p (Test for homogeneity)
Unadjusted OR: 1.44 (0.90 – 2.27)					
Possible confounders	Age group	1.47 (0.93 – 2.34)	0.0982		0.7255
	Province	1.36 (0.89 – 2.08)	0.1528		0.0050
	Eastern Cape			0.00	
	Free State			5.00 (0.31 – 80.57)	
	Gauteng			1.31 (0.78 – 2.20)	
	KwaZulu-Natal			9.33 (1.49 – 58.60)	
	Limpopo			D*	
	Mpumalanga			D*	
	Northern Cape			NO**	
	North West			NO**	
	Western Cape			1.72 (0.44 – 6.69)	
	Sex	1.44 (0.93 – 2.23)	0.1037		0.4781
	HIV status	1.44 (0.91 – 2.27)	0.1197		0.4169
	Antibiotic use in last 2 months	1.22 (0.75 – 1.99)	0.4282		0.3110
	TMP-SMX resistance	0.90 (0.56 – 1.42)	0.6392		0.4098
	ESBL-producing	0.94 (0.56 – 1.59)	0.8315		0.0654
	Other immune compromise	1.41 (0.90 – 2.20)	0.1338		0.4988
	Site of infection	1.43 (0.92 – 2.22)	0.1112	NCU***	
	Serotype	1.50 (0.85 – 2.63)	0.1573		0.1417

*D – dropped as no cases in strata
**NO – no observations in strata
***NCU – no non-invasive cases

3.8.2.4 Mantel-Haenszel analysis of AKSSuT MDR pattern

The analysis for the association of the AKSSuT MDR pattern and TMP-SMX prophylaxis stratified by each explanatory factor is presented in Table 3.14.

The tests of homogeneity for the stratum-specific odds ratios of the association between AKSSuT and TMP-SMX prophylaxis controlling for all other explanatory variables were non-significant. This means that the stratum-specific odds ratios did not differ from each other statistically, and the explanatory variables did not confound the association between the AKSSuT MDR pattern and TMP-SMX prophylaxis. As a result, the pooled odds ratios for the association between AKSSuT and TMP-SMX, taking into account each explanatory variable, are reported.

The pooled odds ratios for age, sex, province, ESBL-producer and site of infection do not differ from the unadjusted odds ratio by more than 10%, while the pooled odds ratios for prior antibiotic use, other immune compromise and serotype do. However, the confidence intervals for each of these odds ratios overlap with that of the unadjusted odds ratio, so they do not differ statistically.

Table 3.14: Odds of association of exposure with outcome AKSSuT and adjustment for possible confounders (Mantel-Haenszel odds ratios)

		Pooled OR (95% CI)	p	p (Test for homogeneity)
Unadjusted OR: 2.00 (1.26 – 3.15)				
Possible confounders	Age group	2.01 (1.28 – 3.15)	0.0019	0.9372
	Province	1.79 (1.16 – 2.78)	0.0082	0.3035
	Sex	2.04 (1.32 – 3.16)	0.0011	0.1893
	HIV status	1.54 (0.99 – 2.41)	0.0560	0.3599
	Antibiotic use in last 2 months	1.64 (1.03 – 2.61)	0.0359	0.3193
	TMP-SMX resistance	1.24 (0.75 – 1.96)	0.3696	0.5122
	ESBL-producer	1.99 (1.17 – 3.38)	0.0092	0.9519
	Other immune compromise	1.74 (1.13 – 2.69)	0.0112	0.0609
	Site of infection	2.00 (1.29 – 3.10)	0.0016	0.4775
	Serotype	1.72 (1.10 – 2.71)	0.0170	0.1851

3.8.2.5 Mantel-Haenszel analysis of ≥1 MDR pattern

The results of the stratified analysis for the association between having at least one MDR pattern with TMP-SMX prophylaxis are presented in Table 3.15.

The tests of homogeneity for the stratum-specific odds ratios of the association between ACKSSuT and TMP-SMX prophylaxis controlling for age, province of origin of isolate, sex, HIV status, ESBL-production, site of infection, antibiotic use in the two months preceding admission and TMP-SMX resistance were all non-significant. As a result, the pooled odds ratios for the association between ACKSSuT and TMP-SMX taking into account these explanatory variables are reported.

The test of homogeneity of stratum-specific odds ratios for serotype produced a significant p value, χ^2p=0.0334. These stratum-specific odds ratios are reported in Table 3.17. As with the ACSSuT MDR pattern, the association between having at least one MDR pattern and TMP-SMX prophylaxis was 6.88 times more likely in S. *enterica* Enteritidis isolates than for other serotypes. This association was not statistically significant, as with the other stratum-specific odds ratios for serotype.

Again, there were no isolates from patients on TMP-SMX prophylaxis from Northern Cape and North West provinces, so these categories were excluded from the analysis. None of the stratum-specific odds ratios for province were significantly different, though.

Table 3.15: Odds of association of exposure with outcome ≥1 MDR pattern and possible confounders (Mantel-Haenszel odds ratios)

		Pooled OR (95% CI)	p	Stratum-specific OR (95% CI)	p (Test for homogeneity)
Unadjusted OR: 1.43 (1.00 – 2.04)					
Possible confounders	Age group	1.48 (1.04 – 2.11)	0.0272		0.9622
	Province	1.39 (0.99 – 1.97)	0.0574		0.5506
	Sex	1.43 (1.01 – 2.02)	0.0403		0.6311
	HIV status	1.33 (0.93 – 1.90)	0.1204		0.7645
	Antibiotic use in last 2 months	1.29 (0.89 – 1.88)	0.1792		0.7559
	TMP-SMX resistance	0.83 (0.57 – 1.22)	0.3376		0.3397
	ESBL-producing	1.04 (0.67 – 1.62)	0.8669		0.2094
	Other immune compromise	1.36 (0.97 – 1.92)	0.0743		0.1558
	Site of infection	1.43 (1.01 – 2.01)	0.0412		0.6253
	Serotype	1.25 (0.86 – 1.82)	0.2331		0.0334
	Typhimurium			1.09 (0.72 – 1.65)	
	Isangi			1.04 (0.18 – 5.95)	
	Enteritidis			6.88 (0.36 – 130.47)	

	Species	2.00 (0.39 – 10.22)
	Dublin	D*
	Other	NUC**

*D – no cases in stratum
**NUC – no unexposed cases in stratum

3.9 Regression modeling of the relationship between use of cotrimoxazole prophylaxis and the MDR patterns in NTS

Univariate and multivariate logistic regression of the ACSSuT resistance pattern with each risk factor in turn was carried out and the results are presented in Table 3.16.

3.9.1 Regression modeling of the ACSSuT MDR pattern

3.9.1.1 Univariate logistic regression – primary association

In univariate modeling, the ACSSuT pattern was not significantly associated with the primary exposure (use of TMP-SMX prophylaxis), having an odds ratio of 1.33 (95% CI 0.93 – 1.90, p=0.118).

3.9.1.2 Univariate logistic regression – demographic factors

- Age: The odds of having the ACSSuT resistance pattern decreased with each category increase in age group as compared to the under-1 year age group (reference group).
- Sex: ACSSuT isolates were less likely to come from women (OR=0.95, 95% CI 0.82 – 1.08, p=0.417) than from men (reference group, OR=1), although this relationship was not statistically significant.
- Province: Isolates with the ACSSuT resistance pattern were less likely to come from provinces other than Eastern Cape (which was the reference group).

3.9.1.3 Univariate logistic regression – clinical factors

- Prior treatment with antibiotics: Isolates from patients who had been on antibiotics in the two months preceding admission were 1.95 times more likely to have the ACSSuT resistance pattern (95% CI 1.33 – 2.86, p=0.001).
- HIV status: Being HIV-negative was suggestive of a protective effect as it had a lower odds of being associated with the ACSSuT resistance pattern (OR=0.81, 95% CI 0.48 – 1.38, p=0.440) compared to the reference group (HIV positive) with an OR of 1, however this association was not statistically significant.
- Site of infection: Invasive isolates also had a lower odds of association with ACSSuT resistance (OR=0.29, 95% CI 0.25 – 0.33) compared to the reference group which was

composed of non-invasive isolates, this association was statistically significant (p=0.000.).

3.9.1.4 Univariate logistic regression – microbiological factors

- Isolates that were already resistant to TMP-SMX were also 35.23 times more likely to have the ACSSuT resistance pattern (95% CI 27.42 – 45.27, p=0.0000).
- ESBL-producing isolates were much more likely to have the ACSSuT resistance pattern, with an odds ratio of 75.99 (95% CI 60.76 – 92.55) compared to those isolates that were not ESBL-producers.
- S. *enterica* Isangi isolates were 70 times more likely to have the ACSSuT resistance pattern than the reference group of S. *enterica* Typhimurium isolates (OR 70.36, 95% CI 52.93 – 93.54). S. *enterica* Enteritidis isolates were less likely to have the ACSSuT resistance pattern compared to the reference group (OR 0.23, 95% CI 0.13 – 0.40, p=0.000). It is important to note that all S. *enterica* Dublin isolates had the outcome, i.e. were ACSSuT resistant.

3.9.1.5 Multivariate regression modeling of the ACSSuT MDR pattern

The outcome of interest and primary explanatory variable were put into a model with those variables that were significant at the 90% level in univariate modeling, namely age, province of origin of isolate, site of infection, antibiotic use in the two months preceding admission, ESBL production, resistance to TMP-SMX and serotype.

In multivariate modeling, TMP-SMX prophylaxis was not significantly associated with the ACSSuT resistance pattern, after controlling for the effects of demographic, clinical and microbiological factors.

ESBL production, TMP-SMX resistance and serotype were significantly associated with the MDR pattern ACSSuT after controlling for the effects of all other variables in the model:

- Isolates which were ESBL-producers were 28.34 (15.43 – 52.05, p=0.000) times more likely to have the ACSSuT MDR pattern than those that were not ESBL-producers
- Patients with NTS isolates resistant to TMP-SMX were 2.47 (1.22 – 4.97, p=0.012) times more likely to have the ACSSuT MDR pattern than those that were susceptible to TMP-SMX

- S. *enterica* Isangi isolates were 9.58 (3.43 – 26.82, p=0.000) times more likely to have the ACSSuT pattern than S. *enterica* Typhimurium, while S. *enterica* Species isolates were 3.20 (1.13 – 9.08, p=0.029) times more likely to have the ACSSuT MDR pattern. Observations for S. *enterica* Dublin isolates were dropped from the analysis as all of these isolates experienced the outcome. All other serotypes were 0.24 (0.06 – 1.02, p=0.052) times less likely to have the ACSSuT MDR pattern than S. *enterica* Typhimurium isolates.

Table 3.16 Univariate and multivariate regression models of ACSSuT MDR pattern

	Univariate model				Multivariate model			
Explanatory var	n	OR	95% CI	p	N	OR	95% CI	p
TMP-SMX use	772				650			
No		1.00	Reference			1.00	Reference	
Yes		1.33	0.93 – 1.90	0.118		0.68	0.38 – 1.24	0.207
Age (grouped)	3930							
<1 year		1.00	Reference					
1-4 years		0.65	0.53 – 0.79	0.000				
5-14 years		0.26	0.20 – 0.36	0.000				
15-24 years		0.14	0.09 – 0.20	0.000				
25-34 years		0.18	0.14 – 0.22	0.000				
35-44 years		0.16	0.12 – 0.20	0.000				
45-54 years		0.12	0.08 – 0.17	0.000				
55-64 years		0.15	0.09 – 0.24	0.000				
>=65 years		0.13	0.07 – 0.23	0.000				
Province	4265							
Eastern Cape		1.00	Reference					
Free State		0.46	0.30 – 0.70	0.000				
Gauteng		0.44	0.36 – 0.55	0.000				
KwaZulu-Natal		0.46	0.36 – 0.60	0.000				
Limpopo		0.26	0.14 – 0.47	0.000				
Mpumalanga		0.05	0.03 – 0.11	0.000				
Northern Cape		0.21	0.05 – 1.01	0.051				
North West		0.47	0.30 – 0.74	0.001				
Western Cape		0.70	0.55 – 0.90	0.005				
Sex	3917							
Male		1.00	Reference			NI**		
Female		0.95	0.82 – 1.08	0.417				
HIV status								
Yes		1.00	Reference			NI**		
No	827	0.81	0.48 – 1.38	0.440				
Antibiotic use in last 2 months	742							
No		1.00	Reference					
Yes		1.95	1.33 – 2.86	0.001				
TMP-SMX resistance					650			
No		1.00	Reference			1.00	Reference	
Yes	4265	35.23	27.42 – 45.27	0.000		2.47	1.22 – 4.97	0.012
ESBL-producer					650			
No		1.00	Reference			1.00	Reference	

Yes	4265	75.99	60.76 – 92.55	0.000		28.34	15.43 – 52.05	0.000
Other immune compromise	501							
None		1.00	Reference			NI**		
TB		1.20	0.62 – 2.29	0.591				
Chronic disease		0.47	0.12 – 1.79	0.268				
Other immune suppression		0.86	0.41 – 1.80	0.685				
Invasive NTS	4265							
No		1.00	Reference					
Yes		0.29	0.25 – 0.33	0.000				
Serotype	4138				650			
Typhimurium		1.00				1.00	Reference	
Isangi		70.36	52.93 – 93.54	0.000		9.58	3.43 – 26.82	0.000
Enteritidis		0.23	0.13 – 0.40	0.000		0.41	0.08 – 1.99	0.266
Species		0.81	0.55 – 1.20	0.295		3.20	1.13 – 9.08	0.029
Dublin		D***	-	-		D***	-	-
Other		0.36	0.26 – 0.50	0.000		0.24	0.06 – 1.02	0.052

*C – collinear – category excluded from analysis
**NI – factor not significant in stratified analysis / univariate modeling – not included in
***D – dropped because cases with this serotype

3.9.2 Univariate regression modeling of the ACKSSuT MDR pattern

Univariate and multivariate logistic regression of the ACKSSuT resistance pattern with each risk factor in turn was carried out and the results are presented in Table 3.17.

3.9.2.1 Univariate logistic regression – primary association

In univariate modeling, the ACKSSuT pattern was significantly associated with the primary exposure (use of TMP-SMX prophylaxis) having an odds ratio of 1.93 (95% CI 1.18 – 3.11, p=0.009).

3.9.2.2 Univariate logistic regression – demographic factors

- Age: The odds of having the ACKSSuT resistance pattern decreased with increasing age as compared to the reference group, i.e. the under-1 year age group (p=0.0000).
- Sex: Isolates with the ACKSSuT MDR pattern were less likely to come from women (OR=0.95, 95% CI 0.79 – 1.13, p=0.556) than from men (OR=1) though this was not statistically significant.
- Isolates with the ACKSSuT resistance pattern were significantly (p=0.0000) less likely to come from provinces other than Eastern Cape (which was the reference group)

3.9.2.3 Univariate logistic regression – clinical factors

- Prior antibiotic treatment: Isolates from patients who had been on antibiotics in the two months preceding admission were 3.24 times more likely to have the ACKSSuT resistance pattern (95% CI 1.94 – 5.42, p=0.000).
- HIV status: Isolates from HIV positive patients were 2.18 times more likely to have the ACKSSuT resistance pattern, however this association was not statistically significant (95% CI 0.85 – 5.60, p=0.105) compared to the reference group (isolates from HIV negative patients).
- Site of infection: Invasive isolates also had a lower odds of association with ACKSSuT resistance (OR=0.39, 95% CI 0.32 – 0.47) compared to the reference group, which was composed of non-invasive isolates; this association was statistically significant (p=0.000.).

3.9.2.4 Univariate logistic regression – microbiological factors

- TMP-SMX resistance: Isolates that were already resistant to TMP-SMX were also 98.52 times more likely to have the ACKSSuT resistance pattern, this was significant even though the confidence interval for this association was very wide (95% CI 48.85 – 198.72, p=0.000).
- ESBL-producers: These isolates were 33.07 times more likely to have the ACKSSuT resistance pattern, compared to those isolates that were not ESBL-producers (95% CI 26.01 – 42.05, p=0.000).
- Serotype: S. *enterica* Isangi isolates were 7.04 times more likely to have the ACKSSuT resistance pattern than the reference group of S. *enterica* Typhimurium isolates (95% CI 5.74 – 8.63, p=0.000). S. *enterica* Enteritidis and Dublin isolates all experienced the outcome of interest (i.e. had the ACKSSuT resistance pattern). All other serotypes were 0.22 times less likely to have the ACKSSuT MDR pattern than S. *enterica* Typhimurium isolates.

3.9.2.5 Multivariate regression modeling of the ACKSSuT MDR pattern

The outcome of interest and primary explanatory variable were put into a model with those variables that were significant at the 90% level in univariate modeling, namely age, province of origin of isolate, site of infection, antibiotic use in the two months preceding admission, ESBL production, resistance to TMP-SMX and serotype. Controlling for these

variables, the ACKSSuT MDR pattern was not significantly associated with TMP-SMX prophylaxis.

The following explanatory variables were significantly associated with the ACKSSuT MDR pattern controlling for all other variables in the model:

- Isolates from patients who had been on antibiotics in the two months preceding admission were 2.19 (0.99 – 4.88, p=0.054) times more likely to have the ACKSSuT MDR pattern.
- Isolates which were ESBL-producers were 17.78 (8.80 – 35.95, p=0.000) times more likely to have the ACKSSuT MDR pattern than those that were not ESBL-producers
- Patients with NTS isolates resistant to TMP-SMX were 7.91 (1.77 – 35.25, p=0.007) times more likely to have the ACKSSuT MDR pattern than those that were susceptible to TMP-SMX

Table 3.17 Univariate and multivariate regression models of ACKSSuT MDR pattern

	Univariate model				Multivariate model			
Explanatory variable	n	OR	95% CI	p	n	OR	95% CI	p
TMP-SMX use	551				388			0.489
No		1.00	Reference			1.00	Reference	
Yes		1.91	1.18 – 3.11	0.009		1.32	0.61 – 2.86	
Age (grouped)	3195							
<1 year		1.00	Reference					
1-4 years		0.78	0.61 – 0.99	0.042				
5-14 years		0.39	0.26 – 0.60	0.000				
15-24 years		0.18	0.10 – 0.32	0.000				
25-34 years		0.31	0.23 – 0.42	0.000				
35-44 years		0.29	0.21 – 0.41	0.000				
45-54 years		0.20	0.12 – 0.33	0.000				
55-64 years		0.17	0.08 – 0.35	0.000				
>=65 years		0.23	0.11 – 0.49	0.000				
Province	3195							
Eastern Cape		1.00	Reference					
Free State		0.33	0.18 – 0.61	0.000				
Gauteng		0.45	0.34 – 0.58	0.000				
KwaZulu-Natal		0.51	0.37 – 0.69	0.000				
Limpopo		0.23	0.10 – 0.51	0.000				
Mpumalanga		0.03	0.01 – 0.11	0.000				
Northern Cape		0.47	0.10 – 2.25	0.343				
North West		0.57	0.32 – 1.02	0.057				
Western Cape		0.42	0.30 – 0.59	0.000				
Sex	3103							
Male		1.00				NI***		
Female		0.95	0.79 – 1.13	0.550				
HIV status	613							
No		1.00	Reference			NI***		

Yes		2.18	0.85 – 5.60	0.105				
Antibiotic use in last 2 months	527				388			
No		1.00	Reference			1.00	Reference	
Yes		3.24	1.94 – 5.42	0.000		2.19	0.99 – 4.88	0.054
TMP-SMX resistance	3195				388			
No		1.00	Reference			1.00	Reference	
Yes		98.52	48.85– 198.72	0.000		7.91	1.77 – 35.25	0.007
ESBL-producer	3195				388			
No		1.00	Reference			1.00		
Yes		33.07	26.01 – 42.05	0.000		17.78	8.80 – 35.95	0.000
Other immune compromise	399							
None		1.00	Reference			NI***		
TB		1.23	0.59 – 2.54	0.582				
Chronic disease		D*	-	-				
Other immune suppression		0.49	0.20 – 1.20	0.116				
Invasive NTS	3195							
No		1.00	Reference			****NUC		
Yes		0.39	0.32 – 0.47	0.000				
Serotype	2898							
Typhimurium		1.00						
Isangi		7.04	5.74 – 8.63	0.000				
Enteritidis		D*	-	-				
Species		0.63	0.38 – 1.03	0.063				
Dublin		D*	-	-				
Other		0.22	0.12 – 0.37	0.000				

*D – dropped because no cases in these strata
**C – dropped because of collinearity
***NI – not included in model
****NUC- no unexposed cases

3.9.3 Univariate and multivariate regression modeling of the ACSSuTNx MDR pattern

Univariate and multivariate logistic regression of the ACSSuTNx resistance pattern with each risk factor in turn was carried out and the results are presented in Table 3.18.

3.9.3.1 Univariate logistic regression – primary association

In univariate modeling, the ACSSuTNx MDR pattern was not significantly associated with the primary exposure (use of TMP-SMX prophylaxis) having an odds ratio of 1.44 (95% CI 0.93 – 2.23, p=0.105).

3.9.3.2 Univariate logistic regression – demographic factors

- Age: The odds of having the ACSSuTNx resistance pattern decreased with increasing age as compared to the reference group (under 1 year age group, p=0.0000).

- Sex: Isolates with This MDR pattern were less likely to come from women (OR=0.94, 95% CI 0.82 – 1.09,) than from men (OR=1), although the latter was not statistically significant (p=0.423).
- Province: Isolates with the ACSSuTNx resistance pattern were significantly (p=0.0000) less likely to come from provinces other than Eastern Cape (which was the reference group)

3.9.3.3 Univariate logistic regression – clinical factors

- Prior antibiotic treatment: Isolates from patients who had been on antibiotics in the two months preceding admission were 1.79 times more likely to have the ACSSuTNx resistance pattern (95% CI 1.12 – 2.85, p=0.000).
- HIV status: Isolates from HIV positive patients were 0.71 times less likely to have the ACSSuTNx resistance pattern, however this association was not statistically significant (95% CI 0.38 – 1.31, p=0.277) compared to the reference group (HIV negative).
- Site of infection: Invasive isolates also had a lower odds of association with ACSSuTNx resistance (OR=0.21, 95% CI 0.18 – 0.25) compared to the reference group, which was composed of non-invasive isolates; this association was statistically significant (p=0.000).

3.9.3.4 Univariate logistic regression – microbiological factors

- TMP-SMX resistance: Isolates that were already resistant to TMP-SMX were also 56.04 times more likely to have the ACSSuTNx resistance pattern, this was significant even though the confidence interval for this association was very wide (95% CI 38.26 – 82.11, p=0.000).
- ESBL-producers: These isolates were 43.66 times more likely to have the ACSSuTNx resistance pattern, compared to those isolates that were not ESBL-producers (95% CI 35.82 – 53.22, p=0.000).
- Serotype: S. *enterica* Isangi isolates were 76.84 times more likely to have the ACSSuTNx resistance pattern than the reference group of S. *enterica* Typhimurium isolates (95% CI 60.37 – 97.80, p=0.000). S. *enterica* Dublin isolates did not experience the outcome of interest (i.e. did not have the ACSSuTNx resistance pattern). All other serotypes were 0.51 times as likely to have the ACSSuTNx MDR pattern than S. *enterica* Typhimurium isolates (p=0.003).

3.9.3.5 Multivariate regression modeling of the ACSSuTNx MDR pattern

The outcome of interest and primary explanatory variable (use of TMP-SMX prophylaxis) were put into a model with those variables that were significant at the 90% level in univariate modeling, namely age, province of origin of isolate, site of infection, antibiotic use in the two months preceding admission, ESBL production, resistance to TMP-SMX and serotype. Controlling for these variables, the ACSSuTNx MDR pattern was not significantly associated with TMP-SMX prophylaxis (OR=0.83, 0.39 – 1.74, p=0.617).

The following explanatory variables were significantly associated with the relationship between the ACSSuTNx MDR pattern controlling for all other variables in the multivariate model:

- Isolates that were ESBL-producers were 32.31 (14.19 – 73.55, p=0.000) times more likely to have the ACSSuTNx MDR pattern
- S. *enterica* Isangi isolates from patients on TMP-SMX prophylaxis were 49.54 (18.72 – 171.53, p=0.000) times more likely to have the ACKSSuT MDR pattern
- NTS isolates serotyped as S. *enterica* Species from patients on TMP-SMX prophylaxis were 7.44 times more likely to have the ACSSuTNx MDR pattern (1.79 – 30.80, p=0.006) than S. *enterica* Typhimurium isolates

Table 3.18 Univariate and multivariate regression model of ACSSuTNx MDR pattern

	Univariate model				Multivariate model			
Explanatory variable	n	OR	95% CI	p	n	OR	95% CI	p
TMP-SMX use	772				515			
No		1.00	Reference			1.00	Reference	
Yes		1.44	0.93 – 2.23	0.105		0.83	0.39 – 1.74	0.617
Age (grouped)	3930							
<1 year		1.00	Reference					
1-4 years		0.70	0.58 – 0.86	0.000				
5-14 years		0.28	0.20 – 0.40	0.000				
15-24 years		0.10	0.06 – 0.17	0.000				
25-34 years		0.15	0.11 – 0.19	0.000				
35-44 years		0.12	0.09 – 0.16	0.000				
45-54 years		0.08	0.05 – 0.14	0.000				
55-64 years		0.14	0.08 – 0.25	0.000				
>=65 years		0.13	0.07 – 0.26	0.000				
Sex	3917							
Male		1.00	Reference			NI***		
Female		0.94	0.82 – 1.09	0.423				
Province	4265							
Eastern Cape		1.00	Reference					
Free State		0.73	0.47 – 1.11	0.148				
Gauteng		0.48	0.38 – 0.60	0.000				

KwaZulu-Natal		0.67	0.51 – 0.87	0.003				
Limpopo		0.03	0 – 0.19	0.000				
Mpumalanga		0.02	0.01- 0.08	0.000				
Northern Cape		0.17	0.02 – 1.35	0.093				
North West		0.50	0.30 – 0.84	0.008				
Western Cape		1.12	0.87 – 1.44	0.370				
Antibiotic use in last 2 months	742							
No		1.00	Reference					
Yes		1.79	1.12 – 2.85	0.015				
HIV status	827							
No		1.00	Reference			NI***		
Yes		0.71	0.38 – 1.31	0.277				
Other immune compromise	476					NI***		
None		1.00	Reference					
TB		0.66	0.32 – 1.34	0.247				
Chronic disease		D*	-	-				
Other immune suppression		0.74	0.33 – 1.64	0.454				
Invasive NTS	4265							
No		1.00	Reference			****NUC		
Yes		0.21	0.18 – 0.25	0.000				
TMP-SMX resistance	4265							
No		1.00	Reference					
Yes		56.04	38.26 – 82.11	0.000				
ESBL-producer	4265				515			
No		1.00	Reference			1.00	Reference	
Yes		43.66	35.82 – 53.22	0.000		32.31	14.19 – 73.55	0.000
Serotype	4138				515			
Typhimurium		1.00	Reference			1.00	Reference	
Isangi		76.84	60.37 – 97.80	0.000		56.66	18.72 – 171.53	0.000
Enteritidis		0.08	0.02 – 0.33	0.000		D*	-	-
Species		1.51	0.96 – 2.37	0.076		7.44	1.79 – 30.80	0.006
Dublin		D*	-	-		D*	-	-
Other		0.51	0.33 – 0.80	0.003		0.68	0.18 – 4.93	0.948

*D – dropped as no cases in strata
**C – dropped because of collinearity
***NI – not included in model
****NUC – no unexposed cases

3.9.4 Univariate and multivariate regression modeling of the AKSSuT MDR pattern

Univariate and multivariate logistic regression of the AKSSuT resistance pattern with each risk factor in turn was carried out and the results are presented in Table 3.18.

3.9.4.1 Univariate logistic regression – primary association

In univariate modeling, the AKSSuT MDR pattern was very significantly associated with the primary exposure (use of TMP-SMX prophylaxis) having an odds ratio of 2.00 (95% CI 1.30 – 3.09, p=0.002).

3.9.4.2 Univariate logistic regression – demographic factors

- Age: The odds of having the AKSSuT resistance pattern decreased with increasing age as compared to the reference group (under 1 year age group, p=0.0000).
- Sex: Isolates with this MDR pattern were less likely to come from women (OR=0.98, 95% CI 0.83 – 1.16) than from men (OR=1), although the latter was not statistically significant (p=0.836).
- Province: Isolates with the AKSSuT resistance pattern were significantly (p=0.0000) less likely to come from provinces other than Eastern Cape (which was the reference group)

3.9.4.3 Univariate logistic regression – clinical factors

- Prior antibiotic treatment: Isolates from patients who had been on antibiotics in the two months preceding admission were 2.32 times more likely to have the AKSSuT resistance pattern (95% CI 1.44 – 3.73, p=0.001).
- HIV status: Isolates from HIV positive patients were 2.65 times more likely to have the AKSSuT resistance pattern, this association was statistically significant (95% CI 1.12 – 6.22, p=0.027) compared to the reference group (isolates from HIV negative patients).
- Site of infection: Invasive isolates also had a lower odds of association with AKSSuT resistance (OR=0.55, 95% CI 0.46 – 0.65) compared to the reference group, which was composed of non-invasive isolates; this association was statistically significant (p=0.000).

3.9.4.4 Univariate logistic regression – microbiological factors

- TMP-SMX resistance: Isolates that were already resistant to TMP-SMX were also 113.49 times more likely to have the AKSSuT resistance pattern, this was statistically significant (95% CI 58.54 – 220.03, p=0.000).

- ESBL-producers: These isolates were 16.46 times more likely to have the AKSSuT resistance pattern, compared to those isolates that were not ESBL-producers (95% CI 13.51 – 20.05, p=0.000).
- Serotype: S. *enterica* Isangi isolates were 4.63 times more likely to have the AKSSuT resistance pattern than the reference group of S. *enterica* Typhimurium isolates (95% CI 3.82 – 5.61, p=0.000). S. *enterica* Dublin and Enteritidis isolates all experienced the outcome of interest (i.e. had the AKSSuT resistance pattern and were excluded from the model). Serotypes grouped as S. *enterica* Species were 0.66 times less likely to have the AKSSuT MDR pattern (0.44 – 0.99, p=0.047). All other serotypes were 0.15 times less likely to have the AKSSuT MDR pattern than S. *enterica* Typhimurium isolates (0.09 – 0.26, p=0.000).

3.9.4.5 Multivariate regression modeling of the AKSSuT MDR pattern

The outcome of interest and primary explanatory variable (use of TMP-SMX prophylaxis) were put into a model with those variables that were significant at the 90% level in univariate modeling, namely age, province of origin of isolate, HIV status, site of infection, antibiotic use in the two months preceding admission, ESBL production, resistance to TMP-SMX and serotype. After controlling for these variables, TMP-SMX prophylaxis was not significantly associated with the AKSSuT MDR pattern (OR=0.92, 0.50 – 1.70, p=0.802). The following were significantly associated with the relationship between the AKSSuT MDR pattern after controlling for the effects of all other variables in the model:

- Isolates that were ESBL-producers were 10.72 (5.60 – 20.52, p=0.000) times more likely to have the AKSSuT MDR pattern
- Isolates from HIV positive patients were 5.89 times more likely to have the AKSSuT MDR pattern; this association was statistically significant (p=0.000) even though the confidence interval around the odds ratio was quite wide (1.61 – 21.60)
- Isolates that were TMP-SMX resistant were 16.90 times more likely to have the AKSSuT MDR pattern (3.95 – 72.26, p=0.000)

Table 3.19 Univariate and multivariate regression model of AKSSuT MDR pattern

	Univariate model				Multivariate model			
Explanatory variable	n	OR	95% CI	p	n	OR	95% CI	p
TMP-SMX use	551				364			
No		1.00	Reference			1.00	Reference	
Yes		2.00	1.30 – 3.09	0.002		0.92	0.50 – 1.70	0.802

Age (grouped)	3028							
<1 year		1.00	Reference					
1-4 years		0.76	0.60 – 0.97	0.018				
5-14 years		0.43	0.29 – 0.64	0.000				
15-24 years		0.29	0.18 – 0.46	0.000				
25-34 years		0.42	0.32 – 0.55	0.000				
35-44 years		0.37	0.27 – 0.49	0.000				
45-54 years		0.32	0.21 – 0.49	0.000				
55-64 years		0.39	0.23 – 0.66	0.000				
>=65 years		0.24	0.12 – 0.49	0.000				
Sex	3103							
Male		1.00	Reference			NI***		
Female		0.98	0.83 – 1.16	0.836				
Province	3195							
Eastern Cape		1.00	Reference					
Free State		0.32	0.18 – 0.60	0.000				
Gauteng		0.59	0.46 – 0.77	0.000				
KwaZulu-Natal		0.62	0.46 – 0.83	0.002				
Limpopo		0.29	0.14 – 0.61	0.001				
Mpumalanga		0.05	0.02 – 0.14	0.000				
Northern Cape		0.46	0.10 – 2.19	0.326				
North West		0.60	0.34 – 1.05	0.075				
Western Cape		0.43	0.31 – 0.59	0.000				
Antibiotic use in last 2 months	527							
No		1.00	Reference					
Yes		2.32	1.44 – 3.73	0.001				
HIV status	613				364			
No		1.00	Reference			1.00	Reference	
Yes		2.65	1.12 – 6.32	0.027		5.89	1.61 – 21.60	0.007
Other immune compromise	416							
None		1.00	Reference			NI***		
TB		1.15	0.60 – 2.21	0.119				
Chronic disease		0.19	0.02 – 1.54	0.670				
Other immune suppression		0.61	0.29 – 1.32	0.210				
Invasive NTS	3195					364		
No		1.00	Reference			1.00	Reference	
Yes		0.55	0.46 – 0.65	0.000		1.05	0.4 – 29.17	0.977
TMP-SMX resistance	3195				364			
No		1.00	Reference			1.00	Reference	
Yes		113.49	58.54– 220.03	0.000		16.90	3.95 – 72.26	0.000
ESBL-producer	3195				364			
No		1.00	Reference			1.00	Reference	
Yes		16.46	13.51 – 20.05	0.000		10.72	5.60 – 20.52	0.000
Serotype	2898							
Typhimurium		1.00	Reference					
Isangi		4.63	3.82 – 5.61	0.000				
Enteritidis		D*	-	-				
Species		0.66	0.44 – 0.99	0.047				
Dublin		D*	-	-				
Other		0.15	0.09 – 0.26	0.000				

*D – dropped as no cases in strata
**C – dropped because of collinearity
***NI – not included in model

3.9.5 Univariate and multivariate regression modeling of isolates with at least one MDR pattern

3.9.5.1 Univariate logistic regression – primary association

In univariate modeling, having at least one multi-drug resistance pattern was significantly associated with the use of TMP-SMX prophylaxis, having an odds ratio of 1.43 (95% CI 1.02 – 2.01, p=0.039).

3.9.5.2 Univariate logistic regression – demographic factors

- Age: The odds of having at least one MDR pattern decreased significantly with increasing age as compared to the reference group (p=0.0000).
- Sex: Isolates with this MDR pattern were less likely to come from women (OR=0.96, 95% CI 0.85 – 1.10) than from men (OR=1), although the latter was not statistically significant (p=0.582).
- Province: Isolates with the AKSSuT resistance pattern were significantly (p=0.0000) less likely to come from provinces other than Eastern Cape (which was the reference group)

3.9.5.3 Univariate logistic regression – clinical factors

- Prior antibiotic treatment: Isolates from patients who were treated with antibiotics in the two years preceding admission were 1.68 times more likely to have at least one of the MDR patterns identified (95% CI 1.16 – 2.44, p=0.006).
- HIV status: Isolates from HIV positive patients and invasive sites were not significantly associated with having at least one MDR pattern,). Other immune compromise factors were not significantly associated with having at least one MDR pattern.
- Site of infection: Isolates from invasive sites were less likely to have at least one MDR pattern (OR=0.34, 95% CI 0.30 – 0.39) compared to the reference group, which was composed of non-invasive isolates; this association was statistically significant (p=0.000).

3.9.5.4 Univariate logistic regression – microbiological factors

- TMP-SMX resistance: Isolates that were already resistant to TMP-SMX were also 42.06 times more likely to have at least one MDR pattern, this was statistically significant (95% CI 32.77 – 53.99, p=0.000).
- ESBL-producers: These isolates were 56.81 times more likely to have at least one MDR pattern, compared to those isolates that were not ESBL-producers (95% CI 46.25 – 69.79, p=0.000).
- Serotype: S. *enterica* Isangi isolates were 55.25 times more likely to have at least one MDR pattern than the reference group of S. *enterica* Typhimurium isolates (95% CI 41.52 – 73.52, p=0.000). S. *enterica* Enteritidis isolates were 0.18 times less likely to have at least one MDR pattern; this association was statistically significant (95% CI 0.10 – 0.30, p=0.000). All S. *enterica* Dublin isolates experienced the outcome of interest (i.e. had at least one MDR pattern and were excluded from the model). All other serotypes were 0.28 times less likely to be associated with any MDR pattern (95% CI 0.20 – 0.39, p=0.000).

3.9.5.5 Multivariate regression modeling of at least one MDR pattern

The outcome of interest and primary explanatory variable (use of TMP-SMX prophylaxis) were put into a model with those variables that were significant at the 90% level in univariate modeling, namely age, province of origin of isolate, site of infection, antibiotic use in the two months preceding admission, ESBL production, resistance to TMP-SMX and serotype. After controlling for these variables, TMP-SMX prophylaxis was not significantly associated with having at least one MDR pattern (OR=0.76, 0.44 – 1.31, p=0.323). The following were significantly associated with having at least one MDR pattern after controlling for the effects of all other variables in the model:

- TMP-SMX resistant isolates were 4.94 times more likely (95% CI 2.50 – 9.74, p=0.000) to have at least one MDR pattern
- ESBL-producing isolates were 15.62 times more likely to have at least one MDR pattern; this association was statistically significant (95% CI 8.58 – 28.42, p=0.000)
- S. *enterica* Isangi isolates were 4.32 times more likely to have at least one MDR pattern (95% CI 1.43 – 13.08, p=0.010), while isolates serotyped as S. *enterica* Species were 3.58 times more likely to have at least one MDR pattern (95% CI 1.37 – 9.39, p=0.009) when compared to *S. enterica* Typhimurium isolates. All other isolates were less likely to have at least one MDR pattern (OR=0.15, 95% CI 0.03 – 0.62, p=0.009)

- Isolates from certain age categories were less likely to have at least one MDR pattern than infants less than one year of age

Table 3.20 Univariate and multivariate regression model of ≥1MDR pattern

	Univariate model				Multivariate model			
Explanatory variable	n	OR	95% CI	p	n	OR	95% CI	p
TMP-SMX use	772				577			
No		1.00	Reference			1.00	Reference	
Yes		1.43	1.02 – 2.01	0.039		0.76	0.44 – 1.31	0.323
Age (grouped)	3930				577			
<1 year		1.00	Reference			1.00	Reference	
1-4 years		0.64	0.52 – 0.78	0.000		0.34	0.12 – 0.97	0.044
5-14 years		0.28	0.20 – 0.38	0.000		0.38	0.09 – 1.56	0.181
15-24 years		0.17	0.12 – 0.24	0.000		0.39	0.12 – 1.26	0.116
25-34 years		0.21	0 17 – 0.26	0.000		0.40	0.18 – 0.91	0.030
35-44 years		0.18	0.14 – 0.23	0.000		0.42	0.19 – 0.93	0.033
45-54 years		0.16	0.11 – 0.22	0.000		0.37	0.13 – 1.06	0.065
55-64 years		0.22	0.15 – 0.33	0.000		0.95	0.25 – 3.70	0.946
>=65 years		0.13	0.08 – 0.23	0.000				
Sex	3917					NI**		
Male		1.00	Reference					
Female		0.96	0.85 – 1.10	0.582				
Province	4265							
Eastern Cape		1.00	Reference					
Free State		0.45	0.30 – 0.69	0.000				
Gauteng		0.51	0.41 – 0.63	0.000				
KwaZulu-Natal		0.53	0.41 – 0.69	0.000				
Limpopo		0.30	0.16 – 0.54	0.000				
Mpumalanga		0.07	0.03 – 0.13	0.000				
Northern Cape		0.21	0.05 – 0.99	0.048				
North West		0.48	0.31 – 0.76	0.002				
Western Cape		0.70	0.55 – 0.90	0.005				
Antibiotic use in last 2 months	742							
No		1.00	Reference					
Yes		1.68	1.16 – 2.44	0.006				
HIV status	827							
No		1.00	Reference			NI**		
Yes		0.97	0.58 – 1.63	0.915				
Other immune compromise	501							
None		1.00	Reference					
TB		1.08	0.59 – 1.97	0.806				
Chronic disease		0.47	0.14 – 1.55	0.212				
Other immune suppression		0.89	0.45 – 1.76	0.735				
Invasive NTS	4265							
No		1.00	Reference					
Yes		0.34	0.30 – 0.39	0.000				
TMP-SMX resistance	4265				577			
No		1.00	Reference			1.00	Reference	
Yes		42.06	32.77– 53.99	0.000		4.94	2.50 – 9.74	0.000
ESBL-producer	4265				577			

No		1.00	Reference			1.00	Reference	
Yes		56.81	46.25 – 69.79	0.000		15.62	8.58 – 28.42	0.000
Serotype	4138				577			
Typhimurium		1.00	Reference			1.00	Reference	
Isangi		55.25	41.52 – 73.52	0.000		4.32	1.43 – 13.08	0.010
Enteritidis		0.18	0.10 – 0.30	0.000		-	-	-
Species		0.86	0.60 – 1.22	0.389		3.58	1.37 – 9.39	0.009
Dublin		NC***	-	-		NC***	-	-
Other		0.28	0.20 – 0.39	0.000		0.15	0.03 – 0.62	0.009

*D – dropped as no cases in strata
**C – dropped because of collinearity
***NI – not included in model

3.10 Relationship between invasive disease and TMP-SMX resistance

A univariate logistic regression model was fitted to investigate the relationship between invasive NTS infection and TMP-SMX resistance. This analysis produced an odds of the association between invasive disease and TMP-SMX resistance of 0.87 (95% CI 0.77 – 0.99, p=0.029), implying that invasive disease is protective against TMP-SMX resistance.

4.0 DISCUSSION

4.1 Annual incidence of NTS

The annual incidence of invasive NTS was consistent with findings reported previously (GERMSSA, 2006). There was insufficient data to assess trends in NTS incidence over time as this analysis covered only three year's of surveillance data. Furthermore, incidence was only calculated for invasive isolates as not all non-invasive isolates are detected by routine / enhanced surveillance or cultured in practice.

4.2 Prevalence of NTS serotypes by year and geographically

S. *enterica* Typhimurium was the most predominant serotype identified, contributing just under 50% of isolates tested during the period of surveillance (i.e. 01/01/2003 – 31/12/2005). This finding is in keeping with what was reported by Helms and others (Helms et al, 2005). Although NTS surveillance data for 2006 has reflected a prevalence of S. *enterica* Typhimurium isolates of 68% (GERMSSA, 2006), analysis for trends over time was not carried out because of insufficient data.

4.3 Prevalence of NTS resistance in South Africa

A very high proportion, 82.84%, of all isolates tested were resistant to more than one of the 15 antibiotics tested. 30.88% of the isolates tested were ESBL-producers, this is in keeping with more recent findings in South Africa (GERMSSA, 2006). 76.31% of ESBL-producing isolates were also resistant to nalidixic acid. This high level of resistance to nalidixic acid means that the patients who contributed these isolates would not be able to be treated effectively with floroquinolones. This also implies that floroquinolones, often a last resort against resistant infections, may no longer be able to be successfully used. The majority of the ESBL-producing isolates (44.04%) were received from Gauteng, with the Western Cape (20.20%) having the next highest number of ESBL-producing isolates. It must be borne in mind that this finding may reflect the fact that access to medical care may be easier and public health infrastructure may be better in these provinces. These results and the high resistance rates observed from these two provinces, in general, may therefore reflect the ease with which cases are detected, as well as higher numbers of NTS cases diagnosed and hospital admissions in these provinces. This study also found that, between 2003 and 2005, there were high rates of resistance to all antibiotics tested except for ciprofloxacin and imipenem. Resistance to imipenem in NTS was not observed as

expected in South Africa (personal communication, K Keddy, 2009). The low resistance to ciprofloxacin is in keeping with previously observed lack of resistance to this drug (Kariuki et al, 2005). This implies that ciprofloxacin may still be effective against NTS. Across all provinces, consistently high rates of resistance (>56% of isolates) were observed to sulfamethoxazole. This finding is similar to that of Mwansa and co-workers (Mwansa et al, 2002) and has implications for the continued use of antibiotics against diseases when these levels of resistance exist.

4.4 Prevalence of MDR patterns

GERMSSA reported that 50.7% of NTS isolates were penta-resistant, this study found that 36.51 percent of isolates exhibited at least one of the MDR penta-resistance patterns (GERMSSA, 2006). Of the NTS isolates tested, 33.93% exhibited the classic penta-resistance pattern (ACSSuT), while 20.03%, 26.14% and 23.47% of isolates exhibited MDR patterns ACKSSuT, ACSSuTNx and AKSSuT respectively, over the period 2003 to 2005. The low rates of AKSSuT and ACKSSuT MDR patterns may be attributable to this data not being collected prior to 2004.

The majority of isolates with the MDR patterns came from younger patients with the mean age of cases ranging from 8.11 to 13.52 years compared to controls (isolates which did not have any of the 4 MDR patterns identified) which ranged in mean age from 21.92 to 24.76 years. There were no significant differences in the distribution of cases and controls between male and female patients, regardless of the MDR pattern exhibited by the isolates.

4.5 Geographic distribution of MDR NTS

Although χ^2 tests showed that there were significant differences in the distribution of cases and controls in the provinces from which isolates were obtained (p=0.000), univariate modeling of each MDR pattern with province showed significant geographical differences only for the MDR pattern ACSSuTNx (OR 5.01 95% CI 1.21 – 20.73; p=0.026) . As the confidence interval around this odds ratio is quite wide, some uncertainty exists as to the validity of this finding. This finding may also reflect differences between provinces, in terms of surveillance systems as well as access to health care, the capacity to detect cases accurately (which may be better in provinces with large academic hospitals). As this is the first such analysis of South African surveillance data for NTS resistance, it will be of interest to see how this compares with data collected in future.

4.6 Association between prophylactic TMP-SMX usage and MDR NTS

This study demonstrated that there are associations between prophylactic usage of trimethoprim-sulfamethoxazole (TMP-SMX) and multi-drug resistance (MDR) patterns in non-typhoidal salmonellae (NTS) isolates tested as part of a national surveillance programme.

These associations were not significant for the MDR patterns ACSSuT and ACSSuTNx, but NTS isolates from patients who had been on prophylactic TMP-SMX were 1.91 times more likely to have the ACKSSuT MDR pattern (95% CI 1.14 – 3.19, p=0.0080) and 2.00 times more likely to have the AKSSuT MDR pattern (95% CI 1.26 – 3.15, p=0.0015). In addition, NTS isolates from patients on prophylactic TMP-SMX were 1.43 times more likely to have at least one of the four MDR patterns investigated (95% CI 1.00 – 2.04, p = 0.0388).

There were no previously published findings against which these observed associations could be measured; although the observation and prevalence of these patterns is in keeping with what was found by other researchers (Glynn et al, 1998; Helms et al, 2002; Helms et al, 2005 and Rabatsky-Ehr et al, 2004).

In multivariate regression analysis, TMP-SMX prophylaxis was not significantly associated with any of the MDR patterns. This could be due to the loss of observations in multivariate regression models to account for the large proportion of missing data on key factors (e.g. TMP-SMX prophylaxis, prior treatment with antibiotics, other immune compromise).

4.7 Other factors associated with MDR NTS

4.7.1 Demographic factors

In univariate logistic regression models, the following factors were significantly associated with each MDR pattern:

- Age: The odds of having either ACSSuT, ACKSSuT, ACSSuTNx, AKSSuT or more than one of these MDR patterns decreased with each category increase in age.
- Province: There was a lower odds of having and MDR pattern if an isolate came from a province other than the Eastern Cape. We were unable to compare this to previous findings, as there were no studies that have looked at the distribution of MDR NTS patterns in NTS in South Africa prior to this.

Neither of these factors was significantly associated with TMP-SMX prophylaxis in multivariate analysis.

4.7.2 Clinical factors

The following factors were significantly associated with TMP-SMX prophylaxis in univariate regression modeling:

- Prior treatment with antibiotics: isolates from patients who had been on antibiotic treatment in the two months preceding admission were more likely to have each of the MDR patterns – this is in keeping with existing knowledge on the association between the use of antibiotics and drug resistance (EMEA, 1999; Glynn et al, 2004; Fisk et al 2005).
- Site of infection: Invasive isolates were less likely to have either one or more of these MDR patterns – this conflicts with earlier evidence that links invasive disease to increased likelihood of drug resistance (Rabatsky-Ehr et al, 2004; Fisk et al, 2005, CDC, 2004). It is possible that there may be some underlying interaction between HIV status, being on TMP-SMX prophylaxis and invasive disease. 91% of invasive isolates were HIV positive, but it was not possible to assess this because all non-invasive isolates were HIVpositive / negative.
- In addition, HIV status was associated with the AKSSuT MDR pattern. This finding is consistent with existing knowledge (Gianella, 1996), although we were unable to explain why it was observed only to the AKSSuT MDR pattern, and there is a lack of published literature to either support or dispute this finding.
- In multivariate modeling, prior antibiotic treatment remained significantly associated with the ACKSSuT MDR pattern only, while HIV status remained significantly associated with the AKSSuT MDR pattern. Site of infection (i.e. whether disease was invasive or not) was dropped from all of the multivariate models as there were no non-invasive cases in the models.

4.7.3 Microbiological factors

In univariate logistic regression, the following microbiologic factors were significantly associated with each MDR pattern:

- ESBL production: ESBL-producing isolates were more likely to have either or more than one of the MDR patterns. This was expected as ESBL-producing bacteria are

known to display resistance to other classes of antibiotics (Kruger et al, 2004 ; Turner, 2005).

- Resistance to TMP-SMX: Isolates that were resistant to TMP-SMX were also more likely to have at least one of the MDR patterns observed and this may be attributable to co-resistance (CDC, 2004). In particular the ACSSuT MDR pattern is also known to be resistant to TMP-SMX and floroquinolones (EMEA, 1999). Isolates that were resistant to TMP-SMX were more likely to have the ACSSuT, ACKSSuT or AKSSuT resistance patterns, these associations were independent of other explanatory factors included in the multivariate model.
- Serotype: S. *enterica* Isangi isolates were more likely to have any one or more of the MDR patterns investigated. S. *enterica* Enteritidis isolates were less likely to have any one or more of the MDR patterns than S. *enterica* Typhimurium isolates. This is consistent with differences in resistance by NTS serotype reported previously (APUA, 2003).

In multivariate regression modeling of the odds of having either one or more of the four MDR patterns:

- ESBL production remained significant in all the models.
- TMP-SMX resistance was significantly associated with the ACSSuT, ACKSSuT and AKSSuT patterns, as well as with having more one or more MDR pattern.
- Serotype remained significantly associated with the ACSSuT and ACSSuTNx MDR patterns, and with having at least one MDR pattern, contrary to what Kariuki et al observed in Kenya (Kariuki et al, 2005).

4.8 Association between invasive disease and TMP-SMX resistance

Invasive disease was associated with an 13% decrease in the odds of an isolate being resistant to TMP-SMX. This is contrary to what is expected from the literature, as invasive disease is known to be associated with resistance (CDC, 2004; Fisk et al, 2005; Rabatsky-Ehr et al, 2004).

4.9 Limitations of this study

There was a large number of isolates (81.90%) for which patients' prophylactic TMP-SMX usage was not ascertained (either recorded as unknown or missing). This is primarily because this information would only be solicited by the enhanced surveillance sites,

effectively causing non-standardised data collection. Similarly, HIV status, antibiotic use in the two months preceding admission and immune compromise was not ascertained for a large proportion of patients who contributed isolates (see Section 3.2). The possibility that this has introduced bias has to be taken into account.

The CLSI breakpoints were not specifically developed for Salmonella, and are therefore not ideal for determining antimicrobial susceptibility to this organism.

It must be also be borne in mind that the database development and data collection processes were completed before the statistical analysis plan was written, certain questions could therefore not be adequately answered.

A possible source of bias may have been the fact that there was no clear sampling strategy. This represents a limitation as the participants who make up the study sample may constitute people who have access to health care / are more likely to exhibit health-seeking behaviour, and because it is expected that provinces with academic centres may be more vigilant as regards disease surveillance. Furthermore, not all cases of salmonella are eventually detected and reported through surveillance (Alos et al, 2004) and so we cannot say with certainty how much the study sample represents the population.

Furthermore, recall bias may be involved as some of the data gathered is reliant on participants' / their relatives' recollections of events post-diagnosis.

As this study was based on cross-sectional data, it was not possible to demonstrate temporality or the direction of relationships.

At the time of revising this report, a literature search was conducted to see if any updates on this subject had been published; nothing additional was found against which to compare the findings of this study.

5.0 CONCLUSIONS

This study has identified high rates of resistance to 13 of the 15 antibiotics tested, with the highest resistance rates observed to sulfamethoxazole.

We have also shown an association between TMP-SMX prophylaxis and two resistance patterns, viz. ACKSSuT and AKSSuT. Patients on TMP-SMX prophylaxis were 1.91 and 2.00 times more likely to have NTS isolates with either the ACKSSuT or AKSSuT resistance patterns, respectively, than those who were not on TMP-SMX prophylaxis.

Age, province of origin of isolate, prior treatment with antibiotics, site of infection, ESBL production, resistance to TMP-SMX and serotype were associated with all MDR patterns in univariate regression modeling. There did not seem to be any significant difference in the proportions of MDR cases and controls from male and female patients in bivariate analysis and univariate regression.

Demographic factors (age and province) were not significantly associated with any of the MDR patterns in multivariate regression. ESBL production remained associated with all MDR patterns in multivariate regression, while age, prior antibiotic treatment, HIV status, serotype and TMP-SMX resistance remained associated with different combinations of the MDR patterns investigated.

TMP-SMX usage for NTS salmonella does not seem to be appropriate in view of the high rates of resistance observed. Although 76.61% of patients on TMP-SMX prophylaxis were resistant to it, we must emphasize that TMP-SMX prophylaxis' usefulness still lies in preventing and treating other bacterial infections (such as pneumonia).

6.0 RECOMMENDATIONS

- Patients presenting with NTS and on TMP-SMX prophylaxis should continue with that regimen and other antibiotics should be considered for the NTS treatment (if clinically indicated).

- Greater awareness is needed on treatment adherence and the importance of completing prescribed regimens to minimize resistance.

- It is important to continue with surveillance (especially in settings where TMP-SMX prophylaxis is available) as it can be used to monitor whether resistance rates are increasing or decreasing and to which antibiotics.

- Improved data collection and quality control procedures to fully ascertain the extent of missing data – i.e. have a response for each question on the CRF, even if that response is unknown, code it as such.

- Double data entry and verification of surveillance data at the EDRU to minimize data entry errors.

- Trend analysis after sufficient data (at least 6 years worth of data) is collected to look at trends over time. It may be useful to repeat this analysis every three years to continue monitoring trends in resistance.

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8.0 APPENDICES

Appendix A – Enhanced surveillance protocol

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

Dr Anne von Gottberg, Ms Linda de Gouveia, Ms Avril Wasas, Dr Shabir Madhi, and Prof Keith Klugman and others

Respiratory and Meningeal Pathogens Research Unit NHLS/MRC/WITS (RMPRU)

National Health Laboratory Service (NHLS) / National Institute for Communicable Diseases (NICD)

Dr Karen Keddy, Ms Arvinda Sooka

Enteric Diseases Reference Unit (EDU)

NHLS / NICD

Dr Anne Schuchat

National Center for Infectious Diseases (NCID)

Centers for Disease Control and Prevention (CDC)

Atlanta

Clinical and laboratory collaborators identified at 10 centers in the Republic of South Africa: to be called **"Enhanced Respiratory and Meningeal Pathogens Surveillance Group"**

Representatives from the Department of Health (DOH): **Dr Lindiwe Makobalo, Ms P. Netshidzivhani and Dr Hans van Heerden**

Introduction:

Surveillance of disease in South Africa includes both clinical and laboratory notification. It is only with the establishment of a truly national network of laboratories under the umbrella of the National Health Laboratory Service (NHLS), together with the commitment to public health issues in the establishment of the National Institute for Communicable Disease (NICD), that the possibility of representative surveillance in our country has been made possible.

At present passive laboratory surveillance has been recommenced over the last five years for the following bacterial organisms: *Salmonella* spp., *Shigella* spp., *Vibrio cholerae, Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae* type b. Susceptibility of these isolates are also being monitored, in line with awareness of international trends of increasing antimicrobial resistance. The information gathered from this surveillance plays an important role in the control and prevention of these diseases in our population, and drug susceptibility data are extremely useful to clinical care providers.

The human immunodeficiency virus (HIV/AIDS) has increased the incidence of some of these diseases significantly, specifically *S. pneumoniae* and *Salmonella* spp.; and reduces vaccine efficacy of others, especially, *H. influenzae* type b disease. Other changes affected by the HIV/AIDS epidemic, including antimicrobial susceptibility in the face of antibiotic prophylaxis, need to be monitored and evaluated in order for us to respond to the needs of our communities.

Project description:

- Over an initial period of one year, from February 2003 to February 2004, national surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae, Neisseria meningitidis* and *Salmonella* spp. causing invasive disease in children and adults in South Africa and isolated from the bloodstream or cerebrospinal fluid is to be enhanced. These data will guide the continuation of such surveillance over a period of 5 years.
- Ten centers are to be identified in the country that will allow for the collection of a large number of episodes (see below for list of centers).
- Data to be collected are age, sex, type of specimen, clinical presentation, HIV status if available, other underlying diseases, vaccination history, previous antibiotic use in the last month, regular attendance at a clinic/day-care center *etc*., therapy, and outcome (see attached data form)
- To assist in capturing these data each center will have a surveillance officer employed for the duration of a year, trained in capturing data and the ethical issues attendant with this.
- All above bacterial isolates associated with invasive disease will be appropriately stored and sent to the RMPRU and EDU for typing and susceptibility testing
- To identify community-based organizations, services and clinics in each center that may offer prophylactic antibiotics, and by means of a questionnaire to assess protocols followed. This will assist in evaluation of differences in trends in the different centers.
- If possible to define the population that each hospital/hospital complex serves and to determine incidence of the each of the abovementioned infections.

- Monthly review of cases identified at each center, completeness of data collected and confirmation of viable isolate for each episode at RMPRU and EDU; and to address any shortcomings highlighted by this review.

Problems to overcome:

- The present system of national surveillance for some infections is laboratory dependent and at present has no formal system of audit.
- Isolates and information are being sent to the central laboratory and there is little feedback. Regional laboratories want more ownership with regard to both isolates and to clinical data being captured.
- A national health laboratory system has only recently been established and a representative laboratory network within South Africa is only now being created.
- Collaboration with the Department of Health is vital and this communication channel is still being optimized.
- Private and public laboratories also have to acknowledge the combined role played in the diagnosis of diseases of public health importance.
- HIV/AIDS is playing an important role in the changing epidemiology of disease in our population, and interventions introduced may change antimicrobial susceptibility patterns of bacteria commonly isolated.

Objectives of this project:

- To strengthen sentinel surveillance in respiratory and diarrhoeal diseases (specifically of the following organisms: *Streptococcus pneumoniae*, *Haemophilus influenzae, Neisseria meningitidis* and non-typhoidal *Salmonella* spp.)

- ❖ To monitor antimicrobial resistance in the above pathogens causing disease in children and adults, in particular as it pertains to the use of trimethoprim-sulfamethoxazole in the care and support of patients with HIV/AIDS
- ❖ To document temporal changes of burden of disease due to HIV/AIDS
- ❖ To facilitate monitoring the use of cotrimoxazole (trimethoprim-sulfamethoxazole)
- ❖ To idenitfy cases of disease due to the above organisms occurring despite cotrimoxazole prophylaxis and the proportion due to resistance

Potential secondary objectives:

- To compare resistance and other characteristics of episodes of pneumococcal and *Salmonella* spp. invasive disease between hospitals in the enhanced surveillance and private hospitals reporting to RMPRU

Strategies to achieve these objectives:

- ❖ To improve substantially the data collected in 10 hospital/complexes in the country by placing increased resources in these centers: staff, training and on-site visits will be included
- ❖ To evaluate some of the reasons for poor data and/or isolate collection
- ❖ To improve the flow of information from hospital to regional laboratory to national surveillance laboratory to national government and back to the source laboratories and hospitals

Results and benefits expected:

- ❖ Improved national surveillance of the above bacteria by addressing issues identified during this project and by improved communications within the networks established

- ❖ Important influences of HIV/AIDS on the above diseases will be recognised and can be addressed
- ❖ Using trends in these opportunistic infections to assess the HIV epidemic and its control
- ❖ Effects of prophylactic use of trimethoprim-sulfamethoxazole can be quantified and may lead to changes in this intervention or better mechanisms to guard against its effects

Plan and proposed time lines:

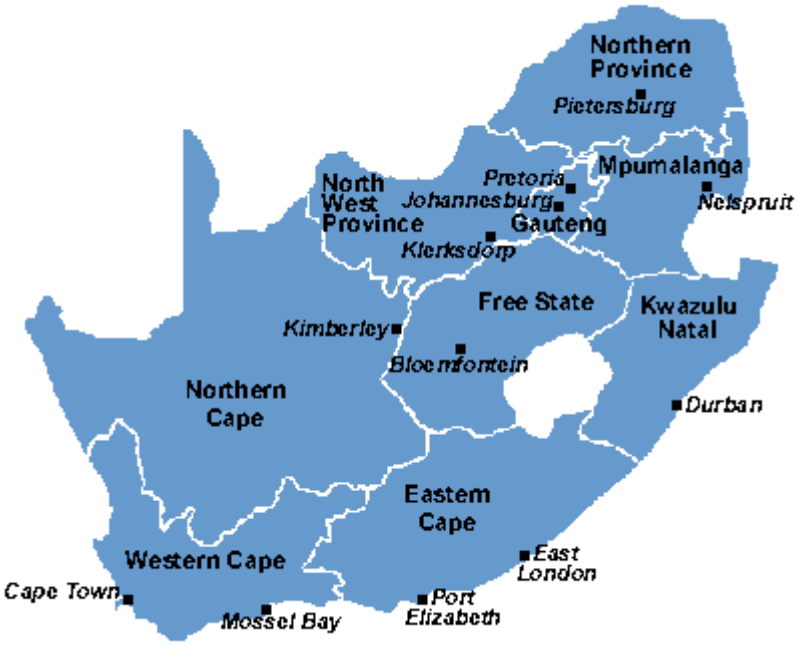
1. Identify 10 hospitals/complexes for enhanced surveillance: in consultation with all relevant parties (November 2002). In each center both clinical and laboratory collaborators will be identified.
2. Finalise data collection forms together with a clear data dictionary that explains/defines each variable and an instruction sheet to assist in the completion of the forms; forms will be piloted with users at different centres (January 2003)
3. Ethics approval for prospective data capture that is not already contained in our surveillance ethics approval (reference number: 00105; granted 8/2/2000) has been granted in October 2002. Each site will need to assess ethics and/or procedural requirements.
4. Hiring of 10 surveillance officers in the regions by the project collaborators identified in those regions (January and February 2003); hiring of 2 project co-ordinators (November/December 2002); hiring of 2 data clerks (November/December 2002)

5. Convene meeting of surveillance officers: education and preparation of surveillance officers with regard to principles and ethics of data capture; familiarization with surveillance procedures including data capture, transmission and audit (March 2002)
6. Provide regular feedback to sentinel sites
7. To commence piloting enhanced surveillance by end of March/April 2003.
8. Follow-up (annual?) meetings (mid to late 2003)

Methods:

- Surveillance officers will review laboratory records to identify all blood and cerebrospinal fluid cultures that are positive for *Salmonella* spp., *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* in adults and children. This will be done almost daily at centers with large numbers of positive cultures, and once or twice weekly at other centers.
- Surveillance officers will oversee the completion of laboratory and clinical data on data capture forms (draft form attached).
- All centers will require evaluation of the hospitals and populations drained by each laboratory (need to define population data in provinces vs cities vs urban centers)
- Weekly planning of travelling between hospital/complexes as required to capture the relevant clinical information.
- Isolates identified in the laboratory will be sent to RMPRU and EDU on an ongoing basis (within days of isolation) through the internal laboratory transport system. These will be processed immediately (serotyping, susceptibility testing) and results will be entered into the database (Regional Laboratory Data Form to accompany isolates).

- On a weekly/monthly basis completed clinical forms will be faxed or sent to the central laboratory and entered into a central database by data clerks (Clinical Report Form). Options for clinical data entry at sentinel complexes to be discussed, once surveillance officers are trained and facilities are available, this will be done at the regional level.
- Study co-ordinators and surveillance officers will evaluate the database on a monthly basis and compare to monthly statistics generated from the DISA laboratory computer system. In addition they will summarize data for regular feedback
- Study co-ordinators will at other times be travelling to each center to assist in setting up the networks required to complete the questionnaires, help the surveillance officers plan their schedule with regard to data collection, laboratory review
- During these visits mini-audits for sensitivity of the surveillance system can be performed.
- Exclude all duplicate entries as identified by name, laboratory of isolation, and date of specimen.
- Repeat specimens from the same patient will be evaluated for type of organism and serotype/serogroup, as well as timing of second specimen *e.g.* same isolate within a period of 4 weeks, may be evaluated as **persistent** (2 to 7 days) versus **recurrent**



Possible hospitals/complexes:

1. Umtata
2. Bloemfontein Complex (initially 1 surveillance officer, workload will determine additional staff)
3. Chris Hani Baragwanath Hospital
4. Johannesburg General Hospital
5. Medunsa
6. Durban Complex (2 surveillance officers)
7. Nelspruit
8. Polokwane
9. Mafikeng
10. Cape Town Complex (2 surveillance officers)

Evaluation:

- Comparison of data collected in the same centers in the year preceding the project.

- ❖ Establish and track surveillance performance indicators (*e.g.* percentage of episodes with isolates tested; percent of cases reported within a threshold time period; percent of cases with data on HIV status and prophylaxis)
- ❖ Evaluate the ease and sustainability of good quality ongoing surveillance in the future: questionnaires to laboratory and clinical staff to improve and facilitate sustainable data capture and submission of forms and isolates; this will be to ensure simplicity, acceptability and flexibility of any future surveillance.
- ❖ Establish advisory committee with internal and external representation
- ❖ Presentation of data to HIV/AIDS clinicians and discuss possible changes in hospital and clinic policies if required.

Measures of Effectiveness:

- Distribution of information gathered to key players involved. Initially at an annual meeting with all involved in the surveillance, this includes the DOH, to carefully assess conclusions drawn and recommendations made. Then to widen the distribution of information to clinicians and laboratories at other centers, the SA HIV Clinicians Society, and other parties to be identified.
- Measures of effectiveness would include reviewing implementation of any changes recommended, acceptability and usefulness of data distributed as determined by the parties receiving the annual reports.

Appendix B – Case record form and informed consent

Clinical Case Report for Surveillance of Invasive *Haemophilus* spp, *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp, *Shigella* spp., *Cryptococcus* spp.

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

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

MYCOLOGY REFERENCE UNIT (MRU) TEL: 011 489 9341 / FAX: 011 489 9361

Regional Laboratory Specimen Number: ☐☐☐☐☐☐☐☐☐☐ Laboratory Name:

Hospital Name:

Hospital Number: | Ward: | Gender: M ☐ Unk ☐ F ☐ | Race: Asian ☐ Coloured ☐ Black ☐ White ☐ Unk ☐ | Date of Birth: d d m m y y y y DOB Unk ☐ Age: ☐☐☐ Units: days ☐ months ☐ yrs ☐

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

Address: Town/City: Province:

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

Have you stayed in SA for the last month: Yes ☐ No ☐ Unk ☐ If no, which country have you come from? _____

Date of admission to Acute Hospital: d d m m y y y y

Outcome at Acute Hospital: Transferred ☐ Discharged ☐ Died ☐ RHT/Absconded ☐ Unk ☐

If patient was transferred, name of hospital transferred to: _____ Date of Transfer: d d m m y y y y

Final outcome of patient at place of discharge: Discharged ☐ Died ☐ RHT/Absconded ☐ Unk ☐ Date of final outcome: d d m m y y y y

DIAGNOSIS

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

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

Site of collection CSF ☐ Blood Culture ☐ Joint Fluid ☐ Other _____

SPECIES ISOLATED

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

Number of children living with you: (<18 years)

None ☐ Number ☐ Place of safety ☐ Unk ☐

Have any of these children been hospitalised recently? (last 3 months) Yes ☐ No ☐ Unk ☐

SEVERITY OF ILLNESS (On the day the positive specimen was taken)

Temperature: ____°C Fever: Yes ☐ No ☐ Unk ☐ BP: ____/____ Unk ☐ Mechanical Ventilation: Yes ☐ No ☐ Unk ☐ Cardiac Arrest: Yes ☐ No ☐ Unk ☐

Mental Status: Alert ☐ Disorientated ☐ Stuporous ☐ Comatosed ☐ Unk ☐ GCS ____/15 E ___ M ___ V ___ Unk ☐

UNDERLYING DISEASES

HIV STATUS PRIOR TO THIS ADMISSION Positive ☐ Negative ☐ Unknown ☐

HIV STATUS AT THIS ADMISSION Positive ☐ Negative ☐ Unknown ☐

Pre & Post test counselling offered by SO Yes ☐ No ☐

If yes, was HIV consent given to SO Yes ☐ No ☐

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

Most recent CD4 count: Absolute ____ ; ____ % Date taken d d m m y y y y Unk ☐

Most recent viral load: _____ Date taken d d m m y y y y Unk ☐

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

- TB ☐
- Oral candidiasis ☐
- PCP ☐
- Wasting secondary to HIV ☐
- Chronic diarrhoea > 10 days ☐
- Kaposis Sarcoma ☐
- Current smoker ☐
- Sickle cell anaemia ☐
- Splanectomy/asplenia ☐
- Immunoglobulin deficiency ☐
- Immunosuppressive therapy (Steroids, Chemotherapy, Radiation) ☐
- Kwashiokor/marasmus ☐
- Asthma ☐
- Emphysema/COPD ☐
- Diabetes mellitus ☐
- Nephrotic syndrome ☐
- Chronic renal failure ☐
- Systemic Lupus Erythematodus (SLE) ☐
- Cirrhosis/Liver failure ☐
- Alcohol dependancy ☐
- Coronary artery disease ☐
- Valvular disease ☐
- Heart failure ☐
- Burns ☐
- Cerebral vascular accident (CVA)/ Stroke ☐
- History of head injury/ head surgery ☐
- Hydrocephalus with VP shunt ☐
- Malignancy (specify) ☐ _____
- Organ transplant (specify) ☐ _____
- Other (specify) ☐ _____

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

Clinical Case Report for Surveillance of Invasive *Haemophilus* spp, *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp, *Shigella* spp., *Cryptococcus* spp.

RESPIRATORY AND MENINGEAL PATHOGENS RESEARCH UNIT (RMPRU) TEL: 011 489 9710 / FAX: 011 489 9715

ENTERIC DISEASES REFERENCE UNIT (EDRU) TEL: 011 489 9333 / FAX: 011 489 9351

MYCOLOGY REFERENCE UNIT (MRU) TEL: 011 489 9341 / FAX: 011 489 9351

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

VACCINATION STATUS FOR *STREPTOCOCCUS PNEUMONIAE*

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

DOSE	DATE GIVEN (d d m m y y y y)	NAME OF CLINIC
1	☐☐☐☐☐☐☐☐	_____
2	☐☐☐☐☐☐☐☐	_____
3	☐☐☐☐☐☐☐☐	_____

Has patient received 23-valent pneumococcal polysaccharide vaccine?

Yes ☐ No ☐ Unk ☐

If YES, list date most recently given and vaccine name

d d m m y y y y ☐☐☐☐☐☐☐☐ _ _ _ _ _ _ _ _ _ _

HAEMOPHILUS INFLUENZAE

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

DOSE	DATE GIVEN (d d m m y y y y)	NAME OF CLINIC
1	☐☐☐☐☐☐☐☐	_____
2	☐☐☐☐☐☐☐☐	_____
3	☐☐☐☐☐☐☐☐	_____

Was there documented proof of vaccination for: *Haemophilus influenzae* type b (Hib) vaccine?

Yes ☐ No ☐ Unk ☐

OTHER VACCINATIONS

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

ANTIBIOTICS PRIOR TO THIS ADMISSION

Cotrimoxazole prophylaxis Yes ☐ No ☐ Unk ☐	Dose: Route:		Date initiated: d d m m y y y y ☐☐☐☐☐☐☐☐	Compliant in last month: Most ☐ Some ☐ None ☐
ABX in 24 hours before specimen: Yes ☐ No ☐	Names: 1 _____ 2 _____ 3 _____ 4 _____	Dose: _____ _____ _____ _____	Route: _____ _____ _____ _____	Date initiated: d d m m y y y y ☐☐☐☐☐☐☐☐
Other ABX in 2 months Yes ☐ No ☐ Unk ☐	Names: 1 _____ 2 _____	Dose: _____ _____	In the last 30 days: Yes ☐ No ☐	In the last 30 to 60 days: Yes ☐ No ☐
TB Rx Yes ☐ No ☐ Unk ☐	Drugs: 1 _____ 2 _____	Dose: _____ _____	Date initiated: d d m m y y y y ☐☐☐☐☐☐☐☐	Compliant is last month: Most ☐ Some ☐ None ☐

ANTIBIOTIC USE IN HOSPITAL DURING THIS ADMISSION (excluding TB therapy)

	Name of antimicrobial	Dose	Route	Date initiated (d d m m y y y y)	Total doses given/no. of days
1	_____	_____	_____	☐☐☐☐☐☐☐☐	_____
2	_____	_____	_____	☐☐☐☐☐☐☐☐	_____
3	_____	_____	_____	☐☐☐☐☐☐☐☐	_____
4	_____	_____	_____	☐☐☐☐☐☐☐☐	_____
5	_____	_____	_____	☐☐☐☐☐☐☐☐	_____

ANTIRETROVIRAL USE

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

Current Antiretroviral therapy: 3TC ☐ D4T ☐ Efavirenz ☐ Nevirapine ☐ AZT ☐ DDI ☐ Kaletra ☐ Unk ☐ Duration: Months _ _ _ _ _ _

Clinical Case Report for Surveillance of Invasive *Haemophilus* spp, *S. pneumoniae*, *N. meningitidis*, *Salmonella* spp, *Shigella* spp., *Cryptococcus* spp.

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MYCOLOGY REFERENCE UNIT (MRU) TEL: 011 489 9341 / FAX: 011 489 9361

ANTIFUNGALS PRIOR TO THIS ADMISSION (For crypto isolates ONLY)

		Date initiated	Dose:
Fluconazole:	Yes ☐ No ☐ Unk ☐	If yes d d m m y y y y	
Amphotericin B	Yes ☐ No ☐ Unk ☐	If yes d d m m y y y y	

ANTIFUNGALS DURING THIS ADMISSION

	Dose	Route	Date initiated	Total doses given/no. of days
Fluconazole:			d d m m y y y y	
Amphotericin B			d d m m y y y y	

On discharge was the patient given fluconazole? Yes ☐ No ☐ Unk ☐

Sources of data: Patient/Guardian ☐ Clinician ☐ Medical Records ☐

Has regional laboratory sent isolate to RMPRU/EDRU/MRU Yes ☐ No ☐ Date isolate sent d d m m y y y y

SURVEILLANCE OFFICER NAME: _____

Title of project: *Enhancement of Surveillance for Trimethoprim-Sulfamethoxazole Resistant Invasive Respiratory and Diarrhoeal Disease in South Africa*

Patient information sheet:

Hello. My name is ___*(name of surveillance officer or clinician at each centre)*____and I would like to ask you for a little of your time to explain something to you, and ask you to please assist us in some work we are doing. As we discuss the information below please feel free to ask any questions.

In South Africa and elsewhere in the world, hospital laboratories and the Department of Health look at specific diseases making people sick. By doing this so-called "surveillance", we count how many people get sick and collect anonymous details about patients to help in the control and prevention of these diseases. Antibiotics/medicines can be used to prevent the diseases, so can vaccines, and when the number of cases increases the health community can be prepared. We are at present doing a study that will be looking at the use of certain antibiotics/medicines in the community and how it affects the germs that infect people. We want to see if there is an increase in germs that will not be treated by antibiotics/medicines that we normally use.

As part of our surveillance we keep information about the infection that you have. We would like to make sure that all the information we have about you is correct and then ask a few more questions about use of antibiotics. We will keep this information confidential, no one else will know that it is about you and all summaries or publications will only refer to group data. We would also like to ask you to volunteer to have an HIV test done, but this will only be done once you have received full pre- and post-test counselling and are given all the details that you require to make a decision about taking a test. Even if you decide not to take the HIV test, we would still like to ask you a few questions, if you agree.

You can make the decision entirely on you own and none of us can force you to take part. If you decide to answer some of these questions, you may also change your mind at any time.

You do not have to agree, and if you decide not to be involved it will not change the way you are treated in the hospital, and your doctor will not do anything differently.

Thank you for your time. Once you have asked any questions you may have, there is a form you need to sign if you agree to take part.

Surveillance officer details.

Details of clinician at centre.

Title of project: *Enhancement of Surveillance for Trimethoprim-Sulfamethoxazole Resistant Invasive Respiratory and Diarrhoeal Disease in South Africa*

Patient assent form:

Hello. My name is ___*(name of surveillance officer or clinician at each centre)*____and I would like to ask you something. I will need to explain what it is we would like to do. You can help us a little if you listen and tell us what you think.

We want to ask you and your mother/father/caregiver some questions about you and the sickness you have. We will ask questions about when you were sick and about what medicines you took before you came to hospital. We want to try and see if these germs are causing more sickness in South Africa and if they are becoming more difficult to treat.

Would it be alright for us to ask the above questions? When we work with this information we leave out your name.

You must not worry if you do not want to answer any questions, nothing will change in how the doctor is taking care of you.

Surveillance officer details.

Details of clinician at centre.

Title of project: *Enhancement of Surveillance for Trimethoprim-Sulfamethoxazole Resistant Invasive Respiratory and Diarrhoeal Disease in South Africa*

Informed consent form:

I have read and/or I understand the contents of the information sheet and understand that I have been invited to participate, that my agreeing is fully voluntary, and that I can withdraw at any time.

Consent given:	Date:
Witness:	Date:

Appendix C – Ethics approval letters

e. | APPROVAL Posted

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\Iain0015\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

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Nanoo

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M071028**
PROJECT	Is There an Association between Trimethoprim-Sulfamethoxazole Data Analysis of Antibiotic
Co_Resistance Data	in South Africa 2003-2005
INVESTIGATORS	Ms A Nanoo
DEPARTMENT	Reproductive health Res Unit
DATE CONSIDERED	07.10.26
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY

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

DATE 07.10.30 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr K Keddy

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